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# Detection and Localization of a Phosphotyrosine-Containing *onc* Gene Product in Feline Tumor Cells*

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## A. Abstract

Protein phosphorylation by a tyrosine-specific kinase is now recognized as a common event in retrovirus-transformed cells. We report in this communication that the feline sarcoma virus (FeSV) encoded transformation-specific proteins (*gag-fes* fusion proteins) and their associated protein kinases are also found in the FeSV in vivo induced tumor preparations, either in the form of fresh tumor homogenate or in the form of cultured cells. With the combined use of subcellular fractionation and detergent extraction we found that the protein kinase activity was present in both the membrane fraction (P100) and the cytosol (S100). The *gag-fes* proteins of two different strains of FeSV were found to associate with the cell framework to different degrees, suggesting that the specific conformational presentation of these proteins may be dictated by the unique portion of each polyprotein. The same *gag-fes* transformation related proteins could be immunoprecipitated with antiserum to phosphotyrosine.

## B. Introduction

Retroviruses that transform cultured fibroblasts usually contain an oncogene which is believed to encode for a protein kinase activity [10]. Such oncogenes are highly conserved across species barriers [4]. As assayed in cells transformed in vitro, such putative oncogene products generally have kinase activity that autophosphorylates and/or heterophosphorylates specifically at tyrosine residues [17]. Tyrosine-specific kinase activities have also been found associated with such diverse proteins as the middle T antigen of polyoma virus [24] and the epidermal growth factor-receptor molecule [30]. Despite the rapidly accumulating information on the function of this class of molecule in cultured cells transformed in vitro by different agents, relatively little attention has been given to the study of such proteins in tumor cells. In the current study we examined cells obtained from tumors induced in vivo with feline sarcoma virus (FeSV) for such activities. We also evaluated the practicability of using antisera specific for phosphotyrosine to detect such proteins.

## C. FeSV-Specific Transformation Proteins

FeSV, a potent tumor-causing agent, induces rapidly proliferating fibrosarcomas in young kittens. FeSV has been isolated from numerous naturally occurring cat fibrosarcomas [15], but only three isolates (Snyder-Theilen-ST, Gardner-Arnstein-GA, and McDonough-SM) have been studied in great detail. These three FeSV isolates share common features in their genomic structure. As is the case with many other replication-defective retroviruses, the FeSV genome consists of a partially deleted *gag* gene, and *onc* gene insert, and a substantially deleted *env* gene. Only the *gag-*

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**Table 1.** Presence of *gag-fes* transformation related proteins in preparations derived from feline tumors

| Tumor type | No. of cats | Inoculum | Method of detection | *gag-fes* protein |
|---|---|---|---|---|
| Fibrosarcoma | 4 | ST-FeSV | Immunoprecipitation: in vivo $^{35}$S-methionine or $^{32}$P-orthophosphate labeled short-term cultured cells | pp 85 |
| Fibrosarcoma | 5 | ST-FeSV | In vitro protein kinase assay on fresh tumor homogenate | pp85 |
| Fibrosarcoma | 6 | GA-FeSV | Immunoprecipitation; in vivo $^{35}$S-methionine labeled cultured cells | p110 |
| Melanoma | 3 | GA-FeSV | Immunoprecipitation; in vivo $^{35}$S-methionine and $^{32}$P-orthosphosphate labeled short-term cultured cells | pp110 |
| Melanoma Fibrosarcoma Neurofibrosarcoma Osteosarcoma Chondrosarcoma | 5 | Unknown, but no FeLV exposure history | Immunoprecipitation; in vivo $^{35}$S-methionine labeled short-term cultured cells | – |

*onc* region has been shown to be transcriptionally active. The *onc* portion, which represents the transforming gene of FeSV [21, 26], was acquired from the cell sequence [13]. Recent studies by nucleic acid hybridization demonstrated that the *onc* portion of ST-FeSV and GA-FeSV share more than 50% homology. This homology, however, was not found in the specific portion of SM-FeSV [14]. The *onc* portion of ST- and GA-FeSV is now designated as *fes* while that of SM-FeSV is designated *fms*, denoting their distinct cellular origin [9]. The translation product from the *gag-fes* or *gag-fms* is a fusion polyprotein containing the antigenic moieties of p15, p12 and part of p27 from the *gag* protein, and the *fes* or *fms* protein. The sizes of polyproteins as measured from SDS-PAGE are 85,000 daltons, 110,000 daltons, and 180,000 daltons for ST-, GA-, and SM-FeSV, respectively [1].

The *gag-fes* or *gag-fms* polyproteins were detected, using metabolic labeling with $^{35}$S-methionine or $^{32}$P-orthophosphate, in cells transformed or transfected in vitro with FeSV. Cells from a broad range of species that were transformed with ST-FeSV and/or GA-FeSV, such as mink [29], goat [1], rat [23], cat [7], and mouse [21], were all shown to contain the *gag-fes* polyprotein. In recent studies we found that the same *gag-fes* polyproteins are present in both fresh tissue homogenate prepared from FeSV-induced firbosarcomas (Table 1) and in tumor cells grown for varying periods of time from a few hours to more than a year [8]. While fibrosarcomas arise when FeSV is inoculated subcutaneously or intramuscularly, the intracutaneous inoculation of GA-FeSV causes the preferential development of melanomas [19]. Although these tumors arise from different embryonic germ layers they express the same *gag-fes* polyprotein when induced by the same virus [7].

