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Viral and Cellular *fos* Proteins Are Complexed with a 39,000-Dalton Cellular Protein

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The structure of viral and cellular *fos* gene products and their association with a 39,000-dalton cellular protein (p39) were investigated by using antisera raised against synthetic peptides. The first peptide, termed M, corresponded to amino acids 127 to 152 of the v-*fos* sequence, a region which is identical in c-*fos*. The second peptide, termed V, corresponded to the nine C-terminal amino acids of v-*fos*; this region is not present in c-*fos*. Rabbit antisera were purified by affinity chromatography against their respective peptides before being used for immunoprecipitation. M peptide antisera precipitated $p55^{v\text{-}fos}$ and $p55^{c\text{-}fos}$, whereas V peptide antisera precipitated only $p55^{v\text{-}fos}$. This observation confirms the prediction from nucleotide sequence analysis that these proteins are distinct at their C termini. p39 was precipitated in association with $p55^{v\text{-}fos}$ and $p55^{c\text{-}fos}$ by M and V peptide antisera. However, V peptide antisera did not precipitate p39 from cells expressing $p55^{c\text{-}fos}$, even though the presence of p39 in such cells was demonstrated with M peptide antisera. Denaturation of cell lysates completely abolished the precipitation of p39, whereas the precipitation of $p55^{v\text{-}fos}$ was unaffected. Taken together, the data demonstrate that p39 exists in a complex with p55.

Approximately 20 different cellular genes have been identified by virtue of homology with oncogenes (v-*onc*) carried by the acutely oncogenic retroviruses (2). Because these genes are the likely progenitors of the viral transforming genes, they have been termed cellular proto-oncogenes, or c-*onc* genes (1). It is now of great interest to determine the function of c-*onc* genes and the mechanisms by which they can cause neoplasia. We have been pursuing this objective with particular reference to the *fos* gene which is responsible for the induction of osteosarcomas by the FBJ murine osteosarcoma virus (FBJ-MSV) (9). Nucleotide sequence analysis predicts that the v-*fos* gene differs from the c-*fos* gene in that it has undergone a deletion (23). The consequence of this alteration is that the C-terminal 48 amino acids of the c-*fos* gene should be different from the C-terminal 49 amino acids of the v-*fos* gene. Both the v-*fos* and the c-*fos* gene products ($p55^{v\text{-}fos}$ and $p55^{c\text{-}fos}$, respectively) migrate with an apparent molecular weight of 55,000 on sodium dodecyl sulfate(SDS)-polyacrylamide gel electrophoresis, and both are nuclear proteins (7). However, $p55^{c\text{-}fos}$ undergoes more extensive post-translational modification than $p55^{v\text{-}fos}$ does, and forms of 57,000, 60,000, and 62,000 daltons can be identified (7). Despite this difference the c-*fos* gene, when linked to a 3′ long terminal repeat, is equally capable of inducing cellular transformation (15). In normal tissues the c-*fos* gene is expressed in relatively large amounts in extraembryonal membranes, particularly the amnion (16), and in some hematopoietic cells (10, 15a). To date, *fos* proteins have been detected by using sera from rats bearing tumors induced by inoculation of FBJ-MSV-transformed nonproducer cells (TBRS) (8, 9). In association with the *fos* proteins, such sera also precipitate a 39,000-dalton cellular protein (p39) (8). Antibodies to synthetic peptides, as reviewed by Walter and Doolittle (25), have proven to be extremely useful reagents in the analysis of various protein-protein interactions. In this report we describe the isolation of antisera specific for synthetic peptides corresponding to regions of the *fos* proteins. Using these sera, we have confirmed the predicted amino acid sequences and demonstrated that the v-*fos* and c-*fos* gene products are complexed to p39.

MATERIALS AND METHODS

Cell culture. Cells were maintained in Dulbecco-Vogt modified Eagle medium supplemented with 10% heat-inactivated calf serum and antibiotics. CHO cells containing amplified levels of a plasmid encoding either the v-*fos* or the c-*fos* gene in association with the dihydrofolate reductase gene (21) were maintained in the same medium supplemented with 10% dialyzed fetal calf serum, 1× nonessential amino acids (GIBCO Diagnostics), and 4×10^{-6} M methotrexate [L-(+)-amethopterin; Aldrich Chemicals Co., Inc.], as described previously (7). 208F cells (18), FBJ-MSV-transformed 208F cells (8), and 208F cells transformed by the pMMV construct (15) have been described previously.

