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The *FER* Gene Is Evolutionarily Conserved and Encodes a Widely Expressed Member of the *FPS/FES* Protein-Tyrosine Kinase Family

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We have recently isolated human and rat cDNAs (designated *FER* and *flk*, respectively) which encode nonreceptor protein-tyrosine kinases which are very similar to one another and related in sequence and domain structure to the c-*fps/fes* gene product. We show that *FER* and *flk* are human and rat counterparts of an evolutionarily conserved gene, hereafter termed *FER* regardless of species. The human and rat *FER* genes encode a widely expressed 94-kilodalton protein-tyrosine kinase which is antigenically related to the *fps/fes* protein-tyrosine kinase. The structural and antigenic similarities between the *FER* and *fps/fes* proteins suggest that they are members of a new family of nonreceptor protein-tyrosine kinases.

The mammalian c-*fps/fes* proto-oncogene encodes a 92-kilodalton (kDa) protein-tyrosine kinase (PTK), $p92^{c\text{-}fes}$, that is specifically expressed during hematopoiesis, principally in cells of the granulocyte-macrophage lineage (2, 4, 9). *fps/fes* gene products are located entirely within the cell and are generally found to be soluble in the cytoplasm or, in oncogenic variants, associated by ionic interactions with the cytoskeleton or plasma membrane (11, 17). Although $p92^{c\text{-}fes}$ can be characterized on this basis as a nonreceptor, cytoplasmic PTK, it is distinguished from PTKs of the *src* and *abl* subfamilies by an individual topology (12). Like $p60^{c\text{-}src}$ and $p145^{c\text{-}abl}$, $p92^{c\text{-}fes}$ contains a tyrosine kinase domain and a noncatalytic SH2 regulatory domain (12, 14). However, $p92^{c\text{-}fes}$ contains only eight residues C terminal to the kinase domain, and these bear no relationship to the regulatory C-terminal tail of $p60^{c\text{-}src}$ (7). In addition, $p92^{c\text{-}fes}$ contains 460 residues N terminal to the SH2 domain which have no obvious counterpart in the *src* and *abl* gene products (1).

We and others have previously described a 94-kDa protein with associated tyrosine kinase activity which is antigenically related to *fps/fes* proteins (2, 3, 9). More recently, we have isolated a full-length human cDNA and a partial rat cDNA, designated *FER* and *flk*, respectively, which encode novel PTKs very similar to one another and closely related to $p92^{c\text{-}fes}$ in both amino acid sequence and overall domain structure (5, 8). Here we show that *FER* and *flk* are cognate human and rat genes and encode the 94-kDa PTK previously identified with anti-*fps* antibodies. c-*fps/fes* is therefore a prototype for a new subfamily of nonreceptor PTKs, of which *FER* is the second member.

***FER* and *flk* represent a single conserved gene.** Comparison of the nucleotide and deduced amino acid sequences of the partial rat *flk* cDNA (8) and human *FER* cDNA (5) indicated substantial homology (Fig. 1). These data strongly suggested that both sequences correspond to the same conserved gene. To investigate this possibility, we hybridized human DNA with both *flk* and *FER* cDNA probes. Analysis of the restriction enzyme maps of both cDNAs demonstrated the presence of conserved *Cla*I and *Sst*I restriction enzyme sites in the *FER* and *flk* cDNAs. Digestion with these enzymes produced a fragment of approximately 900 base pairs encompassing the 3′ coding region of each cDNA. Hybridization of these probes to human DNA (6) gave identical hybridization patterns (data not shown). Although the intensities of the hybridizing fragments differed considerably, different washing conditions in combination with different exposure times revealed that the two probes detected identical fragments. In addition, the hybridization data indicated that the *FER/flk* gene is large and has a complex intron-exon structure. The recent isolation of human genomic clones confirms these findings (unpublished data).