Anti-*fes* serum was generated by repeatedly immunizing young adult cats with their own cells that were biopsied and transformed in culture with FeSV in a nonproductive manner. After the removal of those antibodies reactive to the virus struc-

tural proteins by extensive adsorption with gradient-purified FeLV, the cat anti-*fes* serum retained specificity for the "*fes*" determinants on both $P85^{gag\text{-}fes}$ and $P110^{gag\text{-}fes}$ [7].

## D. *Gag-fes* Protein Kinase Activity in Tumor Cells

*Gag-fes* polyproteins of both p85 and p110 were easily phosphorylated when in vitro protein kinase assays were carried out with the respective immunoprecipitates [2, 5, 28]. The *gag-fes* protein-associated kinase seems to autophosphorylate the *gag-fes* molecules and also to be capable of phosphorylating the heavy chain of the immune IgG. The *gag-fes* protein associated kinase prefers the manganese cation ($Mn^{2+}$) to the magnesium cation ($Mg^{2+}$) and has an optimal pH for enzymatic reaction of about 7.0 [28]. By phosphoamino acid analysis, the in vivo phosphorylated *gag-fes* polyproteins contain phosphotyrosine as well as phosphoserine residues. The phosphoserine appears to reside in the p12 moiety of the *gag* protein [5]. When analyzed by in vitro phosphorylation, the molecule contains primarily phosphotyrosine.

To determine whether or not the protein kinase activity was also found in tumor cells, as opposed to transformed fibroblasts, we examined preparations from FeSV-induced fibrosarcomas. These preparations included fresh tumor homogenates, tumor cells grown for less than 1 day in culture, and long-term cell lines that were originally established from FeSV-induced tumors. Fresh tissue homogenates were examined

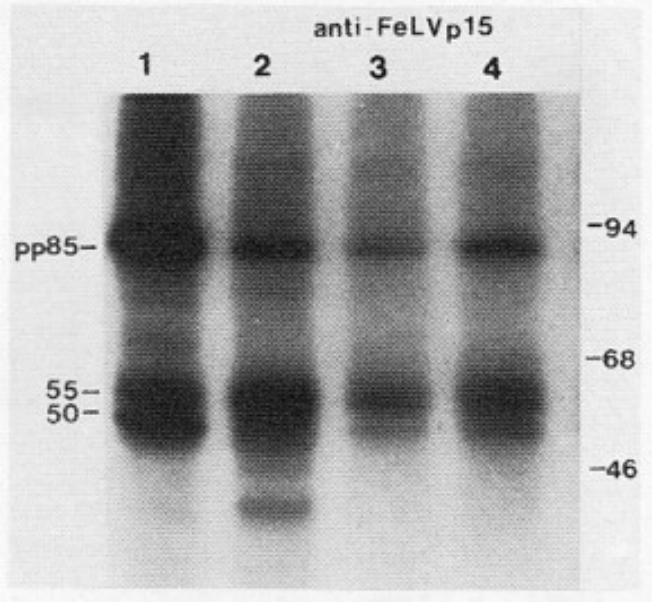


**Fig. 1.** The *gag-fes* polyproteins prepared from fresh cat tumors are phosphorylated in the in vitro protein kinase assay. ST-FeSV-induced fibrosarcomas in young kittens were examined. The fresh fibrosarcoma tissues were minced in cold Hanks balanced salt solution immediately after excision. The minced tissues were then washed with cold PBS and once with washing buffer containing 0.02 *M* Tris-HCl, pH 6.8, 0.137 *M* NaCl, 0.001 *M* $CaCl_2$, 99 kallikrein inhibitor unit/ml aprotinin, and 1 m*M* phenylmethylsuflonyl fluoride. Subsequently, the minced tumor tissue was resuspended in the washing buffer supplemented with 1% (v/v) NP40 and 10% glycerol and then subjected to homogenization in the Sorvall Omnimix at speed 6 for 3 min (on ice). The final homogenate was clarified once at low-speed centrifugation and then at 100,000 g for 30 min. The clarified lysates were then processed for in vitro protein kinase assay [24]. Three FeLV-positive fibrosarcomas from two kittens were examined in this experiment. Lanes 2 and 4 were prepared from two primary fibrosarcomas that occurred on the site of inoculation. Lane 3 was obtained from a secondary fibrosarcoma or metastatic tumors that appeared at a site distant from the original inoculation. Lane 1 was prepared from cat fibroblasts transformed in vitro with ST-FeSV. Goat anti-FeLV p15 serum was used to immunoprecipitate the *gag-fes* polyproteins. PP85 represents the phosphorylated *gag-fes* protein encoded by ST-FeSV. Fifty-five depicts a phosphorylated protein that comigrates with the IgG heavy chain (superimposed with Coomassie blue stained IgG heavy chain). Fifty is another phoshorylated protein. The molecular weight standards are $^{14}C$-labeled phosphorylase b (94,000), bovine serum albumin (68,000), and ovalbumin (46,000)

from five different tumors induced with ST-FeSV, and all were positive for the appropriate kinase activity (Fig. 1). Three proteins were found to be phosphorylated: the $P85^{gag\text{-}fes}$, the heavy chain of immune IgG, and a 50,000-dalton protein of unknown identity.

