were named *Vicia* cf. *faba*, but later *V. narbonensis* or *V.* type *faba*. Van Zeist (*15*) recognized a half seed of *V. faba*/*narbonensis* in the aceramic Neolithic (6000 B.C.) at Cape Andreas-Kastros, Northeast Cyprus. Costantini (*16*) reports two seeds of *Vicia* cf. *faba* from Neolithic (beginning of the fifth millennium B.C.) at Uzzo Cave, northwest Sicily. The earliest, previous positive identifications of horsebeans are reported from a younger Neolithic period (about 4000 B.C.) at sites at Sesklo and Dimini, Greece (*17*). Later Neolithic finds are known from Italy, Spain, Hungary, and Poland; Bronze Age finds are more common (*18*).

Vicia faba is the last important Old World cultivated legume the progenitor of which is still unknown. However, the discovery of cultivated horsebean at Yiftah'el from such an early period may indicate that the ancestor of this plant might have originated in the Levant or its surroundings.

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The Product of the c-*fms* Proto-oncogene: A Glycoprotein with Associated Tyrosine Kinase Activity

Abstract. *The* c-fms *proto-oncogene is a member of a gene family that has been implicated in tumorigenesis. Glycoproteins encoded by* c-fms *were identified in cat spleen cells by means of an immune-complex kinase assay performed with monoclonal antibodies to* v-fms*–coded epitopes. The major form of the normal cellular glycoprotein has an apparent molecular weight of 170,000 and, like the product of the viral oncogene, serves as a substrate for an associated tyrosine-specific protein kinase activity in vitro. The results suggest that the transforming glycoprotein specified by* v-fms *is a truncated form of a* c-fms*–coded growth factor receptor.*

The retroviral oncogene v-*fms* (*1*, *2*) of the McDonough strain of feline sarcoma virus (SM-FeSV) (*3*) encodes an integral transmembrane glycoprotein (*4–6*) whose expression at the cell surface is required for transformation (*5*). The biochemical and topological properties of the transforming glycoprotein are similar to those of a group of cell surface receptors for extracellular growth factors (*7*) and particularly to the v-*erb* B oncogene product, which is now known to be a truncated form of the epidermal growth factor (EGF) receptor (*8*). The mature glycoprotein encoded by v-*fms* is oriented in the plasma membrane with its glycosylated amino terminal portion (~450 amino acids) outside the cell and its carboxyl-terminal portion (~400 amino acids) in the cytoplasm (*5*, *6*, *9*). Nucleotide sequencing has predicted that a 200–amino acid segment of the distal cytoplasmic domain is related to a family of oncogene products that function as tyrosine-specific protein kinases (*9*). Indeed, immune complexes prepared with antibodies to the v-*fms* gene product exhibit a tyrosine kinase activity that phosphorylates the glycoproteins in vitro (*5*, *10*). The product of the cellular proto-oncogene (c-*fms*) has now been identified. We show that antibodies directed to the extracellular amino terminal domain of the v-*fms*–coded polypeptide precipitate glycoproteins of 170 and 130 kilodaltons (kD) from normal cat spleen. These polypeptides are active as substrates in an immune-complex kinase assay.

A species of RNA related to v-*fms* and 3.7 to 4.0 kilobases (kb) in length has been detected in different tissues, including mouse and human placental trophoblasts and choriocarcinoma cell lines (*11*), the murine myeloid cell line WEHI-3B (*12*), human bone marrow cells (*13*), and various human tumors (*14*). Because we were unable to detect a c-*fms* gene product in several murine and human cell lines that express c-*fms* RNA, we used a Northern blot method to analyze RNA from adult cat tissues for transcripts that hybridized to a v-*fms* probe. When equal quantities of polyadenylated RNA from cat spleen, brain, bone marrow, and liver were compared, relatively large amounts of 3.7-kb c-*fms* RNA were detected in spleen, intermediate amounts (~10 percent of spleen) were detected in brain, and substantially smaller amounts were detected in bone marrow and liver. Also seen in spleen cells were small amounts of 4.5- and 5.2-kb transcripts that may correspond to species of RNA detected in WEHI-3B cells (*12*).

