Oligomerization of the Fes Tyrosine Kinase

EVIDENCE FOR A COILED-COIL DOMAIN IN THE UNIQUE N-TERMINAL REGION

Renee D. Read‡, Jack M. Lionberger‡, and Thomas E. Smithgall§

From the Eppley Institute for Research in Cancer and the Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska 68198-6805

The c-fes proto-oncogene encodes a non-receptor tyrosine kinase (Fes) that has been implicated in cytokine receptor signal transduction and myeloid differentiation. Previous work from our laboratory has shown that Fes autophosphorylates via an intramolecular mechanism more commonly associated with growth factor receptor tyrosine kinases. Analysis of the Fes amino acid sequence with the COILS algorithm indicates that the N-terminal region of the protein has a very high probability of forming coiled-coil structures often associated with oligomeric proteins. These findings suggest that oligomerization may be a prerequisite for trans-autophosphorylation and activation of Fes. To establish whether the active form of Fes is oligomeric, we performed gel-filtration experiments with recombinant Fes and found that it eluted as a single symmetrical peak of approximately 500 kDa. No evidence of the monomeric, 93-kDa form of the protein was observed. Deletion of the unique N-terminal domain (amino acids 1–450, including the coiled-coil homology region) completely abolished the formation of oligomers. Furthermore, co-precipitation assays demonstrated that an immobilized glutathione S-transferase fusion protein containing the Fes N-terminal region bound to full-length Fes but not to a mutant lacking the N-terminal region. Similarly, a recombinant Fes N-terminal domain protein was readily cross-linked in vitro, whereas the SH2 and kinase domains were refractory to cross-linking. Incubation of wild-type Fes with a kinase-inactive Fes mutant or with the isolated N-terminal region suppressed Fes autophosphorylation in vitro, suggesting that oligomerization may be essential for autophosphorylation of full-length Fes. The presence of an oligomerization function in the Fes family of tyrosine kinases suggests a novel mechanism for non-receptor protein-tyrosine kinase regulation.

The c-fes proto-oncogene encodes a non-receptor protein-tyrosine kinase (Fes) that is expressed predominantly in hematopoietic cells (1–5). Several lines of evidence suggest that Fes may influence myeloid differentiation commitment. Transfection of the myeloid leukemia cell line K-562 with c-Fes resulted in growth suppression and terminal differentiation (4). K-562 cells are derived from chronic myelogenous leukemia and express the p210 form of Bcr/Abl but not Fes. In this context, restoration of Fes is sufficient to suppress transformation by Bcr/Abl in addition to generating a more differentiated phenotype. Recent studies from our laboratory have shown that normal Bcr is a substrate for Fes and suggest that Fes may interact directly with Bcr/Abl as well (5, 6). In related studies, an activated viral homolog of Fes (v-Fps) was shown to induce differentiation of chicken bone marrow cells to mature macrophages in the absence of CSF-1 (7). Thus, activation of Fes is sufficient to induce terminal differentiation of normal as well as leukemic progenitor cells.

Although Fes was originally thought to be restricted to hematopoietic cells, more recent work has uncovered a broader pattern of expression. First, studies of c-fes expression in developing mouse embryos showed that it was not only present in hematopoietic cells but also in non-hematopoietic tissues where expression is not observed in the adult (8). In particular, c-fes transcripts were detected in embryonic lung, liver, spinal cord, skin, gut, heart, and kidney. These results suggest that Fes may play a critical role in the early development of many tissues in addition to its role in hematopoiesis. A second study reports the surprising finding that Fes may be involved in the regulation of the vascular endothelium. Expression of an activated form of Fes in transgenic mice induced hypervascularity which progressed to multifocal hemangiomas (9). Expression of endogenous Fes was also observed in primary human vascular endothelial cells at levels comparable to those detected in myeloid cells. These results show that in addition to hematopoietic cells, Fes expression is also intrinsic to cells of the vascular endothelial lineage and imply that Fes may play a role in angiogenesis. A more recent study demonstrated Fes expression in other adult tissues, including neuronal and epithelial cells (10). The function of Fes in these tissues is currently unknown.