The *FER/flk* gene encodes a widely distributed, evolutionarily conserved 94-kDa PTK. An antiserum to the N-terminal domain of the human *FER* protein (CH6), raised against a *trpE-FER* bacterial fusion protein containing *FER* amino acid residues 148 to 349, has been described previously (5). We also generated antisera (E and G) to the rat *flk* product by immunizing rabbits with bacterially expressed *trpE-flk* fusion proteins that contain the SH2 or kinase domain (Fig. 1). CH6 anti-*FER* serum immunoprecipitated a 94-kDa protein from HT1080 human fibrosarcoma cells (Fig. 2A, lane 5), which was identified by its apparent ability to autophosphorylate on tyrosine in an immune complex kinase reaction (10, 16, 17). Anti-*flk* sera E and G immunoprecipitated a comigrating 94-kDa protein from HT1080 cells which autophosphorylated in an in vitro kinase reaction (Fig. 2A, lanes 3 and 4). A tyrosine kinase of similar size was immunoprecipitated by anti-*FER* and anti-*flk* sera from all other human, rodent, and avian cells tested (data not shown). An anti-*FER* serum and two distinct anti-*flk* sera, therefore, recognized a 94-kDa protein which is widely distributed in vertebrate species. This protein was most readily distinguished by autophosphorylation on tyrosine. Detection of the 94-kDa protein in immunoprecipitates of cells labeled with [^{35}S]methionine was difficult and irreproducible, suggesting that its level of expression is low (data not shown).

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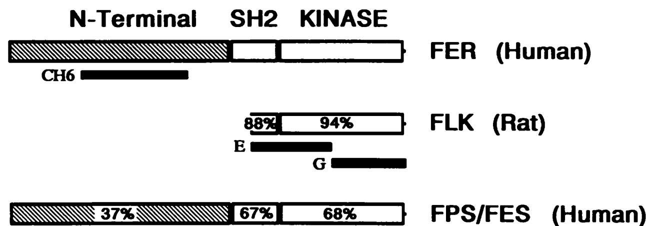

FIG. 1. Structural relationships among *FER*, *flk*, and *FPS/FES* gene products. The kinase, SH2, and N-terminal regions of the human *FER* protein are compared with the partial rat *flk* product and with human $p92^{c\text{-}fps/fes}$. The percent identities of *flk* (8) and *FPS/FES* (15) protein domains with the corresponding regions of the *FER* (5) polypeptide are indicated. The N-terminal regions of the *FER* and *FPS/FES* proteins have no homology with the *src* and *abl* PTKs. The regions used for production of anti-*FER* (CH6) and anti-*flk* (E and G) rabbit sera are illustrated by black boxes. For production of anti-*flk* sera, *trpE-flk* fusion proteins were expressed in *Escherichia coli* with pATH expression vectors, purified, and used for immunization (14). Antiserum E was made against a *trpE-flk* fusion protein containing part of the SH2 domain and the adjoining catalytic domain (residues 1 to 176). Antiserum G was made against the C-terminal portion of the protein (residues 177 to 323).

Antigenic relationship of FER and fps/fes proteins. Rat-1 fibroblasts transformed by Fujinami avian sarcoma virus have been previously used to generate anti-*fps/fes* rat tumor sera which recognize the avian $P130^{gag\text{-}fps}$ oncoprotein, as well as normal avian and mammalian c-*fps/fes* proteins. A few such antisera also recognized a structurally distinct 94-kDa PTK which was present in a number of different human and mouse cell lines (9). The 94-kDa human protein recognized by an anti-*fps/fes* rat tumor serum (J6) used in these previous experiments comigrates with that immunoprecipitated by anti-*FER* serum (Fig. 2B). Furthermore, both antisera recognized a 94-kDa tyrosine kinase in a variety of human cell lines, including cells of fibroblastic, epithelial, neural, and hematopoietic origins.

The simplest interpretation of these results is that *FER* and *flk* encode the human and rat forms of the same 94-kDa PTK, which corresponds to the p94 PTK previously identified with anti-*fps/fes* rat tumor serum. To investigate this possibility, p94 proteins were immunoprecipitated with CH6 anti-*FER*, E anti-*flk*, or J6 anti-*fps/fes* sera from HT1080 cells, radiolabeled with ^{32}P by in vitro autophosphorylation on tyrosine, and incubated during gel electrophoresis with various concentrations of V8 protease. In each case, the resulting partial V8 proteolytic fragments were identical (Fig. 3). These results confirm the common identity of the *FER* and *flk* proteins and prove that the human 94-kDa PTK encoded by this locus is the same as the p94 tyrosine kinase recognized by anti-*fps/fes* sera. Since *FER* and *flk* are clearly the human and rat forms of a conserved gene and encode the same 94-kDa PTK, we will use the designation *FER* for this gene regardless of species.

