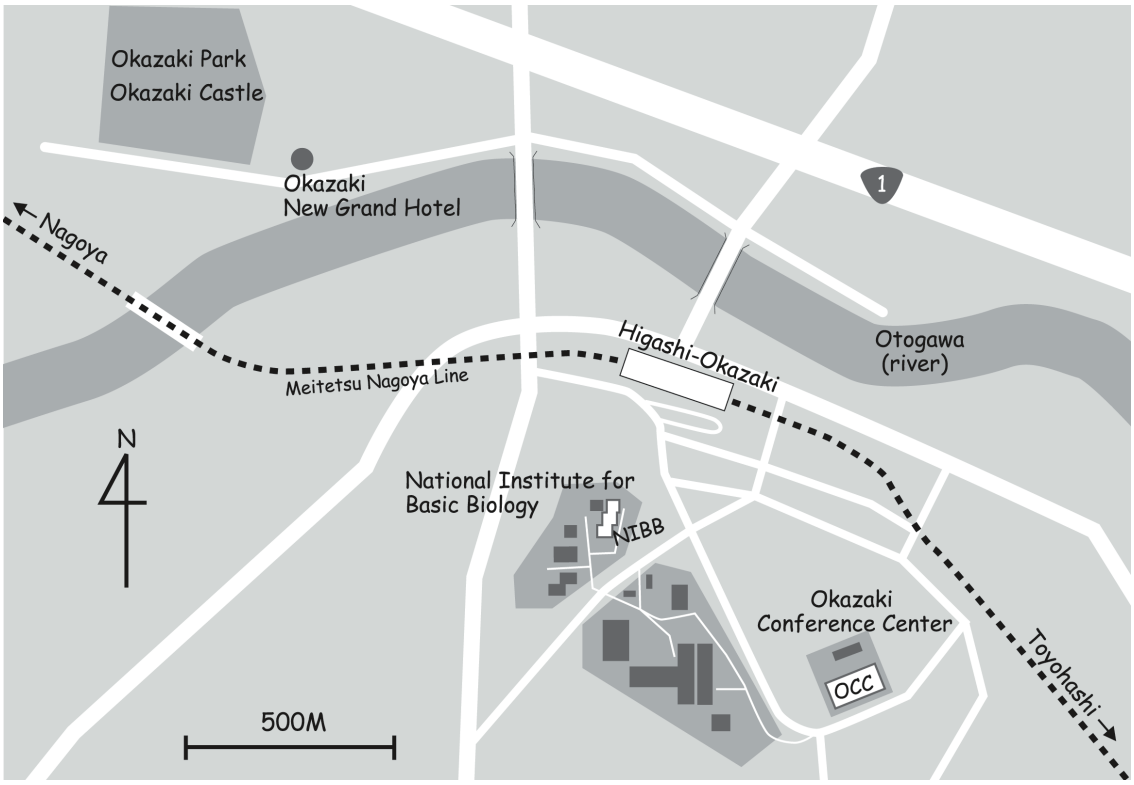
The 57th NIBB Conference

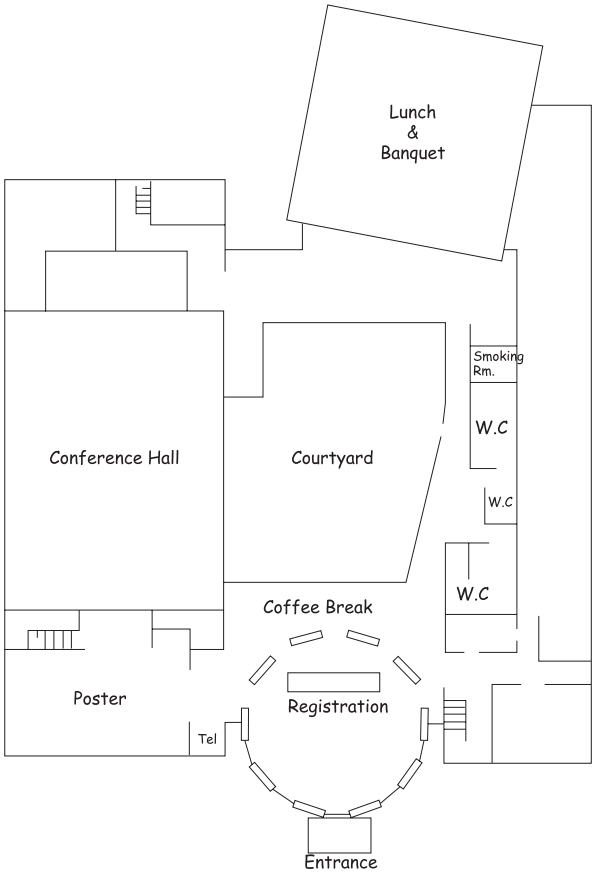
The Dynamic Genome

October 14th (Thu) - 16th (Sat), 2010
Okazaki Conference Center
Aichi, Japan

Okazaki MAP



Okazaki Conference Center (OCC)



Program

October 14 (Thu) at Okazaki Conference Center

09:00-09:30 Registration at the Conference Center

Chair: Takashi Horiuchi

09:30-10:30 Keynote Lecture
"Genomic Stability under Oxidative Stress"
Mutsuo Sekiguchi (Fukuoka Dental College, Japan)

Session 1 Bacterial Genome and Genome Rearrangement

Chair: Katsuki Johzuka

10:30-11:15 **"The boundaries of the Ter Domain in Bacterial Nucleoid"**
Hironori Niki (NIG, Japan)

11:15-11:35 *Coffee Break*

11:35-12:20 **"Molecular Regulation of Recombinase-Mediated Strand Exchange Reactions, A Central Reaction of Homologous Recombination"**
Hiroshi Iwasaki (Tokyo inst. of Tech, Japan)

12:20-14:30 *Lunch & Poster*

Chair: Hiroshi Iwasaki

14:30-15:15 **"Programmed Genome Rearrangements in Tetrahymena: Links to RNAi and Transposons"**
Meng-Chao Yao (IMB, Taiwan)

Session 2 Genome Amplification I

Chair: Meng-Chao Yao

15:15-16:00 **"Regulation of Ribosomal RNA Gene Copy Number and Its Role in Modulating Genome Integrity in Yeast"**
Takehiko Kobayashi (NIG, Japan)

16:00-16:20 *Coffee Break*

16:20-17:05 **"Gene Amplification in Chromosomal and Extrachromosomal Context"**
Noriaki Shimizu (Hiroshima Univ., Japan)