$P85^{gag\text{-}fes}$ is apparently the most preferred acceptor molecule for this enzymatic reaction. Phosphorylation of the tyrosine residue was confirmed by phosphoamino acid analysis (Fig. 2). Phosphoproteins prepared from this in vitro kinase reaction often also contain low levels of phosphoserine, but it is possible that the latter could be due to a contaminating cellular kinase present in the immunoprecipitation. Similar results have been observed by other investigators [5]. In the phosphorylated proteins recovered from both tumor preparations and in vitro transformed cells, the tyrosine is always the most intensely labeled amino acid. It thus seems apparent that the *gag-fes* protein-associated protein kinase exists in its active form in the ST- and GA-FeSV induced tumors.

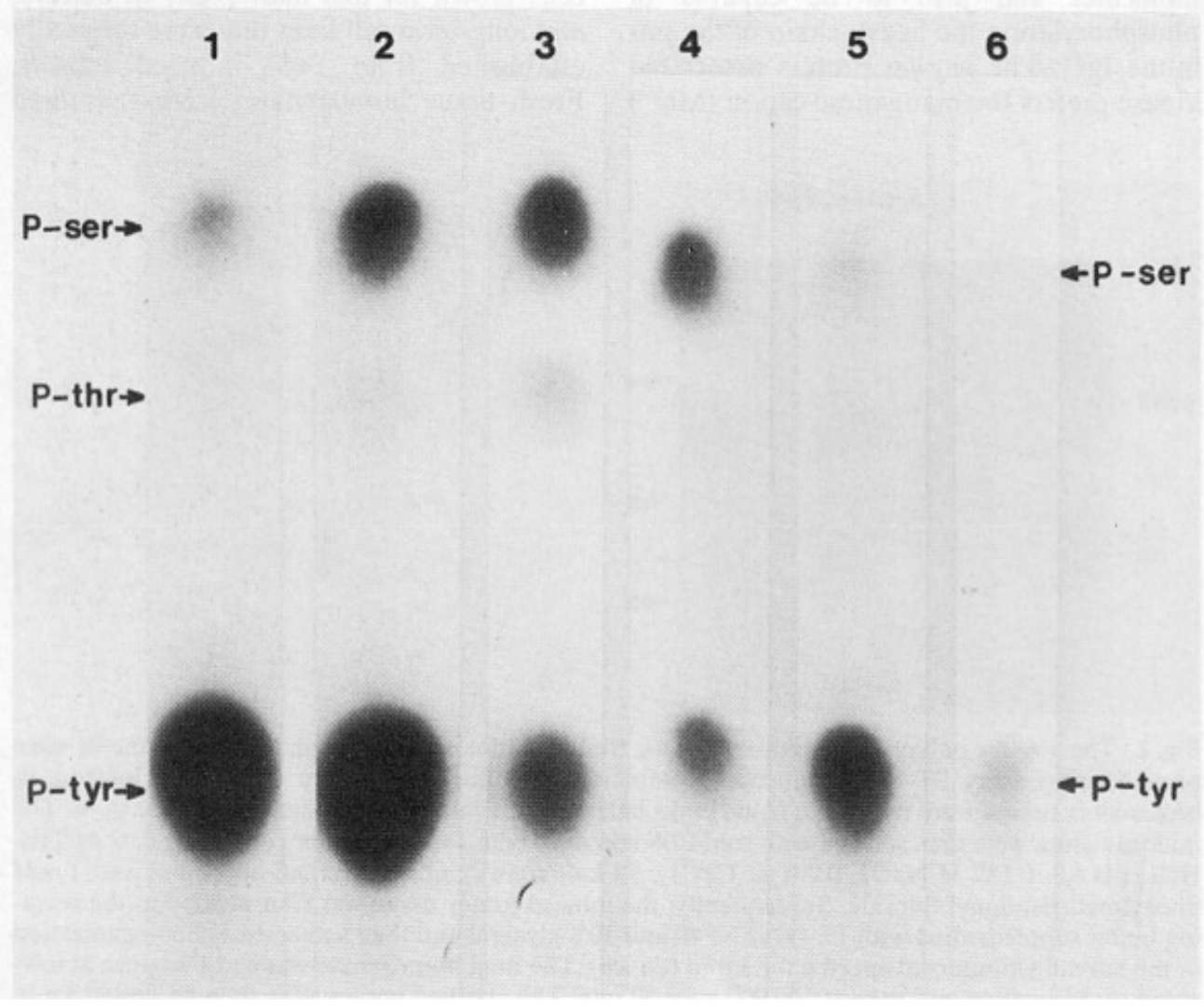


**Fig. 2.** Phosphoamino acid composition of in vitro labeled phosphoproteins. Phosphoproteins obtained by the in vitro protein kinase assay were electroeluted and hdrolyzed in 6 *N* HCl for 2 h at 110 °C according to Beemon et al. [3]. Phosphoamino acids were separated by one dimensional paper electrophoresis in pyridine-acetic acid (0.5 : 5, at pH 3.5) at 2000 V for 2 h. Markers for phosphoserine (*p-ser*), phosphothreonine (*p-thr*), and phosphotyrosine (*p-tyr*) were located by ninhydrin staining. The phosphoproteins analyzed, and approximate radioactive cpm were: *1*, pp85 from a primary culture of an ST-FeSV induced fibrosarcoma, 4800; *2*, pp110 from GA-FeSV transformed fibroblasts, 6700; *3*, pp110 from a cultured GA-FeSV induced melanoma, 2020; *4*, IgG heavy chain from a GA-FeSV induced melanoma, 1300; *5*, IgG heavy chain from a primary culture of a ST-FeSV induced fibrosarcoma, 1090; *6*, pp50 from the same culture as in *1* and *5*, 410

