Fes Tyrosine Kinase Promotes Survival and Terminal Granulocyte Differentiation of Factor-dependent Myeloid Progenitors (32D) and Activates Lineage-specific Transcription Factors

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The c-fps/fes proto-oncogene encodes a 92-kDa protein-tyrosine kinase that is involved in myeloid cell development and function. We have recently shown that expression of an activated allele of Fes (Fesact) in monocytic precursors resulted in their differentiation into functional macrophages through the activation of lineage-specific transcription factors. We now report that this kinase also plays a role in the survival and terminal differentiation of granulocyte progenitors. The expression of Fesact in factor-dependent 32D cells prevented their apoptotic death after interleukin-3 removal, but Fesact-expressing cells remained factor-dependent for proliferation. Removal of interleukin-3 from the Fesact-expressing cells was followed by granulocytic differentiation in the absence of granulocyte colony-stimulating factor within 4–8 days. The differentiated cells had distinctive granulocytic morphology and there was up-regulation of CD11b, Gr-1, and late differentiation markers suggesting that this kinase induced terminal granulocytic differentiation. Concomitantly, Fesact down-regulated the macrophage marker F4/80, indicating that the biological activity of Fes was coordinated in a lineage-specific manner. Further analysis showed that Fesact caused activation of CCAAT/enhancer-binding protein-α and STAT3, two transcription factors that are involved in granulocyte differentiation. Our results provide evidence that Fes may be a key component of the granulocyte differentiation machinery, and suggest a potential mechanism by which this kinase may regulate granulocyte-specific gene expression.

The differentiation of myeloid progenitors into monocyte-macrophages and granulocytes is regulated by the combined action of microenvironmental signals and lineage-specific transcription factors (1). One of the transcription factors involved in granulocytic differentiation is CCAAT/enhancer-binding protein (C/EBP)α; the promoters of G-CSFR and other granulocytic genes contain binding sites for C/EBP-α (1, 2); C/EBP-α knockout mice have no mature neutrophils or eosinophils (3); and conditional expression of C/EBP-α alone can induce granulocytic differentiation in U937, HL-60, and 32D cells (4, 5). The importance of this transcription factor for normal myelopoiesis is underlined by the recent finding that a subset of acute myeloid leukemias exhibit heterozygous mutations in the C/EBP-α protein. This results in truncated C/EBP-α molecules, which act as dominant negative mutants and block the expression of C/EBP-α target genes (6). The mechanism of transcriptional activation of C/EBP-α is not well understood, but recent evidence suggests that it involves its phosphorylation on Ser through a Ras-dependent mechanism (7). Another transcription factor that has been implicated in granulocyte development is signal transducer and activator of transcription (STAT) 3 (8). STAT3 is also activated by phosphorylation. In response to stimulation by a number of cytokines, STAT3 is phosphorylated on tyrosine, and several tyrosine kinases including Jak, Fes, and Src family kinases have been shown to activate STAT3 (9–15).

The c-fps/fes proto-oncogene encodes a 92-kDa cytoplasmic protein-tyrosine kinase that is primarily expressed in vascular endothelial cells (16, 17), monocyte-macrophages and granulocytes (18–20). Expression studies with cloned fps/fes genes have provided considerable evidence that this kinase is involved in macrophage differentiation. The expression of human c-fps/fes in K562 erythroleukemia cells resulted in their differentiation to macrophage-like cells (21), whereas infection of avian bone marrow progenitors with fps/fes viruses led to macrophage differentiation without added M-CSF (22). More recently we reported that a gain-of-function allele of Fes (Fesact) was capable of inducing terminal macrophage differentiation of U937 cells and other monocyte progenitors. This was accompanied by activation of PU.1, a transcription factor that is essential for macrophage development, providing evidence that Fes may regulate macrophage maturation through activation of lineage-specific transcription factors (23).