October 15 (Fri) at Okazaki Conference Center

Session 2 Genome Amplification II

Chair: Takehiko Kobayashi

09:00-09:45 **"A Comprehensive Model of Gene Amplification Centering on Double Rolling-Circle Replication (DRCR)"**
Taka-aki Watanabe (NIBB, Japan)

09:45-10:30 **"Double Rolling Circle Replication (DRCR) is Recombinogenic"**
Takashi Horiuchi (NIBB, Japan)

10:30-10:50 *Coffee Break*

Session 3 Condensin and Cohesin

Chair: Tatsuya Hirano

10:50-11:35 **"Hdac8 is a Cohesin Deacetylase that is Required for Recycling of Cohesion"**
Katsuhiko Shirahige (Univ. Tokyo, Japan)

11:35-12:20 **"Association of Condensin with The Chromosome: Mechanism and Its Effect on Transcriptional Silencing"**
Katsuki Johzuka (NIBB, Japan)

12:20-13:30 *Lunch & Poster*

Chair: Katsuhiko Shirahige

13:30-14:15 **"Mitotic Chromosome Condensation by Condensin-Dependent Intrachromosomal DNA Interactions"**
Sebastian Heeger (LRI, UK)

14:15-15:00 **"A Tale of Two Condensins:**
Differential Regulation and Concerted Actions"
Tatsuya Hirano (RIKEN, Japan)

15:00-15:20 *Coffee Break*

Session 4 mtDNA and cpDNA Dynamics

Chair: Ľubomír Tomáška

15:20-16:05 **"Bases of Break-Induced Replication in Mitochondrial Genome Dynamics"**
Takehiko Shibata (RIKEN, Japan)

16:05-16:50 **"Chromosomal DNA Molecules in Mitochondria and Chloroplasts"**
Arnold J. Bendich (Univ. Washington, USA)

16:50-17:10 *Coffee Break*

Chair: Takehiko Shibata

17:10-17:55 **"Tinkering with Yeast Telomeres"**
Ľubomír Tomáška (Comenius Univ., Slovakia)

18:30 *Reception*

October 16 (Sat) at Okazaki Conference Center

Session 5 Mutation and Gene Evolution

Chair: Joakim Näsvall

09:00-09:45 **"Spontaneous Mutagenesis Associated with Nucleeotide Excision Repair in *Escherichia coli*."**
Hisaji Maki (NAIST, Japan)

09:45-10:30 **"Genome Integrity Preserved by Induction of Apoptosis from DNA Damage"**
Masumi Hidaka (Fukuoka Dental College, Japan)

10:30-10:50 *Coffee Break*

Chair: Hisaji Maki

10:50-11:35 **"DNA Methylation and Heterochromatin Formation in Neurospora Crassa"**
Michael Rountree (Univ., Oregon USA)

11:35-12:20 **"An Experimental Model System for the Evolution of New Genes under Continuous Selection"**
Joakim Näsvall (Uppsala Univ., Sweden)

12:20-12:30 Concluding Remarks
Meng-Chao Yao (IMB, Taiwan)

12:30-13:30 *Lunch*

POSTER PRESENTATION

P01
"Sequential Rearrangement and Eviction of Nucleosomes Allow Interleukin-2 Transcription Following T Cell Activation"
Satoru Ishihara (Fujita Health Univ., Japan)

P02
"Overproduction of *Escherichia coli* DNA Polymerase DinB (Pol IV) Inhibits Replication Fork Progression and is Lethal"
Masahiro Akiyama (NAIST, Japan)

P03
"DDK Phosphorylates Checkpoint Clamp Rad9 and Promotes Its Release from Damaged Chromatin"
Kanji Furuya (NIG, Japan)

P04
"Double Rolling-Circle Replication (DRCR) is Recombinogenic"
Haruko Okamoto (NIBB, Japan)

P05
"APC/C is Involved in Nuclear Envelope Dynamics during Mitosis in Schizosaccharomyces japonicus"
Keita Aoki (NIG, Japan)

October 14th, 2010

Genomic Stability under Oxidative Stress

Mutsuo Sekiguchi

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Nucleic acids as well as their precursor nucleotides may be damaged by internal and environmental agents, including various chemicals and radiations. Particularly, reactive oxygen species, such as superoxide and hydroxyl radicals, which are produced through a normal cellular metabolism, are all threats to genetic materials. These radicals attack nucleic acids to generate various modified bases, among which 7,8-dihydro-8-oxoguanine is the most abundant and plays a critical role in mutagenesis and also gene expression. Organisms are equipped with special mechanisms to repair such lesions and eliminate damaged molecules from the cell. We herein discuss the problems related to (1) the molecular mechanisms for sanitization of the DNA precursor pool, (2) error avoidance mechanisms for gene expression, and (3) the roles of DNA repair and apoptosis in the prevension of cancer.

References:
1. M. Skeiguchi (2006) *Proc. Japan Acad.*, Ser. B, **82**, 278-296.
2. M. Sekiguchi (2006) *DNA Repair*, **5**, 750-758.

The boundaries of the Ter Domain in Bacterial Nucleoid

Hironori Niki

National Institute of Genetics, Mishima 411- 8540, Japan

The whole bacterial chromosome is compacted to form a nucleoid. The nucleoid includes two densely folded domains including replication origin and terminus, respectively. Each domain, the Ori domain and the Ter domain independently located at specific sub-cellular positions throughout the cell division cycle. It is seemed that these domaines play important roles in chromosome replication and segregation coordinately.

The Ter domain contributes for not only termination of replication forks, but also resolution of a chromosomal dimer. There is a site specific recombination site, *dif* at the middle of the Ter domain. Furthermore, recognition sequences of a DNA translocator FtsK are dispersed at the entire chromosome, and their directions are antiparallel along the *oriC-dif* axis. Therefore, FtsK can move *dif* sites of the dimer into the division plan and facilitate resolution into monomers. Recently, it has been known that the Ter domain is organized by an assembly of MatP, which is a specific DNA binding protein. The binding sites, namely matS, are distributed only within the Ter domain. To know contribution of configuration of the domains to chromosome integrity, we have systematically constructed inversion mutants of the *E. coli* chromosome along the *oriC-dif* axis, in which a part of the Ter domain or the entire domain is located at the side of the Ori domain. These inversions caused mutant cells to produce anucleate cells with high frequency. We found that the deficiency of chromosome segregation was not merely depend on gene organization or aberrant termination of replication, but positions of the inversion in the Ter domain markedly affected on deficiency of chromosome segregation. Then, though the inversion mutant that the whole of the Ter domain was located at the side of the Ori domain didn't cause aberrant chromosome segregation, the inversion mutant that the half of the Ter domain did there showed highest frequency of producing anucleate cells. Aberrant chromosome segregation could be suppressed by *matP* or *ftsK*, respectively. These results suggest that a split in the Ter domain is responsible for defects in segregation. Thus, the domain organization on the chromosome is contribute for accurate chromosome segregation. We further carried out the assay of anucleate cells by the large chromosomal inversion, and then we have fond boundaries of the Ter domain at which frequency of producing anucleate cells was remarkably changed. The boundaries were symmetrical located along the *oriC-dif* axis. About 500 kb of the chromosomal segment between the boundaries might be a core in the Ter domain. We would like to discuss on function of the Ter domain in bacteria and how the boundaries are provided in nuceloids

