Prolactin signal transduction mechanisms in the mammary gland: the role of the Jak/Stat pathway

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Prolactin signal transduction in mammary epithelial cells is mediated by a novel, direct signalling system that links the activation of the prolactin receptor at the cell surface to changes in gene transcription in the nucleus. This recently identified pathway is a variant of the Jak/Stat (for Janus kinase/signal transducer and activator of transcription) pathway used by many other growth factors and cytokines. Current data suggest that the key intracellular components of the prolactin signalling pathway are the kinase Jak2 and the transcription factor Stat5. This discovery has exciting implications for the interaction between prolactin and other extracellular signals in both the mammary gland and other tissues. Here we review work that began with attempts to understand the regulation of milk protein gene expression and ultimately demonstrated the central role of the Jak/Stat pathway in prolactin signal transduction in the mammary gland.

Prolactin is a pituitary polypeptide hormone that is involved in a number of biological processes in vertebrates, including reproduction, growth and development, osmoregulation, metabolism and immunoregulation (Nicoll, 1974). The biological actions of prolactin have been most extensively characterized in the mammary gland, where it has an essential role both in the differentiation of the gland during pregnancy and in the regulation of milk protein gene expression (Houdebine et al., 1985). The molecular cloning of the rat prolactin receptor identified a single gene that encodes predominantly two classes of polypeptide, a long form (591 amino acids) and a short form (291 amino acids) that differ in the size of their intracellular domains (Kelly et al., 1992). Significantly, although both forms have a similar capacity to bind prolactin, only the longer form receptor can stimulate transcription from a prolactin responsive promoter. A mutant form of the rat prolactin receptor, cloned from the prolactin-dependent pre-T lymphoma Nb2 cell line, has a deletion within the cytoplasmic domain and retains the ability to stimulate prolactin-induced gene expression (Kelly et al., 1992). These various forms of the prolactin receptor have provided important experimental tools with which to begin mapping regions of the intracellular domains that are essential for prolactin signalling (Lebrun et al., 1995).

Analysis of the prolactin receptor polypeptide sequence revealed that the receptor is a member of the cytokine receptor superfamily (Taniguchi, 1995). This family includes the receptor for growth hormone (GH), to which the prolactin receptor is most closely related, the haematopoietic receptors, interleukin (IL)-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, erythropoietin (EPO), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage CSF (GM-CSF), leukaemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF) and the more distantly related interferon (IFN) receptors. The extracellular domains of these receptors all have conserved motifs including a tryptophan-serine (WSXWS) sequence and four cysteine residues. The intracellular domains are not well conserved, with the exception of a membrane-proximal cytoplasmic region comprising the box 1 and box 2 motifs, which are required for the mitogenic function of the receptors. In common with the other cytokine receptors, the prolactin receptor has no detectable intrinsic kinase activity, and yet activation by prolactin is associated with tyrosine phosphorylation of both the receptor and other intracellular substrates (Lebrun et al., 1995). Establishing the connections between the activation of the prolactin receptor, tyrosine phosphorylation and the induction of gene expression has only been elucidated in the past 3 years through the convergence of studies on transcriptional regulation of prolactin responsive genes and on cytokine signalling pathways.

Identification of prolactin-induced transcription factors in the mammary gland

In mammary epithelial cells, prolactin acts in synergy with other lactogenic hormones, principally glucocorticoid and insulin, to induce differentiation and the expression of mRNAs encoding milk proteins. As a consequence, the promoters for the genes encoding the milk protein genes, β-casein, whey acidic protein (WAP) and β-lactoglobulin (BLG) have been studied extensively to define the transcription factors and DNA sequence elements that mediate the response to lactogenic hormones. A number of transcription factors, including nuclear factor 1 and glucocorticoid receptor, have been shown to be involved in the regulation of milk protein gene expression (Li and Rosen, 1995). In addition, two mammary transcription factors, named MGF (mammary gland factor) or MPBF (milk protein binding factor), were identified independently through their interaction with the β-casein and BLG proximal promoters, respectively (Schmitt-Ney et al., 1991; Watson et al., 1991). The similarity of the MGF, TTCTTGGAA and MPBF, TT/ACCCGGAA binding sites suggested that these transcription factors were related. Experiments performed with the mammary HC11 cell line showed that MGF binding activity increases in response to prolactin, and the MGF site at –90 in the β-casein promoter is essential for the hormone-dependent induction of β-casein...
transcription (Schmitt-Ney et al., 1991). Similarly, mutation of the two proximal MPBF sites in the BLG promoter abrogates the hormonal response of the gene encoding BLG in both HC11 cells (Burdon et al., 1994) and in Chinese hamster ovary cells transfected with the prolactin receptor (Demmer et al., 1995). The first examination of the role of MPBF/MGF in vivo, i.e. in the mammary gland of a lactating animal, was performed by introducing the mutated BLG promoter constructs into transgenic mice (Burdon et al., 1994). This study indicated that MPBF binding was not essential for mammary expression, but was required for maximal activity of the gene encoding BLG in vivo (Burdon et al., 1994). This study indicated that MPBF binding was not essential for mammary expression, but was required for maximal activity of the gene encoding BLG in vivo (Fig. 1). A similar result has subsequently been reported for a rat WAP transgene carrying a mutation in a distal MGF binding site (Li and Rosen, 1995). Together, these transgenic experiments suggest a role for MPBF/MGF in mediating the response of milk protein genes to prolactin in the mammary gland rather than in determining tissue specificity.