To screen cat tissues for a putative c-*fms* gene product, we used a sensitive

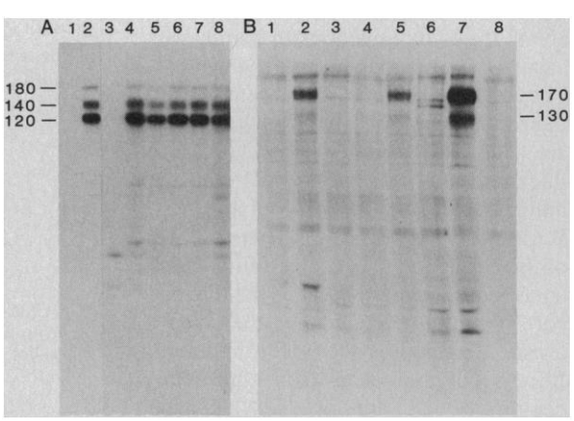

Fig. 1. Endogenous immune-complex kinase assay for products encoded by v-*fms* (A) and c-*fms* (B). Immune complexes were prepared with the antibodies listed below and incubated with [γ-^{32}P]ATP, and the products phosphorylated in vitro were analyzed on gels (*23*). Immune reagents included normal rat serum (lane 1), polyvalent rat antiserum to v-*fms*–coded glycoproteins (lane 2), control myeloma protein (lane 3), and the following rat IgG monoclonal antibodies to v-*fms*–coded epitopes: SM1.32.6 (lane 4), SM2.6.3 (lane 5), SM3.19.4 (lane 6), SM5.15.4 (lane 7), and SM6.2.10 (lane 8). Estimated molecular sizes of the v-*fms*–coded glycoproteins (left) and cat spleen (right) kinase substrates are in kilodaltons. Exposure times for autoradiography were 30 minutes (A) and 8 hours (B).

kinase assay that detects the v-*fms*–coded glycoproteins in vitro in immune complexes prepared with antibodies to the viral transforming gene product (*5, 10*). Three species of phosphorylated v-*fms*–coded glycoproteins are routinely detected in these assays. The largest is the glycosylated polyprotein gP180*gag-fms*, which represents the primary SM-FeSV translation product (*15, 16*). Because the v-*fms* gene was transduced in frame within the viral *gag* gene (*2*), the amino terminus of the SM-FeSV polyprotein is encoded by retroviral sequences, whereas the carboxyl-terminal portion is specified by the oncogene. Cotranslational glycosylation and proteolysis remove the *gag*-coded portion of the polyprotein and generate a v-*fms*–coded glycoprotein, gp120*fms*, that is the predominant form detected in transformed cells (*15, 17*). The gp120*fms* molecules accumulate in the endoplasmic reticulum and contain mannose-rich, asparagine-linked oligosaccharide chains. A small proportion of the molecules is transported through the Golgi complex to the plasma membrane, acquiring complex asparagine-linked oligosaccharides in transit and appearing as glycoproteins of greater apparent molecular weight (gp140*fms*) (*4, 17, 18*). The three forms of the v-*fms*–coded molecules in transformed cells were active as substrates in the immune-complex kinase assay performed with a polyvalent rat antiserum to SM-FeSV gene products (Fig. 1A). No phosphorylation was observed with serum from nonimmune rats or with rat myeloma immunoglobulin G (IgG) as control (Fig. 1A). The same kinase activity was shown with each of five rat monoclonal IgG antibodies to v-*fms*–coded polypeptide epitopes (Fig. 1A) (*19*). The various forms of v-*fms*–coded glycoproteins were phosphorylated in ratios equivalent to their relative steady-state amounts as detected by immunoblotting of transformed cell lysates (*4, 17*).

Detergent homogenates were prepared from samples of an adult cat spleen that was shown in parallel studies to express relatively high levels of c-*fms* RNA. Portions of the tissue homogenate were immunoprecipitated with either the polyvalent rat antiserum or the five rat monoclonal antibodies that detected v-*fms*–coded products in the immune-complex kinase assay. Two phosphorylated species were identified in a kinase assay performed with the monoclonal antibody SM5.15.4 (Fig. 1B). The major phosphorylated species had an apparent molecular size of 170 kD, and the minor phosphorylated species had an apparent molecular size of 130 kD. The same two polypeptides were seen at lower intensity with monoclonal antibody SM2.6.3 and the polyvalent rat antiserum (Fig. 1B). In contrast, these phosphorylated substrates were not identified by assays in vitro with normal rat serum, an unrelated rat immunoglobulin G (IgG) monoclonal antibody, or three other monoclonal antibodies to v-*fms*–coded epitopes (Fig. 1B). Homogenates of brain and liver tissues that expressed lower levels of c-*fms* RNA yielded no detectable 170- and 130-kD proteins in immune-complex kinase assays performed with the same reagents. However, positive results were reproducibly obtained with spleens from several other adult cats (*20*).

The ^{32}P-labeled normal cellular proteins of 170 and 130 kD were eluted from gels, hydrolyzed in acid, and subjected to phosphoamino acid analyses. Forms of v-*fms*–coded glycoproteins phosphorylated in vitro in the assay were analyzed in parallel as controls. Phosphotyrosine was the only phosphoamino acid detected in the v-*fms*–coded products gp120*fms* (Fig. 2A) and gp140*fms* (Fig. 2B). Phosphoamino acid analysis of the viral glycoprotein gP180*gag-fms* yielded the same result. Similarly, phosphotyrosine was the major phosphoamino acid detected in the cat spleen 130-kD (Fig. 2C) and 170-kD (Fig. 2D) proteins, although trace amounts of phosphoserine were also seen. Several background bands from the same gel contained phosphoserine but no phosphotyrosine, showing that immune precipitates prepared from cat spleen homogenates had a small amount of contaminating serine kinase.

