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Friend spleen focus-forming virus glycoprotein gp55 interacts with the erythropoietin receptor in the endoplasmic reticulum and affects receptor metabolism

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ABSTRACT **The Friend spleen focus-forming virus envelope glycoprotein, gp55, binds to the murine erythropoietin receptor (EPO-R) and triggers growth activation in the absence of EPO. Interleukin 3-dependent lymphoid cell lines that have been stably transfected with the EPO-R cDNA grow in the presence of EPO or interleukin 3. In these cells, the EPO-R is synthesized as a minor 62-kDa unglycosylated form and a major 64-kDa form carrying one high-mannose N-linked oligosaccharide. A fraction of the 64-kDa form is processed to a 66-kDa species with complex-type sugars. Very little of the EPO-R is expressed on the cell surface and all three forms of EPO-R are degraded rapidly. Cells transfected with both EPO-R and gp55 cDNAs grow in the absence of EPO. Most of the EPO-R associated with gp55 is endoglycosidase H-sensitive, suggesting that the interactions between these proteins occur in the endoplasmic reticulum. Furthermore, the endoglycosidase H-sensitive EPO-R is more stable than in the absence of gp55, a result suggesting that interaction of gp55 with the EPO-R causes it to remain within the rough endoplasmic reticulum. It is possible that gp55 EPO-R complexes within this compartment send a growth-promoting signal to the cell.**

The Friend spleen focus-forming virus (SFFV) is a defective murine leukemia virus that causes an acute erythroleukemia in mice and erythroblastosis in bone marrow cultures (1–5). The pathological properties of SFFV are caused by the *env* gene, which encodes a 55-kDa glycoprotein, gp55 (6–8). One hypothesis for the mechanism by which gp55 causes increased erythroblastosis and erythroleukemia is that gp55 mimics the action of erythropoietin (EPO) by binding to the EPO receptor (EPO-R), thereby causing proliferation of erythroid cells. Recently, the cDNA encoding the murine EPO-R was cloned (9), and binding of the EPO-R to gp55 was demonstrated (10). Also, an interleukin 3 (IL-3)-dependent cell line can grow in the presence of EPO following cDNA-directed expression of the EPO-R. Coexpression of EPO-R and gp55 allows factor-independent growth of this cell line (10, 11). This suggests that an interaction of gp55 with the EPO-R stimulates the receptor and bypasses the normal requirement for EPO, causing prolonged proliferation of erythroid cells. Ruscetti *et al.* (12) showed that in an EPO-dependent erythroleukemia cell line, SFFV induces factor independence and also a significant decrease in the number of cell surface EPO binding sites.

The SFFV gp55 glycoprotein is transported inefficiently to the cell surface; only 3–5% is expressed on the cell surface in a processed form designated gp65 and the remainder remains in the rough endoplasmic reticulum (ER) (13). These findings raise many important questions. Does the interaction of gp55 with EPO-R occur on the cell surface or inside the cell? Does gp55 affect the biosynthesis and intracellular transport of the EPO-R?

Here we show that interactions of gp55 with the EPO-R occur inside the cell, probably in the ER, and that gp55 causes prolonged retention of newly made EPO-R in this compartment. Our data suggest that the complex of the EPO-R with gp55 can generate a growth signal from within the cell.

MATERIALS AND METHODS

Cells. NIH mouse 3T3 fibroblasts were grown in Dulbecco's modified minimal essential medium (DMEM) supplemented with 10% calf serum. Normal Ba/F3 cells (an IL-3-dependent line derived from mouse bone marrow) were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum and 10% conditioned medium from the WEHI-3B myelomonocytic leukemia cell line (14).

