Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 85–89, January 1984
Biochemistry

Nucleotide sequence of the feline retroviral oncogene v-*fms* shows unexpected homology with oncogenes encoding tyrosine-specific protein kinases

(transforming glycoprotein/signal peptides/feline sarcoma virus)

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Communicated by Peter K. Vogt, September 12, 1983

ABSTRACT **The nucleotide sequence encoding the transforming polyprotein of the McDonough strain of feline sarcoma virus was determined. This sequence includes 231 nucleotides specifying a leader peptide, 1,377 nucleotides encoding most of the feline leukemia virus-derived *gag* gene, and 2,969 nucleotides representing the viral transforming gene v-*fms*. A single open reading frame was predicted to encode a fusion polyprotein of 160,000 daltons (P160*gag-fms*). Fourteen potential sites for glycosylation were predicted within the v-*fms*-encoded portion of the protein, consistent with previous observations that the primary translation product is rapidly glycosylated. The presence of hydrophobic signal peptides within the amino-terminal leader sequence and in the middle of the v-*fms*-encoded moiety suggests that the transforming glycoprotein becomes oriented with its amino terminus within the lumen of the rough endoplasmic reticulum and its carboxyl terminus protruding across the membrane of the rough endoplasmic reticulum into the cytoplasm. The latter portion of the protein shows unexpected homology to tyrosine-specific protein kinases encoded by several of the known retroviral oncogenes.**

Viral oncogenes ("v-*onc* genes") have been derived by recombination between nontransforming retroviruses and proto-oncogene sequences present in normal cells (1, 2). The recombinational event places the acquired transforming gene under the control of strong viral transcriptional promoters that facilitate the expression of the transduced gene in infected cells. The feline sarcoma viruses (FeSVs) represent one such family of sarcoma-inducing viruses formed by recombination between feline leukemia virus (FeLV) and preexisting proto-oncogene sequences present in uninfected cat cells (3). Because FeLV is a natural, horizontally transmitted leukemia virus, as many as five different v-*onc* genes have been independently transduced from cat DNA. These include: (*i*) v-*fes* (3–5), present in the Snyder–Theilen (6) and Gardner–Arnstein (7) strains of FeSV and homologous to the v-*fps* gene of avian Fujinami sarcoma virus (8–10); (*ii*) v-*fms* (3, 11), the transforming gene of McDonough FeSV (12); (*iii*) v-*fgr* (13), a newly identified gene of the Gardner–Rasheed strain (14); and (*iv*) v-*abl* and v-*sis*, found in the Parodi–Irgens and Hardy–Zuckerman FeSV strains (15, 16), which show homology to the independently transduced viral oncogenes of Abelson murine leukemia virus and simian sarcoma virus, respectively. Three of these feline viral oncogenes (v-*fes*, v-*fgr*, and v-*abl*) specify proteins with tyrosine-specific protein kinase activities (13, 17, 18) and are members of an extended multigene family that includes the v-*onc* genes v-*src*, v-*yes*, and v-*ros* (cf. refs. 9 and 10). By contrast, both v-*fms* and v-*sis* encode proteins that lack kinase activity under similar assay conditions; like only one other oncogene, v-*erb*^{B} (19, 20), v-*fms* specifies a transforming glycoprotein (21, 22).

