[Frontiers in Bioscience 16, 3146-3155, June 1, 2011]

Structure and regulation of the c-Fes protein-tyrosine kinase

Sabine Hellwig, Thomas E. Smithgall

Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Pittsburgh, PA 15219, U.S.A.

TABLE OF CONTENTS

1. ABSTRACT

The c-Fes protein-tyrosine kinase is the normal cellular ortholog of several avian and feline retroviral oncoproteins. Unlike its transforming viral counterparts, c-Fes tyrosine kinase activity is tightly regulated *in vivo* through a mechanism involving coiled-coil oligomerization domains and other unique structural features found in its long N-terminal region. This review is focused on the regulatory features and structural biology of c-Fes, which has been implicated in normal cellular growth regulation, the innate immune response, and tumorigenesis.

2. INTRODUCTION

The human c-*fes* proto-oncogene encodes a protein-tyrosine kinase (c-Fes) distinct from c-Src, c-Abl and other non-receptor protein-tyrosine kinases. Although originally identified as the cellular homolog of several transforming retroviral oncoproteins, c-Fes kinase activity is tightly regulated *in vivo*. Unique to c-Fes is its long N-terminal region, which contains a Fer/CIP4 Homology (FCH) region, followed by at least two coiled-coil homology domains which have essential roles in the regulation of kinase activity and mediate c-Fes

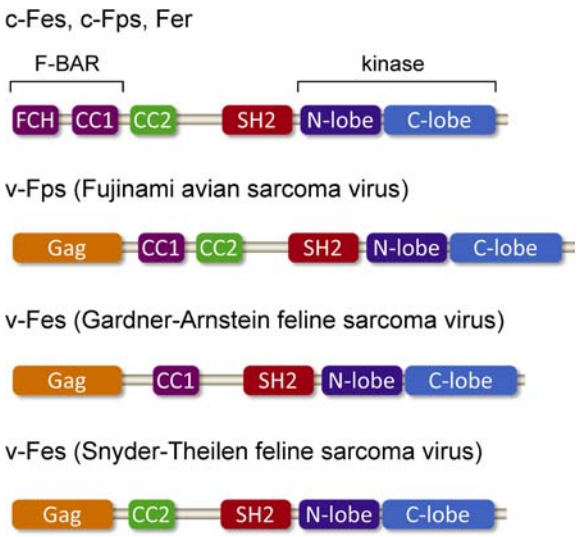

Figure 1. Structural features of c-Fes and representative transforming viral homologs. Mammalian c-Fes, chicken c-Fps and the related tyrosine kinase Fer all share the same overall structural organization, including a C-terminal kinase domain, a central SH2 domain, and a long N-terminal unique region, which includes at least two coiled-coil oligomerization domains (designated CC1 and CC2). The N-terminal region also contains an FCH domain, which together with the first coiled-coil, forms an F-BAR domain. In the case of c-Fps and Fer, analysis of the primary sequence with the COILS algorithm (26) suggests that the second coiled-coil region may actually consist of two closely spaced coiled-coils. Also shown are the structures of three Fes-related transforming tyrosine kinases. These include the v-Fps gene product from Fujinami avian sarcoma virus and two variants of v-Fes derived from the Gardner-Arnstein and Snyder-Theilen feline sarcoma viruses. In all three cases, viral Gag sequences are fused to N-terminal sequences derived from either c-Fps or c-Fes. Note that v-Fps retains both regions of coiled-coil homology, while the GA and ST versions of v-Fes retain only one coiled-coil domain. (Cartoons not drawn to scale.)

oligomerization *in vivo*. The FCH and first coiled-coil motif together define an FCH-Bin/Amphiphysin/Rvsp (F-BAR) domain, which has been associated with the regulation of membrane curvature in other systems (1) and may link c-Fes to the regulation of endocytosis. The remainder of the protein consists of a Src homology 2 (SH2) domain followed by a C-terminal kinase domain. Recent crystallographic studies have provided important insight into the positive regulation of the kinase domain through direct interaction with the SH2 domain, which also influences the subcellular localization of c-Fes. This review traces the discovery of c-Fes and its relationship to transforming retroviral orthologs, and highlights the unique aspects of c-Fes structure and regulation that set it apart from other cytoplasmic protein-tyrosine kinase families.

3. RELATIONSHIP TO TRANSFORMING RETROVIRAL ONCOGENES

Human c-*fes* is the normal cellular ortholog of the transforming oncogenes found in several avian and feline retroviruses. Examples include the v-*fps* oncogenes of Fujinami and PRC-type chicken sarcoma viruses and the v-*fes* oncogenes associated with the Gardner-Arnstein and Snyder-Theilen strains of Feline sarcoma virus (2-6). Both the avian and feline oncogenes encode polyproteins in which viral *gag* sequences are fused to partial c-*fes*- or c-*fps*-derived sequences. (Note that the designation "*fps*" refers to the chicken ortholog of mammalian *fes*.) Transforming Gag-Fps/Fes oncoproteins proteins exhibit constitutive protein-tyrosine kinase activity, which is essential to their transforming function. In contrast, the cellular proto-oncogenes encode protein-tyrosine kinases in which activity is tightly controlled. Structures of representative cellular and viral Fps/Fes proteins are shown in Figure 1.

Using a v-*fes*-derived sequence as a hybridization probe, the human c-*fes* gene was isolated by several groups almost thirty years ago (7-9). Similar approaches subsequently enabled the cloning of the avian and feline c-*fes/fps* proto-oncogenes as well (10,11). All c-*fps/fes* loci share a similar overall organization that includes 19 exons, the first of which is non-coding. The sequence of the human c-*fes* gene was reported in 1985, and was predicted to encode a protein-tyrosine kinase of approximately 93 kDa (12). As described in detail in the next section, biochemical studies completed around the same time identified a 93 kDa protein-tyrosine kinase that cross-reacted with antibodies against the v-Fps transforming protein. Later isolation and sequencing of full-length human c-*fes* cDNA clones confirmed the splicing pattern predicted from the genomic sequence (13).

