The cytokine receptor subunits gp130, leukemia inhibitory factor receptor α (LIFRα), and oncostatin M receptor β (OSMRβ) transduce OSM signals that regulate gene expression and cell proliferation. After ligand binding and activation of the Janus protein-tyrosine kinase/STAT and mitogen-activated protein kinase signal transduction pathways, negative feedback processes are recruited. These processes attenuate receptor action by suppression of cytokine signaling and by down-regulation of receptor protein expression. This study demonstrates that in human fibroblasts or epithelial cells, OSM first decreases the level of gp130, LIFRα, and OSMRβ by ligand-induced receptor degradation and then increases the level of the receptors by enhanced synthesis. The transcriptional induction of gp130 gene by OSM involves STAT3. Various cell lines expressing receptor subunits to the different interleukin-6 class cytokines revealed that only LIFRα degradation is promoted by activated ERK and that degradation of gp130, OSMRβ, and a fraction of LIFRα involves mechanisms that are separate from signal transduction. These mechanisms include ligand-mediated dimerization, internalization, and endosomal/lysosomal degradation. Proteosomal degradation appears to involve a fraction of receptor subunit proteins that are ubiquitinated independently of ligand binding.

Interleukin-6 (IL-6), oncostatin M (OSM), and leukemia inhibitory factor (LIF) are functionally and structurally related and are part of the IL-6 family of cytokines (1–5). Each IL-6 cytokine is recognized by a specific ligand binding receptor subunit. In humans, OSM is exceptional in that it interacts with gp130 and with either LIFRα or OSMRβ to form the high affinity, signaling-competent OSM receptor complex I or II (3, 4). Ligand-induced oligomerization of receptor subunits activates Janus protein-tyrosine kinases (JAKs), which in turn phosphorylate tyrosine residues in the receptor cytoplasmic domain. These phosphorylated tyrosines create docking sites for STAT transcription factors (STAT1, -3, and -5), protein-tyrosine phosphatase SHP-2, and linkers proteins such as Gab-1, Grb2, or SHC, which propagate the signal to other pathways (ERK 1/2, JNK, phosphatidylinositol 3-kinase; Refs. 1–8). Receptor signaling is manifested by the activation of genes such as acute phase proteins (2) or the cyclin-dependent kinase inhibitor p21WAF1, which is primarily activated through STATs (9) and immediate early response genes such as c-fos, c-jun, and egr-1 primarily through ERK 1/2 (6).

Signaling by IL-6 cytokine receptors is transient, often restricted temporally and in magnitude by the action of negative regulators. The SH2 domain-containing protein-tyrosine phosphatases SHP1 and -2, through their catalytic function, attenuate the activity of receptor-associated JAKs and consequently lower the induction of STAT-dependent genes (4, 6). The suppressor of cytokine signaling SOCS1 and -3 are rapidly induced by IL-6 cytokines and, through their SH2 domain, interact and deactivate JAKs or gp130 (4, 10). The protein inhibitors of activated STATs associate with activated STATs, leading to a loss of STAT-DNA binding activity (11). Containment of IL-6 cytokine signaling appears to be directed by two distinct mechanisms, (a) the induction or mobilization of factors that attenuate functions of the cytoplasmic domains of the receptor proteins and (b) the enhanced degradation of receptor proteins. Recently we have also demonstrated that receptor signals acting in trans determine the level of the receptor subunit LIFRα and, thus, the cellular responsiveness to LIF (12). ERK 1/2, activated by IL-6 cytokines or growth factors, phosphorylates serine 1044 (or serine 185 of the cytoplasmic domain) of LIFRα, leading to its lysosomal degradation independent of LIF binding (12, 13). An additional ERK-independent degradation pathway for LIFRα has also been observed in NIH-3T3 cells, but this degradation occurred only after LIF treatment (12).

