CENTER FOR BIO-ENVIRONMENTAL SCIENCE

Interim Head: Hideo Mohri

The center is currently managed by NIBB and is scheduled to be included in a new center for biosciences which will be jointly managed by NIBB and two other institutes in Okazaki, IMS and NIPS. The following projects will be the main focus of the Center: 1) Endocrine disrupters. 2) Other chemicals affecting the environment. 3) Biomolecular sensors of environmental factors. 4) Database of bio-environmental science.

Professor : Teizo Kitagawa

I. Biomolecular sciense

Elucidation of a structure-function relationship of metalloproteins is a current subject of this group. The primary technique used for this project is the stationary and time-resolved resonance Raman spectroscopy monitored by near IR to UV lasers. The main themes that we want to explore are (1) mechanism of oxygen activation by enzymes, (2) mechanism of active proton translocation and its coupling with electron transfer, (3) coupling mechanism of proton- and electron transfers by quinones in photosynthetic reaction center, (4) higher order protein structures and their dynamics, and (5) reactions of biological NO.

In category (1), we have examined a variety of terminal oxidases, cytochrome P450s, and peroxidases, and also treated their enzymatic reaction intermediates by using the mixed flow transient Raman apparatus and the Raman/absorption simultaneous measurement device. For (2) the third generation UV resonance Raman (UVRR) spectrometer was constructed and we are going to use it to the peroxy and ferryl intermediates of cytochrme c oxidase. In (3) we succeeded in observing RR spectra of quinones A and B in bacterial photosynthetic reaction centers for the first time last year, but we focused our attention on tyrosine radical this year. For (4) we developed a novel technique for UV resonance Raman measurements based on the combination of the first/second order dispersions of gratings and applied it successfully to 235-nm excited RR spectra of several proteins including mutant hemoglobins and myoglobins. Nowadays we can carry out time-resolved UVRR experiments with nanosecond resolution to discuss protein dynamics. We have succeeded in isolating the spectrum of β145-Tyr, β35-Tyr and α140-Tyr of Hb A separately and their changes upon quaternary structure transition. For (5) we purified soluble guanylate cyclase from bovine lung and observed its RR spectra. To understand the implication, we examined Raman spectra of NO adducts of various mutant Mbs.

II. Fast dynamics of molecules in a solution phase

Picosecond time-resolved resonance Raman (ps-TR^3) spectroscopy is a promising technique to investigate ultrafast structural changes of molecules. However, this technique has not been used as widely as nanosecond TR^3 spectroscopy, mainly due to the lack of light source which has suitable repetition rates of pulses and wavelength tunability. In order to obtain qualified TR^3 spectra, first we need two independently tunable light sources for pump and probe pulses. Second, the repetition rate should be higher than kilohertz to keep a moderate average laser power without allowing the photon density of probe pulse to be too high. We succeeded in developing light sources for ps-TR^3 spectroscopy having wide tunability and kHz repetition, and applied them to study fast dynamics of photo-excited molecules. For carbonmonoxy myoglobin (MbCO), vibrational relaxation with the time constant of 1.9 ps was observed for CO-photodissociated heme. For Ni-octaethylporphyrin in benzene, appreciable differences in the rise times of population at vibrationally excited levels among various modes were observed in the anti-Stokes spectra for the first time. For the same molecule in piperidine, coordination of two solvent molecules was observed in the transient (d,d) excited state. The ps-TR^3 experiments were also applied to Zn-porphyrin dimers, for which some evidence for the π–π interaction in the S_1 state was obtained. The UV ns-TR^3 experiments on MbCO demonstrated the presence of a transient open form of the ligand pathway.

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Professor (Adjunct):Minoru Kanehisa (Kyoto University)

Though intermediary metabolism common to most organisms has been deeply investigated so far, variety of species specific pathways in secondary metabolism, which may work only in specific environmental conditions, are still unclear. The aim of this laboratory is to develop a database system for environmental biology, which integrates knowledge about organic compounds, chemical reactions between these compounds *in vivo*, enzymes (genes) involved in these reactions, and species whose genomes contain these genes. Through this database combining with data from transcriptome or proteome analyses in various environmental conditions, we intend to elucidate the principle of interactions between organisms and environmental chemical compounds to predict or design novel interactions.

Publication list:

Goto, S., Nishikoka, T., and Kanehisa, M. (1999) LIGAND database for enzymes, compounds, and reactions. *Nucleic Acids Res.* **27**, 377-379.

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Associate Professor : Akihiro Hazama
Research Associate : Kensuke Nakahira
Research Associate : Takuya Takahashi

This division has just started in August, 1999. The cells which constitute our bodies accomadate their environment by sensing the conditions surrounding them. The cells use the special membrane proteins, such as receptors, channels, and transporters, for sensing not only many substances but also heat, osmolarity, and mechanical stimuli. The aim of this division is to understand the sensing mechanims of such membrane proteins sense and how the cells accomodate their environment after sensing the outside conditions.

