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– The role of myoepithelial cells in milk let-down

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Lottie Björkegren & Hanna Johansson

Supervisor: Sabine Ferneborg, Swedish University of Agricultural Sciences
Department of Animal Nutrition and Management
Sabine.Ferneborg@slu.se

Examiner: Sigrid Agenäs, Swedish University of Agricultural Sciences
Department of Animal Nutrition and Management
Sigrid.Agenas@slu.se

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Introduction

Today, international competition causes a high pressure on dairy production to improve efficiency and reduce costs (Swedish Dairy Association, 2011). Two crucial factors for an efficient dairy production are total milking time (Meyer & Burnside, 1987) and the degree of udder emptying at milk removal (Wilde *et al.*, 1989). A high milkability, or ease of milking, among cows decreases the time spent on milking. Milkability is usually measured in milk flow rates, with the goal being a short total milking time (Meyer & Burnside, 1987).

The mammary gland consists of secretory epithelial cells organized in alveoli, the alveoli are in its turn organized in lobes and lobules. From the alveoli, milk ducts of an increasing size leads to gland and teat cisterns. The alveoli and the ducts are surrounded by a capillary network and myoepithelial cells (Sjaastad *et al.*, 2003). As a response to tactile stimulation of the udder the milk ejection reflex, a neuroendocrine reflex, takes place and the hormone oxytocin (OT) is released from the posterior pituitary gland (Svennersten-Sjaunja, 2004). OT is produced both in the hypothalamus and locally in the myoepithelial cells (Cassoni *et al.*, 2006) and affects the myoepithelial cells surrounding the alveoli in the mammary gland (Svennersten-Sjaunja, 2004). When OT binds to receptors on the myoepithelial cells a long chain of reactions takes place which leads to a contraction of the myoepithelial cells (Nakano *et al.*, 2001).When the myoepithelial cells surrounding the alveolus contract, milk let-down takes place (Richardson, 2009).

When a cow is milked without prestimulation the milk ejection reflex and milk let-down is delayed (Bruckmaier & Hilger, 2001) and the total milking time is prolonged. At milk let-down milk is shifted from the alveolar compartments to the ducts and cisternal cavities of the mammary gland which makes it available for being milked out. Lack of milk ejection reflex and the following milk let-down thereby makes it impossible to retrieve a majority of the milk (Knight *et al.*, 1994). Thus, a good milk ejection reflex and following milk let-down is important for an efficient dairy production.

Up until recently myoepithelial cells have not been considered interesting enough for further research but due to their important role in milk let-down this is changing (Moumen *et al.*, 2011). This literature review aims to look further into the role of myoepithelial cells during milk let-down. The objective is to explain how the myoepithelial cell is contracting and which factors that control or influence contraction and relaxation of the myoepithelial cell. In order to fill the gaps left by the lack of studies on myoepithelial cells articles on smooth muscle cells are used.

Literature Review

The myoepithelial cells surrounding the alveoli

The mammary gland development starts already during foetal life in all mammalian species (Svennersten-Sjaunja & Olsson, 2005). During sexual maturation most of the branching of the

ducts takes place and the secretory tissue develops during the pregnancy. The mammary gland is built up by secretory alveoli that are arranged in lobuli and branched out to larger ducts leading to the teats (Moumen *et al.*, 2011). During the whole development of the mammary gland, precursors of myoepithelial cells are present on the branching ducts. In the early development the basal layer of cells can develop into either epithelial or myoepithelial cells and can therefore be thought of as stem cells (Sopel *et al.*, 2010). The mammary epithelium consists of two cell types, the luminal and the basal cells. Already during the embryonic development they are distinctly different both in function and in phenotype. The luminal epithelial cells are secretory cells producing milk, whereas the basal cells are myoepithelial cells able to contract when stimulated (Moumen *et al.*, 2011). The myoepithelial cells are located between the secretory cells and the extracellular matrix and is therefore believed to have an active role in mediating information in the mammary gland (Sopel *el al.*, 2010).The organization of the myoepithelial cells differs between the alveoli and the ducts. The myoepithelial cells are formed in a more continuous way in the ducts and are more star-shaped surrounding the alveoli (Moumen *et al.*, 2011).

Myoepithelial cell morphology. The myoepithelial cell morphology resembles that of smooth muscle cells (Richardson, 2009). The difference between smooth muscle and myoepithelial muscle is the origin. Smooth muscle cells originate from mesoderm and the myoepithelium from the ectoderm (Moumen *et al.*, 2011). The myoepithelial cell contains few cytoplasmic organelles, a dense nuclei, a large portion of myosin and actin filaments and the cell membrane has an irregular shape. Myoepithelial cells isolated from fetal, nonpregnant and nonlactating, pregnant and nonlactating, and nonpregnant lactating cows are all of a similar size, 80-160µ, and morphology (Zavizion *et al.*, 1992).

