

Biosynthesis of the Type I and Type II TGF- β Receptors

IMPLICATIONS FOR COMPLEX FORMATION*

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The TGF- β type I and type II receptors (T β RI and T β RII) are signaling receptors that form heteromeric cell surface complexes with the TGF- β s as one of the earliest events in the cellular response to these multifunctional growth factors. Using TGF- β -responsive mink lung epithelial cells (Mv1Lu), we have determined the half-lives of the endoplasmic reticulum (ER) and mature forms of these receptors. In metabolically labeled cells, approximately 90% of newly synthesized type II receptor undergoes modification of N-linked sugars in the Golgi, with a half-life of 30–35 min; the Golgi-processed form of the receptor has a relatively short metabolic half-life of 2.5 h. In contrast, only 50% of pulse-labeled type I receptor is converted to the Golgi-processed and therefore endoglycosidase H-resistant form, and the endoglycosidase H-sensitive ER form has a half-life of 2.8–3 h. Addition of 100 pM TGF- β 1 causes the Golgi-processed type II receptor to become less stable, with a half-life of 1.7 h, and also destabilizes the Golgi-processed type I receptor. TGF- β 1 binding and cross-linking experiments on cells treated with tunicamycin for various times confirm different ER to cell surface processing times for T β RI and T β RII. Our results, which suggest that stable complexes between type I and II TGF- β receptors do not form until the proteins reach a post-ER compartment (presumably the cell surface), have important implications for our understanding of complex formation and receptor regulation.

The transforming growth factor- β s (TGF- β 1,¹ - β 2, and - β 3) are the prototypical members of a growing superfamily of peptide growth factors that includes the activins and inhibins, bone morphogenetic proteins, Müllerian inhibiting substance, and the product of the *Drosophila decapentaplegic* (*dpp*) gene. Members of the TGF- β subfamily are multifunctional, with wide-ranging effects on growth, differentiation, immune response, and extracellular matrix organization and biogenesis (1–3). They are among the most important and versatile growth

factors yet described.

The three major cell surface receptors for TGF- β are termed types I, II, and III (T β RI, II, and III, respectively) (4–7). T β RI and T β RII are the signaling receptors, whereas T β RIII appears to promote ligand binding to T β RI and T β RII (8, 9). The type I and II receptors are serine/threonine kinases with approximately 40% identity in their kinase domains (7). Like their ligands, they are members of a large superfamily (10–13); receptors with related serine/threonine kinase domains include activin receptors, bone morphogenetic protein receptors, the *Drosophila saxophone*, *thick veins*, and *punt* gene products, and the *Caenorhabditis elegans* Daf-1 and Daf-4 proteins (14–26). Within this superfamily, type I- and type II-like kinases fall into distinct subcategories. T β RI-like receptors have a highly conserved juxtamembrane region rich in glycine and serine residues, termed the GS domain, whereas T β RII and similar receptors have a serine- and threonine-rich C-terminal extension.

When overexpressed in COS cells, all three types of TGF- β receptors form homo-oligomers (likely dimers) on the cell surface even in the absence of TGF- β (27, 28).² It is not known, however, whether they form homo- or heterodimers in the absence of ligand when expressed at lower, more physiologic concentrations on the surface of nontransfected TGF- β -responsive cells. Interactions between the cytoplasmic domains of T β RI and T β RII have been detected in both the yeast two-hybrid system and in COS cells overexpressing the cytoplasmic domains of both receptors (29, 30).³ Studies using chimeric receptors with the extracellular domain of the erythropoietin receptor and the cytoplasmic domains of T β RI or T β RII showed that both homodimerization of the cytoplasmic domain of the type I TGF- β receptor and heterodimerization with the cytoplasmic domain of the type II receptor is required for intracellular signal transduction leading to growth inhibition (31).

The type II receptor is able to bind TGF- β 1 and - β 3 independent of the presence of the type I receptor; T β RI requires the presence of T β RII to bind these ligands (32–34). Both receptors are required for high affinity binding of TGF- β 2, which appears to bind to a preformed complex of T β RI and T β RII (35). Coimmunoprecipitation studies with ligand-bound receptors demonstrated that T β RI, T β RII, and TGF- β ligand form a ternary complex on the cell surface (9, 34). The association between T β RI and T β RII expressed at physiologic concentrations appears to be ligand-dependent, at least when tested with TGF- β 1 (29, 36). Several lines of evidence suggest that this ligand-induced complex may be a heterotetramer, containing at least two copies of each signaling receptor. Weis-

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¹ The abbreviations used are: TGF- β , transforming growth factor- β ; T β RI, type I TGF- β receptor; T β RII, type II TGF- β receptor; ER, endoplasmic reticulum; Endo H, endoglycosidase H; PBS, phosphate-buffered saline.

² L. Gilboa and Y. I. Henis, personal communication.

³ R. Perlman and R. A. Weinberg, personal communication.

Garcia and Massague (37) showed that kinase-deficient and activation-deficient type I receptors can functionally complement each other, implying that the activated complex contains two molecules of T β RI. Additionally, dimers of the T β RI cytoplasmic domain are required for ligand response (31). Henis *et al.* (27) showed that the type II receptor is a homo-oligomer with or without TGF- β . This, in combination with the results of two-dimensional gel analyses (38), makes a heterotetramer the most likely minimum cell surface receptor complex.

