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Biosynthetic and Glycosylation Events of the IL-6 Receptor β-Subunit, gp130

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Abstract It is now recognized that the β-subunit of the interleukin-6 (IL-6) receptor, also known as gp130, is a common signal transducer shared by other cytokines, including ciliary neurotrophic factor, leukemia inhibitor factor, oncostatin M, and IL-11. In this study, the biosynthesis and glycosylation of hepatic gp130 were investigated using a specific polyclonal antibody to the 287 amino acid cytoplasmic domain of gp130. Immunoprecipitation and metabolic labeling experiments demonstrate, in addition to a mature surface expressed gp130, the presence of a major immature form of the molecule within the cell. The immature form can shift to become a functional gp130 only after being terminally glycosylated. The kinetics of gp130 maturation and surface expression were determined. When both forms of gp130 are deglycosylated the resulting core peptides migrate to identical positions in a denatured protein gel, indicating that the principal difference between the two forms resides in the extent of their glycosylation. IL-6 and other members of this cytokine family activate only the mature form, demonstrating its location at the membrane surface. Protein and mRNA turnover studies reveal gp130 to be a stable, slowly renewing population under nonstimulated conditions. These findings provide novel information on the intracellular events leading to the expression of this critically important signal transducing protein.

Key words: biosynthesis, gp130, glycosylation, immunoprecipitation

A large number of proteins expressed on the cell surface are involved in cell communication. Through soluble mediators, such as cytokines, growth factors, or hormones, specific biological functions of different cells are regulated. The formation of a protein complex between the mediator and its specific cell surface receptor is the first step in initiating the signal transduction pathway. One of the hallmarks of both hematopoietic and inflammatory cytokines is their utilization of shared signal-transducing proteins. For example, the KH97 molecule is a signal-transducing subunit shared by interleukin-3 (IL-3), interleukin-5 (IL-5), and granulocyte/macrophage colony-stimulating factor (GM-CSF) [Hayashida et al., 1990; Kitamura et al., 1991; Tavernier et al., 1991]. Similarly, the γ-chain of the IL-2 receptor is a shared functional component for IL-4 and -7 [Kondo et al., 1993; Noguchi et al., 1993; Russell et al., 1993]. IL-15 interacts with the β-subunit of IL-2 receptor [Grabstein et al., 1994]. It has been shown that the β-subunit of the IL-6 receptor complex, gp130, is a shared receptor subunit with ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), oncostatin M (OSM) [Davis et al., 1993; Ip et al., 1992; Liu et al., 1992], and IL-11 [Yin et al., 1993]. Utilization of this common transducing subunit leads to some redundant biological functions within this cytokine subfamily.

Activation of gp130 occurs after the association of the cytokine and its cognate receptor, which then bind gp130, resulting in the formation of a high affinity binding complex ($K_d \sim 10^{-11}$ M) [Taga et al., 1989; Yamasaki et al., 1988]. The post-ligand binding events involve either homodimerization of gp130 brought about by the IL-6 receptor complex [Murakami et al., 1993] or heterodimerization of gp130 with other specific receptors (i.e., CNTF or LIF) [Davis et al., 1993]. Dimerization of gp130 activates a family of cytoplasmic tyrosine kinases, known as Jak kinases. These activated kinases initiate a phosphorylation cascade in which the kinases appear to phosphorylate themselves, gp130 and other specific cytosol proteins. This latter set of molecules belong to a recently described family

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of proteins named STAT for their ability to be signal transducers and activators of transcription [Zhong et al., 1994; Lutticken et al., 1994; Stahl et al., 1994].

Most studies of the signaling pathway for the IL-6 cytokine family have focused on the events that lead to gp130 activation and how it relays signals into the cell [Ihle et al., 1994; Kishimoto et al., 1992]. Little information has been reported exploring the biosynthetic pathway and the surface expression of this important signal transducing molecule. This paucity of information may be due partially to the limited availability of specific gp130 antibody probes. In this study, we developed polyclonal antibodies to rat hepatic gp130 by constructing a recombinant gp130 fusion protein containing its entire cytoplasmic domain. The purified fusion protein was subsequently used as an antigen. The monospecific antibodies generated were purified and used to study the biosynthetic and glycosylation processing of gp130. Two major populations of gp130 were evident from immunoprecipitation and Western blot analysis using this antibody. Pulse-chase experiments and deglycosylation studies demonstrated that the faster migrating species of gp130 is an incompletely glycosylated form. Examining the maturation process of gp130 indicated that the immature form could be converted to the mature form within 4–6 h. Additional studies were carried out to determine the basal turnover of gp130 at the both mRNA and protein levels.