A common feature of receptor tyrosine kinases is that they are activated by oligomerization in response to ligand binding (11, 12). The oligomerized receptor is then capable of trans-autophosphorylation, which is essential for downstream signaling through the recruitment of effector proteins with SH2 domains (13, 14). The mechanism regulating the kinase activity of Fes and other non-receptor tyrosine kinases is less clear. Fes tyrosine kinase activity is tightly regulated in cells, and even under conditions of overexpression, very little tyrosine autophosphorylation is observed (3, 15, 16). Tyrosine autophosphorylation of Fes appears to be essential for biochemical activation and biological function, as c-Fes and v-Fps mutants with Phe substitutions of the major autophosphorylation site show reduced enzymatic and biological activity (16–19). Recently, we demonstrated that Fes autophosphorylation occurs by an in-
Oligomerization of c-Fes

18499

termolecular mechanism, suggesting that Fes activation occurs as a result of transphosphorylation in a manner analogous to receptor tyrosine kinases (16). Consistent with this idea is the observation that Fes associates with multiple hematopoietic cytokine receptors and is activated in response to cytokine binding (20–24), which stimulates the oligomerization of cytokine receptors (25–27). Alternatively, Fes may have an intrinsic oligomerization capacity that may drive autophosphorylation and activation. In this study, we demonstrate that the Fes tyrosine kinase is an oligomeric protein. Amino acid sequences found in the unique N-terminal domain mediate oligomerization and exhibit striking homology to coiled-coil domains associated with other classes of oligomeric proteins (28, 29). Furthermore, we observed that a kinase-inactive mutant of Fes as well as the isolated N-terminal region were able to associate with wild-type Fes and suppress autophosphorylation, consistent with a model for Fes activation that requires oligomerization and trans-autophosphorylation. The presence of an oligomerization domain in Fes suggests a novel mechanism for regulation of its protein-tyrosine kinase activity.

EXPERIMENTAL PROCEDURES

Expression of Wild-type and Mutant Fes Proteins in the Baculovirus System—Expression of Fes wild-type, K590E, Y713F, 

R

I

RI restriction fragment was isolated from the Fes K590E mutant cDNA (17) and swapped with the corresponding fragment in the wild-type ΔN mutant. The resulting ΔN-KE Fes cDNA was subcloned into the baculovirus transfer vector pVL1392 and used to generate a recombinant baculovirus as described elsewhere (16, 30).

Gel Filtration—A Sephacryl S-300 column (1.5 × 75 cm) was equilibrated with S-300 buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 10 mM MgCl₂) and calibrated with thyroglobulin (669 kDa), ferritin (440 kDa), catalase (232 kDa), and bovine serum albumin (67 kDa). SF-9 cells expressing full-length Fes-FLAG (16) were sonicated in S-300 buffer, and the lysate was clarified by centrifugation at 100,000 × g for 1 h at 4 °C. The clarified supernatant (0.5 ml) was applied to the Sephacryl S-300 column and eluted with S-300 buffer at a flow rate of 0.25 ml/min.

Gel-filtration experiments were conducted to determine whether the active form of Fes is oligomeric. Recombinant Fes was expressed in SF-9 insect cells, and clarified lysates from the infected cells were applied to a Sephacryl S-300 column calibrated with thyroglobulin (669 kDa), ferritin (440 kDa), catalase (232 kDa), and bovine serum albumin (67 kDa). The position of Fes in the elution profile was determined by immunoblot analysis. As shown in Fig. 1A, Fes eluted from the column as a single oligomeric peak of approximately 500 kDa. No peak was observed in 93-kDa range, which corresponds to the unit molecular mass of Fes. This result clearly shows that the active form of Fes is oligomeric.

The Fes kinase has a modular structure consisting of unique N-terminal, SH2, and kinase domains. To determine which regions of the protein contribute to oligomerization, Sephacryl S-200 gel-filtration experiments were performed on recombinant Fes proteins either lacking the unique N-terminal domain (ΔN, Fig. 1B) or on the isolated N-terminal region itself without the SH2 and kinase domains (N-term, Fig. 1C). The Fes N-terminal deletion mutant eluted from the column as a monomer of approximately 40 kDa, indicating that the SH2 and kinase domains are not involved in oligomerization. By contrast, the N-terminal region was exclusively oligomeric, with no evidence for the 50-kDa monomeric form of this protein. These data suggest that structural features found within the unique N-terminal domain regulate oligomerization of the Fes molecule.