Once a complex has formed, signaling is initiated by the transphosphorylation of T β RI by T β RII, a constitutively active kinase, on serine and threonine residues in the GS domain (29, 30, 36). This phosphorylation and a functional T β RI kinase are required for downstream signaling (36, 37, 39–41). MADR2, one member of a newly identified class of proteins required for serine/threonine kinase receptor signaling, is a substrate of the TGF- β receptor complex; it becomes phosphorylated and transmits signals directly to the nucleus (42, 43). Several other cytoplasmic proteins that interact with T β RI or T β RII have been isolated, although their role in signaling has yet to be determined (44–49).³

As part of our ongoing studies into early events in the TGF- β response, we have studied the biosynthesis of the type I and II TGF- β receptors in nontransfected, TGF- β -responsive, mink lung epithelial cells (Mv1Lu). We show here that the rates and efficiencies of Golgi processing of newly made T β RI and T β RII are very different, as are the apparent half-lives of the Golgi-processed, mature receptors. We also show that although TGF- β 1 has no effect on Golgi processing of either receptor, it markedly reduces the stability of the mature forms of both. This suggests that the two receptors are synthesized independently and neither arrive on the cell surface as a preformed complex nor, in the presence of ligand, remain on the cell surface as a stable complex. In addition, the rapid turnover of the type II receptor has potential implications for regulation, especially since the Endo H-resistant (and therefore presumably cell surface) form of T β RII is even more unstable in the presence of ligand.

MATERIALS AND METHODS

Cell Lines and Cell Culture—Mv1Lu mink lung epithelial cells were obtained from the American Type Culture Collection and were grown in modified Eagle's medium (Life Technologies, Inc.) with 10% decomplexed fetal calf serum (Life Technologies, Inc.) supplemented with 2 mM glutamine, 100 units/ml penicillin, 0.1 mg/ml streptomycin, and nonessential amino acids (JRH Bioscience) in a 5% humidified CO₂ atmosphere. COS7 cells (American Type Culture Collection) and HYB2 cells (a gift of Dr. A. Geiser (National Institutes of Health) (50)) were grown in Dulbecco's modified Eagle's medium with the same additives except no nonessential amino acids.

Binding and Cross-linking of Iodinated TGF- β —TGF- β 1 (a gift of R & D Systems) was iodinated by the chloramine T method as described (51), with the following modifications. TGF- β 1 (1 μ g) in 10 μ l of 1 M sodium phosphate, pH 7.2, was mixed with 2 μ l of Na¹²⁵I (0.2 μ Ci). The reaction was initiated by the sequential addition of three 2- μ l portions of chloramine T (0.1 mg/ml). The reaction was stopped by the sequential addition of 10 μ l of acetyl tyrosine (100 mM), 100 μ l of potassium iodide (100 mM), and 100 μ l of urea-saturated acetic acid. For the binding and cross-linking experiment shown in Fig. 5, a modification of the method of Wang *et al.* (4) was used. Subconfluent Mv1Lu cells in standard medium were incubated at 37 °C in tunicamycin (Boehringer Mannheim; stock of 3 mg/ml in DMSO) at a concentration of 2 μ g/ml for times ranging from 0 (no tunicamycin added; duplicate plates) to 11 h. Cells were rinsed once with KRH binding buffer (50 mM Hepes, pH 7.5, 128 mM NaCl, 1.3 mM CaCl₂, 5 mM MgSO₄, 5 mM KCl) with 0.5% fatty acid-free bovine serum albumin (Sigma) and then incubated at 37 °C in KRH binding buffer with either 2 μ g/ml tunicamycin or an equivalent volume of DMSO (0 time points). After 30 min, this material was aspirated and 3 ml of ice-cold KRH binding buffer containing 50 pM ¹²⁵I-TGF- β 1 was added. Plates were incubated at 4 °C with rotation for 3 h and then rinsed 3 times

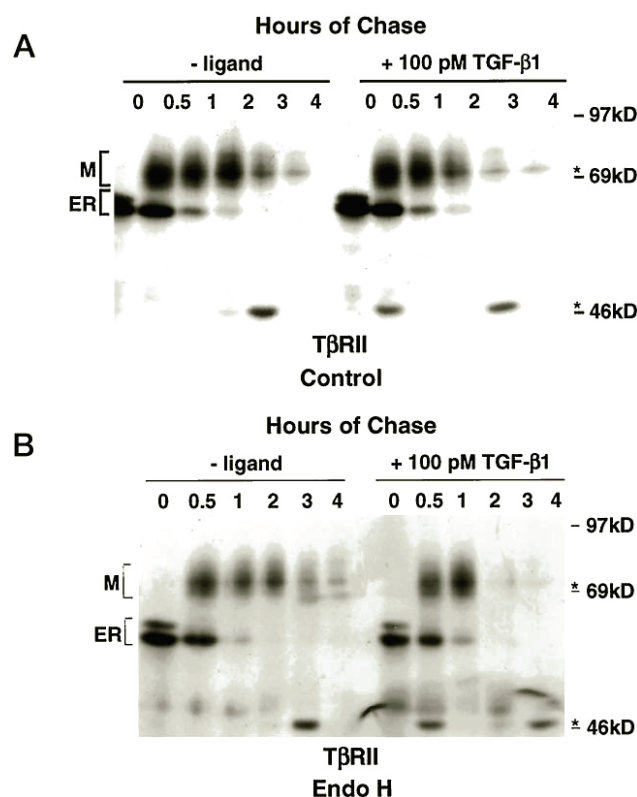


FIG. 1. The ER and mature, Golgi-processed, forms of the type II receptor have relatively short half-lives. Mink lung cells were pulse-labeled for 10 min at 37 °C in 0.5 mCi/ml [³⁵S]methionine/cysteine and then chased at 37 °C for the times indicated in nonradioactive medium containing either 100 pM TGF- β 1 or 50 μ g/ml of a pan-specific TGF- β neutralizing antibody (– ligand). Cells were lysed and immunoprecipitated with α -IIC, an anti-peptide antibody specific for the C terminus of the type II receptor. Eluted material was split in two; one half was treated with Endo H (B), and the other half was left untreated (A). Brackets indicate the Endo H-sensitive (ER) and mature (M) forms of the receptor. The asterisks indicate non-peptide-competable, contaminant bands.