MATERIALS AND METHODS

Cell Culture

Hepatocytes were isolated from adult male Sprague-Dawley rats by a modification of the collagenase perfusion technique and subsequently plated on fibronectin-coated tissue culture plates as previously described [Fuller et al., 1988]. Cells were cultured in William's E media (WE), supplemented with heparin (1 U/ml), insulin (10 mU/ml), penicillin (50 U/ml), streptomycin (50 μg/ml), nicotinamide (1.22 μg/ml), and 5% fetal bovine serum. In experiments in which glycosylation, transcription or translation were blocked, the following concentrations of inhibitors were used: tunicamycin, 2 μg/ml [Elbein, 1981]; actinomycin D, 2.5 μg/ml; cycloheximide, 2.5 μg/ml [Nesbitt and Fuller, 1991].

Generation of a Polyclonal Antibody to Rat Hepatic gp130

The cytoplasmic domain of rat hepatocyte gp130 was amplified by a polymerase chain reaction (PCR) using a forward primer (beginning at nucleotide no. 1935) and reverse primer (beginning at nucleotide no. 2734) [Wang et al., 1992]. The fragment generated was cloned into the GST fusion protein expression vector pGEX-2T (Pharmacia). To improve the efficiency of thrombin cleavage of the gp130 peptide from the glutathione-S-transferase, two glycine codons were added between GST and the gp130 sequences. After induction of the gp130–GST fusion protein expression [Ausubel et al., 1989], the cell lysate was applied to a glutathione–Sepharose affinity column, and the bound fusion protein was eluted with 5 mM-reduced glutathione in 50 mM Tris–HCl (pH 8.0). Removal of the GST part from the fusion protein (GST–gp130) was carried out by 0.5% thrombin digestion; the reaction mixture was then passed through the glutathione–Sepharose column. The flowthrough fraction contained the pure gp130 cytoplasmic peptide (287 amino acids). Subsequently, 200 μg of purified gp130 peptide in 0.5 ml PBS was mixed with equal volume of Freund's complete adjuvant (FCA) and injected intradermally into 10–12 sites along the upper back of a rabbit. After 3 weeks, 100 μg antigen and Freund's incomplete adjuvant mixture was injected subcutaneously into 2–4 additional sites. The antiserum titer was tested on day 31 after the first injection by Western blot. The animal was boosted again with purified gp130 by an intramuscular injection of 100 μg antigen in 900 μl of Freund's incomplete adjuvant. The rabbit was rested for an additional week prior to bleeding and testing of the antibody titer. The high-affinity antibodies to gp130 cytoplasmic domain were obtained by passing the serum over a protein A–Sepharose resin [Ey et al., 1978].

Immunoprecipitation and Western Blot Analysis

Rat hepatocyte cultures were rested for 16–18 h after plating before beginning any experiments. At the start of an experiment, hepatocytes (1.0×10^7 cells) were washed with ice-cold phosphate-buffered saline (PBS), then resuspended in 700 μl of cell lysis buffer (10 mM Tris–HCl pH 7.8, 150 mM NaCl, 1% NP-40, 1 mM PMSF, 1 mM aprotinin, and 1 mM leupep-

tin), and incubated on ice for 30 min. After centrifugation at 10,000*g*, at 4°C, the supernatant was precleared for 2 h at 4°C with protein A–Sepharose (Sigma), preincubated with normal rabbit serum. The precleared supernatant was then immunoprecipitated overnight at 4°C with polyclonal antibodies against the gp130 cytoplasmic domain and pelleted using the solid-phase resin, protein A–Sepharose. The immunoprecipitates were washed gently with lysis buffer six times to remove the nonspecific associated proteins and then boiled in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) sample buffer. The gp130 protein was subjected to SDS–PAGE and detected by an enhanced chemiluminescence (ECL) system (Amersham) with 1 μg/ml of primary antibodies (anti-gp130) and 1:60,000 dilution of anti-rabbit Ig horseradish peroxidase (HRP) conjugate from Promega.

