Erythropoietin Receptor Signal Transduction Requires Protein Geranylgeranylation

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ABSTRACT
Erythropoietin (Epo) acts through the erythropoietin receptor, a member of the type-1 cytokine receptor family, to influence survival, proliferation, and differentiation of erythroid progenitors. Epo stimulation of factor-dependent 32D cells results in phosphorylation of many proteins, including Janus kinase (Jak) 2, signal transducer and activator of transcription (Stat) 5, and extracellular signal-regulated kinase (Erk). Some of Epo-activated signaling proteins require isoprenylation, either farnesylation or geranylgeranylation, for post-translational modification. In this study, we sought to characterize the interplay between protein isoprenylation and Epo signal transduction. Using two different Epo-responsive cell lines, we found that depletion of mevalonate and its isoprenoid derivatives using the 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitor lovastatin impairs Epo signaling as assessed by phosphorylation of cellular substrates and inhibition of apoptosis. Interestingly, the effect of mevalonate depletion was prevented by adding back geranylgeranyl pyrophosphate but not farnesyl pyrophosphate. Furthermore, selective inhibition of protein geranylgeranylation mimicked the effect of lovastatin, whereas selective inhibition of farnesylation had no effect. These results indicate that protein geranylgeranylation and not farnesylation is important for proper Epo signal transduction.

Erythropoietin (Epo) is a hematopoietic growth factor that controls erythrocyte production through regulating proliferation, differentiation, and survival of erythroid progenitors (Krantz, 1991; Youssoufian et al., 1993). Epo exerts these effects by interaction with its cell surface receptor (EpoR) (Koury and Bondurant, 1992; Youssoufian et al., 1993), which is a member of the cytokine receptor superfamily. Binding of Epo to its receptor induces dimerization of two receptor subunits and activation of the associated Jak2 tyrosine kinase (Witthuhn et al., 1993). Jak2 then phosphorylates itself and several tyrosine residues in the cytoplasmic domain of EpoR. These phosphotyrosines then act as recruitment sites for several proteins containing the Src-homology 2 domain (Koury and Bondurant, 1992; Damen et al., 1993, 1995). After binding, these proteins are subsequently phosphorylated and activated to transduce the signal to downstream effectors. The tyrosine most proximal to the plasma membrane acts as a recruitment site for signal transducer and activator of transcription (Stat) 5A/B (Damen et al., 1995). Phosphorylation of Stat5 leads to its dimerization and translocation to the nucleus where it acts as a transcription factor to regulate expression of several Epo-responsive genes (Penta and Sawyer, 1995). The Ras/Raf/mitogen-activated protein kinase pathway is activated by recruitment of Grb2 either directly or indirectly via adaptor proteins, such as Shc (Torti et al., 1992; Damen et al., 1993; Chen and Sytkowski, 2004). This interaction allows the guanine exchange factor Sos, constitutively bound to Grb2, to convert Ras to an active GTP-bound form. Besides Ras, Epo activates other small GTPases, like members of the Ral family (Arai et al., 2002).

The Epo-activated small GTPases mentioned above are isoprenylated. Isoprenylation is an important mechanism of post-translational modification of proteins (Zhang and Castronovo, 2001). It involves the formation of a thioether between the isoprenoid unit in either farnesyl pyrophosphate or geranylgeranyl pyrophosphate and a cysteine residue in the acceptor protein. The reaction is catalyzed by one of the two related isoprenyltransferases, FTase and GGTase (Oitzl et al., 1998; Ziegler and Castronovo, 2001). The isoprenyltransferases are divided into two groups: those that add farnesyl pyrophosphate and those that add geranylgeranyl pyrophosphate. In cells, protein isoprenylation is controlled by a series of regulatory mechanisms that may be combinatorial in nature and involve distinct sets of regulatory proteins. The FTase and GGTase reactions are the key steps in the process of protein isoprenylation. The FTase reaction is regulated by the target protein, whereas the GGTase reaction is regulated by other factors, such as Ras, which acts as a negative regulator of the GGTase reaction (Arai et al., 2002). The GGTase reaction is also regulated by other factors, such as Ras, which acts as a negative regulator of the GGTase reaction (Arai et al., 2002). The GGTase reaction is also regulated by other factors, such as Ras, which acts as a negative regulator of the GGTase reaction (Arai et al., 2002).
rophosphate (FPP) or geranylgeranyl pyrophosphate (GGPP) and a cysteine in the CAAX motif at the carboxyl terminus of the protein (Casey, 1992; Fu and Casey, 1999). Farnesylation and geranylgeranylation are catalyzed by the enzymes farnesyltransferase (FTase) (Manne et al., 1990) and geranylgeranyltransferase (GGTase) (Moomaw and Casey, 1992), respectively. The isoprenoids substrates for these reactions—whether FPP or GGPP—are derived from mevalonate (Fig. 1) (Goldstein and Brown, 1990). Protein isoprenylation is critical for many signaling pathways, such as the Ras/Raf/mitogen-activated protein kinase pathway. Isoprenylation is required for membrane association of signaling proteins, such as Ras, and hence for their proper biological function (Lowy and Willumsen, 1989; Hori et al., 1991; Casey, 1995). As a result, inhibition of protein isoprenylation results in a wide spectrum of biological effects that are probably a consequence of decreased function of these signaling proteins. However, it is possible that there is/are as yet other unidentified mechanism(s) for many of these effects.