Transfectants. The full-length EPO-R cDNA, cloned in the mammalian expression vector pXM (9), was transfected by electroporation into Ba/F3 cells and clones were isolated in medium supplemented with EPO at 0.5 unit/ml (50 pM). A cloned Ba/F3 cell line transfected with the pSFF vector containing EPO-R cDNA and a cloned double transfectant expressing EPO-R and SFFV gp55 were also generated as described (10). The NIH 3T3 EPO-R transfectant was isolated by cotransfection with pSV2-neo and pXM-EPO-R and subsequent screening for G418 resistance and cloning. A cloned NIH 3T3 transfectant expressing EPO-R was designated 3T3-EPO-R, and Ba/F3 cell lines transfected with pXM-EPO-R and pSFF-EPO-R were designated Ba/F3-EPO-R-1 and Ba/F3-EPO-R-2, respectively. The Ba/F3 cell line expressing both EPO-R and SFFV gp55 was designated Ba/F3-EPO-R-gp55. Ba/F3-EPO-R-1 and Ba/F3-EPO-R-2 cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum and containing EPO at 0.1 unit/ml, and Ba/F3-EPO-R-gp55 cells were maintained in normal RPMI-1640 supplemented with 10% fetal bovine serum. Before use in experiments, Ba/F3-EPO-R cells were cultured in 10% WEHI-3B conditioned medium for 2–3 days.

Metabolic Labeling and Immunoadsorption. Metabolic labeling of cells with [^{35}S]methionine and immunoadsorption were performed as described (10, 15). Cell extracts in TBS (10 mM Tris-buffered isotonic saline, pH 7.5) containing 1% (vol/vol) Triton X-100 were mixed with rabbit antiserum against the NH_2-terminal 14-amino acid peptide (Ala-Pro-Ser-Pro-Ser-Leu-Pro-Asp-Pro-Lys-Phe-Glu-Ser-Lys-Cys) of the EPO-R (anti-N) (10) or against the COOH-terminal 16-amino acid peptide (Ser-Leu-Val-Pro-Asp-Ser-Glu-Pro-Leu-His-Pro-Gly-Tyr-Val-Ala-Cys) of the EPO-R (anti-C) or with goat anti-Rauscher murine leukemia virus

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Abbreviations: endo H, endoglycosidase H; EPO, erythropoietin; EPO-R, EPO receptor; ER, endoplasmic reticulum; IL, interleukin; SFFV, spleen focus-forming virus.

gp70 antiserum (anti-gp70) (10), which cross-reacts with gp55. Immunocomplexes with protein A-agarose (Bio-Rad) were washed five times with TBS containing 1% Triton X-100 and 0.1% sodium dodecyl sulfate (SDS), and subjected to SDS/8.5% polyacrylamide gel electrophoresis (PAGE).

For double immunoadsorption, the antigens adsorbed with anti-gp70 were eluted from protein A-agarose and denatured by boiling for 5 min in 50 μl of TBS containing 1% SDS. Samples were diluted 5-fold in TBS containing 1% Triton X-100, 0.5% sodium deoxycholate, and 0.5% bovine serum albumin and then were subjected to reimmunoadsorption with anti-N.

Treatment of Cells with Proteinase K and Preparation of Membranes. After two washes with phosphate-buffered saline, cells (5×10^7) were incubated with proteinase K (1.0 mg/ml) in 15 ml of Hanks' balanced salt solution for 60 min at 4°C. After the cells were extensively washed with RPMI-1640 medium containing 1 mM phenylmethylsulfonyl fluoride, crude cell membranes were prepared (16). Radioiodinated EPO was prepared (11) and binding assays for ^{125}I-EPO were performed (17) as described.

Endoglycosidase H (Endo H) Digestion. Digestion of immunoadsorbed proteins and extracts of cell membranes was carried out as described (18).

Immunoblotting. SDS/PAGE of membrane samples, transfer to nitrocellulose paper, and immunoblotting were described previously (19). ^{125}I-labeled goat anti-rabbit IgG (ICN) at 0.5 μCi/ml (1 μCi = 37 kBq) was used as probe, and antigen was detected with XAR film and an intensifying screen at −70°C.

Cell Proliferation Assay. The colorimetric assay for cell proliferation was processed as described (20) with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma).

