The human c-fes proto-oncogene encodes a cytoplasmic tyrosine kinase (Fes) that is associated with multiple hematopoietic cytokine receptors. Fes tyrosine autophosphorylation sites may regulate kinase activity and recruit downstream signaling proteins with SH2 domains. To localize the Fes autophosphorylation sites, full-length Fes and deletion mutants lacking either the unique N-terminal or SH2 domain were autophosphorylated in vitro and analyzed by CNBr cleavage. Identical phosphopeptides of 10 and 4 kDa were produced with all three proteins, localizing the tyrosine autophosphorylation sites to the C-terminal kinase domain. Substitution of kinase domain tyrosine residues 713 or 811 with phenylalanine resulted in a loss of the 10- and 4-kDa phosphopeptides, respectively, identifying these tyrosines as in vitro autophosphorylation sites. CNBr cleavage analysis of Fes isolated from 32P-O4-labeled 293T cells showed that Tyr-713 and Tyr-811 are also autophosphorylated in vivo. Mutagenesis of Tyr-713 reduced both autophosphorylation of Tyr-811 and transphosphorylation of Bcr, a recently identified Fes substrate, supporting a major regulatory role for Tyr-713. Wild-type Fes transphosphorylated a kinase-inactive Fes mutant on Tyr-713 and Tyr-811, suggesting that Fes autophosphorylation occurs via an intermolecular mechanism analogous to receptor tyrosine kinases.

Autophosphorylation of the Fes Tyrosine Kinase

EVIDENCE FOR AN INTERMOLECULAR MECHANISM INVOLVING TWO KINASE DOMAIN TYROSINE RESIDUES*

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The human c-fes proto-oncogene encodes a cytoplasmic tyrosine kinase (Fes) that is associated with multiple hematopoietic cytokine receptors. Several studies have linked Fes to the receptors for granulocyte-macrophage colony-stimulating factor, erythropoietin, interleukin-3, interleukin-4, and interleukin-6 (7–11). In each case, binding of ligand to the receptor led to stimulation of Fes tyrosine kinase activity. Fes was also demonstrated to associate with the receptors for these cytokines or with the signal transducing component gp130 in the case of IL-6.

Although Fes is often associated with hematopoietic growth regulation, other work suggests a more general physiological role for this tyrosine kinase. Expression of an activated form of Fes in transgenic mice led to hypervascularization, suggesting that Fes may function in angiogenesis (12). Fes also exhibits widespread expression in embryonic tissues, suggestive of an essential role in early development (13). Despite these multiple biological functions, the mechanisms regulating Fes tyrosine kinase activity and the signal transduction pathways in which Fes participates have not been well characterized.

Human Fes is 822 amino acids in length and can be divided into three distinct structural regions: a unique N-terminal domain, a Src homology 2 (SH2) domain, and a C-terminal kinase domain. All three domains have the potential to regulate Fes kinase activity and interaction with signaling partners. The unique N-terminal region has recently been shown to contain a recognition domain for the cellular Bcr protein (14). In the same study, phosphorylation of Bcr by the transforming Fes homolog v-Fps was shown to induce the association of Bcr with the Grb-2/Sos guanine nucleotide exchange complex, suggesting that Bcr may link Fes to the Ras signal transduction pathway (14). The SH2 domains of both c-Fes and v-Fps are critical positive regulators of kinase activity and may provide binding sites for effectors or regulatory proteins (15, 16). The Fes kinase domain contains tyrosine autophosphorylation sites, which may regulate both kinase activity and substrate recognition. Mutagenesis of the homologous autophosphorylation sites shared by c-Fes and v-Fps (Tyr-713) greatly diminishes kinase activity, indicating an important role for these sites in the regulation of enzymatic function (15, 17). Autophosphorylated tyrosine residues in the kinase domain may also represent binding sites for substrates with SH2 domains, such as the Ras GTPase-activating protein (18, 19). In this study, we describe the identification of Tyr-811 as the second principal Fes autophosphorylation site, and demonstrate for the first time that Tyr-811 as well as the other major autophosphorylation site (Tyr-713) are both utilized in vivo. In addition, we provide direct evidence that Fes autophosphorylation is an intermolecular event, suggesting that oligomerization may be required for Fes activation.

