

大学共同利用機関法人  
自然科学研究機構

基礎生物学研究所

外部点検評価報告書  
第二部



2007



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はじめに

平成19年度の自然科学研究機構基礎生物学研究所の外部点検評価報告書別冊（第二部）をお送りします。

第二部には、平成19年度におこなった主要な事業のうち、在職10年を迎えた3名の教授の業績評価の結果を掲載しました。評価対象となった教授は、長濱嘉孝教授、上野直人教授、諸橋憲一郎教授、の3名です。また、この冊子には、参考資料として、平成17年－19年に各研究室において発表した論文リスト、および研究所の活動を分かり易い形で社会と研究者コミュニティに伝える助けとするために新たに作成した和文と英文のパンフレットを綴じ込みました。

第一部には、ほぼ十年ぶりに研究所のすべての研究室主宰者に対して、外国人を含む多数の研究者に依頼して実施した外部評価の実施状況と結果を掲載しています。

平成19年度の外部点検評価報告書の第一部と第二部をともにご一読いただき、基礎生物学研究所の運営と活動についてのご意見ならびにご支援を頂ければ誠にありがたく存じます。

平成20年5月

基礎生物学研究所  
所長 岡田清孝



1-1. 在職10年の教授業績評価について

長濱嘉孝教授







## 長濱嘉孝教授在職10年業績評価

基礎生物学研究所 所長 岡田清孝

### 1. 経緯

- ・平成19年5月 基礎生物学研究所所長および教授3名からなる在職10年教授業績評価実行委員会を設置。
- ・平成19年10月 長濱教授の研究分野に近い所外研究者から海外3名、国内2名を選んで評価委員を委嘱し、資料を送付。送付資料 1) 研究活動の説明、2) 研究業績リスト、3) 主たる業績の論文別刷、4) 略歴
- ・平成20年1月 海外3名、国内2名の評価委員全員から回答を受け取った。

### 2. 評価内容

#### A委員による研究内容のまとめと評価（一部抜粋）

長濱嘉孝教授は、「卵成熟」「性分化」という互いに関連した動物の発生の基本過程について、魚類の生物学的な特性を十二分に活用した、そして厳しい一貫性を保った研究をすすめてきた。研究は、多面にわたるが、決して雑多なものではなく、二課題に収束している。上記の二過程の制御にかかわる分子基盤を徹底的に追求したものであり、科学者の姿勢として範たるものである。

今回の評価は、最近10年間の研究が対象となっているが、長濱教授の1985年の目覚ましい成果、すなわち卵成熟促進因子（MIH）を脊椎動物で初めて同定し、それが  $17\alpha, 29\beta$ -DP であることを示したことが現在の研究の発展の源流である。現在の研究は、その発見を種として育ち繁った、双幹の大樹である。

性分化については、まず、XY型の（雄決定型の）性決定を行う日本メダカの性決定遺伝子がDMYであることを示したのが大きな breakthrough であったが、研究がそこに留まらなかったのが、長濱教授の研究の真骨頂である。近縁メダカを含む他の魚類を研究して、DMYに依存する性決定機構の一般性を問うた。性決定の上位因子は動物種ごとに多様であること、そして性分化制御に一般性を持つ保存された機構は、より下流のプロセスであることを示した。一般性が期待される機構には、DMRTの作用や、教授のグループが発見したGSDF (Gonadal Soma Derived Factor)の発現、またP450c17-II ( $17\alpha, 29\beta$ -DPの産生に関与する)の活性化などである。複数の魚類における対応過程を比較検討して一般性をもった結論を導くという、一貫した研究の姿勢には敬服する。（中略）

長濱教授は、上記の研究を更に発展させて新しい研究に着手している。オキナワベニハゼで魚個体の社会的地位に依存しておきる性転換-自然現象としての可塑的な性転換-と、メダカ・テイラピア個体で人為的に生起させた性転換過程を比較することによって、性転換の基本機構を明らかにすること、更に、それと共役する脳の性分化の機構（性同一性障害などのヒトの社会性をもった問題に直結する）の研究である。これらの研究はずでに佳境に達している。長濱教授がこれらに的を絞って、残任期間に実施する研究目標としているのは慧眼である。斬新な研究成果が期待される。

長濱教授は、もう一つのライフワークである卵形成の多段階機構の解明について、最近の10年間で一つの研究の完成をみた。魚類を用いて、性腺刺激（内分泌）→卵成熟開始因子（濾胞細胞が分泌するMIH）→卵成熟促進因子（卵のMPF）→排卵、という各ステップにかかわるシグナル因子の物質的な実体、その因子の作用に関わる受容体、そのシグナルの作用に関わる遺伝子制御を詳細にわたって解明した。そして、それらの分子過程をヒトデの場合と比較した。（基礎生物学研究所がその歴史に誇る1-メチルアデニンの研究の現代的な復活）。そして、細胞間のシグナル伝達因子には、動物種ごとに異なった物質が採用されるが、（例えばMIHが魚類では $17\alpha, 29\beta$ -DPでいるのに対して、ヒトデでは1-メチルアデニン）、細胞内の過程や細胞種ごとの機能分担は極めて普遍的であることを示した。（中略）

長濱教授の研究成果は、魚類の特性をもとにして、性の成立と卵形成という生物界の一般論を掘り下げたもので、社会的なインパクトは極めて大きい。魚類の養殖、性同一性障害、不妊、環境ホルモンなどの問題とのかかわりは卑近な具体例である。このような実験動物種の選択と問題設定は、基礎生物学研究所の在り方として重要であり、一つの模範である。

#### B委員による評価（一部抜粋）

生殖は子孫を残すための生物の最も基本的で重要な生命活動の一つである。長濱教授は「生殖の制御機構の解明」をライフワークとされ、常に独自の発想からの先進の研究手法を駆使した先導的研究を精力的に推進しておられます。長濱教授は、魚類を主な研究材料として、「性決定と性分化」、「卵の形成と成熟」、「精子の形成と成熟」などを制御する制御因子の同定及びそれら因子の生合成制御機構と作用機構の解明に重点を置き、輝かしい研究成果を挙げておられる。さらに、それらの基礎的知見をもとに、内分泌かく乱化学物質の生殖現象への影響や作用機構についての応用研究も手掛けられ、大変貴重な研究成果を報告されておられる。特に、長濱教授の最近10年間の研究の進展は著しく、研究成果は*Nature*, *Proc. Natl. Acad. Sci. USA*, *J. Biol. Chem.*, *J. Cell Biol.*, *Endocrinology*, *J. Mol.*

*Endocrinol.*など多数の *first rank journals* に合わせて 130 編の原著論文として出版されている。また、国際的に評価が高い国際会議・国際シンポジウムにおいても基調講演・招待公演を数多くなされている。これらの卓越した研究業績に加えて、最近では自然科学研究機構・基礎生物学研究所の副所長として、長濱教授は指導的立場から基礎生物学の中核機関のより一層の充実と発展に尽力されておられる。さらに、長濱教授は Editor-in-Chief あるいは Executive Editor として複数の国際誌の編集に尽力されると共に、国際比較内分泌学会連合の会長、日本動物学会の副会長などの指導的立場から、関連学会の発展にも尽力されておられる。(中略)

以上の素晴らしい研究業績により、長濱教授は 2004 年に Howard A. Bern Lecture Award (Society for Integrative and Comparative Biology, USA)、2007 年に Richard E. Peter Lecture Award (International Symposium on Fish Physiology, Canada) を受賞されている(最近の 10 年間)。

#### C 委員による評価 (一部抜粋)

In summary then I can only praise in my highest words the outstanding contribution that Dr. Nagahama has made in his research field. He has had during the last ten years a major impact on the development of the field of fish reproductive biology. He is an outstanding scientist contributing to all ongoing discussions and certainly a leading expert in the field. His research activities can be summarized in one word, namely outstanding.

#### D 委員による評価 (一部抜粋)

I had personally the opportunity to attend several of the Plenary Lectures given by Prof. Nagahama. It is always a great learning experience listening to his presentations. I feel both respect and admiration for his scientific achievement, and I have no doubt that he will continue to contribute to the advancement of science in many different ways and, as an eminent scientist, to produce ground-breaking work in the future.

Therefore, it is without any hesitation that I give the highest appreciation on Prof. Nagahama's research activity.

#### E 委員による評価 (一部抜粋)

Professor Nagahama has been extraordinarily productive over the past ten years and has brought genuine insights into his chosen fields of research. In addition, he has actively participated in a range of scientific societies and endeavors. I can only conclude by saying he is deserving of the highest commendation for his research achievements.

### 3. まとめ

長濱教授は、昭和 52 年に基礎生物学研究所に赴任して以来一貫して魚類を用いた「卵成熟」および「性分化」の機構の解明に努力してきた。最近の 10 年間においても、メダカの性決定遺伝子を同定し、卵形成の多段階機構を詳細に解明するなど、この分野の世界的な研究リーダーとしての地位を確立した。基礎生物学研究所においても副所長として所内意見のとりまとめに努力された。さらに、National Medaka BioResource Project の第一期運営委員長として努力され、平成 19 年度から基礎生物学研究所が第二期の中核拠点として活動することになった。なお、長濱教授は平成 20 年 3 月末をもって定年退職し、基礎生物学研究所名誉教授および総合研究大学院大学名誉教授の称号を受けた。平成 20 年 4 月より基礎生物学研究所特任教授に任用された。今後も研究の一層の発展と基礎生物学研究所への助力を期待する。

**Research activity  
(1997-2007)**

**Yoshitaka Nagahama**

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## **1. Statement of research activity in the past 10 years**

My research interests are broadly directed at reproductive biology and the endocrine control of reproduction in vertebrates and invertebrates. From a practical point of view, however, my research during the last 10 years has focused on two events: (1) sex determination/gonadal sex differentiation, sex change and sexual plasticity, and (2) oocyte maturation and ovulation.

### **1) Molecular mechanisms of sex determination/gonadal sex differentiation, sex change, and sexual plasticity.**

In vertebrates, sex determination is a key event for the development of either testis or ovary. In fish, sexual characteristics and gonadal development vary from gonochoristic species to several types of hermaphroditism (**Devlin and Nagahama, 2002**). Thus, the sex determination and gonadal sex differentiation in fish will broaden our understanding of these processes beyond the specific details found within the group. We are investigating the molecular mechanisms of sex determination and gonadal sex differentiation using two fish species, the medaka, *Oryzias latipes* (sex determination) and tilapia *Oreochromis niloticus* (gonadal sex differentiation). We are particularly interested in identifying genes or hormonal factors that may regulate the early development of fish gonads. We are also investigating the mechanisms of sex change and sexual plasticity using sex-changing fishes and adult gonochoristic fishes.

#### **A) Sex determination**

In addition to its small size and short generation time, the medaka has two major advantages for genetic research: a large interstrain diversity within the species and the existence of several inbred strains. As in mammals, sex determination in medaka is male heterogametic (a stable genetic XX/XY sex determining system). Using positional cloning and detailed sequence analysis of BAC clones (**Matsuda et al. 2001**) by shotgun sequencing, we located a unique gene in the short sex-determining region on the Y chromosome. This gene consists of six exons and encodes a protein of 267 amino acids including the highly conserved DM domain. The DM domain was named after a related DNA binding motif found in two proteins, doublesex (*dsx*) and *mab-3*, involved in sexual development in *Drosophila* and *C. elegans*, respectively. This Y-specific DM-domain gene was named *DMY* (DM-domain gene on the Y chromosome) (**Matsuda et al. 2002**), or *Dmrt1b* (Nanda et al. 2002).

Two naturally occurring XY female medaka showed different mutations in the *DMY* gene (**Matsuda et al. 2002**). One of these mutants was found to carry a mutation

causing a frameshift and premature termination of the DMY protein. When mated, all XY offspring with the mutant Y were female. The other mutant had a severe depression in *DMY* expression in the embryo and 60% of its XY offspring with the mutant Y developed as females. Taken together, the loss-of-function mutant and the depressed expression mutant strongly suggest that DMY is required for normal testicular development. More recently, we demonstrated that a genomic DNA fragment carrying *DMY*, containing about 56 kb of the coding region, was sufficient to induce testis differentiation and subsequent male development (**Matsuda et al. 2007**). These data demonstrate that DMY is sufficient to induce male development in XX medaka. It is important to note that *DMY* transgenic XX medaka are fully functional and fertile males, whereas *Sry* transgenic mice are sterile (Koopman et al. 1991). Thus, medaka is the first transgenic vertebrate shown to undergo complete sex reversal. Taken together, these loss- and gain-of-function studies indicate that DMY is the sex-determining gene of medaka. *DMY* (**Matsuda et al. 2002**) or *Dmrt1b* (Nanda et al. 2002) is thus the first sex-determining gene to be found in nonmammalian vertebrates.

Y chromosome-linked *DMY* appears to have originated from a duplicate copy of autosomal *DMRT1* (DM-related transcription factor 1), another DM domain gene that is most homologous to *DMY* and is involved in male development in various vertebrates (Lutfalla et al. 2003; Nanda et al. 2002). Then, the duplicated *DMRT1* in the Y chromosome acquired a new function as a sex-determining gene, *DMY* (Matsuda 2005). Further, *DMY* is also found in *Oryzias curvinotus*, which is most closely related to medaka (**Matsuda et al. 2003**), but is not found in other *Oryzias* species or in other fishes (Kondo et al. 2003). Importantly, there is no sequence homology between two known sex-determining genes, *SRY/Sry* and *DMY* (Sinclair et al. 1990; **Matsuda et al. 2002**; Nanda et al. 2002). Taken together, these findings suggest that the factor at the top of the cascades responsible for sex determination exhibits extensive diversity among vertebrates.

Till to date, no targets could be identified for DMY to delineate its mechanism of action. The expression patterns of *DMRT1* suggested that it could be a possible target of DMY, but it starts to display sexually dimorphic expression pattern around 20 dah only (**Kobayashi et al. 2004**). This has ruled out the possibility of *DMRT1* as the target gene of DMY. Therefore, we continued our search for the targets and through cDNA subtraction analysis followed by microarray hybridization we could find a candidate gene, *Gonadal Soma Derived Factor (GSDF)* (**A. Suzuki, H. Kaneko, Y. Nagahama, et al., unpublished**). The initial expression analysis revealed that *GSDF* is sex-specifically up regulated in the males from the day of hatching, while its expression is lower in the females. The expression is found to be specific to the Sertoli cells (in both medaka and tilapia). We are now trying to generate medaka transgenic for *GSDF* to understand more about its function and relationship with DMY, if any.

## **B) Gonadal sex differentiation**

In tilapia, all genetic female (XX) or male (XY) broods can be obtained by artificial fertilization of normal eggs (XX) with sex-reversed, pseudo male sperm (XX), or normal eggs (XX) with super male sperm (YY), respectively (**Nakamura et al. 1998**). Through cDNA subtraction between XX and XY gonads during sex differentiation and microarray hybridization followed by gene expression analyses by RT-PCR and *in situ* hybridization, we have concluded that in tilapia, *Cyp19a1/Foxl2* plays a crucial role in ovarian differentiation and *DMRT1* in testicular differentiation (**Kobayashi et al. 2003; Ijiri et al. 2007**). The transcripts of aromatase (there are two forms of aromatase in teleost fishes; the ovarian form (*Cyp19a1*) and brain form (*Cyp19a2*) and *Foxl2* were expressed only in XX gonads at 5 dph, with a marked elevation in expression during the next two days. Fadrozole (aromatase inhibitor, AI) or tamoxifen (estrogen receptor antagonist) caused complete sex reversal of XX fry to functional males, confirming that endogenous estrogens are critical for directing initial ovarian differentiation in tilapia (**Kobayashi et al. 2003**). In XY tilapia fry, *DMRT1* gene is expressed male-specifically in testicular Sertoli cells prior to and during sex differentiation (**Guan et al. 2000; Kobayashi et al. 2007**). XX tilapia carrying extra copies of tilapia *DMRT1* as a transgene induced various degrees of gonadal changes including complete sex change to testis. It is of great interest to note that some of the sex reserved XX tilapia produced sperm with extremely high motility (**Wang et al. unpublished**).

We then investigated the plausible role of *Foxl2* in ovarian differentiation through transcriptional regulation of aromatase gene (*Cyp19a1*), using mono-sex fry of tilapia (**Wang et al. 2004, 2007**). *Foxl2* expression, like that of *Cyp19a1*, is sexually dimorphic in gonads prior to the occurrence of morphological sex differentiation, co-localizing with *Cyp19a1* and *Ad4BP/SF-1* in the stromal cells and interstitial cells in gonads of normal XX and sex-reversed XY fish. Under *in vitro* conditions, *Foxl2* binds to the sequence, ACAAATA in the promoter region of the *Cyp19a1* gene directly through its forkhead domain (FH), and activates the transcription of *Cyp19a1* with its C-terminus. *Foxl2* can also interact through the FH with the ligand binding domain of *Ad4BP/SF-1* to form a heterodimer and enhance the *Ad4BP/SF-1* mediated *Cyp19a1* transcription. Disruption of endogenous *Foxl2* in XX tilapia by over-expression of its dominant negative mutant induces varying degrees of testicular development with occasional sex reversal from ovary to testis. These results suggest that *Foxl2* plays a decisive role in the ovarian differentiation of tilapia by regulating aromatase expression.

We also investigated the role of *DMRT1* in gonadal sex differentiation. Promoter analysis by luciferase assays revealed that *DMRT1* repressed the basal as well as *Ad4BP/SF-1* activated and *Foxl2* enhanced *Cyp19a1* gene transcription in HEK 293 cells. Luciferase assays with various deletion mutations of *DMRT1* revealed that *DM*



domain is essential for the repression. *In vitro* translated DMRT1 and testis nuclear extraction could directly bind to the palindrome sequences of TACATATGTA on the *Cyp19a1* promoter by EMSAs. DMRT1 can form heterodimers with Ad4BP/SF-1 and Foxl2 by direct physical interaction as revealed by pull down assays and mammalian two-hybrid assays. Transgenic over-expression of *DMRT1* in XX fish resulted in decreased aromatase gene expression, reduced serum estrogen levels, retardation in ovarian cavity formation, varying degrees of follicular degeneration and even partial to complete sex reversal. These findings suggest that *aromatase* is one of the target genes of DMRT1. DMRT1 suppresses the female pathway by repressing aromatase gene transcription and estrogen production in the gonad of tilapia and possibly other vertebrates (**Wang et al. submitted, under revision**).

In addition, we recently discovered a novel type of P450c17 (P450c17-II) which is involved in the production  $17\alpha$ ,  $20\beta$ -DP, the maturation-inducing hormone of fish (**Zhou et al. 2007a**). The expression pattern of P450c17-II transcripts in tilapia and medaka (for example, its first appearance at 10-20 days after hatching (dah) in XX tilapia and 70 dah in XY tilapia) suggests that P450c17-II might also be involved in the initiation of meiosis during early sex differentiation in these fishes (**Zhou et al. 2007b**). Further studies on these lines are expected to reveal whether  $17\alpha$ ,  $20\beta$ -DP or another C-21 steroid is involved in the initiation of meiosis and thus, the sex differentiation.

### **C) Sex change**

Teleost fishes display the greatest diversity of sexual phenotypes. Of the many fish species that are capable of socially mediated sex change, the marine goby (*Trimma okinawae*), having ovarian and testicular tissues simultaneously in its gonad, is one of only four species known to be able to change sex repeatedly in both directions depending on its social surroundings, thus providing an excellent animal model to investigate molecular mechanisms of sex-change (**Kobayashi et al. 2004, 2005a, 2005b**). Recently, we elucidated the involvement of gonadotropins in sex change by determining the changes in gonadotropin receptor (GtHR) gene expression during the onset of sex changes from female to male and male to female (**Kobayashi et al. unpublished**). The expression of the *GtHRs* was found to be confined to the active gonad of the corresponding sexual phase. When the sex change was occurring from female to male, initially the ovary had high levels of *FSHR* and *LHR*, which eventually went up in the testicular tissue once the fish had realized the fact that it was bigger than the other in the aquarium. Opposite of this scenario was observed if another fish bigger than the newly sex-changed male was introduced into this aquarium. Swapping of the gonads started with switching of the *GtHR* expression that was discernible within 8-24 hrs of the visual cue. Further *in vitro* culture of the transitional gonads with a supply of

exogenous gonadotropin (hCG) revealed that the to-be-active gonad acquired the ability to produce the corresponding sex hormone within one day of the activation of GtHR. Conversely, the to-be-regressed gonad did not respond to the exogenous gonadotropin, demonstrating the absence of *GtHR* expression.

These findings suggest that the switching in the *GtHR* expression is an indication of the active gonad's responsiveness to endogenous gonadotropin and the linchpin of successive sex-reversal. With all probabilities the brain of the bigger fish might change its own sex first and then transduce the signal through an unidentified channel to the corresponding gonad to switch the *GtHR* on. We could identify two genes, *Cyp19a2* and *isotocin*, specifically expressed in the adult female and male brains, respectively. Our preliminary experiments on the expression pattern of these genes during the sex change showed that *Cyp19a2* mRNA levels increase within 6 hrs of the initiation of sex change from male to female, suggesting an important role for brain estrogen in the process of sex change. Successive sex changing fish like *T. okinawae* is an excellent animal model to elucidate the mysterious role of brain in bringing out the sexuality of an individual and also for the depiction of sexual plasticity at the organismal level.

In addition to *T. okinawae*, we also use medaka to study the sex determination/differentiation and sexual plasticity of the brain. Through cDNA subtraction between male and female brains and microarray hybridization, we could short-list seven male- and five female-specific genes, important for the sex differentiation of the brain. Among these, the most promising candidates are *DMY* (Ohmuro-Matsuyama et al. 2003) and the Y-specific copy of the gene *Snply*, which is also present in the autosomal chromosome of both males and females (Okubo et al. unpublished). We are investigating the sex differentiation and/sexual plasticity of the juvenile and adult medaka brain using these genes as molecular markers. These genes will be useful tools to approach the problem from various directions. We have also generated medaka transgenics with some of these genes.

#### **D) Sexual plasticity in adult fish**

With the exception of certain hermaphroditic species, most vertebrate species are thought to have lost their sexual plasticity after differentiation of separate gonads/sexes with a single, distinct gamete type (gonochorism) (Devlin and Nagahama, 2002). Recently, we treated adult female tilapia with fadrozole (AI, a non-steroidal aromatase inhibitor) to block the conversion of androgens to estrogens, for two to five months to investigate whether sexual plasticity is retained in the adult gonochoristic fish (Nakamura et al. unpublished). Suppression of estradiol-17 $\beta$  (E2) production via AI treatment caused a rapid degeneration of primary oocytes, leading to testicular germ cell differentiation in the adult ovary. Expression of the genes for the steroidogenic enzymes

like, *3 $\beta$ -HSD* and *P450SCC* and the reduced levels of *Cyp19a1* along with a rise in the expression of the male-specific marker *DMRT1* indicate the differentiation of testicular type of somatic cells in the AI-treated gonads. Sex-changed fish show a typical male pattern of reproductive hormone levels (E2, 11-ketotestosterone) and secondary sex characteristics, producing fertile sperms in the newly formed testes. Additionally, these fish display male-specific territorial behavior, pointing towards the changes that might have occurred to the sex-specific neuronal circuits in the brain. Conversely, co-treatment of E2 inhibited AI-induced sex reversal. Our results demonstrated for the first time in any gonochoristic species that tilapia retains its sexual plasticity even in the adult stage. Furthermore, these data indicate that estrogens are vital to the maintenance of female phenotype in the gonochoristic species.

## 2) Hormonal regulation of oocyte maturation and ovulation

A period of oocyte growth is followed by a process called oocyte maturation (the resumption of meiosis) which occurs prior to ovulation and is a prerequisite for successful fertilization (**Nagahama 1994**). Oocyte maturation has been studied in a variety of vertebrates and invertebrates including mammals, amphibians, fishes, and starfishes, but the endocrine regulation of oocyte maturation has been investigated most extensively in fishes (**Nagahama 1977**). Our studies using vertebrate (fishes) and invertebrate (starfishes) models have revealed that the basic mechanisms involved in oocyte maturation are the same in these two species, despite the differing chemical nature of the hormonal agents involved. In both species, three major mediators have been shown to be involved: gonad-stimulating substance (GSS), 1-methyladenine (1-MeAde) (maturation-inducing hormone, MIH), and maturation-promoting factor (MPF) in starfish, and gonadotropin (GtH, LH), 17 $\alpha$ , 20 $\beta$ -dihydroxy-4-pregnen-3-one (17 $\alpha$ , 20 $\beta$ -DP), and MPF in fish (Fig. 1).

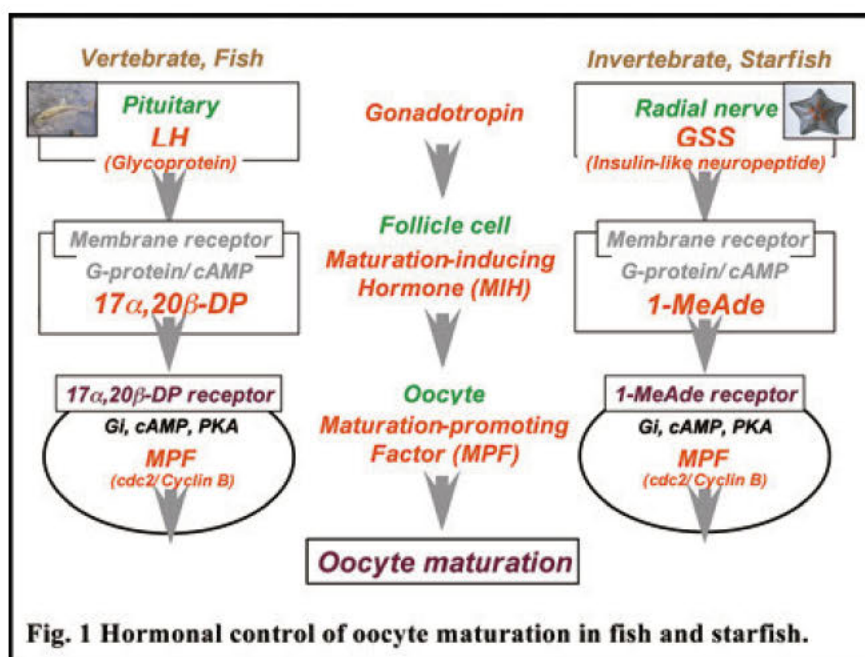
The starfish GSS present in the radial nerves of starfish is the only known invertebrate peptide hormone responsible for final gamete maturation in both ovaries and testis, thus corresponding functionally to the vertebrate gonadotropins. Despite much effort, however, attempts to determine the chemical structure of the GSS have been unsuccessful. We have recently succeeded in purifying and determining the complete amino acid sequence of GSS in starfish, *Asterina pectinifera* (**M. Mita, M. Yoshikuni, Y. Nagahama et al. unpublished**) and showed that starfish GSS is a relaxin-like heterodimeric peptide with a molecular weight of 4737, consisting of A and B chains; the A chain contains 24 residues and the B chain 19 residues. The GSS cDNA encodes a prehormone sequence with a typical cysteine motif of the insulin/IGF/relaxin family in the A-chain. Chemically synthesized GSS is as active as natural GSS in the homologous *in vitro* GVBD assay. Starfish with fully-grown testes

or ovaries started to show spawning behavior and subsequently released fully mature gametes within 30 minutes of the injection of the peptide. A dose-response stimulation of cAMP and 1-MeAde production was observed when isolated follicle cells were incubated with the synthetic heterodimeric GSS peptide. While the precise neural source of these peptides and their physiological roles have not yet been defined, structural identification of starfish GSS opens up a number of avenues of investigation into the neurohormonal control of reproduction in invertebrates including echinoderms.

In fish, LH acts on ovarian follicle cells to produce fish MIH. It was in 1985 that we identified, for the first time in any vertebrate species,  $17\alpha$ ,  $20\beta$ -DP as the MIH of amago salmon, *Oncorhynchus rhodurus* (Adachi and Nagahama, 1985, Dev. Biol.). Later, this progestin was found to be the common MIH among various fish species (Nagahama 1994, 1997).  $17\alpha$ ,  $20\beta$ -DP is synthesized by a two-step process involving two ovarian cell layers, the thecal and granulosa cells. There is a distinct shift in follicular steroidogenesis from estradiol- $17\beta$  (E2) during oocyte growth (vitellogenesis) to  $17\alpha$ ,  $20\beta$ -DP during oocyte maturation. This occurs in two stages, the first being the shift in the synthesis of precursor steroids in thecal cells, while the other is the shift in the final steroidal mediator production in granulosa cells. We have already shown that there is a distinct shift in the steroidogenic enzyme genes from ovarian aromatase (Cyp19a1) to  $20\beta$ -hydroxysteroid dehydrogenase ( $20\beta$ -HSD), occurring in the granulosa cells of ovarian follicles prior to oocyte maturation (Guan et al. 1999; Guan et al. 2000; Tanaka et al. 2002; Senthilkumaran et al. 2002; 2004; Yoshiura et al. 2003). The triggering of the steroidogenic shift by GtHs in granulosa cells occurs through the subjugation of Ad4BP/SF-1 expression with respect to aromatase, followed by an over-expression of  $20\beta$ -HSD probably through CREB (Watanabe et al. 1999; Yoshiura et al. 2003; Senthilkumaran et al. 2004).

The major remaining question was the differential availability of precursor steroid,  $17\alpha$ -hydroxyprogesterone. This was essentially because till to date a single enzyme P450c17, possessing  $17\alpha$ -hydroxylase and 17, 20 lyase activities to mediate the production of estrogen and  $17\alpha$ ,  $20\beta$ -DP has been described among the vertebrates in general. To complicate the scenario further, this enzyme was found to be encoded by a single gene. However, there were some studies that could not correlate the expression pattern of this gene to the production of  $17\alpha$ ,  $20\beta$ -DP in fish, leaving its mechanism of action as a puzzle. Recently, we discovered a novel type of P450c17 (P450c17-II) lacking the lyase activity in several teleost species, and showed in both medaka and tilapia that P450c17-II, but not P450c17-I, is responsible for the shift in precursor steroid from testosterone to  $17\alpha$ -hydroxyprogesterone (Zhou et al. 2007a, 2007b). Thus, our studies have resolved a long-standing question in the field of steroidogenesis with respect to oocyte maturation (Nagahama and Yamashita 2007).

The site of the action of 1-MeAde and  $17\alpha$ ,  $20\beta$ -DP is the surface of the oocytes (Nagahama 1997; Nagahama and Yamashita 2007). A distinct family of G-protein-coupled membrane-bound progestin receptors (mPRs) was identified and characterized, and seems to mediate non-genomic actions of steroids (Zhu et al. 2003; Thomas 2004; Tokumoto et al. 2004; Tokumoto et al. 2006). The MIH signal received on the oocyte surface is transduced into the oocyte cytoplasm for the formation and activation of MPF, the final mediator of oocyte maturation. MPF is present in all eukaryotic cells and functions as the dominant factor to promote M-phase of the cell cycle, irrespective of meiosis and mitosis. Accordingly, MPF is currently known as the M-phase-promoting factor rather than the maturation-promoting factor. Although MPF exhibits a universal molecular structure as a complex of cdc2 and cyclin B in any species, the mechanisms of MPF formation and activation have been modified to various extents from species to species. In fish,  $17\alpha$ ,  $20\beta$ -DP leading to the *de novo* synthesis of cyclin B, the regulatory component of MPF, which activates a preexisting 35-kDa cdc2 kinase via phosphorylation of its threonine 161 by a threonine kinase, thus producing the 34 kDa active cdc2 (Nagahama and Yamashita 2007). We found that poly(A) tail of cyclin B mRNA in mature oocytes is about 100 nucleotides longer than that in immature oocytes. Elongation of the poly(A) tail of cyclin B mRNA takes place at the same time as that of GVBD during oocyte maturation. These results suggest that the MIH-induced cyclin B mRNA translation requires elongation of the poly(A) tail (Katsu et al. 1977; 1999).



To investigate the mechanism of cyclin B degradation upon egg activation, 26S proteasome was purified from goldfish oocytes and its role in the regulation of cyclin B degradation was examined. An initial ubiquitin-independent restricted digestion of cyclin B at K57 by the 26S proteasome allows the truncated 42-kDa cyclin B to be ubiquitinated probably with the aid of APC/C, and then the ubiquitinated cyclin B becomes a target of further complete degradation by ubiquitin-dependent activity of the 26S proteasome, leading to the inactivation of MPF in the eggs (**Tokumoto et al. 1977**).

We have found that an endocrine-disrupting chemical, diethylstilbestrol (DES), a nonsteroidal estrogen, triggers oocyte maturation in two fish species, the goldfish and zebrafish (**Tokumoto et al. 2004**). In both species, the morphology (the time course of the change in germinal vesicle breakdown) and an intracellular molecular event (the *de novo* synthesis of cyclin B) induced by DES were indistinguishable from those induced by  $17\alpha, 20\beta$ -DP. A synergistic action of DES on  $17\alpha, 20\beta$ -DP-induced oocyte maturation was observed. Both  $17\alpha, 20\beta$ -DP- and DES-induced oocyte maturation was inhibited by an antibody against the  $17\alpha, 20\beta$ -DP receptor. These results suggest that DES may act on the  $17\alpha, 20\beta$ -DP receptor as an agonist of  $17\alpha, 20\beta$ -DP.

### **3) Ovulation**

Ovulation is a precisely timed process by which a mature oocyte is released from an ovarian follicle. This process is initiated by the pituitary surge of luteinizing hormone (LH), is temporally associated with transcriptional regulation of several genes. The molecular mechanisms that control the complex process of ovulation are not well understood in vertebrates. Our recent studies (**Shibata et al. unpublished**) on medaka have demonstrated that  $17\alpha, 20\beta$ -DP can induce ovulation (follicle rupture) in the mature follicles *in vitro*. It is particularly important to note that this action of  $17\alpha, 20\beta$ -DP is mediated through its nuclear progesterin receptors (nPRs) expressed in the granulosa cells. We could find that nPR mRNA expression is induced by gonadotropin prior to ovulation. Thus,  $17\alpha, 20\beta$ -DP is the key hormone for the induction of not only maturation (through its membrane receptors), but also ovulation (through its nuclear receptors, **Ikeuchi et al. 2002**).

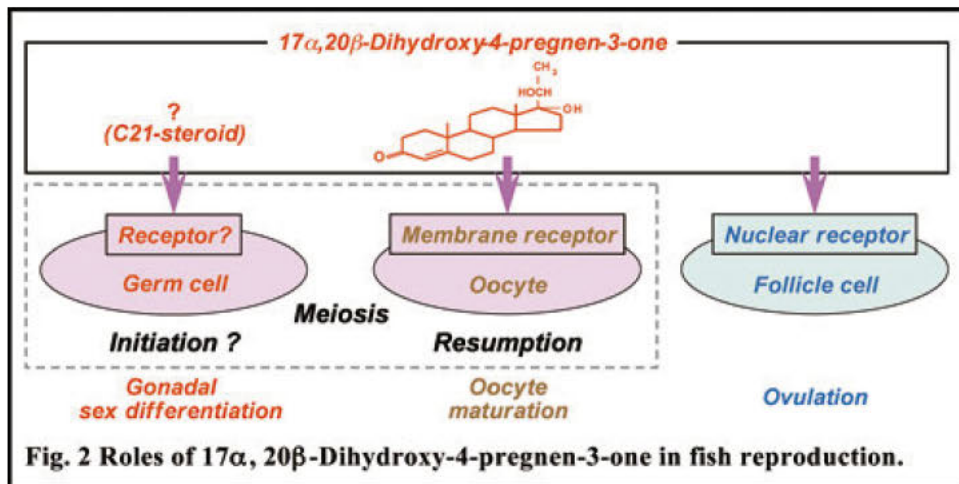
## **2. Perspective of research**

As I have described above, the major areas of my focus have been sex determination/differentiation, sexual plasticity, and oocyte maturation and ovulation. Since I will have limited time, my major area of research will be centered on sex differentiation and sexual plasticity during the rest of my career. To be precise, I wish to

focus my research to understand the role of brain in sexual behavior and gonadal sex change, with an emphasis on the sexual plasticity of brain, using *T. okinawae* and *O. latipes*. Although there are many more important questions remaining unresolved in oocyte maturation and ovulation, I may not be able to concentrate on this field of research due to the lack of time. However, I hope that my collaborators and others will continue their efforts to unravel the mysteries.

**Sex determination/gonadal sex differentiation:**

As afore-mentioned, we could identify several factors crucial for sex determination/gonadal sex differentiation like DMY/DMRT1/GSDF for the male sex and Foxl2/aromatase for the female sex. In future, the first priority will be given to the identification of a target gene of DMY. As already mentioned, *GSDF* expression is found to be specific to the Sertoli cells during the early stages of sex differentiation. Hence, I would like to investigate the role of GSDF in the testicular differentiation. However, the attempts to identify the target of DMY will still be continued. In addition, interaction of two major factors (transcriptional factors), DMRT1 and Foxl2 during the initial stages of testicular and ovarian differentiation needs to be investigated. In parallel with these studies, I will be interested in exploring the scope for the involvement of a C-21 steroid in the initiation of meiosis. Previous experiments in mouse have shown that timing of the initiation of meiosis is critical for the proper differentiation of the gonad. Therefore, I am planning to carry out experiments that will unravel the importance of the stage-dependent sexual dimorphism in the expression pattern of P450c17-II with respect to the initiation of the sex differentiation pathway. The research on these lines may aid in unraveling another aspect of 17 $\alpha$ , 20 $\beta$ -DP's function other than resumption of meiosis (oocyte maturation) and ovulation. Thus, we may be able to conclude that 17 $\alpha$ , 20 $\beta$ -DP is the first all-rounder in the regulation of meiosis, the key event in germ cell differentiation and development (Fig. 2).



### **Sex change:**

Our data from various studies using *T. okinawae* and *O. latipes* have prompted us to hypothesize that brain is an important sex organ orchestrating the whole process of sex differentiation. Sex-changing fish, *Trimma okinawae* can change its sex back and forth from male to female and then to male serially, depending on the social status in comparison with the other fish in the harem. The gonad corresponding to the sexual status of the fish remains functional, while the other is regressed. We could understand that the swapping of the gonads is initiated through a switching in the expression of the gonadotropin hormone receptors, *LHR* and *FSHR*. These two genes act as mediators to convey the information about the change in social status to the to-be-active gonad. Our results suggest that the investigation of the transcriptional regulation of these two genes might reveal the factors and mechanisms involved in the transduction of signals for sex change, from the brain to the gonads. Then further to elucidate the molecular mechanisms in detail, microarray hybridization will be performed. This will aid us in short-listing the genes involved in the brain-gonadal axis. We also aim to study the function of these genes in detail by screening for mutant libraries through TILLING using medaka fish (in collaboration with Professor T. Todo and Dr. M. Matsuda). As goby and medaka genomes are comparable, we expect to obtain precise results on the molecular function of the genes involved in goby sex change. Apart from these genes, we want to expand our knowledge regarding the role of *Cyp19a2* and estrogen in the sex differentiation and sexual plasticity of the brain as our preliminary data suggests that *Cyp19a2* could be a candidate gene associated with the sex-specific actions of the brain.

