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Identification of Cytochrome P450 Genes in Marine Mammals :
Comparative characterization of marine mammal cytochrome P450s

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Marine mammals have been recognized as an animal group long endangered by toxic effects of environmental pollutants. The properties of their xenobiotic metabolizers, cytochrome P450s (CYPs), have been repeatedly studied to understand their metabolic capacities. Those studies, however, have mainly focused on CYP proteins and have not yet been extended to the level of DNAs. In this paper, results from the very first cDNA cloning studies of marine mammal CYPs will be reported.

Six CYP 1 A and five CYP 3 A cDNA fragments were cloned from the livers of five marine mammal species, minke whale (*Balaenoptera acutorostrata*), dall's porpoise (*Phocoenoides dalli*), steller sea lion (*Eumetopias jubatus*), largha seal (*Phoca largha*) and ribbon seal (*Phoca fasciata*), by the method of reverse transcription polymerase chain reaction (RT-PCR). Degenerate primers were first used to amplify the partial CYP fragments from the liver sample. Of these partial cDNAs, two CYP 1 As from ribbon seal and one CYP 1 A from dall's porpoise were fully cloned with complete coding regions, using the technique of rapid amplification of cDNA ends (RACE). The partial and full-length cDNAs belonging in the family of CYP 1 A were classified into two major subfamilies of CYP 1 A 1 and CYP 1 A 2. The remaining five fragments belonging in the family of CYP 3 A were designated CYP 3 A 32, CYP 3 A 33, CYP 3 A 34, CYP 3 A 35, and CYP 3 A 36, respec-

tively, by the P450 Gene Superfamily Nomenclature Committee. The deduced amino acid sequences of the isolated partial and full-length cDNAs were used to construct a molecular phylogeny, along with other vertebrate CYP amino acids in the corresponding regions.

These phylogenetic analyses suggested two distinct evolutionary pathways for aquatic mammalian CYPs, compatible to a conservative taxonomy. Pinniped genes were clustered together with dog gene, forming a carnivore group, and cetaceans formed another branch of cetacean/artiodactyl group. Expression studies of the full-length CYP 1 As from aquatic animals using human embryonic kidney cells, 293T, ascertained the validity of the sequences on the protein function. CYP 1 A genes have been proposed to be one of the effective biological markers, whose induction and the corresponding increase in the enzymatic activities reflect animal's exposure to environmental toxins. Dall's porpoise CYP 1 A 1 s displayed comparable level of enzymatic activities in relation with that of the rat CYP 1 A 1, one of the well-characterized CYP 1 As of terrestrial animals, while ribbon seal CYP 1 A 1, on the other hand, showed lower level of enzymatic activity. This actual difference between animal species in basic activity level emphasizes the importance to know the properties of CYP isozyme, individually in order to correctly estimate the toxic effect of pollutants on the animal through CYP 1 A 1 activity.