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**EXPERIMENTAL PROCEDURES**

Expression of GST-Fes Fusion Proteins and in Vitro Phosphorylation Reactions—Construction of plasmid vectors for the expression of Fes N-terminal amino acids 1-450, 1-347, and 1-126 as fusion proteins with GST is described elsewhere (14). To express Fes amino acids 225-540 and kinase domain residues 551-822 as GST fusion proteins, the coding sequences for these regions of Fes were amplified by PCR and cloned into the expression vector pGEX-2T (20). To generate the GST fusion protein containing the inactive Fes kinase domain, a Fes cDNA containing a point mutation in the ATP-binding site (Lys 590 to Met) was expressed in Escherichia coli and purified with glutathione agarose as described elsewhere (14, 15, 19). The proteins were eluted from the beads with 50 mM Tris-HCl, pH 9.0, containing 6 M urea and dialyzed extensively against 20 mM Hepes, pH 7.4. The concentration of each protein was determined by two-dimensional laser densitometry (Molecular Dynamics). Phosphorylation reactions were conducted in 60 μl of kinase buffer (20 mM Hepes, pH 9.0, 5 mM MgCl2, 5 mM MnCl2) containing 100 μM fusion protein, 10 μCi of [γ-32P]ATP (3,000 Ci/mmol; Du Pont NEN), and recombinant, immunopurified Fes (see below). Phosphorylated proteins were separated by SDS-PAGE and visualized by autoradiography.

Addition of the FLAG and GST Sequences to Fes—PCR was used to add the coding sequence of the 8-amino-acid FLAG epitope (DYKDDDDK) to the C-terminal region of full-length, wild-type Fes. The PCR reaction utilized a forward primer that maps to the 5’ end of the kinase domain and a reverse primer complementary to the C-terminal coding region of Fes. The reverse primer also contained the FLAG coding region and a unique restriction site (EcoRI) that allowed the PCR product to be ligated into the expression vector pGEX-2T (20). The resulting FLAG-Fes cDNA was utilized as the template to generate the mutants described below. A similar PCR-based procedure was used to add the FLAG sequence to the Fes N terminus. In this case, a forward oligonucleotide primer was employed that encoded a unique cloning site, a translational initiation sequence, and the FLAG coding sequence, followed by Fes homologous sequences.

To create a baculovirus construct for the expression of GST fusion proteins, the GST coding sequence, polylinker region, and stop codons of pGEX-2T (Pharmacia Biotech Inc.) were amplified by PCR and subcloned into the baculovirus transfer vector pVL1393 to create the vector pVL-GST. The GST-N-terminal region of Fes was subcloned into pVL-GST from an existing pGEX-2T construct (14). To create a transfer vector containing GST fused to full-length Fes, the DraII-EcoRI fragment of Fes was subcloned into the pVL-GST-N-Fes construct.

**RESULTS AND DISCUSSION**

Localization of Fes Autophosphorylation Sites in the C-Terminal Kinase Domain in Vitro—Previous phosphopeptide mapping studies of autophosphorylated Fes resulted in two phosphopeptides with either trypsin or Staphylococcus aureus V8 protease, suggesting that Fes can autophosphorylate at least two tyrosine residues in vitro (4, 15, 22). We have observed that one of the Fes autophosphorylation sites is located at Tyr-713, within the C-terminal catalytic domain (15). To determine which domain of Fes contains the additional tyrosine autophosphorylation site, we expressed a series of GST fusion proteins containing various combinations of the Fes unique N-terminal, SH2, and kinase domains in E. coli (Fig. 1A). Equimolar amounts of the purified GST-Fes fusion proteins were phosphorylated in vitro with recombinant Fes and [γ-32P]ATP and analyzed by SDS-PAGE and autoradiography. As shown in Fig. 1B, only the fusion proteins containing sequences derived from the Fes kinase domain were strongly phosphorylated by recombinant Fes.