4. ANTIBODIES TO V-FPS/FES ONCOPROTEINS IDENTIFY A NOVEL TYROSINE KINASE IN HEMATOPOIETIC CELLS

Using antibodies raised against v-Fps, a cross-reactive protein-tyrosine kinase of approximately 98 kDa was first discovered in chicken bone marrow cells (14). The strongest expression of this cellular v-Fps homolog (c-

Fps) was later reported for differentiated cells of myeloid lineage, including granulocytes and macrophages (15). Using a similar immunological approach, a 92 kDa cytoplasmic protein-tyrosine kinase was observed in human myeloid cells (16,17). These studies led to the idea that c-Fps/Fes expression was restricted to myeloid hematopoietic cells, and that it may regulate myelopoiesis or myeloid cell function. A major differentiation-related protein-tyrosine kinase observed in differentiated HL-60 promyelocytic leukemia cells was later purified and identified as c-Fes, supporting a role for this kinase in differentiation (18,19). Subsequent work established that re-expression of the c-*fes* gene in the myeloid leukemia cell line K-562, which was derived from a CML erythroid blast crisis and fails to express c-Fes, re-established the differentiation program (20). This study provided some of the first evidence that c-Fes expression is sufficient to induce terminal differentiation of myeloid cells. In addition, by overriding the Bcr-Abl signal for transformation in K-562 cells, these experiments also provided the first hint that Fes may serve a tumor suppressor function in some cellular contexts. Since this time, a wealth of studies have established key roles for c-Fes not only in regulation of myeloid cell fate and function, but in an expanded range of cellular lineages including vascular endothelial cells, breast and gut epithelial cells, and a subset of neuronal cell types. The biological functions of Fes in these and other cellular contexts, both under physiological conditions and in disease, is the focus of several other reviews in this series and will not be detailed here.

5. OVERALL DOMAIN ORGANIZATION

The human c-Fes protein-tyrosine kinase is 822 amino acids in length and can be divided into three major structural regions, a long N-terminal unique region with F-BAR/coiled-coil domains, a central Src homology 2 (SH2) domain and a C-terminal kinase domain (Figure 1). Fes and the related kinase Fer (21) lack regulatory features present in other non-receptor protein-tyrosine kinases, such as SH3 and pleckstrin homology (PH) domains, an N-terminal myristoylation signal sequence, or a negative regulatory C-terminal phosphotyrosine residue. These features distinguish c-Fes from c-Src (22), c-Abl (23), Tec/Btk (24) and other cytoplasmic tyrosine kinases. As described in more detail below, each of the structural regions of c-Fes contributes to the regulation of its kinase activity and interaction with signaling partners.

6. THE UNIQUE N-TERMINAL REGION OF FES CONTAINS F-BAR/COILED-COIL OLIGOMERIZATION DOMAINS INVOLVED IN THE REGULATION OF KINASE ACTIVITY

Previous studies on the mechanism of Fes kinase regulation revealed that the active form of Fes is oligomeric, forming tetrameric or higher-order oligomers *in vitro* (25). Computational analysis of the Fes unique domain with COILS, an algorithm that searches for the amphipathic α-helical heptad repeats associated with coiled-coil oligomerization domains (26-28), revealed the presence of at least two regions with a high probability of forming coiled-coil structures (25). Chemical cross-linking, gel-filtration, and co-precipitation studies all confirmed that the unique region but not the SH2 or kinase domains, is responsible for c-Fes oligomerization.

Several lines of evidence have shown that the unique N-terminal region of c-Fes contributes to the regulation of kinase activity, despite its distance from the kinase domain in the primary sequence (Figure 1). First, a kinase-inactive mutant of Fes suppressed wild-type Fes autophoshorylation *in vitro* in a concentration-dependent manner (25). This inhibitory effect required the Fes N-terminal region but not the SH2 or kinase domains, suggesting an inhibitory mechanism in which mixed oligomers form between active and inactive monomers to interfere with *trans*-autophosphorylation of the kinase domain. Along similar lines, co-expression of a truncation mutant consisting only of the isolated N-terminal region suppressed Fes transforming activity in fibroblasts (29). This finding is consistent with the *in vitro* experiment described above, and suggests that the N-terminal region interacts with active Fes in cells to suppress its function. Along these lines, expression of a similar N-terminal construct in murine embryonic stem cells induced the expansion of myeloid progenitor cell populations *in vitro*, suggestive of a similar suppressor effect that interfered with terminal differentiation (30). Finally, mutation or deletion of the more N-terminal coiled-coil homology domain strongly enhanced Fes autophosphorylation and transforming activities in fibroblasts and differentiation-inducing activity in myeloid leukemia cell lines (29,31). Based on these observations, the N-terminal coiled-coil domain was initially proposed to regulate kinase activity by several mechanisms, including interaction with a cellular inhibitory factor or via intramolecular interaction with the second coiled-coil motif. The latter mechanism was envisioned to hold the molecule in a monomeric state incompatible with *trans*-autophosphorylation (21,32).

More recent work has established that c-Fes exists as a constitutive oligomer in cells, independent of the phosphorylation state of the kinase domain (33). These studies employed the technique of bimolecular fluorescence complementation (34), in which Fes was fused to non-fluorescent, overlapping fragments of the green fluorescent protein variant, YFP. When co-expressed in the same cell, interaction of Fes molecules juxtaposed the fused YFP fragments, resulting in functional complementation and a fluorescent signal. Leucine to proline point mutations previously shown to disrupt the function of the coiled-coil domains (31) dramatically reduced the number of cells exhibiting Fes-mediated YFP fluorescence complementation, providing strong evidence that the coiled-coils are essential for Fes oligomerization *in vivo*. In contrast, inactivating mutations in the SH2 or kinase domains had no effect, providing cell-based evidence that these regions of c-Fes do not contribute to its self-association. These data argue against but do not completely rule out a model in which Fes kinase activity is regulated by interconversion of monomeric and multimeric states. Instead, the N-terminal region may regulate the kinase

domain through steric interference with SH2-kinase domain interaction essential for stabilizing the active conformation of the catalytic site as revealed by X-ray crystallography (35).