One form of actin is smooth muscle alpha-actin (ACTA2) which is a crucial factor for the myoepithelial cell's contractility. Mice lacking ACTA2 has no visible defects in mammary cell structure, but the response to OT is reduced and milk ejection is impaired (Haaksma *et al.*, 2011).

Myosin is composed of four units, two heavy chains, which are of high molecular weight, and two light chains, of low molecular weight. The myosin filament can be described as intertwined coils with protruding globular head regions reappearing in regular intervals. The head regions contain the light chains and make up the catalytic site for binding to actin. When myosin and actin binds through ATP hydrolysis, the cell contracts (Kamm & Stull, 1985).

Contraction of the myoepithelial cell

Contraction of the myoepithelial cells decreases the alveolar volume and thereby forces the milk from the alveolar compartments to the ducts and the cisternal cavities (Nishimori *et al*., 1996). Contraction of the myoepithelial cells is usually considered to be due to the influence of OT, but ATP and vasopressin also influence the myoepithelial cells (Nakano *et al*., 2001, Olsson *et al*., 2003). There are several different pathways within the myoepithelial cell leading to contraction, see Figure 1 in Appendix 1. The different pathways are initiated by the binding of ligands to receptors for OT and ATP (Nakano *et al.*, 2001, Reversi *et al*., 2005,

Raymond *et al*., 2011). In goats vasopressin increases milk flow in a way similar to the way of OT. This similarity indicates that vasopressin can act on the OT receptors on the myoepithelial cells in some species (Olsson *et al*., 2003). The contraction of myoepithelial cells is suggested to be tonic (Nakano *et al.,* 2001).

A study by Moore *et al* (1987) demonstrated that not all myoepithelial cells contract as a response to OT stimuli. Different explanations discussed where if OT did not reach all cells, or if cells where close to undergo apoptosis. The most likely hypothesis this study concluded was that the cells lacked the receptors needed for OT response. From an evolutionary point of view there is no need for the myoepithelial cells to contract if there are not enough milk components in the alveoli. Therefore, only myoepithelial cells surrounding the alveoli ready to secrete milk express the appropriate OT receptor (Moore *et al.,* 1987). The availability for OT response could be due to the placing of specific lipid rafts on the myoepithelial surface which hides the receptors when the alveoli are empty. These specialized membrane components may be connected in some way with the pathways regulating the OT response in the myoepithelial cells (Reversi *et al*., 2006).

Activation of OT and purinergic receptors. The OT and purinergic receptors seem to be G-protein coupled (Nakano et al., 2001). If a receptor is coupled to a G-protein it means that when the receptor structure is changed due to the binding of a ligand the structure of the G-protein changes. The G-protein is located on the inside of the cell membrane and it is the G-protein which starts the intracellular reactions (Stryer *et al*., 2006).

The Phospholipase C/Ca^{2+}pathway. When OT binds to its receptor on the myoepithelial cell it provides a start of the Phospholipase C (PLC)/Ca^{2+} pathways (Reversi et al., 2005). In the PLC/Ca^{2+} pathways the stimulation of OT receptors leads to the activation of the enzyme phospholipase C. Phospholipase C in its turn hydrolyses Phosphatidylinositol 4,5-bisphosphate and releases diacylglycerol and IP3. Diacylglycerol activates protein kinase C (PKC) and IP3 binds to receptorchannels in the endoplasmic reticulum and opens these channels so that intracellular Ca^{2+} is released (Nakano et al., 2001). PKC in its turn phosphorylates the myosin light chain (MLC) (Endo et al., 1982). A higher concentration of Ca^{2+} is associated with activation of myosin light chain kinase (MLCK) which phosphorylates MLC (Somlyo & Somlyo, 1994).

The results of Nakano *et al* (2001) contradict those of Moore *et al* (1987) and Olins & Bremel (1982). Both Moore *et al* (1987) and Olins & Bremel (1982) showed results indicating the importance of extracellular Ca^{2+}. According to Nakano *et al* (2001) the Ca^{2+} concentration increase due to the PLC/Ca^{2+} pathway hyperpolarizes or depolarizes the membrane potential. The reason for the hyper- or depolarization is probably activation of Ca^{2+} sensitive channels (Nakano *et al*., 2001). In smooth muscle cells a depolarization leads to activation of Ca^{2+} membrane channels and increases the influx of extracellular Ca^{2+} (Sjaastad *et al*., 2003). This could provide an explanation for the contradicting results of different studies.