Peptide synthesis. The solid-phase condensation of *t*-BOC amino acids was carried out as previously described (13). Peptides corresponding to the predicted carboxy-terminal nine amino acids of $p55^{v\text{-}fos}$ (V) and 11 amino acids of $p55^{c\text{-}fos}$ (C) were prepared manually, beginning with 2 g of either *t*-BOC–threonine (V) or *t*-BOC–leucine (C) resin. The locations of these peptides in the v-*fos* and c-*fos* proteins are shown in Fig. 1A. A third peptide (M) was selected on the basis of its (i) hydrophilic character, (ii) predicted location on the surface of the protein (4), and (iii) sequence conservation in $p55^{v\text{-}fos}$, $p55^{c\text{-}fos}$ (mouse) (Fig. 1A), and $p55^{c\text{-}fos}$ (human). This 27-amino acid peptide was synthesized on 3 g of *t*-BOC–alanine resin, using a Beckman model 990 peptide synthesizer. A tyrosine residue was added to the N terminus of each peptide for conjugation to carrier protein. The sequence of the M peptide was NH_2-(tyr)-gly-lys-val-glu-gln-leu-ser-pro-glu-glu-glu-glu-lys-arg-ile-arg-arg-glu-arg-asn-lys-met-ala-ala-ala-COOH. The sequence of the C peptide was NH_2-(tyr)-asp-ser-leu-ser-ser-pro-thr-leu-leu-ala-leu-

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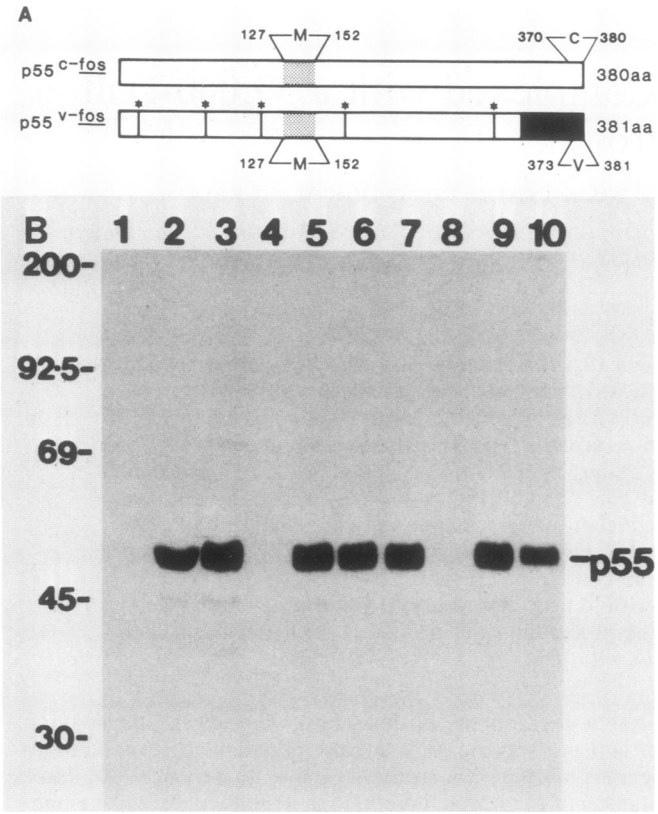

FIG. 1. Immunoprecipitation of $p55^{v\text{-}fos}$, using affinity-purified antisera. (A) The location of the peptides used to raise antisera is indicated diagrammatically on the sequence of $p55^{c\text{-}fos}$ and $p55^{v\text{-}fos}$. The numbers indicate amino acid residues in the indicated protein. The asterisks and the lines through the sequence of $p55^{v\text{-}fos}$ indicate positions at which the protein sequence differs from that of $p55^{c\text{-}fos}$. The closed box at the C terminus of $p55^{v\text{-}fos}$ indicates the altered C terminus of this protein versus $p55^{c\text{-}fos}$ (24). (B) CHO cells containing amplified levels of FBJ-MSV (7) were labeled with [^{35}S]methionine for 20 min. Equivalent amounts of cell lysate were treated with normal serum (lane 1), TBRS (lane 2), 10 μl of affinity-purified M peptide antiserum (lane 3), 10 μl of affinity-purified M2 peptide antiserum preincubated with 10 μg of M peptide (lane 4), 5 μl of affinity-purified M2 peptide antiserum (lane 5), 1 μl of affinity-purified M2 peptide antiserum (lane 6), 10 μl of affinity-purified V peptide antiserum (lane 7), 10 μl of affinity-purified V2 peptide antiserum preincubated with 10 μg of V peptide (lane 8), 5 μl of affinity-purified V2 peptide antiserum (lane 9), and 1 μl of affinity-purified V2 peptide antiserum (lane 10). The products were analyzed by electrophoresis on an 8% SDS-polyacrylamide gel. The numbers on the left indicate the molecular weights ($\times 10^3$) of marker proteins.