As a model system to study the role of brain in sex behavior and plasticity, I will continue to use medaka, especially to see whether the male-specific genes *DMY* and *Snply* have any role in the brain sex differentiation. In addition, efforts will be taken to understand the sexual plasticity of the adult brain. For this purpose, we will use our previously-established aromatase (*Cyp19a2*) transgenic medaka. I believe, the collective data from the investigations on goby and medaka brains will provide a unique insight into the involvement of the brain in sexual plasticity in the vertebrates in general.

### **Sexual plasticity in adult gonochoristic fish:**

Our experiments with tilapia have shown that adult female gonochoristic fish can reverse its sex when aromatase is inhibited by its antagonist fadrozole. Even though we could elucidate that DMRT1 expression and absence of estrogen are involved in the female to male sex-change, the origin of the testicular tissue could not be ascertained. I am particularly interested in understanding the sexual plasticity of somatic cells in the adult ovary by examining whether the granulosa cells transdifferentiate into the Sertoli



cells or whether these Sertoli cells originate from the somatic stem cells that may remain quiescent in adult ovary. Finally, we will also try to induce the male to female sex change by treating XY adult males with estradiol-17 $\beta$ .

We also plan to repeat these experiments using another gonochoristic fish medaka. The advantage of using medaka is the availability of transgenic lines with the markers for germ cells (*vasa* and *nanos*), granulosa cells (*Cyp19a1*) and Sertoli cells (*DMRT1* and *GSDF*) (Tanaka et al. 2001; Lau et al. unpublished). The presence of these transgenes in the gonads of these fish will facilitate the selection of specific cell types. Further these cells will be transplanted to an adult host fish of opposite sex to see whether they can transdifferentiate according to the sex of the host. A recently developed organ culture systems for tilapia and medaka gonads will also be used (Sakai et al. 2007). Furthermore, the gonads undergoing sex change induced by either fadrozole or estradiol-17 $\beta$  treatments will be subjected to microarray hybridization against the EST library of medaka gonads. Thus we will be able to understand the cellular and molecular mechanisms involved behind the sexual plasticity in the adult gonochoristic fish.

### **3. Description of other academic activities (education, service to the academic communities, etc.)**

I have membership in several scientific societies including International Federation of Comparative Endocrinological Societies (IFCES), the Endocrine Society (USA), Society for the Study of Reproduction (USA), Japan Society of Comparative Endocrinology, Zoological Society of Japan, and Japan Society of Developmental Biologists. Among these, I am the President of IFCES since 2005, and in particular, I was involved since the formation of this society. I am also the Vice-President of the Zoological Society of Japan and have been serving this society by holding various positions like council member and editorial board member.

My other contributions to the scientific societies are to hold editorial board positions in various national and international journals, especially I was the Editor-in-Chief of *Zoological Science* for the last two years (2004-2006). During my term *Zoological Science* was made completely online and this helped to augment the popularity of the journal among the scientists all over the world. Since 1993, I have also been holding the office of the Executive Editor responsible for the manuscript submission from the Asia-Oceania region for the journal, *Molecular Reproduction and Development*.

I am involved in many evaluation committees of various government and private funding agencies. One of the most recent of this kind includes the evaluation committee for the CREST project on Development, Differentiation, and Regeneration in Biological Systems. I am a member of the Japanese Council for Science and the chairman of the division for Marine Biology under this council.

I have been involved with the National BioResource Project for medaka, as the chairman of the committee for the first 5 year phase of the project, and from April 2007, I serve as the Principal Investigator of the second phase of this project. Thus, National Institute for Basic Biology has become the centre for dissemination of medaka across the world and we are trying to promote the usage of medaka as a model organism. This is an excellent example for the kind of contributions that NIBB has made to the scientific community.

For the last 20 years, I have been involved with the selection committee for the Japan Students Science Award (JSSA), which is the oldest national science award in the field of science, for middle school and high school students – each year more than 10,000 applications are submitted. Since this year, I am the chairman of the selection committee of the JSSA. This has provided me with excellent opportunities to interact with the young students and educate them.

#### **4. Personal information**

**A) Name:**

Yoshitaka Nagahama

**B) Date of birth:**

██████████

**C) Office address:**

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National Institutes of Natural Sciences, Okazaki 444-8585, Japan  
Tel, 81-564-55-7550; Fax, 81-564-55-7556; e-mail, Nagahama@nibb.ac.jp

**D) Research area:**

Developmental Biology, Reproductive Biology, Endocrinology

**E) Education:**

- B.Sci. – Faculty of Fisheries, Hokkaido University – 1966 – Fisheries
- M.Sc. – Faculty of Fisheries, Hokkaido University – 1968 – Fisheries
- Ph.D. – Faculty of Fisheries, Hokkaido University – 1971 – Fisheries
- 1970 – 1971: Postdoctoral Fellow – Faculty of Fisheries, Hokkaido University
- 1972 – 1974: Postdoctoral Fellow – Department of Zoology, University of California, Berkeley, USA
- 1974 – 1975: Postdoctoral Fellow – Department of Zoology, University of British Columbia, Vancouver, Canada
- 1976 – 1977: Research Associate – Department of Zoology, University of British Columbia, Vancouver, Canada
- 1977: Research Associate, Department of Zoology, University of California, Berkeley, USA

**F) Professional experience:**

- 1977 – 1986: Associate Professor – Laboratory of Reproductive Biology, Department of Developmental Biology, National Institute for Basic Biology
- 1986 – Present: Professor - Laboratory of Reproductive Biology, Department of Developmental Biology, National Institute for Basic Biology
- 1992 – 2000: Chairman – Department of Developmental Biology, National Institute for Basic Biology
- 2004 – Present: Vice Director – National Institute for Basic Biology, National Institutes of Natural Sciences

**G) Awards:**

- 1987: Japan Society of Fisheries Prize
- 1988: Grace Pickford Medal (International Federation of Comparative Endocrinological Societies)
- 1989: Inoue Science Award (Inoue Science Foundation)
- 1989: The Zoological Society Prize (Zoological Society of Japan)
- 2004: Howard A. Bern Lecture Award (Society for Integrative and Comparative Biology, USA)
- 2007: Richard E. Peter Lecture Award (International Symposium on Fish Physiology, Canada)

## H) Professional societies:

American Society of Zoologists  
Endocrine Society (USA)  
International Federation of Comparative Endocrinological Societies  
(Vice President, 2001 – 2005; President, 2005 – )  
Japan Society for Comparative Endocrinology  
Japan Society for Reproductive Endocrinology  
Japan Endocrine Society  
Japanese Society of Developmental Biologists  
Japanese Society of Scientific Fisheries  
Society for the Study of Reproduction (USA)  
The Society for Endocrinology (UK)  
Zoological Society of Japan (Vice President, 2007-)

## I) Publications:

### Class 1: Research articles in peer reviewed journals

1. Chang, X.T., Kobayashi, T., Kajiuira, H., Nakamura, M. and Nagahama, Y. (1997). Isolation and characterization of the cDNA encoding the tilapia (*Oreochromis niloticus*) cytochrome P450 aromatase (P450arom): Changes in P450arom mRNA, protein and enzyme activity in ovarian follicles during oogenesis. *J. Mol. Endocrinol.*, 18, 57-66.
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121. Zhou, L.Y., Wang, D.S., Kobayashi, T., Yano, A., Paul-Prasanth, B., Suzuki, A., Sakai, F. and Nagahama, Y. (2007a). A novel type of *P450c17* lacking the lyase activity is responsible for C21-steroid biosynthesis in the fish ovary and head kidney. *Endocrinology*, 148, 4288-4291.
122. Zhou, L.Y., Wang, D.S., Shibata, Y., Paul-Prasanth, B., Suzuki, A. and Nagahama, Y. (2007b). Characterization, expression and transcriptional regulation of *P450c17-I* and *-II* in the medaka, *Oryzias latipes*. *Biochem. Biophys. Res. Comm.*, 362, 619-624.
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125. Matsuoka, Y., Kobayashi, T., Kihara, K. and Nagahama, Y. Molecular cloning of *Plk1* and *Nek2* and their expression in mature gonads of the teleost fish Nile tilapia (*Oreochromis Niloticus*). *Mol. Reprod. Develop.* in press



126. Ijiri, S., Kaneko, H., Kobayashi, T., Wang, D.S., Sakai, F., Paul-Prasanth, B., Nakamura, M. and Nagahama, Y. Sexual dimorphic expression of genes in gonads during early differentiation of a teleost fish, the Nile tilapia *Oreochromis niloticus*. Biol Reprod. in press.
127. Kobayashi, T., Kajiura-Kobayashi, H., Guan, G. and Nagahama, Y. Sexual dimorphic expression of DMRT1 and Sox9 during gonadal differentiation and hormone-induced sex reversal in the teleost fish Nile tilapia (*Oreochromis niloticus*). Dev. Dyn. in press.
128. Sakai, F., Kobayashi, T., Matsuda, M. and Nagahama, Y. Stability in aromatase immunoreactivity of steroid-producing cells during early development of XX gonads of the Nile tilapia, *Oreochromis niloticus*: An organ culture study. Zool. Sic. in press.

## **Class 2: Invited reviews, book chapters**

1. Nagahama, Y., Miura, T., Kobayashi, T. and Ding, J. (1997). The role of activin in spermatogenesis in fish. *In* Inhibin, Activin and Follistatin (Aono, T., Sugino, H. and Vale, W.W., eds.), pp. 196-203, Springer-Verlag, New York.
2. Nagahama, Y. (1997).  $17\alpha$ ,  $20\beta$ -Dihydroxy-4-pregnen-3-one, a maturation- inducing hormone in fish oocytes: Mechanisms of synthesis and action. Steroids, 62, 190-196.
3. Nakamura, M, Kobayashi, T., Chang, X.T. and Nagahama, Y. (1998). Gonadal sex differentiation in teleost fish. J. Exp. Zool., 281, 362-372.
4. Nagahama, Y. (2000). Gonadal steroid hormones: major regulators of gonadal sex differentiation and gemetogenesis in fish. *In* Reproductive Physiology of Fish (Norberg, B., Kjesbu, O.S., Taranger, G.L., Andersson, E. and Stefansson, S.O. eds.), pp. 211-222, Bergen.
5. Oba, Y., Hirai, T., Yoshiura, Y., Kobayashi, T. and Nagahama, Y. (2001). Fish gonadotropin and thyrotropin receptors: The evolution of glycoprotein hormone receptors in vertebrates. Comp. Physiol. Biochem., Part B 129, 441-448.
6. Devlin, R.H. and Nagahama, Y. (2002). Sex determination and sex differentiation in fish. Aquaculture, 208, 191-366.
7. Matsuda, M. and Nagahama, Y. (2002). Positional cloning of the sex-determining region of medaka using a Y congenic starain. Aquatic Genomes: Steps Toward a Great Future. N. Shimizu, T. Aoki, I. Hirono and F. Takashima (eds.), 236-243, Springer.

8. Nagahama, Y., Nakamura, M., Kitano, T. and Tokumoto, T. (2004). Sexual plasticity in fish: A possible target of endocrine disruptor actions. *Environ. Sci.*, 11, 73-82.
9. Senthilkumaran, B., Yoshikuni, M. and Nagahama, Y. (2004). A shift in steroidogenesis occurring in ovarian follicles prior to oocyte maturation. *Mol. Cell. Endocrinol.*, 215, 11-18.
10. Nagahama, Y. (2005). Molecular mechanisms of sex determination and gonadal sex differentiation in fish. *Fish Physiol. Biochem.*, 31, 105-109.
11. Nagahama, Y. and Yamashita, M. (2008). Hormonal regulation of oocyte maturation and ovulation in fish. *Develop. Growth Differ.* in press.

**J) Symposium presentations/Invited seminars,**

1.  $17\alpha$ ,  $20\beta$ -Dihydroxy-4-pregnen-3-one, a maturation-inducing hormone in fish oocytes: synthesis and action. EMBO Workshop on Meiotic Maturation, Cuenca, Spain.
2. Nagahama, Y. (1998). Hormonal regulation of mitosis and meiosis in fish germ cells. EMBO-Workshop on Reproduction and Early Development, University of Bergen, Norway
3. Nagahama, Y. (1998). Hormonal induction of spermatogenesis in immature eel testis during organ culture. 31<sup>st</sup> Annual Meeting, Society for the Study of Reproduction, College Station, Texas, USA
4. Nagahama, Y. (2000). Gonadal steroid hormones in fish: their roles, synthesis and actions. International Symposium on Molecular Steroidogenesis, Nara, Japan, August 25-28.
5. Nagahama, Y. (2000). Sex steroid hormones and their receptors in fish gonads: Potential sites of environmental endocrine disruptors. Gordon Research Conference on Environmental Endocrine Disruptors, New Hampshire, USA, June 18-23.
6. Nagahama, Y. (2000). Molecular mechanisms of gonadal sex differentiation in fish. 20<sup>th</sup> Conference of European Comparative Endocrinologists, Faro, Portugal, September 5-9, 2000.
7. Nagahama, Y. (2001). Molecular endocrinology of gonadal sex differentiation and gametogenesis in fish. 3<sup>rd</sup> IUBS Symposium on Molecular Aspects of Fish Genomes and Development, February 18-21, 2001, Singapore.
8. Nagahama, Y. (2001). Sex steroid hormones in fish gonads: Synthesis and actions. IMBS 2001, International Marine Biotechnology Symposium, April 12-13, 2001, Taipei, Taiwan.

9. Nagahama, Y., Kobayashi, T., Matsuda, M., Guan, G., Ikeuchi, T., Sakai, F., Sudhakumari, C.C., Wang, D.S., Nakamura, M. (2002). Molecular mechanisms of sex differentiation and gonadal sex differentiation in fish. *Molecular Mechanisms of Sex Differentiation*, 48th NIBB Conference, October 18-20, 2002, Okazaki, Japan.
10. Nagahama, Y. (2002). Effects of environmental factors on sex determination and differentiation. *Minamata International Symposium of Environmental Risk Assessment*, March 21-22, Minamata Moyai-naoshi Center, Minamata, Japan.
11. Nagahama, Y. (2002). Shift in follicular Steroidogenesis in fish ovaries prior to oocyte maturation. *International Congress on Hormonal Steroids and Hormones and Cancer*, October 21-25, 2002, Fukuoka, Japan.
13. Nagahama, Y. (2002). Aromatase and gonadal sex differentiation in fish. *VI International Aromatase Conference*, October 26-30, 2002, Kyoto, Japan.
14. Nagahama, Y. (2002). Molecular mechanisms of sex determination and gonadal sex differentiation in fish. *Fourth Intercongress Symposium of the Asia and Oceania Society for Comparative Endocrinology*, October 8-11, 2002, Guangzhou, China.
15. Nagahama, Y. (2002). Molecular mechanisms of sex determination and gonadal sex differentiation in fish. *2002 Annual Retreat WSU/UI Center for Reproductive Biology*, June 13-14, 2002, Camp Larson – Coeur’Alene Lake, Idaho, USA.
16. Nagahama, Y. (2002). Molecular mechanisms of sex determination and differentiation in fish. *35th Annual Meeting, Society for the Study of Reproduction*, August 28-31, 2002, Baltimore, USA.
17. Nagahama, Y. (2003). Molecular mechanisms of sex determination and differentiation. *International Symposium on Amphibian and Reptilian Endocrinology and Neuroendocrinology*, November 4-6, 2003, Cheju National University Park, Korea.
18. Nagahama, Y. (2003). Sex Determination and Differentiation in Fish. *XXI Symposium of the Society for Reproductive Biology and Comparative Endocrinology. Recent Advances in Endocrinology & Reproduction: Evolutionary, Biotechnological and Clinical Implications*, February 10-13, 2003, Banaras Hindu University, Varanasi, India.
19. Nagahama, Y. (2003). Sex Determination and Gonadal Sex Differentiation in Fish. *The 3<sup>rd</sup> International Medaka Symposium, 2003 – Development of Test Methods with Medaka to Detect Endocrine Disrupting Chemicals*, February 27-28, 2003, Okazaki Conference Center, Okazaki, Japan.
20. Nagahama, Y. (2003). Genetic, hormonal and environmental components of sex determination and differentiation in fish. *Third International Symposium on Vertebrate Sex Determination*, March 24-28, 2003, Kona, Hawaii, USA.

21. Nagahama, Y. (2003). A shift in steroidogenesis occurring in fish ovarian follicles prior to oocyte maturation. Molecular Steroidogenesis Workshop, April 24-27, Bath, UK.
22. Nagahama, Y. (2003). Ontogeny of sex steroid receptors in brain and gonads during sex differentiation in the Nile tilapia, *Oreochromis niloticus*. Third International Symposium on the Biology of Vertebrate Sex Determination, March 24-28, 2003, Kona, Hawaii, USA.
23. Nagahama, Y. (2004). The plasticity of fish gender: an evolutionary basis for sex determination and differentiation in vertebrates. SICB 2004 Annual Meeting, January 5-9, New Orleans, USA
24. Nagahama, Y. (2004). Sex determination and differentiation in fish. International Symposium on The Biology of Sperm Cell - from basic to clinics, February 25-26, 2004, Tokyo.
25. Nagahama, Y. (2004). Sex determination and gonadal sex differentiation in fish. The XIX International Congress of Zoology, August 23-27, Beijing, China.
26. Nagahama, Y. (2004). Sex determination and gonadal sex differentiation in fish. The 5th International Symposium on Fish Endocrinology, September 5-9, 2004, Castellon, Spain.
27. Nagahama, Y. (2004). Molecular mechanisms of sex determination and differentiation in fish. International Workshop on Fish Genetics and Development – Chinese Academy of Sciences, October 11-14, 2004, Wuhan, China.
28. Nagahama, Y. (2004). Molecular mechanisms of sex determination and differentiation in fish. The 4th International Symposium on the Molecular and Cell Biology of Egg- and Embryos-Coats, November 8-13, 2004, Ise-Shima, Japan.
29. Nagahama, Y. (2004). Sex determination and differentiation in fish. Bilateral Symposium Italy and Japan, November 16-19, 2004, Mie, Japan.
30. Nagahama, Y. (2005). Sex determination and differentiation in fish. The first COE Symposium on Potential and Perspective of Marine Bio-Manupulation, February 26-27, 2005, Sapporo, Japan.
31. Nagahama, Y. (2005). *DMY* : The second sex-determining gene in vertebrates. 15th International Congress of Comparative Endocrinology, May 22-27, 2005, Boston, USA.
32. Nagahama, Y. Sex determination and differentiation in fish. 52<sup>nd</sup> NIBB Conference. Okazaki, January 20-23, 2006.

33. Nagahama, Y. Molecular mechanisms of sex differentiation and gonadal sex differentiation in fish. *Frontiers in Molecular Endocrinology*. Hyderabad, India. December 12-15, 2005.
34. Nagahama, Y. (2006). Molecular Mechanisms of Sex Differentiation and differentiation in Fish. International Conference of Ecophysiology in Marine Organisms. Keelung, Taiwan, October 3-6, 2006.
35. Nagahama, Y. (2006). Molecular mechanisms of sex-steroid actions on gonadal differentiation and maturation in fish. The Second Italy-Japan Bilateral Seminar on Responses of Marine Organisms to Physical and Chemical Impacts. Palermo, Area della Ricerca del CNR, November 22- 5, 2006.
36. Nagahama, Y. (2007). Molecular mechanisms of sex determination and differentiation in fish. The International Symposium of *Oryzias* species. Bangkok, Thailand, November 12-14, 2007.

#### **K) Organization of symposium,**

- 1) NIBB conference ‘Molecular Mechanisms of Sex Differentiation’  
October 18-20, 2002, Okazaki, Co-organizer
- 2) International Symposium on ‘Molecular Mechanisms of Sex Determination and Differentiation’ September 21, 2006, Matsue  
Co-organizer

#### **L) Grant awards,**

- 1) 1996-2000 ‘Molecular Mechanisms of Sex Determination and Differentiation in Fish’ JSPS Research for the Future Program  
- ¥ 375, 072, 000
- 2) 1998-1999 ‘Mechanisms of Synthesis of Gamete Maturation-inducing Hormone’ Grant-in-Aid for Scientific Research (B) -  
¥ 11, 800, 000
- 3) 2000-2005 ‘Molecular Mechanisms of Endocrine Disrupter Action on Fish Reproductive System’ Core Research for Evolutional Science and Technology (CREST) - ¥ 496,100,000
- 4) 2000-2001 ‘Function and Expression of Fish Gonadotropin Receptors’ Grant-in-Aid for Scientific Research (B) - ¥ 14, 000, 000
- 5) 2002-2003 ‘Function and Action of Sex-determining Gene of Medaka’ Grant-in-Aid for Scientific Research (B) - ¥ 14,100,000

- 6) 2004-2005 'Molecular Mechanisms of Sex Change in Fish'  
Grant-in-Aid for Scientific Research (B) - ¥ 14,900,000
- 7) 2004-2009 'Molecular Mechanisms of Sex Determination and  
Differentiation in Fish' Grant-in-Aid for Scientific Research  
on Priority Areas - ¥ 74,800,000
- 8) 2005-2009 'Molecular Mechanisms of Sexual Plasticity'  
Solution Oriented Research for Science and Technology  
(SORST) - ¥ 80,000,000

#### **M) Professional activities,**

##### Scientific Journals:

- 1992 – present: *Theriogenology* (Editorial Board)
- 1992 – 2005: *Molecular Marine Biology and Biotechnology* (Editorial Board)
- 1992 – present: *Asian Journal of Zoological Science* (Editorial Board)
- 1993 – present: *Molecular Reproduction and Development* (**Executive Editor**)
- 1994 – present: *Development Growth & Differentiation* (Editorial Board)
- 1994 – 2002: *Zoological Science* (**Division Editor**)
- 1994 – 2004: *Biological Bulletin* (Editorial Board)
- 1994 – present: *Current Topics of Developmental Biology* (Editorial Board)
- 1994 – 2004: *General and Comparative Endocrinology* (Editorial Board)
- 1997 – 2003: *Journal of Molecular Endocrinology* (Editorial Board)
- 1998 – present: *Fish Physiology and Biochemistry* (Editorial Board)
- 1999 – 2004: *Biology of Reproduction* (Editorial Board)
- 2005 – 2006: *Zoological Science* (**Editor-in-Chief**)
- 2007-present: *Sexual Development* (Editorial Board)

##### Societies:

- Committee Member, All Japan Student Science Award Selection Committee  
(1995-present, Chairman since 2007)
- Committee Member, Committee on Grant-in-Aid for Scientific Research,  
Organization of the Science Council (1998-200, 2003-2004, 2005-2007)
- Committee member, Advisory Committee of the JSTA for CREST (Development,  
Differentiation, and Regeneration) (2000-present).
- Committee member (Chairman), Steering Committee of National BioResource  
Project, MEDAKA (2003-2007)

Committee Member, TORAY Development of Science Technology Foundation  
(2004-2006)

Member of Science Council of Japan (Chairman, the Division of Marine Biology)  
(2006-present)

Committee member (Chairman), Advisory Committee of the JSTA for ICORP  
(2006-2007).

Principal Investigator, National BioResource Project, MEDAKA  
(2007-present)

Committee Member, The JSPS Selection Committee for Postdoctoral and Other  
Fellowships

Council Member, International Federation of Comparative Endocrinological  
Society)

Council Member, The Zoological Society of Japan

Council Member, The Japan Society for Comparative Endocrinology

Council Member, The Japanese Society of Developmental Biology

Council Member, Reproductive Endocrinology Society

Council Member, The Society for Pituitary Research

#### **N) Teaching experience (1977-2007),**

Hokkaido University, Tohoku University, Fukui Prefectural University,  
Nagoya University, Toyama University, Kobe University,  
Hiroshima University, Kumamoto University

#### **O) Other activities (if any)**

Postdoctoral fellow supervision (1977-present):

Akihiko Yamaguchi

Yoshinao Katsu

Mika Tokumoto

Takashi Todo

Zuxu Yao (China)

Craig Morrey (USA)

Daisuke Kobayashi

Catherine Dreanno (France)

Yuichi Oba

Balasubramanian Senthilkumaran (India)

Toshitaka Ikeuchi  
Fumie Sakai  
Masatada Watanabe  
Gui-Jin Guan (China)  
Yasushi Shibata  
Masaru Matsuda  
Xiao-Tian Chang (China)  
En-Lieng Lau (Taiwan)  
Cheni Chery Sudhakumari (India)  
De-Shou Wang (China)  
Paul Bindhu (India)  
Mei-Sheng Yi (China)  
Kohei Ohta  
Ramji Bhanbari (Nepal)  
Radha Chaube (India)  
Shigeho Ijiri  
Dipanjan Basu (India)  
Shakuntala Basu (India)  
Hao-Bin Zhao (China)  
Aya Suzuki  
Jian-Zhou Cui (China)  
Jian-Zhong Li (China)  
Lin-Yan Zhou (China)

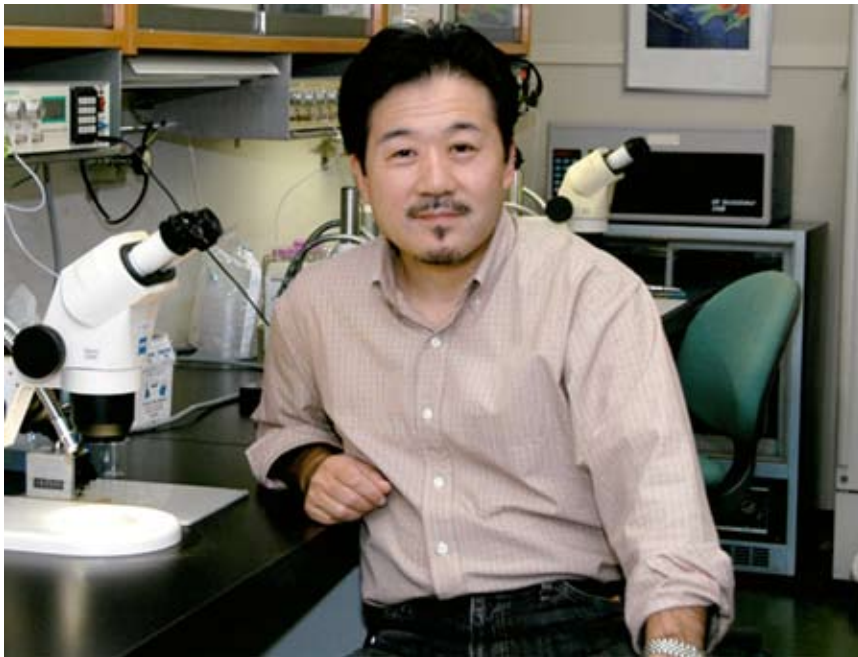
Graduate (Ph.D.) student supervision (1977-2007):

Gui-Jin Guan  
Yuki Ohmuro-Matsuyama  
Ryo Horiuchi  
Yasuhisa Kobayashi  
Lin-Yan Zhou (China)  
Masatada Watanabe



1 - 2. 在職 10 年の教授業績評価について

上野直人教授





## 上野直人教授在職10年業績評価

基礎生物学研究所 所長 岡田清孝

### 1. 経緯

- ・平成19年5月 基礎生物学研究所所長および教授3名からなる在職10年教授業績評価実行委員会を設置。
- ・平成19年10月 上野教授の研究分野に近い所外研究者から海外3名、国内2名を選んで評価委員を委嘱し、資料を送付。送付資料 1) 研究活動の説明、2) 研究業績リスト、3) 主たる業績の論文別刷、4) 略歴
- ・平成20年1月 海外3名、国内2名の評価委員全員から回答を受け取った。

### 2. 評価内容

#### A委員による研究内容のまとめと評価（一部抜粋）

上野博士は、アフリカツメガエル初期胚における背腹軸に沿ったパターン形成に BMP シグナル伝達が重要であることを見いだしたことで、その後のシュペーマンオーガナイザーの研究の流れに大きな影響を与えた。この成果は、「オーガナイザーは BMP 阻害因子を分泌することで中胚葉と外胚葉のパターン形成を行う」という、現在広く受け入れられている概念の確立に大きく貢献をした。上野博士はその後も BMP シグナル伝達の解析を中心に研究を幅広く展開し、最近の10年間においても多くの業績を上げた。注目すべきは、(1) follistatin による BMP の阻害様式を解析し、follistatin は BMP と BMP レセプターとの3者複合体を形成して阻害すること、(2) BMP シグナルの標的遺伝子としてホメオドメイン転写因子 Msx1 が重要な役割を担っていること、(3) BMP2 と BMP4 は分泌性の因子であるが細胞外基質に結合するためその作用範囲は極めて狭いこと、を明らかにしたことである。これらの業績は、脊椎動物初期発生における BMP の役割を理解する上で重要な知見となっている。

上野博士は BMP シグナルの胚発生における役割の一般性を検討するため脊椎動物以外の生物種としてホヤと線虫での解析を行った。特に線虫の解析では、当時としては斬新なマイクロアレイの手法を取り入れて解析し、BMP シグナルの標的遺伝子を同定し体長伸張との関連性について解析した。その後、この網羅的解析をアフリカツメガエルの実験系にも取り入れ、ESTs (expressed sequence tags) の作成と発生過程での遺伝子発現パターンのデータベース XDB を構築した。このデータベースには約 12,000 の unigene セットが含ま

れて入ることから、多くの研究者に利用されている。それらの cDNA リクエストも多数あるにもかかわらず、それを無償で配布するなど、多くの研究者がその恩恵に与っている。

この数年の研究では、初期発生における細胞運動の解析に研究の幅を広げている。Wnt/PCP 経路にかかわる因子である glypican 4 や Prickle、XGAP がアフリカツメガエル胚の収斂伸張運動や原腸形成運動に関することを示し、一流雑誌に発表している。新しい分野に入って直に成果をあげたことは、研究の狙いが的確でありまた研究力量の高さを示すものである。その成果を受け、今後の研究方向も bioimaging を取り入れることで、発生過程の細胞運動を動的に捉えて解析する予定となっており、大きな成果が期待される。

上野博士は、文部科学省の特定領域研究 A「発生システム」の統括として 2005 年までの 5 年間、数十名の発生学の研究者を束ねて大きな成果をもたらした。また基礎生物学研究所で国際シンポジウムやワークショップを開催して最新情報の交換と研究者間の交流を高めることに尽力され、さらに、先に述べたように、cDNA クローンの無償配布をするなど、遅れている研究の基盤整備を補完する活動も行っている。このように、自身の研究活動を超えて、発生学の研究分野全体への貢献は甚大なものがある。

以上のように、上野博士はこの 10 年間、発生学の発展に大きく貢献してきた。したがって、今後も基礎生物学研究所の教授として、研究と教育に携わるに相応しい研究者と考えられる。

B 委員による評価（一部抜粋）

In terms of approaches, it is impressive that Dr. Ueno has been making use of a well-balanced mixture of following approaches: molecular biology, biochemistry, cell biological techniques, functional genomics, and of course classical embryology. Now he is interested in developing novel bioimaging tools and also describing his interest in mechanical stress, which suggests introduction of physics into his developmental studies. I would like to expect that his group would facilitate interdisciplinary researches to make breakthroughs in understanding cellular and developmental processes.

C 委員による評価（一部抜粋）

In sum, Prof. Ueno is a great credit to your institution. He is widely respected in the international arena. He has developed *Xenopus laevis* cDNA and microarray genomic reagents that help the entire community. He serves as Secretary of the Japanese Society of Developmental Biology and is President-elect of the International Society of Differentiation. He has been enormously productive. The NIBB should be very proud of what Prof. Ueno has achieved at your institute.

D委員による評価（一部抜粋）

I have every confidence that he will continue to excel and to be a credit to your organization, and he will continue to be a world-class and world-famous scientist. PLEASE continue to support him, as he has my highest recommendation, and he would receive the same review from everyone I know in the field.

E委員による評価（一部抜粋）

Since joining NIBB ten years ago, Ueno has established a productive and imaginative series of approaches to studying signaling and morphogenesis. His recent approaches in morphogenesis have been extremely interesting, and have contributed to the sense that this is a field whose time is ripe, and where the combination of techniques that Ueno has mastered will lead to seminal developments over the next years. I therefore not only give a very strong review to Prof. Ueno's previous work, but I also am very optimistic that his future work will provide important new insights into animal development, and make contributions in areas that have been difficult to approach.

### 3. まとめ

上野教授は、動物の初期発生システム解析の国際的な研究リーダーと認知されている。特に、マイクロアレイを用いた発現解析やイメージング手法の利用など積極的に新たな研究手法を導入して研究を展開してきたことが高い評価を受けている。研究者コミュニティからの期待も大きい。基礎生物学研究所においても財務担当主幹および国際連携担当として努力された。今後も引き続き国際的リーダーとしての活躍を期待する。

**10 years of achievements at NIBB**

**1997-2006**

**(as of October 1, 2007)**

**Naoto Ueno, Professor**

**National Institute for Basic Biology**

## **1. Research activity in the past 10 years**

### **1.1. Aims and goals**

My laboratory endeavors to understand how organisms develop from a single-celled and spherical egg to a complex adult body with a variety of functionally differentiated cells, tissues, and organs. The fact that embryonic cells not only gradually acquire different fates during early development but that embryo also drastically change their morphology during a limited time of development after the fertilization has been attracting developmental biologists for many years. This embryonic morphogenesis, including dynamic processes such as the remodeling of tissues, lies at the heart of the mystery of life. Understanding the molecular and cellular basis for these complex phenomena, therefore, has been the goal of not only our research but of the entire field for the past 10 years.

### **1.2. Past and current research topics**

#### **1.2.1. Regulation of developmental processes by cell-to-cell interaction**

Until the 1980's, it was understood that polypeptide growth factors mainly regulate cell proliferation and the loss of their control leads to unwanted growth of cells, *e.g.* a cancerous state. In the early 1990's, however, novel roles during early embryogenesis were found for many polypeptide growth factors and they subsequently began to be recognized as a growth/differentiation factor in general; it is now well established that they are used repeatedly to mediate cell-to-cell interactions at different times or places during development.

My laboratory discovered that bone morphogenetic proteins (BMPs) have essential roles in early development. During the course of screening for activin-related genes from an amphibian *Xenopus laevis*, we isolated gene for proteins we now know as BMP2, BMP4, and BMP7, and reported that they are differentially expressed during early *Xenopus* development. Soon after, we attempted to identify the embryonic role of BMPs and found that loss of BMP activity in the ventral side of embryo causes the formation of a secondary body axis with neural tissues reminiscent of the duplicated axis demonstrated by the historical transplantation experiment of a dorsal lip into the ventral region performed by Spemann and Mangold in the 1920's (**Suzuki, A et al. *Proc. Natl. Acad. Sci., USA*, 1994**). This led to the new concept of neural induction whereby neural tissue formation, whose mechanism had been an enigma for nearly 80 years, could be explained as a default state of BMP activity promoting epidermalization. This finding also helped the identification of BMP antagonists Noggin and Chordin, both of which are

expressed in Spemann organizer, being antagonists of BMPs. My group also contributed in demonstrating that Follistatin, another organizer factor, is one such BMP antagonist (13). The new concept of neural induction uncovered the basis of Spemann's experiment and is now widely accepted as a favored model. We also characterized the signaling pathway and demonstrated that Msx1 gene encoding a homeobox protein is an immediate early gene responding to BMP signals and necessary for the activation of further downstream targets such as Vent1, and thus for the ventralization of embryo by BMPs (30, 44). Importantly, we have been able to show that complete shut-down of BMP activity using a dominant negative form of Msx1 leads to the perfect secondary axis formation, exactly what had been seen in Spemann's experiment (44). In addition, using zebrafish (15) and *Xenopus* (49), we have been able to demonstrate that the action of BMP2 or BMP4 is normally short range, that the diffusion is tightly restricted by the physical interaction with negatively charged proteoglycans, and that deletion of the positively charged core sequence at their N-termini dramatically increases their diffusion rate in the extracellular space. These studies enabled a greater understanding of the chemical properties of vertebrate BMP as a morphogen and of how it behaves *in vivo*.

### 1.2.2. Evolution and conservation of the developmental signals

Since after the discovery of the essential developmental functions of BMPs in dorso-ventral patterning, our interest extended to the evolutionary biology aspect of BMP functions in the establishment of body plan.

It was well known that an ortholog of BMP known as Decapentaplegic (Dpp) is present in arthropod such as *Drosophila melanogaster* and that it shares its role in DV patterning with vertebrate BMPs despite the inversion of DV axis in arthropod body plan. Our question was "What is the lowest animal that has BMPs?" and, if they exist in the organism, "What is their role in body patterning?". We isolated BMP-like genes from ascidian *Halocynthia roretzi* (Miya, T. et al, *Development*, 1997) and *Caenorhabditis elegans* (8) and demonstrated for the first time that BMPs exist in the protochordate and nematode. Particularly for nematode BMP Cet1/Db11, we further isolated mutants of the gene and demonstrated that Cet1/Db11 is required for the growth and maintenance of body size. This was somewhat unexpected due to known BMPs functions in other organisms. We also genetically determined that the ligand Cet1/Db11 acts upstream of other components of the signaling pathway such as Sma2 and Sma6, whose mutation also displays shortening of body length. To understand the molecular and cellular basis, we isolated target genes of the pathway using DNA microarray which we applied to *C. elegans* developmental genetics for the first time (28). We



discovered a gene which we eventually identified as a gene responsible for a previously known mutation of body length *lon-1* (50). The gene *lon-1* is normally suppressed by the ligand Cet1/Dbp1 signal and hyper activation of the gene caused by the Cet1/Dbp1 ligand mutation results in a smaller body. We also demonstrated that Cet1/Dbp1 pathway increases chromosome ploidy of hypodermal cells through the inhibition of Lon-1 expression and that the resulting increase of hypodermal cell contributes to the growth and maintenance of body length. This study using *C. elegans* identified a novel mechanism of body size regulation in nematode by the TGF- $\beta$ -like ligand and clarified that the nematode co-opted the signaling system to regulate body length instead of the DV patterning found in arthropods and vertebrates.

### 1.2.3. Morphogenesis as a consequence of cell behavior

In the last several years, we expanded our study to cellular morphogenesis during early embryogenesis.

During gastrulation, an embryo elongates along an anterior-posterior (AP) axis, largely due to a cell movement called convergent extension (CE), in which cells change their morphology to spindle-shape and intercalate each other. This mediolateral intercalation is converted to the force to elongate the mesodermal cell mass along the antero-posterior axis and is believed to be the driving force behind elongating embryo proper from their original spherical shape. It is well known that during CE the mesoderm cells display polarized morphology associated with drastic changes in cytoskeletal arrangement. Recent studies have revealed that the cellular polarization is controlled by a system similar to planar cell polarity (PCP) pathway initially described in *Drosophila* as an essential pathway for wing epithelium to set up cell polarity directing distally oriented wing hair and organized photoreceptor cells in ommatidium. As this PCP pathway shares a known component of Wnt pathway, it is often called Wnt/PCP pathway. We demonstrated that the ortholog of one of *Drosophila* PCP components Prickle is essential for CE in vertebrates (56). We also clarified the detailed mechanism of PCP pathway shedding light on the translocation of Dishevelled, a key component of Wnt/PCP pathway, and showed that PKC $\delta$  is a key enzyme for the translocation as well as for signaling (60). Furthermore, we identified an ARF GAP protein XGAP is required in mesodermal cells for the localization of signaling proteins such as aPKC, Par5 (14-3-3) and Par6, and for the formation of cell protrusions at the ends of the cells (82). These studies highlighted the importance of the coordination of Wnt/PCP pathway with underlying basal systems regulating membrane trafficking and establishing cell polarity and also underlined the intrinsic question as to how the mediolateral cell polarity is actually triggered.