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Associate Professor: *Hirokazu Tsukaya, Ph.D.*
Assistant Professor: *Gyung-Tae Kim, Ph.D.*

Diversity of plant form is mostly attributable to variation of leaf and morphology of floral organs, which are modified, leaves. The leaf is the fundamental unit of the shoot system, which is composed with leaf and stem. So the leaf is the key organ for a full understanding of plant morphogenesis. However, the genetic control of development of these shapes had remained unclear. Recently, studies of leaf morphogenesis has been in a turning point, after our successful application of the techniques of developmental genetics and molecular genetics to it, using model plants such as *Arabidopsis thaliana* (L.) Heynh. Our purpose is to understand Plants from view point of molecular genetic control of leaf morphogenesis.

Focusing on mechanisms that govern polarized growth of leaves in a model plant, *Arabidopsis thaliana*, we found that the two genes act independently to each other on the processes of polar growth of leaves: the *AN* gene regulates width of leaves and the *ROT3* gene regulates length of leaves. The *AN* gene controls the width of leaf blades and the *ROT3* gene controls length. The *AN* gene seems to control orientation of cortical microtubules in leaf cells. Cloning of the *AN* gene revealed that the gene is a member of gene family found from animal kingdom (Tsukaya et al., in prep). The *ROT3* gene was cloned by us in 1998. Transgenic experiments proved that the *ROT3* gene regulates leaf-length without affect on leaf-width (Kim et al., 1999). We are trying to identify molecular function of the above genes which are essential for leaf morphogenesis.

While *ROT3* regulates the length of both leaf blades and petioles, *ACL2* appears to regulate petiole length exclusively. Genes for perception of environmental stimuli such as light and/or phytohormone perception also affect the petiole length relative to the length of the leaf blade. Genetic studies suggested that petioles and leaf blades share some regulatory pathways but petioles also have their own developmental programs that are independent of those of leaf blades (Tsukaya and Kim, submitted).

Apart from polar elongation, we identified the following genes involved in leaf expansion process. The *AS1* and *AS2* genes are needed for proportional growth of the leaf. Molecular and anatomical analysis of the *as2* mutant is now underway, in collaboration with Prof. Machida, Nagoya University.

On the other hand, we are trying to identify molecular mechanisms which distinguish developmental pathway of leaves from that of shoots. For such purposes, we introduced tropical plants having queer developmental program for leaf morphogenesis, namely, *Chisocheton*, *Guarea* and *Monophyllaea*, as materials for molecular studies.

In addition, we are interested in roles of such genes for environmental adaptation. Leaf index, relative length of leaf to width, is one of the most diverse factor of leaf shape. For instance, rheophytes are characterized by narrow leaves, which represent an adaptation to their habitats. Are *AN* and *ROT3* genes are involved in regulation of adaptive change of leaf index in natural condition? Are these genes the responsible for evolution of rheophytes? So called “Evo/Devo” study of leaf morphogenesis is also one of our research project in NIBB.

Publication List:

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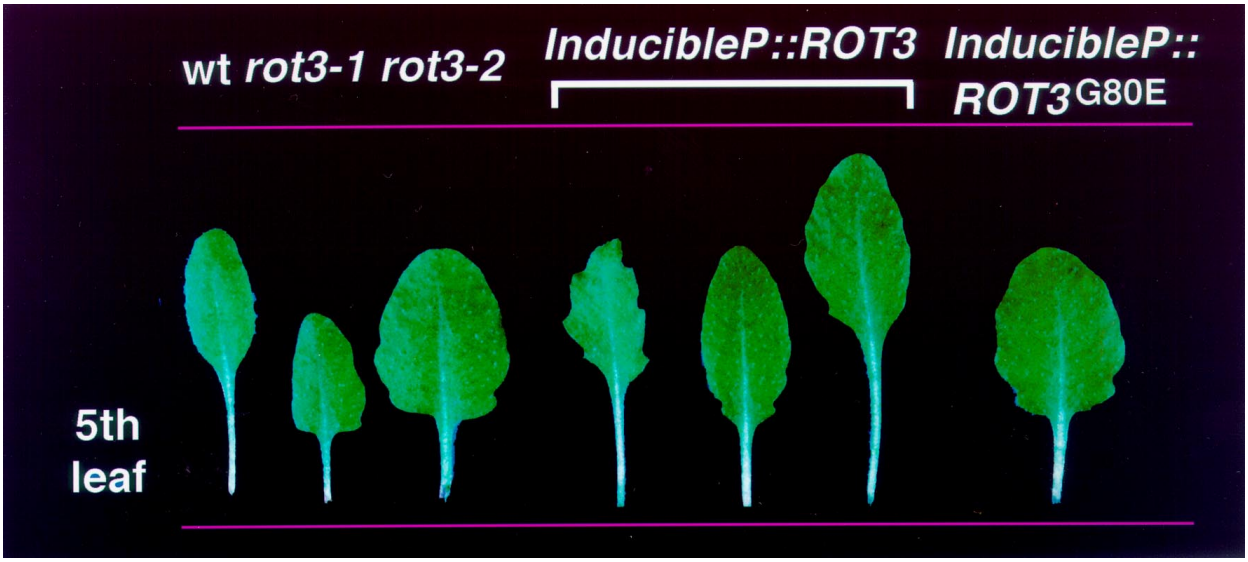

Figure: Single gene, the *ROT3*, controls leaf form (modified from Kim et al., 1999)