Since $p94^{fer}$ was originally identified with anti-*fps/fes* sera, we considered the possibility that anti-*FER* antibodies might in turn recognize *fps/fes* proteins. Within the N-terminal 222 residues used to generate CH6 anti-*FER* serum, only 90 (40%) are identical to the corresponding region of Fujinami avian sarcoma virus v-*fps/fes* oncoprotein $P130^{gag\text{-}fps}$ (15). Nonetheless, CH6 anti-*FER* serum efficiently precipitated $P130^{gag\text{-}fps}$ from a [^{35}S]methionine-labeled Rat-2 cell line that expresses the v-*fps/fes* oncogene (Fig. 4). Heating the cell lysate to 100°C in 0.5% sodium dodecyl sulfate (SDS) did not abolish subsequent immunoprecipitation of $P130^{gag\text{-}fps}$ by

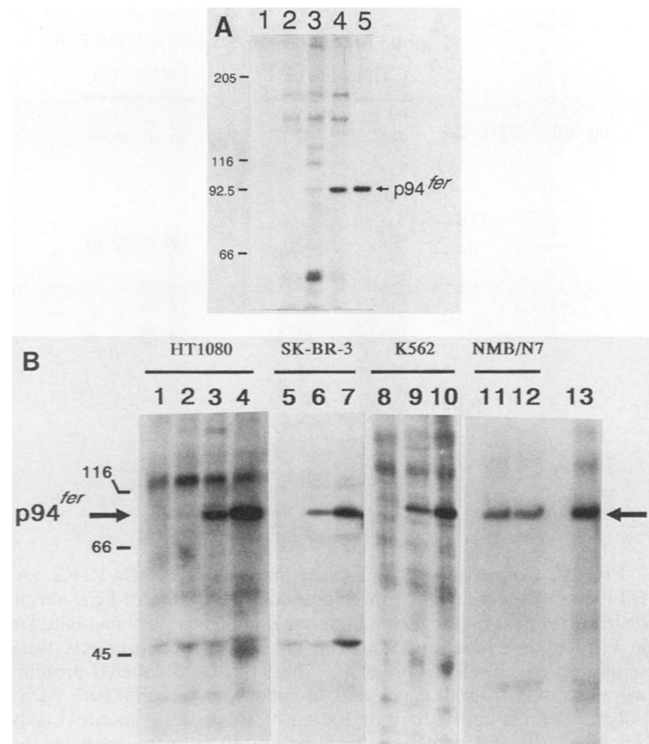

FIG. 2. A 94-kDa PTK recognized by anti-*FER*, anti-*flk*, and anti-*fps* sera. (A) Human HT1080 fibrosarcoma cells were lysed, immunoprecipitated, and incubated in immune complex kinase reactions. Immunoprecipitation was with preimmune G serum (lane 1), preimmune E serum (lane 2), immune G anti-*flk* serum (lane 3), immune E anti-*flk* serum (lane 4), and CH6 anti-*FER* serum (lane 5). (B) Cells were lysed and immunoprecipitated with control antibody (lanes 1, 5, and 8), J2 anti-*fps* rat tumor serum (lane 2), J6 anti-*fps* rat tumor serum (lanes 3, 6, 9, and 11), or CH6 anti-*FER* serum (lanes 4, 7, 10, 12, and 13). J2 is an anti-*fps* serum previously shown to be poorly cross-reactive with the 94-kDa protein. Immunoprecipitates were from human HT1080 fibrosarcoma cells (lanes 1 to 4 and 13), SK-BR-3 mammary carcinoma cells (lanes 5 to 7), K562 chronic myelogenous leukemia cells (lanes 8 to 10), and NMB/N7 neuroblastoma cells (lanes 11 and 12). In both experiments, immunoprecipitates were introduced into in vitro kinase reactions and the phosphorylated products were analyzed by electrophoresis on a 7.5% SDS-polyacrylamide gel. The gels were incubated in 1 M KOH at 55°C for 1.5 h before being dried and autoradiographed. The position of $p94^{fer}$ and the mobilities of size markers (10^3 kDa) are indicated.

