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Generation of a Truncated Hepatocyte Growth Factor Receptor in the Endoplasmic Reticulum*

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The hepatocyte growth factor (HGF) receptor (p190^{MET}) is a tyrosine kinase composed of two disulfide-linked chains, α of 50 kDa and β of 145 kDa. We have previously described an isoform (p140^{MET}) containing a β chain of 85 kDa, lacking the cytoplasmic kinase domain. The two receptor variants originate by post-translational processing of a common single-chain precursor of 170 kDa (Pr170). In the endoplasmic reticulum a fraction of Pr170 is cleaved at the cytosolic side generating an intermediate product of 120 kDa (Pr120). This molecule 1) is already detectable after 15 min of pulse labeling, 2) contains high mannose-branched oligosaccharides, and 3) accumulates upon treatments inhibiting the export from the endoplasmic reticulum. A second cleavage, occurring after 30 min of chase in the trans-Golgi network, converts the single-chain precursors Pr170 and Pr120 into the mature heterodimers p190^{MET} and p140^{MET}. This process is inhibited by brefeldin A treatment. Conditions leading to Pr170 accumulation in the endoplasmic reticulum, such as receptor overexpression, induce kinase activation and overproduction of Pr120. Conversely, cells expressing a kinase-defective HGF receptor lack the truncated isoform. The proteolytic cleavage of the cytoplasmic domain may thus represent a safety mechanism aimed at preventing ligand-independent intracellular activation of the HGF receptor kinase.

The *MET* proto-oncogene encodes the tyrosine kinase receptor for hepatocyte growth factor (HGF)[1] (Bottaro *et al.*, 1991; Naldini *et al.*, 1991a, 1991b), shown to be identical to scatter factor (Furlong *et al.*, 1991; Gherardi and Stoker, 1990; Naldini *et al.*, 1991b; Weidner *et al.*, 1991). HGF is a mitogen (Kan *et al.*, 1991; Miyazwa *et al.*, 1989; Nakamura *et al.*, 1989; Rubin *et al.*, 1991), a morphogen (Montesano *et al.*, 1991), an angiogenic factor (Bussolino *et al.*, 1992), and a potent stimulator of cell motility and invasion (Rosen *et al.*, 1990; Stoker *et al.*, 1987; Weidner *et al.*, 1990). The biological responses to HGF are mediated by the intrinsic tyrosine kinase activity of the receptor (Giordano *et al.*, 1993; Weidner *et al.*, 1993). Uncontrolled tyrosine kinase activity of the HGF receptor, emphasizing its oncogenic potential, has been observed in transformed cells following chromosomal rearrangements (Park *et al.*, 1986), gene overexpression (Giordano *et al.*, 1989a; Di Renzo *et al.*, 1991, 1992; Prat *et al.*, 1991a), defective post-translational processing (Mondino *et al.*, 1991), or autocrine loops (Rong *et al.*, 1992).

The HGF receptor is a transmembrane heterodimer of 190 kDa (p190^{MET}) (Giordano *et al.*, 1989a) composed of two disulfide-linked chains of 50 kDa (p50$^{\alpha}$) and 145 kDa (p145$^{\beta}$). The molecule is synthesized as a single-chain 170-kDa precursor (Pr170), which is co-translationally glycosylated. Terminal glycosylation and proteolytic cleavage generate the mature p190^{MET} heterodimer (Giordano *et al.*, 1989b). The receptor consists of three functionally distinct domains: an extracellular ligand binding domain, comprising p50$^{\alpha}$ and the N-terminal portion of the p145$^{\beta}$; a single transmembrane-anchoring segment; a cytoplasmic domain contributed by the C-terminal portion of p145$^{\beta}$. The latter contains the kinase domain (Gonzatti-Haces *et al.*, 1988; Park *et al.*, 1987; Tempest *et al.*, 1988), the tyrosine autophosphorylation sites involved in kinase activation (Ferracini *et al.*, 1991; Naldini *et al.*, 1991c; Longati *et al.*, 1993) and also serine phosphorylation sites with a negative regulatory role (Gandino *et al.*, 1990, 1991, 1994). The C-terminal region of the cytoplasmic domain contains critical tyrosine residues, which upon phosphorylation become docking sites for binding and activation of intracellular signal transducers (Graziani *et al.*, 1991, 1993; Bardelli *et al.*, 1992; Ponzetto *et al.*, 1993).

