J. Dairy Sci. 90:2726–2734
doi:10.3168/jds.2006-778

Abundance and Phosphorylation State of Translation Initiation Factors in Mammary Glands of Lactating and Nonlactating Dairy Cows

C. A. Toerien and J. P. Cant[1]
Department of Animal and Poultry Science, University of Guelph, Ontario, N1G 2W1 Canada

ABSTRACT

To test if control of mRNA translation is involved in the increase in protein synthesis by mammary glands during lactation, cellular contents and phosphorylation states of translation factors and their upstream regulators were measured in mammary parenchyma from 12 nonpregnant dairy cows. For a 42-d period, 6 cows in late lactation continued to be milked (L) and 6 at the same stage of lactation were dried off (NL). All cows were then slaughtered and mammary glands and tissue samples obtained. Alveoli and lobules tended to be larger in L cows. Mammary parenchymal mass, cell number, cell size, and RNA, DNA, and protein contents were greater in L cows. Increases (3.1- and 1.8-fold) in the abundance of active, phosphorylated ribosomal protein S6 and its kinase, S6K1, respectively, in L vs. NL parenchyma indicated an ability to sustain greater rates of synthesis of translational machinery, which was also evident in the 102% increase in parenchymal RNA:DNA between the 2 groups. Cellular abundances of the main eukaryotic translation initiation factors (eIF), eIF2 and eIF4E, were 2.6- and 3-fold greater, respectively, in L cows. That these differences were greater than the 102% greater RNA:DNA in L mammary parenchyma suggests an elevated translational efficiency in L glands. Abundance of phosphorylated rpS6 was not different between mammary parenchyma and liver, whereas eIF2α was 50% greater in mammary tissue. In semimembranosus muscle, abundances of phosphorylated rpS6 and eIF2α were 3 to 4 times lower than in mammary parenchyma. In both L and NL mammary glands, 11% of eIF2α was in the inhibitory, phosphorylated form and 48 to 60% of eIF4E was complexed with its binding protein, 4EBP1. It is concluded that up-regulation of initiation of mRNA translation occurs in the fully differentiated milk secretory cell and that, where crucial initiation factors are not present in a maximally active form, the initiation rate might be flexible in response to external stimuli.
Key words: involution, lactation, mammary gland, mRNA translation

INTRODUCTION

Terminal differentiation of mammary secretory cells at the onset of lactation is characterized by the expression of milk protein genes. The lactogenic hormones prolactin, insulin, and cortisol synergistically activate transcription through receptor-mediated signaling pathways that converge at the promoters of several milk protein genes (Rosen et al., 1999). Transcriptional regulation clearly plays a role in the increase in fractional protein synthesis rates from 29 %/d in mammary glands of dry, nonpregnant goats to 108 %/d in lactating goats (Baracos et al., 1991). However, insulin and prolactin also up-regulate translation of milk protein mRNA in cultured mouse mammary epithelial cells during differentiation (Choi et al., 2004).

Acute regulation of protein synthesis in cells and tissues is controlled mainly at the point of translation initiation (Hershey, 1991) through phosphorylation of eukaryotic initiation factors (**eIF**) and associated proteins (Raught et al., 2001; Proud, 2005). This provides the organism with a rapid and reversible way to modulate protein synthesis in response to environmental stimuli such as consumption of a meal (Davis et al., 2000), change in amino acid concentrations (Kimball et al., 1991), and Ca^{2+} depletion (Kimball and Jefferson, 1992). The eIF bring the 2 ribosomal subunits, the mRNA and the initiator methionyl-tRNA, together as an initiation complex from which elongation of the peptide chain can proceed. The 2 most important factors for control of translation appear to be eIF2 and eIF4E (Raught et al., 2001; Proud, 2005). In addition, phosphorylation of ribosomal protein S6 (**rpS6**) is linked to an increase in the translation of parts of the translational machinery, including ribosomal proteins, eukaryotic elongation factors (**eEF**) 1A and 2, and poly(A)-binding proteins (Raught et al., 2001), so rpS6 plays a major role in modulating global rates of protein synthesis.

