Leukemia inhibitory factor (LIF) signals via the heterodimeric receptor complex comprising the LIF receptor α subunit (LIFRα) and the common signal transducing subunit for interleukin-6 cytokine receptors, gp130. This study demonstrates that in different cell types, the level of LIFRα decreases during treatment with LIF or the closely related cytokine oncostatin M (OSM). Moreover, insulin and epidermal growth factor induce a similar LIFRα down-regulation. The regulated loss of LIFRα is specific since neither gp130 nor OSM receptor β shows a comparable change in turnover. LIFRα down-regulation correlates with reduced cell responsiveness to LIF. Using protein kinase inhibitors and point mutations in LIFRα, we demonstrate that LIFRα down-regulation depends on activation of extracellular signal-regulated kinase 1/2 and phosphorylation of the cytoplasmic domain of LIFRα at serine 185. This modification appears to promote the endosomal/lysosomal pathway of the LIFRα. These results suggest that extracellular signal-regulated kinase-activating factors like OSM and growth factors have the potential to lower specifically LIF responsiveness in vivo by regulating LIFRα half-life.

Stimulation of Leukemia Inhibitory Factor Receptor Degradation by Extracellular Signal-regulated Kinase*

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Leukemia inhibitory factor (LIF) is one member of the interleukin (IL)-6-type family of cytokines that also include IL-6, IL-11, oncostatin M (OSM), ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1), and neurotrophin-1. LIF is a pleiotropic cytokine that, among other functions, induces differentiation of mouse monocytic leukemia M1 cells, conversion of sympathetic neurons from the adrenergic to cholinergic phenotype, suppresses the differentiation of embryonic stem cells, enhances proliferation of myoblasts, and facilitates endometrial implantation of embryos (reviewed in Refs. 1 and 2).

LIF also plays a role in the systemic inflammatory response, activating the hypothalamic-adrenal axis, and inducing the acute phase reaction of the liver (3). In hepatocytes, LIF, similar to other IL-6 cytokines, stimulates the enhanced expression of a set of plasma proteins, termed acute phase proteins (APP) (4). The precise pattern of APP expression is determined by the action of IL-6 cytokines in combination with various other inflammatory mediators, endocrine hormones, and growth factors (5). For instance, during the acute phase reaction, insulin is increased 3-fold (6) and then modulates the cytokine regulation of APP genes (7, 8). In myoblasts, IGF-1 also reduces LIF action, apparently by down-regulating LIF receptor number (9).

LIFRα is a 190-kDa transmembrane protein with low affinity for LIF. In combination with gp130 subunit, it forms the high affinity LIF receptor complex (10, 11). As described in the human system, OSM also uses LIFRα and gp130 subunits to form a high affinity OSM receptor complex (then termed OSMR complex type I). CNTF, CT-1, and neurotrophin-1 also utilize LIFRα and gp130 subunits (reviewed in Refs. 12 and 13). In addition to the shared LIFRα/gp130 complex used by either LIF or OSM, a specific OSM receptor complex (type II) has been identified that is composed of gp130 and OSMRβ (14). In rodents, OSM seems to act exclusively through the type II receptor complex of gp130/OSMRβ (15, 16).

LIF binding to LIFRα induces heterodimerization with gp130. Janus protein-tyrosine kinases (JAK), constitutively associated with the cytoplasmic domain of these receptors, are then activated by trans- and autophosphorylation, and in turn phosphorylate tyrosine residues in both LIFRα and gp130 intracellular domains. Those phosphorylated tyrosines, within the Box3 sequence context YXXQ, create docking sites primarily for STAT3 but also STAT1. Other sites are recognized by linker proteins which, upon phosphorylation, propagate the signal to other pathways (MAPK, PI3K) (reviewed in Refs. 12, 13, and 17). The Src homology 2 domain-containing protein-tyrosine phosphatase SHP2 functions as such a linker toward Grb2 and MAPK. SHP2 also acts, through its catalytic activity, as a negative regulator of the JAK/STAT signaling by down-regulating JAK activity, and consequently lowering the induction of STAT3-dependent genes (18–20). Other inhibitors of LIF activity are members of the SOCS (suppressor of cytokine signaling) family, especially SOCS3, and the phosphatase SHP1, which, in pituitary cells, is found constitutively associated with JAK. SHP1 decreases JAK and STAT3 phosphoryl-
ERK Stimulates LIFRα Degradation

and SOCS3 has been shown to deactivate JAK within 1 h of LIF treatment (21).