We previously described two heterodimeric isoforms of HGF receptor, one of 130 kDa (p130^{MET}) released from the cells and one of 140 kDa (p140^{MET}) bound to the cell surface. Both isoforms lack the tyrosine kinase domain. The truncated isoforms are generated by post-translational processing and are both detected in cells transfected with the cDNA derived from the major 9-kilobase *MET* mRNA (Prat *et al.*, 1991b). In this paper we show that p140^{MET} originates from an intermediate precursor (Pr120) derived from Pr170. The removal of the kinase domain of Pr170 occurs in the endoplasmic reticulum by proteolytic processing and follows inappropriate tyrosine kinase activation of the receptor precursor in the intracellular compartment.

MATERIALS AND METHODS

Cells and Antibodies—GTL-16 cells are derived from gastric carcinoma (Giordano *et al.*, 1989a). HT-29 colon carcinoma cells were purchased from American Type Culture Collection. NIH/c-*MET* cells are a stable clonal cell line obtained by transfection of NIH 3T3 fibroblasts with a 4.3-kilobase cDNA encompassing the entire human *MET* coding sequence, as described elsewhere (Giordano *et al.*, 1993). NIH/c-*MET*^{K1110A} cells are NIH 3T3 fibroblasts transfected with a pMT2 vector, carrying a Lys^{1110} → Ala mutant. Monoclonal antibodies against the extracellular domain of the HGF receptor (*α-extra* mAbs) were obtained as described elsewhere (Prat *et al.*, 1991b). Antibodies

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[1] The abbreviations used are: HGF, hepatocyte growth factor; Pipes, 1,4-piperazinediethanesulfonic acid; BFA, brefeldin A; PAGE, polyacrylamide gel electrophoresis; endo H, endoglycosidase H; mAbs, monoclonal antibodies; ER, endoplasmic reticulum.

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against the intracellular domain (*α-intra* mAbs) were obtained by immunization against a peptide corresponding to 19 C-terminal amino acids of the human *MET* sequence.

Metabolic Labeling, Immunoprecipitation, and Western Blotting—Cells were pulse-labeled with [^{35}S]methionine (400 μCi/ml; Amersham Corp.) for 15 min in methionine-free RPMI medium, chased with complete culture medium for the indicated times, and extracted with ice-cold buffer containing 10 mM Pipes, pH 7.6, 100 mM NaCl, 5 mM $MgCl_2$, 300 mM sucrose, 5 mM EGTA (DIM buffer), 1% Triton X-100, and inhibitors of proteases (50 μg/ml pepstatin; 500 μg/ml leupeptin; 1 μg/ml aprotinin; 2 mM phenylmethylsulfonyl fluoride (Sigma); 500 μg/ml soybean trypsin inhibitor (Boehringer)). In some experiments, cells were pulse-labeled with [^{35}S]methionine (100 μCi/ml) for 4 h, before chasing. In experiments with brefeldin A (BFA) (Sigma), cells were pretreated for 1 h with 10 μg/ml, and fresh BFA was added to pulse and chase medium. For experiments at a low temperature, the pulse was done at 37 °C, and cells were incubated at 16 °C during chase. Immunoprecipitations, SDS-PAGE analysis, and autoradiography were performed as described previously (Prat *et al.*, 1991b). In Western blots, proteins transferred to nitrocellulose filters (Hi-bond, Amersham) were probed with the indicated antibodies and revealed by the enhanced chemiluminescence system (ECL™, Amersham Corp.).

Endo H Digestion—Immunoprecipitates were boiled in 100 μl of 0.1 M sodium acetate buffer (pH 5), containing 0.1 M 2-β-mercaptoethanol and 0.15% SDS, and incubated for 16 h at 37 °C with 10 milliunits of endo H (Boehringer Mannheim), 0.1 M sodium acetate buffer (pH 5), 20 mM EDTA, 0.1% TX-100, and 1 mM phenylmethylsulfonyl fluoride.

Immunocomplex Kinase Assay—Proteins were extracted in DIM buffer, 1% Triton X-100, and protease inhibitors and immunoprecipitated. Immunocomplexes were phosphorylated in 20 μl of the same buffer in the presence of 7 μCi [γ-^{32}P]ATP (specific activity, 7,000 Ci/mM) (Amersham Corp.) at 30 °C for 5 min. The reaction was stopped by adding 1 ml of ice-cold phosphate-buffered saline, pH 7.2, containing 5 mM EDTA. After SDS-PAGE, gels were dried and exposed to Amersham Hyperfilm for autoradiography.