Molecular Regulation of Recombinase-Mediated Strand Exchange Reactions, A Central Reaction of Homologous Recombination

Hiroshi Iwasaki

Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama 226-8501, Japan

In general, a recombinase binds to single-stranded DNA (ssDNA), leading to a formation of a helical presynaptic filament that promotes homology search and homologous pairing of a complementary DNA strand. This reaction is a fundamental reaction of homologous recombination and the elementary step of it is called as “DNA strand exchange”. We have established an in vitro reconstitution of the central step of DNA stand exchange, which consists of purified proteins, including Rad51 recombinase (which is also called as Rhp51), the two mediators (Rad22 and the Swi5-Sfr1 protein complex) and replication protein A (RPA) from fission yeast and model substrate DNAs. Our genetic and biochemical studies have demonstrated that the Swi5-Sfr1 protein complex acts as an activator and stabilizer of Rad51 recombinase. Rad22 (fission yeast Rad52 homologue) alone cannot activate the Rad51 presynaptic filament, but rather it seems to work as a recruiter to target Rad51 onto RPA-bound ssDNA regions (1, 2, 3). In addition, we have demonstrated in vitro formation of Holliday junction, which is a common DNA intermediate of homologous recombination (4). I will discuss about the molecular mechanisms of these strand-exchange-based processes in this conference.

References:
1. Kurokawa et al., (2008) ***PLoS Biol*** 6, e88.
2. Haruta et al., (2006) ***Nature Struct. Mol. Biol.*** 13. 823-730.
3. Akamatsu et al., (2003) ***Proc. Natl. Acad. Sci. USA.*** 100, 15770-15775.
4. Murayama et al., (2008) ***Nature*** 451, 1018-1021.

Programmed Genome Rearrangements in Tetrahymena: Links to RNAi and Transposons

Chao-Yin Cheng, Julan Chao and Meng-Chao Yao

Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan

Tetrahymena, like other ciliated protozoa, is a single cell eukaryote that contains two types of nuclei: the somatic macronucleus and germline micronucleus. Ciliates are probably the simplest live forms with soma and germ differentiation. This nuclear dualism allows the separation of the two basic genetic functions into two compartments: gene expression in the macronucleus and gene transmission through the micronucleus. When the macronucleus is formed from derivatives of the micronucleus during the sexual process of conjugation, large scaled genome rearrangements occur. One of the rearrangement processes is the deletion of thousands of specific DNA segments that comprise ~15% of the genome and include all transposons.

This deletion process is now known to share mechanistic steps with the RNA interference process. The DNA sequences to be deleted are transcribed into dsRNA, which are processed into siRNA that mark chromatin with similar sequences and prompt the formation of heterochromatin-like structures. This is similar to transcriptional gene-silencing by RNAi in other organisms. However, in ciliates the process takes an extra step – these DNA sequences are not just silenced, they are selectively deleted. Recently we found that a transposase gene is domesticated from a piggyBac transposon to play a key role in this final step. This transposase, TPB2, is selectively expressed just prior to DNA deletion and is localized to heterochromatin. Genetic knock down studies suggested that it is require for the DNA deletion process. *In vitro*, it cuts DNA to generate ends similar to those generated *in vivo*. We are dissecting TPB2 to determine the domain responsible for heterochromatin-targeting. It is interesting that a transposase is captured through evolution to participate in the deletion of all transposons in Tetrahymena, thus providing a defense system against invading genetic elements. Interestingly, the deletion process can also occur to an *E. coli* neomycin-resistant gene when inserted into the genome, which further support the idea of genome defense. It also provides a tool for determining the mechanism through which these sequences are recognized for deletion.

Regulation of Ribosomal RNA Gene Copy Number and Its Role in Modulating Genome Integrity in Yeast

Takehiko Kobayashi

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The genes encoding ribosomal RNA are the most abundant genes in the eukaryotic genome. They are encoded in tandem repetitive clusters, in some cases totaling hundreds of copies. Due to their repetitive structure and highly active transcription, the ribosomal RNA gene repeats are some of the most fragile sites in the chromosome (1). A unique gene amplification system compensates for loss of copies, thus maintaining copy number, albeit with some fluctuations. These unusual natures of ribosomal RNA gene repeats affect cellular functions such as senescence (2). Moreover, we recently found that the repeat number determines sensitivity of the cell to DNA damage (3).

In my talk, I would like to introduce a new aspect of the ribosomal RNA gene repeat as a center of maintenance of genome integrity and discuss its contribution to evolution (4).

References:
1. Kobayashi, T. (2006) *Genes Genet Syst* 81, 155-161.
2. Ganley, A.R.D., Ide, S., Saka, K., Kobayashi, T. (2009) *Mol Cell* 35, 683-693.
3. Ide, S., Miyazaki, T., Maki, H., Kobayashi, T. (2010) *Science* 327, 693–696.
4. Kobayashi, T. (2008) *BioEssay* 30, 267-272.

Gene Amplification in Chromosomal and Extrachromosomal Context

Noriaki Shimizu

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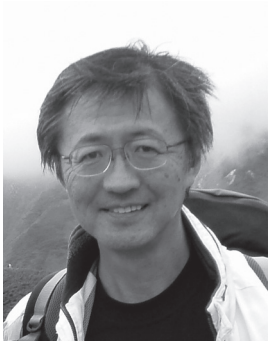

Amplification of oncogenes or drug resistant genes plays a pivotal role in malignant transformation of mammalian cells. Amplified genes localize to the extrachromosomal double minutes (DMs) or the chromosomal homogeneously staining region (HSR). DMs are acentric and atelomeric chromatin composed of circular DNA. We previously found that the elimination of DMs from cancer cells may result in reversion of tumor cell phenotype. The elimination of DMs was linked to their novel intracellular behavior during the cell cycle progression. Namely, the acentric DMs were segregated during the mitosis to the daughter nuclei by sticking to the chromosomes. The normally segregated DMs locate at the nuclear periphery during the G1 and they move to the interior during the early S phase while DMs themselves were replicated. At that time, presence of low concentration of replication inhibitor (HU) resulted in the aggregation of DMs, detachment from the chromosome at the mitosis, and generation of the micronuclei composed of the aggregated DMs. The aggregation of DMs may be caused by the repair-defect in the extrachromosomal context. The DMs in the micronuclei were actively transcribed depending on the presence of the nuclear lamina around the micronuclei, thus it profoundly influences the fate of tumor cells. Such micronuclei may be extruded from the cells by the cytoplasmic membrane blebbing, which was activated by various stimuli.

