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BRIEF COMMUNICATION

Northern analysis of temporal and spatial variation in milk protein expression during early mammary involution in dairy cows

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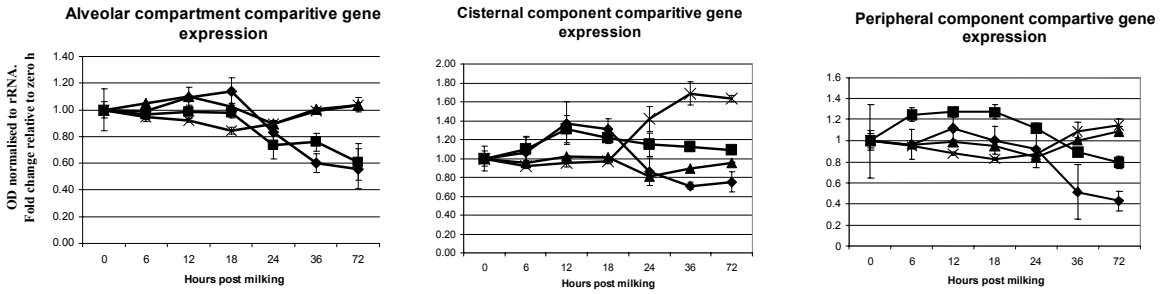
Keywords: Mammary, milk protein gene expression, local spatial variation, northern analysis.

INTRODUCTION

Understanding the mammary responses that occur at the cessation of milking whether natural, forced, or which occur at the end of the milking season and during the subsequent initiation of mammary involution (Holst *et al.*, 1987; Hurley, 1989) has relevance in milk production traits such as milk yield, persistency and extended milking intervals and may suggest ways of improving milk production in later lactation (Stelwagen, 2001) (Vetharaniam *et al.*, 2003). The proportions of secretory, support and milk storage components vary significantly between the alveolar (main milk synthesis), peripheral (a several cm thick layer of secretory tissue surrounding the main alveolar bed) and cisternal (main milk storage area adjoining the teats) compartments of the udder (Farr *et al.*, 1996; Sordillo and Nickerson, 1988; Weber, 1977). In addition, variations in the local proportions of these components has also been observed in the main alveolar areas and dramatic variations of gene expression depending on the local mammary tissue composition have been observed (Molenaar *et al.*, 1992). Ignorance of the effect on gene expression profiles resulting from sampling variations could lead to improper interpretation of experimental results and hence there is a need for more extensive spatial and temporal surveys to be done.

The aim of this experiment was to define the changes in two lactation and two involution/defence associated genes in the bovine udder during early mammary involution and test for the variation in expression at different sampling sites by northern analysis. The lactation genes characterised were α-lactalbumin and α-S1-casein, while the involution/defense genes were lactoferrin and mammary-serum-amyloid-A3 (MSAA). These genes were chosen because work in our laboratory has shown that they are strongly associated with the various mammary activities described above and exhibit differing responses to the same. α-Lactalbumin is one of the two major whey proteins in cow milk (the other being β-lactoglobulin) and its presence is central to the process of milk synthesis. It is a key and limiting protein in the synthesis of lactose and acts to change the substrate specificity of galactosyl transferase by enabling it to combine galactose and glucose in the formation of lactose (Mepham, 1987). Caseins are the most abundant group of milk proteins (McKenzie, 1967; Ginger and Grigor, 1999). They have an amino acid composition that is appropriate for growth and development of the nursing young (Lonnerdal, 1997). α-S1-caseins play an important role in the capacity of milk to transport calcium (Blake and Henning, 1988). Lactoferrin is an iron-binding glycoprotein that is present in milk and other secretions of mammals. Various functions have been ascribed to lactoferrin, including antibacterial, nutrition and iron transport (Sánchez *et al.*, 1992).

FIGURE 1: Changes in milk protein gene mRNAs for α-lactalbumin, α-S1-casein, Lactoferrin and Mammary Serum Amyloid A with increasing time since milking in alveolar, cisternal and peripheral tissues (n=6/time-point normalized to methylene blue staining of ribosomal and showing fold differences relative to immediately after milking (zero hours)).



Lactoferrin mRNA concentration in the mammary gland has been shown to be moderate during pregnancy until parturition, low throughout lactation, and markedly increased at the onset of involution (Schanbacher *et al.*, 1993; Molenaar *et al.*, 1996). The serum amyloid proteins are apolipoproteins (the protein component of a lipid protein complex). The serum amyloid A3 protein is one of the major reactants in the acute-phase response. Proteins of the serum amyloid (SAA) family are mainly synthesised in the liver, with concentrations of SAAs in the plasma rising up to 1000-fold (species dependent) in response to physical stress such as inflammation or infection. This suggests SAAs play an essential role in the immune response (Jensen and Whitehead, 1998). Various SAAs have been detected by *in-situ* hybridisation in a range of other tissues including; the epithelial cells of the intestine, pancreas and mammary glands (Urieli-Shoval *et al.*, 1998; Molenaar *et al.*, 2000).

The middle of the lactation cycle was selected for examination in order to maximise the chance of observing significant changes in genes expression due to the intervention and the specific time points were selected to encompass the defined changes in mammary physiology that have been noted by in our and other workers studies as opening of tight junctions (Stelwagen *et al.*, 1997) and apoptosis (Capuco and Akers, 1999) and gene expression (Schanbacher *et al.*, 1993; Molenaar *et al.*, 1996).