The McDonough strain of feline sarcoma virus (SM-FeSV) has the gene order 5′ Δ-*gag-fms-env* 3′, where only a portion of the FeLV-derived *gag* gene and virtually none of the *pol* gene appear to have been retained (11). Cells transformed by SM-FeSV synthesize a viral polyprotein encoded by the fused *gag* and v-*fms* genes (23–25). The protein initially has an apparent molecular mass of 160 kilodaltons (kDa) but is converted by glycosylation (21, 22) to a heavier molecule designated gP180*gag-fms* (apparent molecular mass, ≈170–180 kDa). The glycosylated polyprotein undergoes proteolytic cleavage, yielding an amino-terminal *gag*-encoded fragment of about 55 kDa, and a carboxyl-terminal v-*fms*-encoded portion of about 120 kDa (gp120*fms*) (21–24). The latter polypeptide is further modified to yield at least one other glycosylated form (gp140*fms*); both gp120*fms* and gp140*fms* comprise the major steady-state species of transforming protein detected in SM-FeSV-transformed cells (22). Perhaps surprisingly, the transforming glycoproteins have not been detected at the cell surface but are localized primarily to juxtanuclear "complexes" containing membranes and intermediate filaments. Although the v-*fms*-encoded glycoproteins can serve as *in vitro* substrates for tyrosine-specific protein kinases, cells transformed by SM-FeSV do not show elevated levels of phosphotyrosine, nor is phosphotyrosine found in the polyprotein metabolically labeled *in vivo* (25, 26). Even when SM-FeSV transformants were superinfected with the Snyder–Theilen strain of FeSV, no phosphorylation of the v-*fms* products was detected in spite of the fact that the total phosphotyrosine levels in transformed cells were grossly elevated in response to v-*fes*-encoded kinase activity (22). Nucleotide sequence assay now predicts that a region of the v-*fms* gene specifies a product with partial homology to v-*onc*-encoded tyrosine kinases.

MATERIALS AND METHODS

The DNA clone used for these analyses is biologically active in DNA transfection assays and yields over 2,000 foci per μg of DNA after transfection of NIH 3T3 cells (11). The nucleotide sequence was determined in accordance with previously published procedures (10) by using the technique of Maxam and Gilbert (27). Both strands were independently analyzed several times from different end-labeled restriction sites. All restriction sites used as starting points were reanalyzed as internal sites within different restriction fragments to assure that small fragments generated by proximal cleav-

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Abbreviations: FeSV, feline sarcoma virus; RER, rough endoplasmic reticulum; v-*onc* genes, viral oncogenes; SM-FeSV, strain McDonough FeSV; kDa, kilodaltons; FeLV, feline leukemia virus.

age events with a single enzyme were not excluded from the analysis.

RESULTS AND DISCUSSION

The nucleotide sequence, numbered with respect to the predicted amino terminus of the *gag* gene, is presented in Fig. 1. The methionine residue at amino acid position +1 was aligned with respect to both previously determined nucleotide (10, 28) and amino acid (29) sequences obtained with the *gag* genes of several FeLV and FeSV strains. Translation of the nucleotide sequence as shown generates no termination codons before amino acid residue 1,434, whereas numerous stop codons are found in the other two reading frames. Because spliced v-*fms*-containing mRNAs were not detected in SM-FeSV transformants (11), the single open reading frame shown in the figure is predicted to encode the SM-FeSV polyprotein.

The illustrated sequence of 4,577 nucleotide pairs shown in Fig. 1 includes a region predicted to encode a *gag* "leader peptide." An open reading frame in the leader sequence is in frame with the ATG codon that specifies the *gag* amino terminus and can encode 77 additional amino acids. The leader sequence, previously detected in both the Snyder–Theilen and Gardner–Arnstein FeSV strains (28), appears to encode a portion of the FeLV *gag* precursor that is removed during translation (30). Because the amino-terminal leader peptide is not represented in completed *gag*-encoded proteins released from polyribosomes (29, 30), its sequence of amino acids was arbitrarily numbered from −77 to −1 to indicate its relative position on the amino-terminal side of the *gag* gene product.