Prolactin induction and the Jak/Stat pathway

The molecular cloning of sheep MGF, in the laboratory of Bernd Groner, provided a crucial advance in understanding prolactin signal transduction (Wakao et al., 1994). The peptide sequence of MGF revealed that it was a novel member of the Stat (signal transducers and activators of transcription) family of transcription factors that were first discovered in studies on interferon regulation of transcription (Schindler and Darnell, 1995). Stat factors are characterized by the presence of src homology 2 and 3 (SH2 and SH3) domains and the requirement for tyrosine phosphorylation for DNA-binding activity. The consensus recognition site for Stat factors, the GAS site (g-IFN activation site) is TTCCNGGAA, although different Stat factors have subtly different site specificities. Since MGF was the fifth of the six currently known Stats to be identified, it has now been renamed Stat5. The identification of the Janus kinase (Jak) family of cytoplasmic protein tyrosine kinases (Wilks et al., 1991) as essential components for IFN signalling (Schindler and Darnell, 1995) placed Jaks and Stats together as essential elements of a novel signalling pathway that is used by all members of the cytokine receptor superfamily, now referred to as the Jak/Stat pathway (Fig. 2).

In a rather simplistic model, the generic Jak/Stat pathway has three components: receptor, Jak kinase and Stat factor. Ligand binding induces dimerization of one or more of the receptor chains, which brings together two Jak kinases, through their association with the membrane proximal intracellular...
The proximity of the two receptor chains plus their associated Jaks results in tyrosine cross-phosphorylation of both the Jaks and specific residues on the receptor. The phosphorylated tyrosine residues on the receptor then may act to recruit Stat proteins, which in unstimulated cells would normally exist as monomers in the cytosol. A transient association of the Stat with the receptor results in tyrosine phosphorylation of the Stat protein, Stat5 and prolactin signal transduction in mammary gland.

**Fig. 2.** Prolactin signal transduction pathways. A schematic representation of the pathways that have been implicated in prolactin signal (PRL) transduction. The Jak (Janus kinase)/Stat (signal transducer and activator of transcription) pathway has an essential role in mediating the response to prolactin in mammary epithelial cells. Engagement of the prolactin receptor induces homodimerization resulting in activation of the associated Jak 2 kinase, which then tyrosine phosphorylates the receptor and leads to activation of Stat5 through specific SH2 (Src-homology 2) domain-phosphotyrosyl interactions. This results in the dimerization of Stat5 which translocates to the nucleus and binds to its recognition site, where it activates transcription. Activated Jak2 may also associate with the SH2 domain of SHC, which then interacts with Grb2 (an adaptor protein) following tyrosine phosphorylation, thereby activating the MAP (mitogen activated protein) kinase pathway. The mechanism of interaction between prolactin signal transduction and glucocorticoid and other signalling events has not been established. Y and H represent tyrosine and glucocorticoid, respectively. GR: glucocorticoid receptor; GAS: TTCCNGGAA.
oligomerization with at least one other Stat protein and subsequent translocation into the nucleus, where the complex binds to GAS sites, thereby activating transcription. Four members of the Jak family, Jaks 1–3 and Tyk2, have been identified and they appear to associate with individual receptors in distinct combinations. However, the specificity of Stat factor activation is most likely determined by the amino acids surrounding particular phosphorylated tyrosines on the receptor (Stahl et al., 1995).

