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Response to erythropoietin in erythroid subclones of the factor-dependent cell line 32D is determined by translocation of the erythropoietin receptor to the cell surface

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ABSTRACT **Regulation of the expression of the erythropoietin (Epo) receptor (EpoR) gene is under the control of transcriptional regulatory factor GATA-1. GATA-1 is expressed widely among the nonerythroid, factor-dependent subclones of the interleukin 3-dependent mouse cell line 32D. Consequently, to determine whether GATA-1 and EpoR gene expression are linked even in nonerythroid cells, we have studied the correlation of GATA-1 expression with expression and function of EpoR in these cell lines. EpoR mRNA (by RNase protection analysis) and EpoR protein (by specific antibody immunoprecipitation of metabolically labeled EpoR protein) were detectable not only in 32D and 32D Epo (an Epo-dependent subclone) but also in 32D GM, a subclone dependent for growth on granulocyte/macrophage colony-stimulating factor. EpoR mRNA also was detectable by PCR in 32D G, a subclone dependent for growth on granulocyte colony-stimulating factor. However, only 32D Epo cells bound ^{125}I-labeled Epo and expressed EpoR protein on the cell surface, as determined by immunoprecipitation of surface-labeled proteins. These results indicate that, in these factor-dependent cell lines, the major regulatory step determining the erythroid-specific response to Epo is the efficiency of EpoR protein translocation to the cell surface. Mechanisms that could affect lineage-specific translocation are the presence of a chaperone protein, erythroid-specific editing of EpoR mRNA, or altered processing of the EpoR protein to the cell surface. In this model, lineage-restricted responses to growth factors such as Epo are determined not by expression of the genes for growth factor receptors but, rather, by appropriate processing of the receptor protein.**

Erythropoietin (Epo) regulates erythropoiesis by binding specific receptors (EpoRs) on the surface of hematopoietic progenitor cells (1). Regulation of EpoR gene expression is mediated, at least in part, by the transcriptional regulatory factor GATA-1 (2). Although GATA-1 was originally thought to be erythroid-specific (3–5), it was found subsequently in mast cells (6) and megakaryocytes (6, 7) and may also be involved in gene regulation in those cells (7).

Previously, we showed that GATA-1 was present in the interleukin 3 (IL-3)-dependent cell line 32D (8). This cell line has predominantly mast cell features (9) but is multipotent in that factor-dependent subclones can be evolved under selective culture conditions *in vitro* (10, 11). Such subclones include 32D GM, 32D G, and 32D Epo, subclones responsive to and dependent for growth upon granulocyte/macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), and Epo, respectively. Epo 1.1 is an IL-3-dependent revertant of 32D Epo.

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Because GATA-1 occurs in the nonerythroid subclones of 32D, it became of interest to determine whether GATA-1 was associated with expression of the EpoR gene. Such an association, even in cell lines incapable of responding to Epo, would provide insight into the cellular and molecular mechanisms by which hematopoietic lineage restriction and growth factor responsiveness are determined.

A number of models exist to explain the role of growth factors, including Epo, in hematopoietic differentiation and maturation. In one model, the interaction of a specific growth factor with its receptor initiates the differentiation process (12). A second model is that the differentiation process is already established (13) and the interaction of growth factor with its receptor results in full maturation and avoidance of programmed cell death (apoptosis) (14). For erythropoiesis, Epo would interact with its receptor on already committed erythroid cells to initiate terminal erythroid maturation.

To assess these models, we evaluated the role of EpoR gene expression in erythroid differentiation by measuring EpoR mRNA levels, EpoR protein, and appearance of EpoR on the cell surface in the factor-dependent subclones of 32D. Results indicate that the major regulatory step determining the erythroid-specific response to Epo is not simply activation of the EpoR gene but, rather, efficiency of EpoR protein translocation to the cell surface. This dependence may result from the total amount of EpoR protein available, erythroid-specific processing of EpoR mRNA or protein, or the presence of an EpoR-specific chaperone protein.

MATERIALS AND METHODS

Cell Lines. Mouse 32D cell line and its subclones (10) were maintained by biweekly passage in McCoy's medium (GIBCO) supplemented with appropriate growth factors. The recombinant hematopoietic growth factors included pure murine IL-3 and GM-CSF (provided by J. J. Mermod, Glaxo) and recombinant human Epo and G-CSF (provided, respectively, by J. Egrie and L. Souza, Amgen). Ability of the cell lines to form colonies with the different growth factors was assessed in semisolid medium under serum-deprived culture conditions, as described (10, 11).

