We have investigated whether Signal Transducing and Activators of Transcription (STAT) proteins become activated following the binding of erythropoietin (EPO) to immature erythroid cells from the spleens of mice infected with the anemia strain of Friend virus. STAT1 and STAT5 proteins are phosphorylated and translocated to the nucleus in EPO-treated cells. STAT1 and STAT5 DNA binding activities were also activated in an EPO-dependent manner. The presence of these STAT proteins in the DNA binding complex was confirmed by Western blot analysis of the proteins bound to the DNA element in the gel mobility shift assays. This EPO-dependent activation of STAT proteins was maximum within 10 min of exposure of the cells to 10 units of EPO/ml, the concentration of EPO required for maximum STAT activation. The magnitude of the EPO-dependent STAT5 activation appeared to be greater than the EPO-dependent activation of STAT1. The significance of STAT protein activation in EPO signal transduction is discussed.

The glycoprotein hormone erythropoietin is the primary regulator of the control of erythroid cell maturation and, therefore, the number of red blood cells that are introduced into the circulation. Factors other than EPO are required for the proliferation of very immature erythroid cells. However, erythroid cells at the proerythroblast stage or colony-forming unit-erythroid stage of differentiation depend on the presence of EPO for continued differentiation and undergo apoptosis or programmed cell death when deprived of EPO in vitro (1–3). EPO-induced signal transduction may be of interest to those studying the control of apoptosis, the transcription of erythrocyte-specific genes, and the proliferation of hematopoietic cells.

The receptor for EPO is a member of the superfamily of cytokine receptors that includes receptors for the interleukins (IL-2, -3, -5, -6, -7, -9, -11, and -12) and other regulators of hematopoiesis such as granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating growth factor (GM-CSF), and thrombopoietin (4, 5). Receptors for factors as diverse as prolactin, growth hormone, and ciliary neurotrophic factor are also related to these cytokine receptors. Recent evidence suggests that these receptors interact with the J anus tyrosine protein kinases (JAK1, -2, and -3 and Tyk2) to transduce intracellular signals through phosphorylation of cytoplasmic proteins (6–8).

The J anus tyrosine protein kinase JAK2 has been implicated in the EPO-dependent signal transduction pathway and itself phosphorylated on tyrosine following EPO treatment of cells (9). A recent report demonstrated that JAK2 associated with the EPO receptor following the binding of EPO (7). J anus kinases are linked to the regulation of gene transcription through the Signal Transducing and Activators of Transcription (STAT) proteins. These cytoplasmic proteins become tyrosine-phosphorylated and are then translocated to the nucleus to bind DNA in response to receptor-mediated signal transduction (10, 11). J anus kinases interact with STAT proteins in the signal transduction pathway of IFN-α/β and γ (12–17). The IFN-γ signal requires the presence of JAK1 and JAK2 for STAT1 α/β activation, while the IFN-α signal requires JAK1 and Tyk2 for STAT1 α/β and STAT2 activation (18, 19). Recent work reveals the involvement of STAT1α/β proteins in the signaling pathways of epidermal growth factor and platelet-derived growth factor (20, 21). Interleukin-6 activates STAT3 by JAK1 (22). STAT3 is also activated by epidermal growth factor treatment (22). Finally, prolactin stimulates the tyrosine phosphorylation of STAT5 (previously known as mammary gland factor) by JAK2. Phosphorylated STAT5 is required for transcription of the sheep β-casein gene (6, 23–26), but many early response genes also have STAT5 binding sites in their promoters (FcyRI, intercellular adhesion molecule, FcRlIb, α2-macroglobulin, and other promoters having the acute phase response element) (28–31).

When this work was initially submitted, only the DNA binding activity of unknown STAT-like proteins induced by EPO were known (32). A report that appeared after the initial submission of our work showed the EPO-dependent tyrosine phosphorylation and activation of DNA binding of STAT1 (33). Very recently, prolactin, growth hormone, EPO, IL-2, IL-3, IL-5, granulocyte-macrophage colony-stimulating growth factor, and epidermal growth factor were shown to induce STAT5 DNA binding activity (27–34). We demonstrate here that STAT1α/β and STAT5 are tyrosine-phosphorylated and translocated to the nucleus following EPO treatment of primary erythroid cells. Furthermore, we demonstrate EPO-dependent binding of the STAT1 and STAT5 proteins to specific DNA elements.
Experimental Procedures