More recently, the N-terminal FCH domain and first coiled-coil motif of c-Fes have been recognized to have significant homology with F-BAR domains, a defining module in the Pombe Cdc15 Homology (PCH) family of proteins (1). In yeast, the PCH protein Cdc15 regulates actomyosin contractile ring closure essential for cytokinesis, while its mammalian counterparts bind to phospholipids and regulate membrane curvature required for diverse processes including endocytosis, vesicular release, motility as well as cytokinesis (1). Recent work from the Craig laboratory developed a structural homology model for the c-Fes F-BAR domain, and established that it indeed functions in phospholipid binding and tubulation of liposomes *in vitro* (36). In mast cells, c-Fes activation downstream of the Fcε receptor was shown to be dependent upon functional F-BAR and SH2 domains, which work in concert to couple Fes to the active, tyrosine-phosphorylated receptor and subsequent phosphorylation of the actin regulatory protein, HS1 (36). Interestingly, LPS-induced internalization of Toll-like receptor 4 (TLR4) is delayed in macrophages from Fes-null mice, suggesting that Fes, through its F-BAR-mediated functions, may have an important role in limiting innate immunity via modulation of TLR4 levels at the cell surface (37).

7. THE FES SH2 DOMAIN IS REQUIRED FOR FULL KINASE AND BIOLOGICAL ACTIVITIES AND MEDIATES SUBSTRATE RECRUITMENT AND SUBCELLULAR LOCALIZATION

SH2 domains are well-known signaling modules that mediate protein-protein interactions during tyrosine kinase signal transduction (38). SH2 domains bind with high affinity and specificity to short peptide sequences within target proteins that are phosphorylated on tyrosine residues. In contrast, these domains have no affinity for the unphosphorylated sequence. Thus, SH2 domains often mediate the assembly of multiprotein complexes in response to tyrosine kinase activation, such as the recruitment of signaling molecules to autophosphorylated growth-factor and immune receptors at the plasma membrane.

Although the role of SH2 domains in growth factor receptor tyrosine kinase signal transduction has been studied in great detail, SH2 domains were first identified within the sequences of non-receptor tyrosine kinases. In fact, the v-Fps SH2 domain was among the first to be described, and was shown to be required for full kinase activity and to influence the host range of transforming Fps/Fes retroviruses (39,40). Subsequent work showed that an intact SH2 domain was also required for v-Fps-induced phosphorylation of specific cellular proteins in transformed fibroblasts (41). The position of the v-Fps SH2 domain immediately N-terminal to the kinase domain led to speculation that it may interact in *cis* with the kinase domain to stabilize the active kinase structure. Consistent with this view are the findings that mild proteolysis of v-Fps releases a stable globular fragment containing both the SH2 and kinase domains and that SH2 mutations that impair kinase function also affect this interaction (41,42). For a review of early work on the role of SH2 and other non-catalytic domains in transforming tyrosine kinase function, see Pawson, 1988 (43).

Subsequent studies have revealed a similar dual role for the human c-Fes SH2 domain in the regulation of kinase activity and biological function. Deletion of the c-Fes SH2 domain greatly reduced kinase activity both in terms of substrate phosphorylation as well as autophosphorylation *in vitro* (44). This study also showed that c-Fes can bind directly to its own SH2 domain, providing further support for the hypothesis that SH2:kinase domain interaction may stabilize the active kinase structure. Direct evidence for regulatory interaction has recently been obtained from a high-resolution X-ray crystal structure of the c-Fes SH2-kinase region, which reveals a regulatory interface between the SH2 domain and the N-terminal lobe of the kinase domain (35). Details of this structure and its implications for overall regulation of c-Fes are presented in Section 9.

In addition to regulating the kinase domain, an intact c-Fes SH2 domain is also required for biological activity. For example, deletion of the SH2 domain completely abolished the fibroblast transforming function of a c-Fes mutant activated by N-terminal addition of the v-Src myristoylation signal sequence (45). Interestingly, changing the binding specificity of the c-Fes SH2 domain by swapping it with the SH2 domain of v-Src led to strong activation of c-Fes tyrosine kinase and transforming activities (45). In addition, the chimeric c-Fes/Src SH2 protein localized to focal adhesions in transformed fibroblasts, suggesting that re-localization of c-Fes to this subcellular compartment may influence its kinase activity. In related work, wild-type c-Fes has been shown to interact with and phosphorylate p130 Cas and other focal adhesion proteins in macrophages (46), suggesting that c-Fes may localize to focal contacts in this physiological site of c-Fes expression. More recently, Fes has been shown to localize to this same compartment in epithelial cells by interacting with Ezrin, a linker protein involved in membrane-cytoskeletal attachment (47). The interaction involves binding of the Fes SH2 domain to an Ezrin phosphotyrosine residue (pTyr477), and is required for HGF-induced cell scattering in this system.

The SH2 domain also regulates c-Fes association with the tubulin cytoskeleton in transfected COS cells (48). When wild-type c-Fes was expressed in this cell line, it remained catalytically repressed and exhibited a predominantly diffuse cytoplasmic distribution. This subcellular localization changed dramatically when c-Fes kinase activity was released by mutation of the N-terminal coiled coil domain (L145P mutant). In this case, active Fes associated strongly with the prominent microtubule network found in these cells. Microtubule association of c-Fes required not only a catalytically active kinase domain but also a functional SH2 domain. An arginine to leucine

point mutation that disrupts the phosphotyrosine binding pocket of the SH2 domain completely reversed the localization of active Fes to microtubules. Furthermore, partially purified preparations of recombinant c-Fes were shown to phosphorylate purified tubulin *in vitro*, and to catalyze tubulin polymerization in the absence of accessory proteins. Taken together, these findings strongly suggest that c-Fes may regulate microtubule dynamics in response to cellular signals that stimulate its kinase activity. Upon activation, c-Fes may catalyze both tubulin polymerization and phosphorylation, followed by association with tyrosine-phosphorylated microtubules through its SH2 domain. In addition, the possibility exists that tyrosine phosphorylation of tubulin by other kinases (e.g., Src-family members) may create recruitment sites for c-Fes via its SH2 domain. Support for this idea comes from the observation that co-expression of wild-type Fes with an active mutant of the Src-family kinase Hck induced Fes localization to microtubules in COS cells (48).