Ligand-induced LIFRα/gp130 heterodimerization increases serine phosphorylation of the LIFRα cytoplasmic domain. ERK1 and ERK2 are implicated in phosphorylating Ser1044 (or Ser185 of the cytoplasmic domain) of LIFRα (22). Moreover, activation of ERK by insulin also increases LIFRα Ser185 phosphorylation. It has been suggested that this modification may contribute to the modulation of insulin of cell responsiveness to LIF (22). However, the mechanism of this LIFRα regulation is unknown. A similar serine phosphorylation of gp130 or OSMRβ by MAPK has not yet been demonstrated, and in contrast to LIFRα, both gp130 and OSMRβ do not contain an overt substrate site for MAPK kinases.

Constitutive, ligand-independent endocytosis of gp130 and LIFRα has been observed that depends in part on dileucine motifs within the cytoplasmic domain of these receptors (23, 25). In the case of gp130, an interaction with the adaptor protein AP2 has been described, which presumably results in a transfer of the receptor into clathrin-coated pits, followed by efficient endocytosis, trafficking from endosomes to lysosomes, and finally lysosomal degradation (24, 25). Most of the current evidence indicates that gp130 half-life is not appreciably modified by ligand binding (i.e. IL-6 binding), suggesting that gp130 signal transduction does not modify internalization/degradation of this receptor subunit.

In this study we show that, in various cell types, LIFRα is specifically down-regulated by treatments with LIF and other effectors that activate ERK 1/2. We suggest that Ser185 phosphorylation of LIFRα promotes lysosomal degradation of this receptor subunit. In contrast, co-activated gp130 or OSMRβ proved to be significantly more stable under the same conditions. Finally, we demonstrate that, in hepatic cells, specific LIFRα down-regulation correlates with a lower LIF induction of signaling and gene expression.

MATERIALS AND METHODS

Tissue Culture Cells—Rat H-35 (clone T-7-18; Ref. 26), H-35 cells stably expressing mOSMRβ (27) or chimeric G-CSFR-gp130 (39), and human HepG2 (clone 86-6; Ref. 41), NIH3T3, and HeLa cells were maintained in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum and antibiotics. Cells used for analyzing signaling or LIFRα down-regulation were incubated for 5–18 h in serum-free Dulbecco’s modified Eagle’s medium. Treatments were carried out in serum-free medium containing 100 μg/ml recombinant human IL-6 and LIF (Genetics Institute, Cambridge, MA), hOSM (Immunex Corp., Seattle, WA), mOSM (prepared in COS-1 cells as described in Ref. 16), EGF (Collaborative Research, Inc., San Jose, MO), 500 ng/ml porcine serum albumin and 5 mM Man-6-P, to avoid binding to the Man-6-P/UDP-glucose glycoprotein 2-epimerase (EC 2.4.99.2) of HepG2 cells.

Radioiodination of LIF and Binding—Chinese hamster ovary cells-derived LIF was iodinated at a specific radioactivity of around 15,000 μCi/nmol according to the chloramine T method as described (29). For binding studies, H-35 monolayers were incubated in phosphate-buffered saline containing 0.05% trypsin and 0.02% EDTA. 0.5 ml/cm² monolayers were washed for 90 min with an increasing concentration of labeled LIF in phosphate-buffered saline, containing 0.5% bovine serum albumin and 5 mM Ca++ to avoid binding to the Man-6-P/UDP-glucose glycoprotein 2-epimerase (EC 2.4.99.2) of HepG2 cells.

