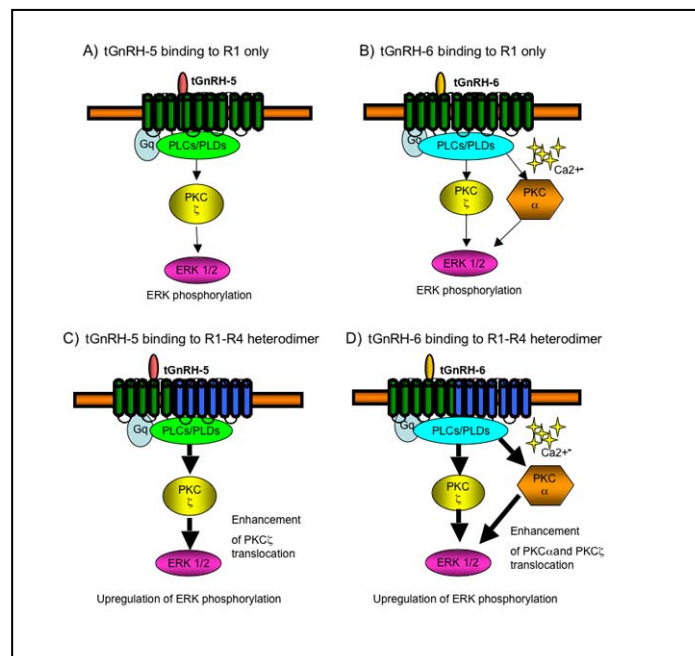


Functional diversity of signaling pathways through G protein-coupled receptor heterodimerization with a species-specific orphan receptor subtype

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Gonadotropin-releasing hormones (GnRHs) play pivotal roles in control of reproduction via a hypothalamic-pituitary-periphery endocrine system and nervous systems of not only vertebrates but also invertebrates. GnRHs trigger several signal transduction cascades via GnRH receptors (GnRHRs), members of the G protein-coupled receptor (GPCR) family. Recently, Six GnRHs (tGnRH-3 to -8) and four GnRHRs (Ci-GnRHR1 to -4), including a species-specific paralog, Ci-GnRHR4 (R4) regarded as an orphan receptor or non-functional receptor, were identified in the protochordate, *Ciona intestinalis*, which lacks the hypothalamic-pituitary system. In present study, we show novel functional modulation of GnRH signaling pathways via GPCR heterodimerization. Immunohistochemical analysis showed co-localization of R1 and R4 in test cells of the ascidian ovary. The native R1-R4 heterodimerization was detected in the *Ciona* ovary by coimmunoprecipitation analysis. The heterodimerization in HEK293 cells co-transfected with R1 and R4 was also observed by coimmunoprecipitation and fluorescent energy transfer analyses. Binding assay revealed that R4 had no affinity for tGnRHs and the heterodimerization did not alter the binding affinity of R1 to the ligands. The R1-R4 elicited 10-fold more potent Ca^{2+} mobilization than R1 exclusively by tGnRH-6, although R1-mediated cAMP production was not affected by any of tGnRHs via the R1-R4 heterodimer. Moreover, the R1-R4 heterodimer potentiated translocation of both Ca^{2+} -dependent $\text{PKC}\alpha$ by tGnRH-6 and Ca^{2+} -independent $\text{PKC}\zeta$ by tGnRH-5 and -6, eventually leading to the up-regulation of ERK phosphorylation, compared with R1 alone. These results provide evidence that the species-specific GnRHR orphan paralog, R4, serves as an endogenous modulator for the fine-tuning of activation of PKC subtype-selective signal transduction via heterodimerization with R1, and that the species-specific GPCR heterodimerization, in concert with multiplication of tGnRHs and Ci-GnRHRs, participates in functional evolution of neuropeptidergic GnRH signaling pathways highly conserved throughout the animal kingdom.



Model of signaling modulation via R1-R4 heterodimerization. The activation of specific PKC subtypes, eventually leading to ERK phosphorylation, is differentially up-regulated by heterodimerization between *Ciona* GnRH receptor, R1 and its orphan receptor paralog, R4 in a ligand-selective manner.