Granulocyte colony-stimulating factor (G-CSF) can elicit responses that include proliferation, granulocytic differentiation, and activation of cellular functions in target cells. The biochemical pathways responsible for transduction of these signals from the G-CSF receptor (G-CSFR) have not been defined. In this report, we show that, in murine (NFS-60) and human (OCI-AML 1) myeloid leukemia cell lines and in murine pro-B-lymphocytic cells, BAF/B03, transfected with the murine G-CSFR, proliferative responses to G-CSF are associated with rapid activation of p42 and p44 MAP kinases and p21. Truncation of the cytoplasmic portion of the murine G-CSFR at residue 646 but not at residue 739 abolished G-CSF-induced stimulation of cellular proliferation as well as activation of MAP kinase and p21 in transfected BAF/B03 cells. G-CSF-induced granulocytic differentiation of the murine leukemic cell line 32DC13(G) occurred in the absence of detectable activation of p42 MAP kinase. Nonproliferative responses to G-CSF in the human promyelocytic cell line HL-60 and in human neutrophils were similarly associated with no MAP kinase activation. These results imply that differing cellular effects of G-CSF may be involved in the recruitment of differing signal transduction pathways with the p21/MAP kinase pathway being limited to proliferative responses.

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MATERIALS AND METHODS

Cell Culture

NFS-60 cells were provided by Dr. J. Ihle (St Jude's Hospital, Memphis, TN), OCI-AML 1 cells were a gift from Dr. E. McCulloch (Ontario Cancer Institute, Toronto, Ontario, Canada), and...
32DC13 cells were obtained from Dr J. Greenberger, NBS-60 cells, 
and BAF/BO3 cells were grown in RPMI 1640 medium supplemented with 
10% fetal calf serum (FCS) and 10% WEHI-3B conditioned medium (WCM) 
as a source of murine IL-3. HL-60 cells were grown in RPMI1640 with 10% FCS. OCI- 
AML1 cells were cultured in a modified concentration of minimum essential 
medium (α-MEM) supplemented with 20% FCS and 10% 5637 
conditioned medium. Human neutrophils were obtained from 
healthy volunteers and were purified by Ficoll-Hypaque density 
gradient centrifugation and dextran sedimentation. They were 
confirmed by morphology to be more than 95% pure before use.

Cytokines

Recombinant human G-CSF (r-hu-G-CSF) was a generous gift of 
Amgen (Cambridge, MA). r-mu-GM-CSF and r-mu-IL-3 were 
purchased from Genzyme (Cambridge, MA).

Stimulation With Cytokines and Cell Lyses

For MAP kinase assays, exponentially growing cells (5 x 10^6 
per point) were made quiescent by deprivation of growth factor 
(IL-3 for NFS-60, BAF-3, and 32DC13 cells; G-CSF for OCI-AML1; FCS 
for HL-60) for 6 to 18 hours depending on cell type. Human 
neutrophils were treated with 100 μL/mL diisopropylfluorophosphate 
before use. The cells were subsequently exposed to r-hu-G-CSF or 
r-mu-IL-3 or r-mu-GM-CSF as indicated. After incubation for the 
specified time at 37°C, the cells were lysed on ice in buffer containing 
20 mM Tris HCl (pH 8.0), 40 mM Na pyrophosphate,  
50 mM NaF, 5 mM MgCl₂, 100 μg/mL Na₃VO₄, 10 mM EDTA, 1% (vol/vol) Triton X-100, 0.5 mM L (wt/vol)  
Na deoxycholate, 10 μg/mL leupeptin, and 3 
mM/mL phenylmethylsulphonyl fluoride (PMSF) (reagents from 
Sigma, St Louis, MO). Cell debris and nuclei were removed by centrifugation 
at 16,000 g for 10 minutes at 4°C. The supernate was 
stored at -70°C until used.

For analysis of p21<sup>ras</sup> bound GTP/GDP, exponentially growing 
cells (2 x 10<sup>6</sup> per point) were deprived of growth stimulus as above 
and metabolically labelled by incubation in phosphate-free Dulbec- 
coll modified Eagle's medium (DMEM) containing [32P] ortho-
phosphate 0.5 μCi/mL (US PBS.11, carrier free; Amersham, Am-
ersham, UK) for 3 hours at 37°C. The cells were then stimulated with G-CSF as for the MAP kinase assays. Cell lysis, immunoprecipita-
tion of p21<sup>ras</sup>, and thin layer chromatography were then per-
formed essentially as described by Downward et al. except that 
Triton X-141 was used as the lysis detergent and, after spinning 
out the nuclei, the lysates were incubated at 37°C for 2 minutes to 
separate the phases of the detergent. The detergent phase containing 
p21<sup>ras</sup> was then diluted 10-fold before immunoprecipitation with 
Y13-259 monoclonal antibody bound to protein G-Sepharose 
(Sigma).