#### **1.2.4. Genome-wide approaches to understand developmental processes**

In order to grasp a global view of early developmental processes in relation to the regulation of gene expression and function, we realized the necessity of describing a gene regulatory network focusing not on a limited number of genes but on the entire set of genes. In the last ten years, whole genome of a variety of organisms has been sequenced and the information made available. Taking advantage of these developments, we started to introduce whole-genome approaches to developmental biology. We first fabricated DNA macroarray using *C. elegans* cDNA set which had been collected and sequenced by the pioneering effort of Dr. Kohara at the National Institute of Genetics. Thousands of unique cDNAs were spotted on filter papers. The aim was to screen target genes of pathways by a differential hybridization using cDNA probes generated from wild type strain and a mutant strain in which a specific pathway of interest is disrupted. Our interest was in the regulation of body length and thus we compared the gene expression profiles between wild type and *Cet1/Db11* and screened for positively or negatively regulated genes (28, 50). The profile was then compared to that of another related mutant of the downstream component *Sma2* in the same pathway; overlapping positive clones were further characterized by in situ hybridization, etc. After the series of experiments using the DNA array, we became convinced that the time and processes of conventional gene expression analysis could be minimized, and that genetic analyses in developmental biology must be revolutionized by this comprehensive approach. This led us to the next step of introducing a similar approach to *Xenopus* developmental biology, taking full advantage, particularly, of the efficacy of *Xenopus* embryo for gain-of-function experiments. Nevertheless, we needed to start by collecting cDNA sets from *Xenopus laevis* embryonic cDNA libraries for producing macro/microarray. After a few years of laborious work, and thanks to the generous support of the Japanese Society of Promotion of Science (JSPS), we have been able to obtain approximately 12,000 unigenes available for micro/macroarray (73, 74, 76). We also profiled developmental expression pattern for approximately 3,000 genes by whole-mount in situ hybridization. After we set up our XDB database, we were able to disclose all of the information on DNA sequence as well as expression pattern, and distribute cDNAs to the scientific community upon request. Using the microarray, not only my group but other groups as well have been able to identify the target genes of a variety of developmental pathways, including FGF and nodal/activin pathways (86, 88).

## **2. Perspective of research**

In the next decade, it will become increasingly more important to understand

morphogenesis at the cellular and organelle levels rather than as a whole, as cells are the fundamental entity of the whole body. It is important, therefore, to ask and to describe how cells behave in developmental contexts. In order to achieve this, bioimaging is obviously an essential technology, as it enables cell observation at higher temporal and spatial resolutions. During CE, the above-mentioned cell movements allowing anterior-posterior elongation of embryo proper, the cellular protrusions are known to be confined to both ends of polarized cells to exert tractive force for the intercalation. Little is known, however, about what triggers the cell polarization and what the polarization causes to control intracellular events enabling CE. To this end, we have been using EB3 (end-binding 3), a member of +TIPs that bind to the plus end of microtubule (MT), to visualize the intracellular polarity of chordamesoderm cells during CE to investigate the trigger of the establishment of cell polarity (**submitted**). Such efforts are essential for an understanding of morphogenesis at cellular levels, and we plan to put a significant effort into improving and refining our bioimaging methodologies. One example is to develop a new probe alternative to fluorescence. Although it is now very common to use fluorescent proteins as markers, they can be inefficient for use with *Xenopus* embryo due to the endogenous auto-fluorescence as a noise. To circumvent this problem, we have been attempting to use bioluminescence, in collaboration with Dr. Ozawa at the Institute for Molecular Science in Okazaki. We hope that the use of such new probes will open new windows on the continuing study of developmental phenomena.

In addition to bioimaging, we consider that taking mechanical forces into account in order to fully understand developmental morphogenesis is very important, and, after observing MT growth using EB3, we have developed the impression that mechanical forces rather than chemical signals may have crucial roles in CE. Characterizing cellular responses to mechanical stress will be an important part of our future studies.

### **3. Other academic activities**

#### **3.1 Teaching**

In addition to teaching at The Graduate University of Advanced studies (SOKENDAI), the graduate school affiliated with NIBB, I have taught at other national and private universities and their graduate schools, including Kyoto University, Hiroshima University, Kobe University, and Keio University. I also participated as a lecturer during a practical course on *Xenopus* embryology at Cold Spring Harbor Laboratory, USA in 2002.

### **3.2 Academic society**

I belong to several Japanese societies including the Japanese Society of Developmental Biologists (JSDB), The Molecular Biology Society of Japan (MBSJ), the Japanese Cancer Association (JCA), and The Japanese Biochemical Society (JBS).

The Japanese Society of Developmental Biologists (JSDB) is the major society for my research activities. I have been the secretary general of the society since 2006, managing the yearly budget, annual meetings, board meetings, and general assemblies.

I also belong to two international societies, the International Society of Developmental Biologists (ISDB), and the International Society of Differentiation (ISD). For ISD, I have been on the board of directors and a publication committee member since 2000, the president-elect from 2006, and will become the president in 2008.

### **3.3 External reviewing and evaluation**

#### **3.3.1 Grants**

I have contributed as a reviewer for Japanese and foreign grants through my involvement with Wellcome trust, the National Science Foundation (NSF), the Biotechnology and Biological Sciences Research Council (BBSRC), the Human Frontier Science Program (HFSP), etc.

#### **3.3.2 Personal evaluations**

I have evaluated the personal achievements of several scientists for their promotions, renewal of employment, tenure, etc. Affiliation of them includes with Johns Hopkins University, School of Medicine, and University of North Carolina.

#### **3.3.3 Evaluation committee**

I acted as an evaluation committee member (“international collaborations” section) for the National Institute of Fusion Science, one of NIBB’s sister institutes in the National Institutes of Natural Sciences (NINS), Japan, in 2006-2007, and for the Institute for Molecular Embryology and Genetics, Kumamoto University, Japan, in 2001.

### **3.4 Editorial board**

I have been on the editorial board of the following international journals and actively involved in editorial decision and reviewing:

Development, Growth, and Differentiation, Wiley-Blackwell

Development, The Company of Biologists Ltd.

Differentiation, Wiley-Blackwell

Birth Defects Research, Embryo Today, Wiley-Blackwell  
Mechanism of Development, Elsevier

### **3.5 Other services**

#### **3.5.1 Operation of XDB, a platform for the *Xenopus laevis* functional genomics**

Since 2000, my laboratory has been operating a large-scale sequencing of *Xenopus laevis* cDNAs and profiling their temporal and spatial expression pattern during development. All of the information is available to the scientific community through the web site and cDNAs are distributed all over the world for free upon request. Thousands of DNA profiles have already been distributed.

#### **3.5.2 Service to Xenbase, a community-shared comprehensive database**

I have been a steering committee member of Xenbase and attempting to integrate the information stored in XDB into Xenbase.

#### **3.5.3 Service to a private company**

I have been on the board of trustees of KIKKOMAN Co., a Japanese company, since 2000, as well as a consultant for their research institute.

## **4. Personal information**

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OKAZAKI 444-8585
- d. Research area** Cell and developmental biology
- e. Education** University of Tsukuba, Doctoral Program, Applied Biochemistry,  
Ph.D. in 1984, Department of Agricultural Chemistry, B.A. in 1979
- f. Professional experience**
- |               |  |
|---------------|--|
| 1997- present | Professor and Chairperson<br>Division for Morphogenesis,<br>Department of Developmental Biology,<br>National Institute for Basic Biology,<br>National Institutes of Natural Sciences |
| 1993-1997     | Professor<br>Faculty of Pharmaceutical Sciences  |

Hokkaido University, Japan  
1988-1993 Assistant Professor  
Institute of Applied Biochemistry  
University of Tsukuba, Japan  
1984-1988 Postdoctoral Fellow/Research Associate  
Laboratories for Neuroendocrinology  
(Professor Roger Guillemin)  
The Salk Institute for Biological Studies  
La Jolla, California, U.S.A.

#### **g. Awards**

None.

#### **h. Professional societies**

Japanese Society of Developmental Biologists (JSDB)

The Molecular Biology Society of Japan (MBSJ)

Japanese Cancer Association (JCA)

The Japanese Biochemical Society (JBS)

#### **i. Publications**

##### **Class 1: Research articles in peer reviewed journals**

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## **Class 2: Invited reviews, book chapters**

1. Ueno, N. BMP signaling in vertebrate embryogenesis. In *Tissue Engineering for Therapeutics use 2*, eds. Ikeda, Y. and Enomoto, S., 1998
2. Iemura, S., Yamamoto, T.S., Takagi, C. and Ueno, N. The analysis of protein-protein interactions in early development: direct binding of follistatin, an organizer factor, to BMPs. In *Real time Analysis of Biomolecular Interactions. Applications of BIACORE* eds. Nagata, K. and Handa, H. Springer, pp105-114, 2000
3. Ohkawara, B. and Ueno, N. Regulation of pattern formation by the interreaction between growth factors and proteoglycans. In *Morphogenesis and Pattern Formation in Biological Systems* eds. Sekimura, T., Noji, S., Ueno, N. and Maini, P.K. Springer pp 69-82, 2003
4. Ueno, N. and Greene, N.D. Planar cell polarity genes and neural tube closure. In *Birth Defects Res. Part C Embryo Today* 69, 318-324, 2004

**j. Symposium presentations/Invited seminars (International)**

1. HFSP Workshop X "Axis formation in the vertebrate embryo"  
"Xmsx-1 in ventralization and head repression"  
2000/5/9 - 2000/5/11
2. "Restriction of BMP diffusion in *Xenopus* ectoderm"  
International Congress on Differentiation and Cell Biology  
2000/9/24 - 2000/9/28
3. Global analysis of gene expression during early development of *Xenopus laevis*  
JDSB 14th International Congress of Developmental Biology  
2001/7/8 - 2001/7/12
4. Induction of apoptosis by BMP 2/4 in the 7th generation of newt spermatogonia. II.  
Possible relationship between prolactin and BMP  
JDSB 14th International Congress of Developmental Biology  
2001/7/8 - 2001/7/12
5. Developmentally regulated expressions of the polyubiquitin receptor Rpn10 family of  
the 26 proteasome  
JDSB 14th International Congress of Developmental Biology  
2001/7/8 - 2001/7/12
6. Ventroptin: A novel BMP-4 antagonist in the retina  
JDSB 14th International Congress of Developmental Biology  
2001/7/8 - 2001/7/12
7. BMP activity gradient as revealed by anti P-Smad  
JDSB 14th International Congress of Developmental Biology  
2001/7/8 - 2001/7/12
8. Regulation of *C. elegans* body length by a member of the TGF- $\beta$ superfamily  
JDSB 14th International Congress of Developmental Biology  
2001/7/8 - 2001/7/12
9. DNA microarray analysis in *Xenopus*  
JDSB 14th International Congress of Developmental Biology  
2001/7/8 - 2001/7/12
10. Induction of apoptosis by BMP 2/4 in the 7th generation of newt spermatogonia. II.  
Possible relationship between prolactin and BMP  
JDSB 14th International Congress of Developmental Biology  
2001/7/8 - 2001/7/12
11. Suppression of head formation by Xmsx-1 through the inhibition of intracellular nodal  
JDSB 14th International Congress of Developmental Biology  
2001/7/8 - 2001/7/1

12. "Role of glypican in gastrulation"  
9th International Xenopus Conference  
2002/8/21 - 2002/8/25
13. "Regulation of Pattern formation by the interaction between growth factors and proteoglycans"  
International Conference on Morphogenesis and Pattern Formation in Biological Systems  
2002/9/24 - 2002/9/27
14. Japan's Xenopus Projects  
Xenopus Genetics and Genomics Workshop – 2003 (NICHD)  
2003/10/21 - 2003/10/21
15. "Molecular Dissection of Cell Movements During Vertebrate Gastrulation"  
The CDB symposium 2004 " Developmental Remodeling"  
2004/3/29 - 2004/3/31
16. "Molecular Dissection of Cell Movements During Vertebrate Gastrulation"  
13th International Conference, International Society of Differentiation  
2004/9/5 - 2004/9/9
17. Molecular dissection of Xenopus gastrulation cell movements  
The 1st NIBB-EMBL symposium (EMBL & NIBB)  
2005/7/1 - 2005/7/2
18. Establishment of cell polarity in Xenopus development  
The 13th CDB meeting -Morphogenetic Signaling: the view from the frog  
2006/2/21
19. The role of Pricke in the mouse development  
The 3rd NIBB-EMBL symposium (EMBL & NIBB)  
2006/4/19 - 2006/4/20
20. Establishment of the cell polarity during Xenopus gastrulation  
Japan/Italy Meeting on Vertebrate Organogenesis  
2006/4/24 - 2006/4/26
21. Xenopus gastrulation: A view from cell polarity  
11th International Xenopus Conference  
2006/5/31 - 2006/6/3
22. Gastrulation cell movements in Xenopus: A view from cell polariy  
14th Conference of the International Society of Differentiation  
2006/10/7 - 2006/10/11
23. Vertebrate gastrulation cell movements: A view from cell polarity



Animal disease model an Stem cell Biology Meeting

2006/11/16 - 2006/11/17

24. Establishment of Cell Polarity in Xenopus Gastrulation

The 5th NIBB-EMBL Meeting “Cell & Developmental Biology”

2007/3/24 - 2007/3/26

**k. Organization of symposium (International)**

1. New Horizons of Developmental Biology

(Makoto Asashima, Atsushi Kuroiwa, Sadao Yasugi, Sumiire Noji & Naoto Ueno )

Okazaki Conference Center, NIBB

2001/3/23 - 2001/3/24

2. The first international workshop on Promotion of Xenopus functional genomics

JSPS Core-to-Core Program

2005/1/7 - 2005/1/8

3. The 1st NIBB-EMBL Symposium “Developmental biology”

EMBL & NIBB (Stephen Cohen & Naoto Ueno)

EMBL, Germany

2005/7/1 - 2005/7/2

4. The 2nd NIBB-EMBL Symposium “Bioimaging”

NIBB & EMBL (Naoto Ueno & Jan Ellenberg)

Okazaki Conference Center, NIBB

2006/3/22 - 2006/3/23

5. The 3rd NIBB-EMBL Symposium “Mouse biology”

EMBL & NIBB (Nadia Rosenthal & Naoto Ueno)

Monterotondo, Italy

2006/4/19 - 2006/4/20

6. 11th International Xenopus Conference

(Makoto Asashima, Masanori Taira & Naoto Ueno )

Kazusa Academia Center

2006/9/12 - 2006/9/16

7. The 5th NIBB-EMBL Meeting "Cell and developmental biology"

National Institute for Basic Biology (Naoto Ueno)

Okazaki Conference Center

2007/3/24 - 2007/3/26

## **l. Grant awards**

Research for the Future Program

Elucidation of the Principles of Development and Regeneration by Systematic Analysis of Genes

2000-2004

Grant-in-Aid for Scientific Research on Priority Areas

Dynamics of Developmental Systems

2000-2005

Grant-in-Aid for Scientific Research, Scientific Research Project “A”

Elucidation of mechanisms underlying the establishment of cell polarity during gastrulation.

2005-2007

## **m. Professional activities**

Development (The Company of Biologists Limited): Editorial Board Member

1999

International Society of Differentiation: Board of Directors

2000

Differentiation (Blackwell): Senior Editor

2000

Development, Growth and Differentiation (Blackwell): Editorial Board Member

2000

Birth Defects Research Part C: Embryo Today: Reviews: Associate Editor

2003

International Society of Differentiation: President-elect

2008, 2007, 2006 (Naoto Ueno)

## **n. Teaching experience**

Adjunct Professor:

Osaka University, Nagoya University, Tokushima University, Hiroshima University, Tokyo Metropolitan University, Kyoto University, Gifu Univ. Medical school, Hokkaido University, Keio University, and so on.

In addition to teaching at SOKENDAI, the graduate school affiliated with NIBB, I have taught at other national and private universities and their graduate schools, including Kyoto University, Hiroshima University, Kobe University, and Keio University. I also participated as a lecturer during a practical course on *Xenopus* embryology at Cold Spring Harbor Laboratory in 2002.

**o. Other activities (if any)**

I have been acting as the director for the promotion of the international collaboration between NIBB and European Molecular Biology Laboratory (EMBL). In 2004, NIBB and EMBL agreed to promote the mutual exchange of academic information, technologies, and researchers, including graduate students. Since then, I have organized joint meetings, technology transfer of the new microscope "SPIM" from EMBL, and a researchers' exchange program.

**5. Selected reprints (5 papers)**

Attached.

1. Ohkawara, B., Iemura, S., ten Dijke, P. and Ueno, N. Action range of BMP is defined by its N-terminal basic amino acid core. *Curr. Biol.* 12, 205-209, 2002
2. Morita, K., Flemming, A., Sugihara, Y., Mochii, M., Suzuki, Y., Yoshida, S., Wood, B., Kohara, Y., Leroi, A.M. and Ueno, N. A *Caenorhabditis elegans* TGF- $\beta$ , DBL-1, controls the expression of LON-1, a PR-related protein, that regulates polyploidization and body length. *EMBO J.* 21, 1063-1073, 2002
3. Takeuchi, M., Nakabayashi, J., Sakaguchi, T., Yamamoto, T.S., Takahashi, H., Takeda, H. and Ueno, N. The prickle-related gene in vertebrates is essential for gastrulation cell movements. *Curr. Biol.* 13, 674-679, 2003
4. Hyodo-Miura, J., Yamamoto, T.S., Hyodo, A.C., Iemura, S., Kusakabe, M., Nishida, E., Natsume, T. and Ueno, N. XGAP, an ArfGAP, is required for polarized localization of PAR proteins and cell polarity in *Xenopus* gastrulation. *Dev. Cell* 11, 69-79, 2006
5. Ogata, S., Morokuma, J., Hayata, T., Kolle, G., Niehrs, C., Ueno, N.\* and Cho, K.W.\* TGF- $\beta$  signaling-mediated morphogenesis: modulation of cell adhesion via cadherin endocytosis. *Genes Dev.* 21, 1817-1831, 2007 (\*corresponding authors)



### 1-3. 在職10年の教授業績評価について

## 諸橋憲一郎教授





## 諸橋憲一郎教授在職10年業績評価

基礎生物学研究所 所長 岡田清孝

### 1. 経緯

- ・平成19年5月 基礎生物学研究所所長および教授3名からなる在職10年教授業績評価実行委員会を設置。
- ・平成19年10月 諸橋教授の研究分野に近い所外研究者から海外3名、国内2名を選んで評価委員を委嘱し、資料を送付。送付資料 1) 研究活動の説明、2) 研究業績リスト、3) 主たる業績の論文別刷、4) 略歴
- ・平成20年1月 海外3名、国内2名の評価委員全員から回答を受け取った。

### 2. 評価内容

#### A委員による研究内容のまとめと評価（一部抜粋）

諸橋教授は哺乳類のステロイドホルモンとくに性ステロイドの合成に関わる器官の発生と性分化を制御する転写因子のカスケードを精力的に研究してきた。その源流は諸橋教授が Ad4BP/SF-1 と呼ばれる転写因子を同定したことに遡るが、彼はこの10年の間この因子を中心に独自の研究を展開し、スケールの大きなものに育て上げた。その成果は、Ad4BP/SF-1 遺伝子の各組織特異的エンハンサーの同定、上流、下流の因子の同定、制御ループの発見、それらの生殖腺をはじめとする各器官の発生や性分化における役割、ダイオキシン受容体との協調作用、Ad4BP/SF-1 制御における SUMO 化の役割など多岐にわたる。これらの成果の多くは分子生物学や内分泌学の一流の専門誌に発表されており、とくに DAX-1 が Ad4BP/SF-1 の標的の一つであることを示した論文 (Mol. Endocrinol. 1999) や、Ad4BP/SF-1 と相互作用する ARX が XLAG（滑脳症と性器の低形成を特徴とする遺伝病）の原因遺伝子であることを示した論文 (Nature Genet. 2002) は高い引用回数を誇る（それぞれ2007年12月時点で65回と148回）。また、1997年に Genes to Cells に発表された総説も47回引用されている。さらに、諸橋教授は国際会議を含む学会に合計63回招待されている。これらの数字は彼の研究がいずれもインパクトの高い独創的なものであることを示しており、その研究業績は高く評価できる。

また、諸橋教授は熱心に学生の教育を行っており、合計9名の総研大博士課程学生を指導し、うち4名が博士号を取得している。さらに、11名の他大学の大学院生の指導・教育を行ったことは、大学共同利用機関としての研究所の役割に大きな貢献をしたといえる。

また、2004年からは特定領域研究「性分化」を領域代表として率い、同分野の発展に大きく貢献している。この特定領域でも若手の会などを通して積極的に後進の育成に努めっていると聞いている。このように、諸橋教授の当該研究分野の発展や後進の指導への貢献は高く評価すべきものである。

B委員による評価（一部抜粋）

I have known Dr. Morohashi since at I met him in the scientific meeting almost 15 years ago when he was at the Kyushu University as an assistant professor. At that time, he was one of the first scientists to clone the cDNA of nuclear receptors far before the idea that 48 nuclear receptors from a gene superfamily. By extending his previous studies on p450 gene promoters, he was trying to identify the master transcription factor, and eventually discovered one of the most physiologically important nuclear receptors, Ad4BP(SF-1). This factor was turned out to be essential for gonad and kidney development by following works by his group and the others. After this work, he moved into and developed a new field related in sex difference and determination. Considering from his publication and contribution to this related field, he is quite outstanding and scientifically successful. Moreover Dr. Morohashi is internationally well known and is invited to major international meetings.

C委員による評価（一部抜粋）

Prof Morohashi joined the National Institute for Basic Biology (NIBB) in 1977. In the last ten years, his research has earned him the reputation of one of the leading researchers in the fields of gonad development, nuclear receptor function, transcriptional regulation, and adrenal development. These areas of work are all tied to the theme of sex differentiation of animal species. (中略)  
In summary, Prof Morohashi is very highly rated as a researcher and leader in Japanese developmental and reproductive biology, and is commended on the quality and depth of his accomplishments at NIBB.

D委員による評価（一部抜粋）

The first phase of Dr. Morohashi's career dealt with the isolation and characterization of Ad4BP/SF-1, which represented a signal accomplishment in molecular steroidogenesis and culminated in his recruitment to the NIBB. Over the 10 years, he has continued to focus on extending these outstanding studies and defining the mechanisms that allow Ad4BP/SF-1 to function in complex networks of tissue-specific development. These studies fall into two main



themes: understanding the transcription factors/co-regulators that determine expression of Ad4BP/SF-1 in the various tissues where it normally is expressed and defining the mechanisms that modulate the function of this transcription factor after it is translated. In recognition of these accomplishments, Prof Morohashi received the Asia and Oceania Medal from the Society for Endocrinology and the British Endocrine Society in 2007. (中略)

There is a high degree of confidence that Prof Morohashi has the talent and resources to bring these studies to a successful conclusion, thereby providing new insights into the mechanisms of tissue-specific differentiation.

E委員による評価（一部抜粋）

Prof. Morohashi remains at the forefront of the field of sexual differentiation and his research plans will continue to push this field forward. Perhaps more importantly, his work can be extrapolated to other broader fields concerned with gene regulation and orphan receptors where his past and future work will also have an important impact.

In addition, I would like to mention that Ken-ichirou is a scientist who is extremely well liked in the field for his kindness, fairness, and generosity. He freely provides his reagents to the international community and is open and considerate in his dealings with others in his field. He has done an outstanding job of the organization of several important meetings in the field of sex determination that have been exciting forums for the exchange of ideas in the field. He is the sort of scientist that we should all strive to be – He is doing exciting, creative, and rigorous science, but he is also kind and generous in his dealings with others. I believe that Ken-ichirou Morohashi is an outstanding scientist.

### 3. まとめ

諸橋教授は、哺乳類の性ステロイドの合成に関わる器官の発生と性分化を制御する転写因子のカスケードを精力的に研究し、この分野の研究リーダーと認知されている。特定領域研究の領域代表者を務めるなど、研究者コミュニティからの期待も大きい。基礎生物学研究所においても庶務および連携担当主幹として努力された。なお、諸橋教授は平成19年4月より九州大学大学院医学研究院教授に転任し、平成19年4月より平成20年7月まで基礎生物学研究所教授を兼任している。国際的リーダーとしての今後の飛躍を期待する。

## **Ken-ichirou Morohashi, Prof. PhD 1997-2007**

- 1. Personal Information***
- 2. Statement of Research Activity in the Past 10 years***
- 3. List of Publication***
- 4. List of Invited Presentation***
- 5. Perspective of Research***
- 6. Description of Other Academic Activities***  
***(Education, Service to the Academic Communities, etc.)***
- 7. Reprints for Selected Papers***

## **1. Personal Information**

- (1) **Name:** Ken-ichirou Morohashi
- (2) **Date of Birth:** ██████████
- (3) **Address (office):** National Institute for Basic Biology  
Higashiyama 5-1, Myodaiji-cho, Okazaki 444-8787, Japan  
TEL; 81-564-59-5865, FAX; 81-564-59-5866  
E-mail; moro@nibb.ac.jp
- (4) **Research Area:** Sexual Differentiation, Transcriptional Regulation, Nuclear Receptor
- (5) **Education:**  
1976-1981; Dept. of Biology, Faculty of Science, Kyushu Univ.  
1981-1986; Dept. of Biology, Graduate School of Science, Kyushu Univ.  
1986; Awarded the degree of D. Sci.
- (6) **Research and Professional Experience:**  
1986-1996 Instructor at Department of Molecular Biology,  
Graduate School of Medical Sciences, Kyushu University  
1996-2003 Professor at Department of Developmental Biology,  
National Institute for Basic Biology  
1996-2006 Professor at The Graduate University for Advanced Studies  
2004-2006 Professor at Division of Sex Differentiation,  
National Institute for Basic Biology,  
National Institute of Natural Sciences  
2007- Professor at Department of Molecular Biology,  
Graduate School of Medical Sciences, Kyushu University
- (7) **Award:** Asia & Oceania Medal from Society for Endocrinology, 2007
- (8) **Professional Societies:**  
Japanese Society of Molecular Biology, Japanese Endocrine Society  
Endocrine Society, Japanese Biochemical Society,  
Japanese Steroid Hormone Society,  
Japanese Reproductive Endocrinology Society
- (9) **Organization of Symposium**  
1. NIBB conference 'Molecular Mechanisms of Sex Differentiation'  
Oct. 18-20, 2002, Okazaki  
Representative Organizer  
2. The 1st International Nuclear Receptor Meeting in Japan  
Feb. 28-Mar. 2, 2002, Kyoto  
Organize with Prof. Shigeaki Kato and Hajime Nawata

3. The 2nd International Nuclear Receptor Meeting in Japan  
Feb 14-16, 2003, Osaka  
Organize with Prof. Shigeaki Kato and Hajime Nawata
4. The 3rd International Nuclear Receptor Meeting in Japan  
April 15-17, 2004, Osaka  
Organize with Prof. Shigeaki Kato and Hajime Nawata
5. The 1st Symposium on Intra- and Extra-Environment and Biological Responses  
July 27-28, Fukuoka  
Representative Organizer
6. International Symposium on 'Molecular Mechanisms of Sex Determination and Differentiation' Sep 21, 2006, Matsue  
Representative Organizer, Coorganized with the Zoological Society of Japan
7. The 4th International Nuclear Receptor Meeting in Japan  
Feb 1-2, 2007, Osaka  
Organize with Prof. Shigeaki Kato and Hajime Nawata

**(10) Grant Awards**

1997-1998	Grant-in-Aid for Scientific Research (B);	12,100,000 yen
1997-1998	Core Research for Evolutional Science and Technology;	25,000,000 yen
1999-2001	Grant-in-Aid for Scientific Research (A);	24,800,000 yen
1999-2003	Core Research for Evolutional Science and Technology ;	474,000,000 yen
2004-2009	Grant-in-Aid for Scientific Research on Priority Areas;	264,000,000 yen
2004	Grant-in-Aid for Scientific Research (S);	35,000,000 yen

**(11) Professional Activities**

Journal of Biochemistry; Advisory Board, Jan, 2000-Dec, 2001  
 Endocrinology; Editorial Board, Jan, 2006-present  
 Sexual Development; Section Editor, 2006- present  
 Molecular Endocrinology; Editorial Board, Jan 1, 2007- present

**(12) Teaching Experiences**

1999 University of Tsukuba School of Medicine  
 2000 Hyogo University of Teacher Education, Nagoya University Faculty of Agriculture  
 Kumamoto University School of Medicine  
 2001 Hokkaido University Faculty of Science  
 2002 Yamaguchi University School of Medicine  
 2003 Yamaguchi University School of Medicine  
 2004 Yamaguchi University School of Medicine, Hyogo University of Teacher Education  
 2005 Yamaguchi University School of Medicine  
 Fukui University School of Medicine, Nagoya University School of Medicine  
 2006 Nagoya University School of Medicine  
 2007 Nagoya University School of Medicine  
 Kanazawa University Faculty of Pharmacology

## **2. Statement of Research Activity in the Past 10 Years**

In the past 10 years, the Division of Sex Differentiation has conducted research for transcriptional regulation, tissue-specific gene expression, nuclear receptor function, gonad development, and adrenal development. These studies are basically related to sex differentiation of animal species.

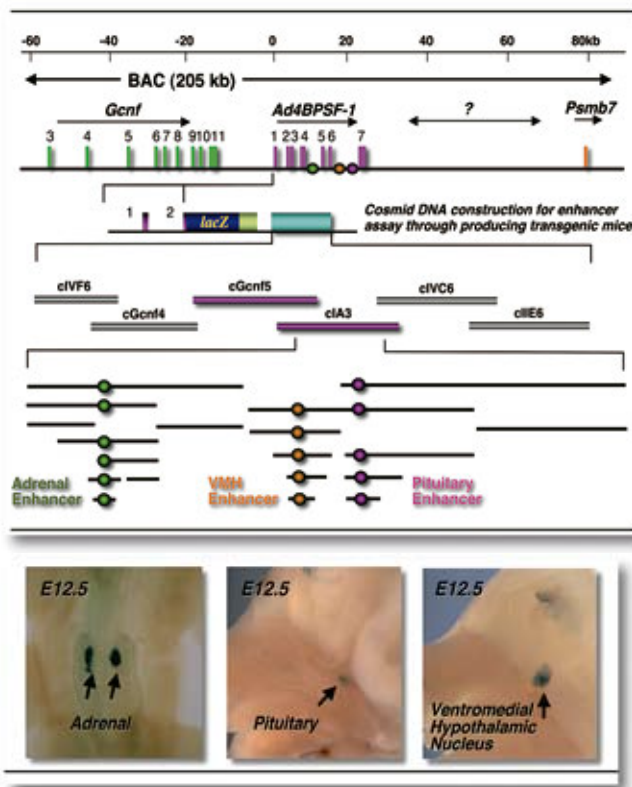
During tissue differentiation, it is conceivable that certain genes encoding transcription factors act as critical components forming a gene regulatory cascade. One component of the cascade required for adrenocortical and gonadal differentiation is the nuclear receptor, Ad4BP/SF-1. The Ad4BP/SF-1 is localized upstream of a set of tissue-specific genes that include steroidogenic *Cyp* genes, and at the same time it is localized downstream of other transcription factors regulating the gene transcription. Given that activation/inactivation of the components occurs in an upstream to downstream direction along the cascade during tissue differentiation, and that Ad4BP/SF-1 is an essential transcription factor of the adrenocortical and gonadal cascades, identification of the components that function with Ad4BP/SF-1 and regulate *Ad4BP/SF-1* gene transcription is quite important to fully understand the entire gene cascade. This is the core of our research program on the molecular mechanisms underlying differentiation of the adrenal cortex and gonad, and gonad sex differentiation.

### **(1) Tissue-Specific Enhancers of *Ad4BP/SF-1* Gene**

Based on the above background, we investigated the regulatory region of the *Ad4BP/SF-1* gene *in vivo*. Our results showed that *Ad4BP/SF-1* is expressed in testicular Leydig and Sertoli cells, ovarian theca and granulosa cells, adrenocortical cells, pituitary gonadotropes, ventromedial hypothalamic nucleus (VMH), and splenic endothelial cells, and that such expression is tightly coupled with tissue development.

It is well accepted that spatial and temporal control of gene expression is essential for establishment of cell fate, and gene transcription is thought to be mediated by appropriate interaction between enhancers and basal promoter. In the case of *Ad4BP/SF-1*, the tissue-specific enhancers are expected to be localized somewhere in the gene locus, and the primary structures of the enhancers are expected to provide information to understand the mechanisms underlying tissue development. Based on this concept, we performed transgenic mouse assays with DNA fragments prepared from a BAC clone containing whole genomic locus of *Ad4BP/SF-1* gene. The BAC clone carried all the elements necessary to reproduce the endogenous expression of the gene (Ref. 41, Fatchya et al, 2006). Eventually, this study succeeded in identifying the tissue-specific enhancers for the fetal adrenal cortex (Ref. 40, Zubair et al, 2006), the VMH (Ref. 31, Shima et al, 2005), pituitary gonadotrope (Ref. 49, Shima et al, submitted), and recently fetal Leydig cells (Figure 1).

In general, it is well known that functional genomic sequences such as exon, basal promoter, and splicing site, are structurally conserved among animal species. Expectedly, sequence comparisons revealed that the enhancers found in the *Ad4BP/SF-1* gene are structurally conserved at least in mammalian species. Since the conserved regions should contain the core sequences recognized by certain transcription factors, the conserved regions were further analyzed by introducing nucleotide substitutions. These constructs were subjected to transgenic mouse assays and thus the functionally active core sequences in the tissue-specific enhancers were finally identified.

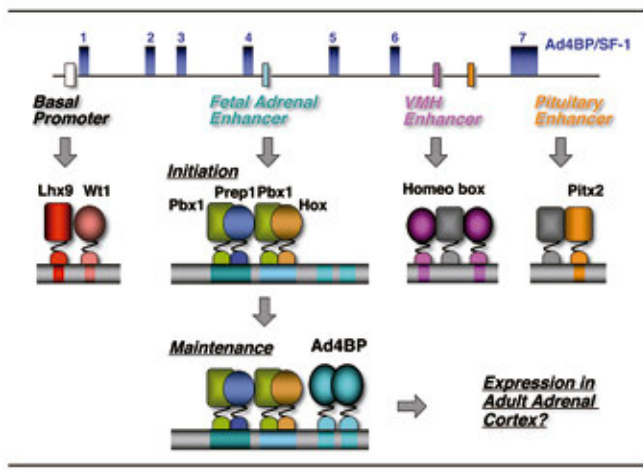


**Figure 1.** Identification of enhancers for the fetal adrenal, ventromedial hypothalamus (VMH), and pituitary of the mouse *Ad4BP/SF-1* gene. (upper panel) *Ad4BP/SF-1* gene consists of seven exons (red boxes). Two other genes; *Nrf6a1* (green boxes) and *Gpr144* (region indicated by ?), are localized 5' upstream and 3' downstream of *Ad4BP/SF-1*, respectively. Various DNA fragments were examined by transgenic assays with *lacZ* as the reporter gene. Among the clones covering the *Ad4BP/SF-1* locus, *cIA3* induced *lacZ* expression in the fetal adrenal, ventral diencephalon (VMH), and pituitary, while *cGcnf5* induced *lacZ* expression only in the fetal adrenal. These active fragments were further fragmented into smaller pieces and subjected to transgenic mouse assays. The enhancers for fetal adrenal were identified in the fourth intron, while those for the VMH and pituitary were in the sixth intron. Lower panel: Representative views of expression of *lacZ* in the fetal adrenal (left), pituitary (middle), and VMH (right) at embryonic day 12.5.

## Fetal Adrenal Enhancer

In the fetal adrenal enhancer conserved among animal species, we noted the presence of two potential *Ad4BP/SF-1* binding sites (*Ad4* sites). Since *Ad4BP/SF-1* activates gene transcription by binding to the recognition sites, the *Ad4* sites in the fetal adrenal enhancer suggested that *Ad4BP/SF-1* gene expression is maintained by autoregulation. This hypothesis was examined further using transgenic mice. These studies demonstrated that the *Ad4* sites in the fetal adrenal enhancer participate in autoregulation of *Ad4BP/SF-1* gene expression in the fetal adrenal. However, initiation of transcription would occur prior to this maintenance step. To address this question, we attempted to identify functional *cis*-elements other than the *Ad4* sites, and found that the potential binding sites for the Pbx/Prep and Pbx/Hox heterodimers are tightly conserved among vertebrate species (Figure 2). RT-PCR revealed that all candidate factors were detected in the early adrenal primordium. Moreover, both Pbx/Hox and Pbx/Prep binding sites were found to be essential for the initiation step of the gene transcription by transgenic mouse studies using *Ad4BP/SF-1* KO mice (Ref. 40, Zubair et al, 2006).

Hox transcription factors direct the patterning of a variety of structures during embryonic development of both vertebrates and invertebrates. Based on the expression profile and binding specificity, we consider that *Hoxb5*, *Hoxb9*, *Hoxc5*, and possibly *Hoxc6* regulate the *Ad4BP/SF-1* expression in the fetal adrenal. The adrenal cortex originates from certain part of the mesoderm. To specify the adrenal region, these *Hox* gene products would induce *Ad4BP/SF-1* expression at the particular region of the mesoderm. Since it has been established that *Hox* genes control axial identity in an anterior-to-posterior direction, the location of the adrenal cortex could be determined by combined expression of the particular set of *Hox* genes.



**Figure 2.** Schematic presentation of structures and functions of the tissue-specific enhancers and basal promoter of the *Ad4BP/SF-1* gene. Transcription factors, Lhx9 and Wt1, are thought to bind to the basal promoter, while Pbx1, Prep1, and Hox proteins bind to the fetal adrenal enhancer as a ternary complex to initiate transcription at the adrenal primordial cells. At a later stage, the gene product, Ad4BP/SF-1, binds to the enhancer to maintain the gene expression. Homeobox-containing proteins together with an unidentified protein bind to the VMH enhancer while Pitx2 acts as an essential factor to activate the pituitary enhancer. Although still unclear, a factor that functionally correlates with Pitx2 is required for the enhancer function.

### VMH Enhancer

Functionally, the VMH enhancer localized in the 6th intron is characterized by its ability to drive VMH-specific expression of the *lacZ* reporter gene. Structurally, the sequence is conserved among vertebrate animal species (mouse, human, and chicken), suggesting again that the conserved sequences function as a VMH-specific enhancer beyond animal species. Detailed transgenic analyses of the enhancer revealed that it contains suppressive and activating elements. Mutation of the former element resulted in ectopic reporter gene expression in an area dorsal to the intrinsic expression domain, while mutations in the latter containing ATTA motifs led to the disappearance of the reporter gene expression. The sequence, ATTA, strongly suggested the involvement of homeobox proteins (Figure 2, Ref. 31, Shima et al, 2005).

