Identification Of Transcripts Showing Differential Expression In The Developing Mammary Gland Using Differential Display

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The mammary gland is a unique organ in that most of its growth, morphogenesis, and differentiation occur in the adult tissue. Development of the mammary epithelial cells is regulated by hormones, growth factors, cell to cell, and cell to substratum interactions [1]. Morphologically, this results in a proliferation of the ductal tree in the early stages of pregnancy. During the later stages of pregnancy the alveoli form lobular structures where the epithelium assumes a secretory function. This is then maintained during lactation, where the epithelial cells become highly specialized and the majority of their capacity is directed to the production of a small number of milk proteins. This process of pregnancy and lactation results in dramatic tissue remodeling, which makes the mammary gland an excellent system for studying questions relating to cell differentiation.

We have used the technique of differential display to study the specific genes which are responsible for the differentiation process in the mammary gland. Differential display RT-PCR was originally developed as a method to identify and analyze changes in gene expression at the mRNA level [2]. The method is based on comparisons of mRNAs expressed within samples in a related cell population by running their RT-PCR products in adjacent lanes on a sequencing gel. Thus RNAs showing altered expression patterns can be detected visually. The primer strategy used is based on a modified oligo dT primer, designed to amplify a subpopulation of the total mRNA, used in combination with a 10mer of arbitrary sequence. The major advantage of using this technique is that it should allow us to analyze changes in gene expression over an 18 day period in mammary gland development.

Differential display RT-PCR was performed on 6 RNA samples from virgin through to day 18 gestation and banding patterns were subsequently analyzed. Five bands were chosen to be analyzed further according to the expression profiles. These bands were cut from the dried gel, and the DNA was then eluted, reamplified and subsequently subcloned. Clone 1 showed an expression profile which increased throughout gestation. Sequence analysis identified this transcript as the milk protein β casein, and its expression profile was confirmed by Northern analysis. This observation mimics the profile documented for this particular milk protein and thus validates the technique [3]. Clone 2 showed an expression pattern which peaked on days 10 and 13 of gestation. This transcript had no homology to any documented sequence nor could its expression profile be confirmed by Northern analysis suggesting that it represented a transcript of low abundance. Clone 3 had a similar expression profile to clone 2, with peaks of expression on days 10 and 13 gestation. Searches of the sequence databases revealed no matches, but Northern analysis duplicated the profile of expression which we had seen on the display gel. Clone 4 represented a transcript which showed a gradual increase in expression from day 10 gestation onwards. Sequence analysis revealed that this transcript had homology to a human protein involved in the ubiquitin dependent protein degradation pathway [4]. The expression profile was again confirmed by Northern analysis. Finally clone 5 showed an expression profile of a transcript which appeared to be present only between days 13 and 15 gestation, with highest expression being on day 15. No sequence homology could be found for this transcript although we could detect a signal by Northern analysis (Fig 1), the expression profile of which matched that shown by differential display.

We are now in the process of isolating full length clones for each of these transcripts to enable us to further analyze their function in the developing mammary gland.

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References