Although much less is known about the biological role of Fes in granulocytes, this kinase is likely to be an important regulatory molecule in this cell type. Antisense inhibition of Fes expression during retinoic acid-induced granulocytic differentiation of HL60 cells leads to apoptosis (24), suggesting a role of this kinase in the survival of differentiating granulocytic cells. More recently, it was reported that both Fes and the Fes-related kinase Fer can be activated after cross-linking of high affinity Fe receptor (FcRI) in mast cells (25). Thus, Fes and Fer may have overlapping functions in leukocytes.

To further investigate the possible functions of Fes in granulocytes we have introduced Fesact into 32D, an IL-3-dependent cell line that undergoes granulocytic differentiation in the presence of G-CSF (26, 27). These cells have a normal karyotype,
are non-tumorigenic, and have characteristics of normal granulocyte progenitors. Therefore, they have been used extensively as a model to study the mechanisms involved in granulocyte differentiation. Here we report that expression of Fes<sup>act</sup> in 32D protected these cells from apoptosis after IL-3 withdrawal, and that this was followed by terminal granulocyte differentiation in the absence of G-CSF. We also found that Fes<sup>act</sup> expression caused the transcriptional activation of C/EBP-α and STAT3. Our results suggest that Fes kinase may contribute to the regulation of myeloid gene expression during granulopoiesis through the activation of specific transcription factors.

**MATERIALS AND METHODS**

Cells—32D and WeHi-3B cells (27) were obtained from Weiquan Li (Georgetown University, Washington, DC). 32D cells were grown in RPMI 1640 medium supplemented with 15% fetal bovine serum, 1% penicillin, 1% streptomycin, and 5% WeHi-3B conditioned medium as a source of murine IL-3. The amphotrophic retroviral packaging cell line Phoenix amphi was grown as described (23). For differentiation with G-CSF, 32D cells were incubated with 20 ng/ml recombinant murine G-CSF for the indicated times in the absence of IL-3.

DNAs—Human c-Fes and Fes<sup>act</sup> cDNAs, the IRES-GFP-containing retroviral vector MigR1, MigR1-c-Fes, MigR1-Fes<sup>act</sup>, and the pCEFL vector were previously described (23). Details of construction of pCEFL-Fes<sup>act</sup> are available upon request. Luciferase reporters containing a 49-bp tandemly repeated C/EBP-α element, a wild type G-CSF promoter that spans bp −1324 to +67, and a mutant C/EBP-α-G-CSF promoter that has a mutation from bp −49 to −45, corresponding to the C/EBP-α binding site, were obtained from Daniel Tenen (Harvard Medical School, Boston, MA) (21). cDNA probes to lactoferrin, lysozyme, and myeloperoxidase were obtained from Daniel Tenen (Johns Hopkins University, Baltimore, MD) (28). Northern blot analysis was carried out as described (29).

**Transfection and Infection**—The transfection of Phoenix amphi cells with retroviral constructs, virus production, and infection of 32D cells were carried out as described previously (23). 32D cells infected with MigR1-Fes<sup>act</sup> virus were selected by incubation without IL-3 for 2 days. After this time dead cells were removed by centrifugation in HistopaqueTM (Sigma). The live cells were then expanded by incubation in IL-3 medium for 2–5 days. To assess the biological properties of Fes<sup>act</sup>, the cells were again resuspended in medium without IL-3 and analyzed as described in the text.

For transient transfection analysis, 32D cells were transfected with 15 μg of total plasmid DNA by electroporation. Transfection efficiency was normalized using a cotransfected promoter-less renilla vector pRL-0 (Promega, Madison, WI). Transactivation of the luciferase-linked C/EBP-α element and G-CSFR promoter were assayed 16 h after transfection using a dual luciferase kit from Promega.

**Flow Cytometry**—Flow cytometric analysis was carried out as previously described (23). For surface marker staining, we used Phycoerythrin-labeled antibodies against murine CD11b and Gr-1 (BD Pharmingen), and Phycoerythrin-labeled antibodies against F4/80 (Caltag Laboratories, Burlingame, CA).