RESULTS

Identification of Pr120 as an Intermediate HGF Receptor Precursor—Previous experiments have shown that NIH 3T3 cells stably transfected with the full size human *MET* cDNA (NIH/c-*MET* cells) express p190^{MET} as well as the truncated p140^{MET} isoform (Prat *et al.*, 1991b). A clone expressing high levels of the latter isoform was used to further characterize the biosynthesis of p140^{MET}. Cells were labeled with [^{35}S] methionine for 15 min, chased for different periods of time, and lysed. Cell extracts were precipitated either with monoclonal antibodies (mAbs) directed against the intracellular C-terminal peptide (*α-intra* mAbs) or with mAbs recognizing an epitope of the extracellular domain (*α-extra* mAbs) of the HGF receptor β chain. Immunoprecipitates were analyzed on SDS-PAGE under nonreducing and reducing conditions. The already described precursor (Pr170) was detectable at early times of chase (Fig. 1). This molecule is already glycosylated (Giordano *et al.*, 1989b). After 60 min of chase, a shift in electrophoretic mobility from 170 to 190 kDa (p190^{MET}) was observed under nonreducing conditions. It is likely that this shift is due to further glycosylation (see below). The molecule was concomitantly cleaved into the mature p50$^{\alpha}$ and p145$^{\beta}$ disulphide-linked subunits, which could be separated upon reduction of disulphide bridges (Fig. 1). When labeled with [^{35}S]methionine the amount of p50$^{\alpha}$ detected was lower than expected on the basis of the stoichiometry with the β chains. This reflects the relative abundance of methionine residues in the α and β chains. At zero time of chase a protein with a molecular mass of 120 kDa (Pr120) was also precipitated by the *α-extra* mAbs (Fig. 1). The lack of reactivity of Pr120 with *α-intra* mAbs and its molecular size indicates that it lacks the cytoplasmic domain. After 1 h of chase, Pr120 matured into the truncated p140^{MET} heterodimer, generating an intact α chain and a truncated p85$^{\beta}$. The processing of Pr120 paralleled that of Pr170.

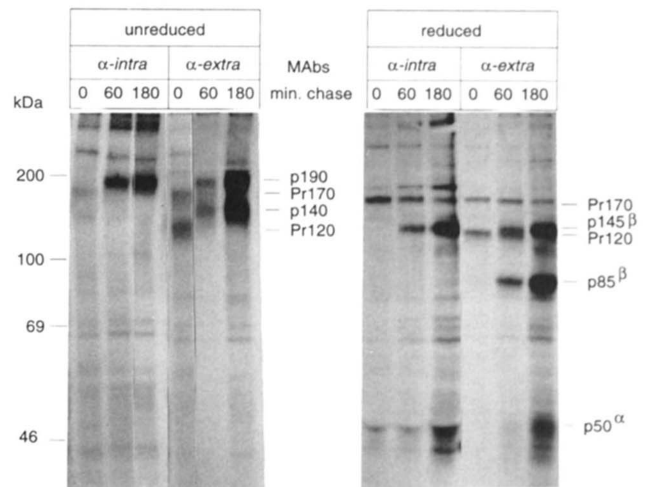

FIG. 1. **Identification of Pr120 as an intermediate HGF receptor precursor.** NIH 3T3 fibroblasts, transfected with the full-length human *MET* cDNA, were labeled with [^{35}S]methionine for 15 min and chased for the indicated periods of time. Solubilized proteins were precipitated with mAbs directed against the intracellular (*α-intra*) or the extracellular (*α-extra*) domains of the p190^{MET} β chain. Immunoprecipitates were analyzed in 8% SDS-PAGE under nonreducing and reducing conditions. In immunoprecipitates obtained with *α-extra* mAbs, two precursors, Pr170 and Pr120, were observed, which were then chased into the mature p190^{MET} and the C-terminal truncated p140^{MET} (*left panel*). Under reducing conditions (*right panel*) the mature heterodimers were resolved into the intact p145$^{\beta}$, the C-terminal truncated p85$^{\beta}$, and the p50$^{\alpha}$ chains.