## E. Cellular Localization of the Tyrosine Kinase Activity

Studies with transformation-defective (*td*) mutants of several transforming retroviruses showed that the ability of such viruses to transform cells was closely correlated with the presence of tyrosine-specific protein kinase activity [5, 20, 27]. However, the subcellular localization or cellular compartmentalization of this enzyme activity has not been conlusively established. To address this issue with cells containing the *gag-fes* protein kinase two

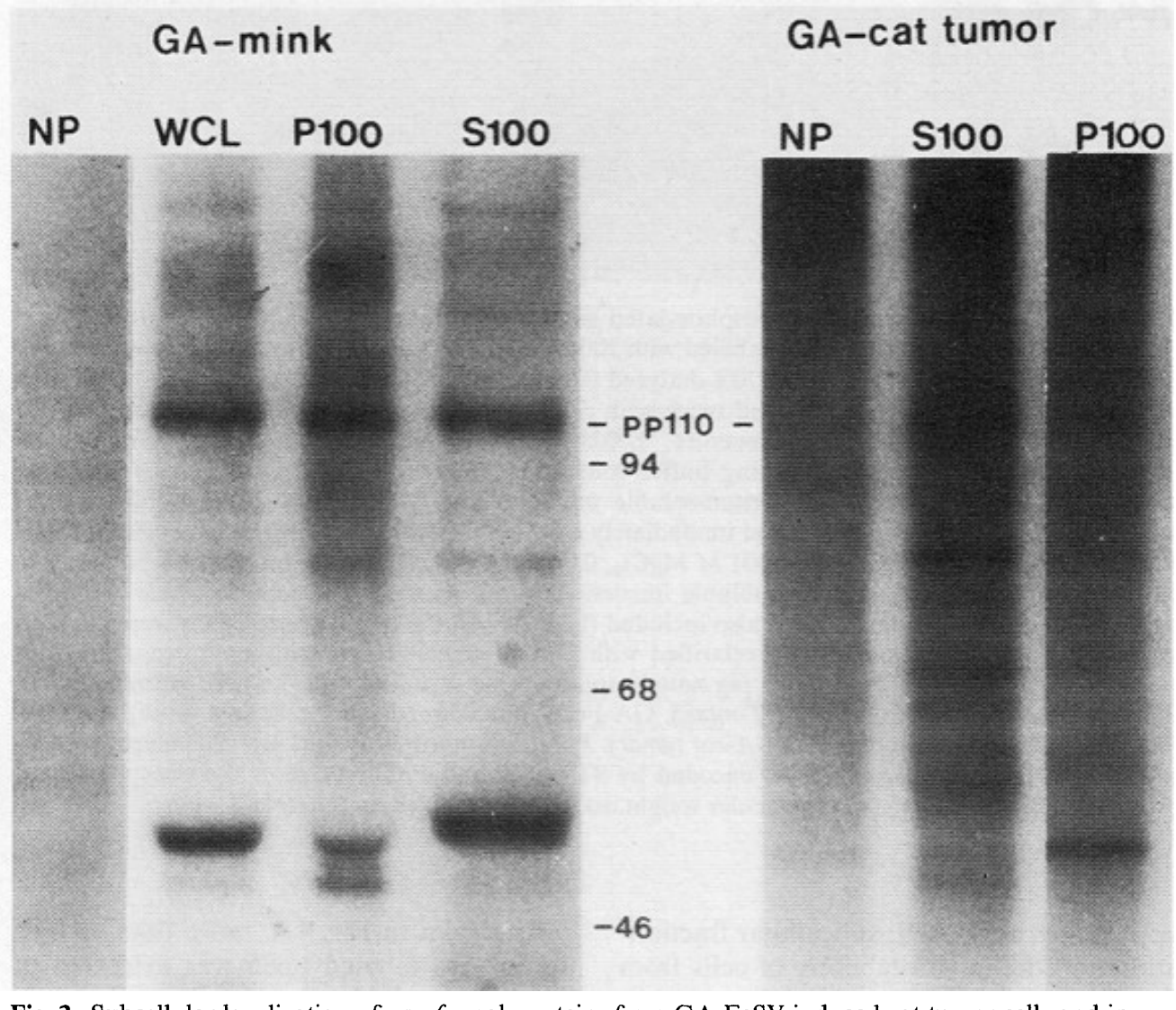


**Fig. 3.** Subcellular localization of *gag-fes* polyproteins from GA-FeSV induced cat tumor cells and in vitro transformed cells. The protocol for cell fractionation was modified from Hay [16] and Courtneidge et al. [12]. Briefly, cells were collected by scraping into cold PBS and spun at 1000 g for 5 min. Washed cells were resuspended in 5 m*M* KCl, 1 m*M* $MgCl_2$, and 20 m*M* Hepes, at pH 7.0 for 40 min on ice. Cells treated with the hypotonic buffer were homogenized with 20–50 strokes in a tight-fitting Dounce homogenizer, which resulted in more than 90% disruption of cells without apparent damage to the nuclei as examined under the phase-contrast microscope. The clear supernatant was obtained by repeated low-speed centrifugation to remove nuclei and partially broken cells. The clear supernatant was spun at 100,000 g for 45 min to separate the supernatant (S100) and pellet (P100). S100 represents the cytosol fraction while P100 is the crude membrane fraction. The nuclear pellet was washed once with 20 m*M* KCl, 20 m*M* NaCl, 0.1 m*M* EDTA, and 20 m*M* PIPES at pH 6.8, resuspended in 20 ml of 60% (w/w) sucrose in the above washing buffer, overlaid on a 10-ml 60% sucrose cushion and spun at 50,000 g for 60 min. The final pellet (NP) is 99% composed of nuclei and is free of visible contamination with membranes or broken cells as checked in the phase-contrast microscope. WCL is whole cell lysate extracted with NP40-containing lysing buffer. All the fractions were processed by the in vitro protein kinase assay. Goat anti-FeSV p15 serum was used throughout the whole experiment. *GA mink*, GA-FeSV transformed mink cells; *GA-cat tumor*, GA-FeSV induced cat melanoma cells