Soluble Human LIFRα Analysis—Parental or hLIFRα-expressing H-35 cells in serum-free medium were treated 4 h with LIF or insulin. Supernatants were collected, dialyzed against 25 mM NH4HCO3, lyophilized, and resuspended in phosphate-buffered saline. The amount of soluble LIFRα in the supernatants was measured by a specific ELISA as reported previously (43).

Plasma Protein Analysis—Synthesis and secretion of APP into the culture medium of cytokine-treated H-35 cells were quantified by immunoelectrophoresis (32). The area under the precipitation peaks (proportional to the amount of antigen) was integrated and expressed in arbitrary units.

RESULTS

LIFRα Is Down-regulated by LIF Treatment—In hepatic cells, the LIF-induced signal transduction, as determined by STAT activation and induction of APP genes, is transient, implying the presence of intracellular negative regulatory mechanisms. In pituitary cells, protein-tyrosine phosphatases SHP1 and SHP2, and SOCS3, are proposed to down-regulate LIF signaling within 30 min to 1 h by inactivation of JAK2 and/or STAT3 (18–21, 33). In myoblasts, it is also suggested that separable mechanisms operate via down-regulation of the LIFRα protein (9). To test the latter possibility, we measured the level of immunodetectable LIFRα after LIF treatment in the rat hepatoma cell line H-35. LIFRα proteins appeared as doublets with 190 and 170 kDa (Fig. 1A). Treatment with monensin, which prevents the transfer of glycoproteins from the endoplasmic reticulum/Golgi ap-
Regulation of LIFRα Turnover in Trans—These first experiments indicated that LIFRα level is regulated in part through LIF binding (autologous regulation). To test the possibility of a regulation of LIFRα level in trans (heterologous regulation), we treated H-35 cells with OSM, IL-6, and insulin. As shown in Fig. 1C, H-35 cells, which do not express endogenous OSMRβ subunit and thus are devoid of an OSMR complex type II (27), respond to human OSM (hOSM) by a reduced LIFRα level (40% level after 2 h) through the action of the OSMR complex type I, formed by LIFRα and gp130 (11, 14, 36). In contrast, IL-6 had little influence (Fig. 1, A and C). To prove that signaling by OSMR complex type II has also the ability to act in trans, we used the recently isolated H-35 cell line stably expressing the transduced murine OSMRβ (27). As shown in Fig. 1 (A and C), mOSM that unlike hOSM does not function through LIFRα (15, 16), induced a down-regulation of LIFRα in mOSMRβ H-35 cells (30% level) but not in parental H-35 cells. Furthermore, treatment of H-35 cells with insulin similarly reduced the level of LIFRα (30% level; Fig. 1, A and C), indicating that LIFRα can also be down-regulated in trans.

Interestingly, insulin in parental H-35 cells or mOSM in mOSMRβ H-35 cells induced the mobility shift of the functional LIFRα (at early time points of treatment). Moreover, both factors induced the appearance of degradation products (after 1 h of treatment), similar to that observed with LIF treatment (Figs. 1A and 5C). The finding of factor-induced reduction of LIFRα suggested that all these factors might affect the turnover process or the synthesis of LIFRα. To discriminate between these two hypotheses, we measured immunodetectable LIFRα in H-35 cells whose protein de novo synthesis was inhibited by cycloheximide (Fig. 1D). Under these conditions, the fully processed LIFRα protein showed an apparent half-life of approximately 2 h and treatment with insulin lowered this value to 1 h. This suggests a factor-induced degradation of the receptor subunit. The small size precursor LIFRα form showed a t1/2 of 30 min to 1 h and appeared insensitive to insulin treatment. Treatment of the cells with monensin, which prevents protein secretion, did not interfere with the turnover of full-length LIFRα but resulted in the accumulation of the short form LIFRα, as expected for a precursor/product relationship (Fig. 1D).