Computer Analysis Reveals a Potential Coiled-coil Oligomerization Motif in the Fes Unique N-terminal Region—Coiled-coil domains often mediate protein oligomerization (31). This structural element is comprised of an amphipathic α-helix with a characteristic heptad repeat in which the first and fourth positions are occupied by hydrophobic amino acids. As a result, these residues are positioned on the same face of the helix and pack together to form a hydrophobic core. The remaining amino acid sequences were analyzed using the COILS algorithm (28, 29). A gliding window of 28 residues was used for the analysis presented. A detailed description of the program is available. Co-precipitation Assay for Fes Oligomerization—SF-9 cells were co-infected with recombinant baculoviruses encoding the Fes N-terminal region fused to GST (GST-N-Fes) and wild-type or mutant Fes proteins with the FLAG epitope fused to their C termini. Forty-eight hours postinfection, cells were lysed by sonication as described (16), and the GST-N-Fes protein was precipitated with glutathione-agarose. The glutathione-agarose beads were washed with RIPA buffer (16), and the presence of associated Fes was detected by immunoblotting with antibodies to the FLAG epitope (M2 antibody). As a negative control, parallel experiments were conducted in which Fes proteins were co-expressed with GST. Expression of GST and GST-N-Fes was verified by SDS-PAGE and Coomassie staining of an aliquot of each glutathione-agarose precipitate.

Chemical Cross-linking—The Fes N-terminal domain (amino acids 1–450) and the SH2/kinase region (amino acids 451–822) were expressed as FLAG fusion proteins in SF-9 cells. Cells were lysed by sonication (16) and diluted 1:10 with lysis buffer, and the bifunctional cross-linking reagent disuccinimidyl suberate (DSS) was added from a concentrated stock solution in Me₆SO to final concentrations ranging from 0.1 to 2.0 mM. The reactions were incubated for 30 min at room temperature and were stopped by heating in SDS-PAGE sample buffer. The effect of the cross-linker on the Fes proteins was analyzed by immunoblotting with antibodies to the FLAG epitope. Parallel incubations with Me₆SO alone were run as a negative control.

Kinase Assays—Lysates from SF-9 cells expressing a GST-Fes fusion protein, a kinase-inactive mutant of Fes (Fes-KE) (17), the isolated Fes N-terminal domain, or a kinase-inactive N-terminal deletion mutant (ΔN-KE) were mixed in various ratios and incubated for 1 h at 4 °C. Fes proteins were immunoprecipitated with the anti-FLAG monoclonal antibody and incubated with 10 μCi of [γ-32P]ATP (3,000 Ci/mmol) in 40 μl of kinase buffer (50 mM Hepes, pH 7.4, 10 mM MgCl₂) for 30 min at 30 °C. Reactions were stopped by heating in SDS-PAGE sample buffer, and phosphorylated proteins were resolved by SDS-PAGE. Relative protein levels were quantitated by twodimensional laser densitometry of the Coomassie-stained gel. The relative extent of autophosphorylation of GST-Fes was analyzed by storage phosphor technology (Molecular Dynamics PhosphorImager). GST-Fes autophosphorylation was corrected for protein levels and is plotted as percent of control activity (16). This finding suggests that Fes activation may require oligomerization in a manner analogous to growth factor receptor tyrosine kinases (11, 12). Gel-filtration experiments were conducted to determine whether the active form of Fes is oligomeric. Recombinant Fes was expressed in SF-9 insect cells, and clarified lysates from the infected cells were applied to a Sephacryl S-300 column calibrated with thyroglobulin (669 kDa), ferritin (440 kDa), catalase (232 kDa), and bovine serum albumin (67 kDa). The position of Fes in the elution profile was determined by immunoblot analysis. As shown in Fig. 1A, Fes eluted from the column as a single oligomeric peak of approximately 500 kDa. No peak was observed in 93-kDa range, which corresponds to the unit molecular mass of Fes. This result clearly shows that the active form of Fes is oligomeric.
acids are often hydrophilic and allow for solvation of the structure. To determine whether a coiled-coil domain exists within the Fes protein, we analyzed the Fes amino acid sequence using the COILS algorithm (28, 29). This program measures the probability that a given amino acid and its surrounding sequence exists as an amphipathic \( \alpha \)-helix capable of forming a coiled-coil and plots the result against the amino acid position within the sequence. As shown in Fig. 2, a region spanning Fes N-terminal amino acids 128–169 is predicted by the program to have a nearly absolute probability of forming a coiled-coil structure, consistent with the gel-filtration results (Fig. 1). Other regions within the N-terminal domain were also predicted to possess coiled-coil forming motifs, although with lower probability relative to the 128–169 region. By contrast, the SH2 and kinase domains are essentially devoid of any predicted coiled-coil domains. As shown in more detail below, the prediction that the unique N-terminal region of Fes confers its oligomeric structure is confirmed by several additional lines of experimental evidence.