Cell Metabolic Labeling of gp130 and Autoradiography

Nearly confluent (~90%) monolayers of primary rat hepatocytes were washed twice with RPMI 1640 lacking methionine and cysteine and incubated in the same medium containing 2.5% dialyzed fetal bovine serum (FBS) for 30 min. The cells were labeled with the addition of [^{35}S]methionine and [^{35}S]cysteine (100 μCi/ml, New England Nuclear) for the indicated times. At the end of the labeling period, cells were washed with PBS (3×) and chased with WE complete medium for the times indicated. Cells were then lysed and immunoprecipitated. In some experiments, cells were chased in the presence of an inhibitor of N-linked glycosylation, tunicamycin (2 μg/ml). The labeled gp130 was immunoprecipitated and applied to 8% SDS–PAGE. Gels were treated with EN^{3}HANCE for 30 min, dried, and exposed to X-ray film.

Enzymatic Deglycosylation of gp130

N-glycosidase F was employed for removal of all N-linked oligosaccharide chains from gp130. The immunoprecipitated protein from 1×10^7 cells was redissolved in 20 μl of 50 mM phosphate, pH 7.8, 0.5% SDS, and 50 μM β-mercaptoethanol. The protein was denatured by boiling 5 min, and 7.5 μl of 10% NP-40 was added before the addition of 0.3 U N-glycanase (Genzyme). Following an overnight incubation at 37°C, the reaction was stopped by the addition of SDS–PAGE sample buffer. The protein was analyzed using the ECL Western detection system as described above.

RNA Extraction and Ribonuclease Protection Assays

Total cellular RNA was isolated from primary hepatocytes using an acid–phenol extraction method [Scherrer, 1969] exactly as described previously [Nesbitt and Fuller, 1991]. A probe to rat hepatic gp130 was made by first subcloning a 1.1-kb cDNA fragment (nucleotide nos. 685–1809) into pBS vector (Stratagene), which was then linearized with Afl II [Wang et al., 1992]. A 700-bp *Pst*I/*Bam*HI fragment of rat cyclophilin [Danielson et al., 1988] was also cloned into the pBS vector. Antisense cRNA probes for rat liver gp130 and rat cyclophilin were obtained by in vitro transcription as described in detail [Nesbitt and Fuller, 1992]. After the cells were treated with 2.5 mg/ml of actinomycin D for the indicated time, the total RNA was extracted and used to hybridize with a mixture of either gp130 or cyclophilin antisense cRNA probes. Hybridization and ribonuclease digestion were performed by following the manufacturer's protocol (Ambion). Ribonuclease-resistant RNA products were analyzed by electrophoresis in a 5% polyacrylamide/8 M urea gel followed by autoradiography and scanning with a densitometer.

Densitometric Scanning

The autoradiography film was scanned using a video densitometer (model 620, BioRad). Data were collected and calculated with 1-D Analyst 1.1 (BioRad) software in a Macintosh computer.

RESULTS

Development of a Polyclonal Antibody That Recognized Recombinant and Native gp130

The functional IL-6 receptor consists of two subunits, a ligand-binding subunit-α (gp80) and a signal transducing subunit β (gp130) [Taga et al., 1989; Hibi et al., 1990]. In order to examine in detail the biosynthetic process of gp130 in the liver cells, we developed a monospecific antibody to the cytoplasmic domain of rat gp130. A cDNA fragment encoding this region was obtained by PCR and cloned into the pGEX-2T vector for recombinant gp130 production. The purified fusion protein was used to immunize a rabbit for

the polyclonal antibody generation. The antibodies recognized both the gp130-GST fusion protein, and gp130 peptide alone, but not the GST peptide (Fig. 1A). When this antibody was used to immunoprecipitate the endogenous gp130 from primary rat hepatocytes, the antibodies specifically recognized native gp130 (Fig. 1B). In this experiment, two different batches of antibodies were used (left two lanes). For specificity controls unrelated antibodies (goat anti-E and rabbit anti-GST) and protein A–Sepharose were also included.