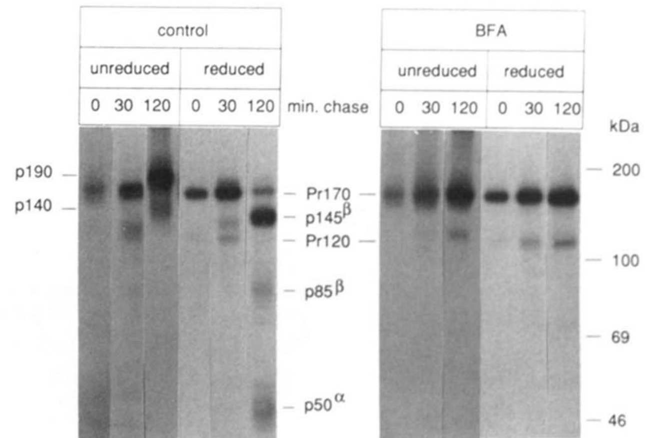

FIG. 2. **Pr120 accumulates in the ER after BFA treatment.** GTL-16 cells, either control or treated with BFA, were pulse-labeled with [^{35}S]methionine for 15 min and chased for the indicated periods of time. Cells were lysed, immunoprecipitated with *α-extra* mAbs, and analyzed in 8% SDS-PAGE under nonreducing or reducing conditions. BFA did not affect the synthesis of Pr170, while it suppressed its further maturation enhancing the production of Pr120.

Similar results were obtained in pulse-chase experiments performed on GTL-16 cells, constitutively overexpressing the HGF receptor (Fig. 2, *left panel*). In this line the ratio of Pr120 to Pr170 was approximately 1:10, a ratio similar to that observed between the two mature heterodimers.

These results show that the mature C-terminal truncated isoform p140^{MET} of the HGF receptor is generated from a C-terminal truncated precursor of 120 kDa through the same post-translational events, namely terminal glycosylation and proteolytic cleavage, which generate p190^{MET} from Pr170 (see the chart drawn in Fig. 11).

Pr120 Is Generated in the Endoplasmic Reticulum—The early detection of Pr120 after chasing suggested that this intermediate precursor might be generated within the endoplasmic reticulum (ER). To test this hypothesis, pulse-chase experiments were done in the presence of BFA, a fungal antibacterial reagent blocking the export of proteins from the ER to the Golgi complex (Misumi *et al.*, 1986). GTL-16 cells were treated with BFA (10 μg/ml) for 1 h before pulsing with [^{35}S]methionine for 15 min; the drug was present during the whole chase period. Cell extracts were immunoprecipitated with *α-extra* mAbs and analyzed on SDS-PAGE under non-reducing and reducing conditions. Fig. 2 shows that the appearance of the mature heterodimers p190^{MET} and p140^{MET} was completely prevented by BFA treatment, over the entire experiment duration (2 h of chase). As a consequence of the BFA-induced block of maturation, both precursors Pr170 and Pr120 accumulated.

Transport between the ER and Golgi is also blocked by low temperatures (Lippincott-Schwartz *et al.*, 1990). GTL-16 and NIH/c-*MET* cells were pulse-labeled with [^{35}S]methionine and chased at 37 or 16 °C. As shown in Fig. 3, Pr170 and Pr120 accumulated at 16 °C and were not processed to the mature forms observed in control cells kept at 37 °C.

It is known that, within the ER, the newly synthesized proteins are glycosylated by mannose-rich oligosaccharide precursors sensitive to endo H. The immature oligosaccharides are trimmed to the mature forms by enzymes localized in the Golgi compartment, becoming endo H-resistant (Kornfeld and Kornfeld, 1985). Pr120 and Pr170 digested with endo H showed a loss in the apparent molecular mass (Fig. 4, *left panel*). As expected, the mature subunits p50$^{\alpha}$, p85$^{\beta}$, and p145$^{\beta}$ were resistant to endo H digestion (Fig. 4, *right panel*). All together, these results indicate that Pr120 is an immature precursor generated in a pre-Golgi compartment, likely to be the endoplasmic reticulum.

Pr120 Derives from the Processing of Pr170—BFA was used to retain both the HGF receptor precursors (Pr170 and Pr120) in the recycling pathway between the ER and the Golgi (Lippincott-Schwartz *et al.*, 1989). The consequent accumulation of precursors was followed by the appearance of Pr120 at a detectable level also in human cell lines, where the expression of the HGF receptor is normally low. HT-29 and GTL-16 cells, pretreated with BFA for 1 h, were pulse-labeled with [^{35}S]methionine for 4 h and chased for different periods