**Immunoprecipitation and Western Blot Analysis**—The preparation of cell lysates, immunoprecipitation, SDS-PAGE, and immunoblotting with specific antibodies were carried out as previously described (23). Monoclonal anti-Fes antibodies have been previously described (30). The antibodies to phosphotyrosine (PY99), C/EBP-α, STAT3, and IL-3Rα were purchased from Santa Cruz (Santa Cruz, CA). Antibodies to phospho-STAT3 (Y705) were purchased from Cell Signaling Technology (Beverly, MA).

**Preparation of Nuclear Extract and Electrophoretic Gel Mobility Shift Assay (EMS)**—The preparation of nuclear protein extracts and gel shift assays (EMSA) were carried out as previously described (23). The oligonucleotide sequences used were: C/EBP consensus (31), sense 5′-TGGGAGGTAGCTCGACGAG-3′, antisense 5′-TGGGAGGTAGCTCGACGAG-3′, STAT3 (size-inducible element) (14), sense 5′-GTGCAGATTTCAAGGATGTAC-3′, antisense 5′-GACAGAATTTCAAGGATGTAC-3′; PU.1-CD11b (32), sense 5′-CTTCTCTGCTCTCTACTCTT-3′, antisense 5′-GGCCAGAAAAGAGAAGAGAAGAG-3′.

**RESULTS**

**Constitutively Active Fes Protects 32D Cells from Apoptosis Induced by IL-3 Withdrawal**—To gain insight into the possible role of Fes in granulocyte development we expressed Fes<sup>act</sup> as a constitutively active form of Fes in 32D cells. The recently described Fes<sup>act</sup> is a chimera between human and avian fes cognate genes in which part of the kinase domain of human c-Fes was replaced with the corresponding region from avian V-fes (Fig. 1A). Fes<sup>act</sup> is isogenic and colinear with both human c-Fes and avian v-Fps (23). Fes<sup>act</sup> was introduced into 32D cells by retrovirus-mediated gene transfer using the IRES-GFP vector MigR1 (33). 32D cells were infected with MigR1, MigR1-c-Fes, or MigR1-Fes<sup>act</sup> viruses (Fig. 1A) in the presence of IL-3 and 2 days later, IL-3 was removed from the culture medium. The uninfected, vector-, and c-Fes-infected 32D cells underwent apoptosis within 2 days of IL-3 withdrawal, but about 30% of the Fes<sup>act</sup>-infected 32D cells survived without IL-3. All of the surviving cells were green fluorescent, indicating that only Fes<sup>act</sup>-expressing cells were protected from apoptosis (Fig. 1B). This enabled us to rapidly isolate a population where 100% of the cells expressed Fes<sup>act</sup>. In the absence of IL-3, Fes<sup>act</sup>-infected cells remained viable for several days but their cell numbers did not appreciably increase (Fig. 2, A and B). This indicated that Fes<sup>act</sup> promoted the survival of 32D cells deprived of IL-3 but had no significant mitogenic effect. In the presence of IL-3, uninfected and Fes-infected cells proliferated at the same rate (Fig. 2B). As shown in Fig. 3, Fes<sup>act</sup> had no significant effect on the levels of expression of IL-3 receptor, as determined by immunoblot analysis using antibodies directed against the α subunit of IL-3 receptor.

We conclude from these results that activated Fes protected 32D cells from apoptosis caused by IL-3 withdrawal. This was not secondary to a mitogenic effect because Fes<sup>act</sup>-expressing cells remained factor-dependent for proliferation.