**Co-precipitation Assays and Chemical Cross-linking Identify an Oligomerization Domain in the Fes N-terminal Region**—As described above, gel-filtration experiments and computer analysis suggest that the Fes N-terminal region is responsible for oligomerization of the protein. To test this hypothesis further, a GST fusion protein containing the N-terminal region of Fes (or GST alone as a negative control) was co-expressed with Fes in a baculovirus/Sf-9 cell system. The GST-N-terminal fusion protein was precipitated from the co-infected cell lysates with glutathione-agarose and the presence of associated Fes was determined by immunoblotting. As shown in Fig. 3, the GST-N-Fes fusion protein readily associated with full-length Fes in the co-precipitation assay, whereas no association was observed with GST itself. This result provides direct evidence that the Fes N-terminal region is capable of protein-protein interactions and is likely to mediate the oligomerization of Fes.

To identify the region of the Fes protein that associates with the GST-N-terminal fusion protein, co-precipitation experiments were conducted using deletion mutants of Fes lacking the N-terminal (\( \Delta N \)), SH2 (\( \Delta SH2 \)), or kinase (\( \Delta KIN \)) domains. As shown in Fig. 3, the \( \Delta SH2 \) and \( \Delta KIN \) mutants readily associated with the Fes N-terminal region, indicating that these domains are dispensable for oligomerization. By contrast, the \( \Delta N \) mutant was unable to associate with the GST-N-terminal domain fusion protein, indicating that the N-terminal region contains the structural elements essential for oligomerization to occur.

We also investigated whether the phosphorylation state of Fes influenced oligomerization using the co-precipitation assay. For these experiments, we used a kinase-inactive mutant of Fes with a glutamic acid residue in place of the critical lysine residue in the ATP binding site (K590E mutant) (17) as well as a mutant with a phenylalanine in place of tyrosine at the major autophosphorylation site (Y713F mutant). As shown in Fig. 3, both of these mutants readily associated with the Fes N-termi-
FIG. 3. Formation of stable complexes between Fes and its unique N-terminal region. A GST fusion protein containing the Fes N-terminal domain (GST-N-Fes; left panels) or GST alone (right) were co-expressed with wild-type Fes (WT) or with Fes mutants lacking an active kinase domain (K590E), autophosphorylation site (Y713F), N-terminal region (ΔN), SH2 domain (ΔSH2), or kinase domain (ΔKIN). All of these Fes proteins are tagged with the FLAG epitope on their C termini. A, GST-N-Fes or GST were precipitated with glutathione-agarose and washed, and associated Fes proteins were visualized by immunoblotting with an anti-FLAG antibody. B, Fes protein levels in the crude cell extracts were determined by immunoblotting. Equivalent expression of GST-N-Fes and GST were verified by Coomassie-staining aliquots of the glutathione-agarose precipitates (data not shown).

Chemical cross-linking experiments were also performed as an alternative method to demonstrate the Fes domain responsible for oligomerization. The Fes N-terminal domain and the SH2/kinase region were expressed independently as FLAG fusion proteins in SF-9 cells. Lysates from the cells were diluted 1:10 and incubated in the presence and absence of the bifunctional cross-linking agent DSS as described in the text. The major 150-kDa cross-linked product observed with the N-terminal region is indicated by the arrow.