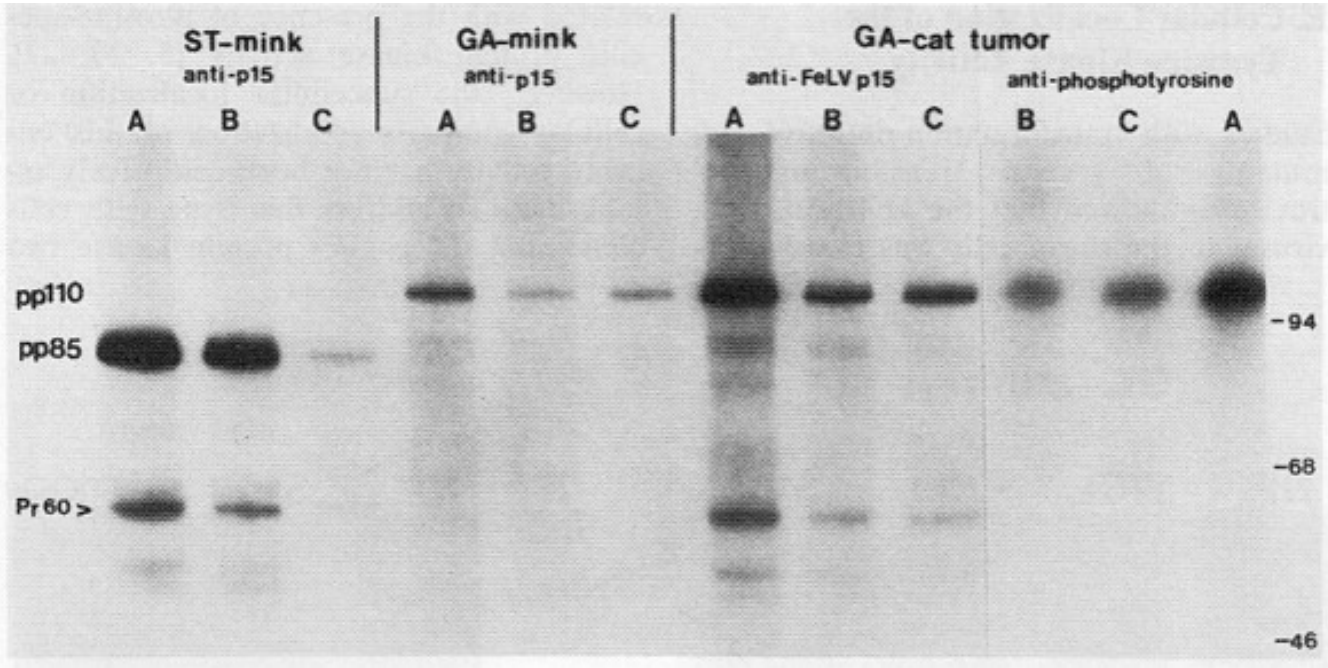


**Fig. 4A.** Immunoprecipitation of phosphorylated *gag-fes* polyproteins from the soluble fraction and the cytoskeletal fraction. Cells were labeled with 200 μCi/ml of $^{32}$P-orthophosphate in phosphate-free Dulbecco-MEM supplemented with 10% dialyzed fetal calf serum for 2 h. The monolayers were then washed four times with cold PBS and twice with cell framework washing buffer containing 0.01 *M* PIPES, pH 6.8, 0.1 *M* KCl, 0.3 *M* sucrose, 0.0025 *M* $MgCl_2$, and 0.001 *M* $CaCl_2$. Subsequently, monolayers were treated with washing buffer containing 1% Triton X-100 at 4 °C for 1 min. The supernatant was collected as the Triton-soluble fraction (lane B). The remaining monolayer was washed twice with washing buffer and immediately lysed with NP40-containing lysing buffer (0.02 *M* Tris-HCl, pH 6.8, 0.137 *M* NaCl, 0.001 *M* $MgCl_2$, 0.001 *M* $CaCl_2$, 10% glycerol, and 1% NP40). The lysate was collected as the Triton-insoluble fraction (lane C). As a positive control, whole cell lysate prepared from NP40 extraction was also included (lane A). All the lysate preparations were clarified at 100,000 *g* for 20 min and were preclarified with normal serum before immunoprecipitation with goat anti-FeLV p15 (5 μl) or guinea pig antiphosphotyrosine serum (2 μl). The cells examined were ST-FeSV transformed mink cells (*ST-mink*), GA-FeSV transformed mink cells (*GA-mink*) and GA-FeSV induced cat melanoma cells (*GA-cat tumor*). *Pp110* is a phosphorylated *gag-fes* polyprotein encoded by GA-FeSV, *pp85* is *gag-fes* encoded by ST-FeSV, and *Pr60* represents the phosphorylated precursor molecule of *gag*. The molecular weight markers are labeled on the *right*

approaches were used: subcellular fractionation and the in situ labeling of cells from the exterior.

Cells were fractionated into a soluble fraction (S100), a crude membrane fraction (P100), and a nuclear pellet (NP) according to Hay et al. [16] and Courtneidge et al. [12]. In vitro protein kinase activity was detected in both the soluble fraction and the membrane fraction of both transformed fibroblasts and the tumor cells (Fig. 3). A nonionic detergent, Triton X-100, when used at low concentrations (0.5%–1%), extracts approximately 80% of the total cell proteins, leaving 20% with the cytoskeleton [6]. This detergent appeared to disrupt the plasma membrane and release the cell contents without rearranging the Triton cytoskeleton.

As shown in Fig. 4A, more than 80% of the in vivo labeled pp85 was extracted in the Triton-soluble fraction (lane B) while less than one-fifth was tightly associated with the Triton cytoskeleton (the insoluble fraction – lane C) in ST-FeSV transformed mink cells. In contrast, equal amounts of pp110 from the GA-FeSV induced tumor cells and the GA-FeSV transformed cells were found in Triton-soluble and insoluble fractions. The profile of in vitro kinase activity correspond to the concentration of phosphorylated *gag-fes* proteins found in each fraction. For example, the Triton-soluble fraction of ST-mink cells contained most of the in vivo phosphorylated pp85 (lane B of Fig. 4A) as well as the majority of the protein kinase activity (lane A of Fig. 4B). An analogous pattern was found