There is also evidence showing that the presence of ATP has a synergistic effect on the

oxytocin response in mice myoepithelial cells. ATP binds to purinergic receptors on the myoepithelial cells and enhances the stimulation of OT on the PLC/Ca^{2+} pathway. It is reasonable to believe that the contraction of the myoepithelial cells cause mechanical stress on the secretory epithelial cells. This mechanical stress could cause the secretory epithelial cells to release ATP and thus stimulate further contraction of the myoepithelial cells (Nakano *et al*., 2001).

The RhoA-ROCK pathway. An increased amount of OT increases the amount of RhoA (Raymond et al., 2011). RhoA is a small enzyme of the type GTPase which together with its antagonist Rac regulates several aspects concerning cell adhesion and motility. The fact that they are antagonists means that when RhoA increases, Rac decreases and vice versa and they have opposite effects on the contractility of the cell. RhoA increases the contractility of the cell and Rac decreases it (Huveneers & Danen, 2009). When the amount of RhoA increases, so does the amount of its effector protein Rho-kinase (ROCK). ROCK in its turn has two important roles in the cell contraction. Firstly ROCK phosphorylates MLC which directly leads to contraction (Amano et al., 1996). On the other hand ROCK inhibits myosin light chain phosphatase (MLCP) which dephosphorylates MLC and thereby inactivates the myosin heads (Somlyo & Somlyo, 2003).

Activation of myosin through phosphorylation. Contraction of myoepithelial cell is dependent on actin and myosin acting together (Kendrick-Jones & Scholey, 1981), and is regulated by phosphorylation of MLC (Hartshorne et al., 1989). In smooth muscles the phosphorylation of the myosin heads activates them and makes binding to the actin possible (Sjaastad et al., 2003). The myoepithelial cell contraction works in a similar way. Myosin needs to be phosphorylated to interact with actin and this binding leads to contraction (Kendrick-Jones & Scholey, 1981).

Relaxation of the myoepithelial cell

As with contraction, there are several different factors which can cause relaxation of the myoepithelial cell (see Figure 1 in Appendix 1). One signaling pathway which causes relaxation is the Focal Adhesion Kinase/Rac (FAK/Rac) pathway. This pathway is strongly intertwined with the RohA/ROCK pathway causing contraction (Raymond *et al*., 2011). It is the balance between RohA and Rac which determines the contractile ability of the cell (Huveneers & Danen, 2009). When FAK is activated through phosphorylation due to α3β1 integrin signalling the balance tips over in favour of Rac. Integrins are receptors which connects the cytoskeleton to the extracellular matrix (Hynes, 2002, Vincente-Manzanares *et al*., 2009). When the level of Rac increases its effectors molecule, p21-activated kinase (PAK) decreases the activity of MLCK (Sanders *et al*., 1999). When there is no longer an effect of MLCK the myoepithelial cell relaxes (Raymond *et al*., 2011).

Another factor involved in relaxation is parathormone related peptide (PTHrP) which is believed to be involved in the discontinuation of Ca^{2+} influx resulting in relaxation (Seitz *et al*., 1993).

Discussion

The majority of the pathways leading to contraction and relaxation are dependent on Rho and Rac and integrins regulating those (Raymond *et al*., 2011). The Rho and Rac pathways are regulating several aspects concerning cell-cell adhesion, contact between the cell and the extra cellular matrix and cell motility (Huveneers & Danen, 2009). This makes it likely that the individual myoepithelial cells are interacting with each other during contraction and relaxation.

According to Nakano *et al* (2001) the contraction of the myoepithelial cell is tonic. This is based on the fact that the shape of the myoepithelial cell was thick as long as a stimulus was provided. Nakano *et al* (2001) is the only study which actually mentions if the contraction is tonic or pulsating. However, no information was found which supports the hypothesis of the contraction being pulsating.

The cells probably do not contract if it is not necessary. So logically, there is some sort of control mechanism. This theory is discussed by Moore *et al* (1987) who provide a hypothesis that the cells surrounding alveoli not containing milk lacks the appropriate receptors. Reversi *et al* (2006) suggests that lipid rafts on the cell surface could regulate the OT response. Another theory could be that all cells in a lobe have to contract in order to prevent milk from contracting alveoli to be pushed into adjacent relaxed alveoli.

It is important that the whole contraction-relaxation cycle is completed. When α3β1-integrins are lacking from the cell surface, the contractile function of the cell is impaired. This is probably due to the antagonists RohA, which is involved in contraction and Rac, which is involved in relaxation. In the contraction-relaxation cycle RohA and Rac are balancing each other so if response from one of them is lacking the cycle becomes incomplete (Raymond *et al*., 2011).