with ice cold KRH. The ligand was then cross-linked to the receptor with the addition of 0.5 mg/ml disuccinimidyl suberate (Pierce) from a 100-fold concentrated stock in DMSO, followed by incubation at 4 °C for 15 min. Glycine was added to 20 mM, and plates were incubated for 10 min at 4 °C. Plates were rinsed twice with ice-cold phosphate-buffered saline (PBS), and lysed at 4 °C for 20–30 min in 1 ml lysis buffer (PBS with 0.5% deoxycholate, 1% Triton X-100, 10 mM EDTA, pH 8.0, and 1 mM phenylmethylsulfonyl fluoride). Insoluble material was pelleted by microcentrifugation at 14,000 rpm for 10 min at 4 °C and discarded. Cleared lysates were immunoprecipitated at 4 °C overnight with 1/200 volume of a polyclonal rabbit anti-serum (α -IIC (9)) raised against the C-terminal 16 amino acids of the human type II receptor; this epitope has 15 of 16 amino acids identical to the mink lung receptor (34). One-twentieth volume of protein A-Sepharose CL-4B beads (Sigma) was added, and the lysates were incubated with rotation for 30 min. Protein A-Sepharose beads were rinsed twice with lysis buffer and once with PBS, and bound protein was eluted by boiling in 0.5% SDS. The eluate from one of the 0 time point plates was treated overnight with *N*-glycosidase F (3000 units/50 μ l; New England Biolabs). Samples were analyzed by 7.5% SDS-polyacrylamide gel electrophoresis. Autoradiographs were quantified with a LaCie Silverscanner II and MacBAS (Fuji) software.

Metabolic Labeling—Mv1Lu cells were grown to subconfluence in standard medium on 10-cm plates. They were rinsed once with PBS and then starved in serum-free Dulbecco's modified Eagle's medium minus cysteine and methionine (ICN) with glutamine and penicillin/streptomycin for 5–5.5 h at 37 °C. The medium was then replaced with fresh medium supplemented with 0.2 mM oxidized glutathione (Boehringer Mannheim) and 0.5 mCi/ml of a mixture of [³⁵S]methionine and [³⁵S]cysteine (Express; New England Nuclear) and incubated at 37 °C for 10 min (pulse-labeling). Plates were quickly rinsed twice with warmed chase medium (serum-free Dulbecco's modified Eagle's me-

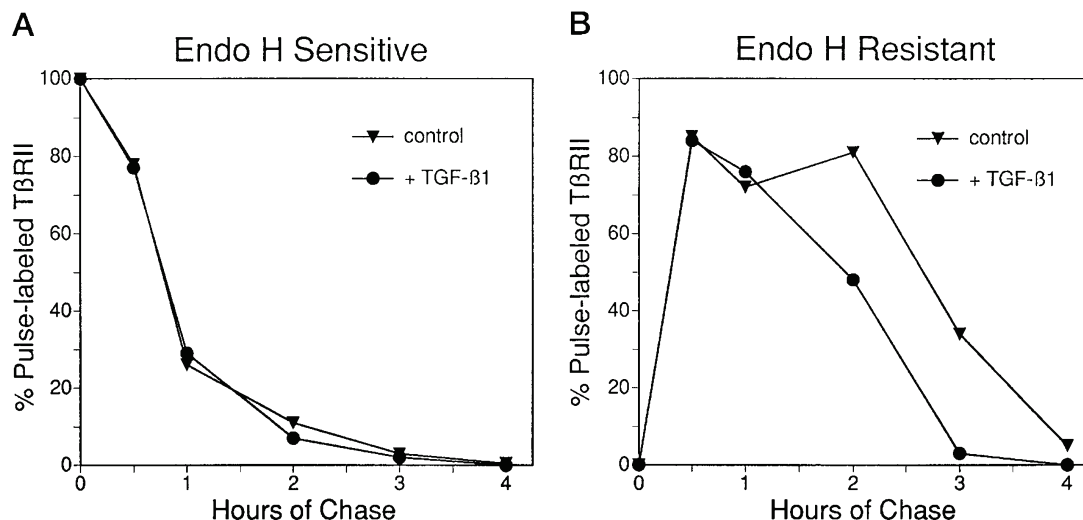


FIG. 2. **Quantitation of the Endo H-sensitive and -resistant forms of newly made type II receptor.** Bands representing Endo H-sensitive (A) and -resistant (B) forms of the type II receptor (from Fig. 1A) were scanned and quantified with MacBAS software and plotted as a percentage of the total amount of labeled protein present at the end of the pulse (time point 0). Control samples (– ligand) represent cells chased in the presence of a pan-specific TGF- β neutralizing antibody.