RNase Protection Analysis of EpoR. Total or poly(A)$^+$ cellular RNA was extracted from cells growing in logarithmic phase by the guanidinium isothiocyanate method (15) and hybridized [41°C in 30 μl of 80% (vol/vol) formamide/0.4 M NaCl/40 mM Pipes, pH 6.4/1 mM EDTA] with a radiolabeled ($\approx$1–5 $\times$ 10^6 cpm) antisense RNA probe. Probe 1 was obtained by transcribing, with T7 polymerase, a pGEM7

Abbreviations: Epo, erythropoietin; EpoR, erythropoietin receptor; IL, interleukin; nt, nucleotide(s); CSF, colony-stimulating factor; GM-CSF, granulocyte/macrophage CSF; G-CSF, granulocyte CSF.

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plasmid, linearized at a *Sca* I site, containing nucleotides (nt) 545–1139 of the EpoR cDNA (ref. 16, provided by G. Wong, Genetics Institute, Cambridge, MA), cloned (after PCR amplification) into the *Eco*RI–*Hin*dIII sites of the polylinker of pGEM7. Probe 2 was obtained by Sp6 polymerase transcription of a pGEM7 plasmid, linearized at the *Xmn* I site, containing the *Xho* I–*Sma* I fragment (nt 272–980) of EpoR cDNA. After RNases A and T1 (40 and 1 μg/ml, final concentration, respectively) digestion, followed by proteinase K digestion and phenol/chloroform extraction, samples were separated by electrophoresis on a 6% acrylamide/8 M urea sequencing gel.

Immunoprecipitation of EpoR Protein. For metabolic labeling, 1–5 × 10^7 cells were preincubated (30 min at 37°C) in 4 ml of methionine-free Dulbecco's modified Eagle's medium (DMEM, GIBCO) and then incubated for 2–4 hr in methionine-free DMEM containing 100 μCi (1 Ci = 37 GBq) of [^{35}S]methionine (specific activity, 1176 Ci/mmol, Amersham). For surface labeling, 10^7 cells were incubated with Na^{125}I (specific activity, 17.4 Ci/mg) (Amersham), and iodination was performed by a lactoperoxidase-catalyzed reaction (17). The cells were then washed three times with cold (4°) phosphate-buffered saline (PBS) and lysed in 1 ml of lysis buffer (0.2 M phosphate buffer, pH 7.4/1% Triton X-100/0.1% SDS/0.1% NaN_3/0.1 M NaCl/2 mM phenylmethylsulfonyl fluoride/100 kallikrein inactivator units of aprotinin). Cell lysates were centrifuged (81,000 × *g* for 30 min); incubated for 1 hr with preimmune serum, 1 hr with protein A-Sepharose, and 1 hr with 9 μl of antiserum; and then incubated overnight with protein A-Sepharose. The Sepharose beads were washed five times with lysis buffer. The immunoprecipitated complex was eluted with Laemmli buffer and loaded on a SDS/10% PAGE gel. The gel was blotted onto nylon membranes, and autoradiography was conducted at −70°C.

Antisera were raised against two synthetic peptides (18) spanning amino acids 25–38 or 490–506 of the EpoR protein (antiserum specific for the NH_2 or the COOH terminus of the EpoR protein, respectively).

Preparation of Cytoplasmic Membranes. Cytoplasmic membranes were prepared as described (19). Approximately 10^8 cells in logarithmic-phase growth were washed in PBS, pH 7.4, resuspended in 4 ml of 10 mM Tris·HCl, pH 7.4/1 mM $MgCl_2$, and incubated for 10 min on ice. Cells were disrupted in a Dounce homogenizer at 4°C, and 16 ml of ice-cold 250 mM sucrose was added. More than 90% of the cells were lysed by this technique. Nuclei and residual undisrupted cells were pelleted by centrifugation at 500 × *g* for 5 min. The supernatant was centrifuged at 25,000 × *g* for 30 min at 4°C to pellet the plasma membranes and microsomes. The pellet was resuspended in 300 μl of 10 mM Tris·HCl, pH 7.4/1 mM $MgCl_2$/1 mM phenylmethylsulfonyl fluoride/aprotinin at 100 kallekrein inhibitor units per ml/leupeptin at 50 μg/ml/25 mM benzamidine. Extracted cellular membrane proteins were stored at −20°C.