Identification of Two Forms of gp130 Protein

It is recognized that the number of IL-6 receptors expressed on the cell surface is relatively low [Bauer et al., 1989; Nesbitt and Fuller, 1992]. In order to detect the gp130 molecule in rat hepatocytes, we carried out immunoprecipitation experiments using anti-gp130. The immunoprecipitated complex was immunoblotted with the same antibody and then visualized by using a sensitive enhanced chemiluminescence detection system. Two distinct bands of gp130 were identified (Fig. 1A), suggesting the existence of two forms of gp130 molecules in primary hepatocytes. For further confirmation of the presence of these two forms, two additional experiments were performed. First, we used varying amounts of gp130 proteins by immunoprecipitating different numbers of cells. As the cell numbers increase, the presence of two forms of gp130 becomes more obvious (Fig. 2A). In addition, the two forms of gp130 protein were increased in the presence of higher concentration of monospecific anti-gp130 antibodies with a constant number of cells (Fig. 2B). The molecular mass of these two forms is approximately 145 and 130 kd, respectively. This observation was consistent with a recent report that showed that the functional form of the β-subunit for IL-6 receptor (even called gp130) migrated to the 145-kd position [Stahl et al., 1994]. We emphasize that the term gp130 has become a specific name for this subunit, rather than an accurate reflection of its actual molecular mass.

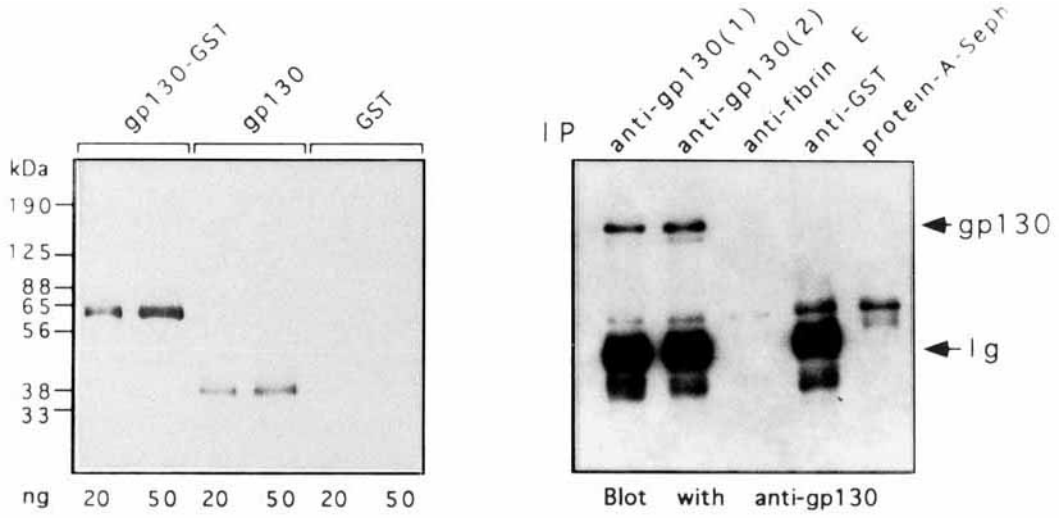

Fig. 1. The generated antibody specifically recognizes recombinant and endogenous gp130 in primary rat hepatocytes. **A:** The first two lanes represent gp130–GST fusion proteins. The middle two lanes are gp130 peptides (after removal of GST from the gp130–GST complex) and the last two lanes are the GST proteins. The filter was immunoblotted with 1 μg/ml of anti-gp130 and developed using antirabbit Ig alkaline phosphatase conjugate. The purified antibody recognizes only the expressed gp130 protein but not the GST protein. **B:** 1×10^7 primary hepatocytes were lysed in 0.7 ml of lysis buffer and precleared with protein A–Sepharose and normal rabbit serum. The antibodies against rat hepatic gp130 (two different batches of antibody preparation, left two lanes) and other unrelated antibodies (goat anti-E and rabbit anti-GST) were used to carry out the immunoprecipitation. The immunoprecipitated proteins were applied to SDS–PAGE, subjected to Western blot with anti-gp130 and detected by using enhanced chemiluminescence (ECL) system. The two arrows indicate the gp130 and Ig heavy chain, respectively.