Gp130 Has a Distinct Turnover Mechanism—Considering the obligatory signal-transducing function of gp130, we determined the influence of cytokine treatments on gp130 protein. We measured the level of gp130 by immunoprecipitation fol-
lowed by immunoblotting (Fig. 2A). LIF, IL-6, or mOSM (in mOSMRβ-H-35 cells) treatment caused a decrease of gp130 level, which was notable after 1 h (65%, 45%, and 70% levels, respectively), but returned to basal values by 2 h (between 85% and 90% level). Treatment with insulin demonstrated a similar low decrease in gp130.

**Down-regulation of LIFRa Contributes to a Reduced Response to LIF**—The results from the experiments illustrated in Figs. 1 and 2 indicate that LIFRa is target for down-regulation by its own, and other, receptor system signals. In contrast, gp130 (Fig. 2) or OSMRβ (Figs. 5A and 8B), as the other members of the IL-6 family of cytokine receptors with signal transducing activity, are less sensitive to signal acting in trans. This finding raises the possibility that LIF, as well as factors such as OSM or insulin, are able to limit LIF responsiveness of cells. Indeed, insulin pretreatment lowered LIF binding (Fig. 3A) and the LIF-inducible tyrosine phosphorylation of LIFRa, gp130, and STAT3 (Fig. 3B, upper panels). In contrast, mOSM or IL-6-inducible STAT3 tyrosine phosphorylation is maintained in insulin-treated cells (Fig. 3B, lower panels).

Insulin has pleiotropic effects, some of which have been demonstrated to reduce IL-6-induced acute phase protein expression (4, 7, 8). However, pretreatment with insulin lowered 2 times more LIF-induced APP expression than did treatment with IL-6 or mOSM (Fig. 3C). This suggests that insulin effect on LIFRa level contributes to the reduced response to LIF.

**Activation of ERK1 and ERK2 Contributes to Down-regulation of LIFRa in H-35 Cells**—As shown in Fig. 1A, the activation of ERK 1/2 but not of STAT3 seems to correlate with enhanced turnover of LIFRa. Although LIF, mOSM, and IL-6 induced STAT3 phosphorylation to approximately the same level, only LIF and mOSM activated ERK 1/2 to the same high level as insulin. To test the relative contribution of ERK to the LIFRa down-regulation, we determined the effect of U0126, an inhibitor of MEK1, on prevention of LIFRa reduction. Pretreatment of H-35 cells with U0126, at a concentration sufficient to suppress activation of ERK but not tyrosine phosphorylation of the insulin receptor, IRS1/2, or STAT3 (Fig. 3A and B, bottom panels), removed the insulin-dependent loss of LIFRa (Fig. 4A, top panel). Similarly, U0126 suppressed LIFRa degradation in cells treated with LIF or mOSM (Fig. 4B).

Schiemann et al. (22) have described, for 3T3-L1 cells, a phosphorylation of the cytoplasmic domain of the LIFRa by ERK1/2. This phosphorylation occurs primarily at the MAPK substrate site Ser185 of the LIFRa and is increased after LIF, insulin, and phorbol 12-myristate 13-acetate treatment. To test the hypothesis that LIFRa degradation is dependent on Ser185 phosphorylation, we established stable H-35 cells expressing either the wild type (WT) or Ser185 to Ala (S/A) mutant human

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**Fig. 2.** gp130 is more stable than LIFRa. A, parental H-35 cells or mOSMRβ-H-35 cells (for mOSM treatment) were treated with the indicated factor and endogenous gp130 levels were analyzed by Western blotting. In order to obtain better signals, gp130 was immunoprecipitated (I.P.) prior to Western blotting (W.B.). B, signals corresponding to the larger size form were quantified by densitometry.

