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Monoclonal Antibodies to the Transforming Protein of Fujinami Avian Sarcoma Virus Discriminate Between Different *fps*-Encoded Proteins

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Two monoclonal antibodies have been obtained that recognize antigenic determinants within the C-terminal *fps*-encoded region of P140$^{gag\text{-}fps}$, the transforming protein of Fujinami avian sarcoma virus (FSV). The hybridomas which secrete these antibodies (termed 88AG and p26C) were isolated after the fusion of NS-1 mouse myeloma cells with B lymphocytes from Fischer rats that had been immunized with FSV-transformed rat-1 cells. FSV P140$^{gag\text{-}fps}$ immunoprecipitated by either antibody is active as a tyrosine-specific kinase and is able to autophosphorylate and to phosphorylate enolase in vitro. The *fps*-encoded proteins of all FSV variants, including the *gag*$^-$ p91^{fps} protein of F36 virus, are recognized by both monoclonal antibodies. However, the product of the avian cellular c-*fps* gene, NCP98, and the transforming proteins of the recently isolated *fps*-containing avian sarcoma viruses 16L and UR1 are recognized only by the p26C antibody. The 88AG antibody therefore defines an epitope specific for FSV *fps*, whereas the epitope for p26C is conserved between cellular and viral *fps* proteins. The P105$^{gag\text{-}fps}$ protein of the PRCII virus is not precipitated by p26C (nor by 88AG), presumably as a consequence of the deletion of N-terminal *fps* sequences. These data indicate that the *fps*-encoded peptide sequences of 16L P142$^{gag\text{-}fps}$ and UR1 P150$^{gag\text{-}fps}$ are more closely related to NCP98 than that of FSV P140$^{gag\text{-}fps}$. This supports the view that 16L and UR1 viruses represent recent retroviral acquisitions of the c-*fps* oncogene. The P85$^{gag\text{-}fes}$ transforming protein of Snyder-Theilen feline sarcoma virus is not precipitated by either monoclonal antibody but is recognized by some antisera from FSV tumor-bearing rats, demonstrating that *fps*-specific antigenic determinants are conserved in *fes*-encoded proteins.

Fujinami avian sarcoma virus (FSV) is one of several acutely oncogenic RNA tumor viruses whose transforming proteins possess tyrosine-specific protein kinase activity. Four distinct avian sarcoma virus (ASV) transforming genes encode such proteins: v-*src*, v-*fps*, v-*yes*, and v-*ros* (3, 6, 7, 15, 24, 27, 34). In addition to these ASV oncogenes, the products of the v-*fes* genes of Gardner-Arnstein feline sarcoma virus (FeSV) and Snyder-Theilen (ST)-FeSV and of the v-*abl* gene of Abelson murine leukemia virus are tyrosine-specific protein kinases (1, 11, 28). A variety of genetic and biochemical studies suggest that this kinase activity is central to the molecular process whereby these viral gene products induce neoplastic cellular transformation (12, 17, 24, 28, 36).

The FSV genome encodes a single protein with an M_r of 140,000 (140K) (P140$^{gag\text{-}fps}$) (13, 18). P140$^{gag\text{-}fps}$ is a chimeric protein with the structure H_2N-p19-p10-Δp27-*fps*-COOH, possessing an N-terminal region of ca. 40K synthesized from a 3′-deleted retroviral *gag* gene and a C-terminal region synthesized from the v-*fps* gene (24, 30, 35). Normal avian cells contain a gene (c-*fps*) which is closely related to FSV v-*fps*, suggesting that FSV originated by retroviral transduction of the c-*fps* proto-oncogene (19, 31). Consistent with this view, a transforming ASV (16L) was recently isolated from a sarcoma which arose in a chicken after infection with the transformation-defective Rous sarcoma virus (RSV) *td*107A; analysis of 16L ASV virion RNA has suggested that this sarcoma virus is a recombinant between the infecting *td*107A leukosis virus and the c-*fps* gene (22). The cellular c-*fps* gene apparently encodes a 98K M_r protein that, like FSV P140$^{gag\text{-}fps}$ and 16L P142$^{gag\text{-}fps}$, is associated in vitro with tyrosine-specific protein kinase activity (21).