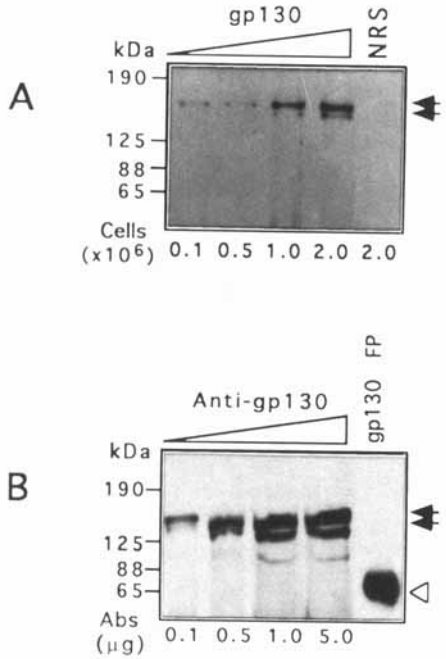

Fig. 2. Identification of the two forms of gp130 molecule. Primary rat hepatocytes were lysed and subjected to immunoprecipitation with anti-gp130, followed by Western blot, using the same antibody. **A:** Various amounts of cell lysates (from 0.1 to 2 × 10^7 cells) were immunoprecipitated with 5 μg antibody to gp130 and normal rabbit serum (NRS). The pelleted proteins were resolved in a 8% SDS–PAGE. **B:** 2 × 10^7 cells were lysed and then immunoprecipitated with different amount of antibody to the rat hepatic gp130. The protein samples were fractionated on a 5–15% gradient SDS–PAGE and detected by ECL system. Solid arrows, the two species of gp130; open arrowhead, gp130 fusion protein (0.2 ng), which was used as an ECL detection control.

Pulse-Chase Analysis of gp130 Biosynthesis

It seemed possible that the faster migration species represented a pool of intracellular forms of gp130. In order to follow the fate of labeled gp130 proteins, we carried out short and long duration pulse-chase experiments using radioactive amino acids. Hepatocytes were metabolically labeled with [^{35}S]methionine and [^{35}S]cysteine for the indicated times and then lysed for immunoprecipitation with anti-gp130. Autoradiography results showed that both forms of gp130 were labeled and accumulated in the cells (Fig. 3A). If cells were pulsed for 3 h and then chased with normal media, the faster migrating form appeared to be gradually converted to the larger form of gp130 (Fig. 3B). Densitometric analysis of the autoradiogram is presented in Figure 3C. The total value of two forms was set as 100% at every time point and the ratio of each band determined. At the end of labeling period, the faster-migrating form accounted for 80% of total labeled gp130 protein, and the slower-migrating band (mature form) was only 20%. These two forms became equivalent (50%) at approximate 3 h. Following the chase period, all labeled gp130 proteins were the mature form (8 h). These data indicate that the faster-migrating form is an intermediate form (or immature form) of gp130. Data presented elsewhere demonstrate that the mature gp130 is removed from the cell surface and degraded following ligand binding [Wang and Fuller, 1994].

Two Forms of gp130 Were Due to Different N-Linked Glycosylation

Information from the pulse-chase experiments implicated the faster-migrating gp130 species as a "precursor or immature form" of the surface expressed protein. Based on the data shown in Figure 3 and on the fact that the hepatocyte gp130 has 11 potential N-linked glycosylation sites in the extracellular domain [Wang et al., 1992], it seems likely that the smaller gp130 species is an incompletely glycosylated protein. To test this hypothesis, the cells were labeled by [^{35}S]methionine/cysteine and chased in medium containing 2 μg/ml of tunicamycin. Tunicamycin, a well-characterized inhibitor of N-linked glycosylation of membrane and secreted glycoprotein, prevents the transfer of N-acetylglucosamine-1-phosphate to dolichol phosphate [Damsky et al., 1979; Elbein, 1981]. When hepatocytes were exposed to tunicamycin, conversion of the 130-kd band to the 145-kd band was significantly diminished (Fig. 4). Overall protein synthesis was not attenuated by tunicamycin used at this concentration [Elbein, 1981]. These observations reinforce the notion that the smaller band is a precursor form of gp130 that has not received its full complement of N-linked carbohydrates.

