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Professor Iwata graduated from the Department of Fisheries Sciences, Faculty of Agriculture, Kyoto University in 1989. He completed the master program in the Graduate School of Agricultural Sciences, Ehime University in 1991, and the doctoral program in the United Graduate School of Agricultural Sciences, Ehime University in 1994. After serving as a Postdoctoral Research Fellow (1994-1995) of Japan Society for the Promotion of Science (JSPS), Japan, he became Assistant Professor of Graduate School of Veterinary Medicine, Hokkaido University in 1995, and Guest Investigator (JSPS Research Fellow to study abroad) of Woods Hole Oceanographic Institution, USA in 1999. He was appointed as an Associate Professor in 2000 and promoted as a Professor in 2004 at the Center for Marine Environmental Studies (CMES), Ehime University. Professor Iwata's research includes studies on the species diversity in hydrophobic ligand receptor-mediated cytochrome P450 signal pathway system and the effects on this system that environmental pollutants exert in wildlife such as fishes, birds and mammals.

Ecotoxicology of Wildlife and Species Diversity of Receptor-CYP Signaling Pathway

Our group addresses the issues on toxic effects and risk assessment of environmental chemicals (dioxins and other persistent organohalogen compounds and trace elements) in wildlife including fish, birds and mammals, and on molecular mechanisms underlying species-specific impacts and susceptibility to chemical exposure, focusing on the functions of xenobiotic metabolizing enzymes and receptors.

Cytochromes P450 (CYP) comprises a large superfamily of heme-thiolate enzymes that are critical for synthesis and degradation of physiologically important endogenous substrates and biotransformation of a vast range of xenobiotics. Expression of certain CYP members is induced by a variety of environmental chemicals. Since such CYP members can play important roles in mediating the biological effects of environmental chemicals including the disruption of the signaling pathways regulated by endogenous substrates, biotransformation of chemicals to harmful intermediates and the production of reactive oxygen species, the chronic induction of the CYPs can act as a potential biomarker of chemical exposure and effect. We have investigated the relationship between concentrations of environmental chemicals and expression levels of CYPs in the wildlife population. To evaluate the metabolic potencies of environmental and

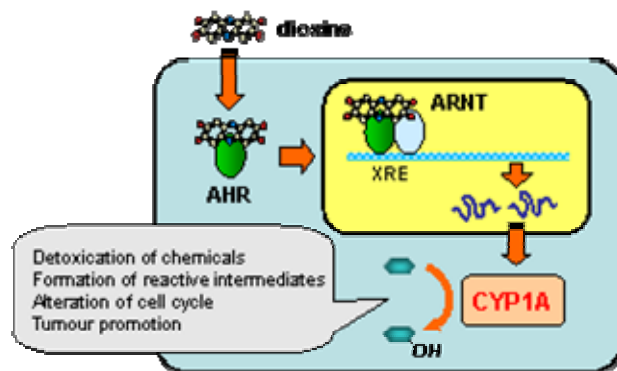
endogenous compounds in wild species, we also have characterized the catalytic functions of individual CYP isozymes, of which the cDNAs were isolated from each species and heterologously expressed.

The toxic effects and susceptibility to chemicals are greatly different among species. The differences in gene coding hydrophobic ligand receptors and metallothioneins may be a factor to account for the interspecies differences. For example, the aryl hydrocarbon receptor (AHR), a dioxin-activated intracellular protein, plays a central role in mediating various toxic effects of dioxins. Interspecies differences in the mechanism of toxic action and sensitivity to dioxins, at least partly, depend on the functional differences of AHRs. Comparative studies on the function of such gene products in key species, representing phylogenetically and ecotoxicologically relevant groups, may lead to more fundamental understanding of diversity of toxicities and sensitivity to chemicals. Therefore, focusing on hydrophobic ligand receptors including AHR, constitutive androstane receptor (CAR), pregnane X receptor (PXR) and peroxisome proliferator-activated receptor (PPAR) and metallothioneins of fish, birds and mammals, we have cloned their cDNAs and developed *in vitro* assay systems in order to evaluate species-specific responses of signal transduction triggered by environmental chemicals. The *in vitro* assay systems can potentially be a valuable tool for assessing the risk of environmental chemicals in wild target species.

Since organisms generally react to exposure of environmental chemicals by altering the expression levels of a variety of genes, monitoring wide range of molecular changes may be a valid approach to predict potential toxic effects and their mechanisms. As the microarray technology has a potential utility in global gene expression analysis, this has been used to evaluate chemical exposure and further toxic effects associated with the alteration of gene expression, in the fields of toxico- and ecotoxicogenomics. However, to date, only a few contaminant-responsive genes are known in wild species. It is imperative to gather further information of these genes in wildlife. In order to screen contaminant-responsive genes, to predict their potential toxic effects and to understand their mechanisms at molecular level in wild birds and aquatic mammals, we have constructed oligo arrays of the target genes from such animals and analyzed gene expression profiles in their tissues. Relationships between concentrations of environmental contaminants and gene expression patterns were also investigated.



Fig.1. Common cormorant (*Phalacrocorax carbo*) from Lake Biwa (left) and Baikal seal (*Pusa sibirica*) from Lake Baikal (right)



- Cloning of receptors and CYPs from wildlife
- Evolution of receptor-CYP signaling
- Ligand-receptor binding
- Receptor-mediated transactivation of CYPs
- Enzymatic function of CYPs
- Induction of CYPs by environmental chemicals in wild population
- Searching for target genes of receptors

Fig.2. Relationships of environmental chemicals, receptors and cytochrome P450s, and research themes related to the signaling pathway