EpoR Binding Analysis. The number of EpoRs and their binding affinity were measured as described (11, 20). Purified recombinant human Epo (129,000 units per mg) and ^{125}I-labeled Epo (300–900 Ci/mmol) (Amersham) were used for the binding experiments. Final concentration of ^{125}I-labeled Epo in the binding mixture varied from 30 pM to 3 nM. Approximately 2 × 10^6 logarithmic-phase growth 32D, 32D Epo, 32D Epo1.1, 32D GM, or 32D G cells or purified cytoplasmic membrane preparations from the same cells were incubated in a premixed solution of ^{125}I-labeled Epo with or without a 100-fold excess of unlabeled Epo in binding buffer. Cell- and membrane-associated Epo was separated by centrifugation through a phthalate oil layer. Equilibrium binding data were analyzed with the ENZFIT computer program.

RESULTS

Growth Response of Cell Lines. The 32D cell line clones with a frequency of 10–15% in cultures supplemented with IL-3 but with a low frequency (<1%) in the presence of Epo (ref. 10 and data not shown). In contrast, 32D Epo clones with 10% efficiency with Epo but less efficiently (2–3%) with IL-3. IL-3-responsive revertants (32D Epo 1.1) of 32D Epo grow equally well in Epo or in IL-3, expressing an erythroid phenotype in both conditions (8). 32D GM and 32D G subclones do not respond to Epo (10, 21).

Expression of EpoR mRNA and Correlation with GATA-1 Levels. Fig. 1 shows that RNase protection analysis, with probes corresponding to either the 5′ or the 3′ portion of EpoR mRNA, detected bands of the expected molecular weight in 32D Epo, as well as in 32D and in 32D GM cells. Intensity comparison of the bands from RNA dilutions allowed quantitation of relative EpoR mRNA contents of the

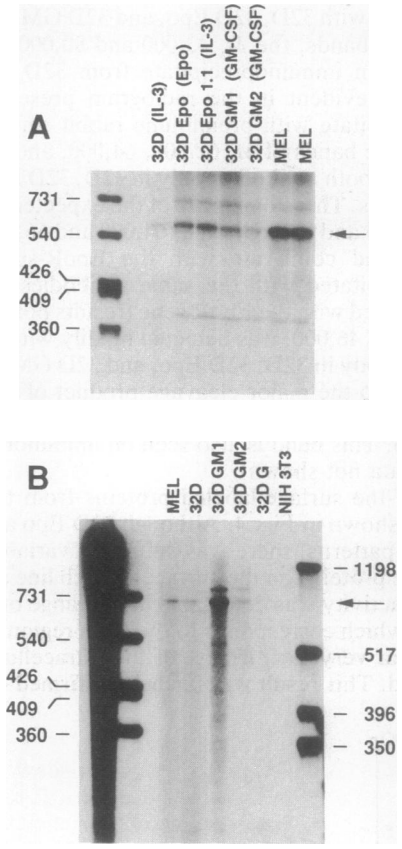

FIG. 1. RNase protection analysis of EpoR mRNA in different 32D clones. (*A*) Total RNA [20 μg per lane except for 32D (5 μg) and control murine erythroleukemia (MEL) cells (15 μg in the first and 20 μg in the second lane)] was hybridized to a 1720-nt-long probe spanning nt 545–1139 of EpoR cDNA. Arrow indicates position of expected band. M_r (× 10^{-3}) size markers are shown at left. (*B*) Poly(A)$^+$ RNA (≈0.3 μg per sample for lanes 32D and 32D GM2 and ≈1.5 μg per sample for 32D GM1, 32D G, and NIH 3T3 lanes), obtained by one cycle of oligo(dT)-cellulose chromatography, was hybridized to a labeled 1830-nt-long probe spanning nt 272–980 of EpoR cDNA. For lane 1 total RNA (5 μg) from MEL cells was analyzed as control. 32D GM1 and GM2 are two separate GM-CSF-dependent subclones of 32D. M_r (×10^{-3}) size markers are shown at left and right. Only the expected EpoR protection fragment was detected, and its position is indicated by an arrow. A positive signal was observed for MEL cells (positive control) and for 32D subclones tested, including 32D, 32D GM, and 32D Epo. No EpoR mRNA was detected with 32D G, although a very low level of EpoR gene expression was found by PCR amplification (data not shown). tRNA and NIH 3T3 cells served as negative controls.