To further characterize the N-linked glycosylation that occurred during the biosynthetic processing of IL-6 receptor β-subunit, the gp130 protein was purified by immunoprecipitation and then denatured for digestion with peptide: N-glycosidase F (N-Glycanase). The digested protein was subjected to SDS–PAGE and immunoblotted with anti-gp130 antibody (Fig. 5). After digestion with N-Glycanase the two bands of gp130 migrated to a single band. These data support the observations that the differences

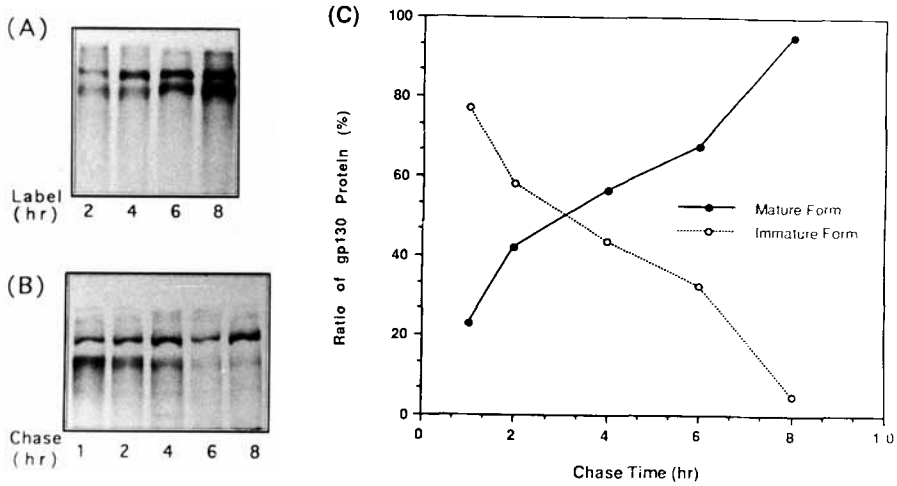

Fig. 3. Pulse-chase analysis of gp130 biosynthesis. **A:** Cells were incubated in methionine and cysteine free RPMI 1640 medium for 30 min prior to pulsing with radiolabeled amino acids. At the end of each pulse period (2–8 h), the cells were washed with PBS and lysed for immunoprecipitation with anti-gp130. Labeled gp130 proteins were applied to 8% SDS–PAGE and exposed to the film. **B:** Cells were pulsed as described above for 3 h, and WE medium was added to each plate for continuing incubation. At the indicated chase times, cells were harvested for immunoprecipitation analysis. **C:** The gel shown in B was scanned with a video densitometer and the bands quantified. The total value of two bands was set as 100%, and the ratio of each band was calculated. The data presented show the percent change of the two forms during dynamic biosynthesis of gp130. The shown data is a typical result from three independent experiments.

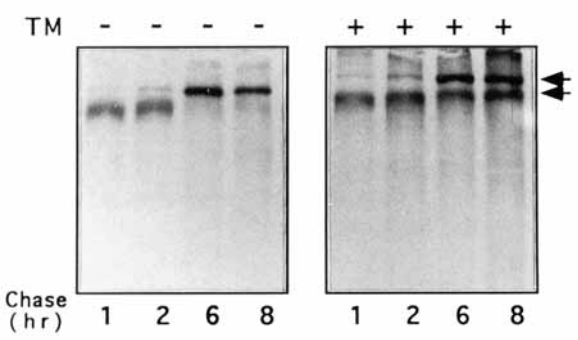

Fig. 4. Tunicamycin inhibits the processing of gp130 protein biosynthesis. Cells were pulsed for 3 h and chased in the presence of tunicamycin (2 μg/ml) for various time period (1, 2, 6, 8 h). The radiolabeled gp130 proteins were analyzed as described in Fig. 3 (two arrowheads).