Association with the tubulin cytoskeleton has also been demonstrated for endogenous c-Fes (49). In whole cell lysates of neonatal rat brain, soluble tubulin was found to co-immunoprecipitate with c-Fes. Immunofluorescence microscopy confirmed that endogenous c-Fes was associated with microtubule fibers in cultured rat hippocampal neurons. When COS cells transfected with c-Fes were treated with colcemid to disrupt the existing microtubular network, Fes was found to aggregate at the microtubule organizing center and co-localize with γ-tubulin, the tubulin isoform essential for microtubule nucleation and polar orientation. During mitotic metaphase, a physiologically relevant context for microtubule disruption and reorganization, a constitutively active coiled-coil mutant of c-Fes (L145P) was found to localize to the mitotic spindle, further hinting at a role for c-Fes in modulating tubulin dynamics (48).

8. KINASE DOMAIN AUTOPHOSPHORYLATION IS REQUIRED FOR THE FULL KINASE AND BIOLOGICAL ACTIVITIES OF FPS/FES PROTEINS

The major site of v-Fps autophosphorylation has been mapped to Tyr1073 in the kinase domain both *in vitro* and *in vivo* (42). Mutagenesis of this tyrosine residue to phenylalanine reduced catalytic activity, resulting in a longer latent period for transformation of fibroblasts. In human c-Fes, the major site of autophosphorylation maps to the homologous residue, Tyr713 (44,50,51). Mutagenesis of this site reduced the catalytic activity of c-Fes toward a model substrate *in vitro* by more than 90% (44). In addition, introduction of the Tyr713 mutation into a transforming variant of c-Fes carrying the membrane-targeting myristoylation signal of v-Src delayed the onset of transformation (J. Rogers and T. Smithgall, unpublished data). However, expression of the c-Fes Tyr713 mutant in K-562 myeloid leukemia cells did not have a major impact on biological activity (differentiation), despite the presumed effect of the mutation on kinase activity (50). One possible explanation for these contrasting results is that two different thresholds of kinase activity are required for transformation vs. differentiation signaling, with differentiation requiring less intrinsic kinase activity for a biological effect. Alternatively, Fes may be able to generate kinase-independent signals in some cellular contexts.

Unlike its transforming counterparts, c-Fes tyrosine kinase activity is tightly regulated *in vivo*, as first demonstrated by phosphoamino acid analysis of c-Fes immunoprecipitated from ^{32}P-labeled cells (17). These early studies revealed only phosphoserine and no phosphotyrosine, consistent with a lack of kinase domain autophosphorylation and activation. Even high-level expression of c-Fes in Rat-2 fibroblasts was not sufficient to release its tyrosine kinase and transforming activities (52). Although the transforming activity of c-Fes was ultimately demonstrated in NIH 3T3 cells, this effect required the use of a highly efficient retroviral vector and subsequent over-expression of Fes to more than 200 times the level observed in a myeloid leukemia cell line (53).

Suppression of Fes kinase activity can be overcome in multiple ways. Fusion of viral Gag sequences to the N-terminal region of c-Fes (as well as its chicken counterpart, c-Fps) results in a chimeric protein with strong tyrosine kinase and transforming activities in fibroblasts (54-56). Viral Gag fusion may interfere with negative regulation, relocalize Fes to a subcellular compartment essential for mitogenic signaling (e.g., plasma membrane, cytoskeleton), or both. As described above, artificial addition of the v-Src myristoylation signal sequence targets c-Fes to the plasma membrane in Rat-2 fibroblasts, resulting in transformation (52). However, myristoylation and plasma membrane targeting alone does not fully stimulate Fes kinase activity in cells (29,45), although it is sufficient to induce a strong hypervascularity phenotype in transgenic mice (57). Targeting of c-Fes to focal adhesions via replacement of the native SH2 domain with that of v-Src also results in strong stimulation of kinase and transforming activities (45) (see Section 7). Finally, mutagenesis or deletion of the first coiled-coil domain causes dramatic upregulation of c-Fes tyrosine kinase activity (Section 6), although the mechanism by which this N-terminal modification influences the C-terminal kinase domain is not clear at present.

9. STRUCTURAL BIOLOGY OF THE C-FES SH2-KINASE REGION PROVIDES A MECHANISTIC BASIS FOR POSITIVE REGULATION OF KINASE ACTIVITY BY DIRECT SH2 DOMAIN INTERACTION

While the structure of full-length c-Fes remains unsolved, the first high-resolution structure of a truncated Fes construct, consisting of the SH2 domain and adjacent kinase domain, was recently solved by X-ray crystallography (Figure 2) (35). Consistent with earlier reports that the SH2 domain positively affects kinase function (see Section 7), the crystal structure revealed an extensive interaction interface between SH2 and the N-lobe of the kinase domain (Figure 2). In contrast to other cytoplasmic tyrosine kinases such as c-Src and c-Abl, c-Fes