different samples. These experiments demonstrated a linear relationship between GATA-1 (ref. 8 and unpublished results) and EpoR mRNA that correlated with lineage, stage of differentiation, and growth factor responsiveness of the 32D-derived cell lines (Fig. 2). The GATA-1 mRNA gradient for cell lines was 32D Epo > 32D > 32D GM >> 32D G (8). The EpoR mRNA gradient was the same, except that no EpoR mRNA was detected in 32D G cells by RNase protection analysis, even when using poly(A)$^+$ mRNA instead of total RNA. However, a very low level of EpoR gene expression was found by PCR amplification of cDNA reverse-transcribed from 32D G mRNA (data not shown).

Control murine erythroleukemia (MEL) cells express very high levels of both GATA-1 and EpoR mRNA (Fig. 1), ≈3- to 4-fold higher than 32D Epo (Fig. 2). Neither GATA-1 nor EpoR mRNA was found in NIH 3T3 fibroblasts.

Immunoprecipitation Studies. When metabolically labeled proteins were immunoprecipitated with antibodies specific for the NH_2 or the COOH terminus of EpoR, six major bands were detected with 32D, 32D Epo, and 32D GM cells (Fig. 3). Two of these bands, the M_r 43,000 and 80,000 bands, were present also in immunoprecipitate from 32D G subclone, although not evident in the radiogram presented, and in immunoprecipitate with preimmune rabbit serum (data not shown). Three bands of M_r 62,000, 64,000, and 66,000 were detected with both antibodies only in 32D, 32D Epo, and 32D GM cell lysates. These bands are of the expected sizes for the native (62,000) and glycosylated (64,000 and 66,000) forms of EpoR (18) and comigrate with the EpoR-specific bands immunoprecipitated with the same antibodies from Ba/F3 cells transfected with the EpoR gene (results not shown). The sixth band, M_r 46,000, was detected readily with the COOH-specific antibody in 32D, 32D Epo, and 32D GM, and its size corresponds to the major cleavage product of EpoR (metabolic labeling was 4 hr long, whereas half-life of EpoR protein is only 1 hr). This band is also seen on immunoblot analysis of EpoR (data not shown).

Profile of the surface-labeled proteins from the different cell lines is shown in Fig. 4. Although 32D Epo and 32D GM had similar patterns, there was sufficient variability to suggest that the proteins on the surface of each line are specific. Little radioactivity was detectable in the range of proteins of M_r 43,000, which corresponds to the actin region. This result indicates that very few, if any, of the intracellular proteins were labeled. This result was further confirmed by immunoprecipitation with antibodies specific for N-ras or β-actin (results not shown). Immunoprecipitation of the surface-labeled proteins with COOH-specific antibody revealed four major bands but only from 32D Epo subclone (Fig. 5, lane 1). No bands were detected from 32D, 32D GM, or 32D G subclones. Of the four bands detected from 32D Epo subclone, two (M_r 64,000 and 66,000 bands) are of expected size for mature forms of EpoR protein, which is translocated to the surface. The M_r 46,000 band once again corresponds to the expected size of the major proteolytic form of EpoR. A new band, M_r 59,000, was not detected in metabolically labeled proteins, and we do not know at present to which protein it corresponds. The M_r 62,000 band, corresponding to the native intracellular form of EpoR, was not detected (Fig. 5, 32D Epo), confirming that intracellular proteins were not labeled with the iodination procedure used.

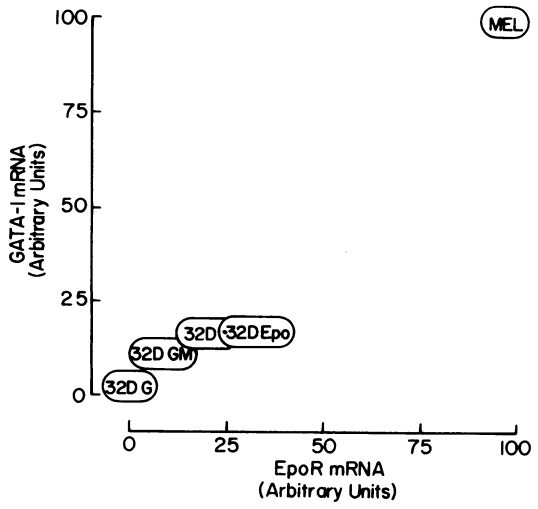

FIG. 2. Correlation between level of expression of GATA-1 (ref. 8 and unpublished results) and EpoR (this paper) mRNA in MEL cells, in the original 32D line, and in its subclones. Relative amounts of mRNA are expressed in arbitrary units.