Immature erythroid cells were prepared from the spleens of CD\(\text{1}\) mice infected with the anemia-inducing strain of Friend virus (FVA cells) as described previously (1, 35). The erythoblasts were washed extensively with I scove's modified Dulbecco's medium and incubated in the same medium supplemented with 30% fetal calf serum for 1 h at 37 °C. Then, either 10 units/ml recombinant human EPO (Amgen) or 5 ng/ml recombinant murine IFN-\(\gamma\) (Genetech) or 100 ng/ml SCF (c-kit ligand) (Genzyme) were added. After 10 min, cells were collected and nuclear extracts were prepared as described by Ruffj-amison et al. (20, 34). Cytoplasmic extracts were prepared by hypotonic lysis as described by Sadowski et al. (36). SDS-PAGE and Western blotting was carried out by standard methods, and detection was accomplished with enhanced chemiluminescence, ECL (Amersham Corp.). Bound antibody was stripped with 2% SDS, 62.5 mM Tris buffer, pH 6.7, at 50 °C for 30 min. Electrophoretic mobility shift assays were done as described by Ruffj-amison et al. (20, 34). The STAT5 binding site of the rat \(\beta\)-casein promoter was used as a probe (5'-gga CTT CCT gga ATT AAg gga-3'). This sequence is sometimes referred to as the prolactin-responsive element (34). The high affinity sequence for the sis-inducible element (SIE) of the fos promoter used as a probe to detect STAT1 binding is 5'-g'g TG CAT TTC CCG TAA ATC TTT g gAT A TT C-3' (20). Antibodies to phosphotyrosine (Tyr(P)) were purchased from UBI, and STAT1 and STAT5 were from Transduction Laboratories.

Results

EPO-dependent Tyrosine Phosphorylation and Nuclear Translocation of STAT1 and STAT5—We have used erythoblasts from mice infected with the anemia strain of Friend virus in this study (FVA cells). Proteins were extracted from purified nuclei from these cells that had been treated with EPO, IFN-\(\gamma\), or SCF. These nuclear proteins were analyzed by Western blotting with antibodies to different STAT proteins. As clearly shown in Fig. 1A, mononodal antibody to STAT5 reacted very strongly with a band that migrated at 95 kDa in the nuclear proteins from EPO-treated cells (lane 2). Moreover, the STAT5 protein was not detectable in the nuclear proteins from untreated cells or the cells treated with IFN-\(\gamma\) and SCF (lanes 1, 3, and 4). This demonstrates the EPO-dependent translocation of STAT5 to the nucleus. Reprobing the immunoblot with monoclonal antibody to phosphotyrosine residues detected a 95-kDa band that comigrated with the STAT5 protein (Fig. 1B, lane 2). Nuclear proteins were probed with the STAT1 monoclonal antibody, and both STAT1\(\alpha\) and -\(\beta\) (p91 and p84) were increased with EPO and IFN-\(\gamma\) treatment (Fig. 1C, lanes 2 and 3). The immunoblot was stripped and reprobed with antibody to the nuclear transcription factor TAL1 (37) as a control for equal loading of the lanes (Fig. 1D, lanes 1-4). IFN-\(\gamma\)-treatment was used as a positive control since it is well established that IFN-\(\gamma\) activates STAT1\(\alpha/\beta\) (15-17). SCF treatment was a negative control.

Characterization of EPO-dependent STAT1 and STAT5 DNA Binding Activity—Based on published information for STAT5 and STAT1\(\alpha/\beta\) DNA binding sequences (38-43), we tested the DNA binding activity of the nuclear proteins used in the experiments shown in Fig. 1. STAT5 binds to the prolactin-responsive or prolactin-inducible element (PIE), which is upstream of the \(\beta\)-casein gene promoter (23-26) and many early response genes (27-31). STAT1 also binds to the PIE element and interacts with a number of DNA elements including the \(\gamma\) response region and SIE that are upstream of several early response genes. In contrast to STAT1, which binds to both the PIE and SIE elements, STAT5 cannot bind to the SIE element (25). Therefore, gel mobility shift assays with both SIE and PIE elements were necessary to discriminate between the DNA binding activity of the STAT1 and STAT5 proteins.