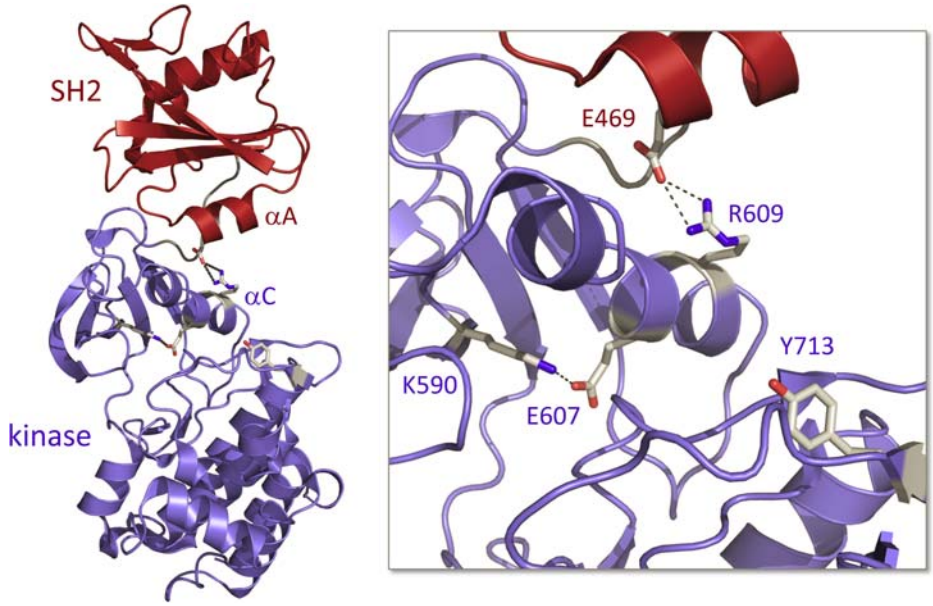

Figure 2. Structure of the c-Fes SH2-kinase region. The overall structure of the c-Fes SH2-kinase unit (amino acids 448-822) is shown on the left, and is modeled on the X-ray crystal coordinates of Filippakopulos, *et al.*, (PDB: 3BKB) (35). The interface of the SH2 and kinase domains is enlarged on the right. Interaction of the SH2 domain and the αC helix of the kinase domain N-lobe is stabilized by interaction between SH2 E469 and αC R609; the adjacent SH2 glutamate residue, E472, may also have a role (side chain not shown). This interaction positions the αC helix in a conformation compatible with catalysis, and is stabilized further by a salt bridge that forms between K590 and E607. Interestingly, replacement of K590 with glutamate completely inactivates c-Fes kinase activity (51), illustrating the importance of this interaction in maintaining an active kinase structure. The side chain of the activation loop autophosphorylation site, Y713, is also shown. While this residue is not phosphorylated in the crystal structure, it coordinates a sulfate ion that mimics phosphorylation and in turn bridges Y713 to kinase domain residue R706 (not shown for clarity). Mutagenesis of residues that contribute to the SH2:kinase interface dramatically reduce kinase activity (see text for details).

contains a much shorter SH2-kinase linker and a unique glycine residue (Gly463) near the N-terminus of the SH2 domain. These features allow for tight SH2 packing against the kinase domain N-lobe, which is stabilized by intercalation of the SH2 N-terminal region between the central SH2 β-sheet (βB) and the loop connecting β4 and β5 of the kinase domain. The electrostatic charge complementarity at the SH2:kinase interface provides a second network of stabilizing interactions. Acidic residues of the SH2 αA helix (Glu469, Glu472) interact with Arg609 in the αC helix of the kinase domain. This interaction stabilizes the αC helix, a key regulatory element in virtually all kinases, in an orientation that allows for engagement of active site Lys590 via a salt bridge to αC Glu607. As a result, the kinase domain adopts a conformation compatible with activity. Mutational analysis confirmed that both packing and electrostatic interactions at the SH2:kinase domain interface are essential for function. Substitution of Gly463 with valine, expected to prevent tight packing of the SH2 against the N-lobe of the kinase domain, completely abolished phosphorylation activity in the context of the full-length protein. In addition, substitution of SH2 domain interface residues Glu469 and Glu472 with lysines prevented formation of the salt bridge network with the N-lobe and strongly diminished kinase function. This defect was rescued by substitution of kinase domain αC helix Arg609 with glutamate, thus restoring charge complementarity at the SH2:kinase interface.

While the activation segment of Fes was found to be relatively stable in the absence of phosphorylation of the activation loop tyrosine (Tyr713), the presence of phospho-Tyr713 is believed to lock the kinase domain in the catalytically competent conformation via a network of salt bridges and hydrogen bonds commonly associated with other active kinase domains. Co-crystallization of the Fes SH2-kinase protein with a synthetic substrate peptide revealed that substrate binding further enhances activation segment stability by induction of a β-sheet between the substrate peptide and a strand in the activation segment. In this way, substrate interaction may induce or maintain positive regulation of kinase activity.

Interestingly, comparison of structures obtained in the absence and presence of substrates or with phosphomimetic salts bound to the SH2 domain revealed that loss of SH2-ligand interaction significantly increased disorder of the SH2:kinase interface and rendered the αC helix highly mobile. In accordance with a role for substrate recognition by the SH2 domain for catalytic activity, introduction of a point mutation that abolished phosphotyrosine binding severely impaired Fes kinase activity in a cell-based assay. These observations suggest that c-Fes kinase activity is induced by a series of

coordinated events. In the absence of phosphorylation at Tyr713 and without ligand binding to the SH2 domain, the activation segment and the kinase domain αC helix remain disordered. Interaction of the phosphotyrosine binding site of the SH2 domain with a peptide ligand primes c-Fes for catalysis by stabilization of the SH2:kinase interface and rotation of αC into an active orientation. Finally, autophosphorylation of Tyr713 leads to further stabilization of the activation segment, rendering the kinase domain fully active.

While the X-ray crystal structure revealed a plausible mechanism for the cooperative interaction of the SH2 and kinase domains in c-Fes activation, the lack of structural data on the long N-terminal region allows only for speculation on the regulation of c-Fes kinase activity by this unique structural feature. Two observations are critical in this regard. First, as detailed in Section 6, deletion or disruption of the first N-terminal coiled-coil domain results in potent activation of the Fes kinase (29,31). This result strongly suggests allosteric interplay between these seemingly disparate regions of the kinase. Second, substitution of the c-Fes SH2 domain with that of v-Src also stimulates kinase activity *in vitro* (45). These findings hint at a model in which the unique N-terminal and SH2 domains interact to repress kinase activity. Such interactions may occur via contact points either overlapping with or distinct from the SH2:kinase interface to prevent the shift to this active conformation. Allosteric repression of c-Fes by such a mechanism may allow for transient kinase activation by interaction of the N-terminal segment with cellular binding partners (58,59), relieving its influence on SH2 and permitting the formation of the active SH2:kinase structure. Evidence for this intriguing possibility will require identification of sequence and structural elements essential for interaction of the unique N-terminal region with the SH2 domain.

