

Human follitropin receptor interaction with adapter proteins

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G-protein coupled receptors signal through multiple pathways. Compartmentalization of receptors (spatial constraints) and residency time (temporal factors) can affect the quality and duration of signaling, provide opportunities for decision making and overcome thermodynamic constraints. It was reasoned that a better understanding of FSH signaling might be possible, if cytoplasmic proteins which interact with FSHR intracellular loops (iL) and potentially govern FSHR actions were identified. FSHR-iL1 when phosphorylated is a key site of interaction with arrestin. FSHR-iL2 contains a motif (ERW) conserved throughout the G-protein coupled receptor family (DRY) and associated with cAMP signaling. Two cytoplasmic proteins that may regulate FSHR-iL 1 and 2 function have been identified. One protein identified was 14-3-3 τ , a member of a family of homodimeric cytoplasmic adapter proteins. The adapter protein 14-3-3 τ associates with FSHR-iL2 in a time and hormone-dependent manner. Since agonist induced phosphorylation occurs on FSHR-iL1 and 3 it is not likely that 14-3-3 τ is recruited to FSHR in response to a phosphorylation signal. The transient association of 14-3-3 τ with receptor suggests a model in which there is a role for 14-3-3 τ in cAMP independent signaling. Another adapter protein which interacts with the FSHR-iL1 was identified, named APPL1 (adaptor protein containing PH domain, PTB domain, and leucine zipper motif). APPL1 is structurally related to another adapter protein APPL2. Both APPL1 and APPL2 contain BAR domains, a signature of proteins that are regulators of endocytosis and N-BAR a domain which is required for binding to lipids. APPL1 and protein kinase B (PKB) co-immunoprecipitate (co-ip). FSHR and PKB also co-ip. FOXO1a a downstream target of PKB does co-ip with FSHR but does not co-ip with APPL1. Interestingly APPL2 also was shown to co-ip with FSHR. As dimerization is a minimal function of both 14-3-3 and BAR domain proteins, it was confirmed that APPL1 and APPL2 form heterodimers. The temporal association of APPL1 with FSHR is sustained suggesting a trafficking function. This was verified by imaging experiments which tracked both receptor and APPL proteins. A working model will be presented which suggests a novel PI3K independent compartmentalized pathway to FOXO1a inactivation. These results suggest that dimeric and heterodimeric adapter proteins serve to recruit proteins to FSHR, perhaps bridge receptors themselves and suggest new targets for pharmacologic regulation of gonadotropin receptor function. Supported by HD-18407.