On the other hand, we found that the plasmid bearing a mammalian replication initiation region (IR) and a nuclear matrix attachment region (MAR) initiate gene amplification quite efficiently in mammalian cells, and it generates DMs and/or HSR. The IR/MAR gene amplification methodology was used for the basic study of chromosome/nucleus, or it was applied to the protein production. Especially, it provided a novel and an efficient system for the analysis on how gene amplification progress in tumor cells. Namely, the IR/MAR-bearing plasmid was initially multimerized to a large circle of plasmid tandem repeat. If the DNA breaks in such extrachromosomal molecule, it will be eliminated from the cells, as above. Alternatively, the broken plasmid repeat may be recombined with the broken end of the chromosome arm, and the chromosomal repeat may be lengthened to generate the HSR. The inverted repeat was frequently detected only in the HSR, suggesting the formation of dicentric chromatid by sister chromatid fusion in the chromosomal context. Consistent with that, we were able to detect anaphase chromatin bridges connected by the plasmid repeat, which were severed in the middle during mitosis. *De novo* HSR generation was observed in live cells, and each HSR was lengthened more rapidly than expected from the classical breakage/fusion/bridge model. We found massive DNA synthesis at the broken anaphase bridge during the G1 to S phase, which could explain the rapid lengthening of the HSR. Taken together, we provide a model on how gene amplification progress differentially in chromosomal and extrachromosomal context.

References (Recent Review Articles):
1. N. Shimizu, (2009) Cytogenetic and Genome Research. 124, 312–326.
2. N. Shimizu, (2010) Kagaku to Seibutu (Japanese), 48, 395-401.
3. N. Shimizu, (2010) Mutagenesis, in press.

October 15th, 2010

A Comprehensive Model of Gene Amplification Centering on Double Rolling-Circle Replication (DRCR)

Taka-aki Watanabe[1] and Takashi Horiuchi[1,2]

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Gene amplification is involved in various biological phenomena, such as drug-resistance, malignant progression, and gene evolution. However, there has not been much progress for over 15 years on the mechanism responsible for oncogene-type amplification. Previous approaches to understand these mechanisms were based on the structural analysis of complex end products. Very few model systems for the amplification are available that allow chromosomal engineering, as is possible in yeast. In response, we are taking a different approach; inducing gene amplification with specifically designed systems and analyzing them. Here we present a pivotal structure that can induce oncogene-type amplification, and propose a comprehensive model of gene amplification in mammalian cells. This model is centering on a rapid amplification mode, namely double rolling-circle replication (DRCR), and can explain the formation of amplification products and the features of amplification seen in mammalian cells.

Reference:
1. T. Watanabe and T. Horiuchi, (2005) EMBO J. 24, 190-198.

Double Rolling Circle Replication (DRCR) is Recombinogenic

Haruko Okamoto[1], Taka-aki Watanabe[1,3], Takashi Horiuchi[1,2,3]

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Homologous recombination frequency is generally much lower than that of site-specific recombination. However, there are several exceptions; totally-unconstrained homologous recombination occurs during DNA replication of chloroplast (cp) DNA or herpes simplex virus (HSV-1) DNA, in which DNA sequences flanked by inverted repeats undergo highly frequent inversion, suggesting hyper-recombinational events. However, the mechanism responsible for the events remains unknown. We previously observed the highly frequent inversion in a designed amplification system based on double rolling circle replication (DRCR). Here we demonstrate that DRCR is closely related to hyper-recombinational events, utilizing yeast 2-micron plasmid and the amplification system. Inverted repeats or direct repeats inserted into these systems frequently caused inversion or deletion/duplication in a DRCR-dependent manner, respectively. These results suggest that DRCR is involved in the replication of cpDNA and HSV genome and chromosome rearrangement associated with gene amplification. We propose a model in which DRCR dramatically stimulates homologous recombination.

Hdac8 is a Cohesin Deacetylase that is required for Recycling of Cohesion

Katsuhiko Shirahige[1], Masashige Bando[1] and Toru Hirota[2]

[1] Laboratory of Genome Structure and Function Research Center for Epigenetic Disease, Institute of Molecular and Cellular Biosciences, The University of Tokyo, Tokyo 113-0032, Japan; [2] Japanese Foundation For Cancer Research, Tokyo 135-8550, Japan

Cohesin is composed of well conserved four subunits among eukaryotes (Smc1, Smc3, Scc1, and SA2) and regulates sister chromatid cohesion during the mitotic cell cycle. Esco1 and Esco2 are Smc3 specific acetyltransferase and acetylate 105K and 106K of Smc3 that has been shown to be essential for establishment of sister chromatids cohesion. We found that Esco2 is required for cohesin acetylation mainly in S-phase, whereas Esco1 is required in both G1 and S-phase. RNA interference–based screening identified a Smc3-specific deacetylase gene (Hdac8). Inhibition of Hdac8 activity resulted in dramatic increase of the amount of Ac-Smc3 in chromatin unbound fraction that is hardly detectable in normal cells, destabilization of cohesin–chromosome interactions, increase in the number of acetylated Smc3 localization sites, and cohesion defects. We propose the model that Hdac8 is important for controlling deacetylated Smc3 pool and therefore important for cohesion establishment. Our study reports the second example of non-histone protein target of Hdac in human.

Association of Condensin with The Chromosome: Mechanism and Its Effect on Transcriptional Silencing

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Mitotic chromosomal condensation is mainly achieved by condensin, a multi-subunit protein complex widely conserved from yeast to human. Despite its conservation and importance for chromosome organization, little is known of the roles of condensin. In budding yeast, *Saccharomyces cerevisiae*, the serious defect in condensin mutants affects the segregation of the chromosomal region containing the tandem repetitive array of the ribosomal RNA gene (rDNA). Microscopic observation indicated the nucleolar localization of condensin. Consistent with this, condensin associates with the replication fork barrier site (RFB) within the rDNA repeat depending on the interaction with multiple recruiter proteins. Interestingly, the association of condensin with the RFB site is observed from early S-phase, long before M-phase during cell cycle, suggesting that condensin plays some role(s) during interphase. Here, I report that condensin contribute for silencing of the RNA polymerase II transcription from the reporter gene inserted near the RFB site in the rDNA repeat. Condensin mutation affects the Sir2 localization pattern along the rDNA region. Consistent with the changes of the Sir2 association pattern, endogenous RNA polymerase II transcriptions along the rDNA region are affected by condensin mutations. We hypothesize that condensin mutation changes the higher-order chromosome structure of the rDNA, resulting in the decrease of Sir2 accessibility to the region, thus contributing to transcriptional silencing.