COOH. The sequence of the V peptide was NH_2-(tyr)-val-phe-pro-gln-arg-phe-pro-ser-thr-COOH. Release from the resin and deblocking yielded 0.5, 1.9, and 2.8 g of the crude V, C, and M peptide preparations, respectively. Aliquots of the V and C peptides were purified by reverse-phase high-pressure liquid chromatography on a C-18 column, using an acetonitrile gradient buffered by 0.25 N triethanolamine phosphate, pH 3.0. The yields were approximately 1.5 and 2.5 mg from 5-mg aliquots of V and C peptide, respectively. The M peptide was purified by gel filtration (Sephadex G-50) and ion-exchange chromatography (Whatman CM-32), yielding approximately 700 mg of peptide. Aliquots of the M peptide were further purified by high-pressure liquid chromatography.

The V and M peptides were readily soluble in water. High-pressure liquid chromatographic purification of the C peptide required the addition of 10% acetic acid and 25% acetonitrile for solubilization.

Immunization and radioimmune assay. For immunization of rabbits, 5.4 μmol of each high-pressure liquid chromatography-purified peptide was conjugated to 40 mg of bovine serum albumin (Pentax fraction V, crystallized; Miles Laboratories), using a stoichiometric amount of diazotized benzidine (11). Aliquots of peptides were similarly conjugated to ovalbumin (grade V; Sigma Chemical Co.) for use in the radioimmune assay. The conjugated proteins were extensively dialyzed against distilled water until the protein precipitated, followed by dialysis against 0.15 M NaCl. Each of nine 3 to 4-month-old rabbits (three for each peptide) was injected at 10 to 15 intradermal sites with a total of 1.5 to 2 mg of conjugated protein emulsified with 50% Freund complete adjuvant (Calbiochem). The rabbits were boosted three or four times with equivalent amounts of conjugate in 50% Freund incomplete adjuvant at intervals of approximately 5 weeks. Blood samples were drawn for radioimmune assay at 3 to 4 weeks after injections. Henceforth, sera taken from the three rabbits injected with the V peptide will be referred to as V1, V2, and V3, respectively, and as C1, C2, and C3 in the case of the C peptide and as M1, M2, and M3 in the case of the M peptide.

The radioimmune assay used was a modification of that described by Mariottini et al. (14). Flat-bottomed, 96-well, polyvinyl chloride microtiter plates were coated with 40 μl of ovalbumin-conjugated peptides (0.2 mg/ml) in phosphate-buffered saline (PBS; 20 mM sodium phosphate, pH 7.0, 0.15 M NaCl) overnight. After three washes with 4 mg of ovalbumin per ml in PBS threefold dilutions of sera (40 μ) were incubated in the wells at 37°C for 1 h. The wells were washed three times with 0.05% Nonidet P-40 in PBS. Aliquots (40 μl) of 1 mg of PBS-ovalbumin per ml, containing about 10^5 cpm of ^{125}I-labeled goat anti-rabbit serum (a gift from Marcia Riley and Russell Doolittle at the University of California, San Diego), were then incubated in the wells at 37°C for 1 h. After three washes with PBS-Nonidet P-40, individual wells were counted. After the final boost, the highest dilutions which retained 50% maximal reactivity (about 25,000 cpm maximum) with the second antibody were as follows: V1, 1/3,200; V2, 1/1,200; V3, 1/1,300; C1, 1/1,200; C2, 1/200; C3, 1/500; M1, 1/2,200; M2, 1/3,000; M3, 1/1,700. The poor immunogenicity of the C peptide may have been due to its poor solubility (see above).