anti-*FER* serum, suggesting that the relevant epitope is not dependent on folding of the native protein. $P130^{gag\text{-}fps}$ immunoprecipitated with anti-*FER* serum showed in vitro tyrosine kinase activity similar to that of $P130^{gag\text{-}fps}$ precipitated with an anti-*gag* monoclonal antibody, which binds to the retrovirally encoded N-terminal region of the Fujinami avian sarcoma virus transforming protein (Fig. 4). Anti-*FER* CH6 serum failed to precipitate human $p92^{c\text{-}fes}$ (data not shown). Interestingly, the human *FPS/FES* protein (13) is less closely related to $p94^{fer}$ in this region than is avian $P130^{gag\text{-}fps}$ (83 [37%] of 222 amino acids identical). Presumably, at least one of the residues shared between $p94^{fer}$ and $P130^{gag\text{-}fps}$ but distinct in human $p92^{c\text{-}fes}$ is crucial for CH6 antibody binding.

A *FPS/FES* PTK subfamily. These data establish that the human *FER* and rat *flk* cDNAs are derived from the same evolutionarily conserved gene, designated *FER*. The rat and

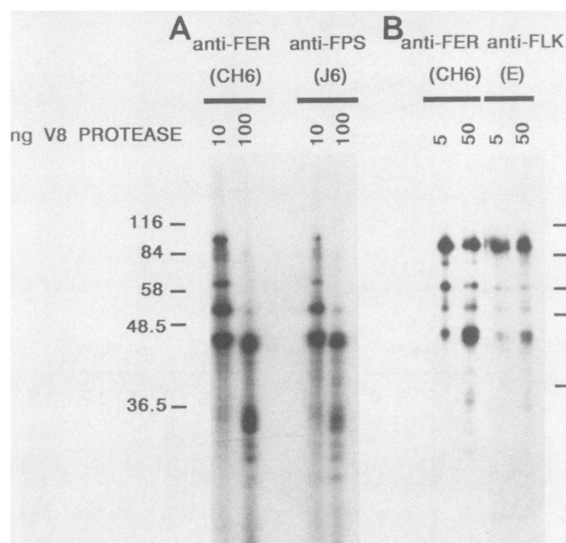

FIG. 3. Comparative V8 protease mapping of 94-kDa PTKs. (A) HT1080 cells were immunoprecipitated with CH6 anti-*FER* serum or J6 anti-*fps* rat tumor serum, immune complexes were incubated in in vitro kinase reactions, and the kinase reaction products were separated by gel electrophoresis. The 94-kDa ^{32}P-labeled proteins were excised from the gel and applied to a second 10% SDS-polyacrylamide gel with 10 or 100 ng of V8 protease as previously described (9). V8 protease digestion products were analyzed by fluorography. The mobilities of size markers and their molecular masses (10^3 kDa) are indicated. (B) Ninety-four-kilodalton proteins were immunoprecipitated from HT1080 cells with CH6 anti-*FER* or E anti-*flk* serum, autophosphorylated in vitro, isolated by gel electrophoresis, and applied to a 10% gel with 5 or 50 ng of V8 protease.