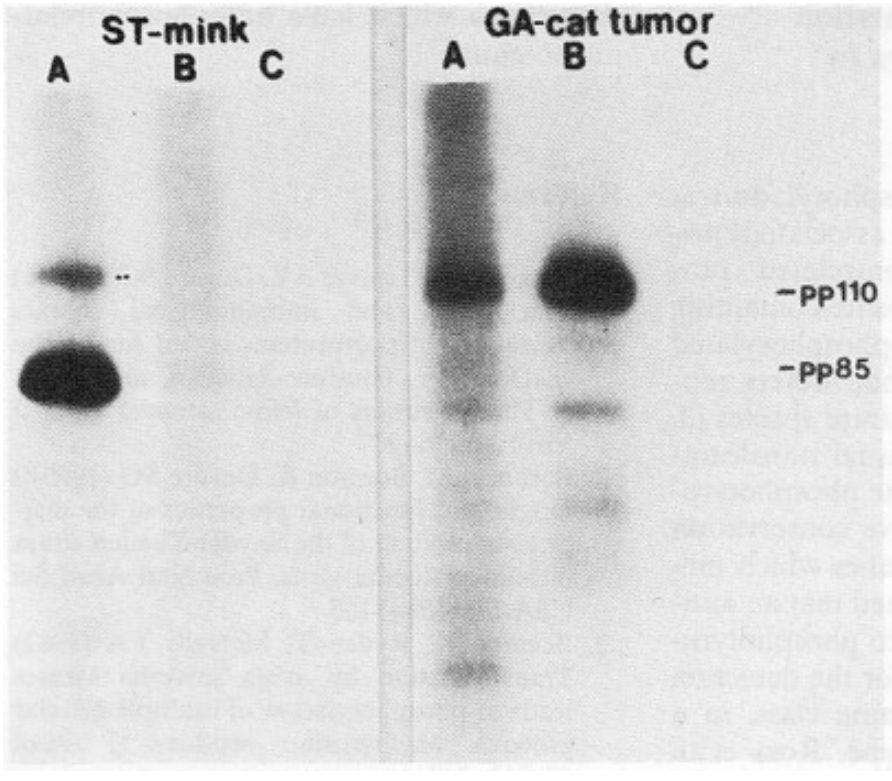


**Fig. 4B.** Phosphorylation of *gag-fes* polyproteins in situ compared with phosphorylation in the soluble fraction. Cells were grown to a density of $3 \times 10^6$ cells per 6.0-cm tissue culture dish, and washed as described in the legend of Fig. 4A. The cells were either treated with Triton X-100 (1%) in washing buffer for 1 min or untreated. The supernatant from Triton X-100 treated cells was saved as the Triton-soluble fraction (lane A) for the in vitro protein kinase assay. Both Triton-pretreated (lane B) and untreated (lane C) culture dishes were incubated with 250 μCi $\gamma$-$^{32}$P-ATP (ICN, Irving, CA) and $2 \times 10^{-5}$ *M* ATP in the cell framework washing buffer at room temperature for 15 min. The cell monolayers were then carefully washed with cold PBS washing buffer and finally were lysed in 0.5 ml of NP40-lysing buffer. Five microliters of goat anti-FeLV p15 serum was used in the immunoprecipitation. The results obtained with ST-FeSV transformed mink cells (*ST-mink*) and GA-FeSV induced melanoma cells (*GA-cat tumor*) are presented. The nonspecific reactivity of the ST-mink cell preparation of goat serum is indicated by dots (. .). *Pp110* and *pp85* are the phosphorylated *gag-fes* polyproteins of GA-FeSV and ST-FeSV, respectively

for GA-FeSV transformed cells as observed in both in vivo and in vitro assays (compare Fig. 4A and 4B).

Attempts to label the cellular protein with exogenously supplied $\gamma$-$^{32}$P-ATP from the cell exterior were unsuccessful (lane C of Fig. 4B), suggesting that the active site of this kinase is not exposed to the exterior of cells and that the plasma membrane is not permeable to $\gamma$-$^{32}$P-ATP. Treatment of the cells for 1 min with Triton X-100 allows the entry of $\gamma$-$^{32}$P-ATP to encounter those cytoskeleton-associated proteins and to allow the active transfer of phosphorus to the substrate, P110$^{gag\text{-}fes}$ (lane B of GA tumor in Fig. 4B). Such activity was not detected in ST-FeSV transformed cells, since more than 80% of the pp85 was extracted with Triton X-100 in the soluble fraction (lane B of ST mink in Fig. 4B).