The GST-Fes fusion proteins used in this experiment were eluted from glutathione-agarose beads in 6 M urea, followed by extensive dialysis (see “Experimental Procedures”). The GST-
phorylated cell lysates with the anti-FLAG monoclonal antibody and phosphorylated Fes were expressed as C-terminal FLAG fusion proteins in Sf-9 cells. The Fes fusion proteins were isolated from infected type Fes were expressed as C-terminal FLAG fusion proteins in Sf-9 cells. The SH2 domains (Fig. 2) are involved mutantsof Fes lacking the unique N-terminal or autophosphorylation sites are localized to the C-terminal regioninvolvedmutants of Fes lacking the unique N-terminal or SH2 domains (Fig. 2A). These mutants and full-length wild-type Fes were expressed as C-terminal FLAG fusion proteins in Sf-9 cells. The C-terminal FLAG epitope was autophosphorylated in vitro, cleaved with CNBr, and the immunoreactivity of the C-terminal fragment was tested using the anti-FLAG monoclonal antibody. As shown in Fig. 3D, the 4-kDa CNBr phosphopeptide was immunoprecipitated by the anti-FLAG antibody, confirming its identity as the C-terminal fragment. The 4-kDa phosphopeptide corresponding to the C-terminal fragment was used in the transphosphorylation reaction containing two candidate tyrosine residues (Tyr-799 and Tyr-811), respectively. To establish that the larger fragment contains Tyr-713, a mutant of Fes containing a Tyr substitution at this position was autophosphorylated in vitro and subjected to CNBr cleavage. As shown in Fig. 3C, cleavage of this mutant produced only the 4-kDa phosphopeptide, identifying the 10-kDa fragment as the one containing Tyr-713. To determine whether the 4-kDa phosphopeptide corresponds to the C-terminal CNBr fragment, we tested for the presence of the FLAG epitope within this fragment. Wild-type Fes containing the C-terminal FLAG epitope was autophosphorylated in vitro, cleaved with CNBr, and the immunoreactivity of the resulting peptides was tested using the anti-FLAG monoclonal antibody. As shown in Fig. 3D, the 4-kDa CNBr phosphopeptide was immunoprecipitated by the anti-FLAG antibody, confirming its identity as the C-terminal fragment. The third phosphopeptide produced in some of these experiments is approximately 14 kDa. Experiments described below indicate that this fragment is a partial digestion product containing the 10- and 4-kDa peptides.

Identification of Tyr-713 and Tyr-811 as Fes Autophosphorylation Sites in Vitro—Data described above identify Tyr-799, Tyr-811, or both as possible candidates sites for Fes autophosphorylation in vitro. To resolve this issue, we generated mutants with individual point mutations at these sites (Y799F and Y811F mutants) and with mutations of both sites (Y799F/
Trans-autophosphorylation of c-Fes

Fig. 3. Localization of Fes autophosphorylation sites to the C-terminal kinase domain by CNBr cleavage analysis. Fes proteins were expressed in Sf-9 cells, immunoprecipitated with the anti-FLAG monoclonal antibody M2, and phosphorylated in vitro with \(\gamma\)-\[^{32}\text{P}\]ATP. The labeled proteins were resolved by SDS-PAGE, excised from the gel, and subjected to cleavage with CNBr. The resulting peptide fragments were separated on 10–20% SDS-Tricine gradient gels and visualized by autoradiography. A, scale diagram of Fes showing the location of all potential CNBr cleavage sites (arrows). The kinase domain is enlarged for clarity, and indicates the positions of Tyr residues 713, 799, and 811. The locations of phosphopeptides 1 and 2 as well as the C-terminal FLAG epitope are also indicated. B, CNBr cleavage analysis of wild-type (WT) Fes and of the deletion mutants \(\Delta N\) and \(\Delta SH2\). Two peptides of 10 kDa (peptide 1) and 4 kDa (peptide 2) were consistently observed and are indicated by the arrows. Additional higher molecular weight peptides were observed in some cases and correspond to incomplete digestion products. C, comparative CNBr cleavage analysis of Fes wild-type and Tyr-713 \(\rightarrow\) Phe (Y713F) auto-phosphorylation site mutant. D, immunoprecipitation of peptide 2 with the anti-FLAG monoclonal antibody. \[^{32}\text{P}\]-Labeled CNBr fragments were prepared from wild-type Fes, lyophilized, and resuspended in immunoprecipitation buffer containing immobilized anti-FLAG monoclonal antibody (M2). Following incubation and washing, the bound peptide was eluted in gel loading buffer and resolved by Tricine gradient gel electrophoresis. An aliquot of the starting peptide mixture is shown for comparison (WT).

Fig. 4. Fes Tyr-811 is autophosphorylated in vitro. Fes mutants with Tyr to Phe substitutions at positions 799, 811, or both were expressed in the baculovirus/Sf-9 cell system, immunoprecipitated, and incubated in vitro with \(\gamma\)-\[^{32}\text{P}\]ATP. The autophosphorylated proteins were subjected to CNBr cleavage analysis as described in the legend to Fig. 3. The resulting CNBr fragments were resolved on a Tricine gradient gel and visualized using storage phosphor technology. Molecular mass standards are shown on the left, and the positions of Fes phosphopeptides 1 and 2 are indicated on the right. The position of the 14-kDa partial cleavage product containing peptides 1 and 2 is also shown (1,2). WT, wild type.