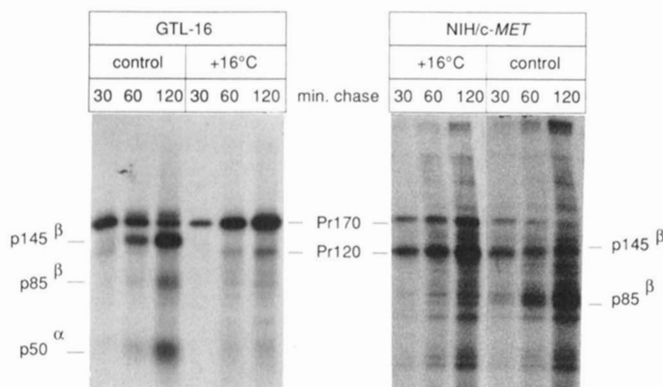

FIG. 3. **Pr120 accumulates in the ER at a low temperature.** GTL-16 cells and fibroblasts transfected with the human *MET* cDNA (NIH/c-*MET*) were pulse-labeled with [^{35}S]methionine for 15 min at 37 °C and chased at 37 °C (*control*) or at 16 °C for the indicated periods of time. Cells were lysed, immunoprecipitated with *α-extra* mAbs, and analyzed in SDS-PAGE under reducing conditions. At low temperatures Pr170 was not translocated to the ER compartment and was therefore prevented from further maturation.

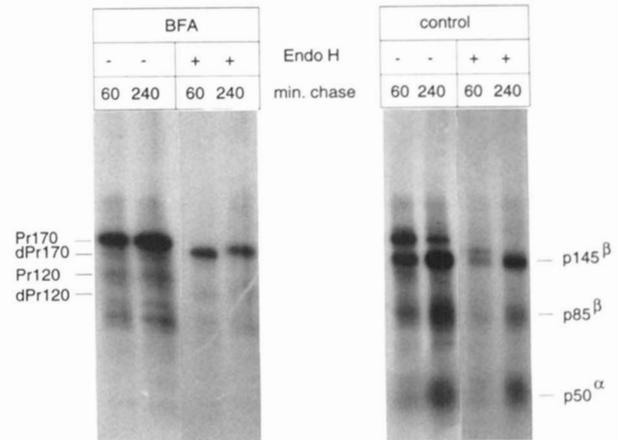

FIG. 4. **Pr170 and Pr120 are endo H sensitive.** GTL-16 cells, either treated with BFA or control, were pulse-labeled with [^{35}S] methionine for 15 min and chased for the indicated periods of time in the presence of BFA. Cells were lysed and immunoprecipitated with *α-extra* mAbs. Immuno complexes were digested with endo H at 37 °C for 16 h and analyzed in 8% SDS-PAGE under reducing conditions. Both Pr170 and Pr120 showed a lower molecular mass when digested by endo H (deglycosylated dPr170 and dPr120, *left panel*), while the mature subunits p50$^{\alpha}$, p85$^{\beta}$, and p145$^{\beta}$ were resistant to endo H digestion (*right panel*). The appearance of partially endo H-resistant forms of both Pr170 and Pr120 at 4 h of chase in BFA-treated cells was due to BFA-induced relocation of Golgi enzymes to the ER.

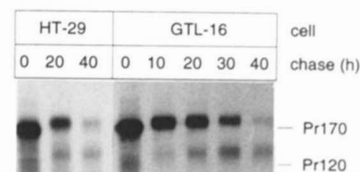

FIG. 5. **Pr170 is chased into Pr120.** HT-29 and GTL-16 cells, pretreated with BFA for 1 h, were pulse-labeled with [^{35}S]methionine for 4 h and chased for the indicated periods of time in the presence of BFA. Cells were lysed, immunoprecipitated with *α-extra* mAbs, and analyzed in 8% SDS-PAGE under reducing conditions. Relocation of *cis*/medial Golgi enzymes to ER, induced by long-term BFA treatment, resulted in terminal glycosylation. This was detectable by a retarded SDS-PAGE migration of both Pr170 and Pr120. Gels were exposed to autoradiography for different periods of time, 2 days (HT29) and 12 h (GTL-16).

of time in the presence of BFA. Fig. 5 shows that Pr170 was highly stable and slowly degraded with a half-life of 25 h. The reduction of Pr170 was accompanied by the subsequent increase in the amount of Pr120. This result indicates that Pr120 originates from Pr170 by a proteolytic process that eliminates a cytoplasmic domain of approximately 50 kDa. Such a fragment was never observed either in control or in BFA-treated cells, suggesting that it has an extremely short half-life (Fig. 6).

The observed increase in molecular weight of the precursors in cells treated with BFA is explained by the BFA-induced relocation into the ER of the glycosyl transferarases that are compartmentalized within the Golgi apparatus in physiological conditions (Lippincott-Schwartz *et al.*, 1990).

The proteolytic cleavage generating the mature $\alpha\beta$ heterodimer receptors (p190^{MET} and p140^{MET}) did not occur in BFA-treated cells, showing that the specific protease is compartmentalized in a most trans-Golgi that does not relocate to the ER in response to BFA (Chege and Pfeffer, 1990; Reaves and Banting, 1992).