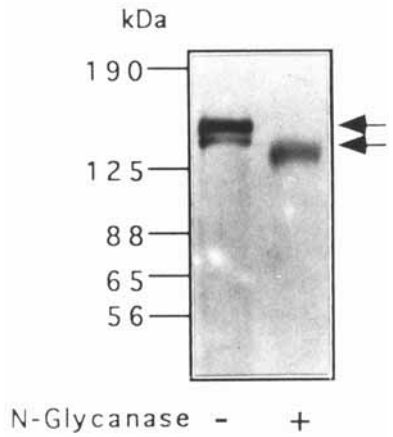

Fig. 5. Deglycosylation analysis of gp130. The immunoprecipitated samples from primary hepatocytes were incubated either with (+) or without (−)*N*-glycanase for overnight digestion as described in method. Following this treatment, the deglycosylated products were detected by using Western blot (ECL system) with anti-gp130 antibody.

seen in the two forms of gp130 are most likely in posttranslationally added sugar residues.

Turnover of gp130 at Both mRNA and Protein Levels

To gain information about the stability of fully formed gp130 protein and its mRNA transcript, we examined the half-life of gp130 in the unstimulated cell. Freshly prepared hepatocytes were incubated under control conditions for 16 h to allow cells to reach a quiescent state. At time 0 h, the transcription inhibitor actinomycin D (2.5 μg/ml) was added and the gp130 mRNA levels were determined using a sensitive ribonuclease protection assay at varying times after blocking transcription (Fig. 6B). The density of gp130 band was normalized with an internal RNA loading control of cyclophilin (as presented in Fig. 6C). The gp130 mRNA half-life was calculated to be 16 h. In measuring the half-life of the gp130 protein, cells were first metabolically labeled overnight with [^{35}S]methionine/cysteine and chased for the indicated times in the media containing cycloheximide (2.5 μg/

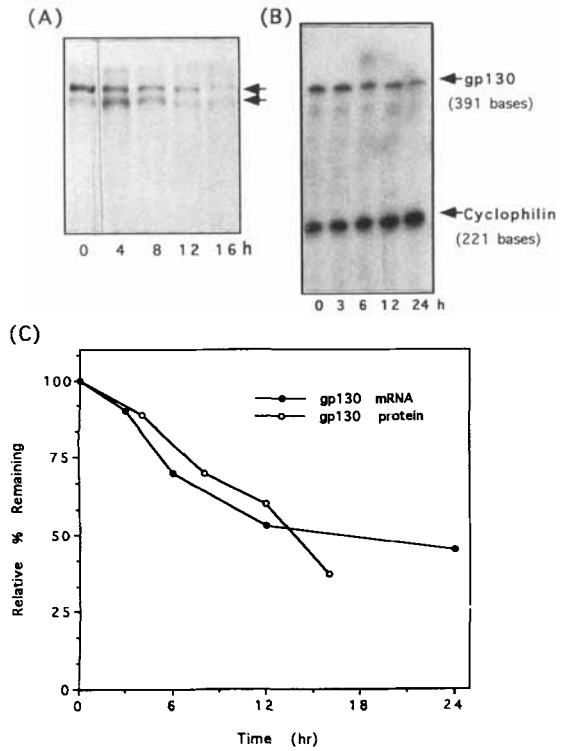

Fig. 6. Half-lives of gp130 mRNA and protein in rat hepatocytes. **A:** Cells were metabolically labeled overnight in RPMI 1640 medium containing [^{35}S]methionine and cysteine and 2.5% dialyzed fetal bovine serum. Then cells were chased with WE medium with cycloheximide (2.5 μg/ml) and tunicamycin (2 μg/ml). After different chase times, the amount of labeled gp130 was determined by immunoprecipitation and SDS–PAGE analysis. **B:** For measuring the half life of gp130 mRNA, cells were treated with the transcription inhibitor actinomycin D for the times indicated (0–24 h). Total cellular RNA was isolated and used for ribonuclease protection assay. This is an autoradiography of protected gp130 and cyclophilin mRNA fragments fractionated in a 5% polyacrylamide/8 M urea gel. **C:** The gels (A, B) were analyzed by using a video densitometer. The gp130 mRNA data represented three different experiments, and the data for gp130 protein represented two separated experiments.

ml). Tunicamycin was also included in the media to block the conversion of the immature to the mature form, which could affect the measurement of protein half-life. Labeled proteins were immunoprecipitated with anti-gp130, followed by electrophoresis and autoradiography (Fig. 6A). The gels were scanned and quantified by densitometry (Fig. 6C). Both redioactive forms (immature and mature) of gp130 gradually disappeared with time. The protein half-life was calculated from the plotted curve to be approximate 14 h. These data provide evidence that gp130 is a relatively stable cytokine receptor subunit at both the mRNA and protein levels. These results of mRNA and protein half-lives were derived from quiescent primary hepatocytes, and therefore likely reflect the cellular events of gp130 in vivo.