Y811F mutant; see Fig. 2). Wild-type and mutant forms of Fes were autophosphorylated in vitro and subjected to CNBr cleavage analysis. As shown in Fig. 4, Phe substitution of Tyr-799 alone did not affect the observed CNBr cleavage pattern. However, substitution of Tyr-811 led to a complete loss of the 4-kDa peptide, identifying this residue as a second c-Fes autophosphorylation site in vitro. As expected, an identical result was obtained with the Y799F/Y811F double mutant. Identification of Tyr-811 as a second site of Fes autophosphorylation is consistent with previous phosphopeptide mapping studies of c-Fes, which consistently produced two phosphopeptides (4, 15, 22).

Analysis of Fes Phosphorylation in Vivo—To investigate the phosphorylation of Fes in living cells, we expressed Fes with a C-terminal FLAG epitope tag in the human embryonic cell line 293T (25) and labeled the cells with \[^{32}\text{P}\]PO\(_4\). Fes was immunoprecipitated, resolved by SDS-PAGE, and analyzed by CNBr cleavage. As shown in Fig. 5A (left panel, C-FLAG lane), the 14-, 10-, and 4-kDa bands were observed, consistent with the in vitro result. Phosphoamino acid analysis shows that each of these peptides contains phosphotyrosine (Fig. 5B). A shorter exposure of this gel revealed that the major labeled fragment resulting from the in vivo analysis is approximately 34 kDa (Fig. 5A, right panel). Phosphoamino acid analysis of the 34-kDa fragment revealed that it contains phosphoserine (Fig. 5B). This result is in good agreement with previous studies of Fes phosphorylation in vivo, in which phosphoserine was the predominant or exclusive amino acid observed (4, 28). Based on the predicted sites for CNBr cleavage of Fes, this 34-kDa serine phosphopeptide is likely to contain the SH2 domain and flanking N- and C-terminal residues (see Fig. 3A). Phosphorylation of this region by a serine/threonine kinase may contribute to the negative regulation of Fes tyrosine kinase activity. Previous studies have shown that Fes has weak transforming and tyrosine kinase activities in vivo (28, 29).

To investigate whether the 4-kDa tyrosine phosphopeptide was derived from the Fes C-terminal region in vivo, we also expressed Fes as an N-terminal FLAG fusion protein in 293T cells (Fig. 5A, N-FLAG lane). Following \[^{32}\text{P}\]PO\(_4\) labeling, the CNBr cleavage pattern of the N-FLAG and C-FLAG Fes proteins were compared. As shown in Fig. 5A, the 4-kDa phosphopeptide produced by CNBr cleavage of the C-FLAG Fes protein shifted down in the gel when digested from N-FLAG Fes. The size of the shifted band is consistent with the C-terminal fragment without the FLAG epitope. A downward shift was also observed with the 14-kDa fragment, consistent with the assignment of this fragment as a partial digestion product containing the 10- and 4-kDa peptides.

To determine whether Tyr-713 and Tyr-811 are phosphorylated in vivo, mutant Fes proteins containing Phe substitutions at these positions were expressed in 293T cells, labeled with \[^{32}\text{PO}_{4}\], and subjected to CNBr cleavage analysis as described above. As shown in Fig. 6, mutation of Tyr-713 led to the complete disappearance of the 10-kDa phosphopeptide, indicating that Tyr-713 is an autophosphorylation site in vivo. Mutagenesis of Tyr-713 also resulted in a loss of the 4-kDa peptide, indicating that phosphorylation of Tyr-713 is required for Tyr-811 autophosphorylation to occur in vivo. Additional evidence supporting this hypothesis is presented below. The CNBr map of the Y713F mutant also lacked the 14-kDa band, consistent with the identity of this fragment as a partial digestion product containing Tyr-713. Mutagenesis of Tyr-811 resulted in the loss of the 4-kDa phosphopeptide, identifying Tyr-811 as an in vivo autophosphorylation site. Mutagenesis of Tyr-799 was without effect, while the Tyr-799/811 double mutant produced a cleavage pattern identical to that observed with the Tyr-811 mutant. These data support the conclusion that the same tyrosine autophosphorylation sites are utilized in vitro and in vivo, and identify for the first time in vivo autophosphorylation sites for Fes. None of these mutations affected Ser phosphorylation of Fes in vivo, as judged by the equal intensities of the 34-kDa fragments from each of the mutants (Fig. 6, peptide 3).
Autophosphorylation of Tyr-713 Is Required for Full Activation of c-Fes—Previous studies of the Fes Y713F mutant indicate that the extent of autophosphorylation of this mutant is reduced by more than 90% relative to the wild-type protein (15, 30). In addition, this mutant shows reduced kinase activity toward model substrates in vitro. To determine whether Tyr-811 is also involved in the regulation of kinase activity, the autophosphorylation capacity of Fes proteins carrying either individual Y713F or Y811F point mutations or both was evaluated in Sf-9 cells. As shown in Fig. 7A, both proteins with Tyr-713 mutations showed greatly reduced autophosphorylation capacity. On the other hand, the Tyr-811 single mutant still reacted strongly with the anti-phosphotyrosine antibody, suggesting that Tyr-811 does not regulate the phosphorylation of Tyr-713.