The other receptor subunits, gp130 and OSMRβ, do not possess a phosphorylation site for ERK 1/2 and, thus, do not appear to be appreciably influenced by activated ERK. However, serine 782 of gp130 located in the cytoplasmic domain has been described as being phosphorylated and directing the cell surface expression of the receptor subunit (14). The kinase for this modification is still unknown. Serine 782 is located immediately N terminus to the di-leucine motif of gp130, which was reported to trigger the constitutive, ligand-independent endocytosis of gp130 (15). The adaptor protein AP2 was noted to interact with...
the di-leucine motif, enabling the transfer of receptors to clathrin-coated pits, endocytosis, and intracellular targeting to lysosomal degradation (15, 16). No corresponding information regarding turnover of the other receptor subunit OSMRβ is available. In this study we asked whether ligand-dependent degradation of gp130 is indeed determined by specific elements in its cytoplasmic domain and whether turnover of receptor complexes that include LIFRs and OSMRβ follow processes that apply to gp130.

MATERIALS AND METHODS

**Tissue Culture Cells**—NIH-3T3 fibroblasts, MCF7 breast carcinoma cells, and clonal lines of H-35 cells stably expressing LIFRβ (12), OSMRβ (8), the chimeric and C-terminal FLAG epitope-tagged G-CSFR-gp130 (60) construct with the full-length 277-residue cytoplasmic domain of gp130 (6), or truncated G-CSFR-gp130(133)wt (containing the 133-residue membrane-proximal cytoplasmic domain of gp130) or the tyrosine-to-phenylalanine mutants of this chimeric construct, Y2F (Tyr579 or tyrosine 117 of the cytoplasmic domain), Y3F (Tyr579 or tyrosine 125 of the cytoplasmic domain), or Y2F,Y3F (tyrosines 117 and 125) (6, 17) were maintained in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum and antibiotics. Primary cultures of human primary fibroblasts and alveolar epithelial cells were prepared from residual lung tissues derived from surgical pneumectomy specimens and provided by the Tissue Procurement Service at Roswell Park Cancer Institute. The proliferating epithelial cells were maintained in serum-free keratinocyte medium supplemented with cholera toxin and epidermal growth factor (Life Technologies, Inc.). The homogeneity of the primary cell cultures was confirmed by immunochromatographic staining for cell type-specific cytkeratins and integrins. To analyze signaling or receptor down-regulation, cells were incubated for 5–18 h in serum-free medium and then treated with 100 ng/ml recombinant IL-6 and LIF (Genetic Institute, Cambridge, MA), human OSM (Immunex Corp., Seattle, WA), or mouse OSM (prepared in COS-1 cells as described in Ref. 8). MEK-1 activity was inhibited by 25 μM U0126 (Promega, Madison, WI), protein synthesis by 10 μg/ml cycloheximide (Sigma), lysosome activity by 100 μg/ml chloroquine (Sigma), and proteasome activity by 1 μM MG132 (Calbiochem).

**Plasmid Constructs**—The following expression vectors have been described previously: wild type and S185A mutant of human LIFRs (12, 13), human OSMRβ (8), human gp130 and chimeric human G-CSFR-gp130 (6) in the vector pDC302, and rat STAT3Δ35C (lacking 55 C-terminal residues; Ref. 18) in pSV-Sport 1. Ubiquitin-HA in the expression vector pMT213 was provided by Nicholas Heintz (University of Vermont; Ref. 19). The chimeric construct G-CSFR-gp130, with deleted cytoplasmic domain but retaining the transmembrane domain of gp130 (residues 599–645 of gp130) and with the FLAG epitope following the remaining 4 cytoplasmic residues (G-CSFR-gp130Δcyto/FLAG), was generated by PCR using the G-CSFR-gp130 in pDC92 as a template. The chimeric receptor construct was transferred into the retroviral vector MINV (6, 8). The retrovirus-producing cells in the packaging PA317 cells were used to transduce H-35 cells. Stable integrants were selected in medium containing 2 mg/ml G418 (6, 8). Clonal lines expressing G-CSFR-gp130Δcyto/FLAG were identified by immunoblotting for the FLAG epitope.

