

## Regulation of gonadotropin receptor gene expression

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Follicle stimulating hormone (FSH) is a critical component of the reproductive axis, which controls gonad function, gametogenesis, and fertility. Its activity depends on expression of its receptor, *Fshr*, which serves as the communicative link between the FSH signal and cellular response and restricts hormonal regulation to cells located only in the gonads. Therefore, understanding how *Fshr* expression is controlled is needed to fully appreciate FSH function and its contributions to reproductive physiology and fertility. Studies on the *Fshr* gene have provided significant insight on the transcriptional mechanisms required for function of its promoter. Notably, an E box located within the proximal promoter is a key regulator of *Fshr* transcription. *In vitro* binding studies previously showed that the transcription factors Usf1 and Usf2 bind the E box. To determine if Usf1 and Usf2 regulate *Fshr in vivo*, we employed chromatin immunoprecipitation (ChIP) to measure Usf binding to endogenous *Fshr* and expression studies in Usf-deficient mice to evaluate their effect on mRNA levels. ChIP revealed that both Usf1 and Usf2 bind the endogenous *Fshr* promoter, while RNase protection analysis showed that *Fshr* transcripts were markedly reduced in the ovary but not testis from Usf1<sup>-/-</sup> and Usf2<sup>-/-</sup> mice. Thus, Usf deficiency impacts *Fshr* expression differently in the testis and ovary. Studies in transgenic mice were used to identify regions within *Fshr* required for proper expression *in vivo*. Analysis of mice carrying transgenes with a 5,000 bp promoter-reporter construct or the entire *Fshr* coding region plus surrounding sequences (~300 kb) indicated that distal regulatory elements, which extend past 50 kb of flanking sequence, are needed for accurate expression of *Fshr*. Comparative genomics and DNase hypersensitivity mapping were used to help identify distal regulatory elements. Characterization of one conserved, hypersensitive site revealed that it acts as a transcriptional silencer predominantly in non-expressing cells and requires GATA4 and OCT1 binding sites. ChIP analysis confirmed that OCT1 associates with the site *in vivo*, but only in the non-expressing cells. Further comparative analysis identified seven non-coding sequences located in the vicinity of *Fshr* that are conserved between the more distantly-related human and chicken genomes. Initial characterization of these highly conserved sites showed they are transcriptionally active in Sertoli cells and implicate them in the regulation of *Fshr in vivo*. This work was supported by HD35217.