### Pituitary Gonadotrope Enhancer

We demonstrated by transgenic mouse assays that the pituitary gonadotrope-specific enhancer is localized within the 6th intron. Functionally, the enhancer recapitulates endogenous *Ad4BP/SF-1* expression in the fetal Rathke's pouch to the adult pituitary gonadotrope. Structurally, the enhancer consists of several elements conserved among animal species. Mutational analyses confirmed the functional significance of these elements. One of these elements interacts both *in vitro* and *in vivo* with Pitx2, suggesting that Pitx2 regulates *Ad4BP/SF-1* gene transcription in the pituitary gonadotrope via direct interaction with the gonadotrope-specific enhancer (Figure 2, Ref. 49, Shima et al, submitted).

## (2) Gonad Development and Gonad Sex Differentiation

Although sexual dimorphism in mammals manifests most obviously in the gonads (testis and ovary), sexual dimorphism can be observed in the entire body of the animal. For instance, it is well known that several tissues such as the external genitalia, muscle, and brain exhibit sexual dimorphisms in terms of their structures and functions. This process of sex differentiation is controlled by sex steroids synthesized in the sexually differentiated gonads. Therefore, the gonad sexes are quite important for sex differentiation of the animal.

Several transcription factors play crucial roles in the process of gonad differentiation. Some of these factors, such as Sry, Wt1, Dax-1, Sox9, and Arx, were identified as products of genes

responsible for human diseases that display structural and functional defects in several tissues including the gonads. The functions of other transcription factors, such as Ad4BP/SF-1, Emx2, M33, and Lhx9, were elucidated by the phenotypes of gene-disrupted mice. In addition, their gonad distribution and sexual dimorphism strongly suggested their functional importance at the early stage of gonad differentiation. However, it remains to be clarified how the above transcription factors regulate their target gene transcription and how the genes encoding the transcription factors are regulated by upstream regulators. Research involving the above two issues is quite important in order to define the gene regulatory cascade and the molecular mechanisms that drive sex differentiation of the gonad. Based on this concept, we examined the functional correlations of *Ad4BP/SF-1*, *M33*, *Emx2*, and *Arx* genes using the gene KO mice.

### **Arx/ARX responsible for Leydig Cell Differentiation and XLAG**

*Arx*, the *aristaless* related homeobox gene, was isolated as a protein interacting with Ad4BP/SF-1. Since *Arx* is expressed in the gonad and brain from the early developmental stage, we investigated the function of *Arx* using gene-disrupted mice. In the fetal testes, *Arx* is expressed in interstitial cells such as the peritubular myoid cells, tunica albuginea, blood vessel endothelial cells, and the cell lining beneath the tunica albuginea, but not in Leydig, Sertoli, or germ cells. Sertoli cells, germ cells, tunica albuginea, and blood vessels were not affected in the mutant testes, whereas the mutant testes were characterized by a dysplastic interstitium. Indeed, *Mis*, a marker for Sertoli, was clearly detected in the testicular cords, whereas  $3\beta$ -Hsd, a marker for Leydig cell, was markedly diminished in the mutant testes, thus implicating *Arx* in Leydig cell differentiation (Ref 15, Kitamura et al, 2002). In order to identify the downstream genes of *Arx*, RNAs prepared from the *Arx* KO and wild type fetal testes were subjected to microarray analyses. Since a few genes showed differential expression between them, we are investigating whether these gene products are involved in Leydig cell differentiation. Since *Arx* is not expressed in Leydig cells, it activates Leydig cell differentiation non-cell autonomously. Thus, we are currently investigating a role of a secretion protein in activating Leydig cell differentiation.

The structural and functional defects observed in the KO mice and the chromosomal localization of human *ARX* on Xp22.13 makes *ARX* a plausible candidate gene for XLAG (X-linked lissencephaly with ambiguous genitalia). In fact, after analysis of the nucleotide sequence, we detected mutations of the *ARX* in all eight XLAG patients examined.

### **Polycomb Component M33 as a Factor bound to the Boundary of *Ad4BP/SF-1* Gene**

#### **Locus**

Mammalian Polycomb (PcG) complexes were recently sub-divided into two distinct types according to their biochemical and functional properties. The first complex, containing Eed and Enhancer of Zeste (*Enx1* and *Enx2*), appears to be required to initiate repression of expression of the target genes in early development, whereas a second complex containing *M33*, *Mpc2*, *rae28/Mph1*, *Bmi1*, and *Mel18* appears to be required for stabilization of the repressed state.

Previously, Yuko Fukui (Research Associate of my laboratory) showed that disruption of *M33* gene induced hypoplasia and sex-reversal of the gonads. In addition to these defects, we identified splenic and adrenal defects in the *M33* KO mice. Histological examination revealed disorganization of the splenic vascular endothelium and its surrounding structures (Ref 32, Fukui et al. 2005). These splenic phenotypes observed in the *M33* KO mice were quite similar to those seen in *Ad4BP/SF-1* KO mice (Ref 7, Morohashi et al. 1999). Moreover, the adrenal gland of *M33* KO and *Ad4BP/SF-1*



heterozygous KO mice are hypoplastic. These phenotypic similarities strongly argue for the presence of genetic and functional interaction between the two genes. In fact, western blotting, immunohistochemistry, and RT-PCR analyses of the *M33* KO showed significantly decreased expression of *Ad4BP/SF-1*, suggesting that *M33* is an essential upstream regulator of *Ad4BP/SF-1* gene. We recently found similar correlation between the two genes during gonad development.

Upstream stimulatory factors (USF-1 and USF-2), Sox9, and Wt1(-KTS form) have been identified as direct-acting positive regulators of *Ad4BP/SF-1* gene transcription. Therefore, we tested whether *M33* directly binds to *Ad4BP/SF-1* gene locus by ChIP assay using antibody to *M33*. Interestingly, the assays with Y-1 adrenocortical cells revealed that *M33*-containing PcG complex are present at the upstream region of the first exon and immediately downstream region from the last exon of *Ad4BP/SF-1* (Ref 32, Fukui et al. 2005).

Our previous study demonstrated that the intergenic region between *Ad4BP/SF-1* and *GCNF* gene contains functional architectures such as *DNase I* hypersensitive sites, a nuclear matrix attachment region (MAR), and CTCF binding sites. Moreover, ChIP assays showed a discontinuous pattern of histone H3 and H4 acetylation over the region. These observations strongly suggested that this region forms a boundary, so-called insulator, between the two transcriptional units, *Ad4BP/SF-1* and *GCNF* (Ref. 36, Ishihara et al. 2005). Therefore, it is important to compare the intergenic structures among various cells and during steroidogenic tissue development.

## Other Genes

*Dax-1* is an essential factor for the development of gonads and adrenal glands. We examined the functions of *Dax-1* and *Dax-1* gene regulation. These studies demonstrated that *Dax-1* acts as a repressor of *Ad4BP/SF-1* through interaction with the LXXLL motif in *Dax-1* (Ref. 23, Suzuki et al. 2003). The interaction with this motif was required for nuclear localization of *Dax-1* as well as transcriptional suppression (Ref. 20, Kawajiri et al. 2003).

We also examined the functions of *vinexine γ* gene during gonad development in *vinexine γ* gene KO mice, and found that this gene is involved in testicular development by providing a platform structure for MAPK signaling (Ref. 34, Matsuyama et al. 2005). We also investigated *Emx2* and *Fkhl18* gene KO mice and showed that *Emx2* is essential for establishment of epithelial cell polarity in the early stages of gonad development (Kusaka et al. manuscript in preparation) while *Fkhl18* is implicated in the development of the testicular vasculature (Sato et al. manuscript in preparation).

## Asymmetric Ovarian Development in Birds

During the sexually-indifferent stage, chick embryonic gonads show no obvious morphological L–R asymmetry. Interestingly, however, genetically ZW female birds develop the ovary at the left side but not in the right side. In contrast, genetically ZZ male birds develop the testis bilaterally. Such asymmetric gonad development has not been described in vertebrates, and the process that regulates avian gonad development is interesting from both evolutionary and developmental perspectives.

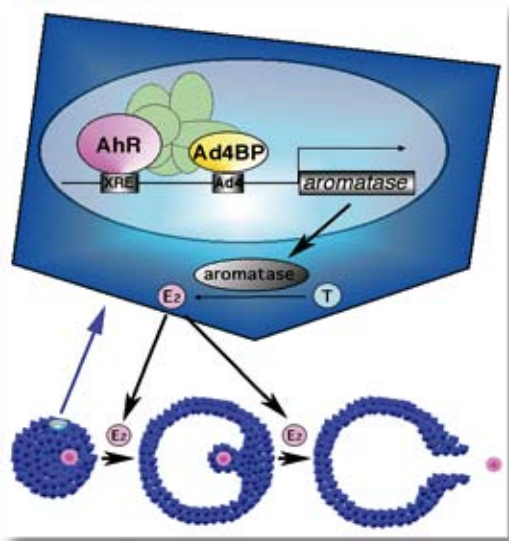
Our study revealed that homeobox gene *PITX2* is expressed asymmetrically in the left presumptive gonad and this asymmetric expression induces the asymmetric expression of the retinoic-acid-catabolizing and -synthesizing enzymes, *CYP26A1* and *RALDH2*, respectively. Subsequently, retinoic acid suppresses the expression of *Ad4BP/SF-1* in the right ovarian

primordium. Conversely, Ad4BP/SF-1 expressed in the left ovarian primordium asymmetrically upregulates *cyclin D1* to stimulate cell proliferation (Ref. 47, Ishimaru et al. in revision).

### (3) Function of AhR (Dioxin Receptor) in the Gonads

It has been well documented that dioxins act as an endocrine disruptor through their estrogenic action. However, the molecular mechanisms underlying this action are largely unknown. To study this, we focused on the reproductive defects displayed by the *AhR* gene KO mouse. The KO female mice showed significantly reduced fertility probably due to disordered estrus cycle, low concentration of ovarian estradiol, and reduced numbers of ovulated eggs. Since estradiol is a representative sex steroid in the female and is produced mainly in the ovary by successive reactions of steroidogenic enzymes, we investigated the expression of these steroidogenic genes. The results showed significantly low expression levels of cytochrome P450 aromatase, which catalyzes the final step of estrogen synthesis, in the KO mouse. Furthermore, estrogen treatment rescued the reduced ovulation capacity. This remarkable phenotype suggested that AhR regulates the *P450 aromatase* gene transcription. Indeed, reporter gene analyses indicated that the mouse and human *P450 aromatase* genes are activated by AhR.

Our previous studies indicated that Ad4BP/SF-1 is specifically expressed in steroidogenic tissues including the ovary, and implicated it in the regulation of the steroidogenic genes. Therefore, we investigated whether AhR activates the *P450 aromatase* gene transcription synergistically with Ad4BP/SF-1. Interestingly, reporter gene assays showed synergy between the two factors. Showing a good correlation, ChIP with anti-AhR and anti-Ad4BP/SF-1 antibodies revealed that both factors bind their recognition sequences on the *aromatase* promoter and form a protein complex in ovarian cells. Thus, together with the *AhR* KO phenotype, we concluded that AhR regulates the *P450 aromatase* gene transcription with Ad4BP/SF-1, thereby regulating estrogen synthesis in the ovary (Figure 3). This finding elucidated the mechanism of the estrogenic action of dioxins (Ref. 30, Baba et al. 2005).

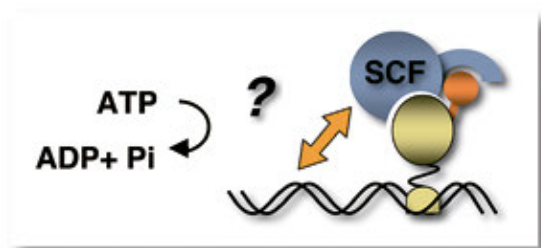


**Figure 3.** Since P450 aromatase is a key enzyme in estrogen synthesis, the process of *P450 aromatase* gene expression is tightly regulated during ovarian estrus cycle. We demonstrated that AhR, together with Ad4BP/SF-1, regulates synergistically *P450 aromatase* gene expression through binding to the upstream elements. Indeed, treatment of animals with the ligand molecule for AhR induced *P450 aromatase* gene expression even when the gene was inactive for transcription. Since estrogen (E<sub>2</sub>) stimulates egg maturation, follicular growth, and rupture, ectopic estrogen synthesis would lead to disruption of endocrine reproductive regulation.

#### (4) Characterization of Factors that interact with Ad4BP/SF-1

In order to define the functional regulation of Ad4BP/SF-1, we screened two-hybrid libraries prepared from mouse fetal gonads. We were able to isolate components of the sumoylation reaction [conjugation of SUMO (small ubiquitin-like modifier)], UBS9, PIAS1, and PIAS3. This finding strongly suggested that Ad4BP/SF-1 is regulated by sumoylation. In fact, Ad4BP/SF-1 is sumoylated *in vivo* and *in vitro* at two lysine residues. Reporter gene assays with wild type and mutant (KR mutant) with lysine-to-arginine substitution at the sumoylation sites demonstrated that sumoylation suppresses the transcriptional activity of Ad4BP/SF-1. In addition to Ad4BP/SF-1, SOX9 has a sumoylation consensus sequence and in fact sumoylated as well. Therefore, we investigated whether sumoylation influences the synergy between the two transcription factors using *Müllerian inhibiting substance (MIS)* gene as a reporter gene, which is regulated by SOX9 synergistically with Ad4BP/SF-1. Expectedly, sumoylation of Ad4BP/SF-1 and SOX9 suppressed MIS gene transcription in this synergistic condition (Ref. 25, Komatsu et al., 2004).

Since the mechanism of SUMO-induced suppression of the transcriptional activity is unknown, we isolated proteins that recognize sumoylated but not non-sumoylated Ad4BP/SF-1. After incubation of nuclear extracts with *in vitro* sumoylated Ad4BP/SF-1, a protein (SCF) was successfully recovered. Interestingly, *in vitro* binding assays demonstrated that SCF recognizes not all but a certain class of nuclear receptors when they are sumoylated. This protein has structurally an ATPase and helicase domain common to chromatin remodeling factors of the SNF2 family. In fact, this protein displayed ATPase activity in the presence of double strand DNA. Moreover, the ATPase activity was enhanced by the presence of sumoylated Ad4BP/SF-1 and double strand DNA containing Ad4 site (Figure 4). This observation strongly suggested that Ad4BP/SF-1, when it is sumoylated, efficiently recruits the chromatin remodeling factor, SCF, and thereby the transcriptional activity is suppressed. In fact, overexpression of SCF suppressed transcription mediated by Ad4BP/SF-1, while SCF-siRNA released the suppressive activity of SCF (Ogawa et al. Manuscript in preparation). Although it is not clear how SCF uses ATP energy, understanding the effect of SCF on chromatin structure is essential for elucidation of SUMO function.



**Figure 4.** The chromatin remodeling factor, SCF, binds to sumoylated Ad4BP/SF-1 more efficiently than non-sumoylated form. When sumoylated Ad4BP/SF-1 binds to DNA, SCF displays ATPase activity. It is not yet clear how SCF uses ATP energy.

### 3. List of Publication

#### **Class 1: Research articles in peer reviewed journals**

1. The Expression of SF-1/Ad4BP is related to Process of Luteinization in the Marmoset (*Callithrix jacchus*) Ovary.  
U. Wehrenberg, C. Wulff, B. Husen, **K. Morohashi**, and G.M. Rune  
**Histochem. Cell Biol.** **107**, 345-350 1997
2. In Vivo Gene Transfer into Blastoderm of Early Developmental Stage of Chicken.  
S. Imada, M.A. Hattori, N. Fujihara, and **K. Morohashi**  
**Reprod. Nutr. Dev.** **37**, 13-20, 1997
3. Regulation of Aldosterone Synthase Cytochrome P450 (CYP11B2) and 11b-Hydroxylase Cytochrome P450 (CYP11B1) Expression in Rat Adrenal Zona Glomerulosa Cells by Low Sodium Diet and Angiotensin II Receptor Antagonists.  
M. Kakiki, **K. Morohashi**, M. Nomura, T. Omura, and T. Horie  
**Biol. Pharm. Bull.** **20**, 962-968, 1997
4. Synergistic Activation of the Human Type II 3b-Hydroxysteroid Dehydrogenase/D<sup>5</sup>D<sup>4</sup> Isomerase Promoter by the Transcription Factor Steroidogenic Factor-1/Adrenal 4-Binding Protein and Phorbol Ester.  
S. Leers-Sucheta, **K. Morohashi**, J.I. Mason, and M.H. Melner  
**J. Biol. Chem.** **272**, 7960-7967, 1997
5. Molecular Cloning and Expression of the SF-1/Ad4BP Gene in the Frog, *Rana rugosa*.  
K. Kawano, I. Miura, **K. Morohashi**, M. Takase, and M. Nakamura  
**Gene** **222**, 169-176, 1998
6. Adrenocortical and Gonadal Expression of the Mammalian *Ftz-F1* Gene Encoding Ad4BP/SF-1 Is Independent of Pituitary Control.  
M. Nomura, K. Kawabe, S. Matsushita, S. Oka, O. Hatano, N. Harada, H. Nawata and **K. Morohashi**  
**J. Biochem.** **124**, 217-224, 1998
7. Structural and Functional Abnormalities in the Spleen of *mFtz-F1* Gene Disrupted Mouse.  
**K. Morohashi**, H. Tsuboi-Asai, S. Matsushita, M. Suda, M. Nakashima, H. Sasano, Y. Hataba, C. Li, J. Fukuta, J. Irie, T. Watanabe, H. Nagura, and E. Li  
**Blood** **93**, 1586-1594, 1999
8. *Saccharomyces cerevisiae* cultured under aerobic and anaerobic conditions: air-level oxygen stress and protein against stress.  
S. Ohmori, Y. Nawata, K. Kiyono, H. Murata, S. Tsuboi, M. Ikeda, R. Akagi, **K. Morohashi**, and B. Ono  
**Biochem. Biophys. Acta** **1472**, 587-594, 1999
9. Sex Reversal Caused by *Mus musculus domesticus* Y Chromosomes Linked to Variant Expression of the Testis-Determining Gene *Sry*.  
C.M. Nagamine, **K. Morohashi**, C. Carlisle, and D.K. Chang  
**Dev. Biol.** **216**, 182-194, 1999
10. The Role of Human MBF1 as a Transcriptional Coactivator.  
Y. Kabe, M. Goto, D. Shima, T. Imai, T. Wada, **K. Morohashi**, M. Shirakawa, S. Hirose and H. Handa  
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11. *Dax-1* as One of the Target Genes of Ad4BP/SF-1.  
K. Kawabe, T. Shikayama, H. Tsuboi, S. Oka, K. Oba, T. Yanase, H. Nawata, and **K. Morohashi**  
**Mol. Endocrinol.** **13**, 1267-1284, 1999
12. Identification of Novel First Exons in Ad4BP/SF-1 (NR5A1) Gene, and Their Tissue- and Species-Specific Usage.  
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**Biochem. Biophys. Res. Commun.** **278**, 63-71, 2000
13. Expression profiles of COUP-TF, DAX-1 and SF-1 in the human adrenal gland and adrenocortical tumors: Possible implications in steroidogenesis.  
H. Shibata, Y. Ikeda, T. Mukai, **K. Morohashi**, I. Kurihara, T. Ando, T. Suzuki, S. Kobayashi, M. Murai, I. Saito, and T. Saruta.  
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14. Comparative localization of *Dax-1* and Ad4BP/SF-1 during development of the hypothalamic-pituitary-gonadal axis implies their closely related and distinct functions.  
Y. Ikeda, Y. Takeda, T. Shikayama, T. Mukai, S. Hisano, and **K. Morohashi**  
**Develop. Dynam.** **220**, 363-376, 2001
15. Mutations of *Arx/ARX* cause abnormal migration and differentiation of GABAergic interneurons and abnormal development of testes in mice, and X-linked lissencephaly with abnormal genitalia in humans.  
K. Kitamura, M. Yanazawa, N. Sugiyama, H. Miura, A. Iizuka-Kogo, M. Kusaka, R. Suzuki, Y. Kato-Fukui, K. Kamiirisa, K. Omichi, M. Kasahara, H. Yoshioka, T. Ogata, T. Fukuda, I. Kondo, M. Kato, W.B. Dobyns, M. Yokoyama and **K. Morohashi**  
**Nature Genetics** **32**, 359-369, 2002
16. Molecular cloning of *DAX-1* and *SHP* cDNAs and their expression patterns in the Nile tilapia, *Oreochromis niloticus*.  
D.S. Wang, T. Kobayashi, B. Senthilkumaran, F. Sakai, C.C. Sudhakumari, T. Suzuki, M. Yoshikuni, M. Matsuda, **K. Morohashi**, and Yoshitaka Nagahama  
**Biochem. Biophys. Res. Commun.** **297**, 632-640, 2002
17. Sexually dimorphic expression of *Dax-1* in the adrenal cortex.  
T. Mukai, M. Kusaka, K. Kawabe, K. Goto, H. Nawata, K. Fujieda, **K. Morohashi**  
**Genes Cells** **7**, 717-729, 2002
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H. Yokoi, T. Kobayashi, M. Tanaka, Y. Nagahama, Y. Wakamatsu, H. Takeda, K. Araki, **K. Morohashi**, and K. Ozato  
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19. Activation of cAMP-dependent Protein Kinase increases the protein level of Steroidogenic Factor-1.  
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21. *Dax-1* gene transcription is regulated by Wnt4 in the female developing gonad.  
H. Mizusaki, K. Kawabe, T. Mukai, E. Ariyoshi, M. Kasahara, H. Yoshioka, A. Swain, and **K. Morohashi**  
**Mol. Endocrinol.** **17**, 507-519, 2003
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J.J. Meeks, S. E. Crawford, T.A. Russell, **K. Morohashi**, B. Capel, J. Weiss, and J.L. Jameson  
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T. Suzuki, M. Kasahara, H. Yoshioka, **K. Morohashi**, and K. Umesono  
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T. Komatsu, H. Mizusaki, T. Mukai, H. Ogawa, D. Baba, M. Shirakawa, S. Hatakeyama, K. Nakayama, H. Yamamoto, A. Kikuchi, and **K. Morohashi**  
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H. Ogawa, R.T. Yu, T. Haraguchi, Y. Hiraoka, Y. Nakatani, **K. Morohashi**, and K. Umesono  
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**Mech. Dev.** **121**, 237-246, 2004
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K. Toda, Y. Okada, M. Zubair, **K. Morohashi**, T. Saibara, and T. Okada  
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**Human Mutation** **23**, 147-159, 2004
  30. Ah (dioxin) receptor as a key factor in the regulation of female reproduction.  
T. Baba, J. Mimura, N. Nakamura, N. Harada, M. Yamamoto, **K. Morohashi\*** and Y.

Fujii-Kuriyama\* (\*; The last two authors equally contributed to this work.)  
**Mol. Cell. Biol.** **25**, 10040-10051, 2005

31. VMH specific enhancer of *Ad4BP/SF-1* gene.  
Y. Shima, M. Zubair, S. Ishihara, Y. Shinohara, S. Oka, S. Kimura, S. Suita, and **K. Morohashi**  
**Mol. Endocrinol.** **19**, 2812-2823, 2005
32. Mouse *Polycomb M33* is required for splenic vascular and adrenal gland formation through regulating *Ad4BP/SF-1* expression.  
Y. Katoh-Fukui, A. Owaki, Y. Sotoyama, M. Kusaka, Y. Shinohara, M. Maekawa, K. Toshimori, and **K. Morohashi**  
**Blood** **106**, 1612-1620, 2005
33. A steroidogenic cell line with differentiation potential from mouse granulosa cells, transfected with *Ad4BP* and *SV40 large T antigen* genes.  
Y. Kamei, Y. Aoyama, T. Fujimoto, N. Kenmotsu, C. Kishi, M. Koushi, S. Sugano, **K. Morohashi**, R. Kamiyama, and R. Asakai  
**J. Endocrinol.** **185**, 187-195, 2005
34. Novel isoform of vinexin, vinexin g, regulates *Sox9* gene expression through activation of MAPK cascade in mouse fetal gonad.  
M. Matsuyama, H. Mizusaki, A. Shimono, T. Mukai, K. Okumura, K. Abe, K. Shimada, and **K. Morohashi**  
**Genes Cells** **10**, 421-434, 2005
35. Mesonephric *FGF* signaling is associated with the development of sexually indifferent gonadal primordium in chick embryos.  
H. Yoshioka, Y. Ishimaru, N. Sugiyama, N. Tsunekawa, T. Noce, M. Kasahara, and **K. Morohashi**  
**Dev. Biol.** **280**, 150-161, 2005
36. Identification of the boundary for histone acetylation between nuclear receptor genes, *Ad4BP/SF-1* and *GCNF*, aligned in tandem.  
S. Ishihara and **K. Morohashi**  
**Biochem. Biophys. Res. Commun.** **329**, 554-562, 2005
37. Expression of the estrogen-inducible EGFP gene in aromatase-deficient mice reveals differential tissue-response to estrogenic compounds.  
K. Toda, Y. Hayashi, T. Okada, **K. Morohashi**, and Toshiji Saibara  
**Mol. Cell. Endocrinol.** **229**, 119-126, 2005
38. *CXorf6* is a causative gene for hypospadias.  
M. Fukami, Y. Wada, K. Miyabayashi, I. Nishino, T. Hasegawa, G. Camerino, C. Kretz, A. Buj-Bello, J. Laporte, G. Yamada, **K. Morohashi**, and Tsutomu Ogata  
**Nature Genetics** **38**, 1369-1371, 2006
39. Role of transcription factors *Ad4BP/SF-1* and *DAX-1* in steroidogenesis and spermatogenesis in human testicular development and idiopathic azospermia.  
Y. Kojima, S. Sakaki, Y. Hayashi, Y. Umemoto, **K. Morohashi**, and Kenjiro Kohri  
**Int. J. Urol.** **13**, 785-793, 2006
40. Two-step regulation of *Ad4BP/SF-1* gene transcription during fetal adrenal development; initiation by a *Hox-Pbx1-Prep1* complex and maintenance via autoregulation by *Ad4BP/SF-1*.

- M. Zubair, S. Ishihara, S. Oka, K. Okumura, and **K. Morohashi**  
**Mol. Cell. Biol.** **26**, 4111-4121, 2006
41. Differential gene dosage effects of Ad4BP/SF-1 on target tissue development.  
Fatchiyah, M. Zubair, Y. Shima, S. Oka, S. Ishihara, Y. Fukui-Katoh, and **K. Morohashi**  
**Biochem. Biophys. Res. Commun.** **341**, 1036-1045, 2006
  42. Atrazine-induced aromatase expression is SF-1-dependent: Implications for endocrine disruption in wildlife and reproductive cancers in humans.  
W. Fan, T. Yanase, H. Morinaga, S. Gondo, T. Okabe, M. Nomura, T. Komatsu, **K. Morohashi**, T.B. Hayes, R. Takayanagi, and H. Nawata  
**Environmental Health Perspectives** **115**, 720-727, 2007
  43. Foxl2 up-regulates aromatase gene transcription in a female-specific manner by binding to the promoter as well as interacting with Ad4BP/SF-1.  
D. Wang, T. Kobayashi, L. Shou, Y. Ohmuro-Matsuyama, G. Guan, S. Ijiri, F. Sakai, M. Matsuda, Y. Shibata, K. Okubo, **K. Morohashi**, and Y. Nagahama  
**Mol. Endocrinol.** **21**, 712-725, 2007
  44. Hypogonadotropic hypogonadism in an adult female with a heterozygous hypomorphic mutation of SOX2.  
N. Sato, Y. Kamachi, H. Kondoh, Y. Shima, K. Morohashi, R. Horikawa, and T. Ogata  
**Eur. J. Endocrinol.** **156**, 169-173, 2007
  45. Clinical and molecular analyses of SRY-negative XX male patients –Correlation between clinical findings and expression of transcription factors implicated in gonadal sex differentiation-  
Y. Kojima, Y. Hayashi, S. Sasaki, P. Koopman, **K. Morohashi**, and K. Kohri  
**Clin. Endocrinol.** In press
  46. Germ cells are essential for sexual dimorphism in the medaka gonad.  
H. Kurokawa, D. Saito, Y. Katoh-Fukui, K. Ohta, T. Baba, **K. Morohashi**, M. Tanaka  
**Proc. Natl. Acad. Sci. UAS** **104**, 16958-16963, 2007
  47. Mechanism of asymmetric ovarian development in birds.Y. Ishimaru, T. Komatsu, M. Kasahara, Y. Katoh-Fukui, Y. Toyama, M. Maekawa, K. Toshimori, R.A.S. Chandraratna, **K. Morohashi**, and H. Yoshioka (The last two authors are equally contributed to the study)  
**Development**, In Revision
  48. Involvement of aryl hydrocarbon receptor (AhR) in maintenance of seminal vesicle through sexually different target gene expression.  
T. Baba, Y. Shima, J. Mimura, M. Oshima, Y. Fujii-Kuriyama, and **K. Morohashi**  
**Sexual Development**, In Revision
  49. Pitx2 directly regulates Ad4BP/SF-1 gene transcription in the pituitary gonadotrope via interaction with the intronic enhancer.  
Y. Shima, M. Zubair, T. Komatsu, S. Oka, C. Yokoyama, T. Tachibana, T.A. Hjalt, and **K. Morohashi**  
**Mol. Endocrinol.** In Revision



## **Class 2: Invited reviews, book chapters**

1. The Ontogenesis of the Steroidogenic Tissues.  
**K. Morohashi**  
Genes to Cells 2, 95-106, 1997
2. Gonadal and Extragonadal Functions of Ad4BP/SF-1-Developmental Aspects-  
**K. Morohashi**  
Trends in Endocrinol. Metab. 10, 169-173, 1999
3. Sex Differentiation of the Gonads–Factors Implicated in Testicular and Ovarian  
Developments.  
**K. Morohashi** Environmental Sciences 9, 13-22, 2002
4. Concerned Regulation of Gonad Differentiation by Transcription Factors and Growth Factors.  
T. Suzuki, H. Mizusaki, K. Kawabe, H. Yoshioka, and **K. Morohashi**  
P68-75 in “The genetics and biology of sex determination” in Novartis Found Symposium  
244, John Wiley & Sons Ltd, UK, 2002

## **4. List of Invited Presentation**

### **1997**

1. Germ Cell development and Meiotic Regulation. Symposium organized by Grant-in-Aid for  
Scientific Research on Priority Areas. (Hakone)  
Invited Speaker, **K. Morohashi**  
‘Ad4BP/SF-1 and DAX-1 in the gonadal development’
2. 10th International Conference on Cytochrome P450: Biochemistry, Biophysics, and Molecular  
Biology. (San Francisco)  
Symposium, Invited Speaker, **K. Morohashi**  
‘Control of Steroidogenic P450 gene expression by orphan nuclear receptors’
3. 16th Annual Meeting for Japan Society of Andrology (Yokohama)  
Symposium, Invited Speaker, **K. Morohashi**  
‘Differentiation of the gonads and functions of Ad4BP/SF-1’

### **1998**

4. 8th Adrenal Cortex Conference (Quebec), 6/13-16  
Invited Speaker, **K. Morohashi**  
‘Expression and regulation of transcription factors implicated in adrenal and gonadal  
development’

### **1999**

5. International Symposium on Environmental Endocrine Disruptors '99 (Kobe) Dec. 9-11,  
Invited speaker, **K. Morohashi**  
‘Expression and Function of Transcription Factors Implicated in Differentiation of the  
Gonads’
6. Japanese-Hungarian Binational Symposium on "Developmental and environmental control of  
cell differentiation" (Szeged, Hungary) Oct. 14-15  
**K. Morohashi**

- ‘Transcription factors implicated in the gonadal and adrenocortical development’
7. The 4th International Symposium on Molecular Steroidogenesis (Nara) Aug. 25-28  
Invited Speaker, **K. Morohashi**  
‘Transcription factors implicated in the gonadal and adrenocortical steroidogenesis’
  8. 22nd the Molecular Biology Society of Japan (Fukuoka), 12/7-12/10  
Workshop, Invited Speaker  
**K. Morohashi**, K. Kawabe, T. Mukai, H. Mizusaki, S. Ishihara  
‘Function of Orphan Nuclear Receptor during Gonad Differentiation’
  9. 2nd Symposium for Reproductive Toxicity (Nagoya) 11/4  
Special Lecture, **K. Morohashi**  
‘Nuclear Receptors required for the Gonad Development’
  10. 72nd Annual Meeting of the Japanese Biochemical Society (Yokohama) 10/6-10/9  
Symposium, Invited Speaker, **K. Morohashi**  
‘Nuclear Receptors required for the Gonad Development’
  11. 18th Annual Meeting for Japan Society of Andrology (Tokyo), 7/2-7/3  
Invited Lecture, **K. Morohashi**  
‘Molecular Mechanisms for Gonad Development’
  12. 72nd Annual Meeting of Japan Endocrine Society (Yokohama) 5/31-6/2  
Symposium coorganized with Science Council of Japan, Invited Speaker, **K. Morohashi**  
‘Effects of Endocrine Disruptors during Gonad Development’
  13. 25th the General Assembly of the Japan Medical Congress (Tokyo) 4/2-4/4  
Symposium, Invited Speaker, **K. Morohashi**  
‘Nuclear Receptors regulating Gonad Development’

## 2000

14. International Symposium on Environmental Endocrine Disruptors 2000 (Yokohama)  
12/16-12/18, Symposium, Invited speaker, **K. Morohashi**  
‘Transcription Factors supporting Gonad Sex Differentiation’
15. 9th Adrenal Cortex Conference (Toronto) 6/17-20  
Symposium, Invited speaker  
**K. Morohashi**, T. Mukai, K. Kawabe, T. Suzuki, H. Mizusaki, S. Ishihara, K. Umesono, K. Kawajiri  
‘Adrenocortical and Gonadal Differentiation Regulated by Transcription Factors, Ad4BP/SF-1 (NR5A1) and Dax-1 (NR0B1)’
16. 2nd International Symposium on the Biology of Vertebrate Sex Determination (Hawaii)  
4/10-4/14  
**K. Morohashi**, K. Kawabe, H. Mizusaki, T. Suzuki, N. Nakamura, M. Kasahara, T. Ikuta, S. Ishihara, H. Yoshika, K. Umesono, K. Kawajiri  
‘Transcriptional Regulation in Differentiating Gonads’
17. 23rd Annual Meeting of the Molecular Biology Society of Japan (Kobe), 12/13-12/16  
Symposium, Invited Speaker  
**K. Morohashi**, H. Yoshioka, K. Kawajiri  
‘Gene Regulation for Sex Differentiation’

18. 53rd Annual Meeting of Japan Society for Cell Biology (Fukuoka) 19/31-11/2  
Symposium, Invited Speaker  
**K. Morohashi**, K. Kawabe, T. Suzuki, T. Mukai, N. Nakamura, H. Mizusaki, M. Kusaka, T. Ikuta, K. Umesono, K. Kawajiri  
'Nuclear Receptors required for the Gonad Development'
19. 73rd Annual Meeting of Japan Endocrine Society (Kyoto) 6/16-6/18  
Symposium, Invited Speaker  
**K. Morohashi**, K. Kawabe, T. Mukai, H. Mizusaki, S. Ishihara  
'Gene regulation for Sex Differentiation'
20. 4th Lecture organized by the Japan Society of Endocrine Disruptor (Tokyo)  
Invited Speaker, **K. Morohashi**  
'Molecular Mechanisms for Sex Differentiation'
21. 18th Takamine Conference (Tokyo) 2/19-2/20  
Invited Speaker, **K. Morohashi**  
'Sex Differentiation and Nuclear Receptor'

### 2001

22. 14th International Congress of Developmental Biology (Kyoto, Japan)  
July 8-12, Symposium /Session organizer, Symposium 'differentiation of sexes'  
**K. Morohashi**, K. Kawabe, T. Suzuki, H. Mizusaki, H. Yoshioka  
'Transcriptional regulation in differentiating gonads'
23. The Endocrine Society's 83rd Annual Meeting (Denver, USA) June 20-23  
Fifth Shionogi Transpacific Symposium -Frontiers in Reproductive Endocrinology,  
Invited speaker, **K. Morohashi**, H. Yoshioka, K. Kawajiri  
'Organogenesis of Gonads and Transcription Factors'
24. Novartis Foundation Symposium No. 244 (London, UK)  
April 30-May 3, Invited speaker, **K. Morohashi**  
The Genetics and Biology of Sex Determination  
"Concerted Regulation of gonad differentiation by transcription Factors and Growth Factors"
25. The 45th International NIBB Conference (Okazaki, Japan) 3/3-3/5  
Invited speaker, **K. Morohashi**  
Recent Progress in Endocrine Disruptor Research  
'Transcription Factors implicated in Gonad Differentiation'
26. 5th Annual Meeting of the Japanese Society for Pediatric Endocrinology (Tokyo) 10/5  
Lecture, Invited Speaker, **K. Morohashi**  
'Molecular Mechanisms for the Gonad Development – Transcription Factor and Growth Factor'
27. CREST Symposium for Endocrine Disruptor (Tokyo) 9/19  
Invited Speaker, **K. Morohashi**  
'Sex Differentiation of the Gonads'

### 2002

28. The 11th International Congress on Hormonal Steroid/7th International Congress on Hormones and Cancer (Fukuoka) 10/ 21-10/25  
Plenary lecture, Invited Speaker  
**K. Morohashi**, M. Zubair, H. Mizusaki, T Suzuki, N. Sugiyama, H. Yoshioka, Y. K. Kitamura,

Katoh-Fukui

‘Molecular Mechanisms underlying Steroidogenic Tissue Differentiation’

29. The 48th NIBB conference “Molecular Mechanisms of Sex Differentiation”  
(Okazaki) 10/ 18-10/20

Organizer

**K. Morohashi**, H. Mizusaki, N. Sugiyama, T. Fukuda, M. Kusaka, T Suzuki, H. Yoshioka, Y. Katoh-Fukui

‘Molecular Mechanism underlying Gonad Differentiation’

30. The 12th Asia-Oceania Congress of Endocrinology (Taipei, Taiwan) 9/20-9/24  
Symposium, Invited Speaker

**K. Morohashi**, M. Zubair, H. Mizusaki, T Suzuki, N. Sugiyama, H. Yoshioka, Y. Katoh-Fukui  
‘Molecular Mechanism underlying Gonad Differentiation’

31. The 1st International Nuclear Receptor Meeting in Japan (Kyoto), 3/1-3/3

Organizer, **K. Morohashi**, T. Suzuki, H. Mizusaki, K. Kawajiri

‘Functions of Orphan Nuclear Receptors in Gonad Differentiation’

32. 25th Annual Meeting of the Molecular Biology Society of Japan (Yokohama), 12/11-12/14  
Symposium, Invited Speaker

**K. Morohashi**, N. Sugiyama, T. Suzuki, H. Mizusaki, M. Kasahara, H. Yoshioka, M. Zubair,  
K. Kawajiri, Y. Fukui-Katoh

‘Functional Regulation of Orphan Nuclear Receptor required for Gonad and Adrenal  
Development’

33. 75nd Annual Meeting of Japan Endocrine Society (Osaka) 6/28-6/30

Symposium, Invited Speaker, **K. Morohashi**

‘Nuclear Receptor and the Cofactors required for Gonad Development’