Generation of Pr120 Correlates with Tyr Phosphorylation of Pr170—The amount of detectable Pr120 correlates with ac-

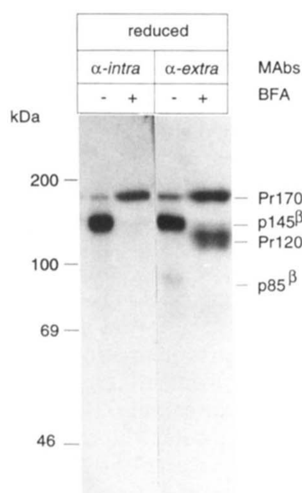

FIG. 6. **The cytoplasmic fragment released from Pr170 is highly unstable.** GTL-16 cells were incubated in the presence (+) or absence (−) of BFA for 36 h, solubilized, and analyzed in Western blots probed with *α-intra* or *α-extra* mAbs under reducing conditions. The cytoplasmic domain of approximately 50 kDa generated after truncation of Pr170 to Pr120 was undetectable.

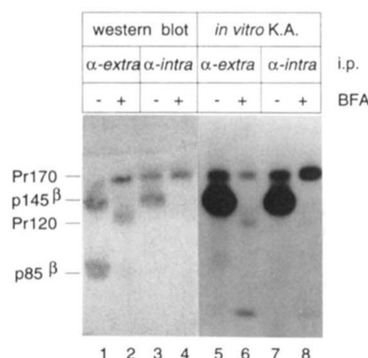

FIG. 7. **Kinase activity of Pr170 *in vitro*.** GTL-16 cells were incubated for 40 h in the presence (+) or in the absence (−) of BFA. The relative amounts of Pr170 and Pr120 generated after BFA treatment were monitored by immunoprecipitation (*i.p.*) with *α-extra* or *α-intra* mAbs, followed by Western blot with *α-extra* mAbs under reducing conditions (*left panel*). Long-term BFA treatment caused accumulation of both Pr170 and Pr120. It should be noted that after 40 h the mature $p145^{\beta}$ completed its turnover and was not renewed in the presence of BFA. The kinase activity of Pr170 was assayed *in vitro* (*K.A.*) by incubation of immunocomplexes with [γ-^{32}P]ATP, as described under "Materials and Methods." Labeled proteins were separated in SDS-PAGE under reducing conditions (*right panel*). Pr170 immunoprecipitated from BFA-treated cells (+) did autophosphorylate in the absence of any detectable mature $p145^{\beta}$.

cumulation of Pr170 in the ER and with the concomitant inappropriate activation of Pr170 kinase. It is well known that overexpression of tyrosine kinase receptors may lead to their activation in the absence of the ligand (Ullrich and Schlessinger, 1990). In GTL-16 cells, where the *MET* gene is overexpressed, the $p190^{MET}$ kinase is activated at the cell surface. The uncleaved Pr170 has intrinsic kinase activity, as shown in a kinase assay *in vitro* (Fig. 7, *lane 8*). *In vivo*, following BFA treatment, Pr170 accumulated in the ER (Fig. 8, *lane 2*) and autophosphorylated in tyrosine as shown in Western blots probed with anti-phosphotyrosine antibodies (Fig. 8, *lane 4*). The appearance of Pr120 correlated with the amount of Pr170 that accumulated in the ER (Figs. 7 and 9). The overall amount of Pr170, detectable in Western blots, reached its maximum 20 h after BFA treatment; autophosphorylation followed the same kinetics. Pr120 appeared after

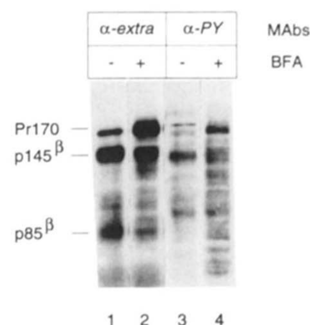

FIG. 8. **Pr170 is tyrosine-phosphorylated *in vivo*.** GTL-16 cells were incubated in the presence (+) or absence (−) of BFA for 24 h, solubilized, and analyzed in Western blots probed with either *α-extra* mAbs or anti-phosphotyrosine (*α-PY*) antibodies. $P145^{\beta}$ is constitutively tyrosine-phosphorylated in these cells. Tyrosine phosphorylation of Pr170 correlated with its accumulation in the ER.