**Transient Transfection**—NIH-3T3 and MCF7 cells were transfected with FuGene6 (Roche Molecular Biochemicals) according to the manufacturer’s recommendation using a ratio of 6 μl of FuGene6 to 4 μg of DNA. In all transfections, 0.25 μg of pEGFP-N1 (Upstate Biotechnology Inc.) was included as marker of transfection efficiency (8). To enrich for NIH-3T3 cells transfected with expression vectors for STAT3Δ35C, GFP−, and GFP+ cells were selected by sterile fluorescence-activated cell sorting as described (8).

**Immunoprecipitation and Western Blotting**—Cell monolayers were lysed in radioimmune precipitation buffer (50 μl/ml 2% monolayers). Lysates were incubated with antibodies and protein-G-conjugated Sepharose (Amersham Biosciences, Inc.). The immunoprecipitates or aliquots of whole cell lysates were separated on 6–12% SDS-polyacrylamide gels, and proteins were transferred to protein membranes (Schleicher & Schuell). The membranes were reacted with antibodies to the extracellular domain of human OSMRβ (Immunex Corp.), to the C-terminal peptide of the cytoplasmic domain of LIFRβ or gp130 (Santa Cruz Biotechnology), and to STAT3, ERK 1/2, SOCS3, SHP-2, FLAG, HA (Santa Cruz Biotechnology), PY-STAT3, PY-STAT5, P-p38, P-ERK (New England Biolabs, Inc.), JAK1, Myc (Upstate Biotechnology, Inc.) and followed with secondary antibodies (ICN Biomedicals, Inc., Aurora, OH) in phosphate-buffered saline containing 0.1% Tween, 5% milk or 3% bovine serum albumin. Immunoreactions were visualized by enhanced chemiluminescence reaction (ECL) according to the manufacturer (Amersham Biosciences, Inc.). From each blot, several x-ray films were prepared before exposing for different lengths of times. The bands on these films were scanned by densitometry and quantified by using the ImageQuant program (Molecular Dynamics).

**RT-PCR and Northern Blot Analysis**—Cellular RNA was extracted by the Trizol method (Life Technologies). For RT-PCR analysis, aliquots of 8 ng to 5 μg of RNA were subjected to cDNA synthesis with 400 units of Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.) and 0.5 μg of oligo(dT) 15-mer. The cDNA present in each reaction mixture was amplified with 0.625 units of Taq polymerase (Promega) and 10 pmol each of sense and antisense primers (20, 21). The thermal cycle profile was as follows: denaturation for 1 min at 94°C, annealing for 1 min at 59°C, and extension for 1 min at 72°C for 30–35 cycles. The ethidium bromide-stained patterns of the electrophoretically separated PCR products were digitally photographed. The staining intensity of the bands was determined by integration of the pixel values using the ImageQuant program. For Northern blot analysis, aliquots of 20 μg of RNA were separated on 1.5% formaldehydeagarose gel, transferred to a nylon membrane (Schleicher & Schuell), and reacted with 32P-labeled cDNA probes for OSMRβ or gp130. The radioactive patterns were visualized by autoradiography and by phosphorimaging (Molecular Dynamics). The ethidium bromide staining pattern of separated ribosomal RNAs was used as a marker for sample loading.

**RESULTS**

**Modification of Receptor Subunits Level during OSM Treatment**—Recently, we showed that rat H-35 hepatoma cells respond to 6 h of treatment with LIF or OSM by down-regulation of LIFRs (12). In contrast, the level of gp130 remains essentially constant with only a slight decrease during the first hour and returns to basal values by 2 h (12). To identify whether the mode of receptor subunit turnover as determined in hepatoma cells is also established in other non-hepatic and non-transformed cell types, we analyzed normal human lung fibroblasts and epithelial cells. Based on the activation profile of STAT3, STAT5, and mitogen-activated protein kinases p38 and ERK 1/2, lung fibroblasts responded strongly to OSM and to a lesser degree to LIF or IL-6 (Fig. 1A). Similar results have been obtained with bronchial and alveolar epithelial cells (data not shown; Refs. 22 and 23). Within 2 h of OSM treatment, the level of activated STAT3 was reduced by 90% in both fibroblasts and epithelial cells, but by 4–6 h of treatment, it increased again (Fig. 1, B and D). During this treatment period, the level of the fully processed form of OSMRβ (Form 1 in Fig. 1B) was reduced to 40%, whereas the level of the precursor form (Form 2) was increased 2-fold. In the same cells, the higher molecular forms of LIFRs and gp130 (Form 1) decreased to 50% by a 2-h OSM treatment followed by recovery of the original level (Fig. 1B).