**Fig. 3.** Insulin down-regulates prominently LIF activity. A, Scatchard plot of the binding of iodinated LIF to H-35 cells incubated 5 h in absence (3,040 sites/cell, Kᵣ = 0.09 nM) or presence (460 sites/cell, Kᵣ = 0.11 nM) of 500 ng/ml insulin. B, parental H-35 cells (LIF and IL-6 treatments) or mOSMRβ-H-35 cells (mOSM treatments) were pretreated with insulin for the indicated time and then treated 10 min with the indicated cytokine. One part of the cell lysates was used for LIFRa immunoprecipitation experiments (Western blotting phosphotyrosine (PY, left panel) or LIFRa (right panel)), and the other part was used for direct PY-STAT3 or STAT3 immunoblotting. I.P., immunoprecipitation; W.B., Western blotting. C, parental or mOSMRβ-H-35 cells were pretreated 8 h with or without insulin and then treated 24 h with the indicated cytokines in the presence of dexamethasone. Conditioned mediums were analyzed by immunoelectrophoresis for thiostatin, α₂-macroglobulin, and α₁-acid glycoprotein.
LIFRα tagged with C-terminal Myc epitope. To evaluate the relative contribution of extracellular domain on LIFRα turnover, we also generated H-35 cells expressing the chimeric receptor comprising the extracellular domain of G-CSFR and the transmembrane/cytoplasmic domain of human LIFRα. Immunodetectable receptor proteins were determined after treatment with LIF (Fig. 5A) or insulin (Fig. 5, A–C). The transduced LIFRα (WT) and chimeric G-CSFR-LIFRα (WT) showed a mobility shift immediately after treatment with LIF or insulin (Fig. 5, B and C). Degradation kinetics, with the appearance of breakdown intermediates (Fig. 5, A and B), were comparable to that established for the endogenous LIFRα (Fig. 1), whereas mOSMRβ level, in mOSMRβ-expressing H-35 cells (27), was stable (Fig. 5A). In the case of insulin-treated cells, LIF binding to the cells was decreased proportionally to the LIFRα protein (Fig. 5D). These results suggested that the information for specific LIFRα down-regulation and degradation is contained in the transmembrane/cytoplasmic domain. The critical role of Ser185 for down-regulation was recognized in LIFRα(S/A), which, despite normal STAT and ERK signaling (data not shown), did not show a ligand-induced mobility shift (Fig. 5C) and was significantly more stable than LIFRα (WT) (Fig. 5, A, B, and D). The remaining ligand-induced decrease of LIFRα(S/A) (75–80% level after 2 h) was in the range of the ligand-induced decrease of endogenous gp130 (Fig. 2) or chimeric G-CSFR-gp130 (Fig. 5A; Ref. 19), and was prevented by pretreatment with U0126 (data not shown).

Taken together, the data support the model that activated ERK1/2 phosphorylate Ser185, and this modification in turn is sufficient to alter the electrophoretic migration and promote degradation of LIFRα. The regulated loss of LIFRα seems to be specific since neither gp130 nor mOSMRβ showed a compara-
ble change in turnover.

The Leu-Ile Motif of LIFRα Is Required for Increased Endocytosis/Lysosomal Degradation—The degradation pathways of LIFRα could be divided into the basic steps: (i) proteolytic shedding of extracellular domain of LIFRα, (ii) endocytosis and localization in the endosomal compartment, and (iii) transfer to the lysosomal compartment (sensitive to PI3K inhibition by wortmannin; Refs. 37 and 44) or, alternatively, recycling to the cell surface.

To identify the contribution of some of the key enzymes to LIFRα turnover, H-35 cells were treated with specific inhibitors. Pretreatment with 1,10-phenanthroline (inhibition of metalloproteases including cell surface enzymes; data not shown), acetyl-Leu-Leu-Met (calpain inhibitor), or proteasome inhibitor I did not prevent LIFRα down-regulation (Fig. 6B). Pretreatment with wortmannin was similarly ineffective in preventing loss of full-length LIFRα but caused an accumulation of LIFRα fragments (Fig. 6, A and B). Pretreatment with chloroquine (lysosome inhibitor) reduced the LIFRα down-regulation after insulin, LIF, and mOSM treatment to 90%, 75%, and 80% levels, respectively, and also promoted accumulation of LIFRα fragments (Fig. 6, A–C).