In this study, we sought to further identify factors important for Epo signal transduction and to characterize the interplay between protein isoprenylation and Epo signaling pathways. To do so, the factor-dependent 32D cells were depleted of mevalonate and its derivatives. This was done using lovastatin, a competitive inhibitor of HMG-CoA reductase, the rate-limiting enzyme in the isoprenoid synthesis pathway (Goldstein and Brown, 1990). Epo signaling was assessed by studying several Epo-induced events, including phosphorylation of cellular substrates and inhibition of apoptosis. The effect of mevalonate depletion was further characterized by adding back select isoprenoids to lovastatin-treated cells. Inhibition of FTase and GGTase and its effects on Epo signaling were also tested. The strictly Epo-dependent leukemia cell line AS-E2 was also used to extend our findings to more than one cell model.

Using this approach, we found that GGPP, a derivative of the mevalonate pathway, is critical for Epo signaling. Specifically, utilization of GGPP as a substrate for geranylgeranylation of cellular proteins is required for proper Epo signal transduction.

**Materials and Methods**

**Cell Lines and Culture Conditions.** The factor-dependent murine myeloid cell line 32D was stably transfected with wild-type EpoR cDNA as described previously (Quelle et al., 1996). The Epo-dependent AS-E2 leukemia cell line was a generous gift from Chugai Pharmaceutical Company (Tokyo, Japan). 32D cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, glutamine, penicillin, streptomycin, and 10 U/ml erythropoietin. AS-E2 cells were cultured in Iscove’s Modified Dulbecco’s Medium supplemented with 20% fetal bovine serum, penicillin, streptomycin, and 10 U/ml erythropoietin. Each culture was maintained at 37°C, 5% CO₂, and saturating humidity. Cells were treated for the indicated time points. During the last 4 h of each treatment, cells were washed of Epo and then stimulated or not with 10 U/ml Epo for 8 min before lysing. FTI-277, GGTI-286, and ubiquinone were purchased from EMD Biosciences (San Diego, CA). Dolichol was obtained from American Radiolabeled Chemicals (St Louis, MO). [3H]Glucosamine was purchased from Moravek Biochemicals (Brea, CA). Lovastatin, isopentenyl pyrophosphate (IPP), geranyl pyrophosphate (GPP), FPP, and GGPP were purchased from Sigma-Aldrich (St Louis, MO).

**Western Blots.** Cells were lysed in NG-lysis buffer [0.5% Nonidet P-40, 10% glycerol, 50 mM Tris (pH 8.0), 0.1 mM EDTA, 150 mM NaCl, protease inhibitor cocktail (Sigma-Aldrich), and phosphatase inhibitor cocktail 1 and 2 (Sigma-Aldrich)]. The cell lysates were clarified by centrifugation at 14,000 rpm for 30 min at 4°C. The supernatants were clarified by centrifugation at 14,000 rpm for 30 min at 4°C. The supernatants were resolved by 10 or 12% SDS-PAGE and transferred to polyvinylidene difluoride membranes. Membranes were probed with the indicated antibody. Jak2, phospho-Jak2, Stat5, and phosphor-Stat5 antibodies were obtained from Upstate Biotechnology (Lake Placid, NY). All of the other antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). The ECL blotting system (GE Healthcare, Little Chalfont, Buckinghamshire, UK) was used for detection.

