The Tammar Wallaby – a Model to Examine Endocrine and Local Control of Lactation

Amelia Brennan*, Julie Sharp, Matthew Digby and Kevin Nicholas
CRC for Innovative Dairy Products, Department of Zoology, University of Melbourne, VIC 3010
*Corresponding author: a.brennan2@pgrad.unimelb.edu.au

The Tammar as a Model System to Study Lactation

Most of our laboratory and livestock species have evolved over millions of years, and in some cases many decades of intensive selective breeding to improve the quality of milk produced – but can the wallaby reveal its ancient secrets of regulating lactation to increase our understanding of the lactation cycle? The increasingly popular approach of bioprospecting for genes and bioactive molecules from native animals and plants provides new resources for the advancement of agriculture and biotechnology. The application of new technology to species with unusual reproductive strategies allows the identification and study of regulatory mechanisms and molecules that are present but not readily apparent in other species.

Marsupials, such as the tammar wallaby (Macropus eugenii), have adopted a reproductive strategy that is very different to eutherians (placental mammals) (1,2). It includes a short gestation of 26 days, birth of an immature young and a relatively long lactation of 300 days. Both the rate of production and the composition of milk, particularly the proteins, change progressively during the lactation cycle to meet the nutritional demands required for the considerable development of the pouch young prior to weaning (Fig. 1a). We know that the lactating tammar regulates these changes in milk composition, which in turn determines the rate of pouch young growth and development. If a pouch young at an early stage of lactation is transferred to the mammary gland of a tammar at a later stage of lactation, the fostered young will grow and develop at an accelerated rate (3). Much of the equivalent development in eutherians occurs in utero. Therefore it is possible that many of the biological factors that regulate development of eutherian embryos are delivered in the milk by the mammary gland of marsupials. This allows researchers to access all the bioactives in milk, to examine the processes by which the mammary gland produces these factors and examine their potential function.

Monotremes (extant egg laying mammals, platypus and echidna) and marsupials both begin consuming milk as a very underdeveloped neonate. Eutherians appear to have developed a more complex placenta for their extended period in utero, giving birth to a more developed neonate. Indeed, eutherians begin to drink large quantities of uterine milk, or amniotic fluid from about mid-term. As all mammals

Fig. 1. The lactation cycle of the tammar wallaby.
(a) Development of the pouch young from day 6 to day 220 of age.
(b) The lactation cycle in the tammar has been divided into four phases characterised by changes in milk composition and the suckling pattern of the pouch young.
(c) Expression of the major milk protein genes during the lactation cycle. The α-casein, β-casein, α-lactalbumin and β-lactoglobulin genes are induced at parturition and expression remains elevated for the entire lactation. The genes for ELP (early lactation protein), WAP (whey acidic protein) and the LLPs (late lactation proteins A and B) are expressed only for specific phases of the lactation cycle.
commence drinking at an early developmental stage, it may be considered that amniotic fluid and colostrum represent a transition from mammary gland-driven development in monotremes and marsupials to in utero driven development in the eutherian foetus. Colostrum is known to contain a large array of growth-promoting activities, which specifically regulate many different physiological processes. Therefore, it is likely that the milk secreted by tammars in early lactation will share some similar active factors with the colostrum of eutherians. In practical terms, the tammar is an excellent and convenient model to discover and assess the physiological roles of specific bioactives. Therefore use of the tammar to study changes in milk composition, and in particular the genes coding for proteins secreted in the milk, is of great interest.

**Milk Protein Gene Expression; Naturally Occurring Gene Knockouts**

Lactation in the tammar has been divided into phases (Fig. 1b; 1,2) that are defined by the composition of the milk and the apparent suckling pattern of the young. Phase 1 is a 26.5-day pregnancy and the subsequent 200 days of phase 2 is characterised by lactogenesis and the secretion of small volumes of dilute milk high in carbohydrate and low in fat and protein. The pouch young remains attached to the teat for the first 100-110 days (phase 2A), after which it relinquishes the teat, suckling less frequently while remaining permanently in the pouch (phase 2B) for an additional 90-100 days. Phase 3 of lactation (200-330 days) is characterised by dramatic changes in milk production and composition with an elevated level of protein and lipid and low levels of carbohydrate (3,4). Broadly there are two groups of milk protein genes expressed during the lactation cycle; one group of genes is induced to high levels around parturition and expressed throughout lactation and a second group of genes is expressed only during specific phases of lactation (Fig. 1c). For example, the genes for the whey proteins β-lactoglobulin and α-lactalbumin, and the α-casein and β-casein genes are induced coordinately and independently of the suckling stimulus at parturition, and are expressed for the duration of lactation. In contrast, the early lactation protein (ELP) gene is expressed at very high levels in phase 2A, the whey acidic protein (WAP) gene most highly in phase 2B and two genes coding for lipocalin-like proteins referred to as late lactation protein (LLP) A and B are highly expressed in phase 3. Orthologues of the proteins are found in other species and the tammar is now emerging as a valuable model to provide a representation of the young or mammary function. Orthologues of the proteins are found in other species and the tammar is now emerging as a valuable model to provide a representation of the young or mammary function. Orthologues of the proteins are found in other species and the tammar is now emerging as a valuable model to provide a representation of the young or mammary function.