Affinity purification of antisera. Peptide-conjugated affinity gels were prepared with Bio-Rad Affi-Gel 10 (which has a neutral 10-atom spacer arm) for all three peptides, as well as with Affi-Gel 15 (which has a cationic 15-atom spacer arm) for the C peptide. The efficiencies of binding, as monitored by absorbance at 280 nm, were as follows: Affi-Gel 10—V, 1.0; C, 0.7; M, 1.0; Affi-Gel 15—C, 1.0. Five milligrams of peptide was added per milliliter of gel bed. Sera were fractionated by two rounds of precipitation with 33% saturation ammonium sulfate, and the pellet was suspended in 0.5 volume of PBS. Two milliliters of fractionated serum was passed over a 1-ml affinity column, which was washed successively with PBS, 0.1 M $NaHCO_3$, and 0.1 M $NaHCO_3$–0.5 M NaCl. Antibody was eluted with 0.1 M glycine-HCl, pH 2.5, into tubes containing sufficient Tris base for neutralization. In general, approximately 10% of the total absorbance units at 280 nm were bound to each column, and 5 to 20% of the bound material eluted with glycine. When assayed by the indirect radioimmue assay,

the yields in activity were 1 and 20% for V1 and M1. There was almost no detectable protein eluting from the columns prepared with the C peptide after passage of C1, C2, or C3 antipeptide serum.

Immunoprecipitation. [^{35}S]methionine and $^{32}P_i$ labeling of cells was essentially as described before (7). Denatured cell lysates were prepared by lysing cells in buffer containing 1 mM dithiothreitol, 0.5% SDS, and 50 mM Tris (pH 8.0), or in buffer containing 5% 2-mercaptoethanol, 0.5% SDS, and 50 mM Tris (pH 8.0), and boiling for 5 min. After boiling, lysates were diluted in RIPA buffer lacking SDS (1% Nonidet P-40, 1% sodium deoxycholate, 150 mM NaCl, 10 mM Tris [pH 7.5], 1 mM dithiothreitol) and clarified as described previously. Lysates were incubated with aliquots of peptide antisera for 1 h at 4°C followed by a further 20 min with 20 μl of a 10% suspension of fixed *Staphylococcus aureus* cells (Pansorbin; Calbiochem). Precipitates were analyzed on 8% polyacrylamide gels (12). The ^{14}C-labeled protein molecular weight markers used (Amersham International) were myosin (molecular weight, 200,000), phosphorylase *a* (92,500), bovine serum albumin (69,000), ovalbumin (45,000), carbonic anhydrase (30,000), and lysozyme (14,300).

Immunofluorescence. Isolation and preparation of normal mouse amnion cells for immunofluorescence were as described previously (7). Affinity-purified M2 and V2 antisera were used at 1:20 dilutions. The second antibody in each case was a 1:100 dilution of fluorescein-conjugated goat anti-rabbit immunoglobulin G (Cappel Laboratories). Staining with TBRS and photography were as described previously (7).

RESULTS

Immunoprecipitation of p55$^{v\text{-}fos}$ by affinity-purified peptide antisera. The level of antipeptide activity in sera of immunized rabbits was monitored with an indirect, solid-phase radioimmune assay (see Materials and Methods). Sera of all rabbits were positive to varying degrees in this assay, but initial attempts at immunoprecipitation, using as much as 10 μl of whole antipeptide serum, failed to detect p55$^{v\text{-}fos}$, even when tested on a cell line which expresses at least 10-fold more p55$^{v\text{-}fos}$ than FBJ-MSV-transformed cells do (7). Since affinity purification of antisera can improve their ability to immunoprecipitate target proteins, the rabbit sera were purified by affinity chromatography (see Materials and Methods). Aliquots of a [^{35}S]methionine-labeled lysate were treated with various dilutions of affinity-purified antisera. Sera directed against the M and V peptides recognized p55$^{v\text{-}fos}$ (Fig. 1B). The identification of this protein as p55$^{v\text{-}fos}$ was confirmed by peptide mapping analysis (data not shown). However, sera directed against the relatively insoluble C peptide failed to bind to, and elute from, two different affinity columns. These sera were not included in any further experiments.

Both M2 and V2 sera precipitated p55$^{v\text{-}fos}$ with similar efficiencies. As little as 1 μl of M2 serum (Fig. 1, lane 6) and 5 μl of V2 serum (Fig. 1, lane 9) precipitated p55 to an extent equivalent to that obtained with 1 μl of TBRS (Fig. 1, lane 2). Preincubation of M2 serum with M peptide (Fig. 1, lane 4) and preincubation of V2 serum with V peptide (Fig. 1, lane 8) completely abolished the precipitation of p55$^{v\text{-}fos}$.