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![Fig. 1. Isoprenoid biosynthetic pathway.](https://example.com/isoprenoid-pathway.png)
Gel-Shift Assays. Nuclear extracts were prepared using nuclear extraction kit (catalog number AY2002; Panomics, Inc., Redwood City, CA) according to manufacturer’s protocol. A 20-μg equivalent of nuclear extract was mixed with 1 ng of 32P-end-labeled Stat5 consensus oligonucleotide (sc-2565; Santa Cruz Biotechnology) in 20 μl of binding buffer (10 mM Tris HCl (pH 7.4), 1 mM MgCl₂, 0.5 mM EDTA, 0.5 mM dithiothreitol, 5% glycerol, 60 mM KCl, and 0.2 μg of poly(dI-dC)). The incubation was carried out at room temperature for 30 min. The products of the binding reaction were separated on a 5% acrylamide gel in 0.2× Tris borate-EDTA and visualized by autoradiography.

Apoptosis Analysis. Apoptosis was detected using ApoAlert Annexin V-FITC apoptosis kit (BD Biosciences, San Jose, CA) according to manufacturer’s protocol. In brief, approximately 1 × 10⁶ cells were collected and washed in 1× phosphate-buffered saline. Cells were stained with Annexin V-FITC and propidium iodide. Stained cells were analyzed by flow cytometry with a BD Biosciences FACScan. For each sample, 1 × 10⁴ events were recorded. Analysis of FACS data was performed using WinMDI software (J. Trotter, The Scripps Institute, La Jolla, CA).

Determination of N-Linked Glycosylation. Total incorporation of [3H]glucosamine into N-linked glycoproteins was measured as described previously (Carson and Lennarz, 1981). In brief, cell cultures were treated as indicated. During the last 4 h of each treatment, cells were labeled with 0.6-[3H]glucosamine (5 μCi/ml, 37.5 Ci/mmol). Thereafter, cells were rinsed three times with phosphate-buffered saline and treated with ice-cold trichloroacetic acid. The precipitate was then washed with 10% trichloroacetic acid and solubilized in 1× phosphate-buffered saline. Radioactivity was determined by scintillation counting and normalized to protein content.

MTT Assay. The MTT assay was performed as suggested by Sigma-Aldrich.

Results

Lovastatin Impairs Epo-Induced Phosphorylation of Jak2, Stat5, and Erk. Lovastatin has been shown to have a wide array of effects on cells including impairment of signaling through different cell surface receptors (Satoh et al., 2001; Mantha et al., 2003). To investigate the effects of lovastatin on Epo signaling, factor-dependent 32D cells cultured in 10 U/ml Epo were treated with increasing concentrations of lovastatin for 24 h. During the last 4 h of treatment, cells were washed free of Epo and then stimulated with 10 U/ml Epo. Western blot analysis was carried out as described under Materials and Methods. Stimulation of control cells with Epo induced phosphorylation of Jak2, Stat5, and Erk (Fig. 2A). Lovastatin treatment caused a dose-dependent decrease in Epo-induced phosphorylation of Jak2, Stat5, and Erk. This decrease in phosphorylation was not due to a decrease in levels of the different proteins (Fig. 2A). Stimulation of IL-3 receptor, a very close cytokine family member, was used as a control to show specificity of the effect to Epo signaling (Fig. 2B).

Lovastatin Inhibits Stat5 Nuclear Translocation and DNA Binding. Because mevalonate depletion impairs Epo-induced phosphorylation of several proteins, we determined the degree to which such impairment affects downstream events. Stat5 nuclear localization and DNA binding upon Epo stimulation were tested in mevalonate-depleted cells. As shown in Fig. 3A, Epo induced nuclear localization of Stat5 in control cells. Interestingly, this effect was totally suppressed in lovastatin-treated cells. This was further characterized in the gel-shift assay using radiolabeled Stat5 probe (Fig. 3B). Nuclear extracts from Epo-stimulated control cells induced a band shift by retarding the mobility of the probe on the gel. This is due to interaction between the nuclear Stat5 and its labeled probe. Upon treating the cells with lovastatin, the shift in band completely disappeared, indicating the absence of Stat5 from those nuclear extracts.