dium with glutamine and penicillin/streptomycin) and then incubated for the designated chase times at 37 °C in the presence of either 100 pM TGF- β 1 (“+ ligand”) or 50 μ g/ml of Pan-Specific TGF- β Neutralizing antibody (R & D Systems; “– ligand”), the IgG fraction of a polyclonal rabbit antiserum that neutralizes TGF- β 1, β 1.2, β 2, β 3, and β 5. Plates were then rinsed three times with ice-cold PBS and lysed in 1 ml of lysis buffer. Cleared lysates were precleared overnight with protein A-Sepharose and then immunoprecipitated with $\frac{1}{100}$ volume of antibody α -IIC or a polyclonal rabbit antiserum raised against the juxtamembrane cytoplasmic domain of the human type I receptor (7) (an epitope with 22 of 22 amino acids identical to the mink lung receptor (37)), at 4 °C for 2 h. After a 30-min incubation with protein A-Sepharose, bound beads were rinsed three times with lysis buffer plus 0.5% SDS and then twice with PBS. Protein was eluted into 0.5% SDS. For type II receptor immunoprecipitates, the eluate was split in two; one half was treated with Endo H (Genzyme, 100 mIU/ml) in the presence of 100 mM sodium citrate, pH 6.0, at 37 °C overnight. For type I receptor immunoprecipitates, one-third of the eluate was treated with Endo H, and another one-third with *N*-glycosidase F. In cases where the type I and II receptors had identical chase times, plates were labeled and chased in parallel; the pairs of lysates from such a time point were pooled and then split before immunoprecipitation to ensure that comparison between the two receptors at a given time point was valid. Samples were analyzed by 7.5% SDS-polyacrylamide gel electrophoresis; gels were fluorographed with 2,5-diphenyloxazole, dried, and placed on Kodak XAR film, which was scanned as above.

Control immunoprecipitations (not shown) were performed in the presence of an excess of the immunizing peptide to confirm the identity of the type I and II receptors. Also, because the type I receptor antibody immunoprecipitates a number of unrelated but peptide-competable proteins, COS7 cells mock transfected or transfected with the ALK-5 (type I receptor) cDNA (7) (a gift of Dr. K. Miyazono, Japanese Foundation for Cancer Research, Tokyo, Japan) were pulse-labeled and analyzed on each gel to confirm the proper size of the type I receptor.

RESULTS

Both the ER and Golgi-processed Forms of the Type II Receptor Have Relatively Short Metabolic Half-lives—To determine the half-life of the native type II receptor, we metabolically labeled TGF- β -responsive mink lung epithelial cells, chased them from 0 to 4 h, and immunoprecipitated detergent extracts with an anti-type II receptor antibody. The newly synthesized receptor (Fig. 1A, left panel, 0 h time point) migrates as a doublet that shifts from 62 and 65 kDa to 58 and 61 kDa after treatment with Endo H (Fig. 1B, left panel), indicating that it is present in the endoplasmic reticulum. This Endo H-sensitive material disappears with a half-life of 30–35 min (Fig. 2A, control). A smear from 67 to 77 kDa, representing the Endo

H-resistant receptor, appears at 0.5 h of chase (Fig. 1, A and B, left panels). This Golgi-processed receptor has a heterogeneous population of complex *N*-linked sugars. More than 50% of the steady state population of cellular receptors, including the majority of the cell surface form, is also *O*-glycosylated (52).⁴ During the chase, approximately 90% of pulse-labeled Endo H-sensitive receptor is converted to the Endo H-resistant, Golgi-processed material (Fig. 2B), demonstrating efficient conversion of the ER to the mature form, with minimal ER degradation.

The Golgi-processed form of T β RII, like the Endo H-sensitive form, disappears rapidly, with a half-life of approximately 2.5 h (Fig. 2B). The smear of Endo H-resistant material represents both intracellular and cell surface receptors. Over half of the total population of Endo-H resistant T β RII, however, is sensitive to digestion of intact cells with proteinase K (data not shown), indicating that approximately half of the total Endo-H resistant T β RII is on the cell surface at steady state. Thus, our data suggest that the half-life of cell surface T β RII is also approximately 2.5 h.

Similar experiments (data not shown) with the HYB2 line of TGF- β -responsive human cells (50) also show rapid metabolism of the type II receptor, with a half-life of the Endo H-sensitive T β RII of 25–30 min and a half-life of the Endo H-resistant form of approximately 3 h.

Golgi-processed Type II Receptor Is Degraded More Rapidly in the Presence of TGF- β —To assess the effect of TGF- β on the stability of the Endo H-sensitive and -resistant forms of T β RII, the above experiments were performed in parallel in the presence of 100 pM TGF- β 1 (Fig. 1, A and B, right panels). The Endo H-sensitive receptor disappeared with a $t_{1/2}$ of 30–35 min, identical to the rate in control cells (Fig. 2A). This indicates that ER stability, processing efficiency, and the rate of ER to Golgi transport of newly made T β RII are unaffected by the presence of TGF- β . Endo H-resistant T β RII, however, is less stable in the presence of TGF- β , with a half-life of 1.7 h compared to 2.5 h in control cells (Fig. 2B). Importantly, all control experiments (– ligand) were performed in the presence of a neutralizing antibody against the TGF- β s (see “Materials and Methods”); thus, it is unlikely that residual or endogenously synthesized TGF- β masked an even more marked effect of

⁴ R. G. Wells and H. F. Lodish, manuscript in preparation.

