Minireview

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Prolactin Signaling in Mammary Gland Development*

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It has now been over 60 years since Riddle *et al.* (1) purified a hormone from the anterior pituitary gland, which stimulated milk secretion in the mammary gland of virgin rabbits. They named it prolactin (PRL).[1] Since then, the synergistic approaches of biochemistry, physiology, molecular biology, and cell biology have unveiled several molecular switches in the PRL signaling cascade (Fig. 1). Loss-of-function studies in the mouse have now provided clear insight into the biology of two components of the PRL pathway. A mandatory role for the prolactin receptor (PRLR) and for the signal transducer and activator of transcription (Stat) 5a in mammopoiesis and lactogenesis was established (2, 3). Although Stat5a is in the line of fire of many signals such as PRL, growth hormone (GH), and several cytokines, its absence *in vivo* reveals an unexpected level of specificity.

Prolactin and Its Receptor

PRL is a 23-kDa peptide, which is mainly synthesized in lactotrophic cells of the anterior pituitary of vertebrates. Many functions have been attributed to this hormone. PRL regulates gonadal functions (4) and behavior such as nest building and the retrieval of offspring (5), and it exerts multiple effects on the immune system (6). The best characterized role of prolactin, however, is its ability to induce lobuloalveolar growth in the mammary gland (7) and to stimulate postpartum lactogenesis. These properties are mediated through the activation of genes involved in growth control and differentiation.

The cloning of the PRLR in 1988 (8) unleashed efforts to elucidate the cascade of molecular switches linking the receptor with the target genes. The PRLR is a single chain transmembrane protein that belongs to the cytokine receptor superfamily and is expressed in a wide variety of tissues. Alternative splice products of the primary transcript yield PRLRs with short and long cytoplasmic tails. Both forms of the PRLR can dimerize upon ligand binding and activate the Janus kinase 2 (Jak2), Fyn, and mitogen-activated protein kinase to promote cell growth (9, 10). However, only the long form does activate transcription of the β-casein gene in transfected tissue culture cells (11). Jak2 in turn phosphorylates specific tyrosine residues in Stat1, -3 and -5 (9, 10). These activated STAT proteins bind to and induce transcription from promoters containing γ-interferon activation sites (GAS) (TTCNNNGAA) (10).

PRL-induced Genes

In a landmark experiment in 1965, Topper and colleagues (12) demonstrated that synergistic signaling by insulin, hydrocortisone, and PRL is required to produce casein in organ explants from mammary tissue. Targeted efforts in the late 1970s and early 1980s led to the cloning of milk protein genes (13–16). Thereafter cell lines derived from mammary tissue and studies using transgenic animals were instrumental in the identification, characterization, and biological verification of PRL response elements in the promoters of genes expressed specifically in the mammary gland (17–22). By 1988 it was clear that promoter sequences from the genes encoding the whey acidic protein (WAP) and β-lactoglobulin contain sufficient genetic information to target transcription exclusively to mammary tissue and to respond to PRL signals (23, 24). In the quest for the identification of PRL response elements, a sequence (GAS) in the promoter of the β-casein gene was identified, which was specifically recognized by a phosphorylated nuclear protein from mammary tissue. This protein was named mammary gland factor (MGF) (25). Transfection experiments of mutated promoter fragments into HC11 cells led to the demonstration that GAS (TTCNNNGAA) convey PRL responsiveness and are recognized by MGF (25). Experiments using transgenic mice demonstrated that GAS in the promoters of the genes encoding WAP (17) and β-lactoglobulin (21, 22) are critical for maximal gene activity and PRL response *in vivo*. MGF by itself, however, is not sufficient for optimal activity, and it may cooperate with juxtaposed transcription factors, such as nuclear factor 1 (17). MGF was cloned in 1994 from sheep mammary tissue and recognized as a new member of the family of STAT proteins (26). In the mouse Stat5 exists as two isoforms (5a and 5b) with a 96% similarity (27).

The JAK-STAT Pathway

PRL, GH, epidermal growth factor, erythropoietin, and many cytokines use STAT proteins to regulate the transcription of specific genes through the JAK-STAT pathway (10). Ligand binding triggers dimerization or oligomerization of receptors. Receptor-associated tyrosine kinases (JAKs) cross-phosphorylate each other as well as the tyrosine residues on the receptors. Subsequently, SH2-containing latent cytoplasmic proteins from the STAT family are recruited to the receptor complex and phosphorylated by the JAKs. Two STAT proteins dimerize, translocate into the nucleus, and activate gene transcription by binding to GAS in gene promoters. The ability of individual receptors (cytokines, GH, PRL) to activate overlapping but distinct sets of homo- and heterodimerizing STAT proteins is thought to contribute to their signal specificity. For example, interferon α activates Stats 1, 2, and 3 and exerts a growth-retarding effect. PRL, in contrast, activates Stats 1, 3, and 5 in many cell lines. It induces transcription from the promoter of the β-casein gene in mammary epithelial cells and causes proliferation of Nb2 lymphoid cells. Stat5a and Stat5b are expressed in most, if not all, tissues, and they can be activated in tissue culture cells by PRL, GH, epidermal growth factor, and many cytokines (9). This suggests that Stat5 transcription factors are components of different signaling pathways leading to cell growth and differentiation. In the mammary gland, Stat5a and -5b are activated by PRL and probably placental lactogen.