The avian v-*fps* gene is closely related to the v-*fes* transforming genes of Gardner-Arnstein- and ST-FeSV, and it is probable that their respective cellular proto-oncogenes (c-*fps* and c-*fes*) represent the avian and feline copies of the same gene (11, 30). In addition, the amino acid sequence of the polypeptide encoded by FSV v-*fps* shows extensive homology within its C-terminal 280 amino acids with the corresponding regions of the RSV v-*src* and Yamaguchi-73 v-*yes* transforming proteins (15, 26, 30). Limited proteolytic digestion of P140$^{gag\text{-}fps}$ in an immune complex yields a soluble C-terminal 45K fragment that retains the kinase activity and substrate specificity of the intact protein, demonstrating that the P140 $^{gag\text{-}fps}$ enzymatic kinase domain is C terminal (35; J. Cooper, F. S. Esch, S. S. Taylor, and T. Hunter, J. Biol. Chem., in press). A similar observation for RSV p60^{src} (20) indicates that the C-terminal amino acid sequence homology noted above corresponds to the location of a conserved tyrosine-specific kinase catalytic domain within these proteins. FSV P140$^{gag\text{-}fps}$ displays a generalized cytoplasmic distribution, possibly with a (nonintegral) membrane and cytoskeletal association (5). Its ability to induce the many phenotypic changes associated with neoplastic transformation can be explained by postulating that it phosphorylates a number of substrates within the cell, some of which are proteins regulating the processes of cell growth, metabolism, and architecture. Of the various proteins phosphorylated at tyrosine as a consequence of transformation by FSV, three have been identified as glycolytic enzymes (4). FSV P140$^{gag\text{-}fps}$ phosphorylates two of these enzymes, enolase and lactate dehydrogenase, at the same tyrosine

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residues in vitro as become phosphorylated in FSV-transformed cells (Cooper et al., in press).

Here we describe antisera and monoclonal antibodies to FSV P140$^{gag\text{-}fps}$ that illuminate the structural relationships between different *fps*- and *fes*-encoded proteins.

MATERIALS AND METHODS

Cells and viruses. The viruses used here and the procedures for infection and growth of gs^- chicken embryo fibroblasts (CEFs) have been described previously (9, 12, 13, 15, 17, 18, 22, 24, 35). The FSV variants *ts*FSV L-5 and *tr*FSV (17, 24, 35) encode 140K M_r proteins (P140$^{gag\text{-}fps}$), whereas a virus isolated from a different FSV stock encodes a 130K M_r protein (12, 30). FSV variants were complexed with the Fujinami-associated helper virus (FAV). 16L, UR1, PRCII, and Yamaguchi-73 ASVs were associated with helper viruses as reported elsewhere (15, 22, 34, 35). The SR-A strain of RSV and the *fps*/SR-A RSV recombinant virus F36 have also been described previously (8, 36).

The Fischer rat-1 cell line was grown in Dulbecco modified Eagle medium with 5% fetal bovine serum. *ts*FSV L-5-transformed rat-1 cells were obtained by direct infection of rat-1 cells with *ts*FSV(FAV). After 4 weeks, foci of transformed cells were picked and grown to mass culture. E26-transformed avian myeloblasts were a gift of G. S. Martin and were maintained in Dulbecco modified Eagle medium containing 10% tryptose phospate broth, 5% calf serum, and 5% chicken serum and supplemented with 2% chicken spleen-conditioned medium. The plasmacytoma cell line P3-NS-1/1-Ag4-1 (NS-1) was grown in Dulbecco modified Eagle medium supplemented with 2 mM glutamine, 2 mM sodium pyruvate, and 10% fetal bovine serum in a 10% CO_2 atmosphere.

Antisera and monoclonal antibodies. Female Fischer, Wistar, or Fischer × Wistar F_1 rats (6 weeks old) were immunized with 5×10^6 *ts*FSV-transformed rat-1 cells, and sera were monitored for the ability to immunoprecipitate P140$^{gag\text{-}fps}$. Antisera raised to FSV P140$^{gag\text{-}fps}$ in this way are designated anti-FST antisera (21). Wistar and F_1 rats were reimmunized at monthly intervals. To obtain monoclonal antibodies, B lymphocytes from Fischer rats with anti-P140$^{gag\text{-}fps}$ serum antibodies were fused with NS-1 mouse myeloma cells, and the rat × mouse hybrid cells were selected on the basis of hypoxanthine-aminopterin-thymidine resistance. Conditions for the formation and selection of hybridomas were essentially as described elsewhere (14, 16). Hybridomas were screened for the production of anti-*fps* antibodies by a solid-phase radioimmunoassay and immunoprecipitation of P140$^{gag\text{-}fps}$, and positive cells were cloned at least twice by limiting dilution. The solid-phase radioimmunoassay was performed as follows. Flexible polyvinyl microtiter plates (Fisher Scientific Co.) were coated for 8 h at 4°C with rabbit anti-rat immunoglobulin serum diluted in carbonate buffer, pH 9.6 (33). The serum was removed, and the wells were incubated with 3% (wt/vol) bovine serum albumin in carbonate buffer (pH 9.6). The plates were washed three times with phosphate-buffered saline (PBS)-Tween, and 50 μl of PBS was added to each well, followed by 50 μl of hybridoma culture supernatant; the plates were then left overnight at 4°C. The plates were washed three times with PBS-Tween, and [^{35}S]methionine-labeled cell-free translation products of heat-denatured polyadenylic acid-selected FSV(FAV) 70S virion RNA (18) were then added to each well as 1 μl (ca. 10^4 trichloroacetic acid-precipitable counts per minute) of messenger-dependent rabbit reticulocyte lysate diluted to 100 μl with PBS-Tween. The plates were incubated for 4 h at 22°C, washed three times with PBS-Tween, air dried, and cut with scissors, and the individual wells were placed in scintillation vials. ACS (Amersham Corp.) scintillation fluid (5 ml) was added to each vial, and the vials were counted in a Beckman LS 7000 liquid scintillation counter. Positive rat × mouse hybridomas were grown in tissue culture, and antibody was precipitated from tissue culture supernatants with 50% ammonium sulfate, resolubilized in PBS, and dialyzed extensively against PBS to effect a 10-fold concentration.