Two-dimensional tryptic peptide analysis showed that ^{32}P-labeled 170- and 130-kD proteins from cat spleen had identical phosphopeptides that comigrated with those of the v-*fms*–coded glycoproteins. We therefore hypothesized that the normal cellular proteins also represented two forms of a single glycoprotein that differed in their carbohydrate moieties. However, the small amounts of these proteins in normal tissues precluded the use of kinetic analyses with metabolic isotopic labeling. As an alternative approach, ^{32}P-labeled proteins phosphorylated in immune complexes were treated with glycosidic enzymes. The v-*fms*–coded glycoproteins and the proteins from cat spleen were sensitive to digestion with endoglycosidase F, which cleaves both mannose-rich and complex oligosaccharide chains (*21*) (Fig. 3A). In the case of the viral transforming gene products, the immature forms gP180*gag-fms* and gp120*fms* were sensitive to endoglycosidase H (Fig. 3B) and resistant to neuraminidase treatment (Fig. 3C), whereas the processed cell surface form gp140*fms* showed a reciprocal pattern of enzyme sensitivity (*4*). Like the mature

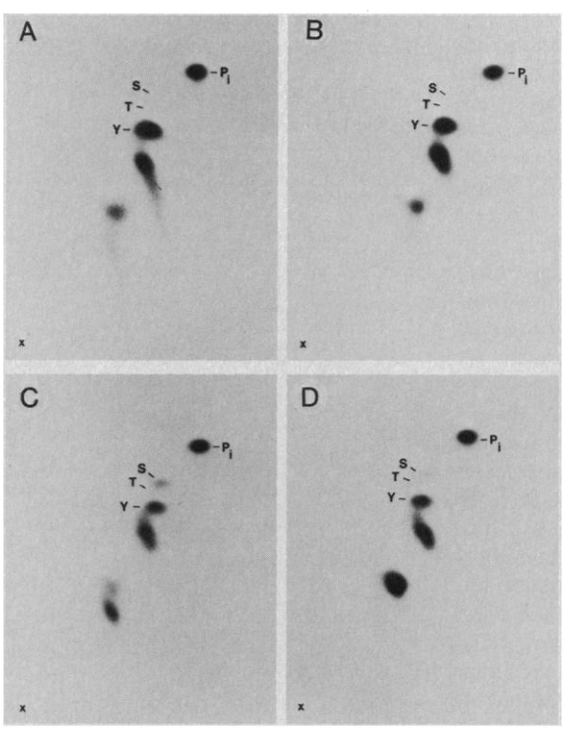

Fig. 2. Phosphoamino acid analysis of substrates encoded by v-*fms* and c-*fms* and isotopically labeled in the immune-complex kinase reaction. Analyses were performed with the transforming viral glycoproteins gp120*fms* (A) and gp140*fms* (B) and the cat spleen 130-kD (C) and 170-kD (D) proteins. Samples were prepared (*24*) and applied to cellulose-coated thin layer plates (origin marked X at the lower left corner of each panel) after suspension in buffer containing authentic phosphotyrosine (Y), phosphoserine (S), and phosphothreonine (T) standards (Sigma). Separation of phosphoamino acids was achieved by electrophoresis in two dimensions at 1000 V. In the first dimension, electrophoresis was from left (cathode) to right in acetic acid–formic acid–water (15:5:80, *p*H 1.9). The plate was then rotated 90° for electrophoresis in the second dimension from bottom (cathode) to top in acetic acid–pyridine–water (5:0.5:94.5, *p*H 3.5). Dried plates were exposed to film for autoradiography, and the autoradiogram spots were aligned with the phosphoamino acid standards visualized by ninhydrin staining. The mobility of [^{32}P]orthophosphate (P_i) is also noted.

gp140*fms* glycoprotein, the 170-kD protein precipitated from cat spleen was resistant to endoglycosidase H (Fig. 3B) but sensitive to neuraminidase digestion (Fig. 3C). In contrast, the minor 130-kD spleen polypeptide was neuraminidase-resistant and endoglycosidase H–sensitive; treatment with the latter enzyme generated a species (arrowhead in Fig. 3B) that was only slightly larger than the corresponding form of the immature viral glycoprotein gp120*fms*. Taken together, these data show that the 130- and 170-kD polypeptides are glycoproteins differing in the composition of their asparagine-linked oligosaccharide chains and suggest that the 130-kD glycoprotein is a precursor to the 170-kD molecule.