Assay of MAP Kinase Activation

Two polyclonal rabbit antisera (122 and 124) generated against 
C-terminal MAP kinase peptides were used for this analysis. 122 is 
an immunoprecipitating antisemur that recognizes p42<sup>mapk</sup>. Anti-
serum 124 recognizes both p42<sup>mapk</sup> and p44<sup>mapk</sup> on immunoblot-
ing. Immunoblot assay. Thirty to 50 μg of lysate protein boiled for 3 minutes in gel-loading buffer was loaded per well onto a 10% 
acrylamide, 0.16% bisacrylamide sodium dodecyl sulfate-polyacryl-
amide gel electrophoresis (SDS-PAGE) gel, subjected to electropho-
resis, and transferred electrophoretically onto a nitrocellulose filter (Schleicher & Schuell, Keene, NH). The filter was then blocked in 
TBST (20 mM Tris HCl, pH 7.5, 150 mM NaCl, 0.05% Tween-20) containing 5% nonfat dried milk for 1 hour at room tem-
perature and probed with 124 for 1 hour at 1:15,000 dilution in blocking buffer. After washing in TBST, the filter was incubated 
with the secondary antibody (goat-antirabbit coupled to horserad-
ish peroxidase; Pierce, Rockford, IL) and developed using the ECL 
detection system (Amersham). Activated (phosphorylated) MAP 
kinease appeared as a band of retarded mobility.

Immunoprecipitate MBK kinase assay (IP kinase assay). 
p42<sup>mapk</sup> was immunoprecipitated from 50 μg of cell lysate protein 
using 10 μL of 122 coupled to protein-A–sepharose (Sigma). After 
washing three times, the immunoprecipitates were suspended in 30 
μL of kinase buffer (30 mM Tris HCl, pH 8.0, 20 mM MgCl₂, 2 mM NaCl, 10 mM ATP, 0.25 mg/mL myelin basic protein [MBP: Sigma], and 33 μCi/mL [γ-32P]ATP [5,000 Ci/ 
mmol; Amersham]). The suspension was incubated with shaking at 
30°C for 30 minutes. The reaction was terminated by the addition 
of 7.5 μL of 5X gel-loading buffer (1X = 62.5 mM Tris HCl, pH 6.8, 2.3% wt/vol SDS, 5 mM EDTA, 100 mM dithiothreitol, 
10% vol/vol glycerol). The mixture was heated at 100°C for 5 
minutes and resolved on 15% SDS-PAGE gel that was dried and 
arautographed.

Construction of mu-G-CSFR Deletion Mutants

mu-G-CSFR cDNA was a generous gift from S. Nagata (Osaka 
Biosciences Institute, Osaka, Japan). The cytoplasmic deletion mu-
tants were constructed using the polymerase chain reaction (PCR) 
with a 5’ (sense) primer corresponding to nucleotides 1817-1838 
containing a unique Kpn l site and 3’ (antisense) primers containing 
in frame termination codons after nucleotides 2195 (Δ 646) and 
2481 (Δ 739), respectively. The deletion mutants and the full-length 
mu-G-CSFR were subcloned into pRcCMV (Invitrogen, San 
Diego, CA), which also contains a neomycin resistance gene under 
the control of the SV40 early promoter. All constructs were subject 
to nucleotide sequence analysis to confirm their identity.

Transfection Into BAF3/BO3 Cells and Isolation 

of Transfected Clones

The mu-G-CSFR and deletion mutants in pRcCMV were 
transfected into BAF/BO3 cells by electroporation in a BioRad gene 
pulsor (BioRad, Hercules, CA). Cells (1 x 10<sup>6</sup>) were transfected 
in 0.8 mL disposable cuvettes (BioRad) with 5 μg of plasmid at 250 V 
and 500 μF capacitance. Transfected cells were plated at low density 
(500/mL) in RPMI/10% FCS/10% WCM in 24-well plates. After 24 
hours, geneticin (G418) was added at 2 mg/mL. Outgrowing clones 
were transferred to tissue culture flasks and grown continuously in 
G418.