Specificities of peptide antisera. To examine the specificities of M and V peptide antisera [^{35}S]methionine-labeled extracts were prepared from normal 208F cells (Fig. 2A), 208F cells transformed by the c-*fos*-expressing construct pMMV (15) (Fig. 2B), and 208F cells transformed by FBJ-MSV (Fig. 2C). These extracts were immunoprecipitated with normal rat serum (lane 1), TBRS (lane 2), affinity-purified sera directed against the M peptide (lanes 3, 4, and 5), and affinity-purified sera directed against the V peptide (lanes 6, 7, and 8). A number of proteins are precipitated by the various peptide sera. One protein of approximately 60,000 daltons is precipitated by M1 sera from all cell lines including normal 208F cells (Fig. 2A, lane 3). This most likely represents a cross-reacting cellular antigen as previously described for other peptide antisera (17). Sera directed against the M peptide precipitate p55$^{c\text{-}fos}$ (Fig. 2B, lanes 3, 4, and 5) and p55$^{v\text{-}fos}$ (Fig. 2C, lanes 3, 4, and 5). The titers of the affinity-purified M2 and M3 antisera appear to be higher

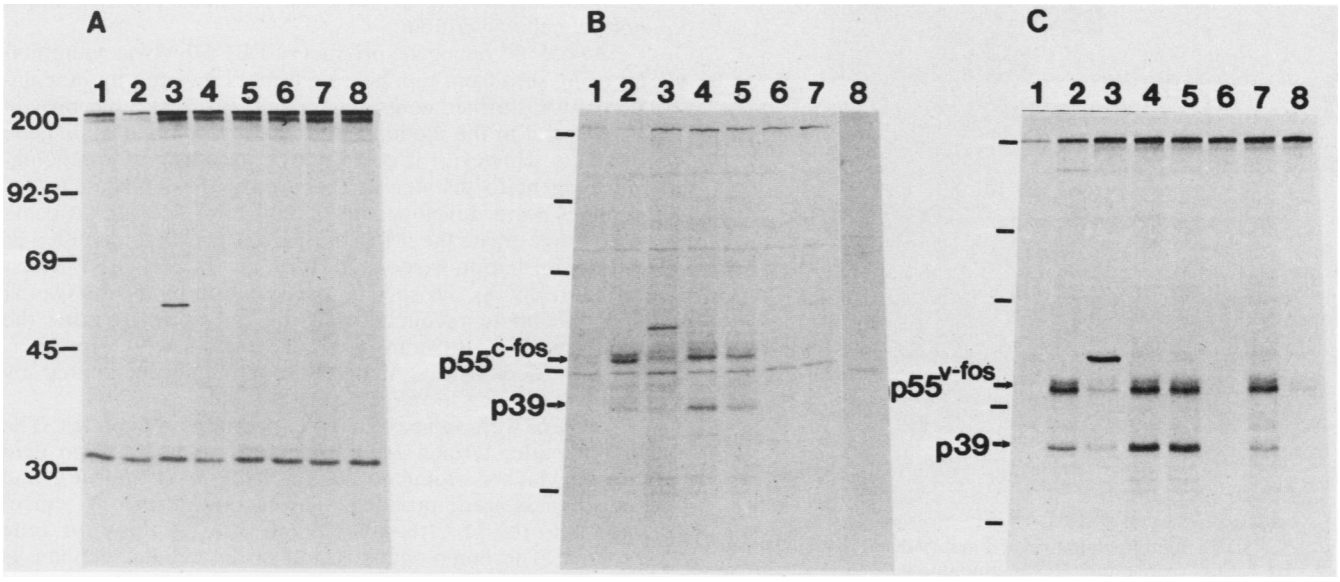

FIG. 2. Immunoprecipitation of p39 in association with p55$^{v\text{-}fos}$ and p55$^{c\text{-}fos}$. [^{35}S]methionine-labeled extracts from normal 208F cells (A), 208F cells transformed by pMMV, a plasmid containing the c-*fos* (mouse) gene linked to a 3′ long terminal repeat (15) (B), and 208F cells transformed by FBJ-MSV (RS2 cells) (C) were treated with normal rat serum (lane 1), TBRS (lane 2), M1 peptide antiserum (lane 3), M2 peptide antiserum (lane 4). M3 peptide antiserum (lane 5), V1 peptide antiserum (lane 6), V2 peptide antiserum (lane 7), and V3 peptide antiserum (lane 8).