RESULTS

Expression of EPO-R in Ba/F3 Transfectants. We generated two types of Ba/F3 transfectants that express the EPO-R; different expression vectors, pXM and pSFF, were used. These transfectants can grow in medium containing EPO or in WEHI-3B-conditioned medium, which contains IL-3. Although the levels of expression of EPO-R protein in the two cell lines were different (see Fig. 3), the EPO dose-dependence curves for their growth were similar (Fig. 1) and also similar to that of mouse EPO-dependent cell lines or of splenic erythroblasts obtained from mice infected with the anemia-inducing strain of Friend virus (12, 21). The Ba/F3 transfectant cell line that expressed both EPO-R and gp55 (Ba/F3-EPO-R-gp55) grew in normal RPMI medium without IL-3 or EPO (Fig. 1).

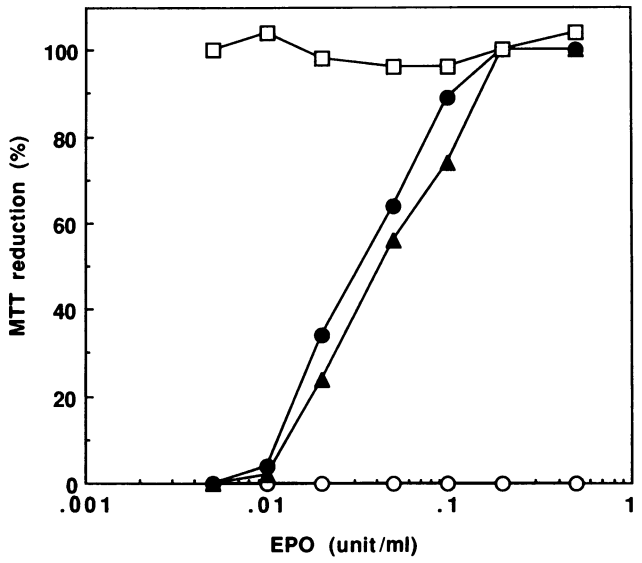

FIG. 1. EPO-dose dependence of cell proliferation. Cells (1000 per well) were cultured for 3 days with medium containing the indicated concentrations of EPO or with 10% WEHI-3B-conditioned medium. The number of viable cells was then measured with a colorimetric assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Measurements were normalized by dividing the optical density at a given EPO concentration by the density obtained in 10% WEHI-3B-conditioned medium. ○, Ba/F3; ●, Ba/F3-EPO-R-1; ▲, Ba/F3-EPO-R-2; □, Ba/F3-EPO-R-gp55.

Expression of EPO-R and gp55 was analyzed by immunoadsorption with specific antibodies following a 60-min incubation with [^{35}S]methionine (Fig. 2). By immunoadsorption with anit-N (specific for the NH_2 terminus of the EPO-R), a major 64-kDa species and a minor 62-kDa species were observed in all transfected cells (3T3-EPO-R, Ba/F3-EPO-R, and Ba/F3-EPO-R-gp55; Fig. 2*A*, lanes 2, 4, and 5) but was not found in nontransfected cells (lanes 1 and 3). These two species were also detected by anti-C (Fig. 2*A*, lanes 6–10), specific for the COOH terminus of the EPO-R. In Ba/F3-EPO-R cells, a diffuse 66-kDa band was also detected (Fig. 2*A*, lane 4, and Fig. 2*B*, lane 2). Also immunoadsorbed by anti-N from Ba/F3-EPO-R-gp55 cells was a 55-kDa protein, the same molecular size as SFFV gp55 (Fig. 2*B*, lane 5). With anti-gp70, several proteins of ≈70 kDa were immunoprecipitated from Ba/F3-EPO-R cells (Fig. 2*B*, lane 1); these