The electrophoretic mobility shift assay shown in Fig. 2A gave a very strong complex with the radiolabeled PIE oligonucleotide with nuclear proteins from EPO-treated versus the control cells (lanes 1 and 2). This specific complex could be eliminated with an excess of unlabeled PIE oligonucleotide (100 ng) (lane 3) (indicating specificity of binding) and to a smaller extent (15-30%) with the unlabeled SIE oligonucleotide (lane 4) that indicated only modest binding of STAT1 to the PIE DNA. The monoclonal antibody to STAT5 used in our experiments only recognized denatured STAT5 protein and hence was unable to supershift the complex (lane 5). Therefore, we cut out the shifted bands (lanes 1 and 2 from the dried gel in Fig. 2A) and separated the protein components with SDS-PAGE, and then Western blotted them with monoclonal antibody to STAT5. As seen in Fig. 2C, STAT5 protein is present only in the EPO-treated sample and not in the control sample (lanes 1 and 2), demonstrating that the STAT5 protein is in the complex binding to the radiolabeled PIE oligonucleotide. Addition of monoclonal antibody to STAT1 did not detectably supershift the complex (Fig. 2A, lane 6). Treatment of the reaction with monoclonal anti-Tyr(P) decreased the binding of STAT5 protein to the PIE sequence (lane 7) showing that tyrosine-phosphorylated STAT5 binds to the DNA element (23). The experiment in Fig. 1C, lane 2, showed the nuclear translocation of STAT1\(\alpha/\beta\) in EPO-treated FVA cells. The above experiment (Fig. 2A, lane C) suggested that there was a small contribution of STAT1 binding to the PIE element. The STAT5-PIE complex was shifted to an area on the gel that is between the two bands resulting from the STAT1-PIE complex. Therefore, the EPO-dependent STAT1 complex with PIE cannot be resolved from the EPO-dependent STAT5 complex. Therefore we carried out experiments to further verify that STAT1 is activated to bind to the appropriate DNA element in EPO-treated cells. In Fig. 3, nuclear proteins from EPO-treated cells (lane 2) gave the characteristic two complexes with labeled SIE oligonucleotide that are expected with STAT1\(\alpha/\beta\) binding (38-40). This can be seen from the IFN-\(\gamma\) control in which only STAT1\(\alpha/\beta\) binds to the SIE oligonucleotide (lane 5). Bands shifted following either EPO or IFN-\(\gamma\)-treatment could be competed out with excess (100 ng) unlabeled SIE oligonucleotide (lanes 3 and 6) and supershifted with the anti-STAT1 monoclonal antibody (lanes 4 and 7). The bands from lanes 1 and 2 (control versus EPO-treated) were also analyzed by SDS-PAGE and Western blotted with antibody to STAT1 and a 91-kDa

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Fig. 1. Characterization of nuclear extracts made from EPO, IFN-\(\gamma\), and SCF-treated FVA proerythroblasts. Cells (6 \times 10^5) were treated with nothing (C, lane 1), EPO (10 units/ml) (E, lane 2), IFN-\(\gamma\) (5 ng/ml) (\(\gamma\), lane 3), or SCF (100 ng/ml) (\(\gamma\), lane 4) for 10 min at 37 °C. Nuclear extracts were prepared as described under “Experimental Procedures.” The immunoblot was probed with monoclonal antibody to TAL1 (\(\gamma\)) (PY) to STAT5 (Fig. 1D) (lane 2), reprobed with monoclonal antibodies to phosphotyrosine (PY) (B) and STAT1 sequentially (C). Finally the membrane was reprobed with affinity-purified polyclonal antibody to TAL1 (D). The 95k and 47k arrows mark the molecular weight and position where the STAT5 and TAL1 proteins migrate on the gel.

Fig. 2. Characterization of nuclear extracts made from EPO, IFN-\(\gamma\), and SCF-treated FVA proerythroblasts. (A) Lane 1, nuclear proteins from EPO-treated cells; lane 2, nuclear proteins from SCF-treated cells; lane 3, nuclear proteins from IFN-\(\gamma\)-treated cells; lane 4, control. (B) Lane 1, nuclear proteins from EPO-treated cells; lane 2, nuclear proteins from IFN-\(\gamma\)-treated cells; lane 3, nuclear proteins from SCF-treated cells; lane 4, control. (C) Lane 1, nuclear proteins from EPO-treated cells; lane 2, nuclear proteins from IFN-\(\gamma\)-treated cells; lane 3, nuclear proteins from SCF-treated cells; lane 4, control.
protein was recognized by anti-STAT1 antiserum in the SIE binding complex (data not shown). Nuclear proteins from SCF-treated cells served as a negative control (lane 8).