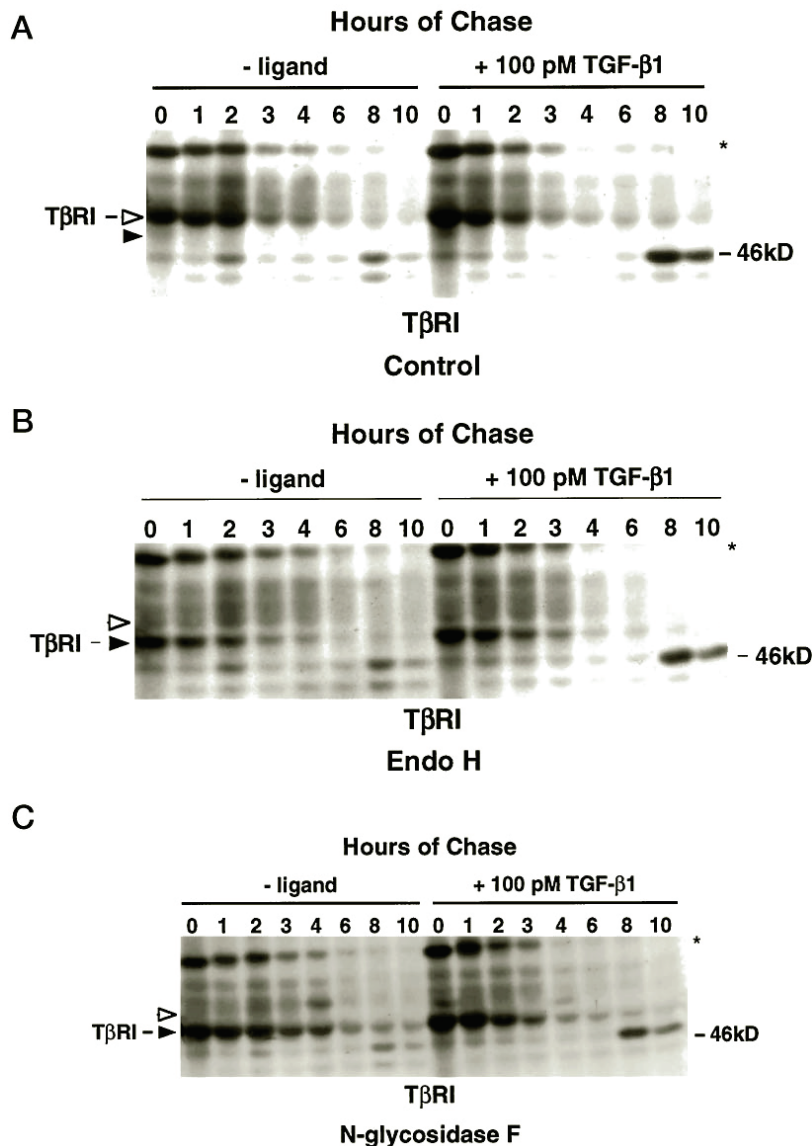


FIG. 3. The type I receptor exits the ER more slowly than the type II. Mink lung cells were ^{35}S -labeled and chased as in Fig. 1, except that chase times ranged from 0 to 10 h. This experiment was performed in parallel with that in Fig. 1. The chase medium contained either 100 pM TGF- β 1 or 50 $\mu\text{g}/\text{ml}$ of a pan-specific TGF- β neutralizing antibody (– ligand). Cells were lysed and immunoprecipitated with an anti-peptide antibody specific for the type I receptor. Eluted material was divided into three portions; one third was treated with Endo H (b), one third was treated with N-glycosidase F (c), and one third was left untreated (a). The *solid arrowhead* shows the location of the deglycosylated receptor at 49 kDa; the *open arrowhead* shows the location of the 52-kDa receptor bearing Endo H-sensitive oligosaccharides that is seen in A. The *asterisk* shows a nonspecific contaminant band.

the ligand.

Performing the same experiment on HYB2 cells, we obtained a similar lack of effect of TGF- β on the fate of the Endo H-sensitive T β RII, and a significant although less marked effect on the stability of the Golgi-processed receptor (data not shown).

The Type I Receptor Is Metabolized More Slowly than the Type II Receptor—The half-life of the type I receptor was determined by metabolically labeling Mv1Lu cells in parallel with the experiments above (Figs. 1 and 2) and immunoprecipitating the cell lysates with an anti-type I receptor antibody. Lysates from similarly labeled, mock- and T β RI-transfected COS7 cells (not shown) were run alongside the Mv1Lu cell material to confirm identification of the T β RI bands. Pulse-labeled T β RI migrates on SDS-polyacrylamide gel electrophoresis as a single species of 52 kDa (Fig. 3A, *left panel*). The gel mobility shifts to 49 kDa after treatment with Endo H, indicating that newly synthesized T β RI contains a high mannose, N-linked oligosaccharide characteristic of the ER (Fig. 3B, *left panel*). The Endo H-sensitive ER form of T β RI disappears with a half-life of 2.8–3 h (Fig. 4A), markedly longer than the half-life of 30–35 min of newly made, Endo H-sensitive type II receptor. At no time during the chase period could we detect a specific higher molecular weight

form of T β RI; we presume that the Golgi-processed, Endo H-resistant form of T β RI migrates within the background “smear” above 52 kDa. By treating the T β RI immunoprecipitates with N-glycosidase F, however, removing all N-linked sugars, we can quantify the total (Endo H-sensitive and -resistant) amount of metabolically labeled T β RI in the cell (Fig. 3C). We calculate the amount of mature (Endo H-resistant) T β RI by subtracting the amount of labeled Endo H-sensitive receptor (Fig. 3B) from the total amount (N-glycosidase F-treated) of T β RI (Fig. 3C). Fig. 4 shows that no more than 50% of pulse-labeled T β RI is converted to an Endo H-resistant form, compared with approximately 90% of T β RII (Fig. 2B).

Because of the slow and incomplete conversion of pulse-labeled T β RI to an Endo H-resistant form, we cannot use the data in Fig. 4B to calculate a precise half-life of Golgi-processed T β RI. Clearly, however, it is significantly longer than the 2.5-h half-life of the mature, Golgi-processed type II receptor (compare control curves in Figs. 2B and 4C).

