Published in final edited form as:
J Mammary Gland Biol Neoplasia. 2008 March ; 13(1): 81–91.

Regulation of prolactin receptor levels and activity in breast cancer

G Swaminathan[1], **B Varghese**[1,2], and **SY Fuchs**[1*]

1*Department of Animal Biology and Mari Lowe Center for Comparative Oncology Research, University of Pennsylvania, Philadelphia, PA 19104, USA*

2*Cell and Molecular Biology Program, Biomedical Graduate School, University of Pennsylvania, PA 19104, USA*

SUMMARY

From its traditional identity as a hormone involved in growth and differentiation of mammary epithelium and in lactation, to having a pertinent role in the development of mammary carcinoma, the peptide hormone/cytokine prolactin (PRL) has emerged as a versatile signaling molecule. There has been significant progress in our understanding of the fine working of PRL in the past several years. Notably, much effort has been concentrated on the mediator of PRL action, namely, the prolactin receptor (PRLr). The causal link between increased PRLr expression and breast cancer is being increasingly appreciated. Considering that the level of the receptor on the surface is a critical determinant of signaling output in response to PRL, the uncovering of regulatory elements that control receptor expression becomes important. The principle focus of this review is on the regulation of PRLr expression and activity in breast cancer with a brief overview of different isoforms of PRLr, their expression, signaling capabilities and the biological outcomes of PRL/PRLr signaling.

Keywords

Prolactin receptor; ubiquitination; breast cancer; endocytosis; regulation; signaling

Structure and signaling capabilities of PRLr isoforms

PRLr signaling

The PRLr is a major mediator of cellular effects of PRL. We briefly describe here these effects mainly to present various elements of PRL signaling as they are transduced by diverse isoforms of PRLr; other reviews in this issue describe all branches of PRL signaling in detail.

The main signaling networks downstream of PRL/PRLr include, the Jak-STAT, RasMAPK and PI3K-Akt pathways. These pathways impact crucial cellular processes like proliferation, survival, cytoskeletal effects and differentiation with well-established roles in the initiation and progression of cancer including mammary tumors. PRLr, analogous to other cytokine receptors, lacks intrinsic kinase activity and the receptor-Jak2 module acts in concert to transmit signals downstream of ligand binding (1,2). PRL-mediated activation of the Jak-STAT signaling results in transcriptional induction of milk protein genes and genes involved in protein proliferation like cyclin D1 (3–5).

*Address correspondence to: Dr. Serge Y. Fuchs, Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, 380 S. University Ave, Hill 316, Philadelphia, PA 19104. (215)-573-6949 (phone), (215)-746-2295 (fax), syfuchs@vet.upenn.edu (email).

PRL has been shown to activate the Ras-Raf-MAPK pathway in several mammary tumor cell lines as well as normal mouse mammary cells, which signals for cell proliferation via multiple mechanisms. This is mediated in some cells by increased association of Shc with Jak2, as well as by the Grb2 and Sos complex (6). PRL has also been implicated in activation of other MAPK such as JNK, which impact proliferation and apoptosis in cell systems like T47D, Nb2 and PC12 (7–9). Other kinases like c-Src that play key roles in normal cellular physiology as well as mammary carcinoma are activated in response to PRL and interface with PRLr-mediated signaling. Src functions upstream of PI3K or focal adhesion kinase (FAK)-Erk activation in PRL stimulated breast cancer cell lines (10).

The activation of PI3K pathway results from either direct binding of p85 subunit to PRLr or downstream of Src or Ras activation. The phosphoinositides generated by PI3K activate Akt, which transmits pro-survival, pro-proliferation signals by modulating cell-cycle regulators and also enable the recruitment of pleckstrin homology containing guanine nucleotide exchange factor (GEF), Vav, which activates the Rho family GTPases, leading to cytoskeletal rearrangements necessary for cell adhesion and migration. The association of Vav with Tec, a tyrosine kinase and Nek3, a Ser-Thr kinase modulate its ability to activate Rac (11–14).

Moreover, PRLr signaling can transactivate other receptors involved in oncogenesis. Thus, PRL treatment has been shown to induce tyrosine phosphorylation of human epidermal growth factor receptor (EGFR), leading to activation of MAPK in breast cancer cell lines, suggesting that these two important pathways can synergize during the development of disease. This has implications for anti-cancer therapy as it has been recently shown that a combination of anti-EGFR mAb, herceptin and the PRL antagonist, G129R PRL additively inhibited cell proliferation in T47D and BT474 cells as well as their growth in xenografts in athymic mice (15).

In addition to positive signal transduction, PRL engagement by PRLr stimulates regulatory molecules capable of attenuating PRL generated signals. Included in this category are the SOCS family proteins, SOCS1 and 3, PIAS, CIS and protein tyrosine phosphatases, PTP1B1 and TC-PTP which target the Jak-STAT pathway (16–20).

PRLr isoforms

The PRLr family encoded by a single gene encompasses five membrane-bound forms generated by alternate splicing and a soluble form, generated by proteolytic cleavage of the extracellular domain (ECD) of the receptor. The membrane-bound forms include long, intermediate, ΔS1 and two short forms and, with the exception of the ΔS1 form, share a common ECD but diverge in their intra-cellular domain (ICD, reviewed in (3)). The different isoforms of PRLr are represented in various species. In addition to the isoforms mentioned above, there are other variants that have been reported to result from alternate splicing and are described under the heading of post-transcriptional regulation.

The prototypic member of the family, the human long form (lPRLr), is 590 amino acids long and represents the entire spectrum of signaling capabilities attributed to PRLr. The lPRLr is a type I transmembrane receptor and structurally resembles the class I cytokine receptor superfamily. The ECD includes two type III fibronectin-like regions, named the S1 and S2 domains and together form the ligand-binding unit. The three-dimensional structure of this domain reveals seven anti-parallel β-strands divided into two β-sheets that are connected by a linker of five amino acids. The S1 region has the sites for N-terminal glycosylation of PRLr and also includes majority of the ligand binding sites/is the primary ligand-binding region. The S2 region has fewer ligand contact sites and is defined by two regions, a Trp-Ser-X-Trp-Ser motif characteristic of cytokine receptor family members and sites involved in dimerization with partner receptors. The ECD is separated from the ICD by a transmembrane (TM) region

of 24 amino acids. The ICD represents the signaling entity/unit of the receptor and contains the Box1 5 and 2 motifs with the variable box (V-box) in between and an extended Box 2 (X-box) (5,21,22). The Box1 region is hydrophobic and has an SH3-like binding domain, which mediates binding to the tyrosine kinase, Janus kinase (Jak2) (23,24). In addition, the C-terminus encompasses several tyrosine residues, the distal residues being crucial for STAT5 binding and activation (25). Other proteins, which are recruited to the receptor in a tyrosine phosphorylation dependent manner, include SHP-2 phosphatase, c-Src, Nek3 and Vav and propagate PRLgenerated signals as outlined above (11,26,27).

The other isoforms of PRLr differ in their signaling properties from the long form due to inherent structural differences, which alter ligand-binding capacity of the receptor or hamper its ability to support the intracellular interactions required for signal transduction. While the intermediate and short forms (S1a and S1b) of PRLr retain the ligand binding region and Jak2 binding Box1 region, they lack most of the C-terminal tyrosines and are hence unable to conduct signaling events downstream of Jak2 activation (e.g. STAT5 recruitment). In addition, the intermediate form of the receptor has the Box2 region and a unique 13 amino acid C-terminal motif of indeterminate function. S1a but not S1b has the Box2 element and a 39 amino acid Cterminal sequence (28–30). Experimental evidence suggests that the short forms, especially S1b, can act in a dominant negative fashion to attenuate the function of the long form of PRLr (31,32). One of the mechanisms by which this can occur is by heterodimerization of long and short that occurs independently of prolactin or relative expression of the different isoforms (33–35).