Lovastatin Induces Apoptosis in Factor-Dependent 32D Cells. To determine the functional outcome induced by lovastatin on Epo signaling, we chose to study cell survival in response to such treatment. Epo is known to mediate the survival of factor-dependent cells partially by inhibition of apoptosis. If Epo signaling is impaired, this will be expected to induce apoptosis in those cells. In fact, this was found to be the case in 32D cells treated with lovastatin. As shown in Fig. 4, lovastatin induced apoptosis in 32D cells in a concentra-
tion dependent manner as measured by Annexin V staining. The effect was more pronounced when cells were incubated for 48 h with lovastatin.

**Mevalonate or GGPP Prevents Lovastatin Effects on Epo Signaling.** Because lovastatin inhibits the rate-limiting step in the synthesis of isoprenoids and thus depletes cells of all isoprenoid intermediates, we sought to identify the specific intermediate(s) whose depletion is responsible for lovastatin effects. To do so, add-back experiments were conducted. 32D cells were cultured in the presence or absence of lovastatin. Lovastatin cultures were additionally supplemented with any of the following: mevalonate, IPP, GPP, FPP, GGPP, or a combination of IPP + GPP or IPP + FPP. As expected, adding mevalonate to lovastatin-treated cells completely restored Epo-induced phosphorylation of Jak2, Stat5, and Erk to control levels (Fig. 5A). Interestingly, besides mevalonate, only GGPP was able to prevent lovastatin effects on Epo signaling. Neither FPP nor GPP had any effect on rescuing Epo-induced phosphorylation of intermediates. The lack of FPP’s effect on Epo signaling was not due to its lack of functionality, which was tested by studying gel migration of Ras, a farnesylated protein (Fig. 5B). Lovastatin treatment led to the emergence of a more slowly migrating band on the SDS gel that represents the unmodified, unfarnesylated Ras (Holstein et al., 2002). When FPP was added along with lovatatoin, this band disappeared and control Ras mobility on the gel was restored (Fig. 5B).

**Inhibition of Protein Geranylgeranylation Impairs Epo Signaling.** Because GGPP is used for the synthesis of dolichol and ubiquinone (Fig. 1) or for post-translational modification of cellular proteins, we wished to know the exact GGPP pathway that is important for Epo signaling. To do so, 32D cells were cultured in the presence or absence of lovastatin. Lovastatin cultures were additionally supplemented with dolichol or ubiquinone. As shown in Fig. 6A, neither dolichol nor ubiquinone was able to prevent lovastatin’s impairment of Epo signaling. The inability of these compounds to prevent the effects of lovastatin was not due to their lack of functionality. Because ubiquinone plays a role in cellular respiration, adding it to cells increased cellular respiration rates as measured by MTT assay. This effect was dose-dependent as shown in Fig. 6B. To test the functionality of dolichol, the sugar carrier in protein N-linked glycosylation, the measurement of [3H]glucosamine incorporation into N-linked glycoproteins was carried out. These experiments showed that dolichol was able to increase protein glycosylation to almost three times that of control levels (Fig. 6C). Lovastatin decreased protein glycosylation, and this effect could be prevented by adding dolichol (Fig. 6C). These results indicate that restoration of ubiquinone and dolichol functions is not sufficient to restore Epo signaling that is inhibited by lovastatin. These results also suggest that GGPP utilization for post-translational protein geranylgeranylation is the critical event for Epo signaling. To test this hypothesis, 32D cells were treated with GGTI-286, a competitive inhibitor for the enzyme geranylgeranyltransferase. Inhibition of protein geranylgeranylation by GGTI-286 impaired Epo-induced phosphorylation of Jak2, Stat5, and Erk in a dose-dependent manner (Fig. 7A). Interestingly, treating the cells with a

![Fig. 4. Lovastatin induces apoptosis in 32D cells. 32D cells were cultured in RPMI 1640 medium with 10 U/ml Epo in the presence of varying concentrations of lovastatin for 24 or 48 h. Cells were collected and stained with Annexin V-FITC and propidium iodide according to manufacturer's instructions (ApoAlert; BD Biosciences Clontech, Palo Alto, CA). Cells were analyzed by flow cytometry, and dot plots were generated using WinMDI software. A, representative dot plot for one experiment. B, means ± S.E. for triplicate experiments.](image-url)
selective FTase inhibitor, FTI-277, had no effect on phosphor-
ylation of Jak2, Stat5, or Erk (Fig. 7B), although it was able
to inhibit Ras farnesylation dose dependently (Fig. 7C). The
selectivity of FTI-277 was confirmed by its lack of effect on
geranylgeranylation of Rap1α, even at high concentrations
(Fig. 7C). Likewise, GGTI-286 selectivity was confirmed by
studying its effect on Ras farnesylation (Fig. 7D). Inhibition
of isoprenylation of several geranylgeranylated proteins by
GGTI-286 was also tested (Fig. 7D).