### 2003

34. The 3rd Meeting on Pathology of Genetically Engineered Mice (Kumamoto) 10/2-10/4  
Invited Speaker

**Morohashi K**, Zubair M, Shima Y, Mizusaki H, Sugiyama N, Ishimaru Y, Yoshioka H,  
Katoh-Fukui Y

‘MOLECULAR MECHANISMS UNDERLYING GONAD DIFFERENTIATION’

35. Workshop on Molecular Steroidogenesis (IV) (Bath, United Kingdom) 4/24-4/27  
Symposium, Invited

H Yoshioka, Y Ishimaru, N Sugiyama, M Kasahara, **K Morohashi**

‘Mesonephric FGF9 is the initiation signal for gonad formation in chick’

36. The 2nd international nuclear receptor meeting in Japan (Osaka), 2/14-2/16

Organizer and Invited Speaker

**Morohashi K**, Zubair M., Mizusaki H., Suzuki T., Sugiyama N., Yoshioka H., Katoh-Fukui Y.

‘Molecular Mechanisms underlying Steroidogenic Tissue Differentiation’

37. 26th Annual Meeting of the Molecular Biology Society of Japan (Kobe), 12/10-12/13  
Symposium organizer, Invited Speaker

H. Ogawa, T. Komatsu, M. Tsuchiya, H. Mizusaki, T. Suzuki, **K. Morohashi**

‘Transcriptional Regulation of Nuclear Receptor implicated in Gonad Development’

38. 26th Annual Meeting of the Molecular Biology Society of Japan (Kobe), 12/10-12/13  
Symposium, Invited Speaker

**K. Morohashi**, N. Sugiyama, M. Kusaka, H. Mizusaki, T. Suzuki, H. Ogawa, H. Yoshioka, Y. Fukui-Katoh

‘Mammalian Gonad Sex Differentiation and Transcriptional Regulation’

39. Keio University Memorial Symposium (Tokyo) 12/4

Invited Speaker, **K. Morohashi**

‘Sex Differentiation of the Gonads-implication of nuclear receptor’

40. 76th Annual Meeting of the Japanese Biochemical Society (Yokohama) 10/15-10/18

Symposium organizer, Invited Speaker

**K. Morohashi**, M. Zubair, H. Mizusaki, K. Masatomo, N. Sugiyama, H. Yoshioka, H. Ogawa, Y. Katoh-Fukui

‘Molecular Mechanisms underlying Gonad Differentiation’

41. 26th the General Assembly of the Japan Medical Congress (Fukuoka) 4/4-4/6

Symposium, Invited Speaker, **K. Morohashi**

‘Molecular Mechanisms for Gonad and Adrenal Cortex’

#### 2004

42. The 12th International Congress of Endocrinology (Lisbon, Portugal) 8/31-9/4,

Symposium Invited, **K. Morohashi**

‘Transcription factors in gonadal sex differentiation’

43. The 11th Adrenal Cortex Conference (New Orleans) 6/12-15

Symposium/Invited

**K. Morohashi**, Y. Ishimaru, N. Sugiyama, H. Yoshioka

‘Growth factors from mesonephros implicated in gonadal and adrenal differentiation’

44. The 3rd International Nuclear Receptor Meeting in Japan (Osaka) 4/15-17,

Organizer and Invited Speaker

**K. Morohashi**, Baba T, Miura J, Nakamura N, Harada N, Yamamoto M, Fujii-Kuriyama Y

‘Ah (dioxin) Receptor as a Key Factor in the Regulation of Female Reproduction’

45. 27th Annual Meeting of the Molecular Biology Society of Japan (Kobe), 12/8-12/11

Workshop, Invited Speaker

**K. Morohashi**, M. Zubair, Y. Shima, K. Masatomo, N. Sugiyama, H. Ogawa, Y. Katoh-Fukui

‘Tissue-specific Expression of Ad4BP/SF-1 Gene’

46. 9th Annual Meeting of Japan Society of Reproductive Endocrinology (Osaka), 11/27

Invited Lecture, **K. Morohashi**

‘Molecular Mechanisms for Sex Differentiation’

47. 77th Annual Meeting of Japan Endocrine Society (Kyoto) 6/24-6/26

Symposium, Invited Speaker

**K. Morohashi**, H. Yoshioka, Y. Katoh-Fukui

‘Molecular Mechanisms for Gonadal Sex Differentiation’

48. 5th Women’s Forum (Kyoto) 6/25

Invited Speaker, **K. Morohashi**

‘Dioxin and Ovarian Function’

#### 2005

49. 78th Annual Meeting of Japan Endocrine Society (Tokyo) 7/1-7/3

Invited Lecture, **K. Morohashi**

‘Molecular Mechanisms for Sex Determination’

50. 78th Annual Meeting of Japan Endocrine Society (Tokyo) 7/1-7/3  
Symposium, Invited

**K. Morohashi**, T. Komatsu, H. Ogawa

‘Transcriptional Regulation of Ad4BP/SF-1 by sumoylation’

## 2006

51. 16th Lake Shirakaba Conference (Grenada) 12/ 6-7

Invited Speaker

**K. Morohashi**, Y. Ishimaru, M. Zubair, S. Oka, K. Miyabayashi, M. Kusaka, Y. Shima, T. Baba, H. Ogawa, H. Yoshioka, Y. Katoh-Fukui

‘Genetic program of gonad differentiation’

52. The Fourth NIBB-EMBL Symposium

Biology of Protein Conjugation – Structure and Function (Okazaki) 12/3-5

Invited Speaker, T. Komatsu, M. Tsuchiya, H. Ogawa, and **K. Morohashi**

‘Regulation of Ad4BP/SF-1 by SUMO’

53. International Symposium on the Environmental Risks of Chemicals (Kushiro) 11/12-14

Invited speaker, **K. Morohashi**

‘Function of AhR in the Ovary’

54. International Symposium on “Molecular Mechanisms of Sex Determination and Differentiation”

Coorganized with the 77th Annual Meeting of the Zoological Society of Japan (Matsue) 9/21  
Organizer, Invited Speaker

H. Yoshioka, Y. Ishimaru, T. Komatsu, M. Kasahara, Y. Katoh-Fukui, **K. Morohashi**

‘Mechanism for asymmetric gonad development in birds’

55. Special developmental biology seminar (Univ. Queensland) 8/9

Invited Speaker, **K. Morohashi**

‘Mechanism for asymmetric development of the avian ovary’

56. 12th Adrenal Cortex and 5th Molecular Steroidogenesis Conference (Boston) 6/20-23

Invited Speaker, **K. Morohashi**

‘Two-step regulation of Ad4BP/SF-1 gene transcription during fetal adrenal development’

57. 17th International Symposium of the Journal of Steroid Biochemistry and Molecular Biology (Seefeld, Austria) 5/31-6/3,

Invited Speaker, Komatsu T, Tsuchiya M, Ogawa H, **K. Morohashi**

‘Regulation of orphan nuclear receptor, Ad4BP/SF-1, by SUMO’

58. Fourth International Symposium on the Biology of Vertebrate Sex Determination, (Hawaii) 4/10-14

Invited Speaker

**K. Morohashi**, M. Kusaka, K. Miyabayashi, Y. Ishimaru, Zubair M., H. Ogawa, H. Yoshioka, Y. Katoh-Fukui

‘Transcriptional Regulation of Genes implicated in Gonad Development’

59. The 52nd NIBB conference, Reproductive Strategies (Okazaki) 6/20-23

Invited Speaker

**K. Morohashi**, Y. Ishimaru, M. Zubair, S. Oka, K. Miyabayashi, M. Kusaka, Y. Shima, T. Baba, H. Ogawa, H. Yoshioka, Y. Katoh-Fukui

‘Genetic Program of Gonad Differentiation’

60. 164th Life Science Forum (Tokyo) 12/11  
Invited Lecture at Japan National Press Club  
**K. Morohashi**  
'The Sexes-their Curiosity and Attractiveness-
61. The 7th Women's Forum (Kobe) 5/20  
Invited Speaker, **K. Morohashi**  
'Unusual Chick Ovary'

### 2007

62. Society for Endocrinology British ES 2007, March 5-8, Birmingham, UK,  
Asia and Oceania Medal lecture, **Ken Morohashi**  
'From Ad4BP/SF-1 to cell and tissue differentiation'
63. The 4th International Nuclear Receptor Meeting in Japan (Osaka), Feb 1-2,  
Organizer, T. Komatsu, H. Ogawa, **Morohashi K**  
Regulation of Ad4BP/SF-1 by SUMO

## 5. Perspective of Research

### **(1) Mechanisms of Leydig Cell Differentiation**

We recently localized fetal Leydig cell-specific enhancer upstream of the *Ad4BP/SF-1* gene, and the structure and function of the enhancer are currently under investigation. Structural and functional analyses of fetal Leydig cell-specific enhancer would clarify the mechanisms for fetal Leydig cell differentiation. Papers published so far and our preliminary results indicate the involvement of several growth factors, such as *Fgf9*, *Pdgf*, and *i*, and transcription factors, such as *Arx*, *Dax-1*, and *Pod1*, in fetal Leydig cell differentiation. Considering that *Ad4BP/SF-1* is highly transcribed in fetal Leydig cells, transcription of *Ad4BP/SF-1* is possibly regulated indirectly by these growth factors and directly/indirectly by these transcription factors. Since the site of action of these factors is probably localized in the fetal Leydig cell-specific enhancer, identification of these sites on the enhancer should be interesting and informative. These studies will be performed using transgenic reporter gene assays with wild type and mutated enhancers on the gene KO background.

As described in the previous chapter, we identified candidate genes downstream of *Arx*, and are investigating the functions of the gene products. Since one of the genes encodes a secretory protein, we have established an assay system to detect the biological functions using cultured fetal gonads. These studies, together with the studies of fetal Leydig cell-specific enhancer, will be performed to elucidate the molecular mechanisms of fetal Leydig cell differentiation.

### **(2) Mechanisms of Enhancer Selection and Enhancer Switching**

The adrenal cortex and Leydig cells share a few common features. Both cells synthesize steroid hormones by the functions of a similar set of steroidogenic genes, suggesting that these two cell types could be derived from a common primordial cell. In fact, we demonstrated previously that a single group of Ad4BP/SF-1-positive cells (Adreno-Gonad Primordium) divided into two groups, one of which develops into the gonad while the other develops into the adrenal cortex. In this process, these two cell types select different enhancers of the *Ad4BP/SF-1* gene. Namely, the

adrenocortical cells select the fetal adrenal enhancer while fetal Leydig cells select the fetal Leydig enhancer.

It is believed that the fetal adrenal cortex and fetal Leydig cells disappear after birth, with the simultaneous emergence of the adult type adrenal cortex and Leydig cells. Although the relationship between fetal and adult cell lineages is not clear, we recently demonstrated that lacZ turned-on by Cre driven by the fetal adrenal enhancer is still active in adult adrenal cells, strongly suggesting that the fetal adrenal cells differentiate into adult adrenal cells. Therefore, the adrenocortical cells switch an active enhancer from the fetal to adult adrenal enhancer during the process of adrenocortical differentiation from fetal type to adult type.

The selection and switching of the enhancers are thought to occur during the process of cell differentiation, and seem to be essential steps in the process. However, the mechanisms that regulate such processes are mostly unknown. To investigate these mechanisms, we recently started to examine the chromatin structure of *Ad4BP/SF-1* gene locus in different cell types by ChIP-on-Chip analyses. This assay has the advantage of detection of target sites of a certain transcription factor or chromatin protein at the whole genomic level. However, as generally pointed out, oligonucleotides used for the DNA chip give variable background binding. Therefore, considering the melting temperature of oligonucleotides, we produced a custom-made chip that covers approximately 40 gene loci of our interest. Unfortunately, however, we found the same problems with the ChIP-on-Chip analyses even with the chips. Accordingly, we plan to continue the ChIP-on-Chip analyses and simultaneously try to analyze chromatin structure using a large-scale on-Chip-sequence analysis. Such analyses should hopefully clarify changes in the chromatin structure at the whole genomic loci of our interest including *Ad4BP/SF-1* gene. These results will allow us understand temporal alterations and cell-specific differences in chromatin structure. I assume that certain regions of the locus will have a characteristic structure, and show structural changes among differentiated cells and during various developmental stages. These regions will be the next target of our investigation.

### **(3) Sex Determining Gene of Chicken**

Although the sex of mammalian species is determined by testicular dominant gene on the Y chromosome (*SRY*), birds are unique in terms of ovarian dominant sex determination. Since individuals carrying the W chromosome develop ovaries, it is thought that the ovarian determining gene is possibly localized on the W chromosome. The genome project determined the entire chick genome sequence, and several genes were found to be localized on the W chromosome-specific region.

We have established techniques to manipulate gene expression in chick embryonic gonads with double strand RNA. Together with the use of retroviral vectors and electroporation, we investigated candidate genes whether they potentially induce the female gonad marker gene, *CYP19* (*P450aromatase*) gene, in the male gonad. Preliminary data suggest that ovarian determination is regulated by both ovarian-dominant and dosage-dependent processes. We expect to obtain definite conclusions from these *in vivo* studies.



#### **(4) Physiological Function of SUMO Modification**

We have identified SCF as a factor that specifically recognizes a class of nuclear receptors when they are sumoylated. SCF is considered a member of the SNF2 family of chromatin remodeling factors and in fact displays ATPase activity in the presence of double stranded DNA. However, we have not elucidated yet the molecular linkage between sumoylation and chromatin remodeling, and sumoylation and transcriptional suppression. We tried to detect the chromatin-remodeling activity of SCF *in vitro* with reconstituted chromatin, however, we could not detect any clear activity of SCF. Considering that other members of the SNF2 family have cofactors and possibly act in combination with them, we assume that a cofactor is required for SCF. Therefore, we tried purification of the protein(s) that interacts with SCF, and fortunately succeeded in purifying a new protein. We anticipate that the newly purified protein is a key factor to uncover the functions of SCF.

In order to examine the functions of sumoylation of Ad4BP/SF-1 and SCF *in vivo*, we plan to establish new mouse lines, in which the two lysine residues of sumoylation targets that reside in Ad4BP/SF-1 are substituted by arginine residues, and in which SCF is floxed to produce conditionally KO mice. Deleter mouse lines to produce the *SCF* conditional KO mouse have been already established with tissues specific enhancers of *Ad4BP/SF-1* for fetal adrenal, pituitary, VMH, and fetal Leydig cells. These two gene disrupted mouse lines will be used in our future research designed to clarify the physiological function of sumoylation.

After spending the past 10 years in NIBB, I have decided to move to the Graduate School of Medical Sciences, Kyushu University from April 2007. I believe that the research environment provided by the NIBB is excellent including the magnificent facilities of the Center for Transgenic Animals and Plants, Center for Analytical Instruments, and others. These facilities have significantly helped the progress of our research. I thoroughly thank the support staff in these centers for their devotion and support. I also thank the faculty members of NIBB. I learned much about developmental biology, reproductive science, sexual differentiation, and many other areas through interesting and interactive discussions with the excellent scientists. The research work performed in this institution provided me with tremendous information and data. Based on these studies, I could formulate a scheme that clarifies the fine mechanisms underlying sex differentiation, gonad development, and steroidogenic cell differentiation that encompasses chromatin remodeling, enhancer selection, and enhancer switching.

## 6. Description of other academic activities

In this past 10 years, our Division supervised 9 PhD students from SOKEN-DAI and 4 of them completed their PhD degrees, 1 is preparing her thesis, and 4 continue their studies at present. In addition to SOKEN-DAI, our Division accepted 2 PhD students from Kyushu University, 1 from Hokkaido University, 1 from Kyoto University, 1 from Tokyo University, 2 from Tohoku University, 1 from Ehime University, 2 from Nagoya University, and 1 from Nagoya City University. All of them obtained their PhD degree after completing their postgraduate studies in this Division.

Based on the importance of the research field of ‘molecular mechanisms of sex differentiation’, I prepared with Prof. Nagahama a grant proposal to the Priority Areas of the Scientific Research by the Ministry of Education, Culture, Sports, Science and Technology, Japan. Fortunately, our research project was selected as one of the programs. Our five-year program started in 2004, and currently 45 domestic research groups are supported by this grant. This program is expected to encourage the establishment of a new scientific section for the study of the mechanisms of sex differentiation.

## 7. Reprints for Selected Papers

Reprints of the five papers below are attached.

1. Mutations of *Arx/ARX* cause abnormal migration and differentiation of GABAergic interneurons and abnormal development of testes in mice, and X-linked lissencephaly with abnormal genitalia in humans.  
K. Kitamura, M. Yanazawa, N. Sugiyama, H. Miura, A. Iizuka-Kogo, M. Kusaka, R. Suzuki, Y. Kato-Fukui, K. Kamiirisa, K. Omichi, M. Kasahara, H. Yoshioka, T. Ogata, T. Fukuda, I. Kondo, M. Kato, W.B. Dobyns, M. Yokoyama and **K. Morohashi**  
**Nature Genetics** **32**, 359-369, 2002
2. Ah (dioxin) receptor as a key factor in the regulation of female reproduction.  
T. Baba, J. Mimura, N. Nakamura, N. Harada, M. Yamamoto, **K. Morohashi\*** and Y. Fujii-Kuriyama\* (\*; The last two authors equally contributed to this work.)  
**Mol. Cell. Biol.** **25**, 10040-10051, 2005
3. Mouse *Polycomb M33* is required for splenic vascular and adrenal gland formation through regulating *Ad4BP/SF-1* expression.  
Y. Katoh-Fukui, A. Owaki, Y. Sotoyama, M. Kusaka, Y. Shinohara, M. Maekawa, K. Toshimori, and **K. Morohashi**  
**Blood** **106**, 1612-1620, 2005
4. *CXorf6* is a causative gene for hypospadias.  
M. Fukami, Y. Wada, K. Miyabayashi, I. Nishino, T. Hasegawa, G. Camerino, C. Kretz, A. Buj-Bello, J. Laporte, G. Yamada, **K. Morohashi**, and Tsutomu Ogata  
**Nature Genetics** **38**, 1369-1371, 2006
5. Two-step regulation of *Ad4BP/SF-1* gene transcription during fetal adrenal development; initiation by a Hox-Pbx1-Prep1 complex and maintenance via autoregulation by Ad4BP/SF-1.  
M. Zubair, S. Ishihara, S. Oka, K. Okumura, and **K. Morohashi**  
**Mol. Cell. Biol.** **26**, 4111-4121, 2006

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Yamada, K., Fukazawa, M., Hayashi, M., Suzuki, I., and Nishimura, M. (2007). Cytosolic HSP90 regulates the heat shock response that is responsible for heat acclimation in *Arabidopsis thaliana*. *J. Biol. Chem.* 282, 37794-37804.

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Li, L., Shimada, T., Takahashi, H., Ueda, H., Fukao, Y., Kondo, M., Nishimura, M., and Hara-Nishimura, I. (2006). MAIGO2 is involved in exit of seed storage proteins from the endoplasmic reticulum in *Arabidopsis thaliana*. *Plant Cell* 18, 3535-3547.

Mano, S., Nakamori, C., Nito, K., Kondo, M. and Nishimura, M. (2006). The *Arabidopsis pex12* and *pex13* mutants are defective in both PTS1- and PTS2-dependent protein transport to peroxisomes. *Plant J.* 47, 604-618.

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大学共同利用機関法人 自然科学研究機構

# 基礎生物学研究所

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基礎生物学研究所長  
岡田清孝

# 基礎生物学の世界へようこそ

岡崎市の丘の上に立つレンガ色の基礎生物学研究所の建物は、東海道新幹線の窓からも、国道一号線の車の中からも遙かに眺めることができます。研究所は昭和52年に創立され、平成19年に30周年を迎えました。研究所の建物はすっかりおなじみになっていますが、研究所の目的や成果が広く知られているとはいえません。研究所の中でどのような研究が行われているのか、どのような人が何を知りたいと考えて日々過ごしているのか、話を聞く機会があるのか、など様々な質問があることでしょう。この冊子には、このような問いかけに対する答が掲載されています。

基礎生物学という学問があるのか、生物学や生命科学とどのように違うのか、という質問を受けることがあります。21世紀になって遺伝子についての理解が進んだために基礎研究と応用が密接に結びつくようになりました。ショウジョウバエやマウスなどの実験動物の遺伝子とヒトの遺伝子がよく似ていることから、実験動物を使った基礎研究の結果が、直ちにヒトの病気を治す方法につながるといった例が続出しています。植物の研究についても同様で、シロイヌナズナなどのモデル植物を用いた基礎研究の成果が悪環境でも育つ作物の開発や穀物の増収に役立っています。生物学を基礎と応用の学問に分けることはあまり意味のないことになってきたのですが、基礎研究が応用研究をリードしていくことには変わりありません。

基礎生物学研究所では生物の基本的な遺伝子の働きや細胞の働きを探ることと、生存環境に適応した生物が多様な形と能力を持つに至ったしくみを調べることをもっとも重要な研究目標にしています。単細胞生物から動物や植物に分かれ、それぞれ様々に進化してきた中で変わらずにきた基本的で普遍的なしくみと、逆に多様な変化を可能にした理由を探求しています。基礎生物学研究所で働いている人々は、生物の中に数十億年の歴史をみて、そこに隠された秘密を明らかにしたいと願っているのです。

基礎生物学研究所は大学共同利用機関としての役割があり、全国の国公立の大学や研究所の研究者や学生さんと共同して研究を進めています。外国からの研究者も頻りに訪れてきています。研究をサポートする職員も頑張っています。また、次の世代の研究や教育を担う人材を養成することも大きな役割です。総合研究大学院大学のキャンパスの一つになっていて、恵まれた環境の中で大学院生が学んでいます。

このように基礎生物学研究所では幅広い活動をおこなっており、研究で得られた成果はもちろん、イベントや生物の写真など様々な情報を発信していこうと考えています。ご意見をお寄せ下さい。



# 沿革

基礎生物学研究所（基生研）は、1977年、岡崎市に生理学研究所と同時に設置され、1981年には、既に設置されていた分子科学研究所とともに岡崎国立共同研究機構を構成するようになりました。基生研は、設立以来、日本を代表する基礎生物学の研究所として国内外から多くの共同研究者が訪れ、論文や国際会議等で「OKAZAKI」発の成果が高く評価されています。また、2002年からは山手地区でも研究が開始されました。2004年には、国立天文台、核融合科学研究所と岡崎の3研究所が、大学共同利用機関法人 自然科学研究機構として、新たな体制で研究を開始しました。

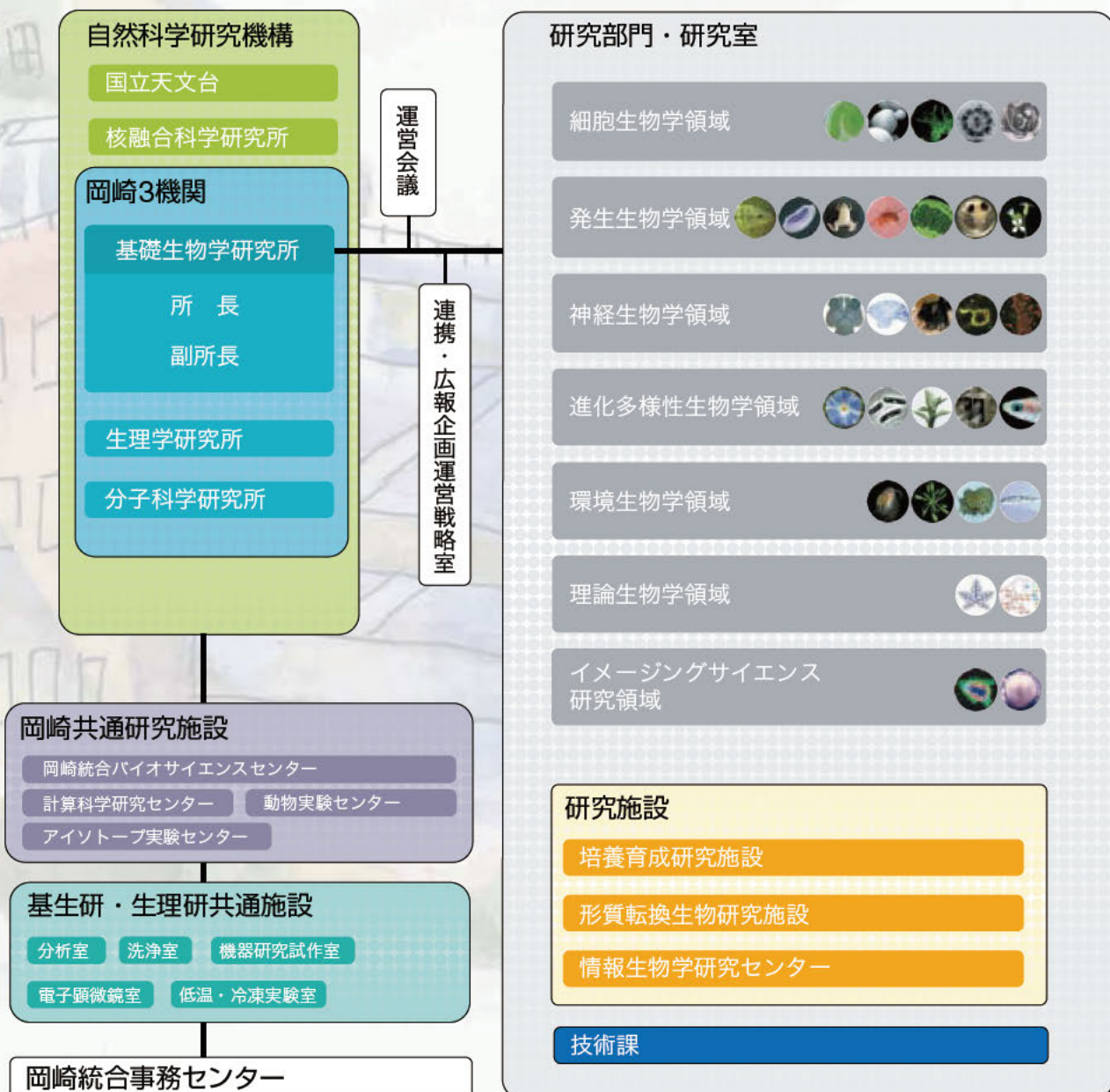


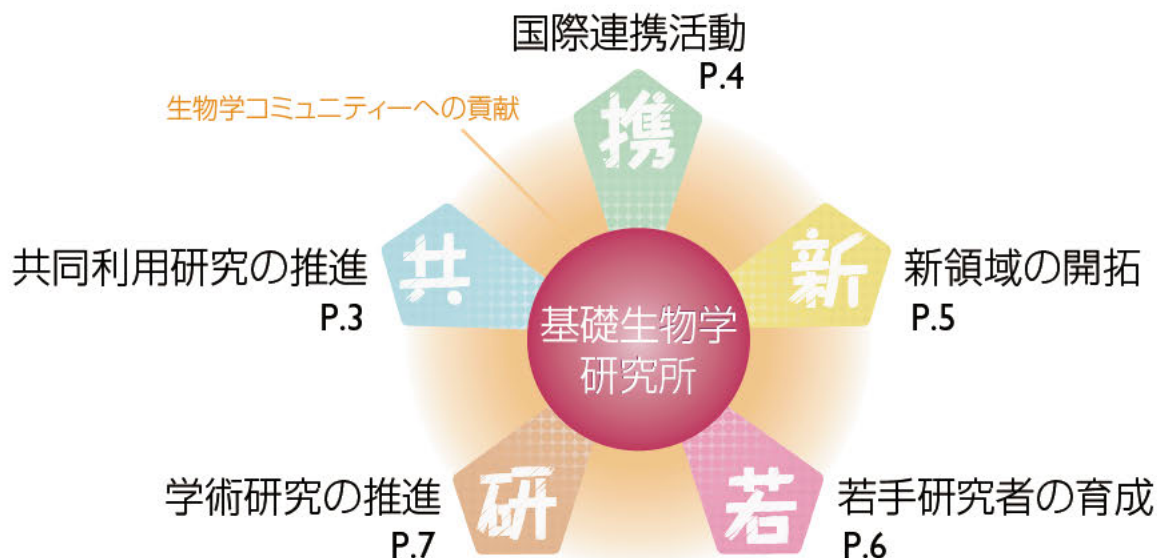
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# 組織図





## 「生物学研究の中心拠点として」

### 共同利用研究

#### 大学共同利用機関

大学共同利用機関は、世界に誇る我が国独自の「研究者コミュニティ」によって運営される研究機関であり、全国の研究者に共同利用・共同研究の場を提供する中核拠点として組織されました。重要な研究課題に関する先導的研究を進めるのみならず、全国の最先端の研究者が一堂に会し、未来の学問分野を切り拓くと共に新しい理念の創出をも目指した活動を行う拠点として、個別の大学では実施困難な機能と場を提供するのがその特色です。

各機関が独自性と多様性を持ちながら、それぞれの研究分野における研究拠点として、我が国の学術研究の発展に重要な貢献をしています。また、海外の研究機関や研究者との協力・交流を推進する国際的拠点としての役割をも果たしています。

#### 共同利用研究

大学・研究機関などに所属する所外の研究者に対し、所内の研究部門・研究室との共同研究、および所内の施設を利用して行われる研究課題を公募しています。従来からの「個別共同利用研究」「大型スペクトログラフ共同利用実験」「基生研研究会」などに加えて、共同利用研究の戦略的組織化を図るため、新たな形の研究公募を開始しました。「重点共同利用研究」は、生物学を先導する研究の創成を目指して、所内外の研究者によるグループ研究として1年以上3年以内で行われるもので、「高等植物の個体制御の分子機構」など、3件が実施されています。また、「モデル生物・技術開発共同利用研究」は、新しいモデル生物の開発と確立をめざすものとして平成19年度から設定されました。「原始的被子植物ハゴロモモにおける形質転換系の開発」など2件が実施されています。基礎生物学研究所では、共同利用研究のあり方は、時代と生物学コミュニティの要請に応じて、変革し続けることが必要だと考え、絶えず検討を重ねています。

共同利用研究受入件数

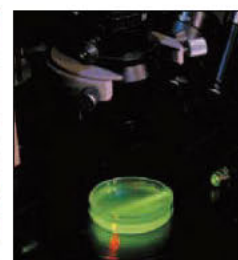
年度	17	18	19
重点共同利用研究	2	3	1
モデル生物・技術開発共同利用研究	—	—	2
個別共同研究	41	37	34
研究会	4	1	1
大型スペクトログラフ共同利用実験	19	18	12
計	64	59	50

#### 大型スペクトログラフの高度化

大型スペクトログラフ室(→P.17)は、1980年に設置されて以来、光生物学研究の世界的な中心施設として、多くの共同利用研究成果を生み出してきました。一層高度な成果をあげるために、制御システムの高性能化、レーザー光源の設置、解析装置の充実などの高度化を推進しています。既に稼働中の波長固定(一部可変)レーザーに加えて、紫外から赤外まで広い範囲をカバーする波長可変レーザーの導入準備を進めています。



紫(390-410nm)および青(440-460nm)のレーザー光源



試料のレーザー照射



# 「世界規模の研究者コミュニティの中核として」

## 国際連携

### EMBLとの国際共同研究

欧州分子生物学研究所 (EMBL) は欧州18ヶ国の出資により1974年に設立された研究所で、世界の分子生物学をリードする高いレベルの基礎研究を総合的に行っています。基礎生物学研究所は、2005年に開始された自然科学研究機構とEMBLとの共同研究の中心となっており、シンポジウムの開催や研究者・大学院生の相互訪問および実験機器の技術導入などを通じて、人的交流と技術交流を行っています。



#### これまでに開催したEMBLシンポジウム

- 第1回 発生生物学 2005年7月・ハイデルベルグ [ドイツ]
- 第2回 バイオイメーキングの最前線 2006年3月・岡崎
- 第3回 マウスの生物学 2006年4月・モンテロトンド [イタリア]
- 第4回 タンパク質複合体の生物学 2006年12月・岡崎
- 第5回 細胞・発生生物学 2007年5月・岡崎



第1回シンポジウム参加者



EMBLでの研究交流



EMBLでのマウス施設見学



第4回シンポジウム参加者

### 基生研コンファレンス (NIBB Conference)

所内の教授等がオーガナイザーとなり、海外からの招待講演者を交えて、年に1~2回開催される国際会議です。研究所創立の1977年に開催された第1回以来、基礎生物学分野の研究者にとって国際交流の貴重な機会となっています。

#### 最近開催された基生研コンファレンス

- 第51回 遺伝子増幅研究の新展開 2005年11月
- 第52回 生殖の戦略 2006年1月
- 第53回 オルガネラの動態から見た植物の生存戦略 2006年6月

### 国際実習コース (International Practical Course)

国内外の研究者の協力のもとに、所内の専用実験室で行われる国際実習コースです。2007年に第一回が「ゼブラフィッシュとメダカの発生遺伝学」をテーマとして開催され、中国、香港、台湾、インドなど東アジア諸国の大学院生に、小型魚類の最新研究技法をトレーニングしました。



専用実験室での実験指導



胚の顕微操作

### バイオリソース

ナショナルバイオリソースプロジェクトは、生物学研究に広く用いられる実験材料としてのバイオリソース (実験動植物、細胞、DNAなどの遺伝子材料) のうち、国が特に重要と認めたものについて、体系的な収集、保存、提供体制を整備することを目的とした国家プロジェクトです。基礎生物学研究所はこのプロジェクトを推進するため、日本オリジナルの脊椎動物モデル生物「メダカ」を担当する中核機関となりました。メダカは、最近その全ゲノム配列が解読され、生物学の研究材料としての有用性が格段に高まっています。基礎生物学研究所はさらに、アサガオおよびゼブラフィッシュ担当のサブ機関となっています。このプロジェクト以外にも、ヒメツリガネゴケ、ミジンコ、アフリカツメガエル、植物オルガネラ、バクテリアゲノムに関する研究情報を掲載したデータベースを提供しています。



上: 全ゲノム配列が決定された近交系Hd-rR系統のメダカ、中: 赤色蛍光を全身に発現する形質転換メダカ、下: 体色が透明なQuintet系統のメダカ





# 「生物学の新領域を切り拓く拠点」

## 新領域開拓

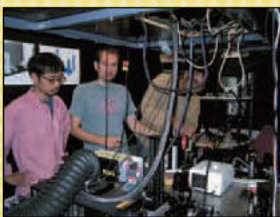
### バイオイメージング

近年の光学顕微鏡性能の著しい向上と、生体光プローブの開発とが相まって、従来は固定した試料から得られる断片的情報から想像するしかなかった生物現象が、生きた材料を使ってリアルタイムで観察できるようになりました。基礎生物学研究所は、このような生物現象の可視化技法（バイオイメージング）の生物学研究への最大限の活用を図るとともに、イメージング新技法の開発を目指しています。

- 1) イメージングサイエンス研究領域の設置 顕微鏡や光プローブの開発拠点を目指します。
- 2) バイオイメージング・アドバイザー委員会の設置 国内を代表する数名のイメージング研究者と定期的に会合を持ち、イメージング研究の推進について諮問します。
- 3) バイオイメージングフォーラムの開催 所内の研究者、アドバイザー委員、企業の開発担当者が参加し、イメージングに関する研究現場の悩みやニーズを率直に討論する研究会です。



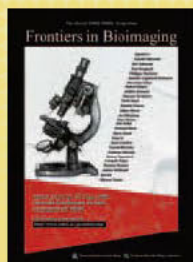
- 4) SPIM顕微鏡の設置 EMBLとの共同研究の一環として、生体の3次元観察に有効なSPIM顕微鏡を日本で初めて導入します。



- 5) バイオイメージングシンポジウム開催 EMBLを中心とした海外の最先端イメージング研究者との研究交流の場としてシンポジウムを開催しています。



シンポジウムの参加者とポスター



### 生物学国際高等コンファレンス (Okazaki Biology Conferences)

基礎生物学研究所では、生物科学学会連合の推薦のもと、生物学における新しい研究課題としての問題発掘を目指し、今後生物学が取り組むべき新たな研究分野の国際的コミュニティ形成を支援するための国際研究集会Okazaki Biology Conferences (略称OBC)を開催しています。国内外を問わず集められた数十人のトップレベルの研究者が、約一週間寝食を共にして議論をつくり、今後重要となる生物学の新たな課題に挑戦するための戦略を検討します。既に開催されたコンファレンスからは、国際的研究者コミュニティが形成されつつあります。

#### これまでに開催されたOBC

- 第1回 絶滅の生物学 2004年1月・岡崎
- 第2回 地球圏微生物学 2004年9月・志摩
- 第3回 絶滅の生物学2 2006年3月・岡崎
- 第4回 地球圏微生物学2 2006年9月・岡崎
- 第5回 種分化と適応 2007年3月・岡崎および掛川



第5回OBCの参加者



オーラルセッション (第4回)



ポスターセッション (第2回)



セッションごとの総合討論 (第5回)



# 「将来の生物学を担う若手の育成」

総合研究大学院大学および他大学院生受入れ

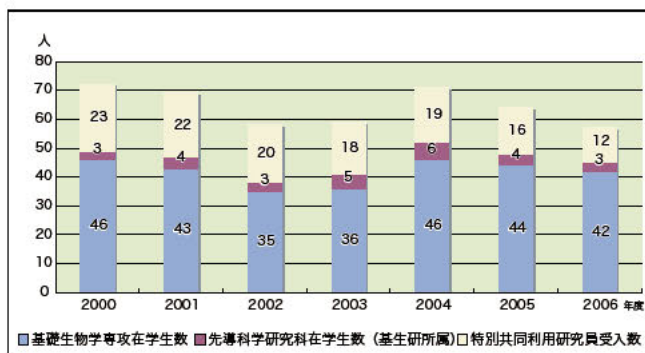
基礎生物学研究所は、我が国の生物学研究の中核の一つとして最先端の施設や設備が整備されているばかりでなく、優れた創造的研究を発信し続けている教授陣を擁し、発表論文の被引用回数は我が国だけでなく世界でもトップクラスに位置しています。この優れた研究環境で将来の生物学におけるリーダーを養成することを目指して、高度な大学院教育を行っています。



基礎生物学研究所での大学院教育のひとつ

## 大学院生として学ぶには

基礎生物学研究所を基盤機関とする、総合研究大学院大学（総研大）基礎生物学専攻に入学するのが一つの方法です。既に他大学の大学院に在籍している場合は、「特別共同利用研究員」になる方法があります。後者は共同利用研究の一つの形として、1年ごとに申請し審査を経て採用されるものです。どちらの大学院生も、同様に研究生生活を送ることができ、RA（リサーチアシスタント）制度による研究所からの経済的支援も同等に受けられます。



基礎生物学研究所が教育している大学院生数

## 総合研究大学院大学とは

総合研究大学院大学は、基礎学術分野の総合的發展を目指した大学院教育を行うために、学部を持たない大学として1988年に設置されました。神奈川県葉山に本部をもち、基盤機関である18の国立学術研究機関に学生を分散配置し大学院教育を行っています。生命科学研究科は基礎生物学専攻と、同じ岡崎にある生理学研究所の生理科学専攻、静岡県三島市の国立遺伝学研究所の遺伝学専攻の3専攻により構

成されています。基礎生物学専攻は、分子生物学を基盤として動植物にかかわる基本的、かつ、高次な生物現象を分子レベルまで掘り下げて解析する高度な研究者の養成課程です。修士課程修了者を対象とする博士後期課程と、学部卒業生を対象とする5年一貫制のコースがあり、いずれも入学時期は4月と10月の2回です。