**Fes<sup>act</sup> Induces Granulocytic Differentiation in the Absence of G-CSF**—We then examined whether Fes<sup>act</sup> had any other biological activity in 32D cells. To this end, 32D cells were infected...
with Fes<sup>act</sup> followed by selection in the absence of IL-3 for 2 days. The surviving Fes<sup>act</sup>-infected population was then allowed to expand for 3 days by supplementing the culture medium with IL-3. No phenotypic changes were detectable at the end of this period. Because IL-3 suppresses the biological activity of G-CSF and may also interfere with that of Fes<sup>act</sup>, IL-3 was removed from the Fes<sup>act</sup>-infected population once again, and the cells were monitored for phenotypic changes. Remarkably, the Fes<sup>act</sup>-expressing cells differentiated into granulocytic cells (Fig. 4A, panels D1 and D2). Differentiation by Fes required IL-3 deprivation for a total of 4–8 days, including the initial selection step. These cells acquired a characteristic mature granulocyte morphology, namely smaller cell size and multilobulated nuclei. Control uninfected 32D cells treated with G-CSF displayed granulocyte morphology within 5 days, and at day 9 more cells showed typical nuclear fragmentation (Fig. 4A, panels B and C). The highly fragmented nuclei seen after 9 days in G-CSF were not readily observed in Fes<sup>act</sup>-expressing 32D cells, indicating that Fes<sup>act</sup>-induced differentiation was not as complete as that obtained with G-CSF. Nevertheless, the mature granulocyte type was found in about 20% of this Fes<sup>act</sup>-expressing population, whereas the rest exhibited intermediate or normal blast morphologies. By comparison, after 5 days in G-CSF-containing medium, about 30% of the treated 32D cells underwent terminal differentiation. IL-3 greatly suppressed both G-CSF- and Fes<sup>act</sup>-dependent granulocytic differentiation (data not shown). Essentially the same results were obtained whether the source of IL-3 used to propagate uninfected or Fes<sup>act</sup>-infected 32D cells was recombinant IL-3 or WeHi-conditioned medium. In the experiments described above, Fes<sup>act</sup>-infected cells were reincubated with IL-3 after the initial IL-3 deprivation step, before removing it again to allow completion of differentiation. The expansion phase in IL-3 was done to obtain enough cells for our biochemical analysis, but it was not necessary to observe Fes-dependent differentiation. If the IL-3 expansion step was omitted, the Fes<sup>act</sup>-expressing cells differentiated to the same extent as those that were expanded in IL-3 (Fig. 4A, panel D3).

**Coordinate Up-regulation and Down-regulation of Granulocyte and Macrophage Markers by Fes<sup>act**

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**Fig. 2. Growth curve and viability of Fes<sup>act</sup>-infected 32D cells.**

32D cells infected with Fes<sup>act</sup> virus were isolated and expanded as described under “Materials and Methods.” After expansion, Fes<sup>act</sup>-infected cells were cultured in the presence or absence of IL-3 for the indicated times. Viability was determined by trypan blue exclusion (A), and cell numbers were determined using a hemocytometer (B). Cell numbers and viability were also followed in 32D cultures grown in the presence or absence of IL-3, and in 32D cultures treated with G-CSF in the absence of IL-3.

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**Fig. 3. Activated Fes does not affect the expression of IL-3 receptor.**

32D cells were infected with MigR1-Fes<sup>act</sup> viruses and the cells were allowed to differentiate in the absence of IL-3 for 4 days after which time, the cells were lysed. Total cell lysates (25 μg) from control 32D cells (lane 1) and from Fes<sup>act</sup>-infected 32D cultures (lane 2) were analyzed by SDS-PAGE (8.5% gel) followed by immunoblotting (WB, Western blot) with specific antibodies to the α subunit of IL-3 receptor. Numbers at the left indicate the position of Mr markers. The position of IL-3Rα is indicated by an arrow.

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**Fes Regulates Granulocyte-specific Gene Expression**
cells. As shown in Fig. 4B, Fes\textsuperscript{act} up-regulated the expression of the common myeloid marker CD11b and the granulocyte-specific marker Gr-1 by severalfold, whereas expression of the macrophage-specific marker F4/80 was down-regulated by this kinase (Fig. 4B). Treatment of control 32D cells with G-CSF for 5 days was more efficient than Fes\textsuperscript{act} in inducing CD11b and Gr-1 expression, but G-CSF was slower than Fes\textsuperscript{act} in down-regulating F4/80. Like G-CSF, Fes\textsuperscript{act} induced the expression of lysozyme and lactoferrin, two markers of late granulocytic differentiation, whereas expression of the early marker myeloperoxidase was down-regulated by Fes (Fig. 5). Although the time kinetics of up- and down-regulation of lineage-specific markers by Fes\textsuperscript{act} and G-CSF was slightly different, their overall biological activities on granulocytic differentiation were comparable.