Rabbit antiserum to avian p19^{gag} was a gift of D. Bolognesi, and caprine antiserum to ST-FeSV P85$^{gag\text{-}fes}$ (anti-ST_{AUT} antiserum [2]) was provided by M. Barbacid. A mouse monoclonal antibody to avian p19^{gag} was obtained by fusion of splenocytes from a BALB/c mouse immunized with purified, disrupted FSV(FAV) virions with NS-1 mouse myeloma cells.

Cell radiolabeling and immunoprecipitation. Cells were labeled for 4 h with [^{35}S]methionine or for 16 h with $^{32}P_i$, lysed, and immunoprecipitated as described previously (24, 25, 35) but with the following modifications. All steps were performed at 4°C. [^{35}S]methionine-labeled cells were lysed in a buffer containing 1% (vol/vol) Nonidet P-40, 0.5% (wt/vol) sodium deoxycholate, 10 mM Tris-hydrochloride (pH 7.5), 100 mM NaCl, and 1 mM EDTA and then precentrifuged at 20,000 × *g*. Cell lysates (0.75 ml from 4×10^5 cells) were incubated for 30 min with 0.1 ml of a 10% (vol/vol) suspension of *Staphylococcus aureus* (1gGsorb; the enzyme center) in lysis buffer. The bacteria were removed by centrifugation, and the operation was repeated two more times. Preabsorbed cell lysates (0.25 ml) were then incubated with the appropriate antibody (rabbit anti-p19^{gag} antiserum [2 μl], goat anti-ST_{AUT} antiserum [5 μl], rat anti-FST antiserum [5 μl], mouse anti-p19^{gag} R254E3 monoclonal antibody as hybridoma culture supernatant [20 μl], rat anti-*fps* 88AG [5 μl] or p26C [20 μl] monoclonal antibodies as ammonium sulfate-concentrated hybridoma culture supernatant) for 2 h. Incubations containing rabbit or goat antisera were then incubated for 1 h with 10 volumes of 10% (vol/vol) *S. aureus*, and those containing rat or mouse antibodies were incubated with 10 volumes of 10% (vol/vol) *S. aureus* precoated with rabbit anti-rat immunoglobulin or rabbit anti-mouse immunoglobulin (Cappell Laboratories) as described previously (10). The immune complexes were washed three to five times in lysis buffer and finally resuspended in sodium dodecyl sulfate-gel sample buffer. The binding of both anti-*fps* monoclonal antibodies is apparently sensitive to denaturation of the antigen and is abolished by inclusion of sodium dodecyl sulfate in immunoprecipitation buffers.

Kinase reactions. Immune complexes for kinase reactions were formed as detailed above, and autophosphorylation reactions were carried out in the presence of [γ-^{32}P]ATP and 10 mM $MnCl_2$ as described previously (24). In vitro phosphorylation of acid-denatured enolase by immunoprecipitated proteins was performed as described by Cooper et al. (in press).

Electrophoresis. Radiolabeled proteins were analyzed by electrophoresis though 7.5% sodium dodecyl sulfate-polyacrylamide gels and detected by autoradiography or fluorography as described previously (35).