The ΔS1 isoform of PRLr, as the name implies, harbors a deletion of the entire S1 region, as a result of which it exhibits a 7-fold reduction in ligand binding affinity in comparison to the long form. However, under conditions of high PRL levels, the receptor displays only a modest delay in the activation of downstream signaling and is equipotent to the long form (36).

The soluble form of PRLr, prolactin binding protein (PRLBP), represents the freecirculating ECD of the PRLr and can be detected in human serum and culture supernatants of human breast cancer cell lines transfected with long form of PRLr. This isoform was shown to be capable of binding 36% of circulating PRL *in vivo* and of antagonizing the effects of PRL *in vitro*(37). The physiological function of this isoform is currently not known. However, considering that this form represents a natural antagonist of PRL, its therapeutic application as a ‘ligand trap’ can be envisioned.

These observations underscore the significance of PRLr signaling in growth control, illustrate the extensive crosstalk between individual pathways downstream of PRLr and its ability to co-operate with other oncogenes implicated in breast cancer. An accumulated wealth of knowledge highlights the pro-tumor capabilities of the PRL/PRLr pathway and the need to delineate the mechanisms, which control PRLr levels that serves as a common, focal point upstream of the myriad pathways outlined above.

Expression of PRLr

PRLr expression has been reported in a wide variety of cells and tissues. The isoforms are differentially expressed, and there does not appear to be a consensus in the relative ratios of the different isoforms in different tissues or in comparisons of normal versus breast cancer. The elements, which affect receptor expression, are discussed in some detail in the subsequent sections of the review.

Control of PRLr levels on the cell surface- a balance between expression and turnover

The amount of PRLr on the cell surface controls both intensity and duration of PRL signals in cells and thereby cellular response to PRL. Alterations in PRLr levels can therefore lead to abberent downstream signaling in response to PRL resulting in disruption of cellular homeostasis. In support of this, early work examining the relative levels of PRLr in different breast cancer lines in comparison to a normal breast cell line indicated that the number of receptors per cell was high as 25,800 in T47D cells versus 1,700 in immortalized HBL-100 cells (38). Subsequently, several studies have reported increased expression of PRLr mRNA in tumor tissue (corresponding to surrounding normal tissue) and in breast cancer cell lines (39–41). Similar results were obtained following analysis of PRLr protein levels by immunohistochemistry (42,43). Conversely, screening of breast cancer profile arrays using probes specific for long and short form of PRLr and absolute quantitation of mRNA levels of long and short isoforms by real-time PCR in normal and breast cancer tissue and cell lines revealed a significant decrease in the ratio of short to long form in tumor tissue and breast cancer lines. The ‘favored’ expression of the long form over the short receptor variant is consistent with the need for stimulatory effects of the long form for driving tumorigenesis as opposed to the signal attenuating short forms (44). Collectively, these observations and other experimental data obtained from cancer cell lines and primary tumor samples have postulated a positive link between increased receptor levels and breast cancer incidence, demonstrating the need to unravel the regulation of PRLr expression on the cell surface. The density of receptor on the surface is a cumulative consequence of events affecting *de novo* synthesis and subsequent fate of the receptor pre- and post-ligand binding. Indeed, several lines of evidence have shown that the availability of long form (as well as other isoforms) of PRLr is modulated at the transcriptional, post-transcriptional as well as post-translational levels. This section will feature reported aspects of these processes and present speculations on other plausible mechanisms by which control can be exerted.

Transcriptional Regulation of PRLr

A number of factors (including hormones and chemotherapeutic agents) have been reported to affect the levels of the PRLr mRNA in normal and breast cancer cells. Treatment of breast cancer cell lines with sex-steroid hormones namely, dihydrotestosterone, organon 2058 or estradiol was observed to cause an increase in PRLr mRNA and protein levels (45). Interestingly, estradiol has been demonstrated to upregulate PRLr expression by acting on specific promoter regions. The *hPRLr* gene has a complex 5′ genomic structure, with multiple (six) alternative non-coding exons 1 and promoter utilization, which include the preferentially utilized, generic promoter 1/exon-1 (PIII/hE13) and five human specific exon-1/promoters (hE1N1-5). This could give rise to diversity in receptor expression based on differential promoter utilization in normal and disease states. It was demonstrated by quantitative competitive RT-PCR analysis that Estradiol (E2), a potent mammary mitogen and growth stimulus for hormone-dependent breast cancer, induced *PRLR* non-coding exon-1 hE13 (generic) mRNA transcripts via promoter III (hPIII) in breast cancer cells. In addition, transfection studies confirmed activation of the hPIII promoter by E2. This effect of E2 was shown to involve a non-classical ERE-independent mechanism (46,47,48,49).

Some studies have demonstrated that PRLr mRNA expression can be potentiated by its ligand PRL via an autocrine/paracrine loop in breast cancer cells. Thus, in MCF7 cells engineered to inducibly over-express PRL, an upregulation of lPRLr was observed in response to ‘induced-endogenous’ PRL but not exogenous PRL. There was also a concomitant increase in estrogen receptor (ER) levels and estrogen responsiveness of these cells. Considering the positive effect of estrogen on PRLr transcription, this mode of reciprocal regulation would serve to amplify

both ER and PRLr signaling in breast cancer (50). It is worth noting that many breast cancer cell lines and tumors co-express PRLr and sex steroid hormone receptors, including ER and that their expression are cross-regulated by their respective ligands, underscoring the synergism between the two signaling pathways in normal development and neoplastic progression (45).

Interestingly, PRL-mediated upregulation of PRLr mRNA in breast cancer cells has been reported to influence their tumorigenic potential in mouse models. It was observed that MDA-MBA-435 breast cancer cells engineered to over-express PRL formed tumors at a higher rate compared to control cells when injected in nude mice; remarkably, these tumors showed significantly higher levels of PRLr (51). This suggests that increased PRLr expression can accelerate tumor development *in vivo* and probably is reflective of what happens in human breast cancer. Indeed, recent observations in PRLr knockout mice have suggested a role for PRLr in promoting cell proliferation in pre-invasive lesions and potentiating the transition to invasive carcinoma (52). Given that PRL stimulates the degradation of PRLr protein, the physiologic role of ligand-stimulated increase in PRLr mRNA seemed counterintuitive at the time of the study, however, later investigations demonstrated that degradation of PRLr protein in breast cancer is impaired as well (see below).

Certain anti-cancer agents have been reported to modulate PRLr mRNA. Tamoxifen has been shown to down-regulate prolactin receptor in breast cancer cells and analysis of biopsy samples from post-menopausal woman before and after initiation of tamoxifen treatment revealed a decline in PRLr mRNA seven days post treatment. Of 28 patients examined the decrease in receptor levels was particularly striking in a sub-group of 11 patients with no clear correlation grade of tumor or other tumor markers (53).

The endocannabinoid system, which plays a role in tumor suppression, has been shown to inhibit cell proliferation, adhesion and migration of breast cancer cells as well. Studies addressing the mechanism of action of anandamide (ANA), the ligand for cannabinoid receptor in breast cancer cells revealed that ANA exerted anti-proliferative action on PRL responsive MCF7 and EFM19 cells by suppression of prolactin receptor synthesis, thereby attenuating PRLr mediated signaling. The decrease in receptor levels following treatment with ANA involved inhibition of cAMP/PKA pathway and stimulation of MAPK (54–57). Interestingly, ANA has been shown to inhibit secretion of PRL by lactotropes in mice, an effect reversed by estrogen and thus could indirectly affect PRLr levels via decreasing circulating PRL levels (58).

