

Multi-level milk protein gene regulation

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Milk proteins are expressed in a tissue- and developmental-stage specific fashion, in functionally differentiated mammary epithelial cells under the influence of lactogenic stimuli. Developmental- and tissue-specific gene expression is regulated at multiple levels: the regulatory elements located in the linear DNA and the transacting-factors that bind to them, the chromatin conformation within which these regions reside, and the position in the nucleus these DNA regions occupy.

In past decades the hormonal signaling that is involved in mammary gland development and milk protein gene expression has been elucidated. Additionally, the factors that can bind the proximal regulatory elements of a number of the milk protein genes as a result of these hormonal cues and their involvement in gene regulation have been identified. However, very little is known about the integration of lactogenic signaling, chromatin conformation and transacting-factor recruitment that occurs during mammary gland development and lactation.

The main goal of this study is to understand the interplay between chromatin structure and transcriptional regulators in milk protein gene expression.

We investigated the chromatin conformation at the beta-casein (b-CSN) promoter and enhancer (-6 kb) in mouse mammary gland tissue. As well as the recruitment of different transacting factors and co-factors to the promoter and enhancer under influence of the lactogenic stimuli of Hydrocortisone (HC) and Prolactin (P) in a mouse mammary epithelial cell line (HC11).

The b-CSN promoter and enhancer are DNase I Hypersensitive in late pregnant and lactating mammary gland tissue but not in liver, indicating an open chromatin conformation in the mammary gland that correlates with casein expression. In addition, the DNA of the b-CSN promoter is methylated, representing a closed chromatin conformation, in liver and brain (non-expressing tissues) but not in expressing mammary gland tissue. Both the promoter and enhancer show lactating mammary gland specific Histone H3 hyperacetylation, a mark for open and actively transcribed chromatin.

Our studies in HC11 cells show that P stimulates the recruitment of STAT5 to the promoter and enhancer, but HC+P are needed for expression and synergistic increase in STAT5 recruitment. P and the recruitment of STAT5 result in the loss of YY1 bound at the promoter correlating with the relief of repression of b-CSN expression. HC stimulates the recruitment of GR to the promoter and histone H3 acetylation at the promoter and enhancer but is not enough to initiate b-CSN expression. P and STAT5 recruitment enhance GR presence on the promoter and are required for b-CSN expression. Each hormone separately increases recruitment of C/EBP β with an additive effect with both hormones. Both hormone recruit P300 and together they have an additive effect. RNA polymerase-II is recruited rapidly to the promoter, while phospho-pol-II appears later correlating with the detection of b-CSN transcripts.

These data suggest a model for the assembly of a multi-protein complex at the beta-casein regulatory regions that helps to understand how the signaling pathways regulated by lactogenic hormones and local growth factors are integrated in the nucleus to direct milk protein gene expression.