RESULTS

Production of anti-*fps* antisera and monoclonal antibodies. To produce an immune response to FSV P140$^{gag\text{-}fps}$, syngeneic 6-week-old female Fischer rats were immunized with 5×10^6 *ts*FSV-transformed rat-1 cells. The rats developed

progressively growing solid tumors at the injection sites within 4 weeks, and half showed an anti-*fps* serum antibody response as measured by immunoprecipitation of P140$^{gag\text{-}fps}$. Sera from responding animals (anti-FST sera) were able to immunoprecipitate both FAV Pr76^{gag} and FSV P140$^{gag\text{-}fps}$ from lysates of radiolabeled FSV(FAV)-infected CEFs and from the products of FSV(FAV) virion RNA translated in a rabbit reticulocyte cell-free protein-synthesizing system. Preincubation of sera with an excess of detergent-disrupted, purified RSV virions to block anti-*gag* antibodies completely inhibited binding of Pr76^{gag}, but it had no effect on the immunoprecipitation of P140$^{gag\text{-}fps}$ (data not shown). We also observed an anti-*fps* response in 50% of Wistar rats repeatedly immunized with *ts*FSV-transformed rat-1 cells, even though no tumors were observed in these nonsyngeneic animals, and in 75% of immunized Fischer × Wistar F_1 rats that typically acquired small regressing tumors. To form hybridomas, the spleens and draining lymph nodes of two positively responding Fischer rats were removed 8 weeks after injection with *ts*FSV-transformed rat-1 cells, and the B lymphocytes were fused with NS-1 mouse myeloma cells. Successfully fused rat × mouse hybrid cells were selected in hypoxanthine-aminopterin-thymidine medium (ca. 2,000 clones) and screened for the secretion of anti-*fps* antibodies by solid-phase radioimmunoassay and immunoprecipitation; in both assays [^{35}S]methionine-labeled in vitro translation products of FSV(FAV) virion RNA were used. Cells from microtiter wells yielding antibody capable of immunoprecipitating P140$^{gag\text{-}fps}$ were subcloned at least twice by limiting dilution, and two independent and stable clones (88AG and p26C) that produced antibodies directed against v-*fps*-encoded antigenic determinants were isolated (Fig. 1).

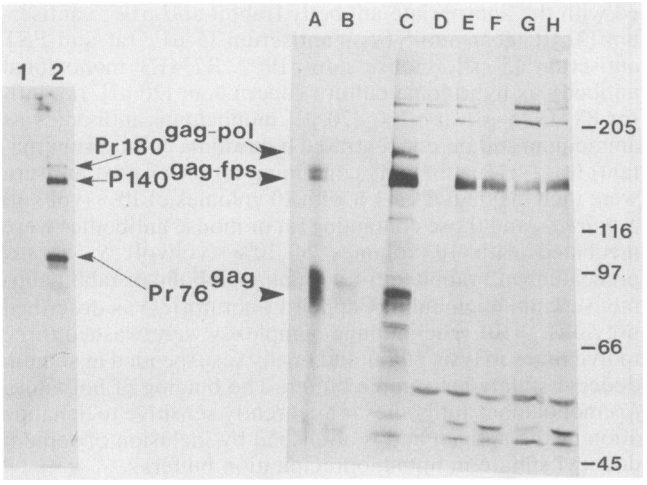

FIG. 1. Specificities of monoclonal antibodies. CEFs were infected and transformed with *ts*FSV(FAV), labeled for 4 h with [^{35}S]methionine, lysed, and subjected to immunoprecipitation with the following antibodies. Lanes: 1 and D, a mouse monoclonal antibody to gastric inhibitory peptide (*gip*) as a control; 2, R254E3 anti-p19^{gag} monoclonal antibody; C, anti-p19^{gag} rabbit antiserum; E, 5 μl of 88AG monoclonal antibody; F, 20 μl of 88AG; G, 5 μl of p26C monoclonal antibody; H, 20 μl of p26C monoclonal antibody. Monoclonal antibodies were used as 50% ammonium sulfate-concentrated hybridoma culture supernatants. Cell-free translation products of FSV-FAV virion RNA (lane A) and FAV virion RNA (lane B) in the messenger-dependent rabbit reticulocyte lysate were used as markers for P140$^{gag\text{-}fps}$ and Pr76^{gag}. Proteins were separated by electrophoresis on a 6.5% polyacrylamide gel, which was stained with Coomassie blue, impregnated with En^{3}Hance (New England Nuclear Corp.) dried, and exposed to X-ray film (Kodak XAR-5) at −80°C. The mobilities of markers of known sizes are shown with their molecular weights (×10^{-3}).