Received November 20, 2006.
Accepted February 26, 2007.
[1]Corresponding author: jcant@uoguelph.ca

The cellular content and phosphorylation state of eIF2, eIF4E, its binding protein 4EBP1, rpS6, and upstream regulators such as the rpS6 kinase (**S6K1**) and mammalian target of rapamycin (**mTOR**) have been demonstrated to be so universally up- or down-regulated in accord with the protein synthetic activity of tissues such as liver, muscle, and adipose as to be considered biomarkers of the process (Kimball et al., 2004). Expression of these biomarkers in mammary tissue has been studied very little. Prolactin increased the phosphorylation state of 4EBP1 in cultured mouse mammary epithelial cells pretreated with insulin (Choi et al., 2004). However, the stimulation of milk protein mRNA translation appeared to occur via lengthening of the poly(A) tail, specifically on milk protein mRNA (Choi et al., 2004), suggesting that mRNA is preferentially translated as opposed to degraded. Removal of all amino acids or only Leu from the culture media of bovine mammary epithelial cells caused a decrease in synthesis rate of the milk protein β-LG that was associated with dephosphorylation of 4EBP1 and S6K1 (Moshel et al., 2006). Furthermore, an inhibitor of the mTOR signaling pathway, by which amino acids are thought to activate protein synthesis (Nobukuni et al., 2005), significantly reduced the degree of reactivation of β-LG translation upon restoration of amino acid concentrations in the media (Moshel et al., 2006). From mammary tissue gathered in vivo, the level of eIF4E mRNA was 8 times greater in lactating cows than in postpubertal heifers (Long et al., 2001). No eIF4E mRNA was detected in mammary glands of prepubertal heifers (Long et al., 2001) and neither the abundance of eIF4E nor its distribution between biologically active and inactive forms were determined. To our knowledge, eIF2 has not been studied in mammary tissue. Christophersen et al. (2002) reported levels of eEF2 in the bovine mammary gland 20 and 50 times greater than those found in liver and skeletal muscle, respectively, and 2 times greater than in the mammary gland of a heifer.

Our hypothesis was that control of mRNA translation is involved in the increase in milk protein synthesis during lactation. According to this hypothesis, we predicted an increase in cell content or phosphorylation state of initiation factors and their associated regulators in secretory cells of the lactating mammary gland relative to nonlactating tissue. To test the hypothesis, we compared mammary content of major initiation factors and associated proteins between cows at the end of a normal lactation cycle and those at a similar stage but not lactating, in the absence of the confounding mammogenic and lactogenic effects of pregnancy.

MATERIALS AND METHODS

Animals and Treatments

All animal procedures were approved by the Animal Care Committee of the University of Guelph. Twelve multiparous cows were allocated randomly to 1 of 2 groups as they approached a daily milk yield of ~15 kg/d. During the next 42 d, the lactating group (**L**; initial DIM 293 ± 18; 17.4 ± 0.7 kg milk/d) continued to be milked twice daily at 0500 and 1500 h, whereas animals in the nonlactating group (**NL**; initial DIM 305 ± 25; 17.6 ± 0.8 kg milk/d) were dried off by abrupt cessation of milking. Cows in both treatments were housed in tie stalls with ad libitum access to hay (60% timothy:40% alfalfa). Lactating cows also received 1.43 kg (DM basis) of a concentrate containing 15.4% CP and 1.58 Mcal of NE_L/kg (Floradale Feed Mill Ltd., Ontario, Canada) following each milking. All cows were slaughtered at the end of their respective 42-d periods.

Blood and Tissue Collection

Three to 4 d before slaughter, blood was collected from the jugular vein of each animal at approximately 1000, 1300, 1600, and 1900 h. Samples were kept on ice until centrifuged for 15 min at 10,000 $\times g$ and plasma was stored at –20°C.

Lactating cows were milked 1 to 2 h before slaughter. At slaughter, the animals were stunned with a captive bolt, exsanguinated, and the mammary glands were removed. Four samples of parenchymal tissue (5 to 10 g each) were removed from the center of the right rear quarter of the mammary gland 2 to 5 cm below the attachment to the ventral body wall. Samples were wrapped in double-layered aluminum foil envelopes and frozen in liquid N_2. During collection, care was taken to avoid visible connective tissue. All samples were collected within 10 min of the start of exsanguination. Samples were double bagged and stored at –70°C. From 3 cows per group, 5 to 10 g of liver and semimembranosus muscle were also collected, processed, and stored in the same way as the mammary tissue samples.

Mammary Gland Composition

After sampling, mammary glands were weighed, double bagged, and stored at –20°C until dissected into skin, parenchyma, adipose, connective tissue, and lymphatic fractions. Each fraction was weighed with corrections for the weight of samples harvested and fluid losses while dissecting.

Plasma Analyses

Plasma samples were pooled for each cow and analyzed spectrophotometrically for glucose by enzyme-linked assay (kit no. 510-A; Sigma Chemical Co., Oakville, Ontario, Canada), α-amino N by the method of Evans et al. (1993), and insulin by RIA (Medicorp, Montreal, Quebec, Canada).