As a control experiment, we also studied STAT1α/β binding to the PIE sequence by activating STAT1 with IFN-γ treatment of FVA cells. Nuclear proteins from IFN-γ-treated cells gave two complexes with the PIE sequence (Fig. 2B, lane 2) that are similar to those obtained with the SIE sequence described above. This experiment verified the ability of STAT1 to bind to the PIE element and demonstrated more activation of STAT5 binding activity by EPO than the smaller effect of IFN-γ on STAT1α/β binding to PIE (panel B of Fig. 2 is a longer exposure than panel A).

Time Course and EPO Concentration Required for STAT5 Activation—We further characterized the EPO-dependent tyrosine phosphorylation, nuclear translocation, and DNA binding activity of STAT5 by comparing these events in a temporal fashion after treating the FVA erythroid cells with EPO. EPO-dependent DNA binding activity was first observed in the cytoplasm. Fig. 4A shows that the EPO-dependent binding of the radiolabeled PIE element in the cytoplasm was maximum within 10 min (lane 2) and then declined sharply (lanes 3–5). Fig. 4B illustrates that in the nuclear fraction of these same cells PIE binding activity was maximum within the first 10 min of EPO treatment (lane 2), remained at a plateau for 60–120 min (lanes 3–5), and then subsequently declined. These results are supportive of the hypothesis that activation of PIE binding proteins by tyrosine phosphorylation in the cytoplasm is followed by the translocation of these proteins to the nucleus. The same nuclear proteins from this experiment were further analyzed by Western blotting for the presence of STAT5 proteins (Fig. 4C) and Tyr(P)-containing proteins (Fig. 4D) following EPO treatment. Both STAT5 and a 95-kDa protein phosphorylated on tyrosine residues appeared in the nucleus at a maximal level in the first 10 min after EPO treatment (lane 2) and were maintained at this level for 60–120 min (lanes 3–5) and declined to approximately 10% of maximal after 4 h (lane 6).

The activation of PIE binding activity was tested in FVA cells exposed to increasing concentrations of EPO as illustrated in Fig. 5. Binding activity was not detected without EPO and was only faintly detected in the presence of 0.1 unit of EPO/ml (lane 2); however, the PIE binding activity increased dramatically with 0.5 unit of EPO (lane 3) and became maximal between 1 and 10 units of EPO/ml (lanes 4 and 5).

Vanadate Increased Basal Activation of STAT5—No basal level of STAT5 in the nucleus or PIE binding activity was detected in FVA cells preincubated for 60 min without EPO. However, as illustrated in Fig. 6, lane 3, treatment of the FVA cells with 0.5 mM Na3VO4, an inhibitor of protein phosphotyrosine phosphatase activity (44, 45), during the 60-min preincubation resulted in detectable PIE binding activity without EPO. These Na3VO4-treated cells still responded to EPO with increased PIE binding activity by approximately 30-fold (lanes 3 and 4), and EPO-dependent PIE binding activity was not increased by the Na3VO4 treatment. STAT5 protein was also detectable in the nucleus in FVA cells after treatment with Na3VO4 (Fig. 4C). It is probable that Na3VO4 maintained the tyrosine-phosphorylated status of the STAT5 protein activated by EPO.

![Fig. 2](http://www.jbc.org/)

**Fig. 2.** Mobility shift assays of the PIE binding nuclear proteins from EPO and IFN-γ treatment. Nuclear protein preparation and gel shift analyses with the PIE sequence were performed as described under “Experimental Procedures.” A, control (C) (lane 1) and EPO-treated extracts (lanes 2–7). Competition experiments with a 100-fold excess of either unlabeled PIE or SIE oligonucleotides were run in lanes 3 and 4. Lanes 5–7 are assays performed with either of the monoclonal antibodies to STAT1, STAT5, and phosphotyrosine (PY) incubated with the extracts for 10 min before the addition of the radiolabeled oligonucleotide. B represents gel shift assay in response to IFN-γ versus no treatment (lane 1). Lanes 2–7 are reactions with IFN-γ-treated extracts run as described for lanes 3–7 in A. A supershift complex with the STAT1 monoclonal antibody is seen in lane 6 of B. C is the SDS-PAGE and Western blot analyses with monoclonal antibody to STAT5 of bands cut out from A, lanes 1 and 2 (C, control).