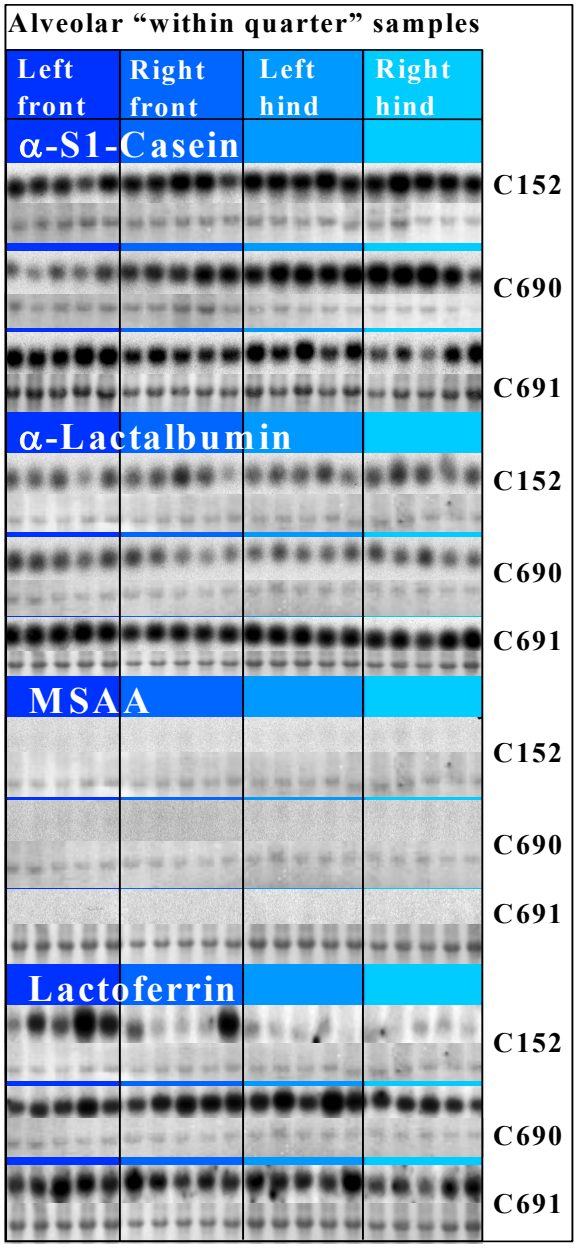
MATERIALS AND METHODS

Mammary tissue was taken from Friesian heifers in mid-lactation (six per time-point), following sacrifice at 0,6,12,18,24,36 and 72 hours (h) after milking. Samples of alveolar, peripheral and cisternal tissue were both snap frozen in liquid nitrogen and taken for histology. Additionally, in order to examine local variations in gene expression in the alveolar compartment, samples of alveolar tissue were taken from five different sites in each udder quarter of three 6 h post milking animals (within quarter). RNA was extracted from the samples and northern analysis performed using probes for the four milk protein mRNAs. Optical densities of the resulting bands after hybridisation were measured and normalised to the total RNA as seen by methlyene blue staining of Ribosomal RNA and then, for the timecourse series, represented graphically as fold differences compared to immediately after milking (zero hours post milking). The within quarter series results are presented as a composite image of the northern blots.

RESULTS

In alveolar tissue (Figure 1), the expression of α-S1-casein and α-lactalbumin was generally constant until 24 h where the expression of both genes rates declined variably between animals over the time-course. By 72 h this variation was pronounced. The raw and normalized

FIGURE 2: Composite northern blots showing changes in milk protein gene mRNAs for α-lactalbumin, α-S1-casein, Lactoferrin and Mammary Serum Amyloid A from 5 different alveolar sites in each quarter from 3 cows (C152, C690, C691) taken at 6 h post milking. The lactoferrin shows a long autoradiograph to better illustrate the variation within the quarters.



results revealed that there were a number of samples where the relative amounts of casein and α-lactalbumin expression varied compared to the majority of other samples in the same time-point. The expression of lactoferrin and MSAA increased around 36 h of non-milking and showed an inverse expression pattern to that of the milk protein genes. The expression profiles of lactoferrin and MSAA showed similar trends to each other, but some within-sample variance was observed. In cisternal and peripheral tissue, gene expression was

quite variable in all samples. In general, α-S1-casein and α-lactalbumin decreased over the time-course while lactoferrin and MSAA increased. The within-sample reciprocal expression relationship of the milk protein and the involution associated genes was maintained and also the frequency of variation in the relative amounts of casein and α-lactalbumin expression was greater than in alveolar tissues from the same time-point.

Figure 2 shows the variations in expression of the four genes in the 5 samples taken 'within quarter'. While they were generally similar, subtle differences were observed.

SUMMARY AND CONCLUSIONS

Significant changes in expression of the major and minor milk mRNAs are apparent by 24 h post milking and there is a clear trend showing the inverse pattern of expression of the two major milk mRNAs and the two involution/defence mRNAs. 'Within-quarter' and tissue-type site variations in gene expression indicates that sampling site effects must be taken into account when measuring any gene expression changes particularly in peripheral and cisternal tissue where there are greater differences in tissue composition such as an increase in the numbers of ducts and inactive alveoli. The observation that occasional variations in the relative within-sample amount of expression of the four genes examined is of interest because it suggests that the regulation of these genes while generally similar, is independently regulated to some degree in different tissue compartments, even in full lactation. This supports data observed in other studies of different lactational stages (Burditt *et al.*, 1981) (Molenaar *et al.*, 1995) (Molenaar *et al.*, 2003).

ACKNOWLEDGEMENT

This study was funded by FRST.

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