

**Evidence for differential regulation of GnRH signaling via heterodimerization among GnRH receptor paralogs in the protochordate, *Ciona intestinalis***

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The endocrine and neuroendocrine systems for reproductive functions have diversified as a result of the generation of species-specific paralogs of peptide hormones and their receptors including gonadotropin-releasing hormones (GnRHs) and their receptors (GnRHRs), which belong to the Class A GPCR family. A protochordate, *Ciona intestinalis*, has been found to possess seven GnRHs (tGnRH-3 to -8 and Ci-GnRH-X) and four GnRHRs (Ci-GnRHR1 to -4). Moreover, Ci-GnRHR4 (R4) does not bind to any *Ciona* GnRHs and activate any signaling pathways. Here, we show novel functional diversification of GnRH signaling pathways via GPCR heterodimerization among *Ciona* GnRHRs. R4 was shown to heterodimerize with R2 specifically in test cells of vitellogenic oocytes by co-immunoprecipitation. The R2-R4 heterodimerization in HEK293 cells co-transfected with R2 and R4 was also observed by co-immunoprecipitation and fluorescent energy transfer analyses. Of particular interest is that the R2-R4 heterodimer decreases the cAMP production in a non-ligand selective manner via shift of activation of Gs protein to Gi protein by R2, compared with R2 monomer/homodimer. Considering that the R1-R4 heterodimer elicits 10-fold more potent  $Ca^{2+}$  mobilization than R1 monomer/homodimer in a ligand-selective manner but does not affect cAMP production, these results indicate that R4 regulates differential GnRH signaling cascades via heterodimerization with R1 and R2 as an endogenous allosteric modulator. Collectively, the present study suggests that the heterodimerization among GnRHR paralogs, including the species-specific orphan receptor subtype, is involved in rigorous and diversified GnRHergic signaling of the protochordate which lacks a hypothalamus-pituitary gonad axis.