![Fig. 3](http://www.jbc.org/)

**Fig. 3.** Mobility shift analyses of SIE binding nuclear proteins from EPO and IFN-γ treatment. Nuclear protein preparation and gel shift analyses were performed as described under “Experimental Procedures.” Nuclear proteins from control (C, lane 1), EPO-treated (E, lanes 2–4), IFN-γ-treated (I, lanes 5–7), and SCF-treated cells (lane 8) were incubated with radiolabeled SIE oligonucleotide. Competition with an excess of unlabeled SIE oligonucleotide with both EPO- and IFN-γ-treated samples is shown in lanes 3 and 6, whereas lanes 4 and 7 represent supershifting of the SIE complex with monoclonal antibody to STAT1. Nuclear proteins from SCF-treated cells, lane 8, are used as a negative control.
by endogenous EPO (or other factors) before the FVA cells were harvested.

DISCUSSION

In this report we show the EPO-dependent tyrosine phosphorylation, nuclear translocation, and DNA binding of both STAT1 and STAT5 in primary erythroid cells. Data were presented that STAT5 binds to the PIE DNA element in an EPO-dependent fashion. These are: 1) the presence of STAT5 protein in the band of PIE shifted in mobility, 2) the reduction of the PIE gel shift by only 15–30% when DNA that binds other STAT proteins was added to the reaction, 3) the identical kinetics of EPO-dependent STAT5 translocation to the nucleus and EPO-dependent PIE binding activity, 4) the equal translocation of STAT5 to the nucleus and activation of PIE binding activity in cells treated with increasing concentrations of EPO, and 5) the simultaneous translocation of STAT5 to the nucleus and activation of PIE binding activity without EPO in Na3VO4-treated cells.

Similarly, data were presented showing that STAT1 is the EPO-dependent binding activity on the SIE DNA element. These are: 1) the presence of STAT1 in the shifted SIE bands, 2) the identical gel mobility shifts that result from treatment of FVA cells with either EPO or IFN-γ (activating only STAT1), and 3) the supershifting of the EPO-dependent SIE band with anti-STAT1 antiserum.

When this report was first submitted there were no reports of EPO-dependent STAT1 or STAT5 activation. During revision of our report, recent publications from Gouilleux et al. (27), Wakao et al. (29), and Pallard et al. (28) appeared also reporting EPO-dependent activation of STAT5. These studies were mostly done in nonerythroid cell lines in which the EPO receptor was artificially expressed. Here we show the activation of STAT5 by EPO in primary erythroid cells that depend on EPO for complete erythroid differentiation (1–3, 45, 46).

In addition to the discovery of the EPO-dependent phosphorylation and activation of STAT5, the DNA binding activity of STAT5 was also found to be activated through the actions of prolactin, growth hormone, granulocyte-macrophage colony-stimulating factor, IL-2, IL-3, IL-5, and epidermal growth fac-
The receptors for these hormones and cytokines are known to interact with one or more of the Janus tyrosine protein kinases, Jak1, Jak2, Jak3, and Tyk2 (except for epidermal growth factor receptor that has intrinsic tyrosine kinase activity). STATs must either interact with the receptor, kinase, or complex of receptor and kinase. In experiments not shown, only Jak2 was tyrosine-phosphorylated in an EPO-dependent fashion in these primary erythroid FVA cells in agreement with previous findings on EPO activation of Janus kinases (7, 9).

Most recently, two murine genes for STAT5 have been identified coding for proteins 96% identical in amino acid sequence and similar in size (30). Both genes are expressed in comparable levels in virtually every tissue, have identical DNA binding activities toward sequences tested thus far, and are so similar that current anti-STAT5 antibodies cannot discriminate the STAT5A and B forms. It should be noted that in our nuclear translocation experiments (Figs. 1A and 4C), at least four cross-reacting bands are detected by Western blotting with the anti-STAT5 monoclonal antibody. It is possible that the most upper and most lower bands are not STAT5 related since they are not affected by EPO treatment; however, the 95-kDa band and a slightly lower molecular weight protein were translocated to the nucleus in response to EPO. The protein isolated from the PIE binding complex was the 95-kDa band, and the other bands were not present (Fig. 2C). Since both STAT5 A and B are expressed in hematopoietic cells, are activated by IL-3, and bind the PIE element, it is likely that the 95-kDa band is a mixture of STAT5A and B. It is possible that some of the bands less than 95 kDa are proteins related to STAT5 that translocate to the nucleus but cannot bind to the PIE element. In this regard, Azam and co-workers (31) have shown 77-, 80-, and 95-kDa forms of STAT5-like proteins in hematopoietic cells.