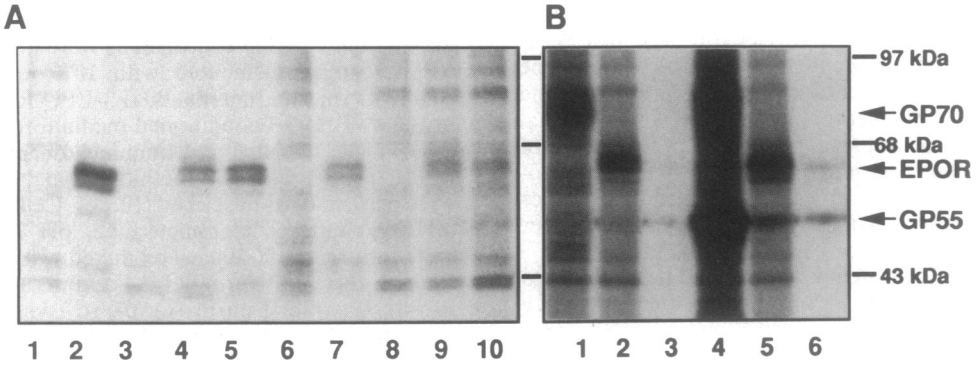

FIG. 2. Expression of EPO-R and gp55. Cells (5×10^6 per sample) were metabolically labeled with 0.2 mCi of [^{35}S]methionine for 60 min. Immunoadsorption was with anti-N (*A* lanes 1–5 and *B* lanes 2 and 5), anti-C (*A* lanes 6–10), or anti-gp70 (*B* lanes 1 and 4). For lanes 3 and 6 of *B* immunoprecipitates with anti-gp70 were reprecipitated with anti-N. Immunocomplexes were subjected to SDS/8.5% PAGE and fluorography. (*A*) NIH 3T3 (lanes 1 and 6), 3T3-EPO-R (lanes 2 and 7), Ba/F3 (lanes 3 and 8), Ba/F3-EPO-R-1 (lanes 4 and 9), and Ba/F3-EPO-R-gp55 (lanes 5 and 10) cells. (*B*) Ba/F3-EPO-R-1 (lanes 1–3) and Ba/F3-EPO-R-gp55 (lanes 4–6) cells. Molecular size markers and the positions for gp70-related protein, gp55, and EPO-R are indicated.

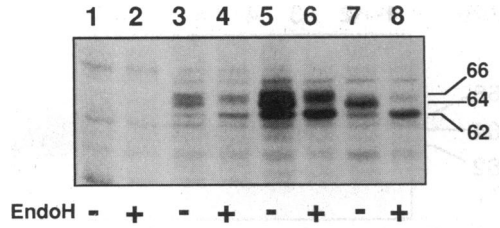

FIG. 3. Endo H digestion of EPO-R. Ba/F3 (lanes 1 and 2), Ba/F3-EPO-R-1 (lanes 3 and 4), Ba/F3-EPO-R-2 (lanes 5 and 6), and Ba/F3-EPO-R-gp55 (lanes 7 and 8) cells were metabolically labeled with [^{35}S]methionine for 60 min. Materials immunoadsorbed with anti-N were treated with (+) or without (−) endo H and subjected to SDS/8.5% PAGE and fluorography. Positions of the endo H-resistant 66-kDa form, endo H-sensitive 64-kDa form, and unglycosylated 62-kDa form are indicated.

were probably products of endogenous retroviruses. However, only Ba/F3-EPO-R-gp55 cells produced large amounts of gp55 (Fig. 2*B*, compare lanes 1 and 4), and only these cells produced gp55 that coprecipitated with the EPO-R (lane 5).

Materials immunoadsorbed with anti-gp70 from Ba/F3-EPO-R-gp55 cells contained 62-kDa and 64-kDa proteins, seen on a lower exposure of lane 4 of Fig. 2*B*. To show that these two proteins were EPO-R, we carried out double immunoadsorptions. Proteins immunoreacted with anti-gp70 were solubilized by boiling with SDS and then reprecipitated with anti-N (Fig. 2*B*, lane 6). A major 64-kDa protein and a minor 62-kDa protein were observed, indicating that these two EPO-R polypeptides were associated with the gp55 protein. These species were not seen in cells expressing only EPO-R (Fig. 2*B*, lane 3), showing that the EPO-R is bound to gp55, not to the endogenous gp70 expressed in both cells (Fig. 2*B*, lanes 1 and 4).