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[1] The abbreviations used are: PRL, prolactin; PRLR, prolactin receptor; STAT, signal transducer and activator of transcription; GH, growth hormone; Jak2, Janus kinase 2; GAS, γ-interferon activation site(s); WAP, whey acidic protein; MGF, mammary gland factor.

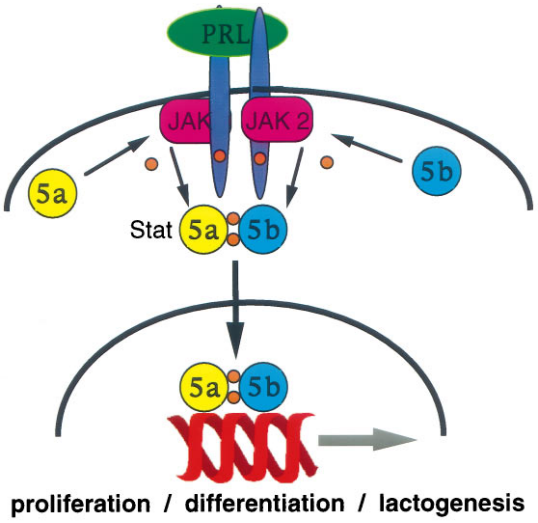

FIG. 1. **Prolactin signaling in the mammary gland.** PRL binds to its receptor and causes the PRLR to dimerize. Receptor-associated tyrosine kinase Jak2 phosphorylates the prolactin receptor and the signal transducers and activators of transcription Stat5a and Stat5b. Activated Stat5a and -5b are transported into the nucleus, bind to GAS sequences (TTCNNNGAA), and induce transcription of target genes that promote proliferation, differentiation, and lactogenesis.

Mammopoiesis and Lactogenesis

Functional differentiation of the mammary gland is a crucial step in the reproductive cycle of mammals (28). The development of the gland proceeds in distinct phases. In newborn mice a rudimentary system of small ducts is present, which grows slowly until the onset of puberty when pronounced ductal growth occurs. Development of the ducts continues in cycling virgins leading to the formation of a ductal tree, which fills the entire mammary fat pad. Terminal differentiation of alveolar epithelial cells is completed at the end of gestation with the onset of milk secretion at parturition. Distinct steps of cellular differentiation take place during this process, which are defined by the sequential activation of genes coding for milk proteins (29). Stricker and Grueter (30) demonstrated in 1928 that pituitary extract injected into castrated virgin rabbits induced milk secretion; this was a harbinger that PRL is essential for mammary function. However, the presence of lactogenic hormones is not sufficient to explain the complex development and temporal regulation of gene expression observed in the emerging gland during pregnancy. Estrogen and progesterone are required for ductal outgrowth (31) and alveolar proliferation (32), respectively. In addition, growth regulators and cell cycle progression are obligatory for alveolar proliferation and differentiation.

PRL is a central player in the cast of characters. Alveolar proliferation and the induction of terminal differentiation, as indicated by the transcriptional activation of milk protein genes, require the presence of prolactin (20, 28, 33, 34). In the functional postpartum gland high levels of activated Stat5 can be found while only small amounts of phosphorylated Stat1 and Stat3 have been detected (35). Phosphorylation of Stat5a and -5b is very low in mammary tissue of virgins and during early pregnancy but rises sharply after day 14 of pregnancy. This led to the hypothesis that the activation of Stat5 is a critical step in the terminal differentiation of mammary secretory epithelium (35).

Genetic Disruptions of the PRL Pathway

Evidence that PRL signaling induces mammopoiesis and lactogenesis comes largely from studies with organ cultures and cell lines. Similarly, our knowledge of the roles of Stat5 in PRL, GH, and cytokine signaling comes primarily from tissue culture cells. Nonetheless, these observations fall short of uncovering the roles of PRL and Stat5a and -5b in normal physiological processes *in vivo*. The generation of mice, from which the genes encoding the PRLR, Stat5a, and Stat5b[2,3] have been inactivated, has now provided clear insight into their biology *in vivo* (2, 3). Since the PRLR is expressed in many organs of the developing fetus and Stat5s are in the line of fire of multiple signaling pathways, late fetal or neonatal lethality could have been expected in the absence of these proteins. In contrast, mice deficient in PRLR or Stat5a or Stat5b were born and survived until adulthood. Defects were confined to a few tissues and specific physiological conditions.