STAT5 was first identified as a mammary gland factor, a gene induced in lactating mammary tissue and later shown to be a transcription factor for the β-casein gene (6, 23–26). Recent evidence suggests that STAT5 binding sites are primarily found in promoters of early response genes induced by cytokines and interferons (27–31). Gouilleux and co-workers (27) have speculated that the primary function of STAT5 may be the regulation of these early response genes. There is circumstantial evidence that EPO-dependent activation of STAT5 is physiologically relevant to EPO-dependent gene transcription. We have used DNA sequences in the gel mobility shift assays that constitute the minimum binding sites for the STAT proteins to assure that only STAT protein binding was detected in the gel mobility shift assays. Mui et al. (30) have shown that this same PIE sequence we have used can drive the in vivo transcription of a reporter gene when STAT5 is activated by IL-3. In addition, the discovery that the EPO stimulates the early and transient transcription of the interferon response factor-1 and virus-like (VL30) genes (STAT5 binding sites in their promoters) in FVA cells is further evidence of the physiological significance of EPO-dependent activation STAT5 (48). It is unlikely, however, that many of the genes known to have a STAT5 binding site in the promoter (β-casein, FcyRI, intercellular adhesion molecule, FcγRIb, α2-macroglobulin and other genes having the acute-phase response element) are transcribed in EPO-treated FVA cells due to the location of these genes in inactive DNA in these highly differentiated proerythroblasts (that are only 36 h from enucleation). Moreover, recent evidence suggests that the transcription of the casin gene is more complicated than the simple binding of STAT5 to the promoter. Guilleux et al. (27) have demonstrated that EPO, prolactin, and growth hormone all activate STAT5 binding to the 20-base pair PIE element of the β-casein promoter. However, when the 344-base pair sequence upstream of the β-casein gene was coupled to a luciferase reporter gene, only prolactin and not EPO or growth hormone could induce transcription (27). This result suggests that either another stimulatory protein or an inhibitor protein may be involved in casein transcription or, alternately, STAT5 may be phosphorylated at a different site in response to growth hormone and EPO. Previous studies have shown that transcription of the α,β-casein genes is restricted to mammary tissue with the exception of the thymus (49). However, casein and other milk proteins are produced by cytotoxic T-lymphocytes (27, 49). This may be due to the unique responsiveness of these lymphoid cells to prolactin and the presence of prolactin receptors that has not been observed in other hematoepoietic cells. Thus, transcription of β-casein seems linked to prolactin signaling and not to STAT5 activation only. Together, all these observations point to a role of STAT5 in the transcription of many genes but possibly in a subordinate rather than dominant role.

The EPO-dependent activation of STAT5 binding was larger in magnitude than the activation of STAT1 binding to the PIE element. However, the SIE gel mobility shift showed that the EPO-dependent gel mobility shift was infinite in magnitude since no base-line binding could be detected. Therefore, the EPO-dependent activation of STAT1 may be critical to erythroid differentiation. STAT1 may play a role in the EPO-dependent transcription of the interferon response factor-1 and guanidine binding protein genes in FVA cells (48), both of which have a STAT1 binding site in their promoters. However, the most studied gene with the SIE element in the promotor, c-fos, is not induced by EPO (50). Thus, the role of EPO-dependent STAT1 activation is not yet clear.

Others have failed to detect an effect of EPO on STAT1 activation in nonerythroid cell systems (27–29, 32). We have also detected the EPO-dependent STAT1 activation in some cell lines that respond to EPO yet do not differentiate (data not shown). In support of our finding of STAT1 activation, a recent paper by Ohashi and co-workers (33) documented the EPO-dependent tyrosine phosphorylation of STAT1 and the activation of STAT1 DNA binding activity in an erythroid cell line. It seems likely that the STAT1 signaling pathway may not function in cells transfected with the EPO receptor or in some transformed cell lines. The observation that IFN-γ inhibits the proliferation of FVA erythroid cells (51) and activates STAT1 is consistent with a possible role for STAT1 in the loss of proliferation during erythroid maturation.

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