The FeLV-derived portion of the SM-FeSV polyprotein comprises 459 *gag*-encoded residues. In FeLV, the *gag* gene precursor Pr65 is about 65 kDa and is post-translationally cleaved to form four small polypeptides whose order in the precursor is NH_2-p15-pp12-p30-p10-COOH (31). By convention, the numbers indicate the apparent kilodaltons of the individual peptides; however, the phosphorylated peptide, pp12, is predicted to be closer to 7 kDa (see ref. 10 and Fig. 1). The SM-FeSV polyprotein contains all of p15, p12, and p30 but only a portion of p10. The p15/p12 junction is located between residues 127 and 128; p12/p30, between residues 196 and 197; and p30/p10, between residues 445 and 446. According to the sequence of the FeLV *gag* gene (unpublished data), the p10 sequence, which is interrupted after 14 amino acid residues, is followed by residues encoded by the acquired v-*fms* gene. In SM-FeSV, the individual *gag* gene-encoded peptides do not undergo the proteolytic cleavage characteristic of the FeLV *gag* gene precursor. Instead, the polyprotein is cleaved at a site (or sites) near the *gag/fms* junction, generating an amino-terminal *gag*-encoded fragment of about 55 kDa and the carboxyl-terminal v-*fms*-encoded portion (23, 24). The proteolytic cleavage of certain other polyproteins, including the *env* gene precursor of Rous sarcoma virus, the hemagglutinin of fowl plague virus, and several peptide hormone precursors, occurs at the carboxyl-terminal side of a configuration of four to six basic amino acid residues (reviewed in ref. 32). A similar potential site of cleavage occurs at residues 435–439 (Lys-Lys-Arg-His-Lys), 20 amino acids to the amino-terminal side of the *gag/fms* junction. Note that residues 416–460 contain 14 basic amino acids sensitive to trypsin-like enzymes.

By heteroduplex analysis performed with molecular clones of SM-FeSV and FeLV, the complexity of the v-*fms* gene was estimated to be about 3 kilobase pairs long (11), close to the 2,969 nucleotide pairs predicted by the DNA sequence. The open reading frame does not include the 44 nucleotides at the 3′ terminus of v-*fms* that precede the 3′ site of recombination with FeLV sequences. The latter site was found to differ from the 3′ site of recombination between v-*fes* and FeLV previously localized within two other FeSV strains (10). The SM-FeSV polyprotein is predicted to contain 1,434 amino acids of which 975 would be encoded by v-*fms*. The deduced molecular size of the primary translation product is 160,098 daltons, in good agreement with the previous estimate of 155 kDa for the unglycosylated polyprotein (22).

The difference in the molecular size of the primary translation product and the polyprotein detected soon after synthesis (gP180*gag-fms*) is due to the addition of carbohydrate residues (21, 22). Because glycosylation was sensitive to the antibiotic tunicamycin (22), the oligosaccharides must be N-linked to asparaginyl residues (33, 34). Predicted sites of linkage are Asn-X-Ser/Thr, where X can be any amino acid with the possible exception of proline (35). These sites are shown by the shaded residues in Fig. 1 and include one within the *gag* p15 sequence (residues 60–62) and 14 within the v-*fms*-encoded region. All detectable glycosylation occurs within the v-*fms*-encoded moiety (22).

A prerequisite for carbohydrate addition is the transport of proteins into the cisternae of the rough endoplasmic reticulum (RER) (36). Translocation across the membrane of the RER is mediated by hydrophobic "signal peptides," which anchor the polypeptide chains in the membrane and facilitate their vectorial transport into the lumen of the RER (37). For secreted proteins like immunoglobulin, a signal peptide located near the amino terminus is proteolytically removed prior to completion of translation, so that the polypeptide is released within the cisternal space (38, 39). In the case of glycoproteins that remain physically associated with membranes, additional signal sequences located nearer to the carboxyl terminus interrupt translocation across the membrane (37). The "stop signals" function as transmembrane anchors, positioning distal carboxyl-terminal residues on the cytoplasmic side of the RER membrane. To localize putative signal sequences, all hydrophobic peptides longer than nine amino acids in length lacking charged residues were identified, and their length was plotted against their hydrophobicity index (40). Two strongly apolar regions were found that shared characteristics with known signal peptides in terms of their size and amino acid composition. The first, located within the *gag* leader peptide at positions −23 to −4, presumably serves to direct nascent polypeptides to the RER membrane (28, 30). The second occurs near the middle of the v-*fms*-encoded portion (see Fig. 1). This suggests that the v-*fms* products are transmembrane proteins, anchored in the RER membrane by residues 1,003–1,028 and oriented with their amino-terminal portion in the cisternae and their carboxyl-terminal moiety in the cytoplasm. If this were the case, 12 of 15 potential glycosylation sites would have access to glycosyl transferases within the RER, whereas only the carboxyl-terminal portion of the transforming glycoprotein could interact with cytoplasmic elements.