We conclude from these results that Fes\textsuperscript{act} is capable of regulating myeloid gene expression in a lineage-specific manner. The extent of differentiation by Fes was not as complete as that induced by G-CSF. This suggests that under physiological conditions, additional cooperating signals are required to complete the granulocyte differentiation program. Nevertheless, the remarkable biological activity of Fes\textsuperscript{act} we observed suggests that Fes kinase may play a key role in granulopoiesis.

**Fes\textsuperscript{act} Activates STAT3 and C/EBP-\alpha but Not PU.1**—Since Fes\textsuperscript{act} was able to induce granulocyte differentiation, we examined whether this kinase activated transcription factors that are important for myeloid development (1). To this end, we carried out EMSA of nuclear extracts from Fes\textsuperscript{act}-infected 32D cells. As shown in Fig. 6A, lanes 3–5, both G-CSF and Fes\textsuperscript{act} induced DNA-binding activity of STAT3. This activity was competed out by excess unlabeled specific oligonucleotide, but not by a nonspecific oligonucleotide (Fig. 6A, lanes 6 and 7). The addition of anti-STAT3 antibodies to the EMSA reaction resulted in a band supershift (Fig. 6A, lane 8), confirming the presence of STAT3 in the DNA-binding complexes induced by Fes.

Transcriptional activation of STAT3 requires its phosphorylation on Tyr-705 (15). Consistent with the results of our EMSA, Fes\textsuperscript{act} induced the phosphorylation of STAT3 on Tyr-705, as determined by immunoblot analysis using phosphospecific antibodies (Fig. 6B, lanes 3 and 4). The phosphorylated species migrated as a doublet, which probably corresponds to STAT3-\alpha and its spliced form STAT3-\beta. Neither G-CSF treatment or Fes\textsuperscript{act} expression had any detectable effect on the expression levels of STAT3 (Fig. 6B).

Fes\textsuperscript{act} also induced binding of nuclear extracts to an oligonu-
cleotide that is specific for C/EBP family members (Fig. 7). This band was competed out by excess unlabeled specific oligonucleotide, but not by a nonspecific oligonucleotide (Fig. 7A, lanes 6 and 7). G-CSF also induced DNA binding to this oligonucleotide (Fig. 7A, lane 3). Although the G-CSF- and Fes-induced complexes had slightly different electrophoretic mobilities, anti-C/EBP-α antibodies caused a supershift of both transcriptional complexes indicating that they contained C/EBP-α (Fig. 7A, lanes 8 and 9). Neither G-CSF treatment nor Fes act expression modified the expression levels of C/EBP-α (data not shown). Whether other C/EBP family members are also activated by Fes will require further analysis. As shown in Fig. 7B, the expression of Fes act in 32D cells had no detectable effect on the DNA binding activity of PU.1. We conclude from these results that in 32D cells, Fes act induced the formation of C/EBP-α and STAT3 DNA-binding complexes.

**Fes act Transactivates C/EBP-α and G-CSFR Reporter Genes**—To further examine the ability of Fes to induce transcriptional activation of C/EBP-α, we analyzed whether Fes act was able to transactivate a minimal luciferase-linked promoter containing 4 tandem repeats of the C/EBP-α-binding site. As shown in Fig. 8, Fes act was able to transactivate this C/EBP-α element. Since C/EBP-α is essential for transcription of the G-CSF gene (2, 34), we also examined whether Fes act would be able to transactivate the G-CSFR promoter. Fes act transactivated the G-CSFR promoter, but not a promoter with a mutation in the C/EBP-α site (Fig. 8). We conclude from these results that Fes act can induce terminal granulocytic differentiation and the transcriptional activation of C/EBP-α and STAT3.

**DISCUSSION**

In this paper we present evidence that Fes kinase can regulate lineage-specific gene expression during granulocytic differentiation. We also identified the transcription factors C/EBP-α and STAT3 as potential Fes targets, which may mediate the biological activity of this kinase.