Fig. 6. Restoring ubiquinone (Ub) and dolichol (Dol) functions is not
sufficient for EpoR signaling. A, 32D cells were cultured in RPMI 1640
medium with 10 U/ml Epo in the presence or absence of 20 μM lovastatin.
Lovastatin-treated cultures were additionally supplemented with 50 µg/ml Dol or 50 µM Ub. After 20 h, cells were washed free of Epo for 4 h.
Subsequently, cells were stimulated with 10 U/ml Epo for 8 min and then
lysed. Lysates were subjected to SDS-PAGE and Western blot analysis
with the indicated antibodies. B, 32D cells were treated with the indi-
cated concentrations (in micromolars) of Ub for 5 h. Subsequently, the
MTT assay was carried out. Means ± S.E. for triplicate experiments are
shown. C, 32D cells were cultured in RPMI 1640 medium with 10 U/ml
Epo in the presence or absence of 20 μM lovastatin. Lovastatin-treated
cultures were additionally supplemented with 50 µg/ml Dol. After 24 h,
[^3H]glucosamine incorporation was determined as described under Ma-
terials and Methods. Means ± S.E. for triplicate experiments are shown.

Fig. 7. Inhibition of protein geranylgeranylation but not farnesylation
impairs EpoR signaling in 32D cells. 32D cells were cultured in RPMI
1640 medium with 10 U/ml Epo in the presence or absence of the indi-
cated concentration of GGTI-286 or FTI-277. After 20 h, cells were
washed free of Epo for 4 h. Subsequently, cells were stimulated with 10
U/ml Epo for 8 min and then lysed. Lysates were subjected to SDS-PAGE
and Western blot analysis with the indicated antibodies. A, cells were
cultured in the presence of varying concentrations of GGTI-286. B, cells
were cultured in the presence of 30 μM FTI-277 or GGTI-286. C, the effect
of FTI-277 treatment on Ras gel migration and Rap1α geranylgeranyla-
tion. The Rap1α antibody that was used only detects the unmodified
(ungeranylgeranylated) Rap1α. D, the effect of inhibition of geranylger-
anylation on several proteins known to be geranylgeranylated is shown.
Also shown is the lack of effect of GGTI-286 treatment on Ras farnesy-
lation.
Finally, we investigated the effect of inhibiting protein geranylgeranylation on Epo signaling in a different cell line, AS-E2. This is an acute myeloid leukemia cell line that is strictly dependent on Epo (Miyazaki et al., 1997), which represents an excellent model for our studies. The effects of lovastatin and GGTI-286 on Epo signaling in these cells are demonstrated in Fig. 8. As shown in Fig. 8A, lovastatin inhibited Epo-induced phosphorylation of Jak2, Stat5, and Erk, although to a lesser extent than that seen in 32D cells. Although the basis for this difference is not clear, it may be the leukemic nature of AS-E2 cells that makes them more resistant to the effects of lovastatin. Inhibition of protein geranylgeranylation by the selective inhibitor GGTI-286 also inhibited Epo signaling in a dose-dependent manner (Fig. 8B). This further confirms our previous findings in 32D cells and corroborates the requirement of protein geranylgeranylation for proper Epo signaling.

**Discussion**

This is the first report to show that protein geranylgeranylation is required for EpoR signal transduction. Two different strategies were used to show this: 1) lovastatin-induced depletion of mevalonate and its downstream intermediates, including GGPP, and 2) the specific inhibition of geranylgeranyltransferase I activity. The impairment of EpoR signal transduction by lovastatin might be a consequence of either depletion of the isoprenoid units in the mevalonate pathway that are required for the synthesis of different intermediates important for cell functioning (i.e., cholesterol, dolichol, and ubiquinone) or a more selective loss of isoprenylation and hence function of a protein necessary for EpoR signaling. Although much is known about the signaling events that follow EpoR, it is not immediately obvious regarding which of these two possibilities is correct. To distinguish these mechanisms, cells were incubated with selective inhibitors for either FTase or GGTase. These experiments clearly demonstrate that GGTase activity is required for EpoR signaling but do not exclude the possibility that isoprenoid depletion per se may play some role.