Human breast cancer cell lines treated with retinoids (ATRA and 9-cis-retinoic acid), another commonly used chemotherapeutic agent showed similar down-regulation of PRLr mRNA within 24h post treatment. The action of retinoids was at the transcriptional level as there was no change in the stability of PRLr mRNA. Moreover, retinoic acid pre-treatment reduced PRL-mediated STAT5 activation (59). In addition, treatment of MCF7 cells with aryl hydrocarbon (Ah) receptor agonist, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) resulted in decrease in PRLr mRNA within 12h. TCDD was also effective in abrogating estradiol induced increase in PRLr in cells co-treated with E2 and TCDD. This suggests that TCDD works through repression of E2/ER activated transcription of target genes, including PRLr (60). These studies highlight the contribution of PRLr signaling in breast cancer and implicate it as an important target of several anti-cancer drugs.

Post-transcriptional regulation of PRLr

Alternate splicing results in the generation of an entire repertoire of PRLr isoforms with differential response to ligand and subsequent downstream signaling. In addition to the long, intermediate, short, ΔS1 forms detailed in a previous section, additional forms have been reported in normal and breast cancer cells. These include Δ7/11, which splices from exon 7 to

exon 11, Δ4-SF1b and Δ4-Δ7/11 that are exon 4 variants of SF1b and Δ7/11 respectively. The Δ7/11 lacks the transmembrane domain and presents as an intracellular or secreted form of PRLr, binds PRL and was found in some primary breast cancer samples in varying amounts (32). It is likely that this form functions as a "ligand trap" similar to PRL-BP.

Post-translational regulation of PRLr levels and function

While there are no reports in literature on translational control of PRLr levels, a substantial progress has been achieved in delineating the post-translational mechanisms of modulation of PRLr levels and activity.

Maturation and surface expression of PRLr—The trafficking of newly synthesised transmembrane receptors from ER to plasma membrane often involves glycosylation. The ECD of PRLr has three glycosylation sites, Asn-35, 80 and 108, capable of N-terminal glycosylation. Studies addressing the requirement of glycosylation by mutational analysis of these sites in rat PRLr revealed that, while N-glycosylation of PRLr might contribute to more efficient targeting to cell membrane, the aglycosylated forms were also present on the membrane, albeit at lower levels and were functionally capable of transmitting PRL-generated signals in a cell-type specific manner. Intriguingly, the ligand binding capacity of the aglycosylated forms was comparable to that of the glycosylated forms. The lower steady-state levels of the aglycosylated forms could stem from their inability to bind chaperones, which aid in correct folding and surface targeting of the receptor or reflect different kinetics of internalization (61).

The role of other factors/chaperones, which might influence the surface expression of PRLr has not been fully elucidated. Jak2 is likely to play a role in maturation of PRLr (62) similar to other cytokine receptors including erythropoietin receptor (EpoR), growth hormone receptor (GHR), thrombopoietin receptor (TPoR) and type I interferon receptor (IFNAR1) (62–65), whose cell surface localization is promoted by an associated Jak. For PRLr and EpoR, the effect of Jak2 was attributed (at least in part) to stabilization of the mature form of the receptor and to stimulation of its trafficking to the cell surface. Alterations of this mechanism in breast cancer are yet to be reported.

Receptor Dimerization—The configuration of receptor present on the cell surface influences both binding affinity and response to ligand. Contrary to the notion that PRL induces receptor dimerization, recent studies have clearly demonstrated that PRLr is capable of ligand independent homo- and heterodimerization and this was found to be largely dependent on its TM domain. The isoform composition of the 'pre-formed' dimer is important for the outcome of PRLr signaling in response to PRL. For example, heterodimers of long and short isoforms have the potential to engage ligand due to an intact ECD but are 'inert' from a signaling perspective as the short form cannot sustain downstream transmission of PRL- stimulated signals. Similarly, upon transfection of T47D cells with different PRLr domains, ECD-TM or TM-ICD, only heterodimers with intact ICD could transmit PRL signals, while dimers containing ECD-TM form were inhibitory (33,35). The existence of an endogenous TM-ICD of PRLr in the breast cancer cell line, T47D, brings forth the possibility of it being a *bona fide* signaling module, which can modulate PRL signaling.

Ligand-induced ubiquitination-dependent down-regulation of PRLr—A common mode of negative regulation of cell-surface receptors including several receptor tyrosine kinases (RTKs) and cytokine receptors following ligand binding/engagement involves ubiquitination of 'activated' receptor, which in turn routes it for lysosomal and in some instances proteasomal degradation (66,67). This serves to limit the duration and intensity of downstream signaling from the receptor, thereby exerting fine control over important cellular processes like proliferation, cell growth, survival, etc. Deregulation of such pathways lead to

pathological conditions including cancer. The long form of PRLr is also subject to such regulation. Pioneering studies in the early 1980s showed that prolactin was capable of facilitating the lysosomal degradation of its receptor (68–71). Subsequent work offered some mechanistic insight into the process. It was demonstrated that following stimulation of lPRLr with its cognate ligand, PRL, the receptor gets phosphorylated on Ser349 within a well-conserved phosphodegron, DSGRGS. This phosphorylation enables the recruitment of $SCF^{\beta TrCP}$ ubiquitin ligase, which catalyzes the ubiquitination of the receptor. The receptor is then sorted for lysosomal degradation (Figure 1). The same group further observed stabilization and accumulation of lPRLr in breast cancer, as a consequence of impaired PRL induced phophorylation of the receptor and its subsequent escape from ubiquitination mediated down-regulation (72,73). This scenario in essence mimics receptor over-expression, the endpoint in both cases being continuous availability of receptor on the surface for ligand engagement and signal propagation.

In this context, it is worth noting that recent work by Lu *et al* demonstrated that inhibitors of both lysosomal pathway and of proteasomes impede the ligand induced degradation of endogenous lPRLr in PRL-deficient MCF7 breast cancer cells (74). While interpretation of these data is confounded by a known fact that many proteasome inhibitors suppress overall protein trafficking into the lysosomes indirectly by depleting the intracellular ubiquitin pool, it was proposed that, in this system, proteosomal function was required for limited cleavage of the receptor and generation of a receptor ECD-containing fragment, post internalization. It is currently unclear if this fragment represents an intermediate degradation product or a signaling unit, or whether this manner of receptor processing is cell-type specific (74).

A more detailed analysis or thorough mapping of the different steps involved in channeling the activated receptor to the proteolytic compartment will help resolve this dichotomy. This will also aid in addressing questions as to whether any of the crucial steps are altered in the cancer milieu during the process of tumorigenesis resulting in receptors refractory to ubiquitination-mediated degradation. There are several precedents from other receptor models, which provide clues as outlined below.