Although 32D cells behave as granulocytic progenitors, these cells are still capable of differentiating along the monocyte/macrophage lineage. Introduction of another tyrosine kinase, M-CSF receptor, into 32D cells allowed them to undergo partial monocytopoietic differentiation in the presence of M-CSF (35). Also, ectopic expression of the transcription factor EGR-1 enabled these cells to undergo macrophage differentiation by GM-CSF treatment (36). Since Fes act was an inducer of EGR-1, we were interested in determining whether in 32D cells, Fes act would induce macrophage or granulocytic differentiation, or both. By contrast with M-CSFR, Fes act was not able to change the lineage commitment of 32D cells but completed their granulocyte differentiation program. The results of these and our previous studies in mononuclear cells (23) suggest that Fes may be involved in regul-
Fes Regulates Granulocyte-specific Gene Expression

How does Fes kinase contribute to the execution of alternative programs of myeloid gene expression? In 32D cells Fes activated STAT3 and C/EBP-α, two important regulators of granulocytic differentiation (3, 8, 37, 38). On the other hand, PU.1, a transcription factor that is essential for macrophage granulocytic differentiation (3, 8, 37, 38), was essentially not dividing. By contrast with Fes act, the expression of v-Src, v-Abl, or Bcr-Abl in 32D cells does not result in differentiation but in their conversion to factor-independent

ence and oncogenic transformation (26, 39–41). Thus, among non-receptor tyrosine kinases, Fes has a unique ability to induce granulocytic differentiation in 32D cells.

The fact that in 32D cells, both Fesmut and G-CSF activated C/EBP-α and STAT3 suggests that Fes kinase may have utilized elements of the G-CSF pathway to drive granulocytic differentiation. There were also other similarities between the biological activities of G-CSF and Fesmut: the time courses of granulocytic differentiation induced by G-CSF and active Fes were similar; G-CSF and Fesmut induced granulocytic differentiation without IL-3, but their biological activity was greatly diminished in the presence of this cytokine; and both G-CSF and Fesmut protected cells from apoptosis after IL-3 removal. Future experiments will be aimed at clarifying the functional relation of Fes to G-CSF and other cytokines that regulate granulocyte development.

Considering the large body of evidence that Fes plays a role in myeloid cell development, the phenotype of Fes knockout mice is milder than expected. Hackenmiller et al. (42) reported that some of the Fes−/− mice were compromised in their innate immunity, had low numbers of B cells, and elevated numbers of macrophages and granulocytes. Fes−/− macrophages exhibited defects in cell adhesion and GM-CSF signaling, pointing to incomplete maturation of their myeloid compartment. However, targeting of the Fes locus using a different strategy did not reveal alterations in the macrophage or granulocyte populations (43). This low penetrance of the Fes−/− phenotype may be a result of functional redundancy. A possible candidate that may compensate for the loss of Fes is the ubiquitously expressed Fer/NCP94 kinase, which has the same overall structure as Fes (18, 19, 44, 45). It was recently reported that in mast cells, Fer and Fes were both activated by FcγRI cross-linking (25). Thus, Fes and Fer may have overlapping functions in mast cells and basophils. These observations are consistent with the idea that in leukocytes, Fer may partially compensate for the absence of Fes. In granulocytes, functional complementation between Src family kinases has masked the phenotypic consequences of deleting single members of the family. For instance, defects in adhesion-dependent degranulation of polymorphonuclear neutrophils were observed only after ablation of both Hck and Fgr kinases (46). And defects in the respiratory burst response were only observed in neutrophils from hck−/−fgr−/−fyn−/− triple knockout mice (47). Since myeloid development can be regulated by multiple pathways (1, 48), it is likely that in addition to Fer there may be other mechanisms capable of compensating for the absence of Fes. For these reasons, we believe that both loss- and gain-of-function genetic approaches will be required to elucidate the biological functions and mechanism of action of Fes.