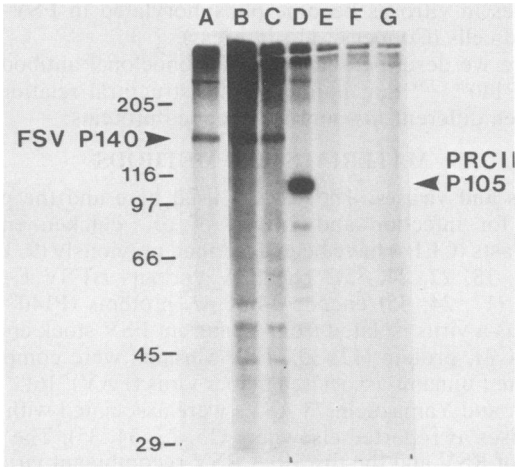

FIG. 2. Reactivity of anti-*fps* monoclonal antibodies with phosphorylated FSV P140$^{gag\text{-}fps}$ and PRCII P105$^{gag\text{-}fps}$. *ts*FSV- and PRCII-transformed CEFs were incubated for 16 h with $^{32}P_i$ (200 μCi/ml) and lysed, and the in vivo radiolabeled proteins from the *ts*FSV CEF lysate (lanes A through C) and from the PRCII CEF lysate (lanes D through G) were subjected to immunoprecipitation with anti-p19^{gag} rabbit antiserum (lanes A and D), anti-*gip* control monoclonal antibody (lane E), 88AG anti-*fps* monoclonal antibody (lanes B and F), and p26C anti-*fps* monoclonal antibody (lanes C and G). Proteins were separated by electrophoresis through a 7.5% polyacrylamide gel. The mobilities of marker proteins of known sizes, together with their molecular weights (×10^{-3}) are indicated.

Characterization of anti-*fps* monoclonal antibodies. To demonstrate the specificities of these monoclonal antibodies, a detergent lysate of [^{35}S]methionine-labeled *ts*FSV(FAV)-infected CEFs was subjected to immunoprecipitation with the 88AG or p26C monoclonal antibodies (Fig. 1). Anti-p19^{gag} antiserum or monoclonal antibody precipitated the FAV-encoded polyproteins Pr76^{gag} and Pr180$^{gag\text{-}pol}$ (18) as well as FSV P140$^{gag\text{-}fps}$, whereas the 88AG and p26C monoclonal antibodies precipitated only FSV P140$^{gag\text{-}fps}$. The addition of competing RSV virions to the immunoprecipitation reactions blocked the binding of P140$^{gag\text{-}fps}$ by anti-p19^{gag} antibodies but not by 88AG or p26C (data not shown). These data show that the monoclonal antibodies 88AG and p26C are each directed against an epitope within the C-terminal *fps*-encoded region of P140$^{gag\text{-}fps}$. When *ts*FSV-transformed CEFs were radiolabeled with $^{32}P_i$ rather than with [^{35}S]methionine, both anti-*fps* monoclonal antibodies proved able to immunoprecipitate the phosphorylated form of P140$^{gag\text{-}fps}$ (Fig. 2).

Enzymatic activity of FSV P140$^{gag\text{-}fps}$ immunoprecipitated by the anti-*fps* monoclonal antibodies. Site-specific antibodies that bind within the extreme C-terminal catalytic domain of FSV P130$^{gag\text{-}fps}$ can inhibit its kinase activity, presumably because the bound antibodies interfere with structures directly involved in the phosphotransfer reaction (29). We therefore investigated whether FSV P140$^{gag\text{-}fps}$ precipitated by 88AG or p26C monoclonal antibodies was active as a kinase. P140$^{gag\text{-}fps}$ precipitated with either anti-*fps* monoclonal antibody was autophosphorylated in the immune complex when incubated with $MnCl_2$ and [γ-^{32}P]ATP (Fig. 3). Subsequent two-dimensional tryptic phosphopeptide mapping revealed that the same P140$^{gag\text{-}fps}$ tyrosine residues were phosphorylated in these instances as when anti-p19^{gag}

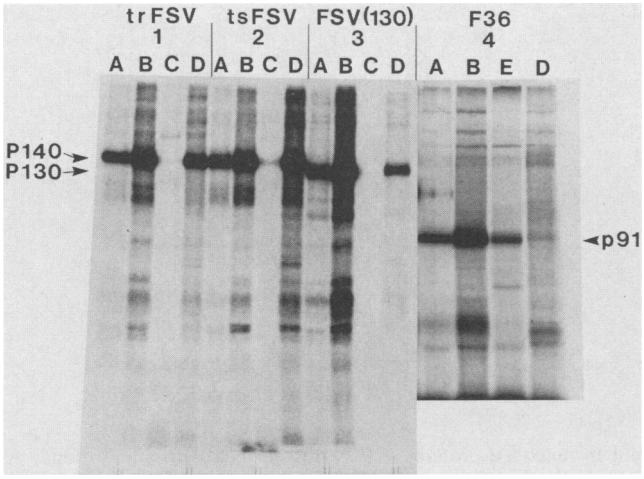

FIG. 3. Reactivity of anti-*fps* monoclonal antibodies with proteins encoded by different FSV variants. Lysates of CEFs transformed with *tr*FSV (section 1), *ts*FSV (section 2), a variant of FSV encoding a 130K protein (section 3), and F36 virus (section 4) were immunoprecipitated with p26C anti-*fps* monoclonal antibody (lane A), 88AG anti-*fps* monoclonal antibody (lane B), anti-*gip* control monoclonal antibody (lane C), anti-p19^{gag} antiserum (lane D), or an anti-FST rat antiserum (lane E). Immunoprecipitates were incubated with [γ-^{32}P]ATP and $MnCl_2$ in an immune complex kinase reaction, and in vitro-phosphorylated proteins were separated by electrophoresis through a 7.5% polyacrylamide gel and identified by autoradiography.