Affinity Cross-Linking With 125I G-CSF

125I-labeled G-CSF was a generous gift from Amersham. Radio-
labeled ligand (1 nmol/L) was incubated with 5 x 10<sup>6</sup> cells for 2 
hours at 4°C and was then cross-linked with bis(sulfosuccinimidyl) 
succinimidy l suberate (BS<sup>S</sup>; Pierce) as described by Larsen et al. 19

Differentiation of 32DC13 Cells

Exponentially growing 32DC13 cells were washed free of WCM 
and incubated in RPMI/10% FCS with 50 U/mL of h-u-G-CSF for 15 
days. Aliquots were sampled on days 0, 5, 7, and 12 and were 
assessed for differentiation by morphology on May-Grunwald-
Giemsa–stained slides myeloperoxidase staining, and expression of 
GR-1 antigen.

Immunoprecipitation and Assay of G-CSFR Using 

Flow Cytometry

Cells were labeled directly with a fluorescein (FITC)-conjugated 
antibody GR-1 (Pharmingen, San Diego, CA), a rat antibody di-
rected against a mouse granulocyte-specific antigen expressed on granulocytes from all mouse strains. Cell surface staining was performed using standard immunofluorescence techniques. An FITC-conjugated rat IgG2b was included as a negative control.

The direct binding of phycoerythrin (PE)-conjugated G-CSF to its receptor was performed using the FCS50P Fluorokine G-CSF-PE flow cytometry system (R&D Systems, Minneapolis, MN). Briefly, 4 x 10^6 cells/mL were washed twice in a wash buffer (provided with the kit) to remove growth factors present in the culture medium and 10 μL of the G-CSF-PE reagent was added to 25 μL of cells and incubated on ice for 60 minutes. A control tube containing cells plus strepavidin-PE at the same concentration as the growth factor was also included. After incubation, cells were washed twice in wash buffer to remove unbound G-CSF-PE and the cells were resuspended in 200 μL phosphate-buffered saline before analysis.

Cells were analyzed using an FACScan (Becton Dickinson, Sunnyvale, CA) with an argon laser operating at 488 nm and 15 mW. The FITC filter had a 530/30 nm band pass and the PE filter a 585/42 nm band pass. Data were collected and analyzed using the LSY-SYS II package on a Hewlett-Packard 340 series.

Myeloperoxidase Staining

Cytospun cells were stained for myeloperoxidase using Sigma Kit 390A. The presence of intense brown-black intracellular granulation was indicative of neutrophils and their precursors.

RESULTS

G-CSF Stimulates a Rapid Increase in GTP Association With p21ras in NFS-60 Cells

Although MAP kinases have been shown to lie downstream of p21ras activation in tyrosine kinase signal cascades and activation of p21ras appears to lead to activation of the MAP kinases, it is possible that MAP kinases may also be activated by p21ras independent alternative pathways. To determine whether the rapid activation of MAP kinases on G-CSF-stimulated cell proliferation is associated with p21ras activation, we measured p21ras bound GTP/GDP ratios after exposure to G-CSF. As indicated in Fig 2, exposure of growth factor starved NFS-60 to G-CSF led to the rapid increase of p21ras in GTP bound form. Maximum activation of p21ras (58% GTP-bound) had occurred...
by 5 minutes of exposure with a subsequent decline so that, at 60 minutes, only 28% of p21\(^{\text{ras}}\) was GTP bound. The kinetics of p21\(^{\text{ras}}\) activation were similar to that of MAP kinase activation, implying that, in these cells, the activation of MAP kinase results at least in part from an increase in cellular p21\(^{\text{ras}}\) bound GTP.

Deletion of the mu-G-CSFR at Amino Acid 646 (Δ646) But Not at Amino Acid 739 (Δ739) Abolishes G-CSF-Induced Activation of MAP Kinase and p21\(^{\text{ras}}\)

It has previously been shown that untransfected BAF/B03 cells do not express the G-CSFR and are unresponsive to G-CSF and that these cells become responsive by proliferation to G-CSF when stably transfected with either the human or murine G-CSFR.\(^{45,47}\) To assess the role of MAP kinase and p21\(^{\text{ras}}\) activation in the G-CSF response of transfected cells, the full-length mu-G-CSFR and mutants deleted at residues 646 (Δ646) and 739 (Δ739) (Fig 3) were subcloned into vector pReCMV containing a neomycin resistance gene and electroporated into BAF/B03 cells. Stably transfected clones were isolated by limiting dilution and the ability to proliferate or incorporate tritiated thymidine in response to G-CSF and failed to remain viable in RPMI/10% FCS with r-G-CSF. If appropriately diluted, full-length and Δ734 transfectedants could grow indefinitely in RPMI/10% FCS/r-G-CSF in the absence of IL-3 with no evidence of differentiation or functional alteration.

