## S 5-2

### GnRH RECEPTORS: MOLECULAR INTERACTION OF GnRHS AND THEIR RECEPTORS

Jae Young Seong

*Laboratory of G Protein-Coupled Receptors, Korea University College of Medicine, Seoul, Korea*

Gonadotropin-releasing hormone (GnRH) synthesized from the hypothalamus (designated GnRH-I) plays a pivotal role in the regulation of vertebrate reproduction. Other GnRH isoforms, in addition to GnRH-I, have been identified from the midbrain (GnRH-II). The presence of two or more forms of GnRH in a single species has proposed the existence of two or more cognate receptors. Recently, we have identified three different GnRH receptors (GnRHRs) in frogs and a type-II mammalian GnRHR in CV1 cells. These receptors exhibited different ligand sensitivity and signal transduction pathway from those of the type-I mammalian GnRHR. The present study elucidates the motifs responsible for ligand selectivity and signal transduction in GnRHRs. An S-E/D-P motif in the extracellular loop 3 of type-I mammalian GnRHRs was found to be responsible for differential ligand selectivity for GnRH-I and GnRH-II as replacement of the S-E/D-P motif by P-X-S/Y that observed in nonmammalian GnRHRs reversed ligand selectivity. Absence of intracellular C-tail in mammalian type-I GnRHRs may account for the preferential coupling to Gq than Gs. The mammalian type-I GnRHR with C-tail from frog GnRHR receptors gained an ability to couple to Gs while frog GnRHRs without C-tail revealed decreased coupling to Gs. Further dissection of the C-tail indicated that HFRK motif in the membrane-proximal region confers the differential signal transduction pathways between mammalian and nonmammalian GnRHRs as the addition of this motif to rat GnRHR or deletion of it from bfGnRHR-1 significantly affected the ability to induce the AC/PKA-linked signaling pathway. Collectively, this study suggests a molecular coevolution of ligands and their receptors and facilitate the understanding of the molecular interaction between GnRHs and their cognate receptors.

## S 5-3

### SINGLE-CELL GENE PROFILES OF GnRH NEURONS

Ishwar S. Parhar

*School of Medicine and Health Sciences, Monash University, Malaysia*

We have developed a novel single cell real-time quantitative PCR technique, which incorporates harvesting marker-identified single cells using laser-capture. Here, for the first time in a vertebrate species, using this innovative single cell gene profiling technique, we report the presence of G-protein coupled receptors in individual gonadotropin-releasing hormone (GnRH) neurons and endocrine cells of the pituitary of the tilapia Oreochromis niloticus. The differential expression of multiple combinations of three GnRH receptor types (R1, R2 and R3) in individual gonadotropic and nongonadotropic cells demonstrates cellular and functional heterogeneity. The differential use of GnRH receptors in corticotropes, melanotropes and thyrotropes during gonadal maturation and reproductive behaviors suggests new roles for these hormones. Further, we provide evidence of the structure of a novel nonmammalian G-protein coupled receptor (GPR54) for kisspeptins, encoded by Kiss-1 gene, which is highly conserved during evolution and expressed in GnRH1, GnRH2 and GnRH3 neurons. We hypothesize GPR54 stimulates GnRH secretion and is crucial for pubertal maturation. In addition, we show an array of genes present in EGFP-labeled single GnRH neurons in rats, which could have multiple implications. We speculate, the use of this method will allow the identification and quantification of known and unknown genes in single cells, which would greatly facilitate our understanding of the complex interactions that govern the physiology of individual cells in vertebrate species.