The levels of LIFRs and gp130 precursor forms (Form 2) were increased approximately 2-fold during the 6-h OSM treatment period (Fig. 1, B and C). None of these changes in receptor expression were evident in cells treated with LIF or IL-6, probably because of the relative low level activity of corresponding receptors in these cells (Fig. 1A and data not shown). Interestingly, in fibroblasts and epithelial cells, an induced expression of SOCS3 could be detected by immunoblotting that peaked at 1 h of OSM treatment (Fig. 1, B and D, lanes marked 60). These results suggest that receptor subunits follow specific turnover mechanisms that are characterized by an initial boost of ligand-induced down-regulation of receptor protein followed by a stimulated synthesis. Together with induced signal-modifying factors such as SOCS3, the regulated expression of receptor proteins appears to determine the temporal profile of activated STAT3 in long term OSM-treated cells.

**Receptors mRNA Levels Are Induced by OSM Treatment**—To address the mechanisms by which OSM induces receptors levels, we first analyzed levels of receptor mRNA by Northern...
blotting for lung fibroblasts (Fig. 2A) and epithelial cells (not shown). Due to technical limitations, only OSMRβ, but not LIFRα or gp130, yielded a significant signal above background with this technique. The results indicate an increase of OSMRβ mRNA by 6 h of OSM treatment and elevated levels maintained for at least 24 h. To assess the effect of OSM on mRNA of the other receptor subunits, transcripts were analyzed by RT-PCR. RT-PCR with serial dilutions of the input total RNA suggested a higher abundance for gp130 mRNA than OSMRβ and LIFRα mRNA (Fig. 2B). After 24 h OSM treatment, the signal for OSMRβ mRNA was increased 7-fold, LIFRα mRNA was increased 5-fold, and gp130 mRNA was increased 3-fold (Fig. 2, C and D). An immediate, but partly transient induction of cytokine-inducible SH2 (CIS), SOCS1, and SOCS3 mRNAs was detected that peaked at 1–2 h of treatment.

Induction of Receptor Subunit Degradation and Synthesis by OSM Is a General Mechanism—The screening of various established cell lines from hepatic, mesenchymal, and epithelial origins confirmed the general features of receptor level modulation by OSM. Among these cell lines, we identified NIH-3T3 fibroblasts as a prominent target for induction of gp130 expression by OSM treatment, but as in primary fibroblasts, not by LIF or IL-6 (Fig. 3A; data not shown for IL-6). Furthermore, like human fibroblasts, 3T3 cells displayed a similar kinetics of STAT3 activation by OSM. The reduction of activated STAT3 correlated with sustained STAT3 activation after OSM treatment and that an effective compensatory synthesis of OSMRβ, gp130, and to a lesser extent LIFRα, occurs that correlates with sustained STAT3 activation after OSM treatment but not after treatment with LIF.

Induction of gp130 Synthesis by OSM Is Mediated by STAT3—The STAT3 and ERK 1/2 pathways are two of the major signaling pathways activated by OSM. To determine their relative contribution to the induction of gp130 synthesis, NIH-3T3 cells were treated with the MEK-1 inhibitor, U0126, or transfected with an expression vector encoding the dominant negative form of STAT3, STAT3Δ55C (18). As shown in Fig. 4, U0126 inhibited the activation of ERK 1/2 by OSM but did not modify the activation of STAT3. Induction of gp130 was maintained in U0126-treated cells, but induction of SOCS3 was reduced by 50%. In contrast, in STAT3Δ55C-overexpressing cells, gp130 was not increased after OSM treatment, and SOCS3 showed attenuated induction.