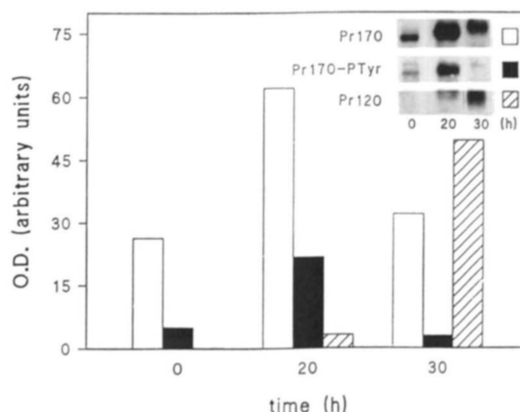

FIG. 9. **Generation of Pr120 follows tyrosine phosphorylation of Pr170.** GTL-16 cells were incubated with BFA for the indicated periods of time, solubilized, and analyzed in Western blots. The amount of Pr170 (□) and Pr120 (▨) was estimated by optical densitometry (*O.D.*) of the bands revealed with *α-extra* mAbs. The extent of tyrosine phosphorylation of Pr170 (■) was estimated by the intensity of the corresponding electrophoretic bands probed with anti-phosphotyrosine mAbs.

a delay, reaching its maximum after 30 h. At that time the autophosphorylation of Pr170 was strongly decreased, suggesting a dominant negative inhibitory effect of Pr120 (Fig. 9).

NIH 3T3 cells transfected with a kinase negative mutant HGF receptor ($Lys^{1110} \rightarrow Ala$) did not express the truncated $p85^{\beta}$, as shown in Western blots probed with *α-extra* mAbs (Fig. 10). The mutant receptor was unable to undergo autophosphorylation (not shown). Thus, tyrosine phosphorylation in the cytoplasmic domain of the receptor is likely to contribute substrate signals for proteolytic generation of the truncated isoform.

DISCUSSION

Tyrosine kinase receptors lacking the cytoplasmic catalytic domain may be generated either by alternative splicing or by post-translational proteolysis (Downing *et al.*, 1989; Klein *et al.*, 1990; Middlemas *et al.*, 1991; Weber and Gill, 1984; Zabrescky *et al.*, 1991). The two previously described C-terminal truncated isoforms of the HGF receptor ($p130^{MET}$ and $p140^{MET}$) originate through proteolytic processing of a common precursor (Pr170). The soluble $p130^{MET}$ is released in the culture medium by proteolytic cleavage at the cell surface (Prat *et al.*, 1991b). We now show that the membrane-bound $p140^{MET}$ originates from the processing of an intermediate

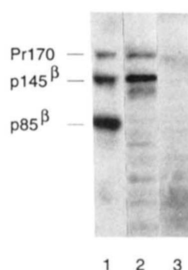

FIG. 10. **The truncated isoform is not detectable in NIH 3T3 cells expressing a kinase-defective receptor.** NIH 3T3 cells transfected either with wild-type (*lane 1*) or with the Ala^{1110} mutant receptor (*lane 2*) and untransfected NIH 3T3 cells (*lane 3*) were solubilized and analyzed in Western blots probed with *α-extra* mAbs under reducing conditions. In cells expressing the mutant, kinase defective receptor, the truncated $p85^{\beta}$ was undetectable.

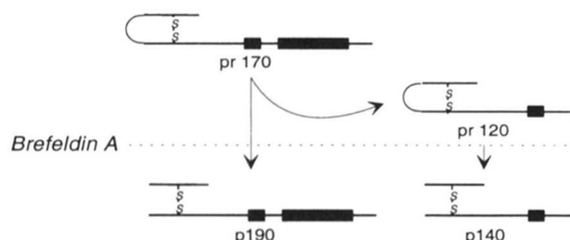

FIG. 11. **Schematic representation of the post-translational processing of the HGF receptor precursor (Pr170).** A fraction of Pr170 is cleaved in the ER, generating an intermediate precursor of 120 kDa (Pr120). Both precursors undergo the same maturation processing in the Golgi compartment producing the heterodimeric $p190^{MET}$ and $p140^{MET}$, respectively. Brefeldin A inhibits the export from the ER and conversion to the mature forms. *Dashed rectangles* indicate the transmembrane domain; *black rectangles* indicate the cytoplasmic tyrosine kinase domain.

precursor of 120 kDA (Pr120). The latter is produced in the ER by a proteolytic event affecting the Pr170 precursor. Pr120 and Pr170 undergo the same maturation process in the Golgi compartment, generating $p140^{MET}$ and $p190^{MET}$ respectively, both exposed at the cell surface (Fig. 11).