10. SUMMARY AND CONCLUSIONS

In this review, we have summarized the major features of the c-Fes kinase that set it apart from other non-receptor protein-tyrosine kinase families in terms of structure and regulation. Like c-Src, c-Abl, and Tec/Btk kinases, c-Fes has a contiguous SH2-kinase domain arrangement that coordinates substrate recruitment, subcellular localization, and kinase function. Unlike Src-family kinases, however, the Fes SH2 domain serves as a critical positive regulator of kinase activity by interacting directly with the small lobe of the kinase domain and positioning the critical αC helix in a conformation compatible with catalysis. Also unique to Fes is its long N-terminal region, which encompasses several structural homology motifs important for kinase domain regulation and overall function. The F-BAR/coiled-coil homology domains are particularly interesting, and not found in any other mammalian tyrosine kinase systems described to date, apart from the homologous Fer kinase (21). The coiled-coils appear to hold Fes in a constitutive oligomeric state both *in vitro* and in cells, regardless of the activation status of the kinase domain. In addition, the first coiled-coil appears to act through an allosteric mechanism to repress kinase activity. The specific mechanism of kinase repression by the coiled-coils or other structural features of the unique domain hold the key to a complete understanding of Fes kinase regulation. Future work directed towards a high resolution structure of this portion of the kinase is essential, as it will inform the discovery or design of small molecules capable of allosteric regulation of c-Fes kinase activity *in vivo*. Such reagents will represent critical tools to evaluate the contributions of c-Fes to both normal biology and disease.

11. ACKNOWLEDGEMENTS

Grant support for the work cited from the Smithgall laboratory was generously provided by the National Institutes of Health. Thanks to all lab members, past and present, whose hard work has helped to provide insight into the structure and regulation of this enigmatic protein kinase.

12. REFERENCES

1. R. H. Roberts-Galbraith and K. L. Gould: Setting the F-BAR: functions and regulation of the F-BAR protein family. *Cell Cycle* 9, 4091-4097 (2010)

2. M. Shibuya and H. Hanafusa: Nucleotide sequence of Fujinami sarcoma virus: Evolutionary relationship of its transforming gene with transforming genes of other sarcoma viruses. *Cell* 30, 787-795 (1982)

3. T. Hanafusa, L. -H. Wang, S. M. Anderson, R. E. Karess, W. S. Hayward and H. Hanafusa: Characterization of the transforming gene of Fujinami sarcoma virus. *Proc. Natl. Acad. Sci. USA* 77, 3009-3013 (1980)

4. W. -H. Lee, K. Bister, A. Pawson, T. Robins, C. Moscovici and P. H. Duesberg: Fujinami sarcoma virus: An avian RNA tumor virus with a unique transforming gene. *Proc. Natl. Acad. Sci. USA* 77, 2018-2022 (1980)

5. A. Hampe, I. Laprevotte, F. Galibert, L. A. Fedele and C. J. Sherr: Nucleotide sequences of feline retroviral oncogenes (v-*fes*) provide evidence for a family of tyrosine-specific protein kinase genes. *Cell* 30, 775-785 (1982)

6. C. -C. Huang, C. Hammond and J. M. Bishop: Nucleotide sequence of v-*fps* in the PRC II strain of avian sarcoma virus. *J. Virol.* 50, 125-131 (1984)

7. J. Groffen, N. Heisterkamp, F. Grosveld, W. J. M. Van de Ven and J. R. Stephenson: Isolation of human oncogene sequences (v-*fes* homolog) from a cosmid library. *Science* 216, 1136-1138 (1982)

8. M. D. Trus, J. G. Sodroski and W. A. Haseltine: Isolation and characterization of a human locus homologous to the transforming gene (v-*fes*) of feline sarcoma virus. *J. Biol. Chem.* 257, 2730-2733 (1982)

9. G. Franchini, E. P. Gelmann, R. Dalla Favera, R. C.

Gallo and F. Wong-Staal: Human gene (c-*fes*) related to the *onc* sequences of Snyder-Theilen feline sarcoma virus. *Mol. Cell. Biol.* 2, 1014-1019 (1982)

10. A. J. M. Roebroek, J. A. Schalken, C. Onnekink, H. P. J. Bloemers and W. J. M. Van de Ven: Structure of the feline c-*fes/fps* proto-oncogene: Genesis of a retroviral oncogene. *J. Virol.* 61, 2009-2016 (1987)

11. C. -C. Huang, C. Hammond and J. M. Bishop: Nucleotide sequence and topography of chicken c-*fps*: Genesis of a retroviral oncogene encoding a tyrosine-specific protein kinase. *J. Mol. Biol.* 181, 175-186 (1985)

12. A. J. M. Roebroek, J. A. Schalken, J. S. Verbeek, A. M. W. Van den Ouweland, C. Onnekink, H. P. J. Bloemers and W. J. M. Van de Ven: The structure of the human c-*fes/fps* proto-oncogene. *EMBO J.* 4, 2897-2903 (1985)

13. M. Alcalay, F. Antolini, W. J. M. Van de Ven, L. Lanfrancone, F. Grignani and P. G. Pelicci: Characterization of human and mouse c-*fes* cDNA clones and identification of the 5' end of the gene. *Oncogene* 5, 267-275 (1990)

14. B. Mathey-Prevot, H. Hanafusa and S. Kawai: A cellular protein is immunologically crossreactive with and functionally homologous to the Fujinami sarcoma virus transforming protein. *Cell* 28, 897-906 (1982)