Activation of MAP kinase and p21\(^{\text{ras}}\) in response to G-CSF in the BAF-3 transfectants correlated with the ability of G-CSF to induce proliferation in these cells. Exposure of IL-3-starved t(8:12) or t(Δ739) to r-G-CSF was associated with rapid activation of both MAP kinase and p21\(^{\text{ras}}\) (Fig 6), whereas t(Δ646) parental BAF/B03 cells and transfectedants expressing pReCMV alone showed no detectable response. The kinetics of the MAP kinase and p21\(^{\text{ras}}\) response to G-CSF in t(8:12) and t(Δ739) was similar to that observed for G-CSF responses in NFS-60 cells. All BAF-3 transfectedants and the parental cell line showed rapid activation of MAP kinase and p21\(^{\text{ras}}\) in response to the readdition of IL-3 to cells starved of this growth factor (data not shown).

MAP Kinases Are Not Activated in the Transduction of Nonproliferative Signals From the G-CSFR

To assess whether MAP kinase activation is a universal component of G-CSFR signal transduction, activation of these enzymes was assessed in cell types that show nonproliferative responses to G-CSF. Human peripheral blood neutrophils are terminally differentiated cells that express the G-CSFR\(^{48}\) and respond to G-CSF with augmentation of effector functions.\(^{45,48}\) Exposure of freshly isolated peripheral blood neutrophils to r-G-CSF was associated with no detectable activation of MAP kinase on the immunoblot assay (Fig 7), whereas exposure to physiologic concentrations of GM-CSF produced a rapidly detectable activation.

To study the role of MAP kinase activation in G-CSF-induced granulocytic differentiation, a variant of the murine myeloid leukemic cell line 32DC13(G) was used. This variant showed no detectable proliferation as estimated by cell counting or tritiated thymidine incorporation when exposed to r-G-CSF in the absence of IL-3 with no evidence of differentiation or functional alteration.

Parental BAF/B03 cells require IL-3 for survival and proliferation and cannot proliferate in response to G-CSF. However, BAF/B03 transfectedants expressing the full-length mu-G-CSFR [t(8:12)] and those expressing Δ739 [t(Δ739)] could proliferate in G-CSF (500 U/mL) in the absence of IL-3 (Fig 5). Transfectants expressing Δ646 [t(Δ646)] or pReCMV alone and parental BAF/B03 cells did not proliferate or incorporate tritiated thymidine in response to G-CSF.
posed to a wide range of concentrations of G-CSF in the absence of IL-3 (data not shown). However, G-CSF treatment (>20 U/mL) in the absence of IL-3 was associated with progressive granulocytic differentiation of these cells with almost complete transformation to neutrophil-like cells by day 12 when assessed by cell morphology (neutrophils: 0% on day 0, 62% on day 7, 89% on day 11), expression of myeloperoxidase (0% on day 0, 92% on day 11), or the murine neutrophil-specific antigen GR-1.

Fig 4. Expression of the mu-G-CSFR by BAF/B03 transfectants. Cells were incubated with 125I G-CSF in the absence (lanes 1, 3, 5, and 7) or presence (lanes 2, 4, 6, and 8) of 200-fold excess of unlabeled G-CSF and cross-linked with BS3. Lanes 1 and 2, t(812); lanes 3 and 4, t(A739); lanes 5 and 6, parental BAF/B03 cells; lanes 7 and 8, NFS-60 cells. The cross-linked murine G-CSFR is indicated by an arrow. The positions of the molecular weight markers used are shown on the left.

Fig 5. Transfectants t(812) and t(A739) but not t(A646) proliferate in response to G-CSF. Transfectants were washed in RPMI alone and then incubated (10^4/mL) in RPMI/10% FCS with G-CSF (500 U/mL) and aliquots were counted on subsequent days. (C) Parental BAF/B03 cells; (a) t(812); (A) t(A739); (O) t(A646).