Post-ligand trafficking/sorting of transmembrane receptors for degradation is a well-orchestrated process involving several players and is best exemplified by the EGFR model. The sequence of events following ligand binding, include receptor internalization, sorting through early and late endosomes, maturation to multi-vesicular bodies leading to the ultimate destination, the lysosomes. The progress through these intra-cellular compartments is dictated by interactions with specific ‘resident’ proteins in the pathway, which in turn is facilitated by modifications, particularly, ubiquitination. However, the initial endocytosis can also occur in a constitutive, ligand independent manner but ubiquitination is required, in most cases, for directing receptor proteolysis. The signals other than ubiquitin, which are recognized by the endocytic machinery, are typically present within the cytoplasmic domains of the target proteins. Some signals are tyrosine-based sorting signals and conform to the NPXY or YXXO consensus sequence while others are referred to as dileucine-based motifs with a [DE]XXXL [LI] or DXXLL consensus sequence. These motifs enable the association of target protein to the AP complex, which drive the formation of clathrin-coated vesicles (75). Thus, activation of EGFR results in its auto-phosphorylation on tyrosines (including a critical Y1045), followed by recruitment of E3 ubiquitin ligase c-Cbl and resultant ubiquitination of this receptor. This enables EGFR recruitment into clathrin-coated pits via interactions with ubiquitin-adaptors like Eps15 or motifs, which bind clathrin adaptors such as AP-2. AP-2 complexes facilitate the formation of clathrin-coated vesicles, which then fuse to form early endosomes, followed by maturation to multi-vesicular bodies, where receptor is sorted to the recycling pathway or to the lysosomes. Sustained ubiquitination is required for targeting to the lysosomes whereas

internalization is uncoupled from this requirement. Alternate means of EGFR degradation exists involving Grb2 and CIN85-endophilin complexes (76,77).

In the case of PRLr down-regulation, the details of the mechanisms are just beginning to emerge. Multiple linear endocytic motifs are located in the cytoplasmic tail of the long form of PRLr and are conserved across species. These motifs, namely, a phenylalanine (F290) in combination with a proximal dileucine (LL) and three dileucines in the vicinity of amino acid 272 facilitated the interaction of bovine PRLr with clathrin and dynamin and its subsequent internalization through these pathways. The endocytosis of the short form is governed by the presence of the proximal dileucine region and proceded along the same route as the long form but at a slower rate. But it is unlikely that the short form is degraded in the same manner as the lPRLr, if at all, as it lacks the S349 phosphorylation site and cytoplasmic lysines i.e. the sites of ubiqutination. Both the motifs were capable of initiating receptor internalization independent of the other (78). These data are consistent with earlier observations that the short form of the rat PRLr was internalized in response to ligand, which involved two motifs between amino acid residues 253–261 and 273–281. Moreover, the short form was shown to interact with alphaadaptin, a component of the AP-2 endocytic adaptor complex (79). However, the contribution of these motifs and of AP-2 to the intracellular sorting of PRLr remains to be elucidated.

Ubiquitination of PRLr is likely to increase the efficiency of receptor internalization by at least two possible means: (i) by enhancing the ability of receptor to interact with endocytic adaptor proteins containing ubiquitin-binding domains and/or (ii) by inducing conformation changes in PRLr that would facilitate its binding to endocytic components. It is possible that the two events are not mutually exclusive but occur simultaneously. Ubiquitination can also enable sorting of PRLr following its internalization, though these issues have not been formally addressed. The relative contribution of the motifs and ubiquitination of cytoplasmic lysines needs to be assessed as well.

In addition to interactions with resident proteins of the endocytic pathway, the response of the cellular machinery to an ubiquitinated target is also governed by other parameters. The manner in which ubiquitin is conjugated to its substrate, the number of ubiquitin molecules and the nature of linkage if receptor is modified with poly-ubiquitin collectively influence the decision-making process in the choice of pathway. Mono-ubiquitination, multi-monoubiquitination or poly-ubiquitination chains linked through K63 often tag the protein for lysosomal degradation, whereas substrates appended with K48-linked ubiquitin are destined for proteolysis in proteasomes (67). Understanding the ubiquitin topology on PRLr is very important considering the potential involvement of two degradation pathways in the modulation of PRLr, which maybe subject to differential regulation. It is worth noting in this regard that analysis of the nature of ubiquitin signal appended to PRLr by AQUA method, which allows for deciphering of chain topology and quantitation of chain type-specific ubiquitination, has indicated that PRLr is modified predominantly by poly-ubiquitin chains. While K63-linked chains were predominant, a smaller fraction of canonical K48-linked poly-ubiquitin chains were found on PRLr as well. In addition, expression of ubiquitin mutants defective in specific type of chain assembly revealed that blocking of receptor modification with K63-linked chains resulted in disruption of interaction between PRLr and adaptin complex as well as in impaired internalization and in stabilization of the receptor (our unpublished data). While these data strongly suggest a predominant role of K63-linked ubiquitination in internalization and degradation of PRLr, it has to be noted that ubiquitination is a dynamic process involving both addition and removal of ubiquitin from the substrate the contribution of deubiquitinating enzymes (DUBs) in chainspecific PRLr ubiquitination and in down-regulation of this receptor would be yet another interesting area for investigation (80).

Moreover, it is likely that proximal signaling events triggered by PRL, involving PRLr interacting proteins would add other tiers of regulation to the process. Observations made with cytokine receptors, GHR, and IFNAR1, which undergo ubiquitination mediated proteolysis point to receptor associated Jaks as being an important modulator of receptor stability (81, 82). This is likely to be plausible for PRLr as activation of Jak2 is one of the first events to occur following stimulation of receptor. Indeed, work done in our laboratory addressing the role of Jak2 in PRLr down-regulation has demonstrated that PRL facilitates the ubiquitination, initial internalization and degradation of its receptor, via catalytic activation of Jak2 (our unpublished data). Thus, Jaks can influence the steady-state surface levels of receptor by fine-tuning two processes; surface targeting of mature receptor and ligand induced endocytosis.

c-Src has also been purported to play a role in PRL-induced internalization of its receptor. One possible mechanism by which c-Src can mediate its effect is by tyrosine phosphorylation of dynamin, a regulator of clathrin coated vesicles, which increases its GTPase activity and facilitate internalization of receptor. In this context, it is worth noting that inhibition of dynamin retards PRLr internalization. The relative contribution of different endocytic components and their activating elements in PRLr trafficking in normal and breast cancer cells needs further studies and will provide useful insights into the regulation of this process (78,83).

The involvement of a ligand-activated hitherto unidentified Ser-Thr kinase, which phophorylates Ser349 (a prerequisite for $SCF^{\beta TrCP}$ ligase recruitment) has also been proposed. Impaired activity of such kinase (or its impaired ability to be recruited to the receptor) in breast cancer cells (73) constitute a probable cause for abrogation of Ser349 phosphorylation and failure to down-modulate PRLr in breast cancer cells and tissues. Ligand activation can also relieve inhibition by a Ser-Thr phosphatase, which dephosphorylates the receptor, thereby preventing its degradation.

Stress induced signals that are common in the tumor setting (such as hypoxia, nutrient deprivation, chemotherapy, etc) can activate stress kinases like p38 MAPK, which might also affect receptor endocytosis. This has been recently observed in the EGFR model system, where activation of p38 in response to treatment with UV, inflammatory cytokines and cytotoxic drugs like cis-platinum accelerated receptor internalization in a clathrin dependent manner. Chronic activation of p38 resulted in receptors getting trapped in endosomes (84,85). Identification of the role of the different candidate kinases, which could contribute to receptor phosphorylation, ubiqutination and degradation would shed more light on the regulation of PRLr stability in normal cells and breast cancer cells.

