

Insights from the LH-beta gene into the LH to CG evolution

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Chorionic gonadotropins (CG) are unique among the glycoprotein hormones because they have on their beta subunits a carboxyl terminal peptide (CTP) extension, bearing multiple mucin type O-glycans. The CG β genes are thought to have evolved from ancestral LH β genes by frame shift mutations that extend the reading frame, yielding a CTP. The glycosylated CTP domain prolongs circulatory survival of CG relative to LH and contains determinants for apical secretion of the placental hormone to the maternal serum in primates. Although the LH β gene is conserved among mammals, and the gain of new hormonal functions is associated with the CTP expression, the extended subunit is seen merely in primates and equids. To address the reason as to why LH β to CG β evolution is restricted in the animal kingdom, we first examined whether LH β genes in non-primate, non-equid species inherently possess DNA sequences that encode CTP domains. Bioinformatics identified a CTP-like sequence that is encrypted in the LH β gene of several mammalian species, but not in birds, amphibians or fish. This suggests that in principle the LH β to CG β transformation could be widespread in placental animals. Based on the computational analysis, we examined whether or not decoding of the cryptic CTP in the bovine LH β gene (boCTP) could be sufficient to generate the LH β homolog of a ruminant with properties typical of the CG β subunit. The bovine carboxyl-extended LH β variant was expressed and N-glycosylated in CHO cells, suggesting that the protein tolerated the mutagenesis. However, unlike human (h) CG β CTP, the cryptic boCTP was devoid of mucin O-glycans. This deficiency was further confirmed when the cryptic domain substituted the genuine CTP in the human CG β subunit. Moreover, this hCG β -boCTP chimera was secreted from the basolateral side of polarized MDCK cells, rather than from the apical compartment, which is the route of the wild type hCG β subunit, a sorting function attributed to the O-glycans attached to the CTP. The absence of the orientation to the maternal circulation, as seen in primates, is a major drawback of the cryptic CTP. These results provide an explanation as to why the LH β to CG β evolution did not occur in ruminants, and perhaps not in other species. Thus, whereas the LH β gene in non-mammalian species is incompatible for development into the CG β gene, the locus in several mammals was modified to embed a CTP-like sequence, thus becoming a tentative precursor for the CG β gene. In primates and equids, further mutations occurred naturally in the progenitor gene and the resulted presence of mucin oligosaccharides in the CTP is crucial for the lutropin to chorionic gonadotropin evolution. The DNA sequence encrypted in mammalian LH β genes can be expressed in order to construct CTP-bearing glycoprotein hormone analogs.

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