than that of M1. In addition to the *fos* proteins, p39 is also precipitated by M antisera (lanes 3, 4, and 5 in both Fig. 2B and C). However, sera directed against the V peptide precipitate p55 and p39 from v-*fos*-transformed cells only (cf. lanes 6, 7, and 8 in Fig. 2B and C). In the case of the V peptide, V2 antisera has a higher titer than V1 or V3. Thus, as predicted from the nucleotide sequence (23), M peptide antisera recognize both the v-*fos* and the c-*fos* gene products, whereas V peptide antisera are specific for the v-*fos* product. Furthermore, p39 is precipitated only in association with the *fos* proteins and is not seen in cells expressing the c-*fos* product treated with V peptide sera (Fig. 2B, lanes 6, 7, and 8). Occasionally, very low levels of *fos* and p39 are detected in 208F cells; this presumably reflects a low basal level of *fos* expression in normal fibroblasts.

Association of *fos* proteins and p39. The precipitation of p39 only in association with p55, as described above, suggests that p39 is in a complex with p55. To determine whether p39 is bound to p55, cell lysates were denatured by boiling in SDS before immunoprecipitation with M2 peptide antisera. $p55^{v\text{-}fos}$ and p39 were clearly detected in a standard cell lysate (Fig. 3, lane 1), but only $p55^{v\text{-}fos}$ was detected in lysates denatured by boiling in SDS (Fig. 3, lane 2) or in lysates denatured and reduced by boiling in SDS plus 2-mercaptoethanol (Fig. 3, lane 3). Many other proteins, seen as minor bands in the immune precipitate, are also precipitated from normal 208F cells and have been discussed previously (9). Thus, the dissociation of p39 from p55 by boiling appears to preclude its direct precipitation by *fos* peptide antisera. The same results were obtained with TBRS and also with cell lines expressing $p55^{c\text{-}fos}$ (data not shown).

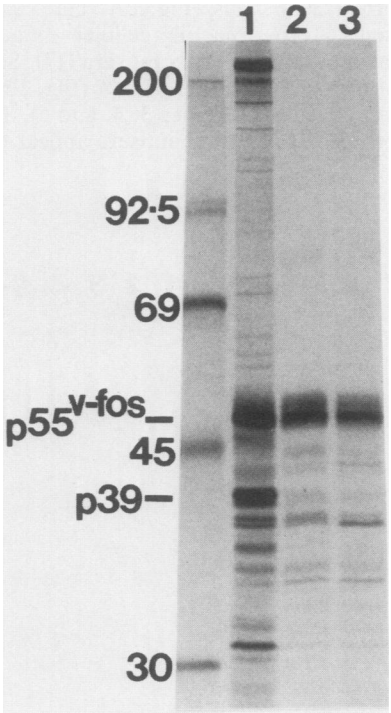

FIG. 3. Immunoprecipitation of denatured and modified forms of p55 by peptide antiserum. [^{35}S]methionine-labeled RS2 cells were prepared in standard RIPA buffer (lane 1), by boiling in 0.5% SDS (lane 2), and by boiling in 0.5% SDS plus 5% 2-mercaptoethanol (lane 3) and were treated with M2 peptide antiserum as described in the text.

Indirect immunofluorescence labeling of normal mouse amnion cells with peptide antisera. Immunoprecipitation of $p55^{v\text{-}fos}$ and $p55^{c\text{-}fos}$ by affinity-purified peptide antisera clearly demonstrated that such sera recognized denatured *fos* proteins in solution. To determine if M peptide antiserum could recognize the cellular *fos* gene product in its natural host cell, normal mouse amnion cells were prepared for immunofluorescence staining as previously described (7). The characteristic nuclear fluorescence of $p55^{c\text{-}fos}$, using TBRS, is seen in Fig. 4a (cf. corresponding Nomarksi image in Fig. 4b). In Fig. 4c a similar pattern, though less intense, was obtained with affinity-purified M2 antiserum. The decreased staining intensity obtained with M2 antiserum versus TBRS probably results from the *fos* protein tertiary structure. Although $p55^{c\text{-}fos}$ undergoes extensive post-translational modifications, such modifications do not interfere with M2 antibody recognition as the highly modified forms of c-*fos* are efficiently immunoprecipitated (data not shown). As expected from the immunoprecipitation analyses, V2 antiserum does not specifically stain amnion cells (Fig. 4e). Thus, M2 peptide antiserum can detect the endogenous $p55^{c\text{-}fos}$, though not as efficiently as TBRS.