FIG. 4. Chemical cross-linking. The Fes N-terminal (N-term) region (~50 kDa) and the SH2-kinase region (~40 kDa) were expressed as FLAG fusion proteins in SF-9 cells and incubated in the presence (+) and absence (−) of the bifunctional cross-linking agent DSS as described in the text. The major 150-kDa cross-linked product observed with the N-terminal region is indicated by the arrow.

kinase activation and the mechanism is intermolecular, then co-expression of Fes with a kinase-inactive mutant would be predicted to suppress autophosphorylation by forming oligomers incapable of maximal transphosphorylation. To test this hypothesis, we expressed full-length Fes as a GST fusion protein as well as the kinase-inactive form of Fes (Fes-KE) independently in SF-9 cells. Clarified lysates from the infected cells were mixed together in various ratios, and the resulting protein complexes were immunoprecipitated and the extent of GST-Fes autophosphorylation was measured by addition of [γ-32P]ATP. As shown in Fig. 5A, the autophosphorylation capacity of Fes decreased in proportion to the amount of the kinase-inactive mutant added to the reaction. This result supports the hypothesis that oligomerization and transphosphorylation are necessary for Fes autophosphorylation to occur.

To demonstrate that the kinase inhibition shown in Fig. 5A is dependent upon the Fes N-terminal domain, similar in vitro kinase assays were performed with GST-Fes following incubation with either the isolated N-terminal domain or a kinase-inactive Fes mutant lacking the N-terminal region (ΔN-KE). As shown in Fig. 5B, the N-terminal region alone was sufficient to suppress GST-Fes autophosphorylation, and the ΔN-KE mutant was without effect.

Data shown in Fig. 5 suggest that GST-Fes and the kinase-inactive mutant form mixed oligomers. To test this possibility, lysates from SF-9 cells co-expressing GST-Fes and Fes-KE, the Fes N-terminal region or ΔN-KE were incubated with glutathione-agarose. Following incubation and washing, GST-Fes-associated proteins were visualized by immunoblotting. As shown in Fig. 6, Fes-KE as well as the isolated N-terminal region readily co-precipitated with GST-Fes, whereas the ΔN-KE mutant did not. None of these proteins bound to GST alone. These results are consistent with the conclusion that mixed oligomer formation is required for the inhibition of kinase activity by the kinase-inactive Fes mutants.

DISCUSSION

Data presented in this paper demonstrate for the first time that the c-Fes tyrosine kinase is an oligomeric protein and are consistent with a model for Fes activation that requires oligomerization and transphosphorylation. Gel-filtration, chemical cross-linking, and co-precipitation experiments all clearly demonstrate the intrinsic capacity of Fes to form oligomers. Gel-filtration experiments suggest that full-length Fes is capable of forming large oligomers containing as many as five Fes molecules (Fig. 1A). Similarly, gel-filtration of the isolated N-
Fig. 5. Suppression of GST-Fes autophosphorylation by a kinase-defective Fes mutant (Fes-KE) and the Fes N-terminal region (N-term). A, Sf-9 cell lysates containing GST-Fes or Fes-KE (both tagged on their C termini with the FLAG epitope) were mixed together in various ratios. Fes protein complexes were immunoprecipitated with the M2 anti-FLAG antibody, incubated with \( ^{32}P \)ATP, and resolved by SDS-PAGE. Protein levels were quantitated by laser densitometry of the Coomassie-stained gel (inset). Autophosphorylation of GST-Fes was quantitated by storage phosphor technology. GST-Fes autophosphorylation was corrected for protein levels and is plotted as percent of control quantitated by storage phosphor technology. GST-Fes autophosphorylation was assayed in immune complexes following incubation with Fes-KE, the isolated Fes N-terminal region (N-term), or a kinase-inactive mutant lacking the N-terminal domain (ΔN-KE) as described in A. Results shown represent the average of two independent determinations. Immunoblots were performed on all cell lysates to verify equivalent expression of the Fes proteins (data not shown).

Oligomerization of c-Fes

Our data clearly indicate that the unique N-terminal region is responsible for the oligomerization of Fes. Computer analysis of this region shows the presence of a heptad repeat pattern of hydrophobic and hydrophilic amino acids typical of coiled-coil oligomerization domains (31). One of these regions (amino acids 128–169) scored with a near absolute probability of being a coiled-coil former. A small FLAG fusion protein containing this putative coiled-coil domain was readily cross-linked by DSS, producing dimers, trimers, and tetramers (data not shown). Other potential coiled-coil-forming sequences were also detected by the COILS program, although with lower probability. The possibility that Fes may contain more than one coiled-coil domain suggests a mode of kinase regulation involving interconversion of a monomeric, inactive form of the protein with the oligomeric, active form of the protein observed here. A precedent for this type of regulation is provided by the heat shock transcription factor, HSF-1 (33). In this case, the latent form of the protein has been proposed to exist as a monomer, in which its multiple coiled-coil domains are occupied by molecular chaperones (34).