生命科学研究科の学生・教員が集まって研究成果を発表しあう「合同セミナー」が毎年開催されます。

## 少数精鋭の大学院教育

多くの大学では、大学院生数に対して教員数が少ない（国立大学では学生一人あたり約0.16人）のに対して、総研大は教員数が圧倒的に多い（約1.8人）ため、個別指導が希薄になるという問題点がありません。現在基礎生物学専攻でも、総研大生42名に対して教員数が55名で、まさに「マンツーマン」の教育を行っています。



研究の進展状況を報告し、多くの教員からアドバイスを受けることができる「生命科学フログレス」

## 質の高いセミナー

基礎生物学研究所では、日常的に所外からの著名な講師によるセミナーが開催されています。また研究所が主催するコンファレンスの多くに参加することができます。これらは研究者としての視野をひろげる良い機会となっています。



EMBL（欧州分子生物学研究所）との共同研究の一環として実施された、EMBL所属の大学院生との交流セミナー

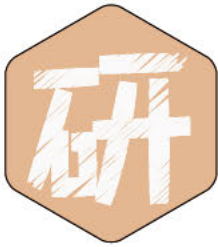
## 高い研究者養成率

基礎生物学専攻では高度な研究者の養成を行っています。過去5年間で約8割の学位取得者が助教、ポスドク等研究者として従事しています。

## 大学院に入学を考えている方へ

基礎生物学研究所ホームページの「大学院教育」ページに詳しい情報があります。また年4回、東京、大阪、岡崎などで大学院説明会を開催しています。実際に研究室で研究生生活を体験できる体験入学（交通費・宿泊費などの補助制度あり）もご利用下さい。

若手研究者の育成



# 最先端生物学研究の推進

## 学術研究

基礎生物学研究所はこの図に示すような研究体制を持ち、最先端レベルの生物学研究を行っています。その内容については、次ページ以降でご紹介します。

### 細胞生物学領域

#### 高次細胞機構 P.9



西村幹夫 教授



林 誠 准教授



#### 分子細胞生物学 P.9



大隅良典 教授



#### 細胞構造 P.9



小川和男 准教授



#### 細胞社会学 P.10



濱田義雄 助教



#### 細胞増殖



酒巻和弘 客員准教授

### 神経生物学領域

#### 行動生物学 P.13



森裕司 客員教授



東村博子 客員准教授



#### 統合神経生物学 P.14



野田昌晴 教授



#### 脳生物学 P.14



山森哲雄 教授



#### 神経生理学 P.14



渡辺英治 准教授



#### 神経生化学 P.14



笹岡俊邦 准教授

### 環境生物学領域

#### 分子環境生物学 P.13



井口泰泉 教授



渡邊肇 准教授



#### 光情報 P.10



和田正三 特任教授



山内大輔 客員准教授



#### 光環境学 P.10



渡辺正勝 客員教授



#### 植物発生遺伝学 P.12



塚谷裕一 客員教授

## 発生生物学領域

### 植物器官形成学 P.12



岡田清孝 所長

### 生殖遺伝学 P.13



田中実 准教授

### 形態形成 P.11



上野直人 教授



木下典行 准教授

### 生殖生物学 P.13



長濱嘉孝 教授

### 性差生物学 P.13



諸橋憲一郎 教授(兼任)

### 発生遺伝学 P.11



小林悟 教授

### 分子発生学 P.11



高田慎治 教授

## イメージングサイエンス研究領域

### 発生ダイナミクス



宮脇敦史 客員教授

### 時空間制御 P.11



野中茂紀 准教授

## 理論生物学領域

### 理論生物学 P.12



望月敦史 准教授

### ゲノム情報 P.15



内山郁夫 助教

## 進化多様性生物学領域

### 生物進化 P.16



長谷部光泰 教授



村田隆 准教授

### 分子遺伝学 P.15



飯田滋 教授

### ゲノム動態 P.15



堀内嵩 教授

### 構造多様性 P.16



児玉隆治 准教授

### バイオリソース P.16



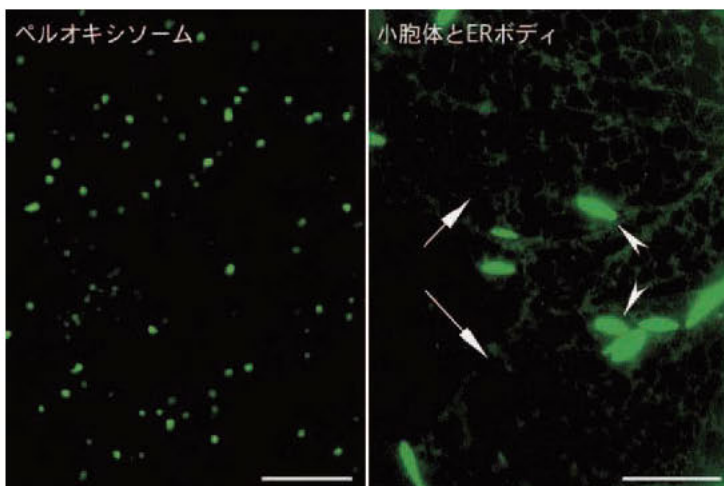
成瀬清 准教授

動物も植物も生きものは細胞という基本単位からできています。この細胞の中には様々な細胞内小器官（オルガネラ）が大きく変化しながら存在しており、これらのオルガネラの統合により生命の基本単位である細胞は生存しているのです。私たちはこうした細胞の千変万化のしくみを明らかにしようとしています。



## 細胞内小器官の機能変換

高次細胞機構研究部門



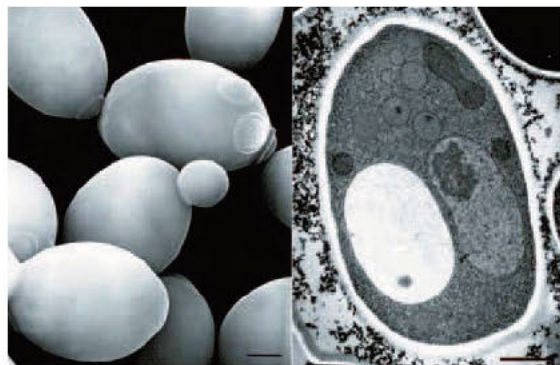
緑色蛍光タンパク質で可視化されたシロイヌナズナ細胞内のペルオキシソームと小胞体ネットワークおよびERボディ。バーは10 $\mu$ m

植物の生育にともない、細胞内小器官（オルガネラ）の動きは大きく変わります。オルガネラの一つで大きさが1 $\mu$ mほどのペルオキシソームは、発芽時には種子の貯蔵脂肪をエネルギーに変える働きを担っていますが、光が当たると光合成を助ける働きへと機能を変えます。これらの機能が失われると、植物の生育が悪くなります。一方、小胞体はタンパク質合成の場となるネットワーク様のオルガネラ（矢印）ですが、虫害により昆虫が嫌がる物質を蓄積する特殊な小胞体（ERボディ：矢じり）が分化してきます。私たちはオルガネラのダイナミックな機能変換を調べることで、植物の生存戦略を明らかにしようとしています。



## 酵母で解かれる細胞のリサイクルシステム

分子細胞生物学研究部門



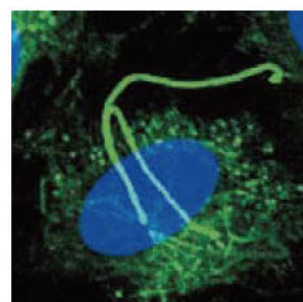
電子顕微鏡で見た酵母の外部構造と内部構造（大隅正子日本女子大名誉教授提供）バーは1 $\mu$ m

酵母（イースト）は、人間がパン作りやお酒などの発酵を通じて最も長い付き合いをしてきた微生物です。それだけではありません。電子顕微鏡で観察すると、酵母細胞の中には細胞小器官と呼ばれる、複雑な構造が詰まっているのがわかります（図）。酵母は、動物や植物の細胞と基本的な構造が共通なのです。このように酵母は、細胞のしくみを研究する上で遺伝学や分子生物学を駆使することができるために、膨大な研究がなされ、多くの基本的な生命現象の謎解きに活躍しているモデル細胞でもあります。当研究部門では、酵母を用いて、細胞内のタンパク質や小器官の分解、リサイクル機構、オートファジーのメカニズムの解明を進めています。



## 細胞の動かない繊毛

細胞構造研究室



マウスの胃臓から分離した細胞をシャーレで培養したもの。核は青く、繊毛は緑に染まっています。

精子の鞭毛や気管支の繊毛は動いています。私たちの体のなかには動かない繊毛もあります。細胞を長い間シャーレで培養していると繊毛が生えてきます。これは有糸分裂の必要がなくなった細胞が、あまった糸の成分であるチューブリンを繊毛へとリサイクルするからです。こうしてできた繊毛は鞭毛や気管支の繊毛のように動きません。普通は中心体から一本の繊毛がでていますが、写真は2本の繊毛がでていたものを撮っています。繊毛の長さは細胞によってまっています。この細胞は比較的長い繊毛が生えています。動かない繊毛は何をしているのでしょうか？





逃避反応により文字を浮かび上げさせた葉



## 葉緑体が動く？

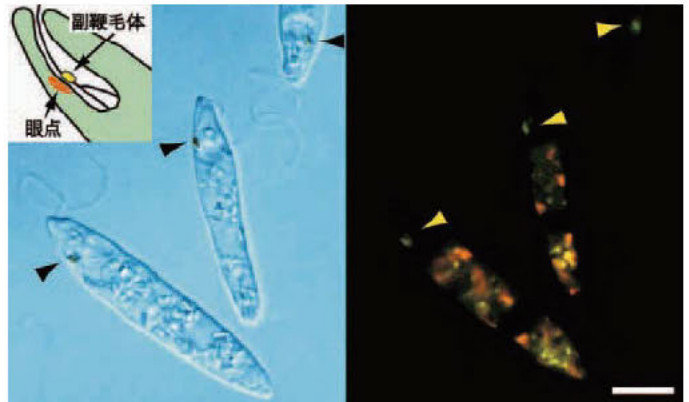
光情報研究部門

植物は光合成により光を生命活動のためのエネルギーに変換しています。光合成は細胞内の葉緑体にある緑色の葉緑素を介して行われます。葉緑体は光をより有効に利用するために弱い光には集まり（集合反応）、光による損傷をさけるために強い光からは逃げる（逃避反応）というように、光環境に応じて細胞内を動いています。この反応を葉緑体光定位反応と呼びます。私たちは葉緑体運動の意味とそのメカニズムを研究しています。葉の緑色は葉緑体によるものですから、葉の一部に弱い光を当てると寄ってきた葉緑体で緑色が濃くなり、強い光を当てると葉緑体が表面から逃げるために緑色が薄くなることから、細胞内で葉緑体が動いていることを肉眼でも観察できます。



## 微生物の光感覚

光環境学研究部門



ミドリムシでは、眼点（左図の褐色の点）ではなく、副鞭毛体（右図の緑色の点）が光を感じる。バーは10μm

植物プランクトンの一種ミドリムシ（Euglena：図）は、水中を泳ぎ回り、また、光合成をするため、明るいところへ集まったり強い光を避けたりします。この光感覚のしくみについて、光を感じる部分である副鞭毛体を分析して光センサーである「光活性化アデニル酸シクラーゼ」（PAC）を発見しました。PACは青色光を感じてサイクリックAMPという信号伝達物質を作ります。これにより、ミドリムシの鞭毛運動の光制御だけでなく、各種動物の神経活動の光による制御などの細胞工学的応用も始まっています。



## 母と子のきずな

細胞社会学研究室



マウスの胎児（左）と胎盤（右の濃いピンクの部分）。胎盤の外側の薄いピンクの皮膜（脱落膜）が母親由来の組織で、生きた状態では胎児と胎盤の全体を覆っています。

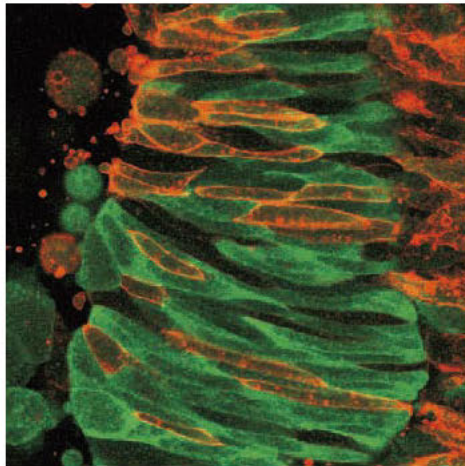
ほ乳類以外の動物は孵化するとすぐに自然にある餌を食べることが出来ます。ほ乳類は卵に貯えられた栄養分が少ないために体作りの初期の段階で孵化し、成長に必要な栄養分を取るために母親に寄生します。母親から栄養や酸素を受け取り、老廃物や二酸化炭素を渡す器官が胎盤です。ほ乳類が生後しばらくの間は栄養を母乳から取らなくてはならないのは、母親に寄生して成長したための必然の結果と考えられます。胎盤は胎児由来の組織ですが、その形成には母親由来の細胞との相互作用が不可欠と考えられます。この研究室では母と子の細胞間相互作用を研究しています。

地球上の生きものは、動物も植物も生きものごとに決まった、様々なかたちをしています。では、生きもののかたちはどのようにつくられるのでしょうか。私たちは、かたち作りをつかさどる遺伝子を見つけてその働きを調べることや、かたちのできる過程を数理モデルで解析することにより、かたち作りのしくみに迫っています。



## 丸い卵からおとなの体へ

形態形成研究部門



アフリカツメガエルの脊索細胞。赤色で標識された細胞が緑色に標識された細胞と互いに滑り込むことによって混じり合っている。

動物の卵の多くは丸いかたちをした一つの細胞です。受精後、細胞数が増え、丸いかたちから次第に前後に長くなり、かたちの変化が始まります。例えばカエルのオタマジャクシが育つときには、脊索（せきさく）と呼ばれる細胞のかたちの変化や移動が、このかたち作りに大切であることがわかっています。脊索細胞はどのようにできるのか？細胞はどうやって動く方向を知るのか？私達はカエル、ホヤ、ショウジョウバエを用いて、かたち作りのしくみを分子や遺伝子のレベルで解明しようとしています。この研究成果は、生物学だけではなくヒト先天異常の原因解明に貢献することも期待されます。



## たまごから赤ちゃんへ

分子発生学研究部門



受精後16時間のゼブラフィッシュ胚

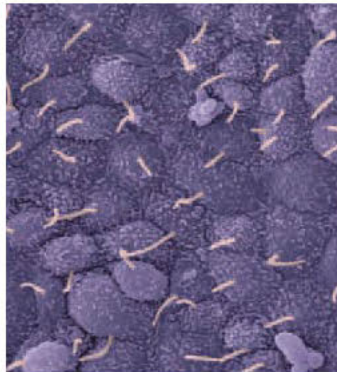
動物の体は1個の受精卵が分裂をくり返すことにより作られます。しかし、むやみやたらに分裂をくり返しても、体の「かたち」ができるわけではありません。そこには「かたち作り」をコントロールするプログラムがあるのです。最近、そのプログラムはさまざまな遺伝子の働きにより成り立っていることがわかって

きました。私たちはヒトをはじめとする脊椎動物のからだの「かたち」ができるしくみを、遺伝子の機能を理解することを通して解明しようと考えています。そのために、遺伝子の機能の解析に適した小型魚類（ゼブラフィッシュ）とマウスをモデルに研究をしています。



## 体の右と左はどう決まるか

時空間制御研究室



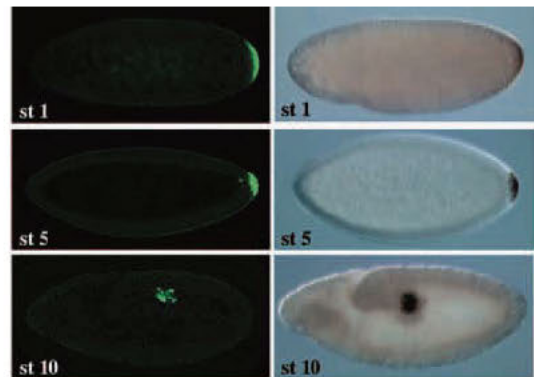
体の表面にある、左右を決める毛（黄色で示す）。

ふつう心臓は左に、肝臓や胆のうは右にあります。でも、どうしてでしょうか？なぜ左右逆になったり、左右対称になったりしないのでしょうか？私たちは、体に左右の区別ができるしくみを調べています。マウスを使った実験で面白いことがわかりました。受精卵から体ができていく途中、体の表面のある部位に長さ1/200ミリ程度のごく小さな毛が生えてきます。その毛が運動して周りの液体をかき回し、体の右から左に向かう流れをつくり出します。人工的に流れの向きを逆にしたところ、左右がひっくり返った体ができることができました。この流れが具体的に何をしているのかを現在調べています。



## 生殖細胞を選び出すしくみ

発生遺伝学研究部門



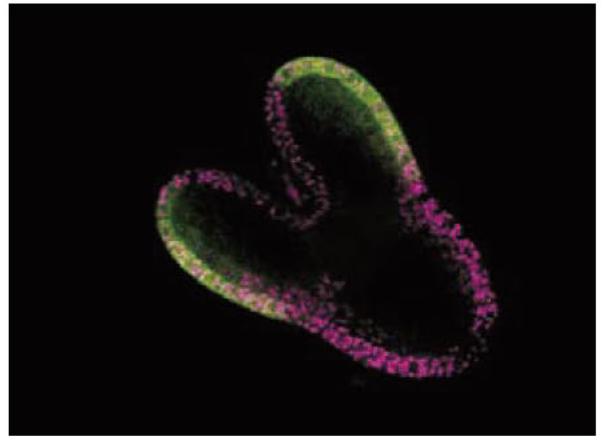
ハエの卵。緑・赤の細胞が将来生殖細胞になる。

生殖細胞は、生物にとって最も重要な能力、すなわち次代の生命を生み出すことのできる能力を持つ細胞です。発生過程において、受精卵は分裂を繰り返して多くの細胞を生み出しますが、そのなかのほんの一部の細胞だけが、生殖細胞として選び出されます。一方残りの細胞群は、個体の体を作る体細胞になります。私たちは、この重要な生殖細胞が選び出されるしくみをショウジョウバエを用いて研究しています。私たちはこれまでにナノスという遺伝子やミトコンドリアがこの過程に重要であることを明らかにしています。



## 植物の体を作るしくみ

植物器官形成学研究室



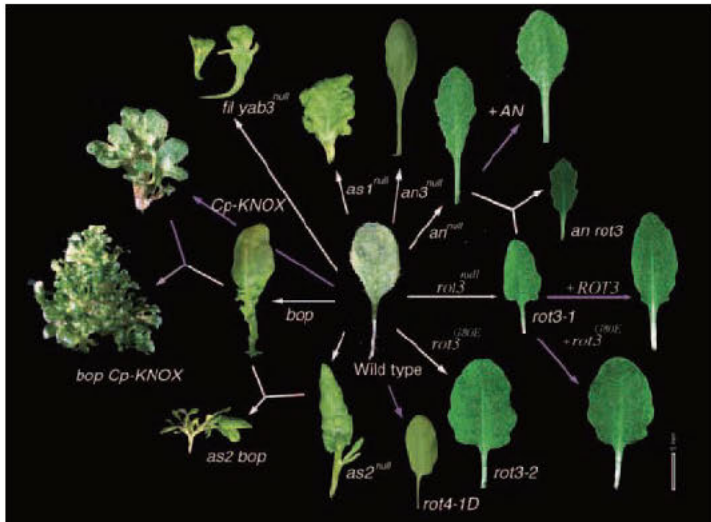
種の中で育っている胚。Y型の上部は子葉に、下部は胚軸と根になる。子葉の裏側を作る細胞が緑色に光るように細工してある。紫色の点は葉緑体。

葉や花、根は対称性を持った印象的なかたちを持っており、内部の組織や細胞は秩序のある美しい配列を示しています。植物の器官は茎や根の先端にある未分化な細胞集団から作られますが、むやみに分裂しても規則的な細胞の配列や対称的なかたちにはなりません。植物の細胞は互いに情報を交換して分裂のタイミングや方向を決めているのではないかと考えられています。私たちは、植物の器官が作られる際に働く細胞間の情報交換のしくみを理解したいと考えています。



## 葉のかたちをつかさどる遺伝子

植物発生遺伝学研究部門



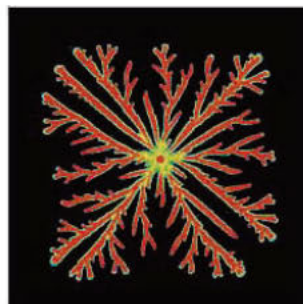
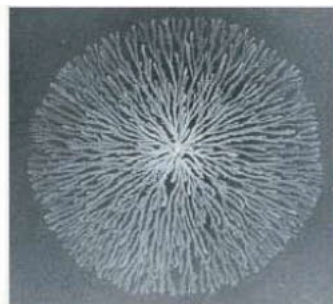
遺伝子が葉形に及ぼす効果

植物の見た目は、葉のかたちやサイズで大きく左右されます。実際、花も葉の集まりにあたります。私たちは葉のかたちやサイズを決めている遺伝子が何で、どのように働いているか、シロイヌナズナという植物を使って研究しています。その結果これまでに、葉の縦の長さや横幅とが別々にコントロールされていることを見だし、それぞれの遺伝子を単離することにも成功してきました。一方、自然界では多様なかたちの葉が進化していますし、各種環境に合わせて葉形が臨機応変に変わることも知られています。そこで私たちは、これらの現象を遺伝子レベルで説明する試みも行なっています。



## 生物現象を数学で解く

理論生物学研究部門



生物が示すさまざまなパターンのコンピュータシミュレーション

発生におけるかたち作りや柔軟な環境応答などの、高度に調節された生物のふるまいは、遺伝子に刻まれた情報から、どのようにして作り出されるのでしょうか？私たちは、計算機や数理モデルを使って、生物学の情報を統合し、本質的な要素をあぶりだすことで、この問いに迫る研究を続けています。左側の図はバクテリアのコロニーや魚の体表で作られる規則的なかたちです。このような秩序は、右側の図に示すように数理モデルを使って再現し、解析することで、作られる条件を定めることができます。

# いのちと遺伝子の継承—性のしくみ

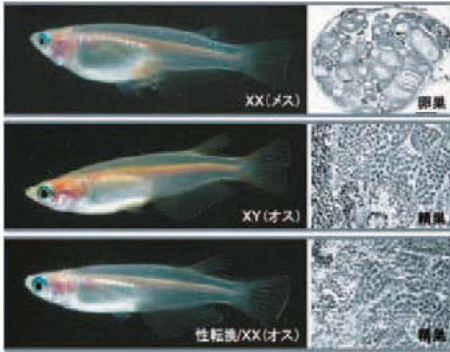
生物は遺伝情報を受け渡すことで、世代を超えていのちを継承しています。そして、そのためには雄と雌の二つの性が作られること、すなわち精子と卵子が作られることが必要です。わたしたちは性を決めるメカニズム、精子と卵子をつくりだすメカニズム、そしてそのメカニズムを破綻させる環境要因などについて研究しています。



## 魚のオスとメス

生殖生物学研究部門

魚の性(オスとメス)は、ヒトなどと同じように、受精時における性染色体の組合せにより決まり、一度決まった性はふつう一生を通じて変わることはありません。ところが、サンゴ礁に棲む熱帯魚のなかには一生の間に性転換する種も存在します。



DMYの遺伝子導入により性転換したXXオス(下)。体色はメスのように白ですが、尾ビレのかたちはオスであり、精巣をもっています。

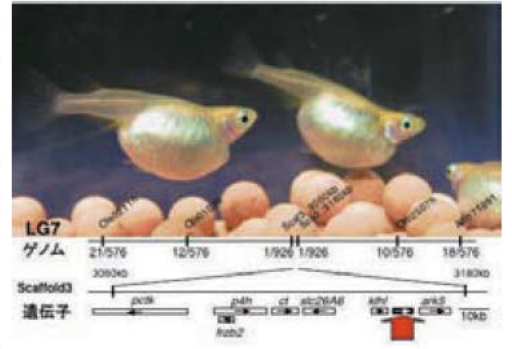
生殖生物学研究部門では、いろいろな魚を用いて、性の決定や転換のしくみを研究しています。私たちは最近、メダカのY染色体にある性決定遺伝子DMY(オスを決める遺伝子)を発見しました。遺伝的にはXX(メス)のメダカ受精卵にDMY遺伝子を導入して育てると、性転換してオスになり、妊娠のある正常な精子を作ります。DMYは脊椎動物で見つかった二番目の性決定遺伝子です。



## 性のしくみを知る

生殖遺伝学研究室

ヒトは受精したときに性が決まり、女性が男性かどちらかにしか生まれません。しかし温度によって性の決まる生物、性が途中で転換してしまう生物、卵巣・精巣の両方を持つ両性の生物などさまざまな生物がいます。すなわち生きものとしてはどのように雌や雄になるうともあまり問題では



(上) 性転換をおこすメダカ、hotel(布袋)。大きく膨らんだお腹は雄でありながら卵巣で満たされている。(下) ゲノム上の赤い遺伝子が変化して性転換をおこすことが判った。

なく、子孫を残すために必要な卵や精子を作り出す卵巣や精巣という器官を環境に応じて作り出すことが大切だと思われます。この性分化や性転換の原理やしくみを、モデル動物メダカを用いて、特定の遺伝子産物や細胞を可視化することにより遺伝子・細胞レベルで解析しています。性転換がおきるメダカを単離してそのしくみも研究しています。



## 性ホルモンと環境ホルモン

分子環境生物学研究部門



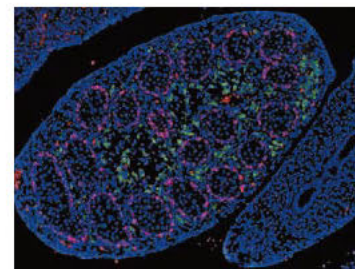
様々な器官に作用するホルモンや化学物質

環境中にはPCBやダイオキシンをはじめとして、農業やプラスチックの原材料など様々な人工的な化学物質が放出されています。この中で性ホルモンに似た働きをするものや、逆に働きを邪魔するものは環境ホルモンと呼ばれています。私たちのグループでは内在性の性ホルモンや生体を取り巻く環境ホルモンが、卵の発生から成体までに及ぼす作用や影響を、ほ乳類・は虫類・両生類・魚類を用いて調べています。これによって、環境と生命体とのかかわりを理解して、よりよい環境を保つための提言にしたいと考えています。



## 動物個体の性を決めるしくみ

性差生物学研究部門



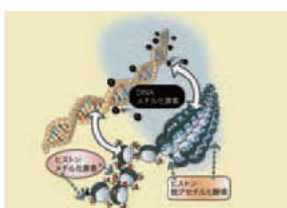
マウス胎仔の精巣での組織構築のようす

生殖腺(精巣と卵巣)は動物個体の性を決める上で最も重要な組織です。多くのほ乳動物の雄では胎仔期に精巣が発達し、男性ホルモンを作りはじめます。そして、このホルモンの影響下に体の他の部分に性差が構築されます。この写真はマウス胎仔の精巣を染めたもので、緑色に染まった細胞が男性ホルモンを産生するライディッチ細胞です。また、ピンク色の輪は精細管構造を示し、この内部に将来精子となる始原生殖細胞が位置しています。このような生殖腺の組織構築を可能にする遺伝子のしくみを研究しています。



## 社会行動の基礎としての性

行動生物学研究部門



DNAメチル化による遺伝子発現不活性化

ほ乳類において、社会行動の多くに性依存性が認められます。ゲノムとしての性の違いは、そのうちの性染色体と呼ばれる場所に位置し、一方、性差を生み出すことに関連する遺伝子の大多数は、オスにもメスにも共通して存在する常染色体にあります。つまり、ゲノムという「配電盤」上のスイッチのON・OFFを固定・維持することで、正常な社会行動を形成すると考えられます。我々の研究室では、そのような分子機構の一つであるDNAメチル化を指標に、行動を生み出す脳の重要な領域について雌雄特異性を調べ、生育環境、体内ホルモン環境との関連を調べています。

# 脳の形成と働きのしくみ

脳・神経系は動物にとっての司令塔です。生きていくための体内環境の調節、食物等の摂取行動の制御、外部環境を知る感覚、記憶や学習、外敵から逃れる為の運動、仲間とのコミュニケーションなどは全て脳・神経系の働きによって可能になります。脳の形成や働きを知ることは、動物の生きるしくみを知る上で欠かせない課題です。



## 脳のできるしくみと働くしくみ

統合神経生物学研究部門



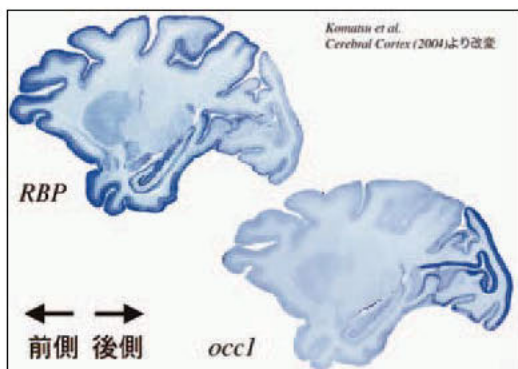
脱水状態になると体液中のナトリウム濃度が上昇します。動物は喉の渇きをおぼえ、水の摂取を行なう一方、塩分の摂取は回避します。当部門では、この行動制御に関わる脳内のナトリウム感知機構を解明しました。

脳は、眼や耳などの感覚器官から様々な外界の情報を取り入れ、それを識別、認知することによって正しい行動を指示します。また脳は、血圧や血糖値などの体内の状態もモニターしており、摂食や排泄などの制御を行っています。これらの脳の機能は、発生の過程で形成される正しい神経回路の働きによって初めて可能となります。私たちは、脳のできるしくみとして視覚系の形成機構を、また、できあがった脳が働くしくみとして体液の恒常性制御の機構と神経伝達の制御機構を研究しています。



## 学習する脳・進化する脳

脳生物学研究部門



サル脳の連合領域と視覚領域に発現する遺伝子

私たちの脳には、まだまだいろいろな謎が秘められています。新しい運動を覚えたり、何かを学習したりするとき、脳の中ではどんなことが起きているのでしょうか？ いろいろな動物の脳、例えばサルとネズミの脳は、どれほど似ていて、どれほど違うのでしょうか？ こういった疑問を解くために、私たちは「遺伝子」をキーワードにして、脳を作る1個1個の細胞が、どんなふうに組み立てられ動くのかを、分子生物学や組織学の方法を使って調べています。遺伝子を使って脳を「見」、その「働きを知る」。そこには今まで誰も見たことのない新しい脳の姿が隠れています。



## 脳はどのようにして塩を感じるか

神経生理学研究室



脳で発現するナトリウムセンサー分子(青色)

塩(ナトリウムイオン)は、動物にとって必要不可欠の電解質です。しかしながら、体液中のナトリウム濃度は一定に保たれる必要があり、過剰な塩分摂取は動物にとってむしろ好ましいものではありません。そのため体液中のナトリウム濃度が高くなると、動物は自然に塩分摂取を控えるという行動をとります。最近の研究から、こうした体液中のナトリウム濃度の検出は

脳が行っていること、また脳では特別なナトリウムセンサー分子が働いていることが明らかになってきました。私たちの研究室ではこうした脳のナトリウム濃度受容機構をマウスなどの動物や培養細胞を用いて研究しています。本プロジェクトは統合神経生物学研究部門と共同で研究を進めています。



## 神経細胞が伝える情報の役割を知る

神経生化学研究室



(A)筋肉の肥大を示すマウス (B)神経系の活動に変化を示すマウス

生物が活動する中で、神経細胞がどのような情報をいかなるしくみで伝えているかを理解するため、神経細胞間で情報を伝える神経伝達物質とその受け手である受容体に着目しています。神経伝達物質のひとつ、ドーパミンは、運動の調節・ホルモン分泌調節・心のはたらきに関わり、ヒトの神経系の病気の原因解明や治療にも重要といわれています。私たちは遺伝子操作技術を用いて神経伝達物質による情報伝達のしくみを変化させたモデルマウスを作成して、成体に発育するまでの時期に細胞・組織・個体に現れる変化を観察し、情報伝達の役割を研究しています。



生き物は膨大な遺伝情報をコンパクトに収納し、細胞から細胞へ、親から子へと伝達しています。また、必要に応じて取り出した遺伝情報にもとづいて生命活動を行っています。いわば遺伝情報は命の設計図です。そのふるまいを調べることは、生き物を知るうえで最も重要なことの一つです。



## 遺伝情報の書き換えと遺伝子の働き

分子遺伝学研究部門



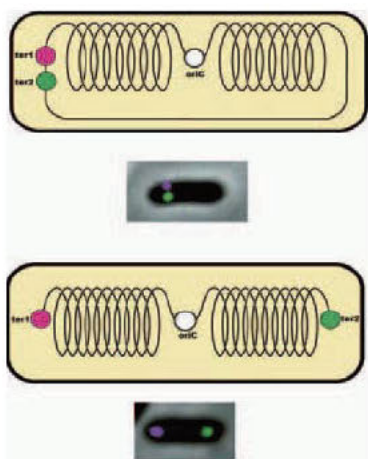
絞り、斑入り模様をもつアサガオの花とイネの葉

ふつう生きものの遺伝情報は、細胞分裂の時に正確にコピーされて新しい細胞に伝えられます。しかし、遺伝情報が細胞や個体によって異なるように書き換えられて、遺伝子の働きに影響する場合も知られています。たとえば植物の斑入りや絞り模様は、着色にかかわる遺伝子でそのような書き換えが頻繁におきたために現れます。このような遺伝情報の書き換え現象と遺伝子の働きとの関係を、私たちに馴染みの深いアサガオとイネを材料にして調べています。イネの染色体DNAの一部を人為的に置き換える技術も開発して、研究に役立てています。



## ダイナミックなゲノムを捕らえる

ゲノム動態研究部門



環状ゲノム大腸菌(上)から線状ゲノム大腸菌(下)の作成

生きものの設計図、遺伝情報は長いヒモ状の物質(DNA)に記録されています。これがゲノムと呼ばれ、細胞の核の中に規則正しく収納されています。ヒトではDNAの全長は2mにもおよびます。でもゲノムは静かにしまい込まれているわけではありません。核の中では設計図を読み出すため、DNA鎖をほどいたり、切ったり、つなげたりといった営みが盛んに行われています。また細胞が増える時、ゲノムも正確に2倍に増えます。ゲノムは常にダイナミックに活動しているのです。私たちは酵母や大腸菌を使い、ゲノムの安定性と進化、さらに病気や寿命との関係を調べています。また人工的改変にも取り組んでいます。



## ゲノムで読み解く微生物の多様性

ゲノム情報研究室



対応する遺伝子がどのゲノムに含まれているかを網羅的に解析した図

近年、様々な生物のゲノム解読が進み、それらを比較することによって生物の進化プロセスを理解することが可能になってきました。一方、私たちの体内や身の回りをはじめ、あらゆる地球環境に無数に存在する微生物については、ゲノム解析を通じて、はじめてその多様性の実体が明らかにされようとしています。ゲノム情報研究室では、主に微生物のゲノムを対象として、大量かつ多様なゲノム情報を系統的に比較するシステムを確立し、情報学的見地からゲノムの多様性の実態と、それを生み出す原動力の解明を目指した研究を行っています。

生きものは進化します。進化を止めることはできません。そして、さまざまな色や形をした花や魚、地味なコケ、そして人などなど、いろいろな生きものが生まれてきました。では、生きものはどうして、どうやって、どんな時に進化するのでしょうか？進化のしくみを調べ、私たちの未来を考えてみましょう。



## なにが進化を引き起こしたか

生物進化研究部門



進化研究の鍵を握るヒメツリガネコケ（基生研などにより全ゲノム配列が決定された）

生物は進化します。人間も十億年ほど前まではカビのような生物でした。では、いったい何が変まることによって進化がおきたのか。遺伝子です。では、どんな遺伝子がどのように変わることによって進化がおこるのか。植物細胞と動物細胞の違いはどう進化したのか、単細胞から多細胞への進化には何が必要だったのか、植物の茎葉や花はどうやって進化したのか、多様な形の花はどのように進化したのか、植物の種分化はどう引き起こされる、どうして植物は簡単に再生できるのかなどについて研究しています。現在の進化学で理解しがたい現象を分子レベルで研究することにより、新しい進化のパラダイムを解明することを目指しています。



## 魚類を用いた比較ゲノム解析とバイオリソースの開発

バイオリソース研究室



バイオリソース研究室から提供されている各種メダカ系統

私たちはメダカを用いて比較ゲノム解析によるゲノム進化に関する研究と生物遺伝資源（バイオリソース）の開発を行ってきました。具体的にはメダカの全塩基配列決定やメダカ遺伝子をくまなく調べ、メダカがどのようなゲノム構成を持つのかという点を明らかにしました。現在は生物材料としてのメダカの利用をさらに促進するために、メダカ遺伝子転写産物の網羅的解析、近縁種比較による進化研究を視野に入れたメダカ近縁種ゲノムソースの開発等を行っています。2007年にメダカバイオリソースプロジェクトの中核機関として基礎生物学研究所が選定されたことにともない、様々な生物遺伝資源の提供も行っています。



## 蝶のハネのかたちの多様性を作り出すしくみ

構造多様性研究室



蝶のハネの断面。左の丸い部分が細胞死を起こしている部分。

チョウやガのハネは、種によって特有のかたちをしています。ところが蛹になったばかりの段階ではほとんどの場合、ハネはつるりとした輪郭をしています。蛹になってすぐ、成虫のハネのかたちの切取線ができて、その外側の細胞が一斉に死んでいくことで、ハネのかたちができることがわかりました。このような「プログラムされた細胞死」はヒトの指ができるときにも見られます。輪郭線の位置やかたちを決めるしくみは、チョウが示す多様性をもたらすしくみとして興味深い課題です。

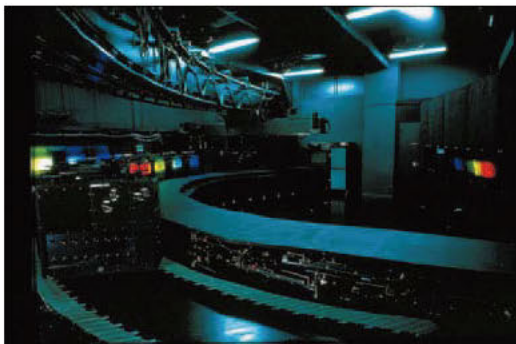


# 研究を支えている施設

基礎生物学研究所では、研究を効率よく推進するために、研究所の研究施設と岡崎3機関共通施設を設けています。実験や研究に用いる種々の生物材料を管理された環境のもとで培養、栽培、飼育する施設や、計測やデータ解析のための中・大型設備、RI施設、高度な解析装置などが整備されています。