Mitotic Chromosome Condensation by Condensin-Dependent Intrachromosomal DNA Interactions

Sebastian Heeger[1], Aengus Stewart[2] and Frank Uhlmann[1]

[1]Chromosome Segregation Laboratory and [2]Bioinformatics and Biostatistics Service, Cancer Research UK London Research Institute, 44 Lincoln's Inn Fields, London WC2A 3PX, UK

Eukaryotic chromosomes reach their stable rod-shaped appearance in mitosis in a reaction dependent on the evolutionarily conserved condensin complex. Little is known about how condensin promotes chromosome condensation. In the budding yeast *S. cerevisiae*, the levels and pattern of condensin binding along chromosome arms remain largely unchanged between interphase and mitosis, suggesting that a cell cycle-dependent change to condensin activity promotes chromosome condensation.

We have implemented chromosome conformation capture followed by high throughput sequencing (4C) to map interaction patterns along budding yeast chromosome 5. This revealed a condensin-dependent pattern of interactions that remains qualitatively similar throughout the cell cycle. However, as a consequence of chromosome condensation in mitotis intrachromosomal interactions quantitatively increase at the expense of interactions with sequences from other chromosomes. The correlation of these changes with the dynamic chromatin binding behaviour of condensin and its posttranslational modifications provides a new opportunity to understand the mechanism underlying mitotic chromosome condensation.

A Tale of Two Condensins: Differential Regulation and Concerted Actions

Tatsuya Hirano

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Condensins are multisubunit protein complexes that play a central role in chromosome condensation and segregation in both mitosis and meiosis (1). Many if not all eukaryotic organisms possess two different condensin complexes, known as condensin I and II (2). The two complexes share the SMC (structural maintenance of chromosomes) core subunits yet contain different sets of non-SMC regulatory subunits. Despite their structural similarities, condensin I and II are subjected to differential regulation, in both space and time, during the cell cycle. The following fundamental questions remain to be answered in the field. Why do two condensins exist? Does each of them have unique functions? How do they work at the mechanistic level? How do they contribute to chromosome inheritance, genome stability and human health? Our laboratory aims to address these questions by using many different experimental systems and approaches.

In this talk, I will focus on recent experiments using *Xenopus* egg cell-free extracts, in which the condensin complex (currently known as condensin I) was originally discovered (3). Firstly, we have devised a series of sophisticated protocols in which the levels or temporal order of actions of condensin I and II can be manipulated precisely in the extracts. This approach allows us to dissect mechanistically how the shape of metaphase chromosomes might be determined by an exquisite balance between forces promoting lateral compaction and axial shortening of chromatids. Secondly, we have used the cell-free extracts to study the molecular action of MCPH1, a putative regulator of chromosome condensation whose mutations cause primary microcephaly in humans (4). Our results demonstrate convincingly that human MCPH1 has a potent activity to inhibit condensin II in a highly specific manner. On the basis of this and other results, I will discuss how loss of MCPH1 (and upregulation of condensin II) might compromise a functional crosstalk between the chromosome and centrosome cycles and how such a defect might potentially contribute to brain size control in humans.

References:
1. Hirano (2005). Curr. Bio. 15:R265-R275 (review).
2. Ono et al (2003). Cell 115: 109-121.
3. Hirano et al (1997). Cell 89:511-521.
4. Trimborn et al (2006). Cell Cycle 5:322-326.

Bases of Break-Induced Replication in Mitochondrial Genome Dynamics

Takehiko Shibata [1, 2]

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Homologous DNA recombination, either crossover or gene conversion, is induced by DNA double-stranded breaks. Gene conversion is the replacement of a DNA sequence flanking a double-stranded break in recipient DNA by a copy of homologous or similar sequence of donor DNA, resulting in accurate repair, if the donor is allele or sister chromatid, or in chimeric gene formation, if the donor is non-allelic sequence. Thus, gene conversion among multiple and mutagenized copies of duplicated genes would rapidly and extensively diversify their sequences. This is the mechanism to generate various antibodies specific to various antigen in chicken (refer 'ADLib system').

Each eukaryotic cell has many copies of mitochondrial DNA (mtDNA) which encodes subunits of oxidative respiration system, and mtDNA suffers a magnitude higher rate of spontaneous mutagenesis. In various organisms including yeasts, mtDNA recombines frequently. Thus, it is expected that mtDNA in a cell is a very heterogeneous population, and mitochondrial genome evolved quickly. Both expectations are not true; mtDNA within a dividing cell is homogeneous, a state called homoplasmy, and except Complex I, mitochondrially encoded proteins are very well conserved between yeast and human.

A type of double-stranded break-induced gene conversion to repair broken chromosomes is break-induced DNA replication (BIR). When BIR occurs between homologous chromosomes, all heterozygous alleles distal to the break are co-converted to the donor genotype, and thus, the genetic diversity is suppressed. Rolling circle DNA replication is a variant of BIR in which donor DNA is circular and the product is a tandem repeat of the donor sequence ("concatemer"). Thus, by this mode of BIR, the copies of a donor DNA molecule dominate in a DNA population. A series of our studies established that rolling circle replication and selective transmission of the concatemers to daughter cells are the mechanism to establish and to maintain homoplasmy [see ref. 1 for a review].

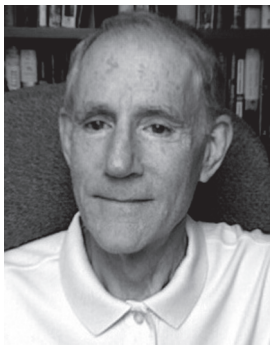
In gene conversion, ends of a double-stranded breakage are resected to generate 3' single-stranded tails, followed by homologous pairing of a tail with a complementary sequence in donor DNA. In normal gene conversion, homologous pairing is catalyzed by Rad51, a RecA homologue, with the aid of Rad52 or other recombination mediators. RecA/Rad51 catalyzes homologous pairing in an ATP-dependent manner, but Rad52 does in the absence of ATP. However, both RecA/Rad51 and Rad52 use the same basic mechanism [2]. Cells with *rad51* mutation repair double-stranded breaks by Rad52-dependent BIR. Interestingly, rolling circle replication in yeast mitochondria depends on ATP-independent homologous pairing protein, Mhr1, and not on RecA/Rad51 homologues. Structural and topological differences between RecA/Rad51 and Mhr1/Rad52 [3,4] may explain the choice of normal gene conversion or BIR, and differences in the genetic features between them.