A functional, cis-acting binding site for activated STAT3 has been reported within the gp130 promoter (24). Therefore, we cloned this gp130 promoter in the pCAT vector and analyzed the effects of OSM treatment on the activity of this promoter. We observed that, depending on the cell line used, OSM induced 2–10-fold the activity of the gp130 promoter, and this induction was prevented by transfection with STAT3Δ55C (data not shown). Together these results strongly suggest that the increased gp130 mRNA and protein in OSM-treated 3T3 cells (Fig. 3 and 4) result from the transcriptional activation of the gp130 gene by STAT3. SOCS3 gene induction appears to depend on STAT3 and ERK pathways.

Down-regulation of gp130 after Ligand Binding Does Not Depend on Cytoplasmic Motif—Previous studies suggest that di-leucine motifs and serine phosphorylation in the gp130 cytoplasmic domain direct degradation of gp130 and LIFRα (12—
To define the requirement of cytoplasmic domain elements for the ligand-induced degradation of receptor subunit, we took advantage of H-35 cells with stable expression of transduced epitope-tagged LIFR\textsubscript{a/H9251}, OSMR\textsubscript{a/H9252}, and chimeric G-CSFR-gp130 (6, 8, 12, 17). In those cell lines, the integrated viral vector is under the transcriptional control of the viral LTR promoter, and its expression is not influenced by cytokine treatment. In OSMR\textsubscript{a/H9252} or G-CSFR-gp130 down-regulation was observed only after OSM or G-CSF treatment, respectively, and was not affected by U0126 (Fig. 5A, a–c).

In contrast to LIFR\textsubscript{a/H9251}, and its expression is not influenced by cytokine treatment. In OSMR\textsubscript{a/H9252} or G-CSFR-gp130 down-regulation was observed only after OSM or G-CSF treatment, respectively, and was not affected by U0126 (Fig. 5A, a–c).

Similarly, treatment with IL-6, insulin, or phorbol 12-myristate 13-acetate induced down-regulation of LIFR\textsubscript{a} in trans but not of OSMR\textsubscript{a} or G-CSFR-gp130 (Fig. 5B). As described previously (12), LIFR\textsubscript{a} with mutation of the ERK substrate site, Ser\textsuperscript{185} to Ala, was no longer subject to down-regulation by ERK action in trans. However, this mutant LIFR\textsubscript{a} retained a LIF-inducible decrease (Fig. 5B).
and gp130 are down-regulated by ligand binding and that only LIFRα is subject to an additional, ERK-sensitive and Ser 185-dependent degradation mechanism.

To characterize the mechanism underlying the prominent down-regulation of gp130, we established H-35 cell lines expressing specific and C-terminally FLAG epitope-tagged G-
CSFR-gp130 forms with truncated cytoplasmic gp130 domains (schematically shown in Fig. 6). These chimeric receptors contain the 133-residue, membrane-proximal domain of gp130 (6.17) and, hence, do not include any of the proposed elements specifically directing endocytosis and degradation. Mutant forms of the chimeric receptor were designed that specifically recruited JAKs, STAT3, and/or SHP2/ERK. The engagement of the STAT3 pathway was eliminated by mutating the tyrosine residue within the single remaining STAT3 binding element (Box3) at position 125 of the cytoplasmic domain (Y3F; Ref. 17). The recruitment of the SHP2/ERK pathway was suppressed by mutating the SHP2 binding element at position 117 (Y2F; Ref. 17). Mutations at both sites (Y2F,Y3F) generated a receptor mutant show only a low level of activated STAT1 and -3 (see Fig. 6, electrophoretic mobility shift assay (EMSA), bottom panel) but a magnified phosphorylation of SHP2 and ERK, and (d) the G-CSFR-gp130 construct with a deleted cytoplasmic domain, G-CSFR-gp130(Δcyto), is turned over with the same kinetics as the G-CSFR-gp130(Y2F,Y3F), which retained a strong JAK1 activation. This suggests that JAK action is not critical for receptor degradation but that extracellular and transmembrane domains are sufficient to direct the ligand-induced degradation.