The results presented in this paper suggest that Fes may play an important role in granulopoiesis. Our observation that C/EBP-α and STAT3 are activated by Fes during this process suggests a potential mechanism by which this kinase may contribute to the regulation of granulocyte-specific gene expression during myeloid development.

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REFERENCES

1. Clarke, S., and Gordon, S. (1998) J. Leukocyte Biol. 63, 153–168
2. Smith, L. T., Hohaus, S., Gonzalez, D. A., Dziemien, S. E., and Tenen, D. G. (1996) Blood 88, 1234–1247
3. Zhang, D. E., Zhang, P., Wang, N. D., Hetherington, C. J., Darlington, G. J., and Tenen, D. G. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 569–574
4. Hadomska, H. S., Huetterer, C. S., Zhang, P., Cheng, T., Scadden, D. T., and Tenen, D. G. (1998) Mol. Cell. Biol. 18, 4301–4314
Fes Regulates Granulocyte-specific Gene Expression

5. Wang, X., Scott, E., Sawyer, C. L., and Friedman, A. D. (1999) *Blood* 94, 560–571

6. Pabst, T., Mueller, B. U., Zhang, P., Radomska, H. S., Narravula, S., Schnittger, S., Behre, G., Hiddemann, W., and Tenen, D. G. (2001) *Nat. Genet.* 27, 263–270

7. Behre, G., Singh, S. M., Liu, H., Bortolin, L. T., Christopeit, M., Radomska, H. S., Ragatia, J., Hiddemann, W., Friedman, A. D., and Tenen, D. G. (2002) *J. Biol. Chem.* 277, 38290–38299

8. McLemore, M. L., Grewal, S., Liu, F., Archambault, A., Foursine-Laurent, J., Haug, J., and Link, D. C. (2001) *Immunity* 14, 193–204

9. Bruzzi, M. F., Aronica, M. G., Rosso, A., Bagnara, G. P., Yarden, Y., and Pegoraro, L. (1996) *J. Biol. Chem.* 271, 3562–3567

10. Nelson, K. L., Rogers, J. A., Bowman, T. L., Jove, R., and Smithgall, T. E. (1998) *J. Biol. Chem.* 273, 7672–7677

11. Park, W. Y., Ahn, J. H., Feldman, R. A., and Seo, J. S. (1998) *Cancer Lett.* 129, 29–37

12. Shimoda, K., Feng, J., Murakami, H., Nagata, S., Watling, D., Rogers, N. C., Stark, G. R., Kerr, I. M., and Bile, J. N. (1998) *Blood* 90, 597–604

13. Wen, Z., Zhong, Z., and Darnell, J. E., Jr. (1995) *Cell* 82, 241–250

14. Yu, C. L., Meyer, D. J., Campbell, G. S., Larner, A. C., Carter-Su, C., Schwartz, J., and Jove, R. (1995) *Science* 269, 81–83

15. Zhong, Z., Wen, Z., and Darnell, J. E., Jr. (1994) *Science* 264, 95–98

16. Greer, P., Haigh, J., Mhamal, G., Kho, W., Bernstein, A., and Pawson, T. (1994) *Mol. Cell. Biol.* 14, 6755–6763

17. Hackenmiller, R., and Simon, M. C. (2002) *Dev. Biol.* 245, 255–269

18. Feldman, R. A., Gabrilove, J. L., Tam, J. P., Moore, M. A., and Hanafusa, H. (1985) *Proc. Natl. Acad. Sci. U. S. A.* 82, 2379–2383

19. MacDonald, I., Levy, J., and Pawson, T. (1985) *Mol. Cell. Biol.* 5, 2543–2551

20. Samarut, J., Mathey-Prevot, B., and Hanafusa, H. (1985) *Mol. Cell. Biol.* 5, 1067–1072

21. Yu, G., Smithgall, T. E., and Glazer, R. I. (1989) *J. Biol. Chem.* 264, 10276–10281

22. Carmier, J. F., and Samarut, J. (1986) *Cell* 44, 159–165

23. Kim, J., and Feldman, R. A. (2002) *Mol. Cell. Biol.* 22, 1903–1918

24. Manfredini, R., Grande, A., Tagliafico, E., Barbieri, D., Zucchini, P., Citro, G., Zapi, G., Franceschi, C., Torelli, U., and Ferrari, S. (1993) *J. Exp. Med.* 178, 381–389