References:
1. T. Shibata and F. Ling, (2007) Mitochondrion 7, 17-23.
2. T. Masuda, Y. Ito, T. Terada, T. Shibata and T. Mikawa, (2009) J. Biol. Chem. 284, 30230-30239.
3. F. Ling, M. Yoshida and T. Shibata, (2009) J. Biol. Chem. 284, 9341-9353.
4. W. Kagawa, A. Kagawa, K. Saito, S. Ikawa, T. Shibata, H. Kurumizaka and S. Yokoyama, (2008) J. Biol. Chem. 283, 24264-24273.

Chromosomal DNA Molecules in Mitochondria and Chloroplasts

Arnold J. Bendich

Department of Biology, University of Washington, Seattle, WA 98195, USA



I will briefly describe the structure and replication of chromosomal DNA in mitochondria and chloroplasts, followed by a more detailed consideration of DNA stability.

For most eukaryotes, chromosomal DNA molecules in the cytoplasmic organelles are not constant among individuals in a population with respect to either size or form. Such non-constancy is also found in bacteria, but not the nucleus where the chromosomes are constant.

Surprisingly little is known about the replication of mitochondrial DNA (mtDNA) and chloroplast DNA (cpDNA) in most eukaryotes. For fungi and plants, it appears that circular DNA does not serve as the main template for replication of wild-type chromosomes. Instead, replication is conducted with linear and branched forms of DNA as found for bacteriophages T4 and T7.

The integrity of chromosomal DNA must be preserved in order for organisms to maintain their identity through the generations. DNA damage leading to extensive mutagenesis can be avoided by either repairing the damage, degrading damaged DNA (feasible for the high-genome-copy cytoplasmic organelles, but not the diploid nucleus), or minimizing the effects of DNA-damaging agents such as reactive oxygen species (ROS). ROS are unavoidable byproducts of respiration and photosynthesis. The instability of mtDNA and cpDNA can be understood as a mutation-avoidance mechanism. Another is the placement of mitochondria and chloroplasts in “quiet” germ line cells so that their DNA is not exposed to the oxidative stress produced by these organelles in “active” somatic cells. The origin of development in animals and plants can thus be traced to protection-by-sequestration of mtDNA and cpDNA.

Tinkering with Yeast Telomeres

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Linearity of DNA molecules generates problems concerning their termini including solving the end-replication problem, protection against nucleolytic attacks and inappropriate DNA repair (1). Yet, all eukaryotes contain linear chromosomes in their nucleus and many, including numerous yeast species, also in their mitochondria (2,3) indicating that having linear chromosomes with defined physical ends can provide for the host cell qualitatively new opportunities not available for circular DNA genomes.

To solve the problems associated with the ends, linear DNA genomes possess specialized nucleo-protein structures (telomeres). Although the list of the problems concerning DNA termini is similar for all linear DNA genophores, the nature of their solutions provided by telomeres greatly varies. This is reflected by very different architecture of particular DNA termini, the composition of protein components as well as molecular mechanisms involved in telomere dynamics and it is of utmost interest to identify and describe these mechanisms. Ascomycetous fungi represent a numerous group of microorganisms resulting from diverse and unique evolutionary trajectories and sharing their common ancestor several hundred millions years ago. This makes them ideal for comparative analysis of telomeres to uncover novel mechanisms involved in the stabilization and replication of linear chromosomes ends in both nuclei and mitochondria. With this rationale we are performing systematic comparative analysis of mitochondrial and nuclear telomeres in nonconventional yeast species. Our studies have demonstrated evolutionary tinkering with sequences of telomeric DNA, their arrangement at the chromosomal ends (3) and proteins involved in their protection and transactions (4). For example, using a group of yeast possessing linear mitochondrial genomes, whose telomeres are composed of tandemly repeated sequences we have shown that their replication is mediated by extragenomic circular molecules (telomeric circles; t-circles; 5), which are also involved in telomere dynamics in nuclei of eukaryotes lacking telomerase, including telomerase-deficient human cancer cells (6). At the same time, comparative analysis of yeast nuclear telomeres (7) demonstrated that, although the changes in telomere architecture is not that dramatic as in case of linear mitochondrial genomes, their protein composition greatly varies, underlining the fact that telomeres underwent dramatical changes in evolution.

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Spontaneous Mutagenesis Associated with Nucleeotide Excision Repair in *Escherichia coli.*

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The vast majority of spontaneous mutations occurring in *Escherichia coli* are thought to be derived from spontaneous DNA lesions, which include oxidative base damage. Systems for removing intrinsic mutagens and repairing DNA lesions contribute to the suppression of spontaneous mutations. Nucleotide excision repair (NER) is a general DNA repair system that eliminates various kinds of lesions from DNA. We therefore predicted that NER might be involved in suppression of spontaneous mutations, and analyzed base substitutions occurring spontaneously within the *rpoB* gene in NER-proficient (wild-type), -deficient and -overproducing *E. coli* strains. Surprisingly, the mutation frequency was lower in NER-deficient strains, and higher in NER-overproducing strains, than in the NER-proficient strain. These results suggest, paradoxically, that NER contributes to the generation of spontaneous mutation rather than to its suppression under normal growth conditions, and that transcription-coupled repair also participates in this process. We hypothesized that unnecessary NER might account for these findings, so that errors introduced during repair DNA synthesis by DNA polymerase I would result in unwanted base substitutions. To test this, we constructed *E. coli* strains that carried an editing exonuclease-deficient *polA* mutation and either an additional NER-deficient mutation or an NER-overproducing plasmid. Results obtained with these constructs indicate that 1) NER can indeed occur unnecessarily; 2) DNA polymerase I makes replication errors at a low but detectable frequency during repair DNA synthesis; and 3) these errors become fixed as spontaneous mutations under normal growth conditions. The repair system itself may thus be is an important generator of spontaneous mutation.