50,000 U/mL or periods of exposure up to 6 hours (data not shown).

DISCUSSION

p21^ras and the MAP kinases are components of a signaling pathway that regulates proliferation and differentiation of eucaryotic cells by receptor tyrosine kinases. Recent investigations have defined a number of the components of this pathway between the RTKs and the MAP kinases. This pathway has also been shown to be involved in signaling from the HRS, but the specific biochemical link between these receptors and the activation of p21^ras and MAP kinases is less well defined. Although G-CSFR belongs to the HRS, it is unusual within this group in functioning as a homodimer. Furthermore, its cytoplasmic portion which is required for signal transduction is homologous to the IL-4R, which can apparently induce cell proliferation without detectable activation of p21^ras or MAP kinases. In this study, we have used cell types showing differing responses to G-CSF to assess the involvement of the p21^ras and MAP kinase activation in signal transduction from the G-CSFR.

We show that, in cells that proliferate in response to G-CSF [NFS-60, OCI-AML1, BAF3/B03 transfectants t(812 and A739)], exposure to G-CSF is associated with a rapid activation of MAP kinase (peak activity, 5 to 10 minutes). The speed of this response implies that MAP kinase activation is a direct consequence of receptor-ligand binding in these cells rather than the result of the secretion of other growth factors with autocrine activity. In NFS-60 cells and t(812 and A739) exposure to G-CSF also led to a rapid increase in cellular p21^ras bound GTP. The kinetics of p21^ras and MAP kinase activation were similar. By analogy, with other systems, it is likely that in these cells p21^ras activation is at least partly responsible for the MAP kinase activation observed with G-CSF. Increase in cytoplasmic p21^ras-GTP has been shown to lead to downstream activation of the MAP kinases, which occurs through a cascade of phosphorylation involving the serine-threonine kinase Raf-1 and the tyrosine/threonine kinase MAP kinase (MAPKKK). The physical association of p21^ras-GTP and Raf-1 has recently been shown, implying direct activation or activation through a multimeric complex. The use of the interfering dominant negative mutant of p21^ras (S17N-Ras) with asparagine substituted for the serine at position 17 has shown that MAP kinase activation in response to...
certain stimuli, eg, phorbol ester, can occur at least partly through a ras-independent pathway.\textsuperscript{42,43} Whether such mechanisms are also involved in G-CSF-induced MAP kinase activation is currently undetermined because we have found it difficult to express S17N-Ras in hematopoietic cells.

In cell types that express the G-CSFR but show nonproliferative responses to G-CSF, MAP kinase activation by G-CSF was either undetectable or very weak, although other ligands, eg, IL-3 and GM-CSF, could activate MAP kinase. In a subclone of the cell line 32DC13(G), G-CSF induced almost complete granulocytic differentiation without detectable proliferation. No p42\textsuperscript{mapk} mobility shift could be demonstrated in response to G-CSF in these cells. The findings for human neutrophils are similar to those of Raines et al\textsuperscript{33} who found that stimulation with G-CSF produced only a minimal enzymatic activation of MAP kinase, whereas GM-CSF induced a 7- to 10-fold increase in activity. This observed disparity between GM-CSF and G-CSF in ability to induce MAP kinase activation in neutrophils is seen despite the considerable overlap in the functional response of these cells to the two ligands. Both GM-CSF and G-CSF increase the survival of granulocytes and enhance chemotaxis, phagocytosis, antibody-dependent cellular toxicity, and priming of the respiratory burst produced by the chemotactic peptide FMLP.\textsuperscript{49,58-60} although GM-CSF additionally stimulates direct triggering of superoxide release and adhesion to cultured endothelium, which are not produced by G-CSF.\textsuperscript{3,61} Whether activation of the p21\textsuperscript{mapk}/MAP kinase pathway is essential in transducing the signal for these differential responses remains to be determined.