Our results indicate that the *gag-fes* polyproteins are found in both cytosol and membrane fractions and are linked to the cell framework. It seems likely that there are two pools of *gag-fes* proteins: one membrane-bound, and the other cytoskeleton associated and subsequently fractionated in the cytosol (S100). Alternatively, it seems possible that the *gag-fes* polyprotein might be anchored partly in the cytoplasmic side of the plasma membrane and partly with the cytoskeleton. Our current evidence, however, cannot distinguish between these two possibilities.

## F. Detection of Transformation Proteins Using Antisera to Phosphotyrosine

As mentioned earlier, phosphorylation at tyrosine residues appears associated primarily with transformation-related proteins. Some phosphotyrosine-containing proteins represent the autophosphorylated enzyme molecule itself, while others represent an independent substrate species [3, 11, 18]. Because of the unusual transformation-associated nature of the phosphotyrosine reaction and the relative conservation of the retrovirus-encoded genes which mediate this activity, we reasoned that an antiserum directed specifically to phosphotyrosine might be a useful tool for the detection of other proteins of this same class. in a previous attempt of this type, Ross et al. [22] used an antiserum to azobenzyl phosphonate to detect the pp120 of cells transformed by the Abelson murine leukemia virus and also a previously undescribed protein designated pp110 which was found in mouse fibroblasts tranformed with the Schmidt-Ruppin strain of Rous sarcoma virus.

We used antiserum made against phosphotyrosine coupled to bovine gamma globulin by means of the carbodiimide method (a generous gift from Drs. Schaffhausen and Benjamin, Harvard Medical School). the antiphosphotyrosine serum specifically interacts with the in vivo $^{32}$P-labeled pp85$^{gag\text{-}fes}$ and pp110$^{gag\text{-}fes}$ of FeSV as well as with the middle T antigen of polyoma virus (Schaffhausen and Benjamin, personal communication). A specificity control for this serum is shown in Fig. 4A. Antiphosphotyrosine-containing serum recognized only the pp110$^{gag\text{-}fes}$ but not the phosphoserine-containing precursor molecule of the gag protein (Pr60). The antiphosphotyrosine also immunoprecipitated several other proteins at molecular weights of approximately 50,000 daltons and 140,000 daltons (data not shown). Our preliminary results demonstrated the potential of this specific antiserum. Subsequent applications may facilitate the identification of phosphoproteins in the context of either distinct substrate(s) for transforming proteins or kinase molecules themselves which have autophosphorylating potential.