The primary nonglycosylated translation product of the *MET* mRNA is a 150-kDa polypeptide that is co-translationally glycosylated to yield Pr170 (Giordano *et al.*, 1989b). Pulse-chase experiments indicated that Pr120 originates from Pr170 by proteolytic removal of the tyrosine kinase domain exposed at the cytoplasmic side of the ER membrane. The expected 50-kDa polypeptide was undetectable, suggesting that it is very unstable. The following data show that Pr120 is generated in the ER. 1) It is detectable after 15 min of pulse; 2) it contains immature high mannose-branched oligosaccharide moieties; 3) it accumulates upon cell treatments inhibiting the export of newly synthesized proteins from the ER.

The fate of Pr120 is the same as that of Pr170; it is translocated from the ER to the Golgi complex, where it matures to the $p140^{MET}$ through the same post-translational processing that generates the intact $p190^{MET}$ from Pr170. Maturation of both precursors requires further glycosylation, followed by proteolytic cleavage giving rise to the αβ dimeric structure. These processes occur in a post-ER compartment. Proteolytic cleavage generating the mature heterodimeric αβ structure was completely blocked by long-term BFA treatment and can thus be localized in the trans-Golgi network, the only Golgi compartment that does not relocate to the ER, in response to BFA (Chege and Pfeffer, 1990; Reaves and Banting, 1992). A canonical consensus sequence of dibasic residues between Lys^{303} and Ser^{308} (Chan *et al.*, 1988; Mark *et al.*, 1993) indicates that the protease responsible for the αβ cleavage of Pr120 and Pr170 belongs to the furin/PACE family (reviewed in Barr (1991)).

The proteolytic process leading to generation of Pr120 remains to be determined. It is known that membrane proteins failing to fold correctly or to assemble into oligomeric complexes undergo rapid degradation in the ER (Amara *et al.*, 1989; Bonifacino *et al.*, 1989; Chen *et al.*, 1988; Lippincott-Schwartz, 1988; Wileman *et al.*, 1990). Pr120 is more abundant in cells overexpressing the *MET* gene; the normal folding of the HGF receptor in these cells may be impaired, and abnormal conformation may trigger the degradation of the cytoplasmic portion of the precursor. However, improper folding does not seem to play a major role, because production of Pr120 is not increased in conditions of artificial stress such as high temperature (data not shown). In most cases degradation within the ER affects the luminal portion of the transmembrane proteins; in the case of Pr170, a protease(s) destroys a protein domain exposed at the cytoplasmic side of the ER membrane. A similar fate occurs to the transmembrane protein hydroxymethylglutaryl-CoA reductase (Gil *et al.*, 1985). Both Pr170 and hydroxymethylglutaryl-CoA reductase contain "PEST" sequences in their cytoplasmic domains; in the HGF receptor precursor this sequence lies between Ser^{988} and Tyr^{1003}, near the boundary between the cytoplasmic and the transmembrane domain. PEST sequences are common to short-lived proteins and confer susceptibility to intracellular proteolysis (Loetscher *et al.*, 1991; Rogers *et al.*, 1986). The HGF receptor, as well as a number of other proteins containing PEST sequences, has a long half-life. In these cases, critical events are required to trigger proteolytic degradation; these include sequence unmasking or phosphorylation of sites nearby or within the PEST sequence (Rogers *et al.*, 1986).

The mechanism(s) triggering the cleavage of the cytoplasmic portion of Pr170 is unclear. Pr170 has potential intrinsic kinase activity, as shown by ligand-independent phosphorylation in baculovirus-infected insect cells and in simian fibroblasts overproducing recombinant HGF receptor (Bardelli *et al.*, 1992). In this work we show that accumulation of Pr170 in the ER results in its phosphorylation, which is followed by the appearance of Pr120. This suggests that inappropriate phosphorylation of Pr170 in intracellular compartments may activate proteolytic degradation, thus providing a safety mechanism aimed at preventing the effects of ligand-independent activation of the kinase. In agreement with this hypothesis is the lack of generation of the truncated isoform in cells expressing a kinase-defective HGF receptor. This safety mechanism has two effects; it eliminates the activated cytoplasmic kinase domain and generates a truncated transmembrane molecule that may function as a dominant negative inhibitor of the intact precursor by forming inactive heterodimers (Basu *et al.*, 1989; Kashles *et al.*, 1991; Ueno *et al.*, 1991). In physiological conditions this process operates in the background, whenever an aliquot of Pr170 forms clusters during its transit in the ER. This event raises the threshold of detection and is manifest in cells overproducing Pr170, as in the case of cells bearing an amplified or a somehow deregulated *MET* oncogene.

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