The expression of milk protein genes is regulated concurrently by endocrine factors, paracrine factors such as the extracellular matrix, and by autocrine factors secreted in the milk. Previous studies using a tammar mammary explant culture model have shown different combinations of insulin, cortisol and prolactin play a role in expression of the α- and β-casein and whey protein genes including α-lactalbumin and β-lactoglobulin (1,2). Interestingly, tammar explants from late pregnant tammars can be induced to express the WAP gene with insulin, cortisol, prolactin, thyroid hormone and estrogen (5). Therefore, the inhibition normally observed in vivo during phase 2A and the subsequent induction of WAP gene expression around 100 days post-partum may be hormonally regulated.
Alternatively, the LLP genes can be down-regulated in explants from phase 3 tammars and then re-stimulated with insulin, cortisol and prolactin, but expression of these genes cannot be induced in mammary explants from pregnant tammars with any hormone combination tested (14). Either the appropriate hormonal milieu was not used or the tissue requires additional factors to express these genes. This conclusion is supported by an earlier study showing that constructs with up to 1.8 kb of the LLP-A promoter did not express a reporter gene following transfection into CHO cells incubated with insulin, cortisol and prolactin. In addition, the same construct was not expressed in transgenic mice (14).

The tammar may perform asynchronous concurrent lactation, whereby the mother provides milk for an older animal which is out of the pouch and at heel, and milk of entirely different composition from an adjacent mammary gland for a newborn pouch young (Fig. 2). This is consistent with mammary function being regulated by factors intrinsic to the gland when both glands are exposed to the same milieu of hormones. This is consistent with mammary gland function being regulated by factors intrinsic to the gland when both glands are exposed to the same milieu of hormones.

There is increasing evidence to suggest that milk plays an important role in regulating mammary epithelial function and survival. Apoptosis was induced preferentially in sealed teats of lactating mice, while the litter suckled successfully on the remainder. This observation indicates cell death is stimulated by an intra-mammary mechanism sensitive to milk accumulation (15). Milk factors involved in the control of mammary gland function have been identified in a number of species. A protein known as the feedback inhibitor of lactation (FIL) is secreted in the milk of the tammar (16) and other species and acts specifically as the accumulation of local factors in the milk, may be an important role in regulating mammary epithelial cells in the tammar wallaby mammary gland. More recent studies using the tammar mammary explant culture model to examine the process of involution have confirmed the role of milk, and putative autocrine factors, for controlling mammary function during involution. Mammary explants from pregnant tammars were cultured for three days with insulin, cortisol and prolactin to induce expression of the milk protein genes and then to mimic involution all hormones were removed from the culture media for 10 days to down-regulate expression of the milk protein genes (Fig. 3). The tissue was then examined for signs of induced apoptosis. Surprisingly, the explants retained the same level of response during a subsequent challenge with lactogenic hormones. Previous studies have shown that there is limited secretion of milk proteins from tammar mammary explants but it is unlikely that milk constituents accumulate to elevated concentrations (20). Therefore, the maintenance of epithelial cell viability and hormone responsiveness in explants cultured in the absence of hormones suggests another mechanism, such as the accumulation of local factors in the milk, may be the primary stimulus for apoptosis of mammary epithelial cells in the tammar wallaby mammary gland.

Approximately three days post-weaning, the ratio of extracellular matrix-degrading proteinases to inhibitors increases and the basement membrane is degraded (19). This irreversible process follows a decline in the concentration of some of the circulating lactogenic hormones, and the accumulation of milk in the mammary gland. More recent studies using the tammar mammary explant culture model to examine the process of involution have confirmed the role of milk, and putative autocrine factors, for controlling mammary function during involution. Mammary explants from pregnant tammars were cultured for three days with insulin, cortisol and prolactin to induce expression of the milk protein genes and then to mimic involution all hormones were removed from the culture media for 10 days to down-regulate expression of the milk protein genes (Fig. 3). The tissue was then examined for signs of induced apoptosis. Surprisingly, the explants retained the same level of response during a subsequent challenge with lactogenic hormones. Previous studies have shown that there is limited secretion of milk proteins from tammar mammary explants but it is unlikely that milk constituents accumulate to elevated concentrations (20). Therefore, the maintenance of epithelial cell viability and hormone responsiveness in explants cultured in the absence of hormones suggests another mechanism, such as the accumulation of local factors in the milk, may be the primary stimulus for apoptosis of mammary epithelial cells in the tammar wallaby mammary gland.
Conclusion

Animal models with extreme adaptation to lactation have different reproductive strategies to most laboratory and livestock models but contribute to an improved understanding of the lactation cycle, and the relationship between milk components and neonatal development. The genome of the opossum has been sequenced and the tammar genome is currently being sequenced. Comparative genomics and bioinformatics are now exploiting these models to provide new opportunities and a selective advantage for Australian industry to remain competitive in a global market.

References