Although normal granulocytic progenitors can be demonstrated to proliferate in response to G-CSF, this ability to proliferate is lost as cells progress down the granulocytic lineage, with later responses to G-CSF being that of enhanced differentiation and the functional activation.\textsuperscript{13} Uncoupling of the activated receptor from the p21\textsuperscript{mapk} and MAP kinase activation could be a means of achieving the loss in proliferative capacity to G-CSF seen in granulocytic progenitors progressing through normal hematopoiesis. The components linking the G-CSFR to the p21\textsuperscript{mapk}/MAP kinase path-
way are not clearly defined. For the β-chain of the IL-2R that shows some homology to the G-CSFR in its cytoplasmic domain, recruitment of the p21<sup>ras</sup>/MAP kinase pathway depends on association of the activated receptor with the nonreceptor tyrosine kinases (NRTKs) p56<sup>ck</sup>, p59<sup>fyn</sup>, or p53<sup>lck</sup> with subsequent enhancement of their tyrosine kinase activity. If an NRTK is also involved in linking the G-CSFR to this pathway, then decreased levels of expression of this NRTK or inactivation by other means may effect uncoupling of the receptor from pathway and from cellular proliferation. BAF/B03 cells stably transfected with full-length murine G-CSFR t(8;12) or with the truncation mutant t(Δ739) but not those transfected with t(Δ646) or vector alone acquired the ability to proliferate indefinitely in the presence of G-CSF. These results are in agreement with those of Fukunaga et al.,<sup>66</sup> who showed that a proliferative response could be obtained with murine G-CSFR truncated at residue 725 but not at residue 654. Similarly, it has recently been shown that the human G-CSFR truncated at residue 688 can still induce BAF/B03 proliferation in response to G-CSF, but that truncated at residue 657 could not.<sup>66</sup> In each case the data suggests that two boxes (boxes 1 and 2) of homology between G-CSFR and other hematopoietin receptors (located at residues 630-643 and 674-683 of the murine G-CSFR) must be retained for the transmission of a proliferative signal to occur. We have shown that BAF/B03 transfecnts [t(8;12) and t(Δ739)] that were capable of proliferation in response to G-CSF also show rapid activation of MAP kinase and p21<sup>ras</sup> on exposure to this ligand, whereas t(Δ646) and control BAF3/B03 cells showed no detectable p21<sup>ras</sup> and MAP kinase activation and could not proliferate in G-CSF-containing medium. However, it remains to be determined whether the region of the G-CSFR responsible for proliferation induction corresponds exactly with that responsible for the activation of p21<sup>ras</sup>/MAP kinase pathway. It has been shown that for the β-chain of the IL-2R transfected into BAF3/B03 cells, activation of p21<sup>ras</sup> and subsequent induction of c-fos and c-jun depends on a domain of the receptor that is also responsible for association of the activated receptor with NRTKs.<sup>65</sup> This domain is known as the acidic region and lies in a C-terminal position to the two boxes of cytoplasmic homology (boxes 1 and 2) between hematopoietin receptors. Deletion of this region abolishes c-fos/c-jun induction by IL-2, but does not abrogate IL-2-induced proliferation. Deletion of a region of the human G-CSFR (residues 687-721) (equivalent to residues 686-720 of the murine G-CSFR) that is similarly placed with respect to conserved boxes 1 and 2 as the acidic region of the IL-2R, although showing only limited homology to it, leads to a suboptimal proliferative response to G-CSF in BAF3/B03 cells and completely abolishes the G-CSF-induced expression of acute-phase proteins in transfected hepatoma cells.<sup>66</sup>

Among the hematopoietin receptor family, G-CSFR is most closely related to gp130,<sup>46</sup> which acts as the signalling chain for the IL-6R, the leukemia inhibitory factor receptor (LIFR) and the receptor for ciliary neurotrophic factor (CNTF).<sup>68</sup> In addition to boxes 1 and 2, G-CSFR and gp130 show a third homologous box (conserved box 3) (amino acids 68% similar) located in a more C-terminal position (resides 738-753 of mu-G-CSFR)<sup>46</sup> (Fig 3). However, we have shown that deletion of this region of the G-CSFR impairs neither the proliferative response to G-CSF nor the associated activation of the p21<sup>ras</sup>/MAP kinase pathway (Fig 6). Like G-CSF, ligands that signal through gp130 are capable of inducing certain target cells to differentiate.<sup>69-71</sup> Furthermore our data suggests that, for G-CSF, this may involve signalling through a pathway not involving activation of p21<sup>ras</sup> or MAP kinase. One possibility to be investigated in the future is that the C-terminal box is specifically involved in generation of nonproliferative signals from these receptors.

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Proliferative but not nonproliferative responses to granulocyte colony-stimulating factor are associated with rapid activation of the p21ras/MAP kinase signalling pathway

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