Conclusions and perspectives: PRLr as a putative therapeutic target in breast cancer

In light of significant recent advances in understanding the mechanisms that regulate PRLr expression and function, an increased appreciation for a causal role of PRL/PRLr signaling axis in the pathogenesis of mammary carcinoma is emerging. Yet, only the development and potential clinical application of a potent anti-PRL/PRLr agent for treatment of breast cancer will ultimately lend credence to the ideas on the important role of PRLr signaling in etiology of this disease. Anti-tumorigenic effects of naturally occurring and mutagenesis-based antagonists of PRLr have been rigorously investigated; these studies are outlined in several outstanding reviews (86,87). Moreover, the growth inhibitory actions of currently available anti-cancer agents like tamoxifen and retinoids have been attributed in part to their ability to down-regulate PRLr expression, thereby identifying PRLr as an attractive target in the treatment of breast cancer. While these drugs are effective against ER-positive breast cancers, fluctuations in PRLr level can occur independently of ER status, illustrating the need to develop alternate interventions targeting PRLr signaling. In addition, the multi-factorial nature of

cancer and the existence of extensive crosstalk between signaling pathways in cancer cells have stressed the need for combination therapy for more effective disease control. This issue becomes crucial in cases where resistance to 'single' treatment is encountered. In this context, PRLr might serve as an important target as evidenced in two studies where fusion proteins containing PRLr antagonist and anti-angiogenic agent endostatin or a combination of PRLr antagonist and anti-EGFR mAb, herceptin exhibited greater tumor inhibitory properties additively compared to individual treatments of breast cancer (15,88).

Moreover, the discovery of regulatory pathways involved in PRLr turnover has spawned new, exciting areas of research. For instance, the loss of negative regulation of growth factor receptors as a result of defective ubiquitination as a driving force in malignancy is underscored in several systems. Such a deregulation is apparent for PRLr as well. This brings forth the possibility of developing novel treatment strategies including antibody-based therapeutics directed to promote lysosomal degradation of PRLr and to inhibit its signaling. It therefore becomes important to unravel the signaling events involved in PRLr down-regulation, including accessory proteins, involvement of additional ubiquitin ligases, and cross-talk with other oncogenic signaling pathways in order to identify new drug targets and improve the efficacy of existing ones targeting PRLr, the central component in PRL-mediated signaling in breast cancer.

Acknowledgements

Work in the Fuchs lab is supported by the Susan G. Komen Breast Cancer Foundation Grant BCTR0504447 (to S.Y.F.) and NCI grant CA115281 (to S.Y.F.)

References

1. Rui H, Lebrun JJ, Kirken RA, Kelly PA, Farrar WL. JAK2 activation and cell proliferation induced by antibody-mediated prolactin receptor dimerization. Endocrinology 1994;135(4):1299–1306. [PubMed: 7925093]
2. Rui H, Kirken RA, Farrar WL. Activation of receptor-associated tyrosine kinase JAK2 by prolactin. J Biol Chem 1994;269(7):5364–5368. [PubMed: 7508935]
3. Clevenger CV, Furth PA, Hankinson SE, Schuler LA. The role of prolactin in mammary carcinoma. Endocr Rev 2003;24(1):1–27. [PubMed: 12588805]
4. Clevenger CV, Kline JB. Prolactin receptor signal transduction. Lupus 2001;10(10):706–718. [PubMed: 11721697]
5. Chilton BS, Hewetson A. Prolactin and growth hormone signaling. Curr Top Dev Biol 2005;68:1–23. [PubMed: 16124994]
6. Das R, Vonderhaar BK. Activation of raf-1, MEK, and MAP kinase in prolactin responsive mammary cells. Breast Cancer Res Treat 1996;40(2):141–149. [PubMed: 8879680]
7. Olazabal I, Munoz J, Ogueta S, Obregon E, Garcia-Ruiz JP. Prolactin (PRL)-PRL receptor system increases cell proliferation involving JNK (c-Jun amino terminal kinase) and AP-1 activation: inhibition by glucocorticoids. Mol Endocrinol 2000;14(4):564–575. [PubMed: 10770493]
8. Cheng Y, Zhizhin I, Perlman RL, Mangoura D. Prolactin-induced cell proliferation in PC12 cells depends on JNK but not ERK activation. J Biol Chem 2000;275(30):23326–23332. [PubMed: 10807911]
9. Schwertfeger KL, Hunter S, Heasley LE, Levresse V, Leon RP, DeGregori J, et al. Prolactin stimulates activation of c-jun N-terminal kinase (JNK). Mol Endocrinol 2000;14(10):1592–1602. [PubMed: 11043575]
10. Acosta JJ, Munoz RM, Gonzalez L, Subtil-Rodriguez A, Dominguez-Caceres MA, Garcia-Martinez JM, et al. Src mediates prolactin-dependent proliferation of T47D and MCF7 cells via the activation of focal adhesion kinase/Erk1/2 and phosphatidylinositol 3-kinase pathways. Mol Endocrinol 2003;17(11):2268–2282. [PubMed: 12907754]

11. Miller SL, DeMaria JE, Freier DO, Riegel AM, Clevenger CV. Novel association of Vav2 and Nek3 modulates signaling through the human prolactin receptor. Mol Endocrinol 2005;19(4):939–949. [PubMed: 15618286]
12. Miller SL, Antico G, Raghunath PN, Tomaszewski JE, Clevenger CV. Nek3 kinase regulates prolactin-mediated cytoskeletal reorganization and motility of breast cancer cells. Oncogene 2007;26 (32):4668–4678. [PubMed: 17297458]
13. Kline JB, Moore DJ, Clevenger CV. Activation and association of the Tec tyrosine kinase with the human prolactin receptor: mapping of a Tec/Vav1-receptor binding site. Mol Endocrinol 2001;15 (5):832–841. [PubMed: 11328862]
14. Berlanga JJ, Gualillo O, Buteau H, Applanat M, Kelly PA, Edery M. Prolactin activates tyrosyl phosphorylation of insulin receptor substrate 1 and phosphatidylinositol-3-OH kinase. J Biol Chem 1997;272(4):2050–2052. [PubMed: 8999900]
15. Scotti ML, Langenheim JF, Tomblyn S, Springs AE, Chen WY. Additive effects of a prolactin receptor antagonist, G129R, and herceptin on inhibition of HER2-overexpressing breast cancer cells. Breast Cancer Res Treat. 2007
16. Tonko-Geymayer S, Goupille O, Tonko M, Soratroi C, Yoshimura A, Streuli C, et al. Regulation and function of the cytokine-inducible SH-2 domain proteins, CIS and SOCS3, in mammary epithelial cells. Mol Endocrinol 2002;16(7):1680–1695. [PubMed: 12089360]
17. Dif F, Saunier E, Demeneix B, Kelly PA, Edery M. Cytokine-inducible SH2-containing protein suppresses PRL signaling by binding the PRL receptor. Endocrinology 2001;142(12):5286–5293. [PubMed: 11713228]
18. Aoki N, Matsuda TA. A nuclear protein tyrosine phosphatase TC-PTP is a potential negative regulator of the PRL-mediated signaling pathway: dephosphorylation and deactivation of signal transducer and activator of transcription 5a and 5b by TC-PTP in nucleus. Mol Endocrinol 2002;16(1):58–69. [PubMed: 11773439]
19. Aoki N, Matsuda T. A cytosolic protein-tyrosine phosphatase PTP1B specifically dephosphorylates and deactivates prolactin-activated STAT5a and STAT5b. J Biol Chem 2000;275(50):39718–39726. [PubMed: 10993888]
20. Tomic S, Chughtai N, Ali S. SOCS-1, -2, -3: selective targets and functions downstream of the prolactin receptor. Mol Cell Endocrinol 1999;158(1–2):45–54. [PubMed: 10630404]
21. Boutin JM, Edery M, Shirota M, Jolicoeur C, Lesueur L, Ali S, et al. Identification of a cDNA encoding a long form of prolactin receptor in human hepatoma and breast cancer cells. Mol Endocrinol 1999;3 (9):1455–1461. [PubMed: 2558309]
22. Somers W, Ultsch M, De Vos AM, Kossiakoff AA. The X-ray structure of a growth hormone-prolactin receptor complex. Nature 1994;372(6505):478–481. [PubMed: 7984244]
23. Lebrun JJ, Ali S, Ullrich A, Kelly PA. Proline-rich sequence-mediated Jak2 association to the prolactin receptor is required but not sufficient for signal transduction. J Biol Chem 1995;270(18):10664–10670. [PubMed: 7537736]
24. Pezet A, Buteau H, Kelly PA, Edery M. The last proline of Box 1 is essential for association with JAK2 and functional activation of the prolactin receptor. Mol Cell Endocrinol 1997;129(2):199–208. [PubMed: 9202403]
25. Pezet A, Ferrag F, Kelly PA, Edery M. Tyrosine docking sites of the rat prolactin receptor required for association and activation of stat5. J Biol Chem 1997;272(40):25043–25050. [PubMed: 9312112]
26. Ali S. Recruitment of the protein-tyrosine phosphatase SHP-2 to the C-terminal tyrosine of the prolactin receptor and to the adaptor protein Gab2. J Biol Chem 2000;275(50):39073–39080. [PubMed: 10991949]
27. Ali S, Nouhi Z, Chughtai N. SHP-2 regulates SOCS-1-mediated Janus kinase-2 ubiquitination/ degradation downstream of the prolactin receptor. J Biol Chem 2003;278(52):52021–52031. [PubMed: 14522994]
28. Hu ZZ, Meng J, Dufau ML. Isolation and characterization of two novel forms of the human prolactin receptor generated by alternative splicing of a newly identified exon 11. J Biol Chem 2001;276(44): 41086–41094. [PubMed: 11518703]
29. Kline JB, Roehrs H, Clevenger CV. Functional characterization of the intermediate isoform of the human prolactin receptor. J Biol Chem 1999;274(50):35461–35468. [PubMed: 10585417]