The most surprising feature of the predicted amino acid sequence of v-*fms* was the homology of its carboxyl-terminal portion to regions of other v-*onc*-encoded products that exhibit associated tyrosine-specific protein kinase activities. Fig. 2 shows a representative comparison between the predicted carboxyl-terminal regions encoded by v-*fms* (residues 1,201–1,400), v-*fes* (Gardner–Arnstein strain) (10), and v-*src* (Prague C Rous sarcoma virus) (41). These regions (*i*) were predicted previously to have close homology with other members of the tyrosine kinase gene family (reviewed in refs. 9 and 10); (*ii*) include the regions of homology to the catalytic subunit of cyclic AMP-dependent protein kinase (42); and (*iii*) appear to represent the portions of these proteins responsible for enzyme activity (43–46). In particular, a 29-kDa peptide representing the carboxyl-terminal portion of pp60*src* retains tyrosine kinase activity *in vitro* (43).

Fig. 2 shows that there is 45% amino acid homology be-

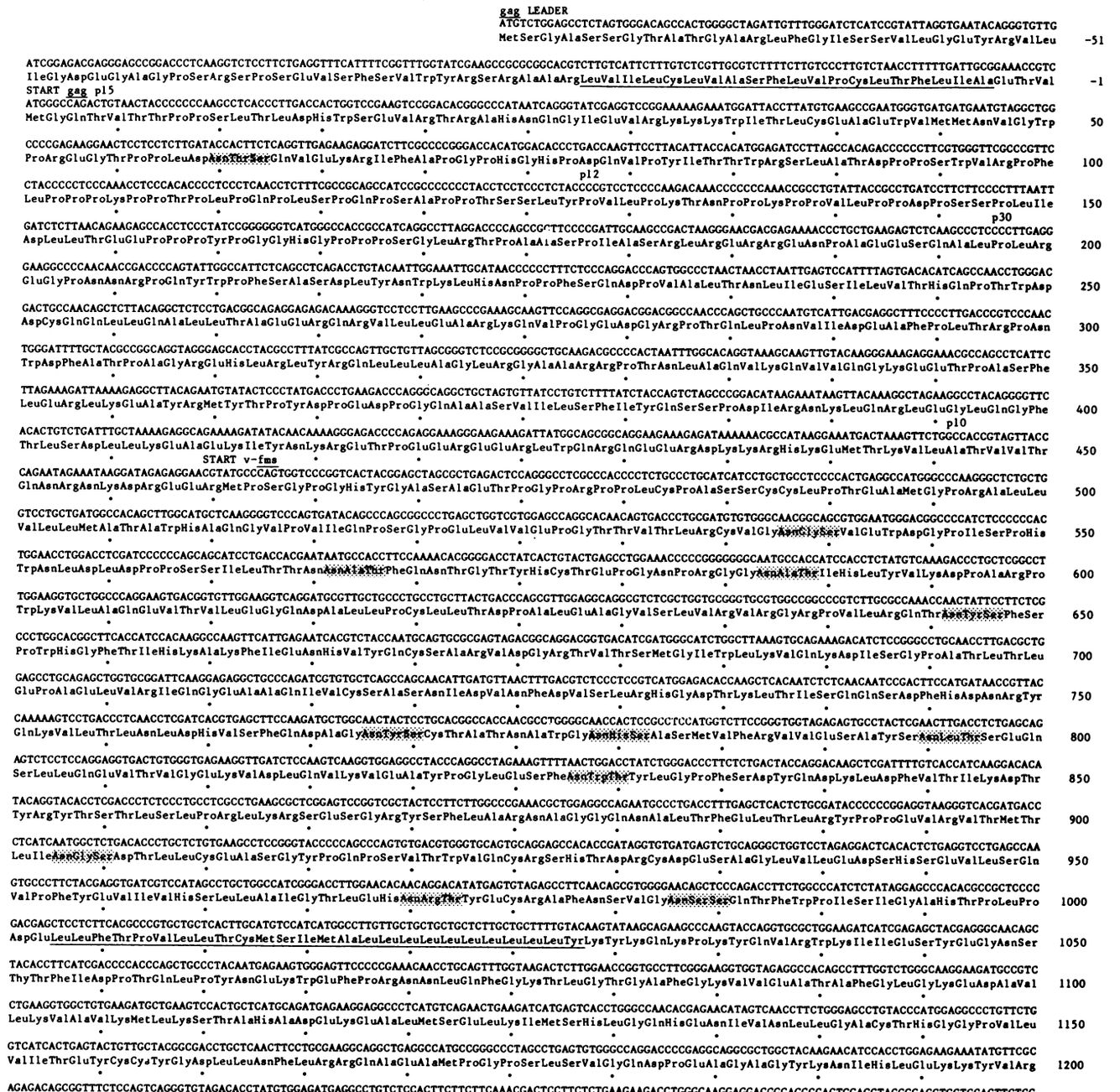

FIG. 1. Nucleotide and predicted amino acid sequence of the polyprotein-encoding region of SM-FeSV. The numbers at the end of each line refer to the position of the predicted amino acid residues, with position +1 defining the *gag* gene amino-terminal methionine residue. The *gag* leader sequence includes 77 amino acids not found in the complete *gag* gene products; these have been arbitrarily assigned positions −77 to −1 in the sequence to indicate their derivation from nucleotides 5′ to the polyprotein-encoding region. The junctions of individual *gag* peptides are shown after residues 127, 196, and 445. The *gag-fms* junction is indicated after residue 459. The 5′ site of recombination between FeLV and v-*fms* sequences occurs in the *gag* p10 sequence and differs from the crossover sites in Snyder–Theilen and Gardner–Arnstein FeSVs, which occur in *gag* p30 sequences. Similarly, the 3′ site of recombination between FeLV and v-*fms* is in the 3′ end of the *pol* gene, upstream of the recombination site seen in Snyder–Theilen and Gardner–Arnstein FeSVs. Potential sites of glycosylation are shaded, and the two hydrophobic "signal peptides" are underlined. The 3′ v-*fms* noncoding sequences include 41 nucleotide pairs distal to the TAG stop codon.