To document more precisely the contribution of proteolytic shedding of LIFRα extracellular domain to LIFRα down-regulation, hLIFRα(WT)-transduced H-35 cells were treated for 4 h with LIF or insulin, and the concentration of soluble hLIFRα in the culture supernatant was determined (Fig. 6D). The ELISA used for this measurement is specific for human LIFRα and did not cross-react with any material from parental H-35 cells (Fig. 6D, left panel). A constitutive basal rate of soluble human LIFRα release was detected, which amounted in three separate experiments to 100 pg/h/10⁶ cells. LIF treatment did not change significantly this LIFRα shedding, but in the presence of insulin, the value was enhanced between 20% and 70% (Fig. 6D, right panel). Under these conditions, the membrane-associated hLIFRα protein was effectively reduced (Fig. 6D, lower panels). By taking into consideration a LIFRα concentration of 2,000 molecules/cell, and the receptor having a turnover of 2 h, we calculated that approximately 5–10% of cellular LIFRα is shed. Since the loss of cell-associated hLIFRα protein exceeded appreciably that accounted for by shedding, a prominent role of intracellular degradation (chloroquine-sensitive) as part of the observed LIFRα turnover is predicted.

To visualize a possible subcellular redistribution of LIFRα as a function of autologous or heterologous treatments, we employed HepG2 cells transiently transfected with expression vectors for GFP-tagged WT, S/A mutant, or LI/AA endocytosis-deficient mutant (23) human LIFRα (Fig. 7). Wild type LIFRα was predominantly detected at the plasma surface (uniform green staining), and in the endoplasmic reticulum/Golgi compartment (punctate staining, probably representing the LIFR-GFP precursor form). After LIF or OSM treatment for 24 h, the plasma membrane staining disappeared, while the punctate, most probably endosomal/lysosomal, intracellular staining has intensified. S/A and LI/AA mutants LIFRα did not prominently respond to LIF and OSM treatment by a redistribution of tagged LIFRα to the intracellular structures, in contrast to wild type LIFRα. Western blot analysis (data not shown) confirmed the retention of mature LIFRα protein in S/A and LI/AA LIFRα-expressing cells.

Taken together, these results suggest that increased lysosomal degradation of LIFRα is responsible for down-regulation of the wild type receptor subunit, and that LI-mediated endocytosis and ERK-dependent tagging are necessary for factor-regulated LIFRα degradation.

LIFRα Down-regulation/Degradation Is Not Restricted to Hepatoma Cells—Since all the studies presented so far have used hepatoma cells as experimental tools, the question arises whether similar LIFRα regulation occurs in other cell types. Therefore, similar analyses were performed on NIH3T3 mouse fibroblasts and HeLa human epithelial cells. As shown in Fig. 8A, LIF or mOSM on NIH3T3 cells induced an early mobility shift and down-regulation of LIFRα. Degradation products of LIFRα were not detected, presumably because of a lower LIFRα expression in these cells. Other factors, namely IL-6 and insulin, did not significantly activate ERK and did not modify LIFRα level (data not shown). Interestingly, chloroquine, but not U0126, prevented the effect of LIF on LIFRα. In contrast, both reagents suppressed the effect of mOSM, as already observed in H-35 cells (see Figs. 4 and 6). NIH3T3 cells also confirmed the subunit-specific effect by an unaltered expression level of gp130 (Fig. 8A). Western blot analyses of mOSMRβ6 could not be performed due to the unavailability of immune reagents to this receptor subunit.
A LIFRα regulation in *trans* could be also demonstrated by EGF treatment in HeLa cells (Fig. 8B). This ERK-activating growth factor (data not shown) induced a down-regulation of LIFRα to the same level as with LIF (60% level after 2 h), whereas the same treatment did not appreciably affect levels of gp130 or OSMRβ.