To investigate the extent of Y811F autophosphorylation in comparison to wild-type Fes more quantitatively, an equal amount of each immunopurified protein was incubated in vitro with [γ-32P]ATP and the extent of autophosphorylation was monitored over time. In two separate experiments, the extent of Y811F autophosphorylation was observed to be 60–70% of the wild-type level (data not shown). This result is consistent with the distribution of 32P in the 10-kDa and 4-kDa phosphoamino acids observed both in vivo and in vitro (approximately a 2:1 Tyr-713:Tyr-811 ratio). These results support the conclusion that Tyr-811 does not influence the autophosphorylation of Tyr-713.

To determine the effect of autophosphorylation site mutations on substrate phosphorylation, these mutants were co-expressed with the Bcr protein, which has recently been identified as a substrate for c-Fes and its transforming homolog, v-Fps(14). As shown in Fig. 7B, mutagenesis of Tyr-713 greatly reduced Tyr phosphorylation of Bcr, whereas mutagenesis of Tyr-811 was without effect. Taken together, these results indicate that autophosphorylation of Tyr-713 is required for autophosphorylation of Tyr-811 and for maximal substrate phosphorylation, as observed previously with the model substrate enolase in vitro (15). Autophosphorylation of Tyr-811 may create a docking site for signaling proteins with SH2 domains.
Autophosphorylation of Fes is an intermolecular event—a final mechanistic question that we wished to address was whether autophosphorylation occurs by an intra- or intermolecular mechanism. Demonstration that Fes autophosphorylates in an intermolecular fashion would support a model for activation similar to that proposed for growth factor receptor tyrosine kinases (26, 27) and for non-receptor tyrosine kinases that associate with cytokine receptors such as those of the Jak kinase family (31, 32). To answer this question, we co-expressed a kinase-inactive form of full-length Fes (K590E mutant; see Fig. 2) with a catalytically active GST/Fes fusion protein in Sf-9 cells. The GST/Fes fusion protein was used because it can be readily distinguished from the K590E mutant on Western blots (120 kDa versus 93 kDa, respectively). The GST/Fes and Fes-KE proteins were immunoprecipitated and analyzed for the presence of phosphotyrosine by immunoblotting. As shown in Fig. 8, expression of the Fes kinase-inactive mutant alone resulted in no detectable tyrosine autophosphorylation. However, co-expression of the kinase-inactive mutant with the active GST/Fes fusion protein resulted in transphosphorylation of the mutant to approximately the same extent as the wild-type protein. CNBr cleavage analysis of Fes-KE following transphosphorylation by GST-Fes produced a cleavage pattern identical to that observed with autophosphorylated wild-type Fes (data not shown). Although this experiment does not rule out the possibility of intramolecular autophosphorylation, it is consistent with a model of Fes activation by oligomerization and transphosphorylation.