The presence of TGF- β has no effect on the metabolic stability of the Endo H-sensitive form of T β RI (Fig. 4A), nor on the rate by which this material is converted into an Endo H-resistant form (Fig. 4C). The Golgi-processed, Endo H-resistant form of T β RI, however, is less stable in the presence than the

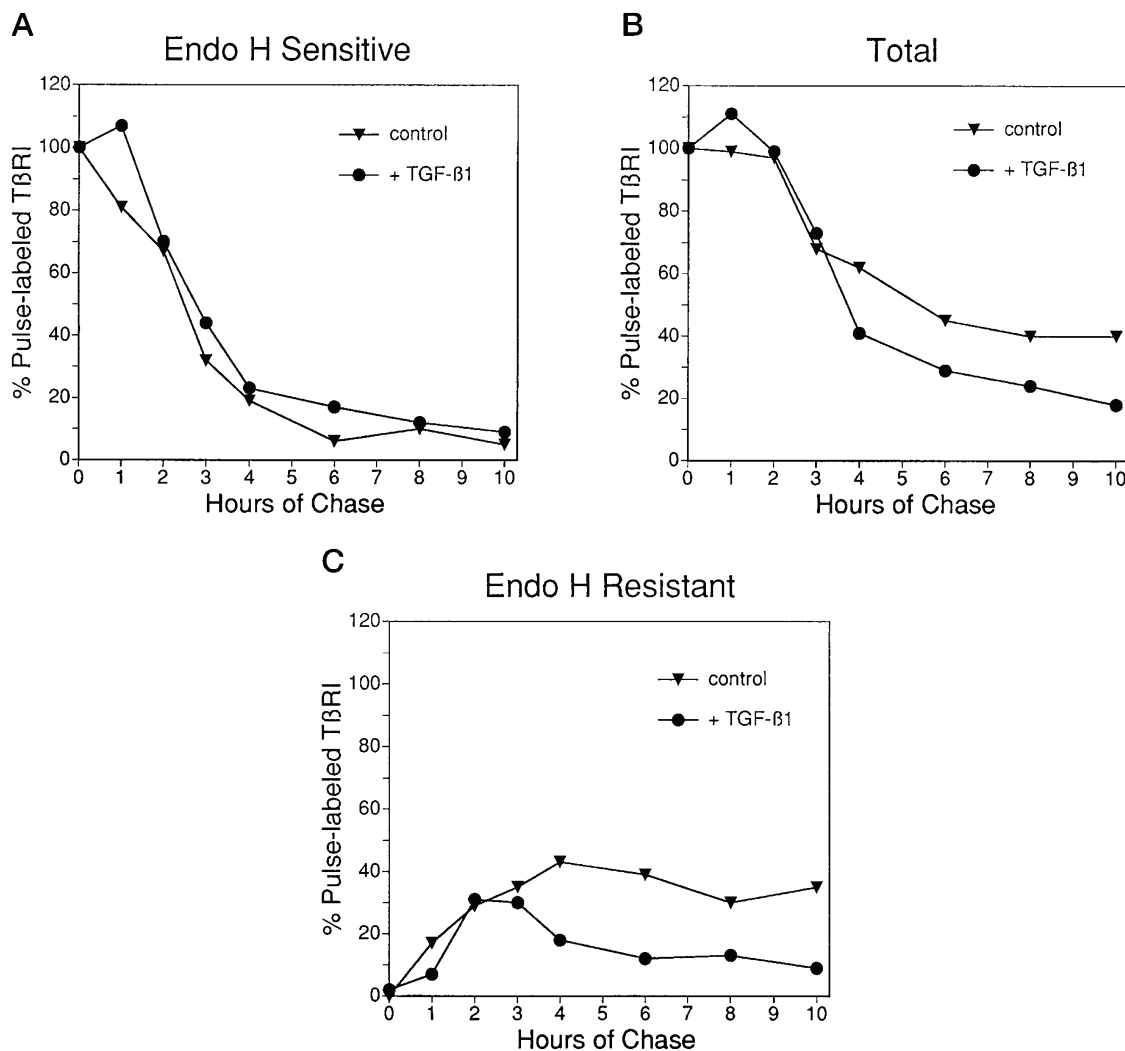


FIG. 4. **Quantitation of the Endo H-sensitive and -resistant forms of newly made type I receptor.** Labeled Endo H-sensitive (Fig. 3B) and total cellular T β RI (Fig. 3C) were quantified as in Fig. 2 and were plotted as a percentage of the amount of (Endo H-sensitive) labeled T β RI present at the end of the pulse labeling (0 h time point). The difference between the total cellular (B) and Endo H-sensitive (A) curves was assumed to represent Endo H-resistant material and is plotted in C. Control cells (— ligand) represent cells chased in the presence of a pan-specific TGF- β neutralizing antibody.

absence of TGF- β 1 (Fig. 4C); this is similar to the decrease in stability of the Golgi-processed type II receptor in the presence of ligand (Fig. 2B).

Ligand-binding and Cross-linking Experiments Confirm Different ER to Cell-surface Processing Times for the Type I and Type II Receptors—We performed ligand-binding and cross-linking experiments to confirm that the ER to Golgi half-lives calculated from pulse-chase experiments (above) correlate with ER to cell surface half-lives. In the experiment depicted in Fig. 5, Mv1Lu cells were incubated in tunicamycin for times ranging from 0 to 11 h. The cells were chilled, and ^{125} I-TGF- β 1 was bound and then cross-linked to cell surface TGF- β receptors. Tunicamycin blocks the synthesis of dolichol pyrophosphoryl *N*-acetyl glucosamine, an essential intermediate in the addition of *N*-linked sugars to newly synthesized polypeptides. In preliminary experiments, we showed that the concentration of tunicamycin used, 2 μ g/ml, completely inhibited *N*-linked glycosylation of both receptors.