Direct evidence of the role of Stat5 in prolactin induction has been provided by reconstructing the prolactin signalling system in COS cells, through the co-transfection of prolactin receptor and Stat5 expression vectors. In these experiments, it was shown that prolactin treatment results in phosphorylation of Stat5 and induction of DNA-binding activity in cells transfected with the long form prolactin receptor but not in those transfected with the short form (Gouilleux et al., 1994; Wakao et al., 1994). Importantly, stimulation of a β-casein promoter (-344-1)/luciferase reporter construct in these cells requires both Stat5 and the prolactin receptor (Gouilleux et al., 1994). Phosphorylation of the single tyrosine residue at position 694 in sheep Stat5, a site conserved in all six known Stats, is essential for activation of DNA-binding activity (Gouilleux et al., 1994; Azam et al., 1995). The association of Jak2 with the prolactin receptor in both Nb2 cells and in mouse mammary gland explants suggests that Jak2 is likely to be involved in the activation of Stat5 (Campbell et al., 1994). The demonstration that Jak2, but not fyn, lyn and lck kinases, can phosphorylate Stat5 in vitro would also support this model (Gouilleux et al., 1994).

Additional factors in prolactin signal transduction

It is clear from the experiments described above that Stat5 and probably Jak2 play a central role in prolactin signal transduction in the mammary gland. However, results from previous studies and an increasing understanding of the Jak/Stat pathway imply the involvement of other factors and interrupt with distinct prolactin-signalling mechanisms. For example, there is evidence that prolactin may also activate the mitogen-activated protein kinase pathway (Clevenger and Medaglia, 1994) and mediate effects directly by the translocation of the prolactin polypeptide into the nucleus (Clevenger et al., 1991). It is important to note that these effects were obtained with non-mammary cell lines, which raises the possibility that prolactin may signal through different routes in different cell types.

It is established that milk protein gene expression is not coordinately regulated during mammary gland development. Expression of the gene encoding β-casein (and BLG transgenes) is induced around mid-gestation in the mouse at day 10 of pregnancy, WAP at day 15 and α-lactalbumin even later, just before birth. All these genes are induced by prolactin, and yet the different temporal patterns of expression indicate that signals from systemic hormones, local growth factors and cell–cell contacts converge with Stat5 on the regulatory elements of these genes. In a similar vein, despite the value of experiments with non-mammary cell lines in identifying key components in prolactin signalling, the efficient induction of Stat5 and endogenous milk protein transcription in mammary cells depends on cellular differentiation. This can be promoted by confluence and removal of growth factors in established cell cultures or, in primary mammary cultures, induced through the addition of components present in extracellular matrix (ECM). Recent work on the role of ECM in regulating BLG expression in primary cultures from transgenic mammary gland cultures has shown that Stat5 DNA binding activity cannot be induced by prolactin in cells grown on plastic. In contrast, significant amounts of tyrosine phosphorylated Stat5 are found in nuclear extracts from cells grown on ECM (Streuli et al., 1995). Also, the stimulation of BLG expression in response to growth on ECM is abolished when the Stat5-binding sites in the BLG promoter are mutated. The mechanism of Stat5 activation by ECM is not clear.

Specificity of the prolactin response

Further complexity has been added by the discovery that Stat5 becomes tyrosine phosphorylated in response not only to prolactin but also to IL-2, IL-3, IL-5, EPO and GH (Azam et al., 1995; Mui et al., 1995). For activation of specific genes in a particular cell type expressing multiple receptors, which can potentially activate the same Stat factor, it is necessary to invoke additional mechanisms to control the specificity of the response. This notion is supported by the intriguing observation that in transfected COS cells, Stat5 can be activated by the GH and EPO receptors as well as the prolactin receptor, but only the prolactin receptor can stimulate transcription from a β-casein reporter gene (Gouilleux et al., 1995). In addition to tyrosine phosphorylation, serine phosphorylation of Stat1 and Stat3 can also play a role in modulating signalling through the Jak/Stat pathway (Zhang et al., 1995), opening up further possibilities for regulating specificity.

The expression of mRNA encoding Stat5 is regulated during mammary gland development and the DNA-binding activity reaches a maximum during lactation. Modulation of the transcriptional activation potential of Stat5 could also be a control point in the prolactin signalling pathway. The existence of two closely related genes encoding Stat5 that differ mainly at the carboxy terminus, together with the use of alternative splicing to generate at least two polypeptides from each gene, increases the heterogeneity of Stat5 transcription factors (Azam et al., 1995; Mui et al., 1995). It seems likely that the activation potential of these variants could be substantially different. Further elucidation of the role of Stat5 in both the development of the mammary gland and the transcriptional regulation of milk protein genes requires the deletion of the Stat5 genes by homologous recombination. Since Stat5 is expressed in most tissues and is a component of many cytokine signal transduction pathways, an embryonic lethal phenotype might be anticipated for mice lacking Stat5. In such circumstances, deletion of Stat5 only in mammary epithelial cells, through a tissue-specific knockout, would be an elegant approach for determining the function of Stat5 in the mammary gland. We await the Stat5 knockouts with great interest!

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