Biosynthesis and Processing of EPO-R in Transfected Cells. To determine the type of glycosylation on each form of the EPO-R, endo H digestion was carried out (Fig. 3). In both Ba/F3-EPO-R and Ba/F3-EPO-R-gp55 cells, endo H converted a major 64-kDa form of EPO-R to a 62-kDa form (lanes 4, 6, and 8). Since the predicted EPO-R polypeptide sequence has only one potential N-glycosylation site (9), this suggests that the 64-kDa form has one high-mannose-type N-linked oligosaccharide. Tunicamycin-treated cells produced only the 62-kDa form (data not shown), suggesting that the 62-kDa polypeptide was an unglycosylated form of the EPO-R. The 66-kDa diffuse band in Ba/F3-EPO-R cells was endo H-resistant (lanes 4 and 6) and neuraminidase-sensitive (data not shown), indicating that it contained one complex-type oligosaccharide, and thus that the protein had matured through the trans-Golgi compartment.

Very little endo H-resistant EPO-R was seen in Ba/F3-EPO-R-gp55 cells (Fig. 3, lane 8); after a 1-hr labeling, Ba/F3-EPO-R cells produced much more endo H-resistant (mature) EPO-R than Ba/F3-EPO-R-gp55 cells. This was confirmed in other Ba/F3-EPO-R or Ba/F3-EPO-R-gp55 clones, which expressed different levels of EPO-R (data not shown), and with immunoblotting of membranes from each cell line (see Fig. 7).

We next studied the processing and half-life of EPO-R in 3T3-EPO-R, Ba/F3-EPO-R, and Ba/F3-EPO-R-gp55 cells. Cells were labeled for 30 min with [^{35}S]methionine and "chased" by incubation with nonradioactive methionine for 0–4 hr (Fig. 4). In all cells, the EPO-R was synthesized as major 64-kDa and minor 62-kDa forms. After a 30- to 60-min chase, a mature, endo H-resistant 66-kDa form was seen in Ba/F3-EPO-R cells; in 3T3-EPO-R cells, an endo H-resistant 64- to 66-kDa form was also observed. In contrast, in Ba/F3-EPO-R-gp55 cells very little endo H-resistant mature 66-kDa form was observed at any chase time.

In Ba/F3-EPO-R cells, all three forms of the EPO-R were degraded very quickly; the half-life, estimated by densitometric scanning, was about 70 min. This rate of degradation was not affected by the presence of EPO (data not shown). The EPO-R synthesized in the 3T3 fibroblast transfectant also had a short half-life, about 45 min. In Ba/F3-EPO-R-gp55 cells, in contrast, the half-life of EPO-R was about 120 min. The difference in half-life of EPO-R in Ba/F3-EPO-R and Ba/F3-EPO-R-gp55 cells was most apparent after a 2-hr chase (Fig. 4).

EPO-R Is Associated with gp55 in the ER. To determine which form of EPO-R associates with gp55 in Ba/F3-EPO-R-gp55 cells, [^{35}S]methionine-labeled cell extracts were immunoadsorbed with anti-gp70 or anti-N and digested with endo H. The EPO-R species coprecipitated with gp55 were the glycosylated, endo H-sensitive 64-kDa form and the unglycosylated minor 62-kDa form (Fig. 5, lanes 1 and 2). The gp55 coprecipitated with EPO-R by the anti-N antibody was also endo H-sensitive (lanes 3 and 4). Anti-gp70 immunocomplexes were reprecipitated with anti-N; this showed directly that the EPO-R polypeptides associated with gp55