30. Clevenger CV, Chang WP, Ngo W, Pasha TL, Montone KT, Tomaszewski JE. Expression of prolactin and prolactin receptor in human breast carcinoma. Evidence for an autocrine/paracrine loop. Am J Pathol 1995;146(3):695–705. [PubMed: 7534043]
31. Chang WP, Ye Y, Clevenger CV. Stoichiometric structure-function analysis of the prolactin receptor signaling domain by receptor chimeras. Mol Cell Biol 1998;18(2):896–905. [PubMed: 9447986]
32. Trott JF, Hovey RC, Koduri S, Vonderhaar BK. Alternative splicing to exon 11 of human prolactin receptor gene results in multiple isoforms including a secreted prolactin-binding protein. J Mol Endocrinol 2003;30(1):31–47. [PubMed: 12580759]
33. Qazi AM, Tsai-Morris CH, Dufau ML. Ligand-independent homo- and heterodimerization of human prolactin receptor variants: inhibitory action of the short forms by heterodimerization. Mol Endocrinol 2006;20(8):1912–1923. [PubMed: 16556730]
34. Tan D, Johnson DA, Wu W, Zeng L, Chen YH, Chen WY, et al. Unmodified prolactin (PRL) and S179D PRL-initiated bioluminescence resonance energy transfer between homo- and hetero-pairs of long and short human PRL receptors in living human cells. Mol Endocrinol 2005;19(5):1291–1303. [PubMed: 15695371]
35. Gadd SL, Clevenger CV. Ligand-independent dimerization of the human prolactin receptor isoforms: functional implications. Mol Endocrinol 2006;20(11):2734–2746. [PubMed: 16840534]
36. Kline JB, Rycyzyn MA, Clevenger CV. Characterization of a novel and functional human prolactin receptor isoform (deltaS1PRLr) containing only one extracellular fibronectin-like domain. Mol Endocrinol 2002;16(10):2310–2322. [PubMed: 12351696]
37. Kline JB, Clevenger CV. Identification and characterization of the prolactin-binding protein in human serum and milk. J Biol Chem 2001;276(27):24760–24766. [PubMed: 11337493]
38. Shiu RP. Prolactin receptors in human breast cancer cells in long-term tissue culture. Cancer Res 1979;39(11):4381–4386. [PubMed: 227585]
39. Peirce SK, Chen WY. Quantification of prolactin receptor mRNA in multiple human tissues and cancer cell lines by real time RT-PCR. J Endocrinol 2001;171(1):R1–R4. [PubMed: 11572805]
40. Touraine P, Martini JF, Zafrani B, Durand JC, Labaille F, Malet C, et al. Increased expression of prolactin receptor gene assessed by quantitative polymerase chain reaction in human breast tumors versus normal breast tissues. J Clin Endocrinol Metab 1998;83(2):667–674. [PubMed: 9467590]
41. Laud K, Gourdou I, Belair L, Peyrat JP, Djiane J. Characterization and modulation of a prolactin receptor mRNA isoform in normal and tumoral human breast tissues. Int J Cancer 2000;85(6):771–776. [PubMed: 10709093]
42. Reynolds C, Montone KT, Powell CM, Tomaszewski JE, Clevenger CV. Expression of prolactin and its receptor in human breast carcinoma. Endocrinology 1997;138(12):5555–5560. [PubMed: 9389544]
43. Gill S, Peston D, Vonderhaar BK, Shousha S. Expression of prolactin receptors in normal, benign, and malignant breast tissue: an immunohistological study. J Clin Pathol 2001;54(12):956–960. [PubMed: 11729217]
44. Meng J, Tsai-Morris CH, Dufau ML. Human prolactin receptor variants in breast cancer: low ratio of short forms to the long-form human prolactin receptor associated with mammary carcinoma. Cancer Res 2004;64(16):5677–5682. [PubMed: 15313907]
45. Ormandy CJ, Hall RE, Manning DL, Robertson JF, Blamey RW, Kelly PA, et al. Coexpression and cross-regulation of the prolactin receptor and sex steroid hormone receptors in breast cancer. J Clin Endocrinol Metab 1997;82(11):3692–3699. [PubMed: 9360527]
46. Leondires MP, Hu ZZ, Dong J, Tsai-Morris CH, Dufau ML. Estradiol stimulates expression of two human prolactin receptor isoforms with alternative exons-1 in T47D breast cancer cells. J Steroid Biochem Mol Biol 2002;82(2–3):263–268. [PubMed: 12477494]
47. Hu ZZ, Zhuang L, Meng J, Tsai-Morris CH, Dufau ML. Complex 5′ genomic structure of the human prolactin receptor: multiple alternative exons 1 and promoter utilization. Endocrinology 2002;143(6):2139–2142. [PubMed: 12021177]
48. Hu ZZ, Zhuang L, Meng J, Leondires M, Dufau ML. The human prolactin receptor gene structure and alternative promoter utilization: the generic promoter hPIII and a novel human promoter hP(N). J Clin Endocrinol Metab 1999;84(3):1153–1156. [PubMed: 10084611]