Data presented here show that the Fes tyrosine kinase autophosphorylates two kinase domain Tyr residues (713 and 811) both in vitro and in vivo. By analogy to growth factor receptor tyrosine kinases, autophosphorylation of these Fes tyrosine residues may form binding sites for substrate proteins with SH2 domains. Structural studies of the SH2 domains of Src and other proteins show that SH2 binding specificity is often conferred by the amino acids immediately C-terminal to the phosphotyrosine residue (33). These findings led Songyang et al. (34, 35), to predict possible SH2 domain binding specificities using a degenerate phosphopeptide library in which every amino acid (except Trp or Cys) was represented at the +1, +2, and +3 positions relative to the Tyr(P) residue. Phosphopeptides were selected from the library with recombinant SH2 domains, and their sequences were determined directly. Comparison of these predicted SH2-binding motifs with the amino acid sequences immediately adjacent to Fes Tyr-811 (Gln-Glu-Leu) revealed partial matches to ten predicted SH2 binding motifs. A particularly striking example was the C-terminal SH2 domain of Syk, which is a perfect match for the Fes Tyr-811 SH2 binding sequence and one predicted by Songyang et al. (35). Syk is a member of a unique tyrosine kinase family with tandem N-terminal SH2 domains that exhibits hematopoietic expression and has been implicated in integrin, cytokine, and antigen receptor signal transduction (36–38). Recent evidence suggests that multiple cytoplasmic tyrosine kinases are recruited to antigen and cytokine receptors in response to ligand binding (32). SH2-phosphotyrosine interactions may allow for interaction among the tyrosine kinases involved in the receptor-kinase complex (39), which may include Fes in the case of several hematopoietic cytokine receptors (see Introduction).

By contrast to Tyr-811, the +1, +2, +3 motif adjacent to Tyr-713 (Ala-Ala-Ser) showed very little resemblance to any of the predicted SH2 domain binding motifs. Thus, Tyr-713 may function primarily to regulate kinase activation rather than substrate recruitment. Data shown in Fig. 7 are consistent with this hypothesis; mutants of Fes containing the Tyr-713 to Phe substitution showed greatly reduced autophosphorylation capacity and ability to transphosphorylate Bcr.

Figs. 1 and 8 provide evidence that Fes can autophosphorylate via an intermolecular mechanism. These results are significant in the context of possible physiological mechanisms of Fes activation by cytokines. Recent studies of cytokine receptors suggest a general mechanism of activation that involves ligand-induced receptor dimerization or oligomerization, followed by activation of multiple receptor-associated tyrosine kinases of the cytoplasmic class (31, 40). Receptor oligomerization is likely to activate the associated kinases by a transphosphorylation mechanism reminiscent of growth factor receptor tyrosine kinases (26, 32, 41). Recruitment of Fes into an activated cytokine receptor complex could lead to Fes activation by this mechanism. Because cytokines control pleiotropic responses in myeloid cells (growth, differentiation, and function), their receptors must be able to activate a diverse array of signal transduction pathways that control these responses. The ultimate biological effect of a given cytokine is likely to be dependent upon the complement of signaling molecules present in the cell at the time of challenge with the factor. Activation of Fes in the context of a myeloid progenitor may contribute to differentiation signal transduction.

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REFERENCES

1. Groffen, J., Heisterkamp, N., Shibuya, M., Hanafusa, H., and Stephenson, J. (1983) Virology 125, 480–486
2. Smithgall, T. E., Yu, G., and Glazer, R. I. (1988) J. Biol. Chem. 263, 15050–15055
3. Feldman, R. A., Gabriolev, J. L., Tan, J. P., Moore, M. A. S., and Hanafusa, H. (1985) Proc. Natl. Acad. Sci. U.S.A. 82, 2379–2383
4. MacDonald, I., Levy, J., and Pawson, T. (1985) Mol. Cell. Biol. 5, 2543–2551
5. Yu, G., Smithgall, T. E., and Glazer, R. I. (1989) J. Biol. Chem. 264, 10276–10281
6. Carmier, J. F., and Samarut, J. (1987) Cell 44, 359–365
7. Hanazono, Y., Shibuya, S., Sasaki, K., Mano, H., Yazaki, Y., and Hirai, H. (1993) Blood 81, 3103–3116
8. Hanazono, Y., Shibuya, S., Sasaki, K., Mano, H., Miyajima, A., Arai, K., Yazaki, Y., and Hirai, H. (1994) EMBO J. 13, 1641–1646
9. Izaurralde, K., Feldman, R. A., Greer, P., and Harada, N. (1994) J. Biol. Chem. 269, 16823–16829
10. Matsuda, T., Fukuda, T., Takahashi-Titaka, M., Okuizama, Y., Fujitani, Y., Hanazono, Y., Hirai, H., and Hirano, T. (1995) J. Biol. Chem. 270, 11037–11039
11. Rao, P., and Mufson, R. A. (1995) J. Biol. Chem. 270, 6886–6893