## 生命を探る虹の架橋

培養育成研究施設（大型スペクトログラフ室）

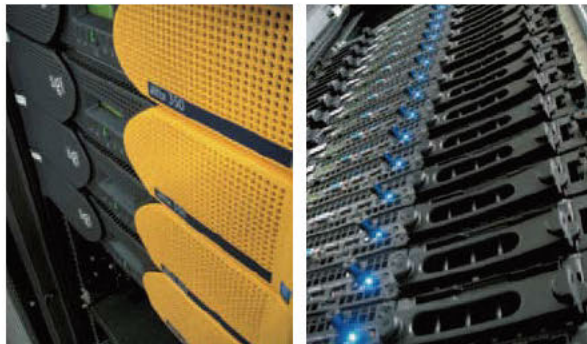


大型スペクトログラフ室の内部風景

生命の誕生した頃の地球には大量の紫外線が降り注いでいました。この紫外線から逃避するために原始生物が発達させた紫外線感覚が、現在の生物の光感覚のもとだと考えられます。例えば、植物は青色と赤色・遠赤色を感じ、動物は緑色を中心に各色を感じます。また、現存する微生物にはこれらの先祖型のような各色の光感覚があります。大型スペクトログラフ室では世界最大の巨大な人工虹を作って、これらの光感覚のほか、光の有益な作用や有害な作用のしくみを調べています。

## コンピュータでゲノムを探る

培養育成研究施設（電子計算機室）



生物情報解析システム

電子計算機室では、高速・大容量計算機を利用した研究支援を行っています。ここでは、遺伝子やタンパク質の配列データベースを構築し、これを利用した配列解析、発現データ解析、画像処理解析を主な柱として研究を支えています。また、解析用プログラム、Webを介したデータベース公開プログラムの開発を行い、モデル生物の遺伝子解析結果を全世界に配信しています。計算機を利用した解析に加え、超高速ネットワークシステムの維持管理を行うと共に、計算機・ネットワークに関する相談対応、新しいサービスの導入にも力を入れて所内の情報交換基盤を支えています。

培養育成研究施設にはこの他に、実験植物の生育を管理する人工気象室と動物組織・細胞の培養を行う細胞器官培養室があります。

## 形質転換生物の開発と解析

形質転換生物研究施設



研究に用いられるモデル生物

生物の生きるしくみを理解するためには生物のもつ設計図(ゲノム)の1つ1つの遺伝子に着目して研究をする必要があります。

マウス・メダカ・ゼブラフィッシュ・ニワトリなどのモデル生物を用いると、遺伝子・細胞工学技術により目印を付けた遺伝子操作(形質転換)生物を作ることができ、遺伝子や細胞機能などの研究を詳細に行うことができます。これらモデル生物は短期間で成体にまで发育するので、細胞・器官・個体全体に見られる変化を効率よく調べることができます。形質転換生物研究施設ではこのような生物を、安全に、効率よく、適切に維持できるよう機器・設備とスタッフが配置されています。

## 放射性物質で生物のしくみを知る

アイソトープ実験センター



放射性物質を用いた実験及び管理(左下)の様子

生物学の研究では、遺伝子の機能やタンパク質の性質を解析するなど、生体内の物質の所在を調べる必要が多くあります。アイソトープ実験センターは、放射線を発生する物質=ラジオアイソトープ(放射性同位元素)を用いて物質の挙動を調べるための施設で、ラジオアイソトープを安全に取り扱うために厳密に管理しています。

## 最先端の研究を支える機器

分析室



分析機器を操作する様子

分析室は生物学や生理学の研究を推進するのに必要な分析機器を設置しています。およそ60種類、約100台の分析装置を備えており、生体内のタンパク質や遺伝子の解析、生理活性物質などの分離、精製、同定と構造解析や、画像解析まで広く研究に活用されています。分析機器は系統的に以下のように分類され、職員が機器の維持管理や依頼測定をしています。

1. タンパク質・遺伝子解析装置
2. 分離分析装置
3. 物理化学的解析装置
4. 分光分析装置
5. 顕微鏡・画像解析装置



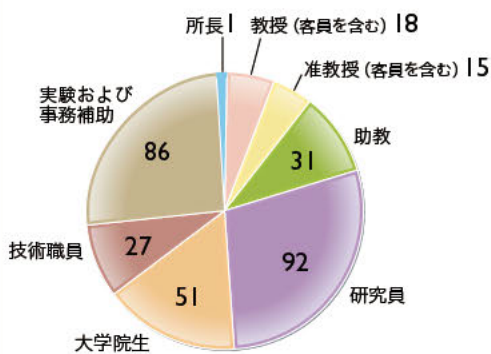
## 建物の配置図



基礎生物学研究所の研究室・研究施設の一部は山手地区実験研究棟にあります。

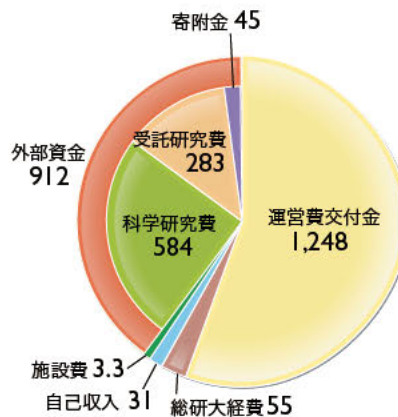
## 研究所で働く人たち (2007年4月1日現在)

数字は人数



## 研究所の財政規模 (2006年度決算額)

数字は百万円



基礎生物学研究所では国からの補助(運営費交付金、総研大経費)に加え、各研究者の努力により科学研究費補助金、受託研究費など多くの競争的資金を獲得して研究を行っています。

## 一般公開・情報発信など

### 研究所一般公開

岡崎の3研究所は、毎年1研究所ずつ秋に一般公開を開催します。各研究所は3年に1回の公開になります。研究内容の紹介、研究材料や機器の展示、講演会など、いろいろな企画があります。基礎生物学研究所は2007年、2010年に公開する予定です。

### 広報誌「OKAZAKI」

岡崎の3研究所の研究活動やイベント開催の様子をお知らせする広報誌を年4回発行しています。

PDF形式で研究所ホームページ (<http://www.nibb.ac.jp/okazaki/>) からダウンロードできます。



### 自然科学研究機構シンポジウム

自然科学研究機構の5研究所が、自然科学の最新研究を一般に紹介するシンポジウムが、春と秋に東京で開催されています。詳しくは研究所のホームページをご覧ください。

## 交通アクセス



### 東京方面から

新幹線で豊橋駅下車、名鉄本線（豊橋駅）に乗り換えて、東岡崎駅下車（豊橋—東岡崎間約20分）。

### 大阪方面から

新幹線で名古屋駅下車。名鉄本線（名鉄名古屋駅）に乗り換えて、東岡崎駅下車（名鉄名古屋—東岡崎間約30分）。

### 中部国際空港から

名鉄バスJR岡崎駅行きを利用、東岡崎下車。所要時間約60分。  
または、名鉄空港線で名古屋方面へ向かい、神宮前駅で豊橋方面へ乗り換えて、東岡崎駅下車。所要時間約70分。



### 東岡崎駅から各地区へ

明大寺地区へは、東岡崎駅南口より徒歩7分。  
山手地区へは、南口バスターミナルより名鉄バス「竜美丘循環」に乗り「竜美北1丁目」下車（所要時間5分）、さらに徒歩で3分。

### 自動車利用の場合

東名高速道路の岡崎ICを下りて国道1号線を名古屋方面に約1.5km、「市役所南東」信号を左折。ICから約10分。



自然科学研究機構

**基礎生物学研究所**

〒444-8585 愛知県岡崎市明大寺町字西郷中38番地

Tel: 0564-55-7000 Fax: 0564-53-7400

<http://www.nibb.ac.jp/>

2007年9月発行

編集：連携・広報企画運営戦略室



基礎生物學研究所 · 基礎生物學研究所 · 基礎生物學研究所 · 基礎生物學研究所 · 基礎生物學研究所 · 基礎生物學研究所

Inter-University Research Institute Cooperation  
National Institutes of Natural Sciences

# National Institute for Basic Biology



[www.nibb.ac.jp/en](http://www.nibb.ac.jp/en)



Director-General of  
the National Institute for Basic Biology

**Kiyotaka Okada**

## "Welcome to the world of basic biology"

The National Institute for Basic Biology (NIBB), a brick-colored building overlooking the city of Okazaki, can be viewed from the windows of the Tokaido Shinkansen and the vehicles driving on Route 1. The NIBB was established in 1977 and celebrated its 30th anniversary in 2007. Our building is already well-known to the people of Okazaki, but our purposes and achievements are not yet widely known. Various questions might arise, for example: what kind of research is conducted in it? What kinds of people spend their days there, and what do they want to know about? Is there any chance to meet them to ask about their work? This brochure will answer these kinds of questions.

I am often asked questions like: what is basic biology and how does it differ from biology and life science? Since entering the 21st century, our understanding of genes has progressed, and basic and applied research are closely bound together. There have been many cases in which the results of basic research using experimental animals have quickly lead to cures for human diseases because the genes of the experimental animals, such as drosophilas and mice, are quite similar to those of humans. In addition, the achievements of basic research using model plants, such as Arabidopsis, have contributed to the development of crops that can grow even in adverse environments and have allowed for increases in cereal yields. Dividing biology into basic biology and applied biology may seem of little significance, but it will remain the case that basic research will lead to applied research.

The NIBB sets as its most significant goals the study of the functions of the basic genes of living organisms and cells and research into the mechanisms that allow living organisms to fit into a living environment and obtain various shapes and abilities. The NIBB researches the basic and universal mechanisms that have been unchanging throughout the evolutionary process and the reasons that various changes have occurred in unicellular organisms, animals, and plants. The researchers uncover the billion-year history in living organisms and struggle to reveal the secrets hidden in them.

The NIBB, according to its role as an Inter-University Research Institute, promotes joint study between researchers and students from universities and institutes across Japan. Researchers from abroad have frequently visited the NIBB, and the staff has supported their research effectively. Cultivating researchers capable of leading future research and fostering the education of the next generation are also a major role. As one of the campuses of SOKENDAI, the NIBB have provided a favorable environment in which graduate students may conduct research.

With these goals and roles in mind, the NIBB has dedicated itself to a wide range of activities. We will provide not only the results of research, but also various kinds of information, such as pictures of events and living organisms. Your comments or suggestions are appreciated.

# "History"

The National Institute for Basic Biology (NIBB) was established in the city of Okazaki in 1977 along with the National Institute for Physiological Sciences (NIPS). Since 1981, the NIBB and NIPS have constituted the Okazaki National Research Institutes with the previously established Institute for Molecular Science. As a leading research institute for basic biology in Japan, the NIBB, since its establishment, has accepted many joint researchers from Japan and abroad, and its achievements, as products of "OKAZAKI," are highly praised in treatises and at international conferences. Research also commenced in the Yamate area in 2002. Since 2004, the National Astronomical Observatory of Japan, the National Institute for Fusion Science, and three institutes in Okazaki have been conducting research under a new framework called the National Institutes of Natural Sciences.

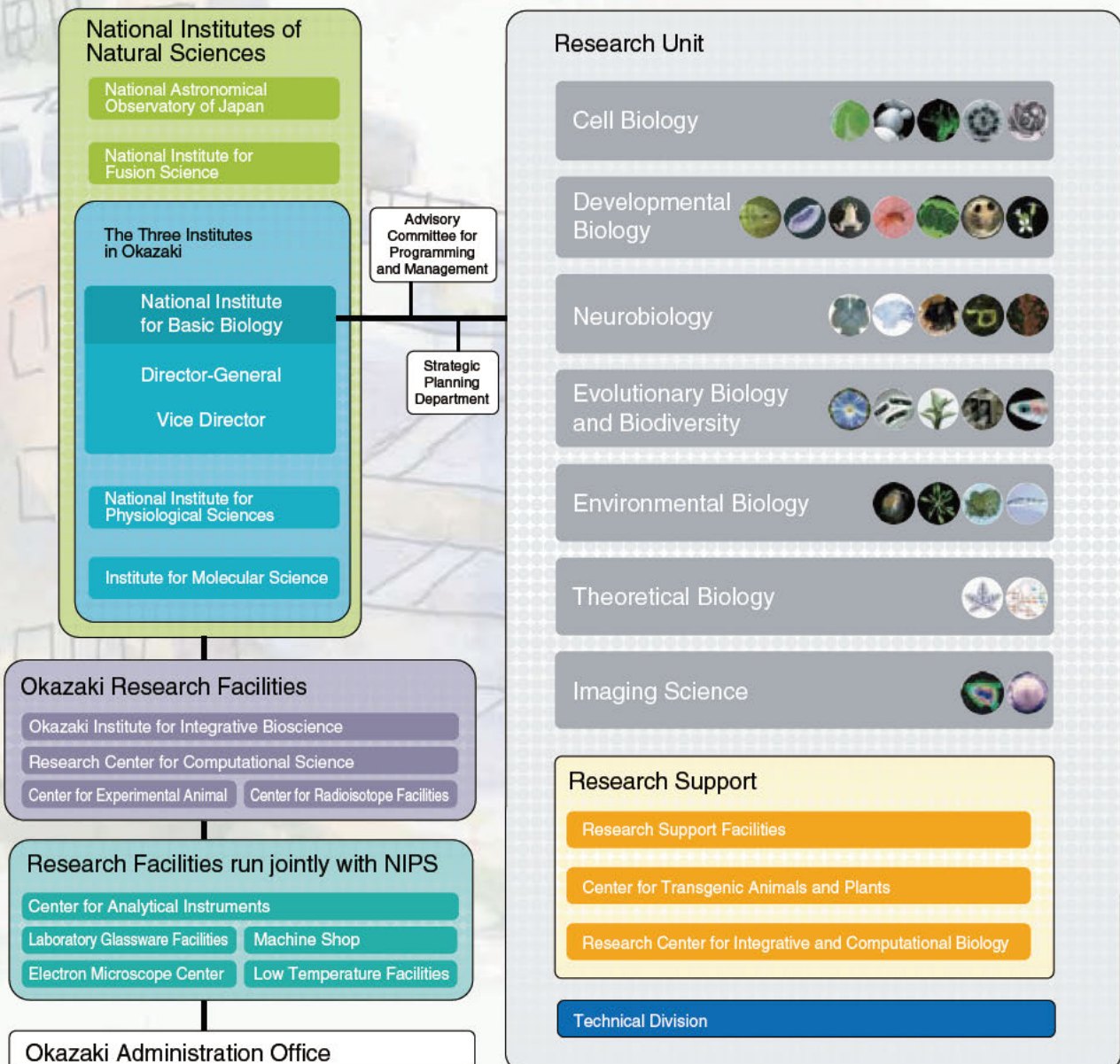


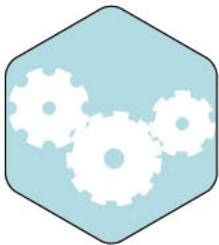
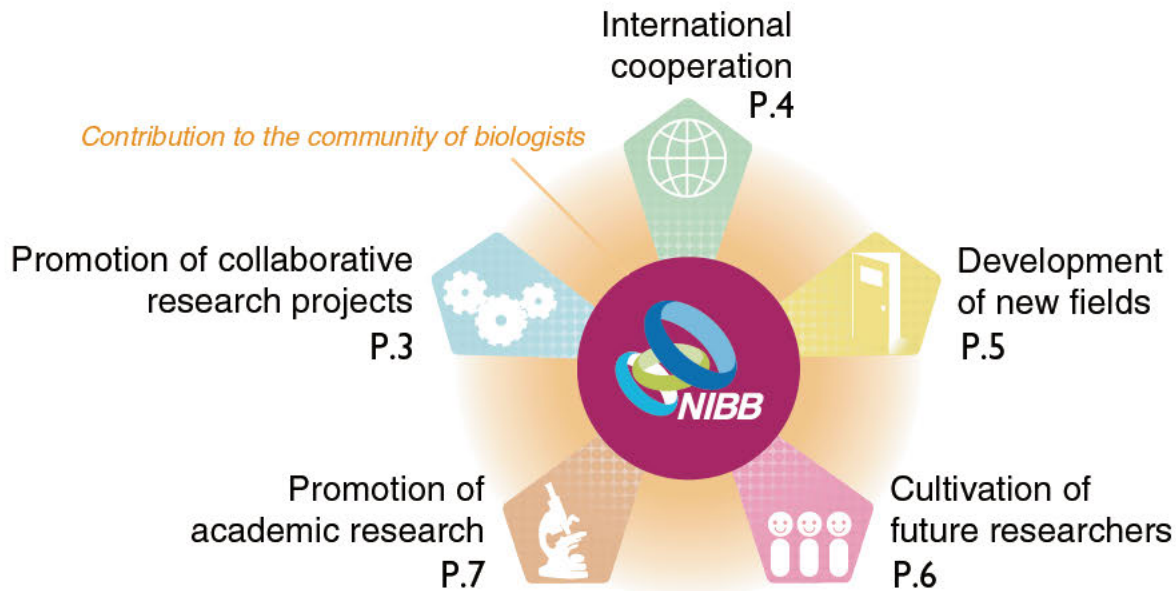
Myodaiji area



Yamate area

# "Organization"





## “As the center of excellence for biological research”

### Collaborative research projects

#### Inter-University Research Institutes

An inter-university research institute is a 'research institute operated by the research community', a type of world-class organization unique to Japan. The inter-university institute was organized as a core base to provide a place for joint research and extramural use by researchers across Japan. The inter-university research institute not only promotes pioneering studies on important research issues, but also provides opportunities for cutting-edge researchers throughout Japan to gather and engage in activities aimed at exploring future academic fields and creating new principles.

While maintaining its uniqueness and diversity, each institution makes a great contribution in the development of academic research in Japan as a Center of Excellence (COE) in each research field. Together, they also serve as an international core base to promote cooperation and exchange with research institutes and researchers abroad.

#### Collaborative research projects

Research projects to collaborate with the NIBB's divisions/laboratories and research activities to be conducted using facilities in the NIBB are solicited from external researchers at other universities and institutes. In addition to conventional "individual collaborative research projects," "collaborative experiments using the Large Spectrograph," and "NIBB workshops," new types of research projects are solicited that will facilitate the strategic organization of collaborative research projects. "Priority collaborative research projects" are carried out in one to three years as group research by internal and external researchers with the purpose of developing pioneering research fields in biology. Three projects have already been carried out, including "Molecular mechanism for controlling the individuals of higher plants." Also, the category of "collaborative research projects for model organisms/technology development" has been set since 2007 with the aim of developing and establishing new model organisms. Two projects have already been carried out, including "Development of the transgenic strain of *Cabombaceae* (primitive angiosperm)." In the belief that the methods of conducting collaborative research projects must be constantly modified according to the demands of the age and the biology community, the NIBB always encourages discussion on such projects.

#### Collaborative research projects by year

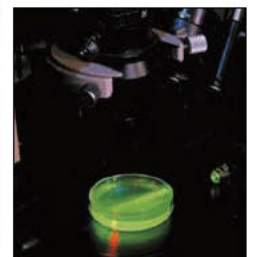
year	2005	2006	2007
Priority collaborative research projects	2	3	1
Collaborative research projects for model organisms/technology development	—	—	2
Individual collaborative research projects	41	37	34
NIBB workshops	4	1	1
Collaborative experiments using the Large Spectrograph	19	18	12
total	64	59	50

#### Enhancement of the Large Spectrograph

The Large Spectrograph Laboratory (see p. 17), as a world-leading research facility in photobiology, has succeeded in a large number of collaborative research projects since its foundation in 1980. To ensure further higher-level achievements, the Laboratory has promoted enhancement of its laboratory equipment, including advanced control systems, the use of laser sources, and sophisticated analysis equipment. To supplement the currently operating fixed (partly tunable) wavelength laser sources, the introduction of tunable laser sources covering a wide range of wavelengths from UV to IR is in preparation.



Violet laser (390 to 410 nm) and blue laser (440 to 460 nm) sources

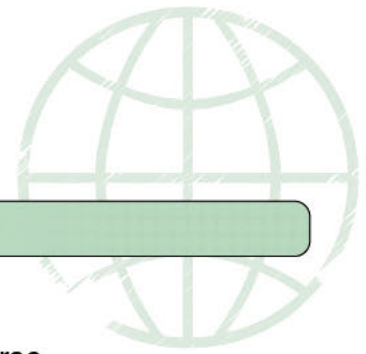


Laser irradiation of a sample



# "As the core of the worldwide community of researchers"

International cooperation



## Collaborative research projects with the EMBL

The European Molecular Biology Laboratory (EMBL) is a research institute funded by 18 European states and established in 1974. It conducts comprehensive, high-level basic research programs, leading the world in the field of molecular biology. The NIBB takes the leading role in collaborative research programs between the EMBL and the National Institutes of Natural Sciences (NINS), which were launched in 2005, and promotes personal and technological exchange through symposia, exchange between researchers and graduate students, and introduction of experimental equipment.



Past EMBL Symposia			
1st	Developmental Biology	Jul. 2005	Heidelberg (Germany)
2nd	Frontiers in Bioimaging	Mar. 2006	Okazaki (Japan)
3rd	Mouse Biology	Apr. 2006	Monterotondo (Italy)
4th	Biology of Protein Conjugation	Dec. 2006	Okazaki (Japan)
5th	Cell and Developmental Biology	May 2007	Okazaki (Japan)

## International Practical Course

With the cooperation of researchers from Japan and abroad, the NIBB international practical course, a practical training course, is given at the dedicated laboratory specifically prepared for the course in the NIBB. The first course, titled "Developmental Genetics of Zebrafish and Medaka," was held in 2007. Graduate students from various East Asian nations and areas, including China, Hong Kong, Taiwan, and India, were provided with training in state-of-the-art techniques for small fish research.



Practical training at the dedicated laboratory



Micro-manipulation of the embryo



Participants of the 1st symposium



Academic exchange at the EMBL



Study tour of a mice facility at the EMBL



Participants of the 4th symposium

## Bioresources

The National BioResource Project (NBRP) is a national project for the systematic accumulation, storage, and supply system of nationally recognized bioresources (experimental animals and plants, cells, DNA, and other genetic resources), which are widely used as materials in life science research. To promote this national project, the NIBB has been appointed as a research center for research on "Medaka (*Oryzia latipes*)" whose usefulness as a vertebrate model first developed in Japan. The usability of Medaka as a research material in biology has drawn increasing attention since its full genome sequence recently became available. The NIBB also works as a sub-center for this national project for research on Japanese morning glory and zebrafish. In addition, the NIBB provides databases containing research data on the moss *Physcomitrella patens*, *Daphnia*, *Xenopus laevis*, plant cell organelles, and bacterial genomes.



Top: Inbred Hd-rR strain of Medaka with the full genome sequence determined, Middle: Transgenic Medaka with red fluorescence emerging from the whole body, Bottom: Quintet strain of Medaka with transparent body.

## NIBB Conference

The NIBB Conference is an international conference organized by the NIBB's professors once or twice a year with the participation of a guest lecturer from abroad. Since the first conference in 1977 (the year of the NIBB's foundation), the NIBB Conference has provided researchers in basic biology with valuable opportunities for international exchange.

Recent NIBB Conferences		
51st	New Aspects of Gene Amplification	Nov. 2005
52nd	Reproductive Strategies	Jan. 2006
53rd	Dynamic Organelles in Plants	Jun. 2006





# "Center for developing new fields of biology"

## Development of new fields

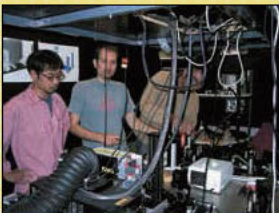
### Bioimaging

Recently, the capability of optical microscopes has greatly improved, and biophotonics probes have also been developed. The combination of these technologies allows us to use living samples and observe biological phenomena in real time, which, in the past, could only be estimated based on fragmentary information from fixed samples. The NIBB aims to maximize the application of these techniques for visualizing biological phenomena (bioimaging) in biological research and to develop new imaging techniques.

- 1) **Establishment of the imaging science laboratories** The NIBB aims to be a center for developing microscopes and biophotonics probes.
- 2) **Setup of the Advisory Committee on Bioimaging** Regular meetings are held with several leading researchers in the bioimaging field in Japan to formulate advice on imaging research.
- 3) **Holding of the Bioimaging Forum** This Forum provides an opportunity for researchers in the NIBB, members of the Advisory Committee, and company engineers to frankly discuss practical difficulties and needs regarding imaging.



- 4) **Introduction of SPIM (Selective Plane Illumination Microscopy)** As part of collaborative work with the EMBL, the NIBB introduces SPIM, which is effective for the three-dimensional observation of living samples, first in Japan.



- 5) **Holding of the Bioimaging Symposium** This Symposium provides an opportunity for academic exchanges with overseas cutting-edge researchers in the imaging field, mainly from the EMBL.



Participants of symposium and a poster



### Okazaki Biology Conferences

The NIBB holds Okazaki Biology Conferences (OBC) that, under the endorsement of the Union of Japanese Societies for Biological Science, support the formation of international communities in future biological research fields with the goal of identifying new research issues in biology. Dozens of top-level researchers from Japan and abroad spend about one week together in exhaustive discussions seeking strategies for addressing future critical issues in biology. The past conferences have promoted the formation of international researcher communities.

#### Past OBCs

OBC1	The Biology of Extinction	Jan. 2004
OBC2	Terra Microbiology	Sep. 2004
OBC3	The Biology of Extinction 2	Mar. 2006
OBC4	Terra Microbiology 2	Sep. 2006
OBC5	Speciation and Adaptation	Mar. 2007



Participants of the OBC5



Oral session (OBC4)



Poster session (OBC2)



General Discussion (OBC5)





# “Cultivation of future researchers in biology”

## Admission of graduate students: SOKENDAI and other universities

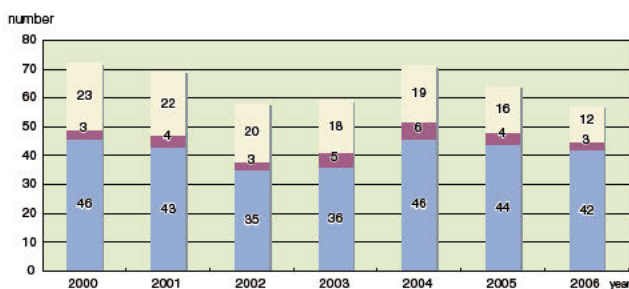
As a center of biological research in Japan, the NIBB has cutting-edge facilities and equipment. It also retains excellent faculty members who have continuously produced outstanding creative research results; the number of citations of their published papers is at the top level in Japan and in the world. The NIBB offers advanced graduate education in this excellent research environment with the goal of cultivating future leaders in biology.



Education of graduate students in NIBB

### Admission of graduate students

One way to enter the NIBB is to enroll in the Department of Basic Biology in the School of Life Science of the Graduate University for Advanced Studies (SOKENDAI), which is affiliated with the NIBB. Another way for graduate students already enrolled in the graduate schools of other universities is to become a “special research student” under collaborative research projects. To become a special research student, graduate students must apply for enrollment in the NIBB and undergo an assessment each year. In both cases above, graduate students can live an academic life and receive financial support from the NIBB based on the research assistant (RA) system in the same way.



■ Department of Basic Biology ■ School of Advanced Science (educated in NIBB) □ Special research student

Graduate students educated by NIBB

### About SOKENDAI

SOKENDAI was established in 1988 as a university without undergraduate courses that provides graduate education with a view to the comprehensive development of basic academic fields. It has headquarters in Hayama, Kanagawa Prefecture, offering graduate education with students separately assigned to 18 Inter-University Research Institutes (national institutes). Its School of Life Science consists of three departments: Department of Basic Biology, Department of Physiological Sciences (National Institute for Physiological Sciences in Okazaki, Aichi Prefecture), and Department of Genetics (National Institute of Genetics in Mishima, Shizuoka Prefecture).

The Department of Basic Biology provides advanced research training courses in which fundamental and advanced biological phenomena related to plants and animals are analyzed at the molecular level. There are two courses available: a three-year doctoral course for graduate students with master’s degrees and a five-year course for university graduates. For both courses, students are admitted in April and October every year.



A “joint seminar” is held every year for students and faculty members in the School of Life Science to gather and present research results.

### Graduate education with a high ratio of staff to students

In most universities, the number of faculty members is small in relation to that of graduate students (approximately 0.16 per graduate student for national universities). In contrast, SOKENDAI has a significantly higher ratio of faculty members to graduate students (approximately 1.8 per graduate student); there is no concern about lack of student mentoring. Currently, the Department of Basic Biology has 42 SOKENDAI students and 55 faculty members, providing the ideal “one-to-one” education.



In “Life Science Progress Report” sessions, students report the progress of research activities and, in turn, receive advice from many faculty members.

### High-quality seminars

The NIBB holds many seminars with external distinguished guests as lecturers. It also hosts conferences, most of which students in the NIBB can attend. These seminars and conferences offer good opportunities for future researchers to widen their vision.



Exchange seminar with graduate students in the EMBL (European Molecular Biology Laboratory) as part of collaborative work with the EMBL.

### High rate of students becoming researchers

The Department of Basic Biology is engaged in the cultivation of advanced researchers. In the last five years, approximately 80 % of those who finished the courses became researchers, including associate professors and postdoctoral researchers.

### How to enroll in SOKENDAI

Detailed information is available at “Graduate School” in the homepage of the NIBB. In addition, four admission information sessions are held in Tokyo, Osaka, Okazaki, etc. every year. An open laboratory program is also available, which allows students to experience actual academic life in the NIBB (a support system for transportation and accommodation costs is available).



# “Promotion of cutting-edge biological research”

## Academic Research

The NIBB is organized as follows for conducting cutting-edge biological research. The details of its research activities are given in the following pages.

### Cell Biology

#### Division of Cell Mechanisms P.9



Prof. Mikio Nishimura



Assoc. Prof. Makoto Hayashi

#### Division of Molecular Cell Biology P.9



Prof. Yoshinori Ohsumi

#### Laboratory of Cell Structure P.9



Assoc. Prof. Kazuo Ogawa

#### Laboratory of Cell Sociology P.10



Assist. Prof. Yoshio Hamada

#### Division of Cell Proliferation



Adj. Assoc. Prof. Kazuhiro Sakamaki

### Neurobiology

#### Division of Behavioral Biology P.13



Adj. Prof. Yuji Mori



Adj. Assoc. Prof. Hiroko Tsukamura

#### Division of Molecular Neurobiology P.14



Prof. Masaharu Noda

#### Division of Brain Biology P.14



Prof. Tetsuo Yamamori

#### Laboratory of Neurophysiology P.14



Assoc. Prof. Eiji Watanabe

#### Laboratory of Neurochemistry P.14



Assoc. Prof. Toshikuni Sasaoka

### Environmental Biology

#### Division of Molecular Environmental Endocrinology P.13



Prof. Taisen Iguchi



Assoc. Prof. Hajime Watanabe

#### Division of Photobiology P.10



Prof. Masamitsu Wada



Adj. Assoc. Prof. Daisuke Yamauchi

#### Laboratory of Photoenvironmental Biology P.10



Adj. Prof. Masakatsu Watanabe

#### Division of Plant Developmental Genetics P.12



Adj. Prof. Hirokazu Tsukaya

## Developmental Biology

Laboratory of Plant Organ Development P.12



Director-General  
Kiyotaka Okada

Laboratory of Molecular Genetics for Reproduction P.13



Assoc. Prof.  
Minoru Tanaka

Division of Morphogenesis P.11



Prof.  
Naoto Ueno



Assoc. Prof.  
Noriyuki Kinoshita

Division of Reproductive Biology P.13



Prof.  
Yoshitaka Nagahama

Division for Sex Differentiation P.13



Prof.  
Ken-ichirou Morohashi  
(Concur. with Kyushu Univ.)

Division of Developmental Genetics P.11



Prof.  
Satoru Kobayashi

Division of Molecular and Developmental Biology P.11



Prof.  
Shinji Takada

## Imaging Science

Division of Developmental Dynamics



Adj. Prof.  
Atsushi Miyawaki

Laboratory for Spatiotemporal Regulations P.11



Assoc. Prof.  
Shigenori Nonaka

## Theoretical Biology

Division of Theoretical Biology P.12



Assoc. Prof.  
Atsushi Mochizuki

Laboratory of Genome Informatics P.15



Assist. Prof.  
Ikuo Uchiyama

## Evolutionary Biology and Biodiversity

Division of Evolutionary Biology P.16



Prof.  
Mitsuyasu Hasebe



Assoc. Prof.  
Takashi Murata

Division of Molecular Genetics P.15



Prof.  
Shigeru Iida

Division of Genome Dynamics P.15



Prof.  
Takashi Horiuchi

Laboratory of Morphodiversity P.16



Assoc. Prof.  
Ryuji Kodama

Laboratory of Bioresource P.16



Assoc. Prof.  
Kiyoshi Naruse

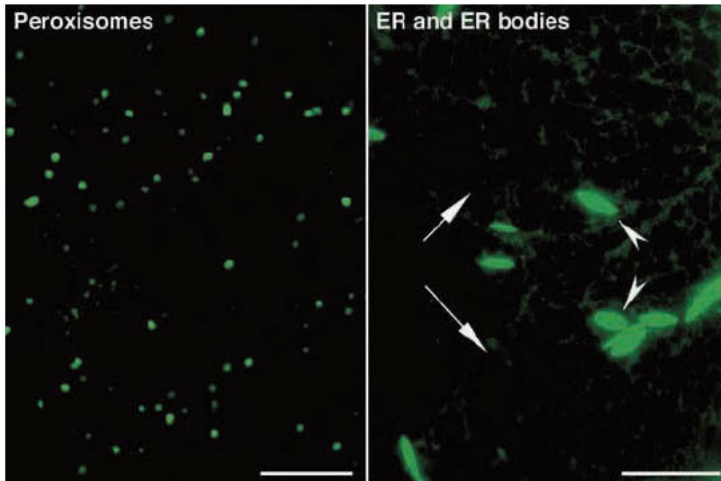
# Living unit: the cell

All organisms are made up of a basic unit called the cell. Within each cell there are various small organs known as organelles. Organelles change their form and function dynamically and the life of a cell depends on the integrative function of the organelles. We are attempting to elucidate the mechanism behind the dynamic changes of cells.



## Functional change of organelles

Division of cell mechanisms



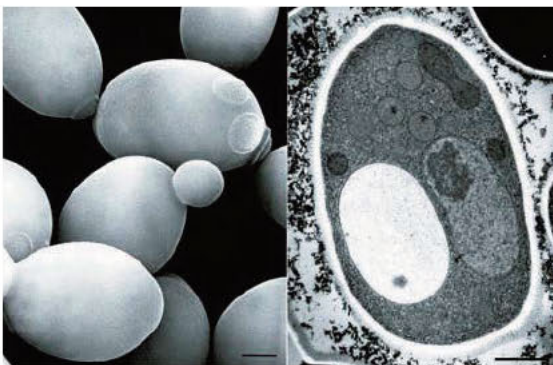
Peroxisomes and ER network (arrows) with ER bodies (arrowheads) of an *Arabidopsis* cell visualized by a green fluorescent protein Bar: 10  $\mu\text{m}$

The functions of organelles change significantly during the growth of a plant. Peroxisomes are organelles of approximately 1  $\mu\text{m}$  in size. When a plant seed germinates, peroxisomes change the stored fat into energy, but when the plant is exposed to light, peroxisomes change their function and assist the subsequent photosynthesis. A plant will show poor growth when the functions of the peroxisomes are impaired. The endoplasmic reticulum (ER), on the other hand, is a network-shaped organelle where the protein synthesis occurs. If the plant is attacked by insects, a special form of the ER called the ER body is formed where an insect repelling substance is stored. We are endeavoring to elucidate the living strategies of plants by studying the dynamic functional changes of organelles.



## The recycling system of cells studied using yeast

Division of molecular cell biology



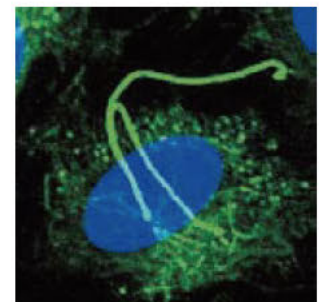
The external and internal structure of yeast observed using electron microscopes (provided by emeritus professor Masako Osumi of the Japan Women's University) Bar: 1  $\mu\text{m}$

Yeast is a microbe with which we have had a long acquaintance through the fermentation processes in the manufacture of bread, wine, etc. The simple appearance of yeast is deceptive. An electron microscope will reveal that there are intricate structures called organelles within yeast cells (Figure). The yeast cell therefore shares a common basic structure with the cells of animals and plants. Thanks to the applicability of genetic and molecular biological techniques, yeast is used as a model cell in a vast amount of scientific studies and has contributed extensively in solving some of the mysteries underlying the basic living processes of plants and animals. Our division uses yeast to clarify the mechanisms of autophagy, which are the degradation and recycling processes of the intracellular proteins and organelles.



## An immotile cilium of a cell

Laboratory of cell structure



Cells isolated from mouse kidney and cultured in a petri dish. The nucleus is stained in blue and the cilia in green.

The flagellum of the sperm and the cilia of the bronchus are motile. There are, however, immotile cilia in our body. When cells are cultured in a petri dish for a long period, cilia start to grow on the cell. This is because the tubulin, a protein needed in the mitosis, is of no further use when the cell ceases proliferation and the cell recycles the tubulin to form the cilium. The cilium thus formed is immotile, in contrast to the flagellum or the cilia of the bronchus. In most cases, a cilium stems from the centrosome, but the figure above shows a case wherein two cilia grow on a cell. The length of the cilium varies from cell to cell; the cell in the figure shows long cilia. We are interested in the function of these immotile cilia.



A leaf showing a Chinese character meaning "light" by the avoidance response



## Can the chloroplast move?

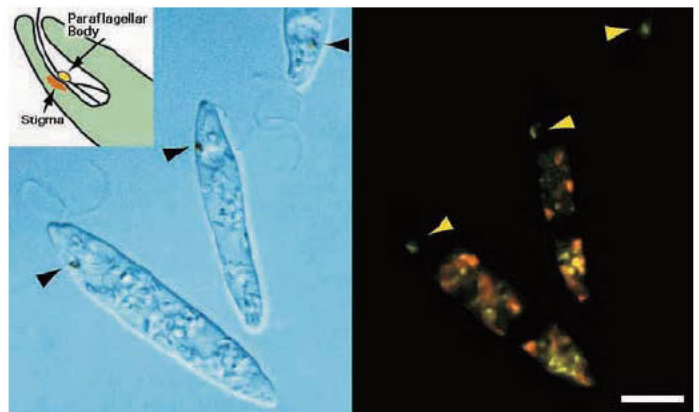
Division of photobiology

Plants transform light into the energy necessary for the plant's life processes using photosynthesis, which proceeds through the function of the green-colored chlorophyll within the chloroplasts of the plant's cells. The chloroplasts move within the cell according to existing light conditions, gathering at locations illuminated with weak light in order to fully utilize it (accumulation response) and avoiding areas illuminated with strong light to avoid damage (avoidance response). We termed these reactions chloroplast photorelocation movements. We are studying the significance and the mechanism of chloroplast movement. The green color of a leaf is made possible by the chloroplasts within the leaf's cells. We can manipulate a leaf's color to a dark green by shedding a weak light on the leaf to trigger the accumulation response or, alternately, we can change a leaf's color to a light green by shedding a strong light in order to make the chloroplasts escape from the leaf's surface. The movement of the chloroplasts within the cell becomes evident to the naked eye (figure).