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v-fms  RDSGFSSQGVDTYVEMRPVSTSSSNDS FSEEDLGKEDG RPLELRDLLHFSSQVAQGMAFLASKNCIHRDVAARNVLLTSGRVAKIGDFGLARDIMNDSNY  1300
v-fes  HPNIVRLIGVCTQKQPIYIVMELVQGGDFL TFLRTE GAR LRMKTLLQMVGDAAAGMEYLESKCCIHRDLAARNCLVTEKNVLKISDFGMSREAA DGIY   848
v-src  RHEKLVQLYAVVSEEPIYIVIEYMSKGSLL DFLKGEMGKY LRLPQLVDMAAQIASGMAYVERMNYVHRDLRAANILVGENLVCKVADFGLARLIE DQEY   416

v-fms  IVKGNARL PVKWMAPESIFDCVYTVQSDVWSYGILLWEIFSLGLNPYPGILVNSKFYKLV KDGYQMAQPAFAPKNIYSIM QACWALEPTRRPTFQQICSL 1400
v-fes  AASGGLRQVPVKWTAPEALNYGRYSSESDVWSFGILLWETFSLGASPYPN LSNQQTREFVEKGGRLPC PELCPDAVFRLMEQ CWAYEPGQRPSFSAIYQE  948
v-src  TARQGAKF PIKWTAPEAALYGRFTIKSDVWSFGILLTELTTKGRVPYPG MVNREVLDQVERGYRMPCPPE CPESLHDLMCQ CWRKDPEERPTFKYLQAQ  515
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FIG. 2. Homology between the predicted carboxyl-terminal portions of transforming proteins encoded by v-*fms*, v-*fes*, and v-*src*. The v-*fms* residues correspond to amino acids 1,201–1,400 in Fig. 1. The v-*fes* residues are numbered from the *gag-fes* amino terminus and correspond to the predicted sequence of the polyprotein of the Gardner–Arnstein FeSV strain (10). The v-*src* sequence is derived from the Prague C strain of Rous sarcoma virus (41). The sequences were aligned without computer assistance, and the percentage of homology was calculated in the exact region shown. The single underlined residue marks the tyrosine phosphoacceptor in the v-*src*- and v-*fes*-encoded proteins. A, alanine; R, arginine; D, aspartic acid; N, asparagine; C, cysteine; E, glutamic acid; Q, glutamine; G, glycine; H, histidine; I, isoleucine; L, leucine; K, lysine; M, methionine; F, phenylalanine; P, proline; S, serine; T, threonine; W, tryptophan; Y, tyrosine; and V, valine.