**DISCUSSION**

This study demonstrates that LIFRα is down-regulated as the result of ligand binding (autologous regulation), or the action of OSM, insulin, and EGF in *trans*. The suggested mode of regulation is by increased endocytosis/trafficking and lysosomal degradation. In contrast, the constitutive turnover of gp130 is largely unaffected by cytokine and hormone treatment, a phenomenon that has also been observed in other experimental systems (24, 25).

The results have revealed different molecular mechanisms of LIFRα down-regulation. The one described here is dependent on activated ERK, which phosphorylates Ser185 in the cytoplasmic domain of the LIFRα (22). A possible consequence of this tagging by Ser phosphorylation could be a facilitated interaction between the LI motif in LIFRα and α-adaptin (AP2) in clathrin-coated pits, resulting in endocytosis, and further lysosomal degradation (23). If so, the Ser185-dependent “regulated” pathway for LIFRα could mechanistically resemble the “constitutive” turnover of gp130 that depends on Ser139, located six amino acids upstream of the LL motif in gp130 (24, 25). However, our attempts to demonstrate a physical association between LIFRα and AP2 by the experimental approach of co-immunoprecipitation, as applied by Thiel *et al.* (24), were unsuccessful.2 An alternative mode of Ser185-dependent pathway could be proposed that is comparable to the pathway demonstrated for insulin receptor down-regulation (37). This mode proposes that the endocytosis of LIFRα is not affected by Ser185 modification but that the tagged LIFRα is transferred more efficiently from endosomes to lysosomes and/or subject to reduced recycling to the cell surface. The subcellular transfer of LIFRα could also be promoted by ERK affecting cytoskeletal functions such as myosin light chain kinase activity (46). Either of the two mechanisms of regulated turnover will result in

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2 Frédéric Blanchard and Heinz Baumann, unpublished data.
a persistent reduction of LIFRα at the cell surface as shown in Figs. 3A and 7.

The results with NIH3T3 cells indicated yet another mechanism of LIFRα down-regulation that appears to be independent of activated ERK (insensitive to U0126). This down-regulation occurs only after autologous stimulation with LIF. Since inhibitors to ERK, PI3K, or p38 MAPK did not prevent this type of receptor regulation2 (Fig. 8A), two possible alternative mechanisms are possible. One mechanism would propose involvement of other kinases: 1) by Ser phosphorylation through a non-ERK mechanism, such as casein kinase II, implicated in Man-6-P/IGF-II, CD3-γ chain, and CD4 down-regulation (47–49); 2) by tyrosine phosphorylation, like that for EGFR, to enhance the interaction with α-adaptin (AP2; Ref. 50); or 3) by ubiquitination of the cytoplasmic domain, to increase its internalization, as seen with the GHR (51, 52). In this case, the ubiquitin conjugation system (E3) would assist in an adaptor role between the receptor and α-adaptin (AP2). The other mechanism proposes that the decrease in receptor level is independent of signal transduction, but relies on ligand-induced receptor heterodimerization. The close proximity of the suggested α-adaptin (AP2) binding site in the cytoplasmic domain of the LIFRα and gp130 in the heteromeric receptor complex would result in a higher recruitment in clathrin-coated pits than the corresponding monomeric receptor chains in the absence of the ligand. This form of turnover is assumed to be cell type-specific, i.e. highly effective in NIH3T3 cells. Yet, this intracellular process of lysosomal degradation seems to be restricted to LIFRα and accounts for the differential turnover of LIFRα and gp130 (Fig. 8A).

LIF response can be divided into early receptor-initiated reactions (ligand binding, JAK activation, and protein recruitment to receptor), and receptor downstream events that affect receptor endocytosis/degradation and attenuate signaling reactions. The latter process involves regulatory mechanisms such as dephosphorylation of JAK or STAT3, by SHP1/2 or inhibition of the Jak cascade, and CD4 down-regulation (47–

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