49. Dong J, Tsai-Morris CH, Dufau ML. A novel estradiol/estrogen receptor alpha-dependent transcriptional mechanism controls expression of the human prolactin receptor. J Biol Chem 2006;281(27):18825–18836. [PubMed: 16651265]
50. Gutzman JH, Miller KK, Schuler LA. Endogenous human prolactin and not exogenous human prolactin induces estrogen receptor alpha and prolactin receptor expression and increases estrogen responsiveness in breast cancer cells. J Steroid Biochem Mol Biol 2004;88(1):69–77. [PubMed: 15026085]
51. Liby K, Neltner B, Mohamet L, Menchen L, Ben-Jonathan N. Prolactin overexpression by MDA-MB-435 human breast cancer cells accelerates tumor growth. Breast Cancer Res Treat 2003;79(2): 241–252. [PubMed: 12825859]
52. Oakes SR, Robertson FG, Kench JG, Gardiner-Garden M, Wand MP, Green JE, et al. Loss of mammary epithelial prolactin receptor delays tumor formation by reducing cell proliferation in low-grade preinvasive lesions. Oncogene 2007;26(4):543–553. [PubMed: 16862169]
53. de Castillo B, Cawthorn S, Moppett J, Shere M, Norman M. Expression of prolactin receptor mRNA in oestrogen receptor positive breast cancers pre- and post-tamoxifen therapy. Eur J Surg Oncol 2004;30(5):515–519. [PubMed: 15135479]
54. De Petrocellis L, Melck D, Palmisano A, Bisogno T, Laezza C, Bifulco M, et al. The endogenous cannabinoid anandamide inhibits human breast cancer cell proliferation. Proc Natl Acad Sci U S A 1998;95(14):8375–8380. [PubMed: 9653194]
55. Melck D, Rueda D, Galve-Roperh I, De Petrocellis L, Guzman M, Di Marzo V. Involvement of the cAMP/protein kinase A pathway and of mitogen-activated protein kinase in the anti-proliferative effects of anandamide in human breast cancer cells. FEBS Lett 1999;463(3):235–240. [PubMed: 10606728]
56. Melck D, De Petrocellis L, Orlando P, Bisogno T, Laezza C, Bifulco M, et al. Suppression of nerve growth factor Trk receptors and prolactin receptors by endocannabinoids leads to inhibition of human breast and prostate cancer cell proliferation. Endocrinology 2000;141(1):118–126. [PubMed: 10614630]
57. Grimaldi C, Pisanti S, Laezza C, Malfitano AM, Santoro A, Vitale M, et al. Anandamide inhibits adhesion and migration of breast cancer cells. Exp Cell Res 2006;312(4):363–373. [PubMed: 16343481]
58. Scorticati C, Mohn C, De Laurentiis A, Vissio P, Fernandez Solari J, Seilicovich A, et al. The effect of anandamide on prolactin secretion is modulated by estrogen. Proc Natl Acad Sci U S A 2003;100 (4):2134–2139. [PubMed: 12578974]
59. Widschwendter M, Widschwendter A, Welte T, Daxenbichler G, Zeimet AG, Bergant A, et al. Retinoic acid modulates prolactin receptor expression and prolactin-induced STAT-5 activation in breast cancer cells in vitro. Br J Cancer 1999;79(2):204–210. [PubMed: 9888458]
60. Lu YF, Sun G, Wang X, Safe S. Inhibition of prolactin receptor gene expression by 2,3,7,8-tetrachlorodibenzo-p-dioxin in MCF-7 human breast cancer cells. Arch Biochem Biophys 1996;332 (1):35–40. [PubMed: 8806706]
61. Buteau H, Pezet A, Ferrag F, Perrot-Applanat M, Kelly PA, Edery M. N-glycosylation of the prolactin receptor is not required for activation of gene transcription but is crucial for its cell surface targeting. Mol Endocrinol 1998;12(4):544–555. [PubMed: 9544990]
62. Huang LJ. Constantinescu SN, Lodish HF. The N-terminal domain of Janus kinase 2 is required for Golgi processing and cell surface expression of erythropoietin receptor. Mol Cell 2001;8(6):1327–1338. [PubMed: 11779507]
63. He K, Loesch K, Cowan JW, Li X, Deng L, Wang X, et al. Janus kinase 2 enhances the stability of the mature growth hormone receptor. Endocrinology 2005;146(11):4755–4765. [PubMed: 16081639]
64. Royer Y, Staerk J, Costuleanu M, Courtoy PJ, Constantinescu SN. Janus kinases affect thrombopoietin receptor cell surface localization and stability. J Biol Chem 2005;280(29):27251–27261. [PubMed: 15899890]
65. Ragimbeau J, Dondi E, Alcover A, Eid P, Uze G, Pellegrini S. The tyrosine kinase Tyk2 controls IFNAR1 cell surface expression. Embo J 2003;22(3):537–547. [PubMed: 12554654]

66. Mukhopadhyay D, Riezman H. Proteasome-independent functions of ubiquitin in endocytosis and signaling. Science 2007;315(5809):201–205. [PubMed: 17218518]

67. Hicke L. Gettin' down with ubiquitin: turning off cell-surface receptors, transporters and channels. Trends Cell Biol 1999;9(3):107–112. [PubMed: 10201076]

68. Djiane J, Houdebine LM, Kelly PA. Down-regulation of prolactin receptors in rabbit mammary gland: differential subcellular localization. Proc Soc Exp Biol Med 1981;168(3):378–381. [PubMed: 6275396]

69. Djiane J, Delouis C, Kelly PA. Prolactin receptor turnover in explants of pseudopregnant rabbit mammary gland. Mol Cell Endocrinol 1982;25(2):163–170. [PubMed: 6276249]

70. Djiane J, Clauser H, Kelly PA. Rapid down-regulation of prolactin receptors in mammary gland and liver. Biochem Biophys Res Commun 1979;90(4):1371–1378. [PubMed: 229837]

71. Genty N, Paly J, Edery M, Kelly PA, Djiane J, Salesse R. Endocytosis and degradation of prolactin and its receptor in Chinese hamster ovary cells stably transfected with prolactin receptor cDNA. Mol Cell Endocrinol 1994;99(2):221–228. [PubMed: 8206330]

72. Li Y, Kumar KG, Tang W, Spiegelman VS, Fuchs SY. Negative regulation of prolactin receptor stability and signaling mediated by SCF(beta-TrCP) E3 ubiquitin ligase. Mol Cell Biol 2004;24(9): 4038–4048. [PubMed: 15082796]

73. Li Y, Clevenger CV, Minkovsky N, Kumar KG, Raghunath PN, Tomaszewski JE, et al. Stabilization of prolactin receptor in breast cancer cells. Oncogene 2006;25(13):1896–1902. [PubMed: 16278670]

74. Lu JC, Piazza TM, Schuler LA. Proteasomes Mediate Prolactin-induced Receptor Down-regulation and Fragment Generation in Breast Cancer Cells. J Biol Chem 2005;280(40):33909–33916. [PubMed: 16103113]

75. Bonifacino JS, Traub LM. Signals for sorting of transmembrane proteins to endosomes and lysosomes. Annu Rev Biochem 2003;72:395–447. [PubMed: 12651740]

76. Thien CB, Langdon WY. Negative regulation of PTK signalling by Cbl proteins. Growth Factors 2005;23(2):161–167. [PubMed: 16019438]

77. Thien CB, Langdon WY. c-Cbl and Cbl-b ubiquitin ligases: substrate diversity and the negative regulation of signalling responses. Biochem J 2005;391(Pt 2):153–166. [PubMed: 16212556]

78. Lu JC, Scott P, Strous GJ, Schuler LA. Multiple internalization motifs differentially used by prolactin receptor isoforms mediate similar endocytic pathways. Mol Endocrinol 2002;16(11):2515–2527. [PubMed: 12403840]

79. Vincent V, Goffin V, Rozakis-Adcock M, Mornon JP, Kelly PA. Identification of cytoplasmic motifs required for short prolactin receptor internalization. J Biol Chem 1997;272(11):7062–7068. [PubMed: 9054398]

80. Clague MJ, Urbe S. Endocytosis: the DUB version. Trends Cell Biol 2006;16(11):551–559. [PubMed: 16996268]

81. Marijanovic Z, Ragimbeau J, Kumar KG, Fuchs SY, Pellegrini S. TYK2 activity promotes ligand-induced IFNAR1 proteolysis. Biochem J 2006;397(1):31–38. [PubMed: 16551269]