25. Craig, A. W., and Greer, P. A. (2002) *Mol. Cell. Biol.* 22, 6363–6374

26. Rivera, G., Valtieri, M., Mavilo, F., and Reddy, E. P. (1987) *Oncogene* 1, 29–35

27. Valtieri, M., Tweardy, D. J., Caracioli, D., Johnson, K., Mavilo, F., Altman, S., Santoli, D., and Rivera, G. (1997) *J. Immunol.* 158, 3829–3835

28. Friedman, A. D., Krieder, B. L., Venturelli, D., and Rovera, G. (1991) *Blood* 78, 2426–2432

29. Areces, L. B., Delio Sharba, P., Jucker, M., Stanley, E. R., and Feldman, R. A. (1994) *Mol. Cell. Biol.* 14, 4606–4615

30. Jucker, M., McKenna, K., da Silva, J. A., Rudd, C. E., and Feldman, R. A. (1997) *J. Biol. Chem.* 272, 2104–2109

31. Choi, B. H., Park, G. T., and Rho, H. M. (1999) *J. Biol. Chem.* 274, 2858–2865

32. Pahl, H. L., Scheibe, R. J., Zhang, D. E., Chen, H. M., Galson, D. L., Maki, R. A., and Tenen, D. G. (1993) *J. Biol. Chem.* 268, 5014–5020

33. Pear, W. S., Miller, J. P., Xu, L., Pau, J. C., Soffer, B., Quackenbush, R. C., Pendergast, A. M., Bronson, R., Aster, J. C., Scott, M. L., and Baltimore, D. (1998) *Blood* 92, 3780–3792

34. Zirngibl, R. A., Senis, Y., and Greer, P. A. (2002) *Mol. Cell. Biol.* 22, 2472–2486

35. Areces, L. B., Dello Sbarba, P., Jucker, M., Stanley, E. R., and Feldman, R. A. (1993) *J. Biol. Chem.* 268, 26293–26299

36. Shimmura, K., Nakajima, K., Hirano, T., and Nagata, S. (1997) *J. Biol. Chem.* 272, 25184–25189

37. Zupi, G., Franceschi, C., Torelli, U., and Ferrari, S. (1993) *Mol. Cell. Biol.* 13, 397–407

38. Aaronson, S. A. (1990) *Science* 242, 29–35

39. Cleveland, J. L., Dean, M., Rosenberg, N., Wang, J. Y., and Rapp, U. R. (1989) *Mol. Cell. Biol.* 9, 5685–5695

40. Cortez, D., Reuther, G., and Pendergast, A. M. (1997) *Oncogene* 15, 2333–2342

41. Zsolt, G., and Anderson, S. M. (1991) *Oncogene* 6, 245–256

42. Hackenmiller, R., Kim, J., Feldman, R. A., and Simon, M. C. (2000) *Immunity* 13, 397–407

43. Zirngibl, R. A., Senis, Y., and Greer, P. A. (2002) *Mol. Cell. Biol.* 22, 2472–2486

44. Feldman, R. A., Tam, J. P., and Hanafusa, H. (1986) *Mol. Cell. Biol.* 6, 1065–1073

45. Borden, P. L., Heisterkamp, N., and Giglio, M. (1988) *Mol. Cell. Biol.* 8, 1587–1593

46. Mocsai, A., Ligi, E., Lowell, C. A., and Berton, G. (1999) *J. Immunol.* 162, 1120–1126

47. Peccia, S., Zhou, M., Mocsai, A., and Lowell, C. (2001) *J. Immunol.* 166, 4115–4123

48. Zhang, P., Nelson, E., Radomska, H. S., Iwasaki-Arai, J., Akashi, K., Friedman, A. D., and Tenen, D. G. (2002) *Blood* 99, 4406–4412