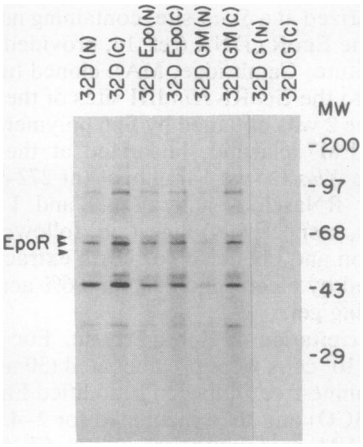

FIG. 3. Immunoprecipitation with antiserum specific for sequences near the NH_2 (N) or COOH (C) terminus of EpoR protein from metabolically labeled [^{35}S]methionine proteins of 32D, 32D Epo, 32D GM, and 32D G cells. Protein from 3 × 10^6 cells was loaded in each lane. M_r (MW) markers (× 10^{-3}) are indicated at right.

Epo Binding by Factor-Dependent Subclones of 32D. 32D Epo cells express a single class of low-affinity EpoRs (Fig. 6 *Upper*). These cells exhibit 400–800 receptors per cell with a K_d from 0.4 to 0.7 nM (Table 1). 32D Epo 1.1 cells, although maintained for >3 mo in IL-3, exhibit numbers of EpoRs per cell similar to 32D Epo cells (Fig. 6 *Lower*); the receptors also have a similar affinity (Table 1).

The original 32D cells displayed little Epo binding (Fig. 6, *Upper*) with a maximal number of 10 receptors per cell. 32D GM and 32D G subclones failed to bind Epo (Table 1).

When purified cytoplasmic membrane preparations were used, ^{125}I-labeled Epo binding was seen not only with 32D Epo

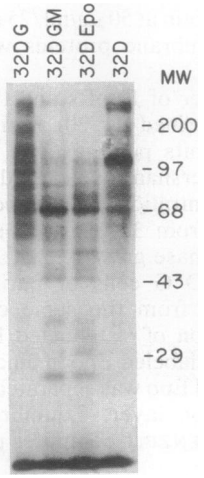

FIG. 4. Fluorography of total ^{125}I-surface-labeled proteins from 32D G, 32D GM, 32D Epo, and 32D cells separated by SDS/PAGE. Heterogeneity of proteins on the surfaces of the four cell lines is evident. M_r (MW) markers (× 10^{-3}) are indicated at right.

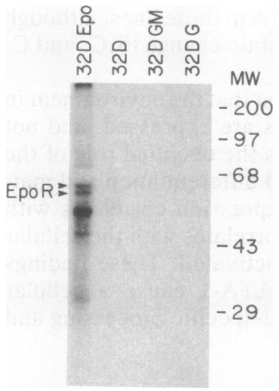

FIG. 5. Immunoprecipitation with antiserum specific for the COOH terminus of EpoR protein from ^{125}I-surface-labeled proteins of 32D Epo, 32D, 32D GM, and 32D G cells. Radioactivity (as cpm) equivalent to 10^6 cells (32D Epo) or 4×10^6 cells (32D, 32D GM, and 32D G) was loaded in the appropriate lane. Only 32D Epo had EpoR protein detectable by this technique. M_r (MW) markers ($\times 10^{-3}$) are indicated at right.

but also with 32D and 32D GM (Table 2). Again, no binding of Epo to 32D G plasma membrane preparations was seen.

DISCUSSION

Epo is the hormone that regulates day-to-day production of erythrocytes (22). Recently, the murine (16) and human (23, 24) EpoR genes have been cloned and expressed. These genes encode a single polypeptide with a transmembrane-spanning region and both cytoplasmic and extracytoplasmic domains. The EpoR, structurally, is a member of a superfamily of receptor genes with properties shared by the receptors for IL-1, IL-6, GM-CSF, and other cytokines (1).

Important to our understanding of how growth factors work is their role in promoting terminal differentiation and maturation of their respective lineages. For instance, does Epo activate the erythroid differentiation program or, as suggested (25), does it simply prevent programmed death of progenitor cells already poised to complete erythroid differentiation. And, in either case, what is the relationship of expression of the EpoR gene to erythroid differentiation?