antiserum was used (35) (data not shown), suggesting that neither monoclonal antibody binds to a phosphoacceptor site. When acid-denatured enolase was added to the immune complex kinase reactions as an exogenous substrate, it was phosphorylated regardless of whether anti-p19^{gag} antiserum, anti-FST antiserum, or the 88AG or p26C anti-*fps* monoclonal antibody was employed to precipitate P140$^{gag\text{-}fps}$ (Fig. 4). Thus neither monoclonal antibody has an inhibitory effect on P140$^{gag\text{-}fps}$ tyrosine-specific kinase activity.

Reactivity of monoclonal antibodies with different *fps*-encoded proteins. A number of distinct FSV variants have been reported, all of which apparently derive from the original isolate of Fujinami in 1914 (9). The *ts*FSV L-5 virus described above has yielded a temperature-resistant (*tr*FSV) virus, upon passage in a chicken, which also encodes a P140$^{gag\text{-}fps}$ protein (17). A stock of FSV with a different passage history has been described (13) from which a spontaneous variant encoding a P130$^{gag\text{-}fps}$ protein has been isolated (12, 30). The *fps* sequences of this latter FSV have been used to construct a transforming virus (F36) in which the *src* oncogene of SR-A RSV has been replaced with v-*fps* (8). F36 expresses a 91K M_r protein (p91^{fps}) which has no covalently linked *gag* peptide. The 88AG and p26C anti-*fps* monoclonal antibodies precipitate all of these FSV-derived *fps*-encoded proteins (*ts*FSV P140$^{gag\text{-}fps}$, *tr*FSV P140$^{gag\text{-}fps}$, FSV P130$^{gag\text{-}fps}$, and F36 p91^{fps}) from lysates of virally transformed CEFs (Fig. 3). The ability of 88AG and p26C to recognize F36 p91^{fps} proves that these antibodies are directed to *fps*-encoded determinants.

The relationship between viral oncogenes and their cellular progenitors is of considerable interest, and we therefore investigated whether the anti-*fps* monoclonal antibodies recognized the NCP98 protein believed to be the product of the avian c-*fps* gene. E26-transformed avian myeloblasts were lysed and immunoprecipitated with anti-FST antiserum or with the 88AG or p26C anti-*fps* monoclonal antibodies (Fig. 5). NCP98 was precipitated by the anti-FST antiserum and by the p26C monoclonal antibody but not by the 88AG monoclonal antibody. Thus, p26C recognizes an antigenic determinant which is shared between c-*fps*-encoded NCP98 and FSV P140$^{gag\text{-}fps}$, whereas the epitope for 88AG is found on FSV P140$^{gag\text{-}fps}$ but not on NCP98. The *fps*-containing 16L ASV has been postulated to result from a very recent recombination event between an avian leukosis virus and c-*fps* (22). When 16L-transformed CEFs were immunoprecipitated with the anti-*fps* monoclonal antibodies, 16L P142$^{gag\text{-}fps}$ was precipitated by p26C but not by 88AG. 16L P142$^{gag\text{-}fps}$ therefore shows the same reactivity with the anti-*fps* monoclonal antibodies as does NCP98. UR1, another *fps*-containing ASV isolated in 1969, encodes a P150$^{gag\text{-}fps}$ protein (34), and this is also recognized by p26C antibody but not by 88AG (Fig. 5). A series of *fps*-containing viruses have been described which are apparently derived from a single isolate in Mill Hill, England (PRCIV, PRCII-p, and PRCII) (23, 37). The PRCII RNA genome is of particular interest since it lacks nearly half the *fps* sequences found in FSV, as reflected in the size of its P105$^{gag\text{-}fps}$ gene product (23, 37). Oligonucleotide mapping, comparative hybridization, and, recently, DNA sequencing of the PRCII genome have shown that PRCII P105$^{gag\text{-}fps}$ is lacking a sequence of ca. 340 amino acids found in the N-terminal half of the *fps* region of FSV P140$^{gag\text{-}fps}$ (32, 37; C.-C. Huang and J. M. Bishop, personal communication). Other than this, the *fps* sequences of PRCII P105$^{gag\text{-}fps}$ and FSV P130$^{gag\text{-}fps}$ show very few amino acid differences (C.-C. Huang and J. M. Bishop, personal communication). PRCII P105$^{gag\text{-}fps}$ is not precipitated by either the 88AG or p26C antibodies from lysates of [^{35}S]methionine- or ^{32}P-labeled CEFs transformed with PRCII (Fig. 2), nor is autophosphorylating protein kinase activity precipitable from PRCII-transformed CEFs by these