tween proteins encoded by v-*fes* and v-*src* in the region shown. For the same region, there is 45% homology between the v-*fes* and v-*fms* products and 35% homology between the v-*src*- and v-*fms*-encoded proteins. A site-directed point mutation that substituted threonine for alanine at position 433 in pp60*src* inactivated its kinase activity and rendered the mutant nontransforming (44). The v-*src*- and v-*fes*-encoded products contain the peptide Lys-Trp-Thr-Ala-Pro-Glu-Ala (at pp60*src* residue 433). The v-*fms* sequence in the analogous region predicts the peptide Lys-Trp-Met-Ala-Pro-Glu-Ser in which two amino acids are substituted. The conserved regions of these proteins also include the sites of tyrosine phosphorylation within the products themselves (underlined in Fig. 2). The v-*src* and v-*fes* sequences predict arginine and aspartic acid seven and three residues, respectively, to the amino-terminal side of the tyrosine phosphoacceptor. This configuration of amino acids was postulated to represent a consensus sequence conserved among different tyrosine phosphoacceptors (47). In the predicted v-*fms*-encoded protein, arginine is eight amino acids amino-terminal to the homologous tyrosine residue. Although this sequence may serve as a phosphoacceptor *in vitro* (26), alterations could unmask secondary *in vitro* phosphoacceptor sites (48, 51). The v-*fms*/v-*src* homology region does not include amino acid residues near the lysine at position 295 in pp60, which is postulated to represent the binding site for ATP (41, 42). The differences in the v-*fms*-encoded protein are consistent with findings that the product lacks an intrinsic kinase activity and is not itself phosphorylated in tyrosine *in vivo* (25, 26).

Another v-*onc* gene, v-*mos*, was predicted previously to encode a protein homologous to the conserved region of pp60*src* (49), even though tyrosine kinase activity was not found in association with the precipitated gene product (50). More recently, v-*erb*^{B} which, like v-*fms*, specifies a glycoprotein (19, 20), was predicted to encode a protein homologous to that of pp60*src* (52). Thus, portions of many v-*onc* genes may be descended from a single primordial sequence, which has evolved to specify functionally distinct proteins. One possibility is that v-*onc* gene products currently thought to lack tyrosine kinase activity may be shown upon further examination to specify this activity under different assay conditions. Alternatively, the proteins encoded by v-*fms*, v-*mos*, and v-*erb*^{B} might transform cells by entirely different mechanisms. The homology between these different v-*onc* gene products raises provocative questions concerning the role of tyrosine-specific protein kinases in cell transformation. Is kinase activity *per se* necessary for transformation or are kinase activity and other cryptic "transforming functions" encoded by the same regions of these molecules? Are there indeed physiological targets for protein kinases that must be phosphorylated in tyrosine in order to trigger the transformation process? These ambiguities emphasize the need for cellular mutants, refractory to transformation by certain oncogenes, which will be crucial in defining relevant physiological targets of transforming proteins.

We thank Dr. Eric Hunter for discussions concerning signal peptides and Drs. Gilbert Jay and Douglas Lowy for their critical reading of the manuscript. This work was supported by an ongoing collaborative agreement between the participating laboratories, initiated through the Institut Nationale de la Santé et de la Recherche Médicale, France, and the Carcinogenesis Intramural Program of the National Cancer Institute.

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