The length of the polyadenylated c-*fms* messenger RNA is somewhat longer than the transduced 3.0-kb v-*fms* gene of SM-FeSV (*2, 9*), indicating that v-*fms* includes most of the information specified by the proto-oncogene. On the basis of (i) the size of the normal cellular proteins, (ii) their detection in tissues expressing high levels of c-*fms* RNA, (iii) their cross-reactivity with polyvalent and monoclonal antibodies to the viral transforming proteins, (iv) their functional similarity as substrates for an associated tyrosine kinase in vitro, and (v) their patterns of glycosylation and tyrosine phosphorylation, these proteins represent the products of the feline c-*fms* locus. By convention, we propose that gp170c-*fms* and gp130c-*fms* be used to designate these glycoproteins.

Each of the monoclonal antibodies used in these studies recognizes epitopes that have been mapped to the amino-terminal domain of gp140v-*fms* and that are expressed on the surfaces of live SM-FeSV–transformed cells (*5, 6, 17*). All five of the antibodies were active in immune-complex kinase assays performed with v-*fms* gene products; however, only two of the antibodies functioned in enzyme assays with immunoprecipitates from cat spleen lysates. We have not yet detected the c-*fms* gene products with these antibodies by immunoblotting or immunofluorescence procedures, possibly because their levels of expression are too low to be detected by these less sensitive methods. It is possible that the tyrosine kinase activity associated with the v-*fms* product acts constitutively, whereas that of the c-*fms* product does not. Monoclonal antibodies SM5.15.4 and SM2.6.3 may recognize epitopes in the putative ligand-binding site of a c-*fms*–coded receptor, thereby activating its associated tyrosine kinase in immune complexes. Antibodies that stimulate the tyrosine kinase activities

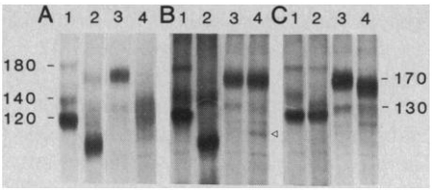

Fig. 3. Sensitivity to endoglycosidase F (A), endoglycosidase H (B), and neuraminidase (C) of ^{32}P-labeled glycoproteins encoded by v-*fms* (lanes 1 and 2) and c-*fms* (lanes 3 and 4). Polypeptides were isotopically labeled in immune-complex kinase assays. Immune precipitates were then incubated (*25*) in either the absence (lanes 1 and 3) or presence (lanes 2 and 4) of enzyme. Products were analyzed by electrophoresis in 6 percent polyacrylamide slab gels. Each panel is a composite of lanes run on the same gel. The mobilities of undigested phosphoproteins encoded by v-*fms* (left) and c-*fms* (right) are in kilodaltons. The arrowhead (B, lane 4) indicates the band from the 130-kD normal cellular glycoprotein after treatment with endoglycosidase H.

and in some cases mimic the biologic effects of ligand binding have been reported for the EGF and insulin receptors (*22*). Whatever the mechanism, monoclonal antibodies that precipitate the c-*fms*–coded glycoprotein should facilitate the identification of putative ligands capable of activating the kinase in vitro.

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24. Phosphoproteins were identified in dried polyacrylamide gel slabs by autoradiography. The regions of the gel containing isotopically labeled bands were excised, washed in 10 percent methanol, and lyophilized to dryness. The gel slices were incubated for 18 hours at 37°C in 0.6 ml of 50 m*M* ammonium bicarbonate containing 30 μg of L-(tosylamido 2-phenyl)ethyl chloromethyl ketone–treated trypsin. The gel slice was then removed, and the supernatant was lyophilized to dryness. The dried material was resuspended in 0.5 ml of 6*N* HCl, heated in a boiling-water bath for 2 hours in a sealed plastic tube, and lyophilized.
25. The ^{32}P-labeled proteins in immune precipitates were incubated for 20 hours at 37°C in 25 μl of (i) 100 m*M* sodium phosphate (*p*H 6.1) containing 50 m*M* EDTA, 1 percent Triton X-100, 0.1 percent SDS, 1 percent 2-mercaptoethanol, and 0.2 U of *Flavobacterium meningosepticum* endo-β-*N*-acetylglucosaminidase F (New England Nuclear) or (ii) 150 m*M* sodium citrate (*p*H 5.3) containing 7.5 mU of *Streptomyces griseus* endo-β-*N*-acetylglucosaminidase H (E.C. 3.2.1.96) (Sigma) or (iii) 50 m*M* sodium acetate (*p*H 5.5) containing 150 m*M* NaCl, 4 m*M* $CaCl_2$, and 0.025 U of *Vibrio cholerae* neuraminidase (E.C. 3.2.1.18) (Calbiochem-Behring). Matched control incubations were performed in the same buffers without enzymes. Reactions were terminated by addition of electrophoresis sample buffer.

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