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Genome Integrity Preserved by Induction of Apoptosis from DNA Damage

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O^6-Methylguanine (O^6-mG), produced in DNA by the action of simple alkylating agents, induces base mispairing during DNA replication and thus is responsible for the induction of mutations as well as tumors. To prevent such an outcome, organisms possess a mechanism to eliminate cells carrying O^6-mG by inducing apoptosis in a mismatch repair (MMR) proteins-dependent manner. To understand the molecular mechanism of O^6-mG-induced apoptosis, we carried out retrovirus-mediated gene-trap mutagenesis, followed by the selection of N-methyl-N-nitrosourea (MNU)-resistant clones from MNU-sensitive *Mgmt*$^{-/-}$ cells. One of the mutants isolated was unable to induce mitochondrial membrane depolarization and caspase-3 activation, hallmarks for the induction of apoptosis, after treatment with MNU, although it still retains the ability to undergo cell death caused by interstrand-crosslinks and double-stranded breaks in DNA. The mutant has an insertional mutation in a new gene, designated *Mapo1* (O^6-methylguanine-induced apoptosis 1), which is highly conserved in various organisms ranging from nematodes to humans. The Flag-tagged MAPO1 protein expressed in cells was immunoprecipitated and was revealed to be associated with FLCN and AMPK. By the introduction of siRNAs specific for these genes, the production of sub-G_1 population of cells following the treatment with MNU was severely suppressed, suggesting the important role of MAPO1-containing protein complex in the induction of apoptosis triggered by O^6-mG.

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DNA Methylation and Heterochromatin Formation in Neurospora Crassa

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Neurospora possesses a potent homology-based genome defense system called RIP (repeat-induced point mutation) that detects duplicated sequences premeiotically leaving both mutated with C:G to T:A mutations and silenced by DNA methylation. In fact, almost all regions of DNA methylation in the Neurospora genome are relics of transposons inactivated by RIP. Our genetic and biochemical studies have revealed clear ties between DNA methylation and histone modifications. DNA methylation in Neurospora is dependent on trimethylation of histone H3 lysine 9 (H3K9me3) by the histone methyltransferase, DIM-5. DIM-5 is sensitive to modifications of histones including methylation and phosphorylation and is found in a complex with several other proteins that are essential for DNA methylation: DIM-7, DIM-8 (DDB1), DIM-9 and CUL4. The linkage between H3K9me3 and DNA methylation is facilitated by heterochromatin protein 1 (HP1), which serves as an adapter protein. HP1 binds to the H3K9me3 and recruits the DNA methyltransferase, DIM-2. Moreover, H3K9me3 and DNA methylation are rapidly and fully reestablished after these marks are stripped off genetically indicating that the mutations generated by RIP serve as a powerful de novo methylation signal. Although HP1 links H3K9me3 to DNA methylation, it also serves to recruit DNA methylation modulator-1 (DMM-1), a JmjC domain protein, to the edges of heterochromatin regions, which prevents the spreading of DNA methylation and H3K9me3 from RIP inactivated transposons into nearby genes. Mutants defective in *dmm*-1 grow poorly but growth can be restored by reduction or elimination of DNA methylation using the drug 5-azacytosine or by mutation of the DNA methyltransferase gene, *dim*-2. Our recent progress towards the elucidation of mechanisms controlling heterochromatin and DNA methylation in Neurospora will be presented.

An Experimental Model System for the Evolution of New Genes under Continuous Selection

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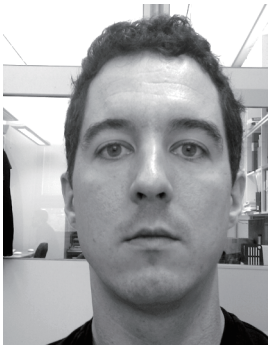

It is a generally accepted idea that new genes can evolve from a duplication of an ancestral gene, freeing one copy from the constraints of purifying selection (1). Through random mutations and natural selection one of the copies may acquire a new function, while the other copy retains the original function.

Tandem duplications are very common, reaching steady-state frequencies of 10^{-4}-10^{-2} for different loci in bacterial cultures (2). Despite this, there are several factors that make neofunctionalization an apparently improbable outcome of duplication (sometimes referred to as "Ohno's dilemma"). Tandem duplications are usually unstable and segregate by homologous recombination with a rate that is much higher than their rate of formation. Duplications often have fitness costs associated to them, which leads to their loss from the population through counter selection. Deleterious mutations by far outnumber beneficial ones, making pseudogenization the most likely outcome, if the duplication would remain in the population long enough to acquire any mutation (3).

The innovation, amplification and divergence (IAD) model for evolution of new genes under continuous selection is based on the observation that many enzymes have weak secondary activities. A change in the environment, such as the presence of a toxic compound, a new nutrient or fixation of a deleterious mutation, can make such a minor activity beneficial, causing a selective pressure to increase the activity. Individuals with duplications and higher-order amplifications of the gene encoding such a weak beneficial activity express more of the beneficial activity, and will therefore have a selective advantage and are selectively enriched in the population (3).

The structurally similar enzymes HisA and TrpF catalyze the same chemical reaction on their respective substrates in the biosynthesis of the amino acids histidine and tryptophan. To study the evolution of new gene function under continuous selection we have isolated spontaneous mutants of the *Salmonella enterica hisA* gene that converts the HisA enzyme into a poorly functional "new TrpF" or a bifunctional "HisA/TrpF". When grown in medium lacking histidine and tryptophan these mutants show signs of enrichment for duplications or amplifications, and acquire additional mutations in *hisA* that improves the biochemical activities.

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Poster Session

Sequential Rearrangement and Eviction of Nucleosomes Allow Interleukin-2 Transcription Following T Cell Activation

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Chromatin is assembled into structures such as primary nucleosome arrays, secondary 30-nm fibers, and chromatin loops. These structures must be unraveled step-by-step in order to decode genetic information. We observed two sequential chromatin structural changes at the Interleukin-2 (IL-2) promoter. A new sedimentation velocity method designated the "SEVENS" assay (1) revealed local rearrangement of nucleosomes at the activated promoter prior to their eviction. The initial change was temporally correlated with the binding of NFAT1 and AP-1 (Fos/Jun heterodimer), while the second step occurred in parallel with the recruitment of other transcription factors and RNA polymerase II. Although pharmacologic inhibitors for activation of NFAT1 or the induction of Fos proteins blocked the nucleosome rearrangement, this step was not affected by inhibition of c-Jun phosphorylation; instead, the subsequent eviction and IL-2 mRNA production were still blocked. Thus, the "early-binding" proteins and their modifications perform two sequential steps in chromatin remodeling at the IL-2 promoter for the induction of IL-2 transcription.