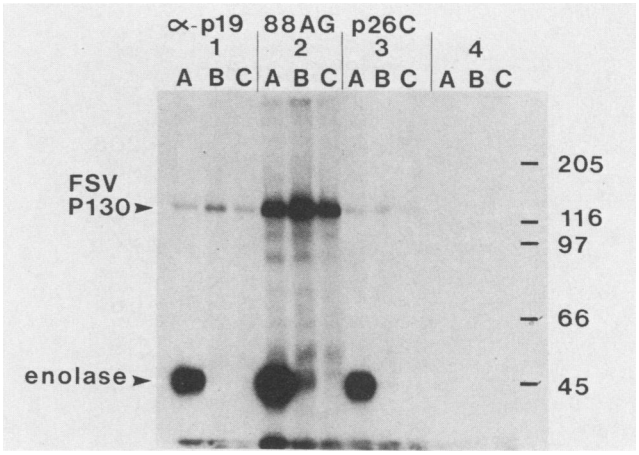

FIG. 4. In vitro phosphorylation of enolase by FSV P130$^{gag\text{-}fps}$ precipitated by anti-*fps* monoclonal antibodies. Lysates of FSV-transformed CEFs were immunoprecipitated with anti-p19^{gag} antiserum (section 1), 88AG anti-*fps* monoclonal antibody (section 2), p26C anti-*fps* monoclonal antibody (section 3), or rabbit anti-rat immunoglobulin serum alone as a control (section 4). Immune complexes were incubated with 5 μg of acid-denatured enolase (lane A), 5 μg of untreated enolase (lane B), or in the absence of exogenous substrate (lane C) in the presence of $MnCl_2$ and [γ-^{32}P]ATP. Labeled proteins were detected by electrophoretic separation in a 7.5% polyacrylamide gel, followed by autoradiography. The mobilities of size markers and their molecular weights ($\times 10^{-3}$) are indicated.

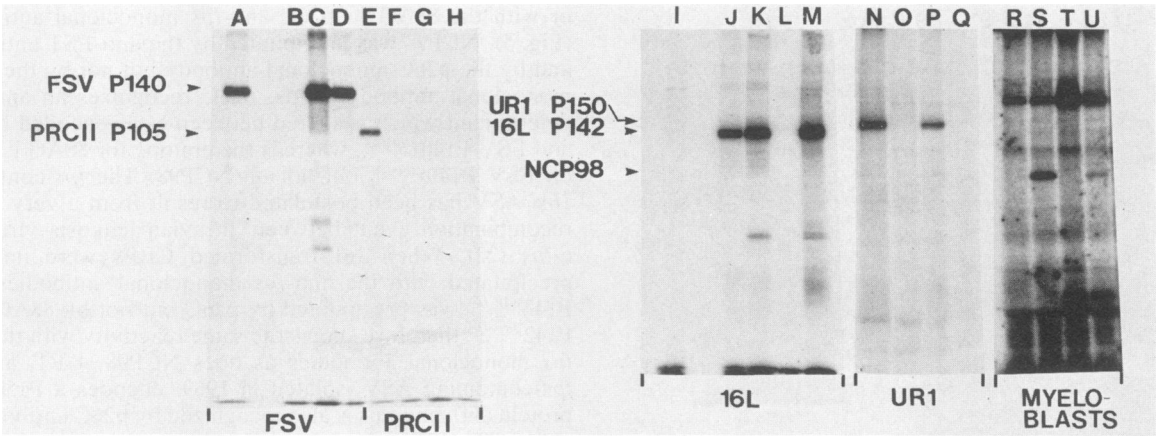

FIG. 5. Cross-reactivity of anti-*fps* monoclonal antibodies with different *fps*-encoded proteins. CEFs transformed with *ts*FSV (lanes A through D), PRCII (lanes E through H), 16L (lanes I through M), UR1 (lanes N through Q), or avian E26-transformed myeloblasts (lanes R through U) were immunoprecipitated with anti-p19^{gag} antiserum (lanes A, E, M, and N), 88AG anti-*fps* monoclonal antibody (lanes C, G, L, O, and T), p26C anti-*fps* monoclonal antibody (lanes D, H, J, P, and U), anti-FST rat antiserum (lanes K and S), or normal rat serum (lanes B, F, I, Q, and R). Immunoprecipitates were incubated with $MnCl_2$ and [γ-^{32}P]ATP in autophosphorylation reactions and analyzed by electrophoresis through a 7.5% polyacrylamide gel.

monoclonal antibodies (Fig. 5). The epitope recognized by p26C, which is otherwise conserved in those *fps*-encoded proteins examined, is therefore comprised of peptide sequences which are either lost or structurally impaired in PRCII P105$^{gag\text{-}fps}$.

