**D107** Three distinct types of gonadotropin-releasing hormone (GnRH) receptor in a single diploid species bullfrog (*Rana catesbeiana*): molecular cloning and functional characterization.

Li Wang*, Hueng-Sik Choi, Sang-Yang. Chun, and Hyunk-Bang Kwon
Hormone Research Center and Department of Biology, Chonnam National University

Three distinct full length cDNAs, encoding three distinct types of GnRH receptor (GnRHR), were isolated from pituitary and hindbrain of bullfrog using reverse transcriptase-polymerase chain reaction (RT-PCR), followed by rapid amplification of cDNA ends (RACE). The first type of cDNA consists of 2086 bp and encodes a putative 369-amino acid protein, designated BF I. The second type of cDNA consists of 1772 bp, which encodes a putative 424-amino acid protein, designated BF II, that represents the first full-length cDNA encoding a type II GnRHR. The third type of cDNA consists of 1839 bp, which encodes a putative 407 amino acid protein, designated BF III. This cDNA represents a novel type of GnRHR, and therefore designated a type III GnRHR. Hydropathy analysis revealed that three receptor proteins contain seven putative transmembrane domains as observed in typical GTP-binding protein -coupled receptors. The amino acid identity of BF I with BF II is 41%, and with BF III 40%. BF II and BF III share 53% amino acid identity with each other. Comparison with mammalian GnRHRs, the homology is about 30%, and around 40% with non-mammalian species. In contrast to mammalian GnRHRs, the BF I, II and III contain a carboxyl-terminal cytoplasmic tail of 57 a.a, 79 a.a. and 74 a.a., respectively. Northern blot analysis revealed that BF I, II and III mRNAs are expressed in a tissue specific way and with a seasonal variation. Southern blot analysis indicated that each of the three receptors is encoded by separate gene. The isolated cDNAs encoded functional receptors since their transient expression in Cos-7 cells resulted in a ligand-dependent increase in $IP_3$ production. Pharmacological studies of these transfected receptor cDNAs revealed distinct difference in their ligand selectivity. Thus, we have cloned three full-length cDNAs that correspond to three distinct GnRH receptors. Both BF II and BF III are novel types of GnRHRs which have not identified in other vertebrates yet.

**D108** Cloning and characterization of two gonadotropin-releasing hormone (GnRH) cDNAs in bullfrog *Rana catesbeiana* brain

Li Wang*, Myoung-Sik Yoo, Hueng-Sik Choi, Hae-Mook Kang, Wook-Bin Im, and Hyunk-Bang kwon
Hormone Research center* and Department of Biology, Chonnam National University

Gonadotropin–releasing hormone plays an important role in vertebrate reproduction. In amphibians, only limited information on the GnRH gene is available although extensive biochemical studies of GnRH have been carried out during the past twenty years. Thus, we have isolated the cDNAs that encode two gonadotropin-releasing hormone (GnRH) precursors, mammalian GnRH (mGnRH) and chicken GnRH II (cGnRH II) in bullfrog (*Rana catesbeiana*) brain. The full length mGnRH precursor cDNA consists of 652 bp, encoding an open reading frame of 90 a.a.; while the cGnRH II precursor cDNA consists of 1075 bp, encoding an open reading frame of 85 a.a. The deduced amino acid sequence revealed that bullfrog mGnRH decapeptide is the same as in mammalian and *Xenopus laevis*, and bullfrog cGnRH II decapeptide is the same compared with other reported species. But in Amphibian this is the first report on cloning it. The bullfrog mGnRH precursor exhibits an identity of 63% with *Xenopus*, 41% with human, and 25% with fish. For cGnRH II presursor it is around 50% ~ 60 % with fish. Northern blot analysis detected a single mRNA transcript of approximately 0.6 kb in frog forebrain for mGnRH and about 1.4 kb in hindbrain for cGnRH II. Genomic Southern blot analysis indicated that both bullfrog GnRH genes exist in genomic DNA as a single copy. BY RT-PCR quantitation, a high level of mGnRH expression was observed in olfactory bulb and telencephalon region of the brain and low level in other brain region, while it is reversed for cGnRH II. This study demonstrates that both the GnRH molecules are evolutionary well conserved in terrestrial vertebrates.