15. J. Samarut, B. Mathey-Prevot and H. Hanafusa: Preferential expression of the c-*fps* protein in chicken macrophages and granulocytic cells. *Mol. Cell. Biol.* 5, 1067-1072 (1985)

16. R. A. Feldman, J. L. Gabrilove, J. P. Tam, M. A. S. Moore and H. Hanafusa: Specific expression of the human cellular *fps/fes*-encoded protein NCP92 in normal and leukemic myeloid cells. *Proc. Natl. Acad. Sci. USA* 82, 2379-2383 (1985)

17. I. MacDonald, J. Levy and T. Pawson: Expression of the mammalian c-*fes* protein in hematopoietic cells and identification of a distinct *fes*-related protein. *Mol. Cell. Biol.* 5, 2543-2551 (1985)

18. T. E. Smithgall, G. Yu and R. I. Glazer: Identification of the differentiation-associated p93 tyrosine protein kinase of HL-60 leukemia cells as the product of the human c-*fes* locus and its expression in myelomonocytic cells. *J. Biol. Chem.* 263, 15050-15055 (1988)

19. G. Yu and R. I. Glazer: Purification and characterization of $p93^{fes}$ - and $p60^{src}$ -related tyrosine protein kinase activities in differentiated HL-60 leukemia cells. *J. Biol. Chem.* 262, 17543-17548 (1987)

20. G. Yu, T. E. Smithgall and R. I. Glazer: K562 leukemia cells transfected with the human c-*fes* gene acquire the ability to undergo myeloid differentiation. *J. Biol. Chem.* 264, 10276-10281 (1989)

21. P. A. Greer: Closing in on the biological functions of Fps/Fes and Fer. *Nature Rev. Mol. Cell Biol.* 3, 278-289 (2002)

22. J. R. Engen, T. E. Wales, J. M. Hochrein, M. A. Meyn, III, O. S. Banu, I. Bahar and T. E. Smithgall: Structure and dynamic regulation of Src-family kinases. *Cell Mol. Life Sci.* 65, 3058-3073 (2008)

23. O. Hantschel and G. Superti-Furga: Regulation of the c-Abl and Bcr-Abl tyrosine kinases. *Nat. Rev. Mol. Cell Biol.* 5, 33-44 (2004)

24. J. M. Bradshaw: The Src, Syk, and Tec family kinases: distinct types of molecular switches. *Cell Signal.* 22, 1175-1184 (2010)

25. R. D. Read, J. M. Lionberger and T. E. Smithgall: Oligomerization of the Fes tyrosine kinase: Evidence for a coiled-coil domain in the unique N-terminal region. *J. Biol. Chem.* 272, 18498-18503 (1997)

26. A. Lupas: Prediction and analysis of coiled-coil structures. *Meth. Enzymol.* 266, 513-525 (1996)

27. A. Lupas: Coiled coils: New structures and new functions. *Trends Biochem. Sci.* 21, 375-382 (1996)

28. A. Lupas, M. Van Dyke and J. Stock: Predicting coiled coils from protein sequences. *Science* 252, 1162-1164 (1991)

29. H. Y. Cheng, J. A. Rogers, N. A. Dunham and T. E. Smithgall: Regulation of c-Fes tyrosine kinase and biological activities by N-terminal coiled-coil oligomerization domains. *Mol. Cell Biol.* 19, 8335-8343 (1999)

30. R. Hackenmiller and M. C. Simon: Truncation of c-fes via gene targeting results in embryonic lethality and hyperproliferation of hematopoietic cells. *Dev. Biol.* 245, 255-269 (2002)

31. H. Y. Cheng, A. P. Schiavone and T. E. Smithgall: A point mutation in the N-terminal coiled-coil domain releases c-Fes tyrosine kinase activity and survival signaling in myeloid leukemia cells. *Mol. Cell Biol.* 21, 6170-6180 (2001)

32. T. E. Smithgall, J. A. Rogers, K. L. Peters, J. Li, S. D. Briggs, J. M. Lionberger, H. Cheng, A. Shibata, B. Scholtz, S. Schreiner and N. A. Dunham: The c-Fes Family of Protein-Tyrosine Kinases. *Critical Rev. Oncogenesis* 9, 43-62 (1998)

33. J. M. Shaffer, S. Hellwig and T. E. Smithgall: Bimolecular fluorescence complementation demonstrates that the c-Fes protein-tyrosine kinase forms constitutive oligomers in living cells. *Biochemistry* 48, 4780-4788 (2009)

34. T. K. Kerppola: Bimolecular fluorescence complementation (BiFC) analysis as a probe of protein

interactions in living cells. *Annu. Rev. Biophys.* 37, 465-487 (2008)

35. P. Filippakopoulos, M. Kofler, O. Hantschel, G. D. Gish, F. Grebien, E. Salah, P. Neudecker, L. E. Kay, B. E. Turk, G. Superti-Furga, T. Pawson and S. Knapp: Structural coupling of SH2-kinase domains links Fes and Abl substrate recognition and kinase activation. *Cell* 134, 793-803 (2008)

36. V. A. McPherson, S. Everingham, R. Karisch, J. A. Smith, C. M. Udell, J. Zheng, Z. Jia and A. W. Craig: Contributions of F-BAR and SH2 domains of Fes protein tyrosine kinase for coupling to the FcepsilonRI pathway in mast cells. *Mol. Cell Biol.* 29, 389-401 (2009)

37. S. A. Parsons and P. A. Greer: The Fps/Fes kinase regulates the inflammatory response to endotoxin through down-regulation of TLR4, NF-kappaB activation, and TNF-alpha secretion in macrophages. *J. Leukoc. Biol.* 80, 1522-1528 (2006)

38. T. Pawson: Specificity in signal transduction: from phosphotyrosine-SH2 domain interactions to complex cellular systems. *Cell* 116, 191-203 (2004)

39. J. E. DeClue, I. Sadowski, G. S. Martin and T. Pawson: A conserved domain regulates interactions of the v-fps protein-tyrosine kinase with the host cell. *Proc. Natl. Acad. Sci. USA* 84, 9064-9068 (1987)