Lysosomal and Proteosomal Degradation of Receptor Subunits—We next determined the relative contribution of different degradation pathways to receptor turnover as part of ligand-induced down-regulation. Treatment of primary fibroblasts for 6 h with the proteosome inhibitor MG132 or the lysosome inhibitor chloroquine alone or together with cycloheximide indicated an increased half-life of both LIFRα and gp130 (Fig. 7A). This implies that proteosomal and lysosomal pathways contribute to the normal turnover of the receptor subunits. Chloroquine treatment also induced an accumulation of gp130 degradation products (Fig. 7A, gp130 fragments; fragments derived from LIFRα are not shown; see Ref. 12). MG132 and chloroquine have a more important effect on LIFRα than gp130, as evident from the severalfold higher level of receptor protein after 6 h of treatment with these drugs (Fig. 7A, lanes

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**Fig. 7. Down-regulation of receptor subunits depends on lysosomal and proteosomal degradation.** A, α, human pulmonary fibroblasts were treated for 360 min with medium alone (Control) or with medium containing 1 μM proteosome inhibitor MG132 (MG) or 100 μM lysosome inhibitor chloroquine (6-min lane in each panel). Parallel cultures were treated with these reagents in combinations with 10 μM cycloheximide for 120–360 min (CHX). Whole cell lysates were analyzed as indicated (NS, nonspecific). b, signals corresponding to the higher molecular size forms of the full-length receptor proteins detected in the gel patterns of a were quantified by densitometry and expressed relative to the values of the untreated control cultures. Chl., chloroquine. B, H-35 cells expressing G-CSFR-gp130(277) wild type were treated with medium alone (Control) or with medium containing MG132, chloroquine, and G-CSF for the times indicated. Cell lysates were analyzed by immunoblotting for the indicated proteins. C, MCF7 cells were transfected with expression vector for HA-tagged ubiquitin (Ub-HA) together with expression vectors for OSMRα (a), LIFRα (b), gp130 (c), or STAT3 (d) or empty vector (Control) as indicated at the top of each panel set. Cells were then treated with OSM or LIF and 1 μM MG132 for 6 h. Receptor subunits or STAT3 were immunoprecipitated and analyzed by immunoblotting for ubiquitin-HA (upper panels) and receptor subunit or STAT3 protein (lower panels).
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marked with 0-min cycloheximide treatment. An equivalent involvement of the two degradation pathways in G-CSF-induced G-CSFR-gp130 down-regulation is demonstrated in G-CSFR-gp130-transduced cells treated with MG132 or chloroquine (Fig. 7B).

To better define the role of ubiquitination as part of the proteosomal degradation of receptor subunits, we assessed the level of ubiquitination of gp130, OSMRβ, and LIFRα. Expression vectors for these receptor subunits together with that for HA-tagged ubiquitin were transfected in MCF7 cells (19). The receptor subunits were immunoprecipitated, and the presence of ubiquitin was identified by HA immunoblotting. Based on the pattern of immunodetectable signals in Fig. 7C, a–c, immunoprecipitated gp130, OSMRβ, and LIFRα appeared to include polyubiquitinated species. Transfected STAT3 served as a control for an overexpressed protein that was not affected by ubiquitin (Fig. 7C, d). Treatment with the corresponding ligands did not modify the intensity of receptor ubiquitination, but treatment with MG132 caused an accumulation of ubiquitinated receptor subunits. This suggests that a fraction of gp130, OSMRβ, and LIFRα in the cells is ubiquitinated independently of cytokine treatment and that this fraction may be a target for proteosomal degradation.