DISCUSSION

Understanding the interaction of viral and cellular oncogene products with the components of normal and transformed cells is of prime importance in elucidating the nature of neoplastic transformation. In this way we hope to unravel the function of c-*onc* genes and the manner whereby their perverted function results in tumorigenesis.

Studies on $pp60^{src}$, the oncogene product of Rous sarcoma virus, have identified a number of possible protein substrates (5, 19) and some phospholipid substrates (22) for the action of its protein kinase activity. In addition, proteins of 90,000 and 50,000 daltons have been identified which bind to $pp60^{scr}$ and which may be important in its subcellular transport or regulation (3). Similarly, evidence has been presented which suggests that polyoma middle-T antigen forms a complex with the product of the c-*src* gene (6). We have investigated such an interaction between the *fos* gene product and a normal cellular protein.

The $p55^{v\text{-}fos}$ oncogene product of FBJ-MSV was identified by using sera from rats bearing tumors induced by inoculation of transformed nonproducer cells (9). TBRS has proved very useful in the identification of the v-*fos* and c-*fos* gene products. However, it could not be used as an immunological reagent to distinguish these proteins. Antisera to *fos* peptides were developed to obtain more specific reagents and to investigate the origin of p39. p39 was first identified as a transformation-associated protein in FBJ-MSV-transformed cells (8). Its specific precipitation by TBRS was at first difficult to reconcile as it did not appear among the products of in vitro translation of viral RNA (9). However, we now demonstrate that p39 is immunoprecipitated by virtue of binding to the *fos* gene product.

Antisera were raised against two synthetic peptides. The first peptide, termed M, corresponds to a 26-amino acid region which is common to the v-*fos*, c-*fos*(mouse), and c-*fos*(human) gene products. The second, termed V, corresponds to the 11 C-terminal amino acids of the v-*fos* gene product. The homologous region in the cellular genome is not normally translated since it is from an alternate reading frame and does not have the required signals for expression. Using these antisera, we have confirmed the gene structures of v-*fos* and c-*fos* as derived from previous nucleotide