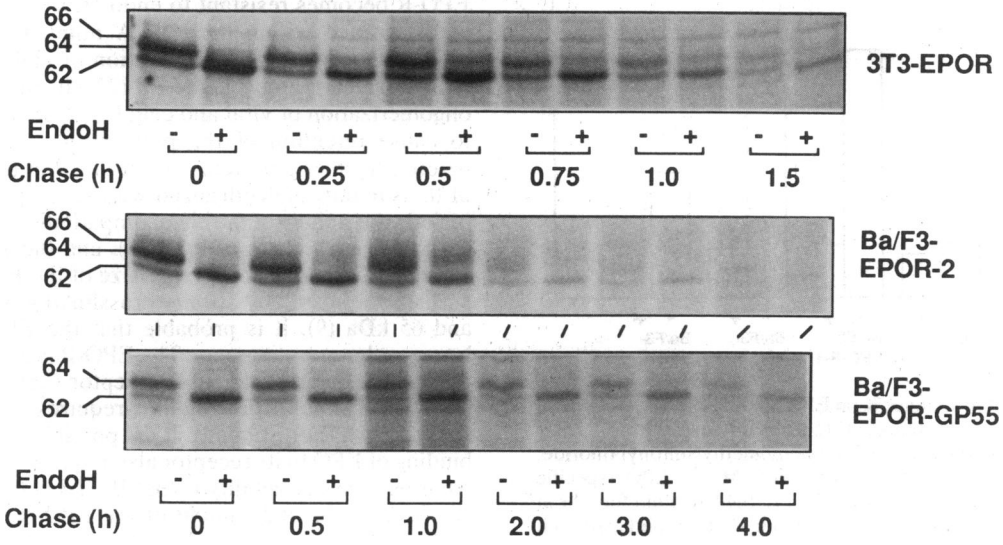

FIG. 4. Processing and degradation of EPO-R. 3T3-EPO-R, Ba/F3-EPO-R-2, and Ba/F3-EPO-R-gp55 cells were pulse labeled with [^{35}S]methionine for 30 min and then chased in 10% WEHI-3B-conditioned medium for the indicated periods (h, hours). Immunocomplexes were incubated with (+) or without (−) endo H and subjected to SDS/8.5% PAGE and fluorography.

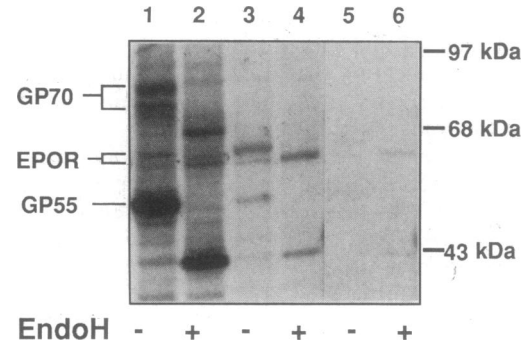

FIG. 5. Endo H digestion of EPO-R and gp55 from Ba/F3-EPO-R-gp55 cells. Ba/F3-EPO-R-gp55 cells were labeled with [^{35}S]methionine for 60 min and detergent-solubilized proteins were immunoadsorbed with anti-gp70 (lanes 1 and 2) or anti-N (lane 3 and 4). Double immunoadsorption was with anti-gp70 and then anti-N (lanes 5 and 6). Immunocomplexes were digested with (+) or without (−) endo H and subjected to SDS/8.5% PAGE and fluorography.

were mainly the endo H-sensitive 64-kDa and the unglycosylated 62-kDa species (lanes 5 and 6). These data are consistent with the hypothesis that the interaction of EPO-R with gp55 occurs mainly in the ER.

Cell Localization of EPO-R. We next measured binding of EPO to the surface of Ba/F3 transfectant cells (Fig. 6). Approximately 500 molecules of EPO were specifically bound to each Ba/F3-EPO-R cell when the cells were incubated with 500 pM ^{125}I-labeled EPO. Binding of EPO to the two Ba/F3-EPO-R cell lines was similar, although the expression levels of EPO-R polypeptide were different (Fig. 3). Specific binding of EPO to Ba/F3-EPO-R-gp55 cells was less than half that to Ba/F3-EPO-R cells. The EPO-binding activity of Ba/F3 transfectants was comparable to that of MEL erythroleukemia cells (9), even though the transfected cells produced a much higher amount of EPO-R polypeptide than the MEL cells (data not shown). Under our conditions, cell surface binding activity for EPO was completely abolished by proteinase K digestion (Fig. 6). No degradation of ^{125}I-EPO itself was observed after incubation with protease-treated or untreated cells (data not shown). To determine whether any of the forms of EPO-R were predominantly on the cell surface, immunoblotting of proteinase K-digested cells was carried out. Immunoblotting of membrane extracts