Deglycosylated T β RII appears on the cell surface as early as 1 hour after addition of tunicamycin, indicating that it is transported from the ER to cell surface in less than this time. The half-time for appearance of the maximum amount of deglycosylated type II receptor on the cell surface is approximately 2 h; the actual half-time for its formation is less than this, since the

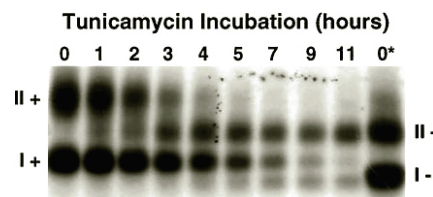


FIG. 5. **Binding of radioiodinated TGF- β to tunicamycin-treated Mv1Lu cells.** Mv1Lu cells were incubated with 2 μ g/ml tunicamycin at 37 °C for the times noted. Cells were then cooled to 4 °C and allowed to bind and subsequently become cross-linked to ^{125} I-TGF- β . Labeled cells were lysed and immunoprecipitated with α -IIC. The 0 h time point was prepared in duplicate; one half of the combined immunoprecipitated eluant was treated with *N*-glycosidase F before SDS-polyacrylamide gel electrophoresis (0*) and the other half was untreated (0). II+, II-, I+, and I- indicate the type II and I receptors with (+) or without (—) *N*-linked sugars.

time from addition of tunicamycin to depletion of dolichol pyrophosphoryl *N*-acetyl glucosamine is unknown. In contrast, deglycosylated type I receptor does not appear on the cell surface until 3 h after addition of tunicamycin; the half-time for appearance of the maximum amount of deglycosylated type I receptor is approximately 4 h. The half-lives of the glycosylated forms of the cell surface receptors cannot be readily determined

from this type of experiment, because at the time of addition of tunicamycin there may be substantial intracellular (ER or Golgi) pools of T β RI or T β RII that can subsequently move to and then be removed from the cell surface. Thus, the fact that glycosylated cell surface T β RII is depleted much more rapidly than T β RI (Fig. 5, compare *II+* and *I+*) provides only an indirect indication that their actual half-lives are different. After 4–5 h of treatment with tunicamycin, however, all cell surface T β RII is nonglycosylated, whereas all cell surface T β RI is fully glycosylated. (We see the same results regardless of whether lysates are immunoprecipitated with antibodies against T β RI or T β RII or are not immunoprecipitated; data not shown.) These results are consistent with the different rates of processing from the ER of newly made types I and II receptors depicted in Figs. 2 and 4. They also indicate that complexes between types I and II TGF- β receptors do not occur until the proteins have reached a post-ER compartment (presumably the cell surface) and have important implications for our understanding of formation of receptor complexes, as discussed below.

The decreased amount of cell surface receptors after long incubations in tunicamycin may represent decreased protein synthesis (a known consequence of tunicamycin treatment, affecting some proteins more than others). Receptors lacking *N*-linked oligosaccharides bind ligand and are immunoprecipitated as efficiently as fully glycosylated receptors,⁴ so the lower signal is not the result of inefficient binding and cross-linking or immunoprecipitation. Decreased formation or stability of nonglycosylated type I/II receptor complexes is unlikely, given that our findings are similar for nonimmunoprecipitated lysates.

DISCUSSION

Our principal finding is that the types I and II TGF- β receptors are metabolized very differently. The ER form of the type II receptor has a half-life of 30–35 min and is efficiently (approximately 90%) processed by Golgi enzymes; the Golgi-processed forms of the type II receptor have a relatively short metabolic half-life, about 2.5 h. In contrast, the Endo H-sensitive, ER form of the type I receptor has a half-life of 2.8–3 h, and less than 50% of pulse-labeled type I receptor is converted to an Endo H-resistant form. The exact half-life of Golgi-processed type I receptor is difficult to determine because of the large ER pool of receptor subunits being slowly processed to the Golgi at the same time that Golgi-processed receptors are being degraded (Fig. 4, A and C). Nonetheless, the half-life is considerably longer than that of the type II receptor. Addition of TGF- β 1 to cells causes a decrease in the stability of the Endo H-resistant forms of both receptors. These conclusions, derived from experiments in which TGF- β -responsive mink lung epithelial cells were metabolically pulse-chase labeled, were confirmed by TGF- β 1 binding and cross-linking experiments on cells treated with tunicamycin for various times. In unpublished studies, similar results were obtained using a TGF- β -responsive human cell line (HYB2). Because these cell lines express native, rather than transfected, receptors, it ensures that the concentrations and ratios of the types I and II TGF- β receptors are physiologic.

Our results are consistent with recent studies on the metabolism of the type II receptor in bone-forming cells, which show that after cycloheximide treatment, the amount of cell surface Type II receptor able to bind to iodinated ligand decreased with a half-life of 2 h (53). This is an indirect measure of receptor biosynthesis and stability, however, and provides no information about the type I receptor, which requires cell surface type II receptor for binding to TGF- β . To the best of our knowledge,

biosynthetic studies have not been undertaken for any other members of the receptor superfamily, and there are no published studies of metabolically labeled receptors in nontransfected cell lines.

Our pulse-chase experiments indicate that newly made type II receptor moves quickly and efficiently through the ER after synthesis. This suggests rapid and efficient folding within the ER because only correctly folded and processed proteins are transported to the Golgi apparatus (54). Similarly, the soluble extracellular domain of the human TGF- β type II receptor is efficiently synthesized in COS-7 cells and binds ¹²⁵I-TGF- β with the same specificity and affinity as the normal cell surface T β RII (52).⁵ Thus, the rapid folding and efficient processing of the type II receptor is a property mainly of its extracellular domain, which contains six potential disulfide bonds and two (three in human) *N*-linked oligosaccharides, as well as an undetermined number of *O*-linked sugars.

