

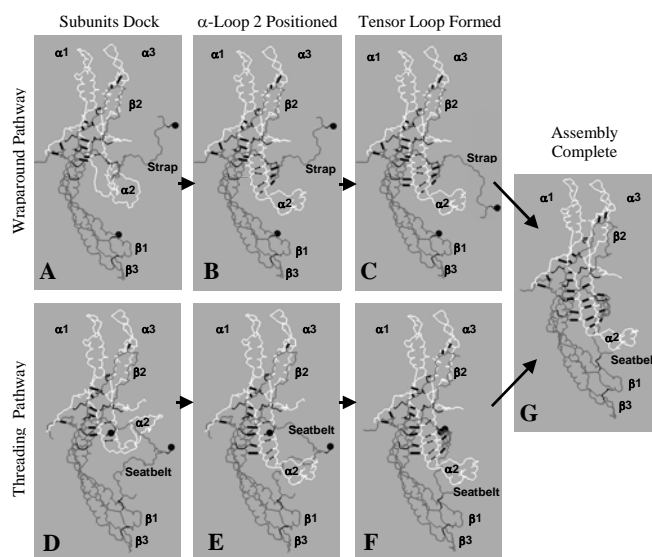
Assembly of glycoprotein hormones in cells.

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Introduction: Glycoprotein hormones are heterodimers in which α -subunit loop 2 is surrounded by 20 residues of the β -subunit that are commonly termed the seatbelt. Studies described here were initiated to learn if these heterodimers are assembled by mechanisms wherein the subunits dock before or after the seatbelt is latched by a disulfide to the β -subunit. **Methods:** Analogs produced by site directed mutagenesis enabled us to deduce pathways of heterodimer assembly and to devise new methods of monitoring hormone receptor interaction (see JBC 279: 35426-35468 & 44427-44459 (2004)). **Results & Discussion:** hCG, hFSH, and hTSH are assembled by a threading mechanism in which α -subunit loop 2 passes beneath the seatbelt after it is latched (bottom pathway). hLH is formed by an alternate mechanism in which the subunits dock before the seatbelt is wrapped around α -subunit loop 2 and latched (top pathway). Insights from these findings enabled us to build hCG, hFSH, and hTSH analogs having various sized "knobs" at specific sites. The knobbed analogs permitted us to probe distances between sites of hCG, hFSH, and hTSH and their membrane bound G-protein coupled receptors. This information enabled us to build models of the membrane bound complexes that explain ligand binding and signaling. By combining this information with that gained from other aspects of assembly, we devised a new class of high affinity antagonist analogs that contain all but one oligosaccharide.



Mechanisms of heterodimer assembly. Two mechanisms account for heterodimer assembly. In the wraparound pathway (Panels A, B, C, G), the subunits dock before the seatbelt is latched. In the threading pathway (Panels D, E, F, G), the subunits dock after the seatbelt is latched to the β -subunit. The formation of hydrogen bonds drives assembly in both pathways. Code: α -subunit, white; β -subunit, gray; black bars, selected hydrogen bonds; black spheres, sulfur atoms in cysteines of disrupted disulfides.

Conclusions: An understanding of hormone folding and assembly has provided important insights into the manner of hormone action and should also provide new significant insights into hormone sorting and secretion. **Support:** NICHD