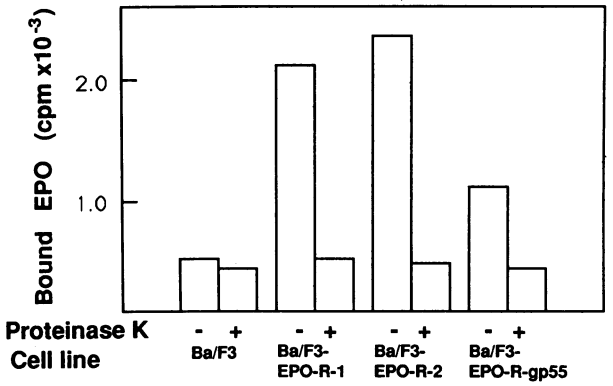

FIG. 6. Effect of proteinase K on EPO binding. Cells were treated with (+) or without (−) proteinase K (1.0 mg/ml) for 1 hr at 4°C. After extensive washing in the presence of phenylmethylsulfonyl fluoride, 2.5 × 10^6 cells were incubated with 500 pM ^{125}I-EPO (specific activity, 1000 cpm/fmol) in 100 μl of RPMI-1640 medium for 4 hr at 4°C. After sedimentation of cells through dibutyl phthalate oil, cell-associated radioactivity was measured. Nonspecific binding (^{125}I-EPO binding in the presence of 10 nM native EPO) was about 500 cpm for all cell lines, which was similar to the level of EPO bound to Ba/F3 cells and to all proteinase K-treated cells.

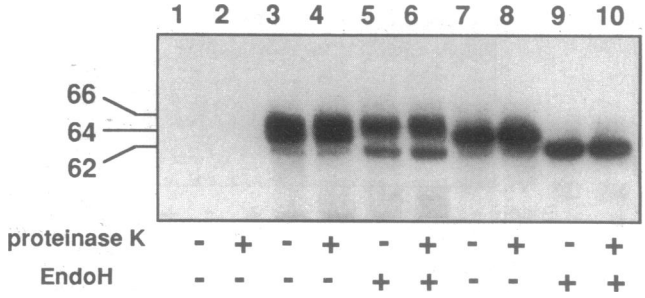

FIG. 7. Effect of proteinase K on EPO-R. Ba/F3 (lanes 1 and 2), Ba/F3-EPO-R-2 (lanes 3–6), and Ba/F3-EPO-R-gp55 (lanes 7–10) cells were treated with (+) or without (−) proteinase K. Membrane fractions from cells were treated with (+) or without (−) Endo H and subjected to SDS/7.5% PAGE and immunoblotting with anti-N.

with anti-N showed that most of each of the three forms of EPO-R was proteinase K-resistant (Fig. 7). These data suggest that very little EPO-R, even the mature endo H-resistant 66-kDa species, is expressed on the cell surface in Ba/F3 transfectants.

DISCUSSION

We have elucidated several surprising aspects of the biosynthesis of the EPO-R and of its interaction with the SFFV gp55 glycoprotein. In Ba/F3 transfected cells expressing only the EPO-R, the EPO-R is synthesized as a 64-kDa precursor with a high-mannose, endo H-sensitive oligosaccharide and as a 62-kDa unglycosylated species. Within 60 min about 25% of the polypeptide has matured to a 66-kDa species with an endo H-resistant oligosaccharide. Within an additional 60 min most of the EPO-R has been degraded. Furthermore, most of the cellular content of the 66-kDa species is not on the cell surface; presumably it is either en route from the trans-Golgi compartment to the plasma membrane or to a lysosome or has been endocytosed from the cell surface and is en route to a degradative organelle, possibly a lysosome. As a result, these transfectants have on their cell surface only about 1000 EPO binding sites, even though the mRNA encoding the EPO-R is highly expressed. At least some of the surface EPO-R molecules on these transfected cells must be functional, since the cells grow in the presence of EPO.