DISCUSSION

In the cytokine receptor superfamily, the gp130 (IL-6 receptor β-subunit) is the common signal-transducing protein shared by IL-6, LIF, CNTF, OSM, and IL-11 [for review, see Kishimoto et al., 1992]. Human, mouse, and rat gp130 cDNAs have been cloned and sequenced [Hibi et al., 1990; Saito et al., 1992; Wang et al., 1992]. Most reported studies of gp130 have focused on its dimerization, phosphorylation, and interaction with other cellular kinases [Boulton et al., 1994; Ihle et al., 1994; Lutticken et al., 1994; Murakami et al., 1991; Stahl et al., 1994; Zhong et al., 1994]. The studies outlined here describe another aspect of this important molecule, its biosynthesis and posttranslational processing. Using newly developed monospecific antibodies to the cytoplasmic domain of rat hepatic gp130, we provide the first evidence that the IL-6 receptor β-subunit is synthesized as a distinct precursor (~130 kd), based on the following data: it is immunoprecipitated by an antibody raised against the purified cytoplasmic protein of gp130; it is converted to the higher-molecular-weight form shown by pulse-chase experiments; and tunicamycin, a glycosylation inhibitor, is able to block the shift of this faster-migrating gp130 species to the slower-migrating form. In other experiments, different members of IL-6 cytokine family (IL-6, CNTF, LIF, and OSM) induce only one tyrosine-phosphorylated band, which migrates to the same position of larger species of gp130 (data not shown), suggesting that the higher-molecular-weight form is capable of responding to a cytokine signal, representing the mature form.

Current information on the relationship between the biosynthesis of cytokine receptors and the mechanism of cytokine signaling is not fully understood. Growth factor receptor systems have been better characterized in this regard. The epidermal growth factor receptor (EGFR) acquires tyrosine kinase activity independent of the N-linked glycosylation process, whereas the EGF binding requires N-linked glycosylation [Slieker and Lane, 1985; Slieker et al., 1986]. On the other hand, platelet-derived growth factor receptor (PDGFR) is able to be activated by its ligand cotranslationally or imme-

diately after translation, demonstrating that the addition of either N- or O-linked oligosaccharides is not required for ligand binding or tyrosine kinase activity [Daniel et al., 1987; Keating et al., 1989]. The newly synthesized PDGFRs could be processed and become surface active pool within 30–45 min. During this process, three PDGFR species have been identified, 145-, 165-, and 175-kd proteins [Claesson-Welsh et al., 1987; Daniel et al., 1987]. Our data show that two prominent gp130 species are synthesized in hepatocytes: one is the mature form that migrates to the 145- to 150-kd position, consistent with findings of other studies [Stahl et al., 1994] and is able to be activated by tyrosine phosphorylation upon cytokine stimulation; the other major form appears to be an incompletely glycosylated precursor of gp130 (or immature form) (Fig. 5). These data indicate that the two gp130 species are not different translation products of two receptor mRNA transcripts found in the liver cell [Wang et al., 1992]. However, we are not yet able to determine completely whether the immature form is transported to the cell surface or if it just lacks ligand-binding ability.

The basal turnover of gp130 was determined by pulse-chase experiments. Transcription inhibition experiments showed that the gp130 mRNA has a long half-life (16 h), which is in contrast with the relative short half-life of IL-6 receptor α-subunit (approximately 3 h) [Nesbitt and Fuller, 1992]. In keeping with the stable gp130 mRNA is the fact that gp130 protein seems to be long-lived in the unstimulated cell (14 h). We should point out, however, that gp130 is rapidly activated and removed from the cell surface when cells are stimulated by IL-6. Also, new gp130 synthesis is required for another round of activation [Wang and Fuller, 1994].

Overall, this study provides new information on the biosynthetic processing of the IL-6 receptor β-subunit, gp130. An important goal for future studies will be to determine whether the dominant gp130 precursor is able to bind IL-6/IL-6R complex and transduce cytokine signal.

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