Two enzymes which both phosphorylate the myosin light chain and thereby enable contraction are PKC and MLCK. Whether or not PKC and MLCK in fact is the same enzyme is unclear.

Conclusions

The contraction and relaxation of myoepithelial cells are under strict control of several different signaling pathways dependent on many factors. OT is often mentioned as the regulating factor for contraction of myoepithelial cells and milk let-down, but it is not the only factor. The contraction is likely to be tonic and it is unlikely that a cell contracts if it is not necessary. There are probably pathways involved in the contraction and relaxation of myoepithelial cells which remains unknown. More studies are needed to fully understand this topic.

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Appendix 1

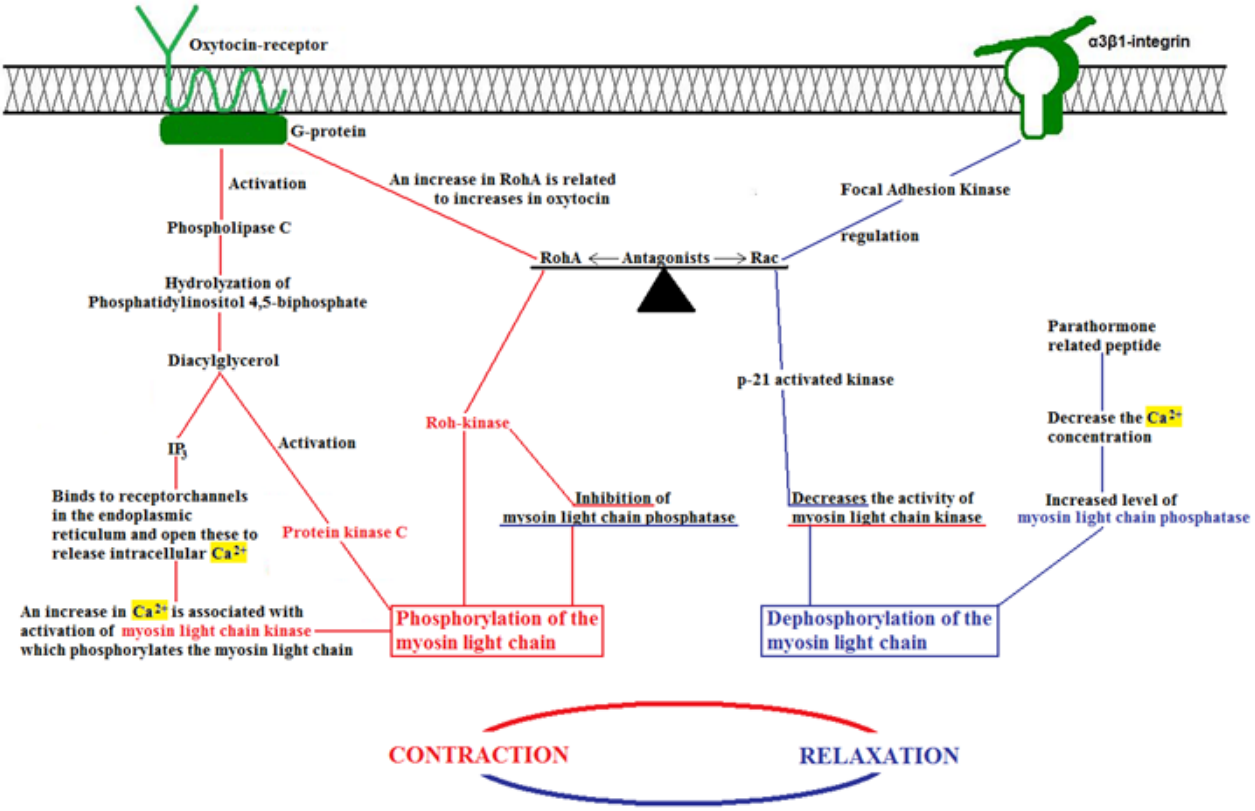

Figure 1. The cellular pathways leading to contraction and relaxation.

Words marked in red are factors directly associated with contraction, while words underlined with red are actions that lead to contraction by affecting a factor important for relaxation (underlined with blue). Words marked in blue are factors directly associated with relaxation, while words underlined with blue are actions that lead to relaxation by affecting a factor important for contraction (underlined with red).

The figure is modified from Raymond *et al.*, 2011, with additional information from Endo *et al.*, 1982, Seitz *et al.*, 1993, Somlyo & Somlyo, 1994, Amano *et al.*, 1996, Sanders *et al.*, 1999, Nakano *et al.*, 2001, Somlyo & Somlyo, 2003 and Huveneers & Danen, 2009.