**DISCUSSION**

Numerous studies have addressed the mechanisms by which the signaling of hematopoietic cytokines is restricted in time and magnitude. In the example of IL-6 cytokines, two basic processes have emerged. One process is the moderating action of specific suppressors such as SHP1/2, SOCS1/3, and the protein inhibitor of activated STATs on signals activated by gp130, LIFRα, and OSMRβ (3, 4, 6, 10, 11). The other process is the adjustment of the expression levels of receptor subunits and the regulation of downstream signal transduction pathways. Recent studies by us (12) and others (13–15) identify regulated degradation of receptor subunits as significant factors that determine cellular responsiveness to IL-6 cytokines. We have demonstrated that differences in the control of receptor turnover exist that affect individual receptor proteins. LIFRα is unique in that phosphorylation by activated ERK 1/2 induces the lysosomal degradation of the protein (12, 13). In the present study, we addressed the process by which the expression of the more stable gp130 and OSMRβ is controlled. We determined that ligand binding enhances degradation of these subunits, in part as noted for LIFRα, but that compensatory mechanisms, through increased receptor synthesis, reestablish and maintain levels of cytokine responsiveness close to pretreatment. We suggest that ligand-induced synthesis is primarily mediated by a STAT3-dependent induction of the receptor genes. The ligand-induced degradation of functional receptor proteins is not critically dependent on JAKs, SHP2/ERK, or STAT3 signaling or any specific cytoplasmic domain structure. We propose that ligand-induced dimerization of receptor subunits, through the extracellular and transmembrane domains alone, initiates endocytosis and that degradation involves lysosomal as well as proteosomal ubiquitin-mediated pathways. Regulated synthesis and degradation are effective mechanisms by which cells adjust their IL-6-type cytokine responses.

The availability of cytokines and receptor expression represent two of the most fundamental targets for determining cytokine responsiveness of cells. The next level of importance includes control of cytokine receptor action as a function of cytokine treatment. Two components for this control are identified here; they are (a) expression of receptor subunits is inducible by the receptor signals, and (b) the ligand-recruited receptor complex is tagged for degradation beyond the constitutive turnover process. The balance of the two processes establishes the temporal profile of potential cytokine receptor action.

Previous analyses indicate that IL-6 or OSM treatment increases gp130 promoter activity (24). Because a functional STAT binding element is present and necessary within the gp130 promoter to mediate this effect, it has been suggested that activation of gp130 by its ligands may stimulate the production of new gp130 to replenish receptors consumed upon ligand activation (24). Indeed, in normal lung fibroblasts and epithelial cells as well as in various cell lines we detected an increased production of gp130, but also OSMRβ and, to a lower extent, LIFRα. Only OSM treatment appears to be able to trigger these effects, correlating with the finding that OSM is a more effective inducer of signaling than IL-6 or LIF in these cells (22, 23). Receptor protein levels increase proportional to the mRNA for the gene products. Transfection of dominant negative STAT3 into 3T3 cells confirmed the predicted transcriptional role of STAT3 for regulation of the gp130 gene. Whether a similar STAT3-dependent mechanism is responsible for ligand-induced synthesis of OSMRβ and LIFRα in normal fibroblasts (Fig. 1–2) remained to be determined.