In Ba/F3-EPO-R cells, only a portion of newly made EPO-R becomes resistant to endo H, while most is retained and degraded within the ER. A number of reports show selective retention and degradation of unfolded, misfolded, or aggregated proteins in the ER (22). In particular, defective oligomerization of viral and cellular glycoproteins is thought to cause retention of the polypeptides in the ER. Some hetero-oligomeric proteins are retained in the ER when one of the subunits is synthesized without its partner; examples include the γ chain of the histocompatibility antigen HLA-DR (23), the heavy chain of IgM (24), and the T-cell receptor α chain (25). Since the molecular size of the EPO-R on the cell surface, estimated by affinity crosslinking, is about 105 kDa and 65 kDa (9), it is probable that the EPO-R consists of homo- or hetero-oligomers. The EPO-R is highly homologous to the β subunit of the IL-2 receptor (26) and high-affinity binding of IL-2 to this subunit requires the presence of a second, α (Tac) subunit; it is possible that high-affinity binding of EPO to its receptor also requires a second subunit. An interesting possibility is that Ba/F3 cells express at very low levels a second subunit of the EPO-R, causing the cell surface expression of the EPO-R to be very low.

Metabolism of the EPO-R is profoundly affected by coexpression of SFFV gp55: extremely little EPO-R matures to an endo H-resistant form, and the ER (i.e., endo H-sensitive)

form becomes more stable. The interaction of EPO-R and gp55 occurs within the cell, most likely in the rough ER, since both polypeptides in the complex bear endo H-sensitive oligosaccharides (Fig. 5). This is not surprising, because 95–97% of SFFV gp55 is retained in the ER (11), and most of the gp55 in Ba/F3-EPO-R-gp55 cells is also endo H-sensitive. Our data raise the possibility that signal(s) for growth might be produced inside the cell. Signal generation from within the cell has been reported in a novel IL-3 autocrine system in which IL-3 has the Lys-Asp-Glu-Leu ER retention signal (27) and in transformation of fibroblasts by the v-*sis* oncogene, a homolog of the platelet-derived growth factor B-chain gene (28).

Ruscetti *et al.* (12) reported that SFFV infection of an EPO-dependent cell line causes marked reduction in the number of cell surface EPO binding sites. We observed that there are fewer cell surface EPO binding sites in Ba/F3-EPO-R-gp55 cells than in Ba/F3-EPO-R cells (Fig. 6). Expression of gp55 causes a reduction in the amount of the endo H-resistant form of the EPO-R, presumably due to retention of EPO-R within the ER. This results in a decrease of cell surface expression of EPO-R. This would be analogous to the interaction of adenovirus E19 protein with murine class I (H-2) or human (HLA) major histocompatibility molecules, which anchors them in the ER, also resulting in low cell surface expression (29, 30). Stabilization of EPO-R by association with gp55 may be advantageous in producing and maintaining signal(s) for cell growth.

Expression of gp55, in the absence of the EPO-R, has no effect on the growth of the IL-3-requiring Ba/F3 cells (10), even though the IL-3 receptor is homologous to the EPO-R (31). This is consistent with evidence that SFFV affects proliferation of erythroid cells only and that gp55 binds to no receptor other than the EPO-R. The strain of SFFV gp55 used in our studies causes polycythemia and EPO-independent proliferation of erythroid cells. Other strains of SFFV cause anemia; infected spleen cells require EPO for proliferation. Chimeras of the anemia and polycythemia variants of gp55 show that the COOH-terminal membrane-spanning region of gp55 confers the polycythemia phenotype (21). This suggests that the transmembrane domain of gp55 is important for functional stimulation with EPO-R. The difference in the interaction of these two types of gp55 with the EPO-R is a key unsolved problem.

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