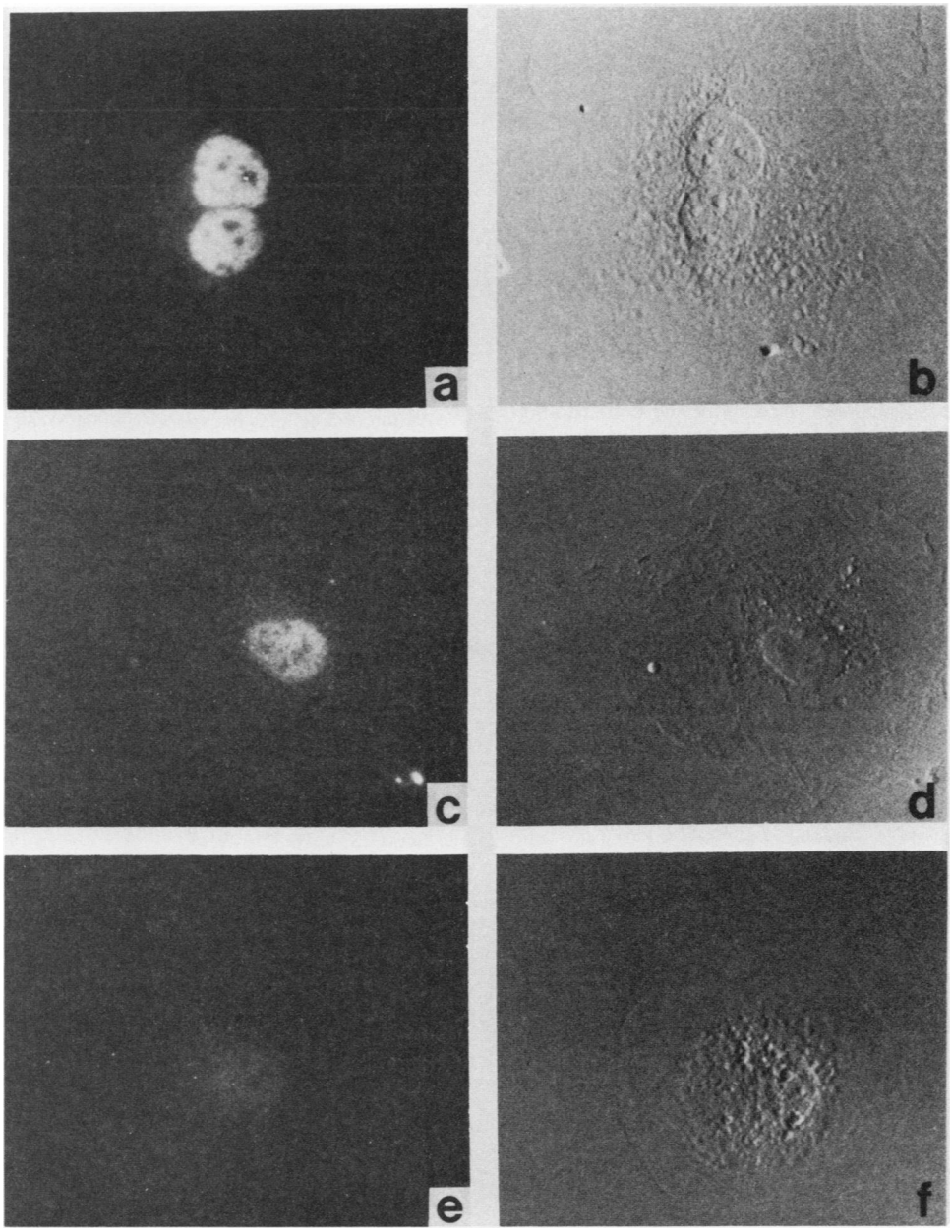

FIG. 4. Indirect immunofluorescence labeling of normal mouse amnion cells, using peptide antiserum. Cells were grown on cover slips and processed for indirect immunofluorescence as described in the text. Amnion cells stained with TBRS (a), same field as in (a) viewed under Nomarski optics (b), amnion cells stained with M2 peptide antiserum (c), same field as in (b) viewed under Nomarksi optics (d), amnion cells stained with V2 peptide antiserum (e), same field as in (e) viewed under Nomarski optics (f). The second antibody in (a) was rhodamine-conjugated rabbit anti-rat immunoglobulin G and that in (c) and (e) was fluorescein-conjugated goat anti-rabbit immunoglobulin G.

sequence analyses (23, 24). Antisera directed against the conserved peptide (M) recognize $p55^{v\text{-}fos}$ and $p55^{c\text{-}fos}$ in immunoprecipitation (Fig. 1 and 2) and immunofluoresence (Fig. 4) assays. In contrast, antisera against the v-*fos* C-terminal peptide recognize $p55^{v\text{-}fos}$ but not $p55^{c\text{-}fos}$ (Fig. 2 and 4). Thus, the v-*fos* and c-*fos* gene products can be differentiated by their distinct C-terminal regions.

Previous peptide mapping studies indicated no major similarities between p55 and p39. Thus, the precipitation of p39 by two distinct *fos* peptide antisera is, in itself, strong evidence that this protein is bound to p55, particularly in view of the fact that the V peptide is not present in the c-*fos* protein. In addition, p39 was not precipitated from cells expressing $p55^{c\text{-}fos}$, using V peptide antiserum, even though the presence of p39 in the cell lysate was demonstrated with M peptide antiserum (Fig. 2B). Preliminary peptide mapping analysis indicates that the p39 detected in cells expressing $p55^{v\text{-}fos}$ is identical to that detected in cells expressing $p55^{c\text{-}fos}$. Denaturation of cell lysates by boiling in SDS abrogates the precipitation of p39 by M peptide antiserum (Fig. 3). This observation is true independent of the *fos* antibody or the cell line used (data not shown). Thus, the association of p39 and p55 is noncovalent and does not appear to involve disulfide bonding. Analysis of the p55/p39 complex by glycerol gradient sedimentation indicated that, although p39 and p55 did comigrate, the complex did not

resolve very well and the majority sedimented close to the monomer range.

Previously, we demonstrated that the v-*fos* and c-*fos* proteins are primarily located in the nucleus (7). In the present report we confirm the nuclear location of $p55^{c\text{-}fos}$ in mouse amnion cells, using M peptide antiserum (Fig. 4). In earlier cell fractionation studies p39 was detected only in the nuclear fractions, although some p55 could be detected in cytoplasmic fractions (7). Thus, it appears that p39 complexes with p55 exclusively in the nucleus. The function of p39 remains unknown; however, it is a potential target or cofactor of the *fos* gene product.

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