40. I. Sadowski, J. C. Stone and T. Pawson: A noncatalytic domain conserved among cytoplasmic protein-tyrosine kinases modifies the kinase function and transforming activity of Fujinami sarcoma virus $p130^{gag\text{-}fps}$. *Mol. Cell. Biol.* 6, 4396-4408 (1986)

41. C. A. Koch, M. Moran, I. Sadowski and T. Pawson: The common *src* homology region 2 domain of cytoplasmic signaling proteins is a positive effector of v-*fps* tyrosine kinase function. *Mol. Cell. Biol.* 9, 4131-4140 (1989)

42. G. Weinmaster, E. Hinze and T. Pawson: Mapping of multiple phosphorylation sites within the structural and catalytic domains of the Fujinami avian sarcoma virus transforming protein. *J. Virol.* 46, 29-41 (1983)

43. T. Pawson: Non-catalytic domains of cytoplasmic protein-tyrosine kinases: regulatory elements in signal transduction. *Oncogene* 3, 491-495 (1988)

44. S. J. Hjermstad, K. L. Peters, S. D. Briggs, R. I. Glazer and T. E. Smithgall: Regulation of the human c-*fes* protein-tyrosine kinase ($p93^{c\text{-}fes}$) by its *src* homology 2 domain and major autophosphorylation site (tyr 713). *Oncogene* 8, 2283-2292 (1993)

45. J. A. Rogers, H. Y. Cheng and T. E. Smithgall: Src homology 2 domain substitution modulates the kinase and transforming activities of the Fes protein-tyrosine kinase. *Cell Growth Differ.* 11, 581-592 (2000)

46. M. Jucker, K. McKenna, A. J. Da Silva, C. E. Rudd and R. A. Feldman: The Fes protein-tyrosine kinase phosphorylates a subset of macrophage proteins that are involved in cell adhesion and cell-cell signaling. *J. Biol. Chem.* 272, 2104-2109 (1997)

47. A. Naba, C. Reverdy, D. Louvard and M. Arpin: Spatial recruitment and activation of the Fes kinase by ezrin promotes HGF-induced cell scattering. *EMBO J.* 27, 38-50 (2008)

48. C. E. Laurent, F. J. Delfino, H. Y. Cheng and T. E. Smithgall: The human c-Fes tyrosine kinase binds tubulin and microtubules through separate domains and promotes microtubule assembly. *Mol. Cell Biol.* 24, 9351-9358 (2004)

49. S. Takahashi, R. Inatome, A. Hotta, Q. Qin, R. Hackenmiller, M. C. Simon, H. Yamamura and S. Yanagi: Role for Fes/Fps tyrosine kinase in microtubule nucleation through is Fes/CIP4 homology domain. *J. Biol. Chem.* 278, 49129-49133 (2003)

50. F. Fang, S. Ahmad, J. Lei, R. W. Klecker, J. B. Trepel, T. E. Smithgall and R. I. Glazer: The effect of mutation of tyrosine 713 in $p93^{c\text{-}fes}$ on its catalytic activity and ability to promote myeloid differentiation in K-562 cells. *Biochemistry* 32, 6995-7001 (1993)

51. J. A. Rogers, R. D. Read, J. Li, K. L. Peters and T. E. Smithgall: Autophosphorylation of the Fes tyrosine kinase: Evidence for an intermolecular mechanism involving two kinase domain tyrosine residues. *J. Biol. Chem.* 271, 17519-17525 (1996)

52. P. A. Greer, K. Meckling-Hansen and T. Pawson: The human c-*fps/fes* gene product expressed ectopically in rat fibroblasts is nontransforming and has restrained protein-tyrosine kinase activity. *Mol. Cell. Biol.* 8, 578-587 (1988)

53. R. A. Feldman, D. R. Lowy, W. C. Vass and T. J. Velu: A highly efficient retroviral vector allows detection of the transforming activity of the human c-*fps/fes* proto-oncogene. *J. Virol.* 63, 5469-5474 (1989)

54. D. A. Foster, M. Shibuya and H. Hanafusa: Activation of the transforming potential of the cellular fps gene. *Cell* 42, 105-115 (1985)

55. R. A. Feldman, W. C. Vass and P. E. Tambourin: Human cellular *fps/fes* cDNA rescued via retroviral shuttle vector encodes myeloid cell NCP92 and has transforming potential. *Oncogene Res.* 1, 441-458 (1987)

56. J. G. Sodroski, W. C. Goh and W. A. Haseltine: Transforming potential of a human proto-oncogene (c-*fps/fes*) locus. *Proc. Natl. Acad. Sci. USA* 81, 3039-3043 (1984)

57. P. Greer, J. Haigh, G. Mbamalu, W. Khoo, A. Bernstein and T. Pawson: The Fps/Fes protein-tyrosine kinase promotes angiogenesis in transgenic mice. *Mol. Cell. Biol.*

14, 6755-6763 (1994)

58. F. J. Delfino, J. M. Shaffer and T. E. Smithgall: The KRAB-associated co-repressor KAP-1 is a coiled-coil binding partner, substrate, and activator of the c-Fes protein-tyrosine kinase. *Biochem. J.* 399, 141-150 (2006)

59. Y. Maru, K. L. Peters, D. E. H. Afar, M. Shibuya, O. N. Witte and T. E. Smithgall: Tyrosine phosphorylation of BCR by FPS/FES protein-tyrosine kinases induces association of BCR with GRB-2/SOS. *Mol. Cell. Biol.* 15, 835-842 (1995)

Key Words: c-Fes, tyrosine kinase, SH2, coiled-coil, F-BAR, Review

Send correspondence to: Thomas E. Smithgall, Department of Microbiology and Molecular Genetics, University of Pittsburgh School of Medicine, Bridgeside Point II, Suite 523, 450 Technology Drive, Pittsburgh, PA 15219, USA, Tel: 412-648-8106, Fax: 412-624-8997, E-mail: tsmithga@pitt.edu

http://www.bioscience.org/current/vol16.htm