Oligomerization domains have also been implicated in the biological activation of the c-Abl tyrosine kinase in various human leukemias. The best characterized example is Bcr/Abl, the chimeric oncoprotein that results from the Philadelphia chromosome translocation first associated with chronic myelogenous leukemia (35). The N-terminal, Bcr-derived portion of Bcr/Abl mediates the formation of large oligomeric Bcr/Abl complexes (36, 37). A specific Bcr domain with homology to coiled-coil oligomerization sequences (amino acids 1–63) has been shown to mediate homotrimer formation in vitro (36). Tetramerization of Bcr/Abl via this domain correlates with F-actin binding, leading to association of Bcr/Abl with the actin cytoskeleton. Oligomerization has been proposed to enhance the affinity of Bcr/Abl for F-actin via a triple-stranded coiled-coil structure; this trimeric form of the protein shows greatly enhanced affinity for DNA. In the case of Fes, interaction with a host factor may suppress kinase activity in intact cells by preventing formation of the active oligomer. As with HSF-1, Fes has recently been shown to interact with HSP70 and other molecular chaperones (34).
leukemia has been reported recently that activates Abl by a similar mechanism (40). This translocation event fuses c-Abl to the N-terminal region of the TEL transcription factor, resulting in oligomerization, kinase activation, and cytoskeletal localization. Thus, oligomerization may represent an important activating mechanism for both normal and transforming tyrosine kinases of the non-receptor class.

REFERENCES
1. Smithgall, T. E., Yu, G., and Glazer, R. I. (1988) J. Biol. Chem. 263, 15050–15055
2. Feldman, R. A., Gabrilove, J. L., Tam, J. P., Moore, M. A. S., and Hanafusa, H. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 2379–2383
3. MacDonald, I., Levy, J., and Pawson, T. (1985) Mol. Cell. Biol. 5, 2543–2551
4. Yu, G., Smithgall, T. E., and Glazer, R. I. (1989) J. Biol. Chem. 264, 10276–10281
5. Maru, Y., Peters, K. L., Afar, D. E. H., Shibuya, M., Witte, O. N., and Smithgall, T. E. (1995) Mol. Cell. Biol. 15, 835–842
6. Li, J., and Smithgall, T. E. (1996) J. Biol. Chem. 271, 32930–32936
7. Carmier, J. F., and Samarut, J. (1986) Cell 44, 159–165
8. Care`, A., Mattia, G., Montesoro, E., Parolini, I., Russo, G., Colombo, M. P., and Peschle, C. (1994) Oncogene 9, 2543–2551
9. Fantl, W. J., Johnson, D. E., and Williams, L. T. (1993) Annu. Rev. Biochem. 62, 453–481
10. Ihle, J. N., Witthuhn, B. A., Quelle, F. W., Yamamoto, K., Thierfelder, W. E., Kreider, B., and Silvennoinen, O. (1994) Trends Biochem. Sci. 19, 222–227
11. Hanazono, Y., Chiba, S., Sasaki, K., Mano, H., Miyajima, A., Arai, K., Yazaki, Y., and Hirai, H. (1993) Blood 81, 3193–3196
12. Hanazono, Y., Chiba, S., Sasaki, K., Mano, H., Miyajima, A., Arai, K., Yazaki, Y., and Hirai, H. (1993) EMBO J. 12, 1641–1646
13. Hjermstad, S., Peters, K. L., Briggs, S. D., Glazer, R. I., and Smithgall, T. E. (1993) Oncogene 8, 2283–2292
14. Fang, F., Ahmad, S., Lei, J., Klecker, R. W., Trepel, J. B., Smithgall, T. E., and Glazer, R. I. (1993) Biochemistry 32, 6995–7001
15. Weinmaster, G., Zoller, M. J., Smith, M., Hinze, E., and Pawson, T. (1984) Cell 37, 559–568
16. Golub, T. R., Goga, A., Barker, G. F., Afar, D. E. H., McLaughlin, J., Bohlander, S. K., Rowley, J. D., Witte, O. N., and Gilliland, D. G. (1996) Mol. Cell. Biol. 16, 4107–4116