LIFRα, gp130, and JAKs have half-lives of 2–4 h (12, 25). In contrast, STATs and SHP2 have a slower turnover rate, and SOCS1/3 are very short-lived (12, 25). In fact, the immunodetectable level of these proteins is not always a direct reflection of their functional contribution to the cytokine effects. Although the signal-mediating molecules STATs and SHP2 are usually expressed at relatively high levels, only that fraction of protein physically recruited by receptors is functionally relevant, and the activity of these proteins is strictly regulated by post-translational modification (i.e. phosphorylation). SOCS proteins, the functions of which are not strictly directed by post-translational modifications, are largely regulated at the transcriptional level by, among other factors, activated STATs (Fig. 4; Refs. 3, 10, and 25). Receptor subunits appear to be regulated by two processes; they are (a) immediately after initial ligand binding, the activity of most if not all cell surface-exposed receptors is induced by phosphorylation, unless there is a limited amount of JAKs to react with all available receptor proteins, and (b) subsequently, continued function of receptors is dependent on the rate of synthesis, which in the case of gp130 includes a STAT-specific stimulation of transcription and on the rate of degradation. We have previously demonstrated that LIFRα degradation is enhanced by the ERK-dependent phosphorylation of LIFRα cytoplasmic domain at Ser185 (12). Because phosphorylation of gp130 at Ser782 also regulates cell surface expression of gp130 (14), it was conceivable that ligand-induced activation of cytoplasmic protein kinases mediates Ser-phosphorylation of receptor subunits to regulate their turnover. However, we observed that truncated gp130 with 133 residues of the cytoplasmic domain, hence devoid of the Ser782-phosphorylation site, is still down-regulated. This down-regulation is compatible with that observed for LIFRα with the Ser185 to Ala mutation that is no longer the target of ERK-directed down-regulation (Fig. 5B). The study of truncated receptor subunits also indicated that the di-leucine motifs (Leu786 Leu787) implicated in receptor internalization (15, 16, 29), and the sites for STAT, SHP2, and even for JAKs activation are dispensable for ligand-induced down-regulation of G-CSFR-gp130 (Fig. 6). Therefore, we conclude that dimerization of receptor subunits without intracellular signal transduction is sufficient to trigger the process of receptor degradation. Similar conclusions were reached in other receptor systems as follows. (a) Monoclonal antibodies against the EGF receptor act as inhibitors and induce receptor down-regulation only in their dimerizing forms (26) and (b) a point mutation...
within the fibroblast growth factor receptor 3 transmembrane domain leads to a selective delay in the down-regulation and ligand-induced internalization of the receptor (27).

Kinetic studies reveal that IL-6 treatment does not appreciably modify the rate of gp130 internalization, which is largely constitutive (3, 12, 16, 28). Because truncated gp130 without the di-leucine motif and even without the cytoplasmic domain is still down-regulated after ligand binding, internalization mechanisms other than those proposed to engage the di-leucine motif and AP-2-dependent processes remain effective. Based on mechanisms other than those proposed to engage the di-leucine motif and even without the cytoplasmic domain, it is conceivable that gp130, LIFRα, and OSMRβ are targets for lysosomal degradation, where intact receptors as well as cytoplasmic receptor fragments, products of endoproteolytic release of the extracellular domain, accumulate. Moreover, we have also observed an accumulation of receptor subunits with intact cytoplasmic domains after inhibition of proteosome activity. Therefore, it is conceivable that gp130, LIFRα, and OSMRβ are targets for proteosomal degradation by their direct ubiquitination or by association with other ubiquitinated proteins such as SOCS/CIS proteins (30, 31). The former hypothesis is supported by the finding that gp130, LIFRα, and OSMRβ are directly ubiquitinated, and ubiquitinated receptor proteins accumulate after proteosome inhibition (Fig. 7C). Similar observations have been made previously with the growth hormone receptor (32, 33). However, truncated gp130, with no cytoplasmic domain and no identifiable direct ubiquitination, is still effectively down-regulated after ligand binding, indicating that gp130 down-regulation does not strictly depend on ubiquitin or proteosomal degradation.

Together our results suggest that in addition to other mechanisms reported to determine the constitutive receptor degradation, ligand-induced dimerization enhances gp130, LIFRα, and OSMRβ turnover by a process that depends on di-leucine-independent internalization, endosomes to lysosomes trafficking, and/or lysosomal degradation. Additionally, our data indicate that ERK-specific down-regulation of receptor protein is limited to LIFRα. A compensatory mechanism retaining specific cytokine responsiveness is the enhanced synthesis of receptor subunits, especially gp130 and OSMRβ.

Acknowledgments—We are grateful to Immunex Corp. for providing cloned human cytokine receptors and anti-sera against human OSMRβ, Immunex Corp. and Genetic Institute for cytokines, Dr. A. Miyajima for the mouse OSM expression vector, Dr. G. Fey and Dr. J. Ripperger for the STAT3 expression vector, and Dr. G. Loewen for bronchial brushings to prepare epithelial cells.

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J. Biol. Chem. 2001, 276:47038-47045.
doi: 10.1074/jbc.276.50.47038 originally published online October 15, 2001

Access the most updated version of this article at doi: 10.1074/jbc.276.50.47038

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