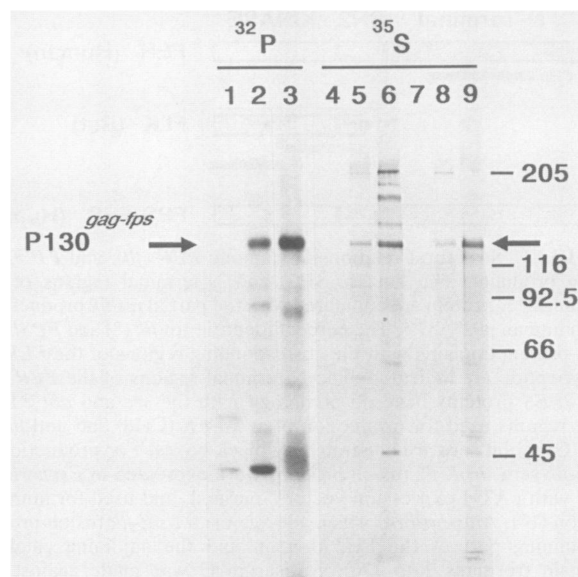

FIG. 4. Anti-*FER* serum recognizes the v-*fps* PTK. Rat-2 cells transformed by the *gag-fps* oncogene of Fujinami avian sarcoma virus were lysed and immunoprecipitated with normal rabbit serum (lane 1), anti-*FER* serum (lane 2), or anti-*gag* mouse monoclonal antibody (lane 3). Immunoprecipitates were incubated in immune complex kinase reactions and analyzed for autophosphorylated P130$^{gag\text{-}fps}$. *gag-fps*-transformed Rat-2 cells were metabolically labeled for 4 h with [^{35}S]methionine, lysed, and immunoprecipitated with control antiserum (lanes 4 and 7), anti-*FER* serum (lanes 5 and 8), or anti-*gag* monoclonal antibody (lanes 6 and 9) as previously described (16). Cells were lysed either in RIPA buffer (50 mM Tris hydrochloride [pH 7.5], 150 mM NaCl, 0.1% [wt/vol] SDS, 1% [vol/vol] sodium deoxycholate, 1% [vol/vol] Triton X-100, 2 mM phenylmethylsulfonyl fluoride, 100 μg of leupeptin per ml, 100 μM sodium orthovanadate) on ice (lanes 4 to 6) or in boiling 0.5% SDS buffer (10 mM $NaPO_4$ [pH 7.0], 0.5% SDS, 1 mM EDTA, 1 mM dithiothreitol), followed by dilution with RIPA buffer. The mobilities of P130$^{gag\text{-}fps}$ and size markers (10^3 kDa) are indicated.

human *FER* genes encode a 94-kDa PTK indistinguishable from a polypeptide previously identified by its immunological cross-reactivity with anti-*fps/fes* rat tumor serum (9). Several facets of p94^{fer} structure and antigenicity indicate that it is a close relative of p92$^{c\text{-}fes}$. Both p94^{fer} and p92$^{c\text{-}fes}$ share a short, basic C-terminal tail and a large N-terminal hydrophilic domain which are unique to these proteins and absent from *src* and *abl* nonreceptor PTKs (1, 12). We have shown here that an antiserum raised to the N-terminal domain of human p94^{fer} recognized avian P130$^{gag\text{-}fps}$. In all likelihood, p94^{fer} is the same 94-kDa protein, NCP94, precipitated by an anti-*fps/fes* peptide serum raised against a 12-amino-acid segment of the *fps/fes* kinase domain (3). Therefore, both the N-terminal and catalytic domains of *fps/fes* and *FER* proteins are antigenically cross-reactive.

The most striking difference between human *FER* and *FPS/FES* is in the extent of their expression. Although p92$^{c\text{-}fes}$ is synthesized only in some hematopoietic cells, p94^{fer} was detected in a diverse range of cell types, consistent with the broad distribution of a 2.9-kilobase *fer* transcript in a Northern (RNA) analysis of RNA from rat tissues (8). A similarly sized 3.0-kilobase *FER* RNA is present in all human cell lines examined (5). It therefore appears that p94^{fer} is coexpressed with a number of other nonreceptor PTKs, including p60$^{c\text{-}src}$ and p145$^{c\text{-}abl}$, which are both widely distributed. It is unlikely that these PTKs are entirely redundant in their functions. More probably, they couple distinct external signals to an interrelated set of effector pathways. The broad distribution of p94^{fer} suggests that it is of general and widespread importance in intracellular signaling. In addition to the widely expressed 2.9-kilobase *fer* mRNA, very abundant transcripts of 1.3 and 2.4 kilobases have been detected in rat testes with a *fer* probe (8). It will be of interest to know whether testis-specific *fer* transcripts encode a protein distinct from that found in somatic tissue.

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