82. Deng L, He K, Wang X, Yang N, Thangavel C, Jiang J, et al. Determinants of growth hormone receptor down-regulation. Mol Endocrinol 2007;21(7):1537–1551. [PubMed: 17488973]

83. Ahn S, Kim J, Lucaveche CL, Reedy MC, Luttrell LM, Lefkowitz RJ, et al. Src-dependent tyrosine phosphorylation regulates dynamin self-assembly and ligand-induced endocytosis of the epidermal growth factor receptor. J Biol Chem 2002;277(29):26642–26651. [PubMed: 12011079]

84. Frey MR, Dise RS, Edelblum KL, Polk DB. p38 kinase regulates epidermal growth factor receptor downregulation and cellular migration. Embo J 2006;25(24):5683–5692. [PubMed: 17139251]

85. Zwang Y, Yarden Y. p38 MAP kinase mediates stress-induced internalization of EGFR: implications for cancer chemotherapy. Embo J 2006;25(18):4195–4206. [PubMed: 16932740]

86. Walker AM. S179D prolactin: antagonistic agony! Mol Cell Endocrinol 2007;276(1–2):1–9. [PubMed: 17669587]

87. Goffin V, Bernichtein S, Touraine P, Kelly PA. Development and potential clinical uses of human prolactin receptor antagonists. Endocr Rev 2005;26(3):400–422. [PubMed: 15814850]

88. Beck MT, Chen NY, Franek KJ, Chen WY. Prolactin antagonist-endostatin fusion protein as a targeted dual-functional therapeutic agent for breast cancer. Cancer Res 2003;63(13):3598–3604. [PubMed: 12839947]

Abbreviations

PRLr, prolactin receptor
PRL, prolactin
Jak, Janus kinase
GEF, guanine nucleotide exchange factor
ECD, extracellular domain
ICD, intracellular domain
TM, transmembrane domain
FAK, focal adhesion kinase
EGFR, epidermal growth factor receptor
PRLBP, Prolactin receptor binding protein
E2, estradiol
ER, estrogen receptor
ANA, anandamide
TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin
Ah, aryl hydrocarbon
GHR, growth hormone receptor
EpoR, erythropoietin receptor
IFNAR1, type I interferon receptor
TpoR, thrombopoietin receptor
RTK, receptor tyrosine kinase

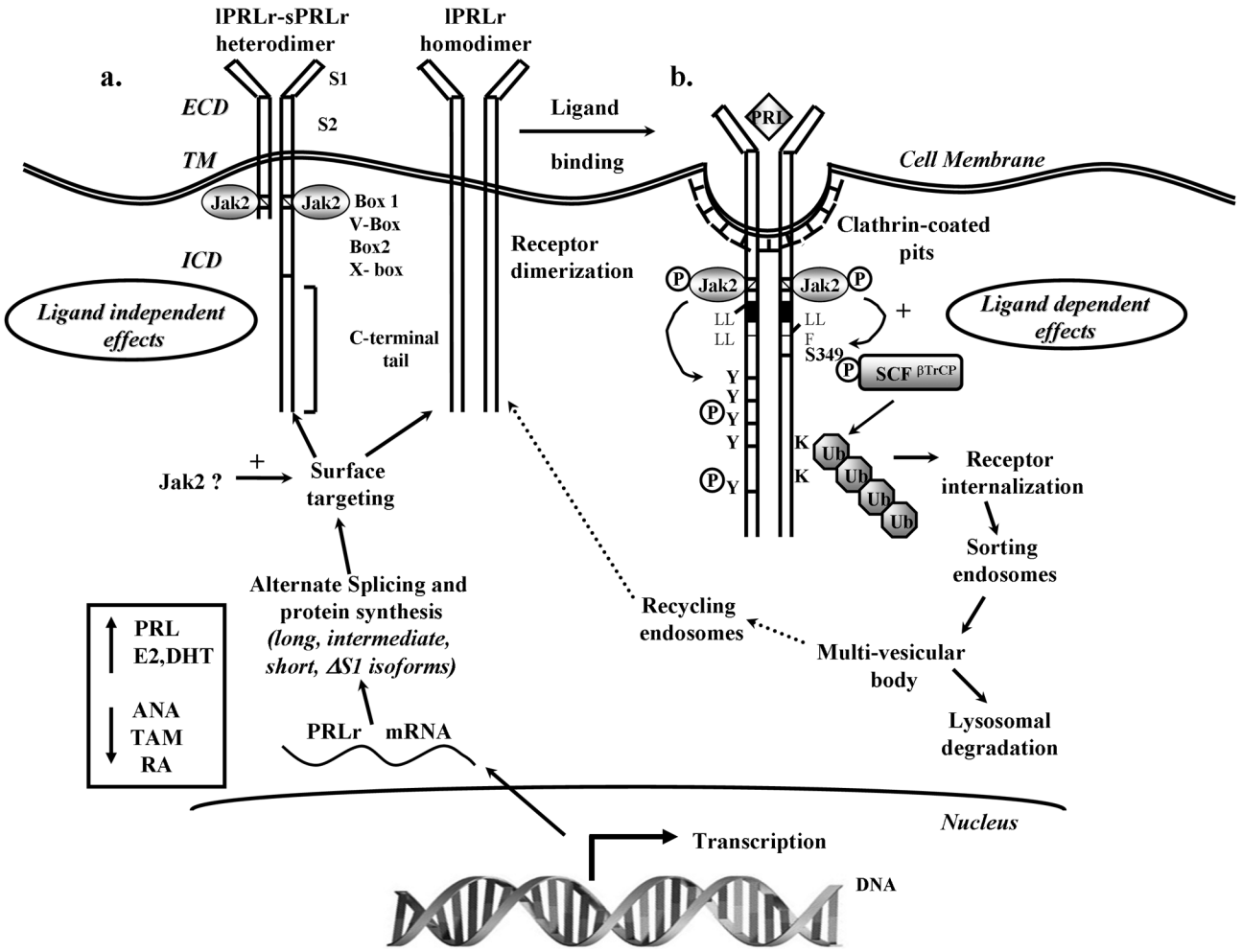

Figure 1. Regulation of surface levels of prolactin receptor

The steady state levels of PRLr are a result of a balance between ligand independent trafficking of newly synthesised receptor to the surface and ligand dependent turnover. **A.** Formation of different isoforms is controlled at the transcriptional, post-transcriptional and post-translational level. Several factors like PRL and hormones upregulate PRLr mRNA, while anti-cancer agents like retinoic acid (RA) down-regulate PRLr trancription. Alternate splicing results in the generation of multiple isoforms, which differ in structure and signaling abilities. The long form of PRLr (lPRLr) has an extracellular domain (ECD) that includes the S1 and S2 region, a transmembrane (TM) domain and an intra-cellular domain (ICD) containing different regions. Jak2 is constitutively bound to PRLr and gets phosphorylated following ligand engagement. The different forms of PRLr are capable of ligand independent homo- and hetero-dimerization, which in turn modulates functional response to ligand. Details are provided in the text. **B.** Ligand binding triggers ubiquitination dependent lysosomal degradation of lPRLr. This involves receptor phosphorylation on Ser349, recruitment of $SCF^{\beta TrCP}$ E3 ubiquitin ligase and subsequent ubiquitination of PRLr on the intracellular lysine(s). These steps are positively regulated by Jak2. PRLr also harbors internalization motifs in the C-terminus (two di-leucines, LL and a di-leucine with adjacent tyrosine (LLY), which contributes to receptor endocytosis. Ubiquitination is expected to promote receptor internalization (via clathrin coated pits), post-internalization sorting, and, ultimately, lysosomal degradation of PRLr.