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Overproduction of *Escherichia coli* DNA Polymerase DinB (Pol IV) Inhibits Replication Fork Progression and is Lethal

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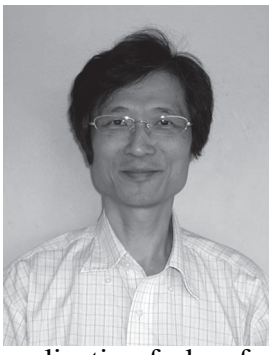

Escherichia coli has five DNA polymerases, DNA polymerase I-V (Pol I-V). Pol III holoenzyme (Pol III HE) is the replicative enzyme, and is composed of a Pol III* subcomplex and a sliding b-clamp. DinB (Pol IV) is a specialized Y-family DNA polymerase encoded by *dinB*; it is induced as part of the SOS stress-response system, and functions in translesion synthesis (TLS) to directly copy damaged DNA in place of the replicative Pol III HE when the latter is stalled at DNA lesions. Pol III HE exists at 10-20 molecules per cell, a number that is adequate for efficient DNA synthesis at the 2-8 replication forks of an exponentially growing cell. However, the number of DinB molecules is 250 per cell even in unstressed cells, which is greater than that required to accomplish TLS. Thus, it is thought that *dinB* plays some additional physiological role.

Newly identified functions of DinB *in vitro* is to rapidly release Pol III* from its stable complex with the β-clamp at a primer-template junction and to slow down a moving Pol III HE that is catalyzing rapid chain elongation on the template (Furukohri *et al*., *J. Biol. Chem*., 2008). A possible inference from these data is that *dinB* overexpression might impede DNA replication by providing a large excess of DinB over Pol III HE. Here, we overexpressed *dinB* under the tightly regulable arabinose promoter and looked for a distinct phenotype (Uchida *et al., Mol. Microbiol*., 2008). Upon induction of *dinB* expression, progression of the replication fork was immediately inhibited at random genomic positions, and the colony-forming ability of the cells was markedly reduced. Overexpression of mutated *dinB* alleles revealed that the structural requirements for these two inhibitory effects and for TLS were distinct. The extent of *in vivo* inhibition displayed by a mutant DinB matched the extent of its *in vitro* impedance, at near-physiological concentration, of the moving Pol III HE. We suggest that DinB targets Pol III, thereby acting as a brake on replication fork progression. Because the brake operates when cells have excess DinB, as they do under stress conditions, it may serve as a checkpoint to prevent potentially harmful replication until the cellular stress is alleviated.

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DDK Phosphorylates Checkpoint Clamp Rad9 and Promotes Its Release from Damaged Chromatin

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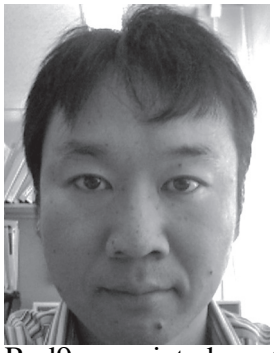

When inappropriate DNA structures arise, they are sensed by DNA structure-dependent checkpoint pathways and subsequently repaired. Recruitment of checkpoint proteins to such structures precedes recruitment of proteins involved in DNA metabolism. Thus, checkpoints can regulate DNA metabolism. We show that fission yeast Rad9, a 9-1-1 heterotrimeric checkpoint clamp component, is phosphorylated by Hsk1^{Cdc7}, the *S. pombe* Dbf4-Dependent Kinase (DDK) homolog, in response to replication-induced DNA damage. Phosphorylation occurs after 9-1-1 chromatin loading, depends on the Rad9-associated protein Cut5^{TopBP1} and disrupts interactions between Rad9 and RPA. *rad9* mutants defective in DDK phosphorylation show wild-type checkpoint responses but abnormal DNA repair protein foci and increased inviability following replication stress. We propose that Rad9 phosphorylation by DDK releases Rad9 from DNA damage sites to facilitate DNA repair.

Double Rolling-Circle Replication (DRCR) is Recombinogenic

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Oncogene-type gene amplification is well known to be associated with genomic rearrangements. We have found that double rolling-circle replication (DRCR) is a central mechanism to oncogene-type gene amplification, and that sequences flanked by inverted repeats were subject to frequent inversion during DRCR. To examine the relationship between DRCR and the associated recombination, we constructed a system able to turn DRCR on or off using yeast 2-micron plasmid. An inverted or direct repeat was inserted into the plasmid to detect inversion or deletion/duplication, respectively. As a result, we observed highly frequent recombination events depending on DRCR, including not only inversion but also deletion/duplication. Furthermore, similar results were also found when DRCR was induced in a chromosome in yeast. From these results, we concluded that DRCR is a recombinogenic process. We will discuss relationship between the DRCR-dependent recombination and extensive genomic rearrangement occurred during amplification of oncogene or drug-resistant gene.

APC/C is Involved in Nuclear Envelope Dynamics during Mitosis in *Schizosaccharomyces japonicus*

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Most of higher eukaryotes show nuclear envelope-break down before chromosome segregation, or open mitosis. By contrast, most of lower eukaryotes show chromosome segregation within their nuclei, or closed mitosis. In yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, molecular mechanisms of chromosome segregation in closed mitosis is similar to higher eukaryotes. However, biological significance of nuclear envelope in each mitosis is not fully understood until now.

Interestingly, it has been suggested that extension and breakage of nuclear envelope are occurred in *Sz. japonicus* cells, which is a fission yeast. We have focused on nuclear and chromosome dynamics of *Sz japonicus* by using Cut11-GFP that is a component of nuclear pore in living cells and an electron microscopy in fixed cells. We confirmed that *Sz. japonicus* undergo 'semi-open mitosis' in which nuclear envelope made fusiform-shape and finally broke around the equatorial plane of the nucleus during anaphase. Furthermore, dynamics of nuclear envelope showed that distribution of nuclear pores changed before breakage of nuclear envelope. Nuclear pores were located on entire nuclei in interphase, but did on both sides of fusiform-shaped nuclei in anaphase. Fortunately we succeeded to find temperature-sensitive mutants in which nuclear envelope was not broken but separated as closed-mitosis, and distribution of nuclear pores did not change before nuclear division. Three alleles among the mutants were further analyzed genetically, and then we determined that each three mutant was defective in APC/C factors, Apc2, Cut23 and Nuc2, respectively. APC/C is a well-known E3-ubiquitin ligase. In the APC/C mutants, though chromosomes were separated equally, separation of nuclei and nucleoli were unequal. These results suggest that APC/C ensures semi-open mitosis and equal separation of nucleolus in *Sz. japonicus*. And the distribution of nuclear pores along nuclear envelope may be also regulated by APC/C. To understand the significance of semi-open mitosis in *Sz. japonicus*, we would like to discuss on how APC/C is involved in separation of nuclear envelope and chromosomes.

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