## References


1. Barbacid M, Lauver AV, Devare SG (1980a) Biochemical and immunological characterizatioin of polyproteins coded for by the McDonough, Gardner-Arnstein, and Snyder-Theilen strains of feline sarcoma virus. J Virol 33:196–207
2. Barbacid M, Beemon K, Devare SG (1980b) Origin and functional properties of the major gene product of the Snyder-Theilen strain of feline sarcoma virus. Proc Natl Acad Sci USA 77:5158–5163
3. Beemon K, Ryden T, McNelly EA (1982) Transformation by avian sarcoma viruses leads to phosphorylation of multiple cellular proteins on tyrosine residues. J Virol 42:742–747
4. Bishop JM (1982) Oncogenes. Sci Amer 246:80–92
5. Blomberg J, Van de Ven WJM, Reynolds FH Jr, Nalewaik RP, Stephenson JR (1981) Snyder-Theilen feline sarcoma virus P85 contains a single phosphotyrosine acceptor site recognized by its associated protein kinase. J Virol 38:886–894
6. Brown S, Levinson W, Spudick JA (1976) Cytoskeletal elements of chick embryo fibroblasts revealed by detergent extraction. J Supramol Struct 5:119–130
7. Chen AP, Essex M, Shadduck JA, Niederkorn JY, Albert D (1981) Retrovirus-encoded transformation-specific polyproteins: Expression coordinated with malignant phenotype in cells from different germ layers. Proc Natl Acad Sci USA 78:3915–3919
8. Chen AP, Essex M, Kelliher M, deNoronha F, Shadduck JA, Niederkorn JY, Albert D (1983) Feline sarcoma virus-specific transformation related proteins and protein kinase activity in tumor cells. Virol 124:274–285
9. Coffin J, Varmus HE, Bishop JM, Essex M, Hardy WD Jr, Martin S, Rosenberg NE, Scolnick EM, Weinberg RA, Vogt PK (1981) Proposal for naming host cell-derived inserts in retrovirus genomes. J Virol 40:953–957
10. Collett M and Erikson RL (1978) Protein kinase activity associated with avian sarcoma virus *src* gene product. Proc Natl Acad Sci USA 75:2020–2024
11. Cooper JA and Hunter T (1981) Changes in proein phosphorylation in Rous sarcom virus transformed chicken embryo cells. Molecular and Cellular Biology 1:165–178

12. Courtneidge SA, Levison AD, Bishop JM (1980) The protein encoded by the transforming gene of avian sarcoma virus ($pp60^{src}$) and a homologous protein in normal cells ($pp60^{proto\text{-}src}$) are associated with the plasma membrane. Proc Natl Acad Sci USA 77:3783–3787
13. Franchini G, Even J, Sherr CJ, Wong-Staal F (1981) *Onc* sequences (v-*fes*) of Snyder-Theilen feline sarcoma virus are derived from noncontiguous regions of a cat cellular gene (c-*fes*). Nature 290:154–157
14. Frankel AE, Gilbert JH, Porzig KJ, Scolnick EM, Aarsonson SA (1979) Nature and distribution of feline sarcom virus nucleotide sequences. J Virol 30:821–827
15. Hardy WD Jr (1980) The biology and virology of the feline sarcoma virus. In: Hardy WD Jr, Essex M, McClelland J (eds) Feline leukemia and sarcoma viruses. Elsevier, New York pp 79–118
16. Hay AJ (1974) Studies on the formation of the influenza virus envelope. Virol 60:398–418
17. Hunter T and Sefton BM (1980) Transforming gene product of Rous sarcoma virus phosphorylates tyrosiine. Proc Natl Acad Sci USA 77:1311–1315
18. Martinez R, Nakamura KD, Weber MJ (1982) Identification of phosphotyrosine-containing proteins in untransformed and Rous sarcoma virus-transformed chicken embryo fibroblasts. Mol Cell Biol 2:653–665
19. McCullough B, Shaller J, Shadduck JA, Yohn DS (1972) Induction of malignant melanoma associated with fibrosaromas in genotobiotic cats inoculated with Gardner-feline fibrobsarcoma virus. J Natl Cancer Inst 48:1893–1896
20. Reynolds FH Jr, Van de Ven WJM, Stephenson JR (1980) Abelson murine leukemia virus transforming-defective mutants with impaired P120-asociated protein kinase activity. J Virol 36:375–386
21. Rosenberg Z, and Haseltine W (1980) A transfection assay for transformation by feline sarcoma virus proviral DNA. Virol 102:240–244
22. Ross AH, Baltimore D, Eisen H (1981) Phosphotyrosne-containingg proteins isolated by affinity chromatography with antibodies to a synthetic hapten. Nature 294:654–656
23. Ruscetti SK, Turek LK, Sherr CJ (1980) Three independent isolates of feline sarcoma virus code for three distinct gag-x polyproteins. J Virol 35:259–264
24. Schaffhausen B and Benjamin TL (1979) Phosphorylation of polyoma T antigen. Cell 18:935–946
25. Schaffhausen B, Benjamin TL (1981) Protein kinase activity associated with polyoma virus middle T antigen. Cold Spring Harbor Conf on Cell Prolif 8:1281–1298
26. Sherr CJ, Fedele LA, Oskarsson M, Maizel J, Vande Woude G (1980) Molecular cloning of Snyder-Theilen feline leukemine and sarcoma viruses: Comparative studies of feline sarcoma virus with its natural helper virus and with Moloney murine sarcoma virus. J Virol 34:200–212
27. Sefton BM, Hunter T, Beemon K, Eckhart W (1980) Evidence that the phosphorylation of tyrosine is essential for cellular transformation by Rous sarcoma virus. Cell 20:806–816
28. Snyder H (1982) Biochemical characterization of protein kinase activities associated with transforming gene produts of the Snyder-Theilen and Gardner-Arnstein strain of feline sarcoma virus. Virol 117:165–172
29. Stephenson JR, Khan AS, Sliski AH, Essex M (1977) Feline oncornavirus-associated cell membrane antigen: Evidence for immunologically cross-reactive feline sarcoma virus coded protein. Proc Natl Acad Sci USA 75:5608–5612
30. Ushiro H and Cohen S (1980) Identification of phosphotyrosine as a product of epidermal growth factor-activated protein kinase in A-431 cell membranes. J Biol Chem 255:8363–8365