Although it would be expected for IPP to overcome HMG-CoA reductase inhibition and restore GGPP levels, this was not the case. This might be due to the inability of IPP to achieve sufficiently high intracellular concentration to restore isoprenoids synthesis. IPP, unlike FPP or GGPP, is a charged molecule at pH 7.4 without the hydrophobic isoprenoid tail that may be necessary for internalization. This is consistent with our results using this compound in other cell types. This is also consistent with the finding that adding IPP with FPP was not sufficient to achieve the same effect as GGPP. With HMG-CoA reductase inhibition and the addition of FPP, there is still a requirement for IPP to form GGPP (Fig. 1). This can be achieved only with external addition of IPP.

Geranylgeranylation plays an important role in modifying many cellular proteins, mostly notably, the members of the Rho family (Mackay and Hall, 1998). These are small G protein that function as molecular switches, cycling between an active GTP-bound state and an inactive GDP-bound state (Bar-Sagi and Hall, 2000). The attachment of a geranylgeranyl moiety to the CAAX motif in the carboxyl terminus of the small G protein is important for membrane localization and hence biological function (Hori et al., 1991).

The functional consequences of inhibition of protein geranylgeranylation are diverse. Many studies have shown that inhibition of geranylgeranylation induces apoptosis in several cell types, including myeloma plasma cells (Van de Donk et al., 2003), acute myeloid leukemia cells (Xia et al., 2001), pulmonary vascular smooth muscle cells (Stark et al., 1998), and anaplastic thyroid cancer cells (Zhong et al., 2003). In pulmonary vascular smooth muscle cells, the exact mechanism underlying this effect is largely unknown. Our finding of the consequence of inhibition of geranylgeranylation on signal transduction of growth factors, such as Epo, might partially explain the apoptotic effect of geranylgeranylation inhibition in many cells.

Interestingly, farnesylation was shown not to be required for phosphorylation of Erk, which is believed to be a downstream target of Ras, a farnesylated GTPase. This can be explained in that Erk might be activated in a Ras-independent manner. In fact, a recent study has shown evidence for the existence of such a mechanism in Epo-responsive cells (Chen and Sytkowski, 2004). Alternatively, a geranylgeranylated form of Ras might be responsible for Erk activation in these cells. Although Ras is generally farnesylated, one of its isoforms, K-Ras, can also be geranylgeranylated (Rowell et al., 1997). Finally, geranylgeranylation may be required for events upstream of Ras activation, such as Jak2 phosphorylation, and this would explain why adding back FPP alone to mevalonate-depleted cells was not enough to restore Erk phosphorylation.
Previous studies from our group have shown that mevalonate depletion by lovastatin alters the expression of small G proteins like Ras, Rap1, RhoA, and RhoB (Holstein et al., 2002). It is unknown to this point whether the effect of mevalonate depletion on EpoR signaling is at the level of transcription. However, the finding that protein levels of EpoR, Jak2, Stat5, and Erk were not affected by mevalonate depletion probably excludes an effect of mevalonate depletion on the transcriptional regulation of these proteins.

It is unclear which geranylgeranylated target protein(s) is/are involved in Epo signal transduction. Possible candidate geranylgeranylated proteins include RhoA, RhoB, and Rac1 or Rac2. These small G proteins play crucial roles in diverse cellular events, such as cytoskeleton organization, membrane trafficking, transcriptional regulation, growth control, and oncosogenesis (Khosravi-Far et al., 1995; Qiu et al., 1995; Hall, 1998; Mackay and Hall, 1998; Bar-Sagi and Hall, 2000). Recently, a role has been established for Rac in Epo signal transduction. It has been shown that Rac is rapidly and transiently activated by Epo or IL-3 in hematopoietic cell lines 32D/EpoR(wild type) and UT-7 and that this activation is required for activation of the Erk signaling pathway (Arai et al., 2002). This is consistent with a previous finding of Rac involved in survival signaling of cytokines to hematopoietic cells (Nishida et al., 1999). Moreover, Rac2 deficiency is associated with myeloid cell dysfunction in both human and mouse (Gu and Williams, 2002). A constitutively active form of Rac1 has been shown to induce phosphorylation and activation of Jak2 (Simon et al., 2000). Other Rho family members, such as RhoA and B, are also possible. The unraveling of new roles that these proteins play in several cellular processes, including signaling (Pelletier et al., 2003) and cell transformation (Qiu et al., 1995), makes them strong candidates.

References


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