Females with only one intact PRLR allele failed to lactate after their first pregnancy due to greatly reduced mammary development (3). Clearly epithelial cell proliferation during pregnancy depends on a threshold of PRLR, which cannot be obtained with just one functional allele. However, mammary gland development after the second pregnancy was sufficient for a successful lactation, demonstrating that continued hormonal stimuli will eventually lead to functional development. It is currently not clear whether other growth pathways will compensate for a decreased PRLR population. In contrast, the progesterone (32) and estrogen (31) receptors, although essential for mammopoiesis, mediate functional development in the presence of only one functional allele.

The only noticeable phenotype of Stat5a-deficient mice is their inability to lactate due to a failure of the gland to develop and differentiate during pregnancy (2). Whether this is due to a unique property of Stat5a relative to Stat5b is unclear. Stat5a and Stat5b exhibit superimposable expression patterns during mammary gland development (35) and have a 96% similarity, although the majority of the differences lie in the transcriptional activation domain at the carboxyl termini (27, 36). The observation that mice heterozygous for the PRLR deficiency exhibit a comparable phenotype suggests that a 50% reduction in signaling capability can cause a lactation deficiency. In addition, the disruption of the Stat5a locus also clearly had an effect on the amount of phosphorylated Stat5b (2). Whether the Stat5 proteins have unique functions will also be addressed by the derivation of Stat5b-deficient mice. Two groups[2,3] have recently obtained such mice although the preliminary results appear different. In one case[2] the phenotype of the Stat5b-deficient mice is distinct from that of the Stat5a-deficient mice. These mice exhibit reduced growth of males and a severely compromised fertility in females. Interestingly, the reproductive lesions in the Stat5b-deficient mice were similar to those observed in the PRLR-deficient mice. The Stat5b-deficient males were characterized by a decrease in body growth profile to the slower rate of wild type females.[2] This growth defect first emerges at puberty and is apparently due to a loss of responsiveness to plasma growth hormone pulses, proposed to be a male-specific Stat5b-mediated signaling pathway in rodents. A second group[3] obtained a phenotype that was comparable with the Stat5a-deficient phenotype in exhibiting only a lactation deficiency. The basis for the difference in phenotypes is currently unclear although it is essential to characterize multiple, independently derived strains of mice. It will also be important to obtain mice that are deficient in both genes. Such mice have recently been generated,[3] and the phenotype of these mice should be reported soon. Although both the WAP and β-casein genes contain GAS, only WAP gene

[2] G. B. Udy, R. P. Towers, R. G. Snell, R. J. Wilkins, S.-H. Park, D. J. Waxman, and H. W. Davey, submitted for publication.

[3] J. Ihle, personal communication.

expression was reduced in Stat5a-deficient mice, suggesting that β-casein gene expression is primarily controlled by other transcription factors. Finally, mice with an inactive PRL gene have been generated,[4] and the physiological consequences should be known soon.

It is intriguing to speculate why widespread and general signaling cascades involving the PRLR and Stat5a are critical for mammopoiesis and lactogenesis, while their requirements for the development of other tissues appear to be far less stringent. Since the mammary gland is a recent acquisition in the phylogenetic scale of organ evolution, it may well be possible that redundant signaling pathways have not been developed.

The Next Steps

Future progress in understanding the cell- and ligand-specific effects of signaling molecules with a widespread distribution, such as the PRLR and the transcription factors Stat5a and Stat5b, will depend on our ability to further manipulate the genetic components of the mouse. Aberrant mammary development and function observed in the absence of Stat5a and the PRLR are likely the result of accumulated consequences of misguided signaling during puberty and pregnancy. To understand the molecular basis of these compound lesions, it will be necessary to identify the cell type and record the time point at which the initial impact of aberrant signaling occurs.

Experimental approaches to identify cell types and developmentally crucial time windows are becoming available. These include the time-sensitive activation of genes (38, 39), and the cell- and development-specific inactivation of genes by site-specific recombination, such as the Cre-lox technique (40). The time-sensitive activation of a Stat5a transgene in a Stat5a-deficient background could help in the identification of developmental steps during puberty and pregnancy. This could be accomplished either with the classical *tetracycline*-responsive system (39) or by adenovirus-mediated gene transfer into mammary epithelial cells. Alternatively, the combination of the Cre-lox and the tetracycline-responsive expression system will permit the deletion of the Stat5 gene in a tissue- and temporal-specific fashion (40).

The deletion of more than one gene in the PRL pathway gene will provide further insight into both the specificity and redundancy of PRL switches. Considering the variety of signals that utilize Stat5a and -5b, it is likely that the simultaneous deletion of both genes will have complex consequences. The tools of tissue- and temporal-specific deletions of genes will find an application. Finally, the mammary gland lends itself to transplantation studies, which permits the analysis of wild-type epithelium in a mutant host and vice versa. Such experiments allow the identification of the tissue affected by elimination of one component in the pathway.

The past 60 years of PRL research were characterized by a synergy of biochemistry, genetics, and physiology, which culminated in the identification of molecular switches in the PRL signaling pathway. Future research will focus on how these switches control the physiology of the mammary gland.

[4] N. Horseman, personal communication.

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