The Golgi-processed form of T β RII is degraded with a half-life of approximately 2.5 h (Fig. 2B). Because more than half of the total population of Endo-H resistant T β RII is sensitive to digestion of intact cells with proteinase K and thus at steady state is on the cell surface, our data suggest that the half-life of cell surface T β RII also is approximately 2.5 h. This short half-life is similar to the half-lives reported for the mature forms of the nonrecycling interferon receptor (3 h) (55), the IL-2 receptor (1 h) (56), and the erythropoietin receptor (45 min) (57). In contrast, other receptors have longer half-lives: the asialoglycoprotein receptor (20 h) (58), the low density lipoprotein receptor (11 h) (59), and the transferrin receptor (20 h) (60). After exiting the cell surface, type II receptors do not appear to undergo proteolytic cleavage or storage within the cell, because experiments with ¹²⁵I-TGF- β -bound receptors show no release of smaller labeled fragments into the medium (data not shown). Furthermore, by analysis of immunoprecipitates of metabolically labeled cells, we have not seen specific smaller proteolytic degradation products of T β RII (data not shown).

We speculate that the short metabolic half-life of the type II receptor has implications for regulation of TGF- β signaling that go beyond its impact on complex formation. The rapid turnover of the type II receptor potentially allows receptor numbers to change quickly in response to various stimuli, an important characteristic for a growth factor receptor with wide-ranging effects on differentiation and development. The decreased stability of the mature form of T β RII in response to ligand suggests that TGF- β itself may regulate the numbers of its receptor and that the type II receptor may be a limiting factor in the cellular response to TGF- β . Phosphorylation of T β RII following ligand binding may be a signal for receptor degradation, in a manner analogous to many receptors spanning seven membranes, which undergo internalization and down-modulation after ligand binding (61).

The newly made ER form of the type I receptor disappears from cells with a half-time of nearly 3 h, 5–6 times as long as for the type II receptor. Less than one-half of the newly made receptors ever acquire Endo-H-resistant oligosaccharides. Processing of the type I receptor thus is similar to that of the erythropoietin receptor, which likely folds inefficiently (57, 62, 63). A mutant erythropoietin receptor is transported from the ER more efficiently than the wild type receptor and is expressed in elevated numbers at the cell surface (63); this is the first example of a mutant polypeptide that is processed in the ER more efficiently than its wild type counterpart. We speculate that inefficient folding and processing of the wild type erythropoietin receptor and potentially the type I TGF- β recep-

⁵ H. Yankeliev, R. G. Wells, and H. F. Lodish, unpublished results.

tor in the ER is one mechanism for controlling the number of plasma membrane receptors.

The fact that the types I and II TGF- β receptors exit the ER at different rates implies that they do so separately. Fig. 5 provides perhaps the most vivid demonstration of the different rates of ER exit of the two receptors: after 4 h of treatment with tunicamycin, all cell surface T β R_{II} was nonglycosylated, whereas all cell surface T β R_I remained fully glycosylated. Because the rates and extents of ER to Golgi processing of the two receptors are different and because the mature forms of the two receptors are degraded with different half-lives, our results imply that stable complexes between the types I and II TGF- β receptors do not occur until the proteins have reached a post-ER compartment, presumably the cell surface. Given our results, it would be possible to conclude the opposite—that hetero-oligomers form in the ER—only if one receptor was synthesized in excess and accumulated in the ER awaiting production of the other receptor. This scenario would imply that the first receptor could not become correctly folded and mature to the Golgi unless complexed to the second receptor. Both the type I and type II receptors, however, mature normally to the cell surface even when expressed alone in COS cells (36).⁶ Furthermore, the soluble exoplasmic domain of the type I receptor, like that of the type II, is efficiently secreted by transiently transfected COS cells, as well as by stably transfected Chinese hamster ovary cells,⁵ again implying that folding of the exoplasmic domain of T β R_I occurs efficiently in the absence of T β R_{II}. Our results showing different rates of degradation of Golgi-processed T β R_I and T β R_{II} are also consistent with the idea that stable complexes between the two receptors do not occur in the ER; indeed, they suggest that even cell surface complexes are short-lived.

Studies with TGF- β 1 showed that formation of a T β R_I/T β R_{II} heteromeric complex is dependent on the presence of TGF- β (29, 36). TGF- β 2, however, binds to neither cell surface signaling receptor expressed in the absence of the other, suggesting the existence of a preformed T β R_I/T β R_{II} complex (35). Our present experiments imply that if T β R_I/T β R_{II} complexes do occur on the cell surface in the absence of TGF- β , they are relatively unstable and do not form in the ER. Although our preliminary data suggest that the type II receptor forms dimers cotranslationally,⁶ it is unlikely that heteromeric complexes with the type I receptor also form at this point.

The cytoplasmic domains of T β R_I and T β R_{II} may have an intrinsic but weak affinity for each other, as interactions between the two have been detected in both the yeast two-hybrid system and COS cells overexpressing the cytoplasmic domains of both receptors (29, 30).³ Such interactions, however, have not been detected in TGF- β -responsive cells, and we do not know whether they affect receptor *trans*-activation and signal transduction. Although weak interactions between the cytoplasmic domains of T β R_I and T β R_{II} expressed at physiologic concentrations are not, in themselves, sufficient for generating growth inhibitory signals, they could, if they exist, serve to initiate formation of heterodimers of T β R_I and T β R_{II} that become stabilized by the binding of a TGF- β ligand.

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Note Added in Proof—Koli and Arteaga (Koli, M., and Arteaga, C. L. (1997) *J. Biol. Chem.* **272**, 6423–6427) recently published similar data.

Their data agree with ours in showing a reduction in the half-life of T β R_{II} in the presence of TGF- β . They see no effect of ligand, however, on the half life of the type I receptor. This difference may be due to different assumptions about the identity of a 55-kDa form of the type I receptor. We have identified it as an Endo H-sensitive, ER form, whereas Koli and Arteaga identify it as the mature, Endo H-resistant receptor.

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