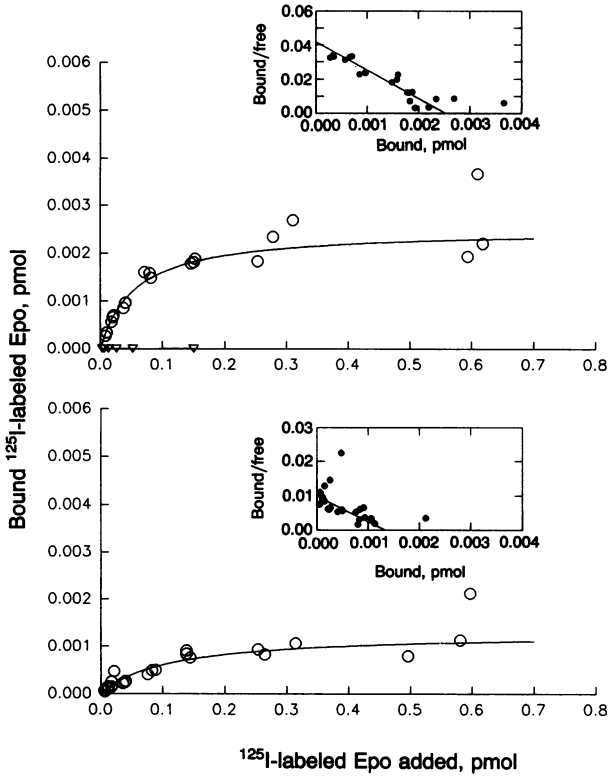

FIG. 6. ^{125}I-labeled Epo binding analysis on 32D Epo (*Upper*, ○), 32D (*Upper*, ▽), and 32D Epo 1.1 (*Lower*). (*Insets*) Scatchard analyses of results on 32D Epo and 32D Epo 1.1 subclones appear in *Upper* and *Lower*, respectively. For both 32D Epo and 32D Epo 1.1 cells, a single class of low-affinity binding sites was seen. No binding was observed on 32D cells in this experiment.

Table 1. ^{125}I-labeled Epo-binding analysis of the factor-dependent subclones of 32D

Cell line	Binding sites/cell	K_d, nM
MEL*	300–400	0.1–3.3
32D	0–10	
32D Epo1	700–900	0.4–0.7
32D Epo1.1	400	1.3
32D GM	0	
32D G	0	

*Data are from ref. 1.

To explore this question, we have used factor-dependent subclones of the IL-3-dependent parent cell 32D. These cells are highly plastic in their ability to differentiate, and GM-CSF-, G-CSF-, and Epo-dependent subclones have been successfully maintained for over 2 yr in culture under serum-deprived conditions (10). The subclones take on the properties of the lineages characteristic of the growth factors supporting them, and, interestingly, the most terminally differentiated cell lines (the G-CSF- and Epo-dependent subclones) cannot be interconverted—i.e., Epo-dependent subclones will not give rise to G-CSF-dependent clones and vice versa. Thus, the environments in which the relevant growth factors work appear fixed, although 32D Epo cells will give rise with low frequency to IL-3-dependent revertants. Nevertheless, the IL-3-dependent revertants retain their erythroid phenotype.

The EpoR gene, as well as almost all erythroid-specific genes, contains in its promoter region a consensus sequence for the transcriptional regulatory factor GATA-1 (2). Originally believed to be found only in erythroid-specific genes, GATA-1 consensus sequences are also found in megakaryocyte-specific genes (7), and GATA-1 is expressed not only in erythroid cells but also in megakaryocytes (6, 7) and mast cells (6).

In previous studies (8), we showed that GATA-1 was expressed in 32D cells but became progressively extinguished as cells differentiated and matured down the granulocyte/monocyte pathway. Thus, the relative expression of GATA-1 in cell lines was of the order: 32D Epo > 32D > 32D GM; GATA-1 was barely detectable by RNase protection analysis in 32D G subclone.