## Light sensing of the microbes

Laboratory of photoenvironmental biology



The euglena senses the light not by the stigma (the brown dots in the left figure) but by the paraflagellar body (the green dots in the right figure).

The euglena (figure) is a kind of plant plankton. In order to raise the efficiency of the photosynthesis that they require to live, euglena gather where light is available and escape from light that is too strong. To elucidate the mechanisms of light sense, we analyzed the paraflagellar body, which is a light-sensing organ, and found that the photoactivated adenylate cyclase (PAC) is the light-sensing molecule. PAC senses blue light and produce a signaling molecule called the cyclic AMP. This finding is not only crucial in understanding the light controlling mechanisms of the flagellum movement of the euglena but is also applicable to cytotechnological methods, such as controlling the nerve activity of various animal tissues using light signals.



## The tie between mother and child

Laboratory of cell sociology



The mouse embryo (left) and the placenta (right, dark pink). The light pink membrane surrounding the placenta (the decidua) is the tissue derived from mother and surrounds the entire embryo and the placenta when intact.

All animals except mammals start eating food immediately after they hatch. Mammals have little nourishment within the egg and, as soon as they are hatched in the early stages of the formation of the body, mammalian embryos become parasitic to the maternal body in order to intake the nourishment necessary for continued growth. The placenta is an organ through which the embryo receives nourishment and oxygen from the mother and returns wastes and carbon dioxide back to the maternal body. As a necessary consequence of their parasitic growth process, mammalian children continue to receive nourishment from their mothers in the form of breast milk. The placenta is an organ made up of the cells of the embryo, but its organogenesis is considered to be heavily dependent on the interaction with the mother-derived cells. Our laboratory studies the cellular interaction between mother and child.

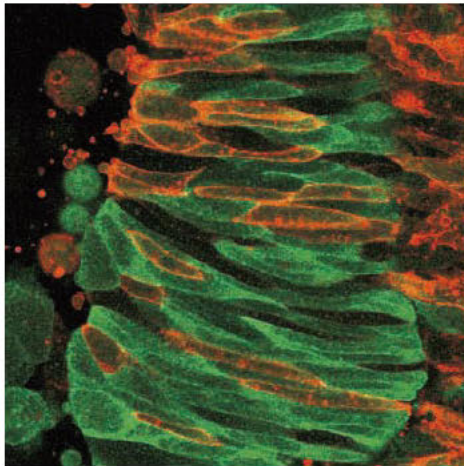
# Formation of the shape of living organisms

Living organisms, both animals and plants, have various shapes that differ from species to species. How are living things formed? We are endeavoring to discover the mechanisms of morphogenesis by searching for the genes controlling shape formation and studying their function as well as by analyzing morphogenetic processes using mathematical models.



## From a round shaped egg to an adult body

Division of morphogenesis



The notochord cells of the African clawed toad (*Xenopus*). Cells labeled with red fluorescence and those with green mix by sliding by each other.

Most animal eggs begin as a spherical-shaped cell. After fertilization, as the cell number increases, the embryo gradually changes its shape, elongating from a sphere to an oblong from head to tail. In a frog embryo, for example, it is known that the movement and modification of cells in the notochord are important to the elongation process. How are the cells of the notochord formed? How do the cells know which way to move? We are trying to clarify the molecular and genetical mechanisms of morphogenesis using frogs, ascidians, and fruit flies. The results of such studies are expected to contribute to the analysis of human inherent malformations.



## From eggs to a baby

Division of molecular and developmental biology



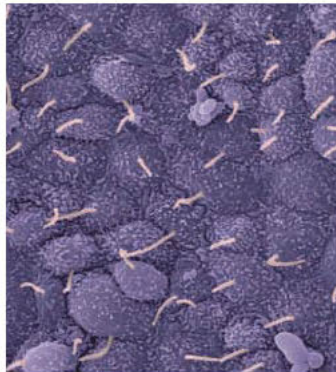
A zebrafish embryo 16 hours after fertilization

An animal's body is formed by the continual cell divisions of a single fertilized egg. An uncontrolled repetition of this cell division, however, would not result in the formation of the body shape. A program controlling the morphogenetic processes is at work. Recently, the program has been found to be comprised of the working of various genes. We are attempting to elucidate the mechanisms of the formation of the body shape of vertebrates, including humans, by understanding the function of the genes. We use small fish, such as the zebrafish, and the mouse as model organisms for the functional studies of genes.



## How the right and the left sides of the body are determined

Laboratory for spatiotemporal regulations



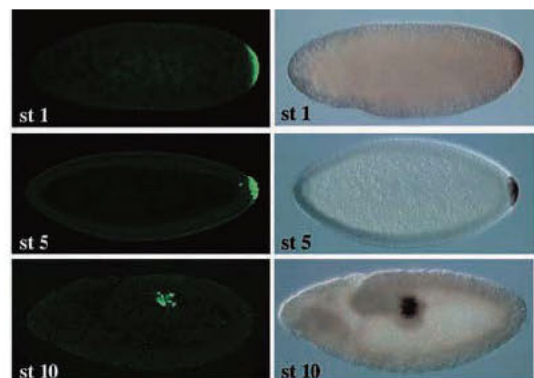
Hairs on the body surface which determines the left-right asymmetry (colored in yellow).

The heart is on the left-hand side and the liver and the gall bladder are on the right-hand side of the body. Why is this so? Why doesn't the body have a mirrored or a symmetrical plan? We are studying the mechanisms behind the body's left-right asymmetry. Experiments using mouse embryos have achieved interesting results. In the course of body formation from a fertilized egg, tiny hairs of approximately one two hundredth of a millimeter in length grow on a specific area of the body surface. The movements of these hairs rotate the surrounding fluid, causing the flow from the right to the left-hand side of the body. When the flow is artificially reversed, there arises an embryo with a mirrored body plan. We are now studying what this flow actually does.



## A mechanism for selecting reproductive cells

Division of developmental genetics



Fly eggs. Cells colored green (left figures) and brown (right figures) later become reproductive cells.

Reproductive cells have one of the essential abilities of a living organism: the ability to give rise to the next generation of life. In the process of embryonic development, the fertilized egg continually divides and many cells appear. A tiny number of them are selected as reproductive cells, while the rest of the cells become somatic cells, forming the body. Using fruit flies (*Drosophila*), we are studying the mechanisms responsible for selecting these important reproductive cells. We have clarified that a gene called nanos and the mitochondria are both important in this process.



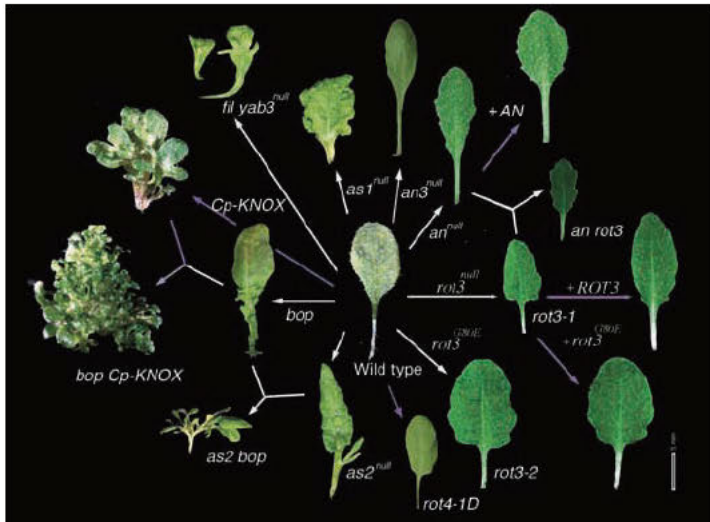
## Mechanisms forming the shape of the plant

Laboratory of Plant Organ Development



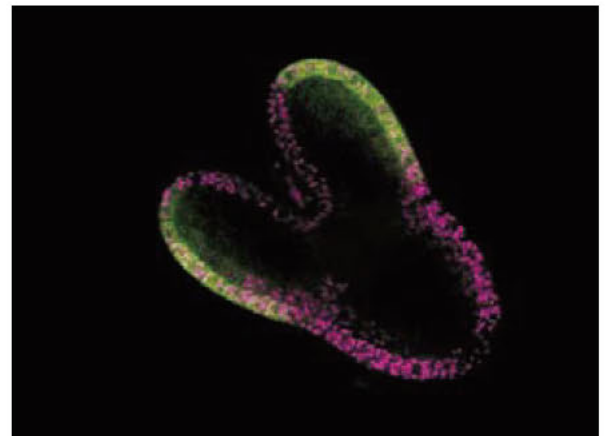
### Genes controlling the shape of a leaf

Division of plant developmental genetics



The effect of each gene on leaf shape

The appearance of a plant is greatly affected by the shape and the size of its leaves. A plant's flower is also considered to be a special accumulation of its leaves. Using a plant called the Arabidopsis, we are studying which genes control the shape and the size of the leaves and how they work. We have found that the length and the width of a leaf are independently controlled and we have succeeded in isolating some of the genes controlling each dimension. In a natural environment, there evolve various leaf shapes and these shapes vary greatly according to changes in the environment. We are studying how these phenomena are linked to the functions of the plant's genes.



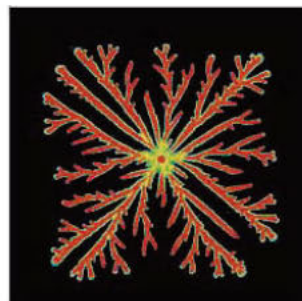
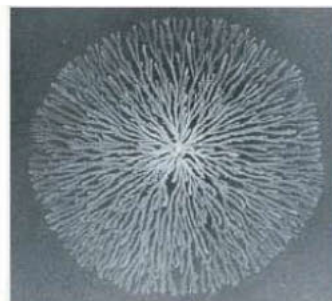
A plant embryo growing in the seed. The upper part with a Y shape becomes the cotyledon, while the bottom part becomes the embryonic stem and the root. Cells becoming the back surface of the cotyledon are made to show a yellow fluorescence. The purple dots are the chloroplasts.

Leaves, flowers and roots show impressive, symmetrical shapes and the tissues and cells within bear an ordered and beautiful arrangement. The organs of a plant are formed by a group of undifferentiated cells at the tip of the stem or the root. This process does not result in an ordered cell arrangement or a symmetrical shape without the minute control of cell divisions. The plant cells are believed to exchange information with each other, thus determining the timing and the direction of cell divisions. We are trying to understand the mechanisms of the informational exchange between cells working during the organogenesis of the plant.



### Analyzing biological phenomena using mathematics

Division of theoretical biology



Computer simulations of the formation of various biological patterns.

How does the information inscribed in the genome evolve into the highly regulated behavior of organisms found in morphogenesis during development or in flexible adaptation to the environment? We investigate this question through the integration of essential elements of biological information using computer calculations and mathematical models. The figures on the left show regular shapes found in the bacterial colonies and on the body surface of a fish. Using mathematical models, such ordered shapes can be reproduced (as shown on the right) and can be analyzed to reveal the underlying conditions.

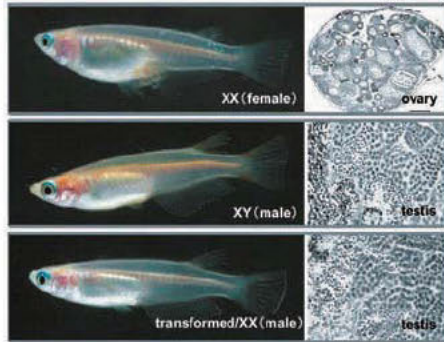
Living organisms inherit life from one generation to the next by passing on genetic information. The formation of two sexes, male and female, and thus the formation of the sperm and the egg, is indispensable to this process. We are studying the mechanisms determining sex, the mechanisms producing sperms and eggs, and the environmental factors capable of disturbing such mechanisms.



## The differentiation of male and female in fish

Division of reproductive biology

Like humans, the sex of a fish (male or female) is determined by the combination of sex chromosomes resulting from fertilization and, once determined, does not usually change for the entire life of the individual. Some tropical fish living in coral reefs, however, can change their sex several times during their lifetime. Our division studies the mechanisms of the original determination and the subsequent sex change using various types of fish. We have recently discovered a male determining gene (*DMY*) on the Y chromosome of the medaka fish. When a genetically female egg bearing two X chromosomes is transformed using the *DMY* gene, the embryo grows to become a male fish producing normal sperms capable of fertilization. The *DMY* gene is the second sex-determining gene found in the vertebrates.



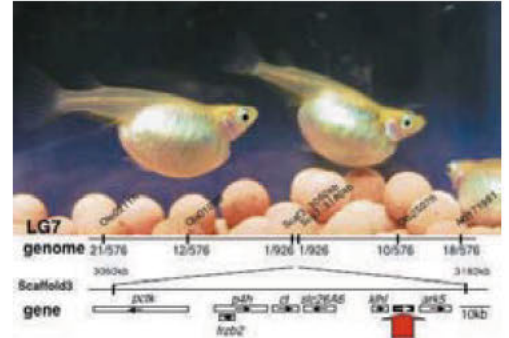
The transformed XX male fish after introducing the *DMY* gene (bottom). The body color is white like the female, while the shape of the tail fin is male-shaped and it has testes.



## To know the mechanisms of sex determination

Laboratory of molecular genetics for reproduction

The sex, male or female, of a human individual is determined at fertilization. However, it is possible to find organisms whose sex is determined by temperature, organisms whose sex changes in the middle of their life, or even hermaphrodite organisms bearing both ovaries and testes. This seems to indicate that what is important for living organisms is to form the ovaries and testes, each producing the eggs and sperms necessary for reproduction, according to environmental cues and that the mechanisms giving rise to males and females is of little importance. We are analyzing the genetic and cellular mechanisms of sex differentiation and sex transformation using medaka as model organisms and by visualizing specific gene products and cells. We have also isolated medaka mutants showing sex transformation and are analyzing the mechanisms.

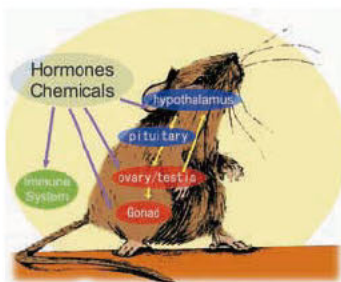


(Top) A medaka showing sex transformation, hermaphroditism. Though the fish is male, ovaries fill the expanded body (Bottom). The mutation on the gene indicated by a red arrow on the genome is known to cause the sex transformation.



## Sex hormones and environmental hormones

Division of molecular environmental endocrinology



Hormones and chemical substances affecting various organs.

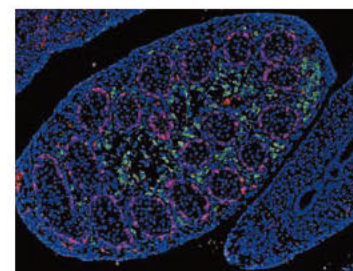
Various artificial chemical substances such as PCB, dioxins, agricultural chemicals, and the raw materials of the plastic industries are continually released into the environment. Among these substances, those showing an effect similar to sex hormones or those inhibiting the effects of sex hormones are known as the environmental hormones. Using mammals, reptiles, amphibians, and fish, our group studies the effect of intrinsic sex hormones and the environmental hormones on the development of eggs and on adults. We are trying to understand the interactions between

living organisms and the environment with a hope that the resulting knowledge will be useful in proposing how we can maintain a satisfactory environmental condition.



## Mechanisms determining the sex of an individual animal

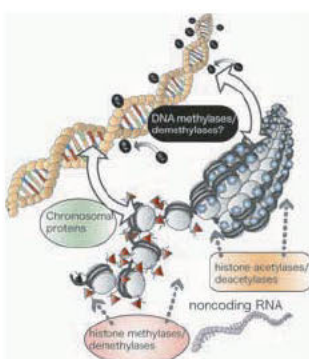
Division for sex differentiation



The process of the tissue construction of the testes of a mouse embryo

Gonads (testes and ovaries) are the most important organs in determining the sex of an animal. In most male mammals, the testes develop during the fetal period and start production of male hormones; subsequently, the construction of sex differences in other parts of the body begins. The photograph shows the testis

of a mouse fetus; the green cells are the Leydig cells producing male hormones. The rings in pink are seminiferous tubules within which are located primordial germ cells which will differentiate into sperms later. In the ovary of a female at the same stage, there are no such developed structures nor any specialized cells.



## The sex as the basis of social behaviors

Division of behavioral biology

Many of the social behaviors of mammals are sex-dependent. The difference between male and female genomes is located on the sex chromosome. Most of the genes related to the realization of sex differences are, in contrast, located on the autosomes, which are common between males and females. Thus, normal social behavior is formed by fixing and maintaining the positions of switches on the switchboard, i.e. the genome. Our laboratory utilizes the methylation of DNA as an indicator of such switches and studies the male-female specificity of regions of the brain important in determining behaviors in order to clarify the relationship between the brain condition and the rearing environment or between the brain and the internal hormonal environment.

Inactivation of the gene expression by DNA methylation



# The mechanisms of the formation and the function of the brain

The brain and the nervous system act as the control tower of an animal, enabling animal functions such as the regulation of the internal environment, the control of feeding behaviors, the sensation of external environment, memory and learning, and behaviors for escaping from enemies and communicating with friends. It is indispensable to investigate the formation and the function of the brain in order to elucidate the living mechanisms of animals.



## How the brain is formed and how it works

Division of molecular neurobiology



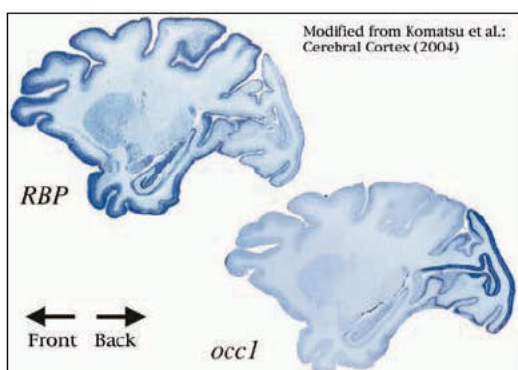
Dehydration of the body causes a rise in the sodium ion concentration of the body fluid. The animal becomes thirsty and drinks water, avoiding salty water. We have clarified the sodium sensing mechanisms in the brain that control such drinking behaviors.

The brain receives various types of information about the external world through sense organs such as the eyes and the ears and instructs the individual concerning the right behavior through the processing and the recognition of that information. The brain is also continually monitoring internal conditions such as blood pressure and blood sugar levels, thereby controlling feeding and excretion behaviors. These brain functions are possible only through the function of the correct and appropriate wiring of the nervous circuit formed during the embryonic development process. We are studying the mechanism of the formation of the visual system as an example of how the brain is formed and the mechanism controlling the homeostasis of the body fluid and the control mechanism of neurotransmission as examples of how the brain works.



## Learning brain, evolving brain

Division of brain biology



The genes each expressed in the association area (left) and the visual cortex (right) of the monkey brain.

There remain various mysteries within our brain. What is happening in the brain when we become skilled in a new sport or when we learn something new? How similar and how different are the brains of various animals, such as monkeys and mice? In order to answer these questions, we focused on the gene and are studying how individual cells building the brain are combined and how they work using the methods of molecular biology and histology. "To visualize the brain and to analyze its function using the gene" is what we are attempting to do, and we expect to get a glimpse of a new aspect of the brain and its functions that has heretofore eluded scientists.



## How the brain senses salt

Laboratory of neurophysiology



The sodium-sensing molecule existing in the brain (stained in blue).

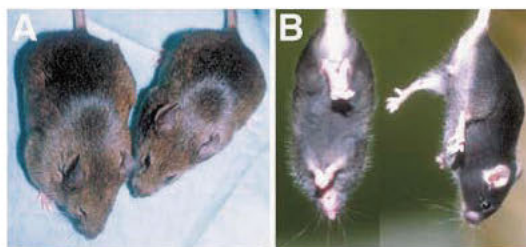
Salt (sodium ion) is an indispensable electrolyte for animals, yet its concentration in the body fluid must be kept constant and an excessive intake of salt is deleterious for an animal. An animal, therefore, instinctively avoids the intake of salt when the sodium concentration of the body fluid rises. Recent studies showed that the detection of the sodium concentration of the body fluid is done by the brain and that a specific sodium-sensing molecule is responsible.

Our laboratory studies the mechanism of sensing the sodium ion concentration in the brain using mice and cultured cells. This project is run in collaboration with the division of molecular neurobiology.



## To know the role of information transferred by the nerve cells

Laboratory of neurochemistry



(A) Mice showing hypertrophy of the muscles (B) Mice showing changes in the activity of the nervous system

In order to understand what information the nerve cells transfer and how they do it during the body activity of an organism, we study the neurotransmitter substances that transfer information between nerve cells and their receptor molecules. Dopamin, one of the neurotransmitter substances, is related to the control of body movement, the regulation of hormonal secretion, and the function of the mind. It is also believed to be important in the causal analysis and the remedy of human dysfunction of the nervous system. We utilize genetic manipulating techniques to produce model mice which bear changes in the mechanisms of information transfer through neurotransmitter substances so that we can observe the changes appearing in the cells, tissues and individuals in the course of the development into adults and study the role of the information transfer therein.



# The behavior of genetic information

Living organisms store a vast amount of genetic information compactly and transfer it from one cell to another and from a parent to children. The information is accessed when necessary and used to orchestrate life activities. The genetic information is, so to speak, a master plan of life. The study of its behavior is crucial in understanding the essence of life.



## Rewriting of genetic information and the movement of genes

Division of molecular genetics



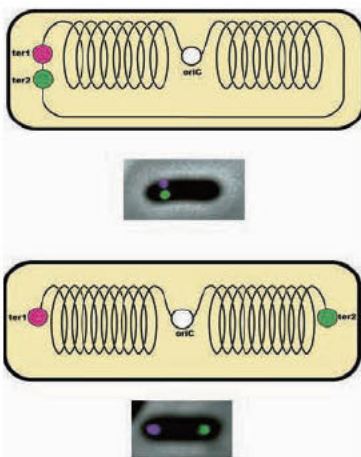
Flowers of the morning glory and leaves of the rice with dappled or spotted patterns.

The genetic information of living organisms is usually copied exactly at the time of a cell division and distributed to new cells. There are, however, cases where the genetic information is rewritten in some cells or in some individuals, which results in a modified activity for some genes. Dappled or spotted patterns seen in some plants, for example, are caused when the genes related to the coloring are repeatedly rewritten. We are studying how this rewriting of genes affects the activity of the genes using familiar plants such as the morning glory and rice. We have also developed a useful technology which enables an artificial exchange of part of the DNA on the rice chromosome.



## Catching a dynamic face of the genome

Division of genome dynamics



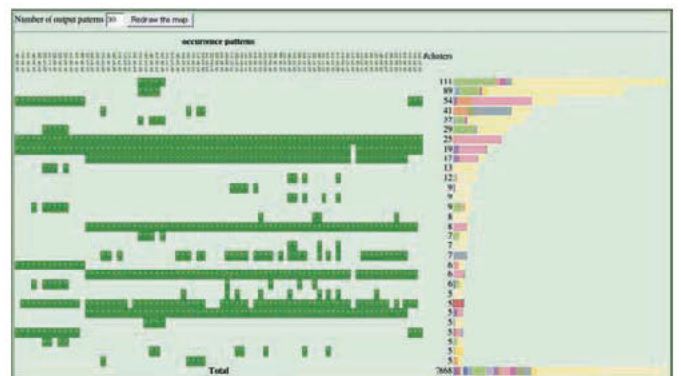
Modification of *E. coli* with a circular genome (top) into one with a linear genome (bottom).

The genetic information - the life plan - of living organisms is stored in the organism's long, string-like substance known as DNA. The entire mass of genetic information tidily stored in the cellular nucleus is called the genome. The total length of the DNA of the human genome is as long as two meters. The genome is not stored quietly; rather it is ceaselessly dynamic. To read the plan, the DNA chain is incessantly unwound, cut and spliced within the nucleus. The genome is exactly doubled when the cell divides. We are studying the stability and the evolution of the genome in relation to diseases and life span using yeast and *E. coli*. An artificial modification of the genome is also one of our research targets.



## The diversity of microbes explored using the genome

Laboratory of genome informatics



A chart showing the result of an extensive analysis of how corresponding genes are included in the genome of various microbial species.

Thanks to the decoding of the genome which has recently been done for various living organisms, scientists have been able to better understand the evolutionary processes of different species by comparing the decoded genomes. The genome analysis is meanwhile disclosing the true diversity of microbes, which exist in limitless numbers in every part of the earth, including in our bodies and our surroundings. Our laboratory is developing a method to systematically compare the vast amount of diversified genome information of the microbial genomes and explore the diversity of the genome and the motive force which brought about this diversity using informatics.

# The evolution of living organisms

Organisms always evolve. Evolution can not be interrupted, and it results in the emergence of flowers and fish of various shapes and colors, mosses with an unremarkable appearance, human beings, and countless other kinds of organisms. Why, how and when do organisms evolve? We study the mechanisms of evolution with the hope of discovering clues to our own past and future.



## What caused evolution?

Division of evolutionary biology



A key organism in the study of evolution, *Physcomitrella*. (The entire genome was decoded by NIBB and others)

Living things evolve. The ancestor of humanity was a creature resembling mold that existed about one billion years ago. What changes, then, caused the evolution? Changes in the genes. What kind of changes on which genes, then, can cause evolution? In order to answer this question, we must ask others, such as: How did plant cells and animal cells evolve differently? What changes were necessary for the evolution from a unicellular organism to a multicellular one? How did the stems, leaves and flowers of plants evolve? How did flowers of various shapes evolve? What caused species differentiation in plants? Why is it that plants can easily regenerate? These are some of the questions we ask in our laboratory. Through the analyses of heretofore insolvable questions of evolutionary biology with molecular methodologies, we are attempting to establish a new paradigm for understanding evolution.



## Mechanisms creating the diversity of the shape of butterfly wings

Laboratory of morphodiversity



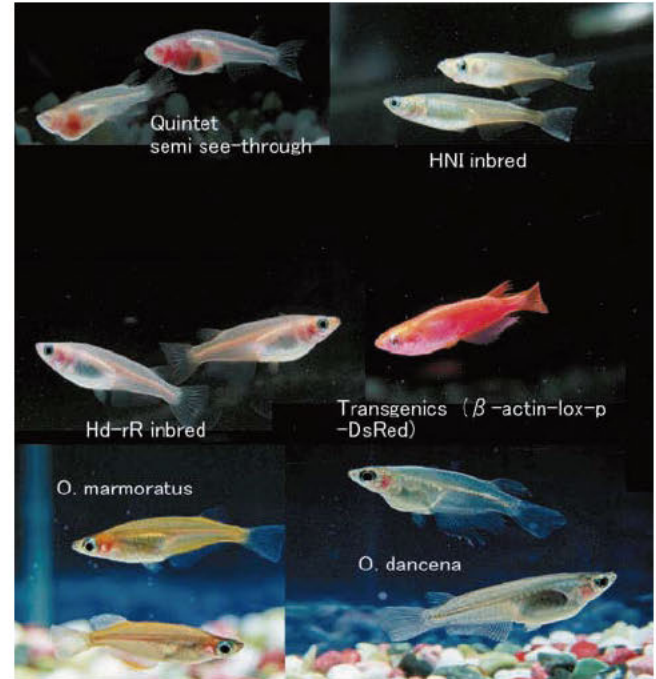
A cross section of the pupal wing. The round shaped part on the left is where the cell deaths are occurring.

The wings of butterflies and moths have a different shape unique to each species. The wings just after puparization, however, have a smooth outline common to most species. A contour line appears with the shape of the adult wing just after this stage and the deaths of cells outside of this line bring about the shape of the adult wings. A similar kind of 'programmed cell death' is also seen in the process of finger formation in human embryos. We study the mechanisms determining the position and the shape of the contour line as an example of the mechanisms enabling the diversity seen in butterflies.



## Comparative genome analyses and development of bioresources using fish

Laboratory of bioresource



Various medaka strains supplied by our laboratory.

We have studied the evolution of the genome through comparative genome analyses and exploited a bioresource using medaka fish. We decided the entire sequence of the medaka genome and analysed many genes, clarifying the whole genome structure of medaka. In order to enhance the use of medaka as a research model organism, we are exploiting the genome resource of medaka-related species, anticipating its use in an extensive comparison of medaka gene transcripts and in an evolutionary study comparing related species. NIBB was selected as the resource center for medaka in the National BioResource Project in 2007, and the supply of various genetic resource is managed by us.

# Research support facilities

To efficiently conduct research activities, the NIBB has its own facilities and shares research facilities with the National Institute for Physiological Sciences and the Institute for Molecular Science in Okazaki. There are facilities for the controlled cultivation, growth, and breeding of living samples for experiments and research activities, mid/large-scale equipment for measurement and data analysis, radioisotope (RI) facilities, and advanced analysis systems.

## An artificial rainbow to study biological phenomena

Research Support Facilities (The Large Spectrograph Laboratory)

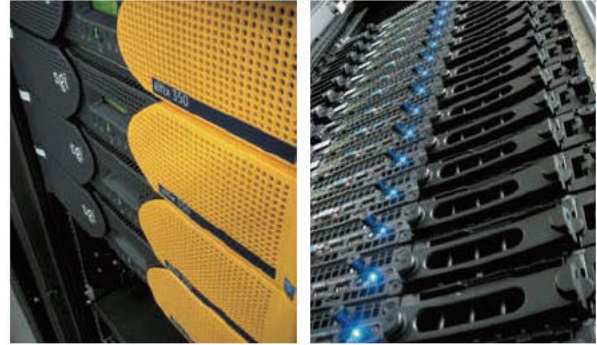


The interior of the large spectrograph laboratory

The Earth had been heavily exposed to ultraviolet radiation since life began. Primitive life forms developed ultraviolet sensitivity that enabled them to avoid exposure to ultraviolet radiation; it is supposed that this ultraviolet sensitivity evolved into the light sensitivities of contemporary organisms. For example, plants sense blue, red, and dark red light while animals sense a range of colors with a peak in green. An evolutionary connection between microbes, plants and animals is suggested by the similarity of their existing light-sensing abilities. The Large Spectrograph Laboratory makes the world's largest artificial rainbows to study light sensitivity and investigate the mechanisms of the beneficial and harmful effects of light.

## Computer-based genome exploration

Research Support Facilities (Computer Laboratory)



NIBB Biological Information Analysis System

The Computer Laboratory supports research activities using high-speed large-capacity computers. Building gene and protein sequence databases, it supports research mainly in terms of sequence analysis, expression data analysis, and imaging analysis. It has also developed analysis programs and programs for database publication via the Web to distribute the results of gene analysis for model organisms worldwide. In addition to computer-based analysis, the Computer Laboratory supports the NIBB's information-sharing infrastructure through the maintenance of an ultra high-speed network system, computer/network-related consultation, and introduction of new services.

The NIBB's research support facilities further include the Plant Culture Laboratory for the controlled growth of plants for experiments and the Tissue and Cell Culture Laboratory for the cultivation of animal tissues and cells.

## Development and analysis of transgenic organisms

Center for Transgenic Animals and Plants



Model organisms used in biological research

To understand the mechanisms of living organisms, studies require focusing on each gene in the design of life (genome). The use of model animals, such as mice, Medaka

(*Oryzias latipes*), zebrafish, and chickens, makes it possible to produce genetically controlled (transgenic) organisms with markers placed by genetic and cell engineering technology. Such marking allows detailed studies of genes and cell functions. The model animals mature in a short period of time; therefore, changes in cells, organs, and individuals can be totally and efficiently observed. The Center for Transgenic Animals and Plants has equipment, facilities, and staff to maintain such organisms safely, efficiently, and appropriately.

## Studying living mechanisms with radioisotopes

Center for Radioisotope Facilities



Radioisotope experiment and control (inset)

Biological studies for the analysis of genetic functions and protein characteristics often require locating substances in living organisms. The Center for Radioisotope Facilities is a facility that uses materials emitting radiation, or radioisotopes, to investigate the material behavior; thus, the Center strictly controls radioisotopes to ensure safe handling.

## Equipment that makes advanced research possible

Center for Analytical Instruments

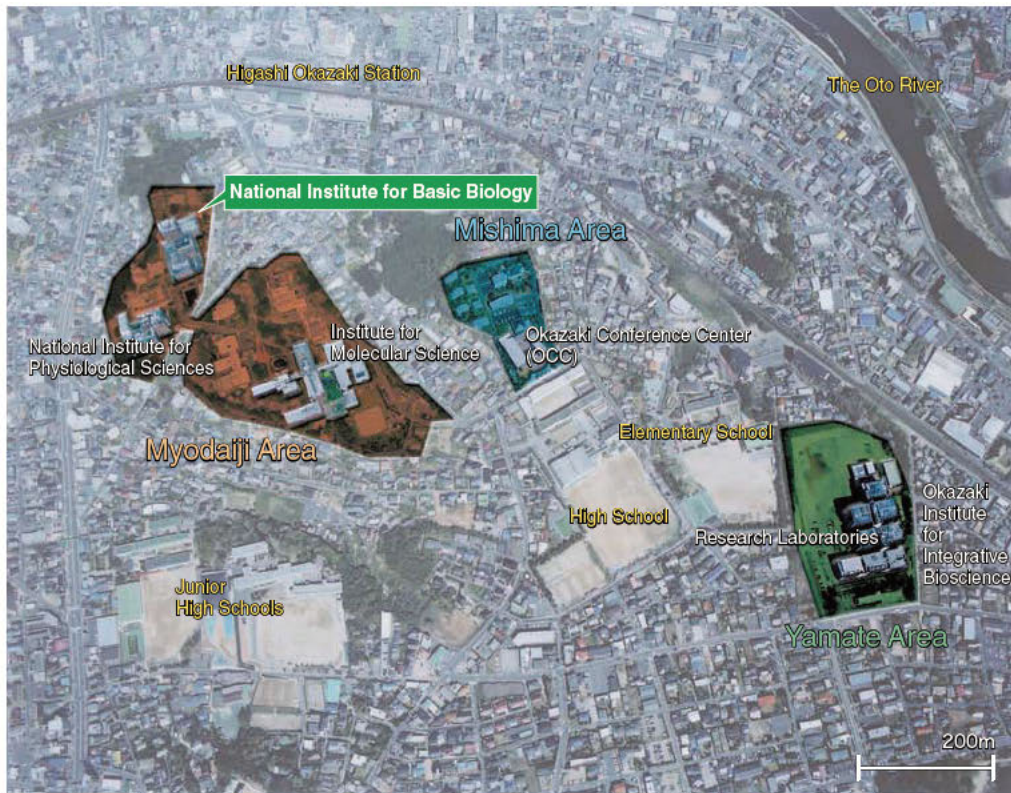


Staff operating analytical instruments

The Center for Analytical Instruments provides the analytical instruments that are required to conduct research in biology and physiology. About 60 different types of equipment and 100 individual units are available. They are used in a wide range of research: protein and genetic analysis, separation, purification, identification, and structural analysis of physiologically active substances, and imaging analysis of living organisms. The analytical instruments are methodologically categorized as below, and the staff is responsible for the maintenance and management of the instruments and conducting requested measurements.

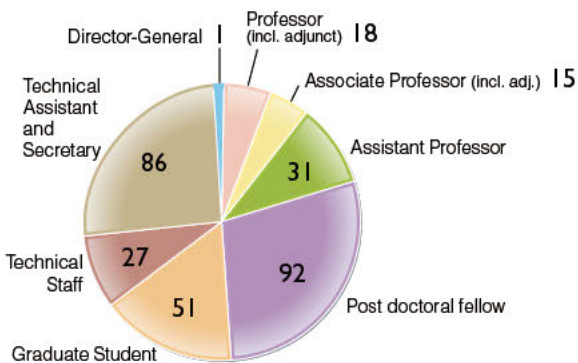
1. Protein/genetic analysis systems
2. Analytical ultracentrifuges
3. Physicochemical analysis systems
4. Spectrophotometers
5. Microscopes and imaging analyzers

## Location of the NIBB



Laboratories of NIBB are located both in Myodaiji and Yamate areas.

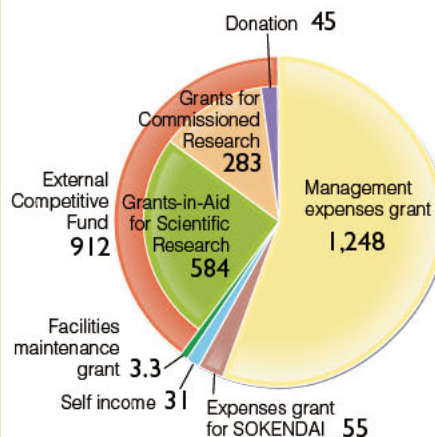
## Members in the NIBB (as of 1st April 2007)



## Financial configuration of the NIBB

(settled for April 2006 - March 2007)

numbers are in million yen



The NIBB acquires numerous competitive funds in an effort made by individual researchers, including Grants-in-Aid for Scientific Research and Grants for Commissioned Research, in addition to national subsidies (Management Expenses Grants and Expenses Grants for SOKENDAI).

## Open house and public relations

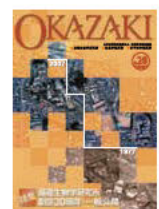
### Open house of the NIBB and other Institutes

Each year, one of the three Institutes in Okazaki opens to the public in the fall. They welcome the public with a variety of events, such as introductions of research contents, exhibitions of research materials and equipment, lectures, etc. The next NIBB open house will be in 2010.

### Public relations magazine "OKAZAKI"

A public relations magazine is published quarterly to report recent research activities and events hosted by the three Institutes in Okazaki. This publication is currently only available in Japanese.

You can download the issues in PDF format from the NIBB homepage (<http://www.nibb.ac.jp/okazaki/>).



### NINS symposia

The five Institutes of NINS semiannually host symposia in the spring and the fall in Tokyo to introduce the latest achievements of research in natural science. Please visit the homepage of each Institute for details.

## Access



### From Tokyo

Take Tokaido Shinkansen and get off at Toyohashi, change to the Meitetsu Line and get off at Higashi Okazaki Station (approximately 20 minutes from Toyohashi).

### From Osaka

Take Tokaido Shinkansen and get off at Nagoya, change to the Meitetsu Line and get off at Higashi Okazaki Station (approximately 30 minutes from Nagoya).

### From Central Japan International Airport

Get on the Meitetsu bus bound for JR Okazaki Station and get off at Higashi Okazaki Station (approximately one hour from the airport). Alternatively, take the Meitetsu Line for Nagoya and change at Jingu-mae Station to a Toyohashi-bound train and get off at Higashi Okazaki Station (approximately 70 minutes from the airport).



### From Higashi Okazaki Station to Each Area

Turn left (south) at the ticket barrier and exit the station. The Institute is a 7-minute walk up the hill (Myodaiji-area) or 20-minute walk (Yamate-area).

### By car

Take the Tomei Expressway to the Okazaki Exit. Turn left at the City Office S.E. signal (approximately 10 minutes from the Exit).



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issued in November 2007

edited by the Strategic Planning Department, NIBB



大学共同利用機関法人  
自然科学研究機構  
基礎生物学研究所

## 外部点検評価報告書

### 第二部

発行日 平成20年5月  
発行者 大学共同利用機関法人 自然科学研究機構  
基礎生物学研究所点検評価委員会  
〒444-8585  
岡崎市明大寺町字西郷中3-8