

## Specificity and promiscuity amongst glycoprotein hormone receptors

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The dichotomy between hormone recognition, by the ectodomain containing leucine-rich repeats (LRR), and activation of the G protein, by the rhodopsin-like serpentine portion, is a well established property of glycoprotein hormone receptors (GpHRs). Extensive site-directed mutagenesis experiments and, more recently, direct structural data have fully confirmed that high affinity recognition of the hormones by their receptors was essentially built within the structure of the LRRs. It is possible to transform a TSH receptor (displaying minimal sensitivity to hCG) into a fully hCG responsive receptor by introducing only 8 aminoacid substitutions at selected positions in TSHr ectodomain. Similarly, mutating two residues of the ectodomain of the FSHr renders it highly responsive to hCG. The serpentine portions of GpHRs share many structural and functional characteristics with other rhodopsin-like GPCRs. Upon mutation of specific residues in the transmembrane helices, or in the connecting loops, they may display increase in basal activity. It was initially thought that the ability to be activated by mutations was a property of TSH and LH/CG receptors, the FSH receptor being more refractory to activation in the absence of the hormone.

In the past two years, mutations responsible for rare spontaneous cases of ovarian hyperstimulation syndromes (sOHSS) have partially modified this simple view. Five naturally occurring mutations [D6.30<sup>567</sup>N and D6.30<sup>567</sup>G (unpublished), T3.32<sup>449</sup>I, T3.32<sup>449</sup>A and I5.54<sup>545</sup>T (unpublished)] have now been identified in the TM3, TM5 and TM6 of the serpentine region of the FSH receptor, which cause increase in sensitivity to hCG. Surprisingly these mutations caused also an increase in sensitivity of the FSHR to TSH and were responsible for detectable constitutive activity. The unexpected location of these mutations in the serpentine portion of the receptor, together with the relaxation of specificity towards both hCG and TSH suggested the existence of a correlation between constitutive activity and loss of specificity.

This hypothesis has been verified by extensive site-directed mutagenesis of the FSHR and by evolutionary studies. As a rule, most mutations of the FSHR causing increase in constitutive activity (whether in the serpentine portion, or in the ectodomain) were shown to allow promiscuous activation of the mutants by hCG and TSH. A series of chimeric FSHRs, with an ectodomain of human origin and serpentine portions of a variety of mammals, displayed essentially the same phenotype (i.e. detectable basal activity and relaxed specificity). Interestingly, when transposed on the TSHR, the same mutations, while causing increase in constitutive activity, were without effect on its specificity.

Together, our results suggest that during evolution of primates, different paths have been followed to avoid spurious stimulation of the TSH or FSH receptors by progressively increasing concentrations of chorionic gonadotropin. In the TSH receptor, the specificity barrier evolved within the hormone recognition portion of the ectodomain, while the receptor kept detectable basal activity (a peculiarity of the TSH receptor). In the FSH receptor, the serpentine domain became strongly locked (with no detectable basal activity in human), making it only sensitive to activation following high affinity interaction of the ectodomain with FSH. This view fits well with our current model of GpHR activation, in which the immediate agonist of the serpentine domain of the receptor would be the hormone-ectodomain complex.