Because only 32D Epo and 32D cells bound Epo on the cell surface (and the latter minimally), it became of interest whether presence of GATA-1 correlated in any way with expression of the EpoR gene, or if GATA-1 could be present but not activate the EpoR gene. In the studies reported here, we have found that presence of GATA-1 in the various 32D subclones correlates with level of expression of the EpoR gene, as reflected by EpoR mRNA determined by RNase

Table 2. ^{125}I-labeled Epo binding to membrane preparations of the 32D subclones

Membrane preparation*	Binding, cpm	
	Labeled	Labeled + unlabeled
32D	1200	340
32D Epo	2100	350
32D GM	650	390
32D G	130	240

*Membranes equivalent to 10^7 cells were incubated with 3 nM of ^{125}I-labeled Epo in the absence (Labeled) or presence (Labeled + unlabeled) of 100-fold excess of noniodinated Epo. Results from one experiment done in triplicate are presented; similar results were obtained in two additional experiments.

protection assay. Thus, even in nonerythroid cells, when GATA-1 is expressed, EpoR mRNA is present.

In further experiments, we determined whether there was immunoprecipitable EpoR protein. We used antisera raised to two regions of the EpoR protein. Results of the immunoprecipitation experiments showed EpoR protein of the expected size in 32D Epo, 32D, and 32D GM cells; EpoR protein was undetectable in 32D G cells. Thus, despite substantial EpoR protein in 32D and 32D GM cells, there was minimal Epo binding to 32D, and no Epo binding to 32D GM cells. There were no apparent differences in size and processing of EpoR in the different cell lines.

When Epo-binding experiments were done with plasma membrane preparations from the various cell lines, specific binding was demonstrable with all cell lines except 32D G. This result indicated that the EpoR protein in the nonerythroid subclones of 32D has a functional Epo-binding domain but was not expressed on the cell surface, as reflected by virtual absence of Epo binding to 32D and no binding to 32D GM. Absence of specific binding was confirmed by immunoprecipitation of surface-labeled EpoR. Only 32D Epo had the expected M_r 64,000 and 66,000 precipitable bands. These results agree with those of Yoshimura *et al.* (18). However, in addition, another band of M_r 59,000 appeared.

These results provide several insights into regulation of the erythroid-differentiation program. Although the exact function of GATA-1 in erythropoiesis remains to be determined, its expression correlates with expression of the EpoR gene. However, in nonerythroid cells, the low levels of EpoR mRNA are translated into low levels of EpoR protein, but this protein is not properly translocated to and inserted into the cell membrane.

There are several explanations. (*i*) In nonerythroid cells, EpoR may be retained and degraded in the endoplasmic reticulum. In Ba/F3 cells, which express high levels of a transfected EpoR gene, most EpoRs are retained and broken down in the endoplasmic reticulum (18).

(*ii*) Some complex proteins are retained in the endoplasmic reticulum when one subunit is synthesized without its partner, which functions as a chaperone; examples include the γ chain of the histocompatibility antigen HLA-DR (26) and the heavy chain of IgM (27). Because the M_r of the EpoR on the cell surface, estimated by affinity cross-linking, is $\approx M_r$ 105,000 and 65,000, the EpoR probably consists of homo- or heterooligomers. Given that other members of the receptor gene superfamily to which EpoR gene belongs have surface receptors with multiple subunits, one explanation for inefficient EpoR expression would be lack of expression of a functional subunit. Thus, the M_r 59,000 protein detected by surface labeling could represent an accessory subunit of the EpoR required for cell-surface localization. It is noteworthy that 32D cells are infected with the Friend murine leukemia virus F-MuLV but not with the polycythemic strain of the Friend spleen-focus-forming virus F-SFFVp, and, therefore, no gp55 was immunoprecipitable from cell lysates of the 32D subclones (18).

(*iii*) Erythroid-specific editing of EpoR mRNA could result in a more functional EpoR. Alternatively spliced forms of EpoR, such as those recently described (28), could have greater or lesser likelihood for entrapment in the endoplasmic reticulum or give rise to a protein having more or less likelihood of associating with other receptor subunits required for insertion and function of the receptor on the cell surface. However, from analysis of PCR-amplified EpoR DNA fragments from 32D and 32D GM cDNAs, we can rule out a major alteration of EpoR mRNA in these lines, although we cannot as yet exclude a more subtle change (S.C. and C. Barron, unpublished work).

In conclusion, these results suggest that the environment in which growth factor receptor genes are expressed, and not simply their expression, determines the eventual role of the growth factor in promoting terminal differentiation and maturation. For the EpoR gene, its expression correlates with GATA-1 activity, but its function correlates with the cellular environment in which the gene is activated. These findings suggest that genes other than GATA-1 cause a cellular environment conducive to erythroid-specific processing and function of the EpoR.

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