Reactivity of anti-*fps* antisera and monoclonal antibodies with ST-FeSV P85$^{gag\text{-}fes}$. Antisera to the transforming proteins of the Gardner-Arnstein- and ST-FeSVs can recognize FSV P140$^{gag\text{-}fps}$ (2), supporting the hypothesis that the avian c-*fps* gene is homologous to the feline c-*fes* gene. However, anti-*fps* antibodies have not previously shown the reciprocal ability to recognize v-*fes*-encoded proteins. We tested a number of rat anti-FST antisera and the anti-*fps* monoclonal antibodies for their ability to precipitate ST-FeSV P85$^{gag\text{-}fes}$ from ST-FeSV-transformed NIH 3T3 cells and found that both monoclonal antibodies and six of eight anti-FST antisera failed to react with P85$^{gag\text{-}fes}$. However, two of the anti-FST rat antisera (from a Fischer and an F_1 rat) were cross-reactive with ST-FeSV P85$^{gag\text{-}fes}$ (Fig. 6). The avian and feline retrovirus group-specific antigens are not immunologically cross-reactive (2), and these results therefore show that some *fps*-encoded antigenic determinants are present in the ST-FeSV *fes* sequence.

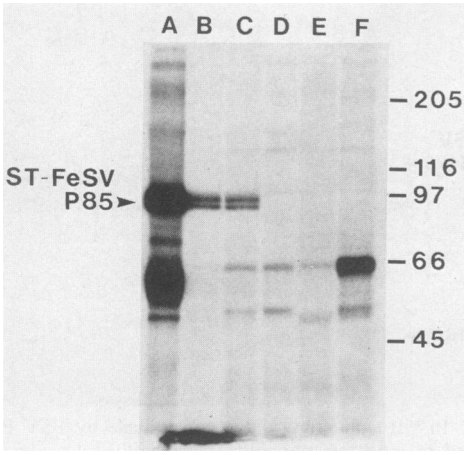

FIG. 6. Reactivity of rat antisera and monoclonal antibodies to FSV P140$^{gag\text{-}fps}$ with the P85$^{gag\text{-}fes}$ protein of ST-FeSV. Detergent lysates of ST-FeSV-transformed NIH 3T3 cells were incubated with caprine anti-ST_{AUT} antiserum to ST-FeSV P85$^{gag\text{-}fes}$ (lane A), J6 Fischer rat anti-FST antiserum (lane B), F_1-1 rat anti-FST antiserum (lane C), 88AG McAb (lane D), p26C McAb (lane E), or normal rat serum (lane F). Immunoprecipitates were incubated with $MnCl_2$ and [γ-^{32}P]ATP in autophosphorylation reactions and were subsequently analyzed by electrophoresis through 7.5% polyacrylamide gels. The mobilities of size markers and their molecular weights ($\times 10^{-3}$) are indicated.

DISCUSSION

The 88AG and p26C anti-FSV *fps* monoclonal antibodies clearly have different binding activities for distinct *fps*-encoded proteins (Table 1) and must, therefore, recognize separate antigenic determinants. The NCP98 protein synthesized from the c-*fps* proto-oncogene is precipitated by p26C but not by 88AG, and this same binding specificity is displayed by 16L P142$^{gag\text{-}fps}$ and UR1 P150$^{gag\text{-}fps}$. In contrast, all proteins encoded by FSV *fps* are recognized by both antibodies. These data have interesting implications for the formation of *fps*-containing ASVs, and suggest that the *fps* peptide sequence of 16L P142$^{gag\text{-}fps}$ is more closely related to the c-*fps* protein NCP98 than is FSV P140$^{gag\text{-}fps}$. This supports the contention that 16L P142$^{gag\text{-}fps}$ resulted

TABLE 1. Reactivity of anti-*fps* monoclonal antibodies with different *fps*-encoded proteins

Viral or cellular *fps* proteins	Monoclonal antibodies	
	88AG	p26C
*ts*FSV L-5 P140$^{gag\text{-}fps}$	+	+
*tr*FSV P140$^{gag\text{-}fps}$	+	+
FSV P130$^{gag\text{-}fps}$	+	+
F36 P91^{fps}	+	+
16L P142$^{gag\text{-}fps}$	−	+
UR1 P150$^{gag\text{-}fps}$	−	+
c-*fps* NCP98	−	+
PRCII P105$^{gag\text{-}fps}$	−	−
ST-FeSV P85$^{gag\text{-}fes}$	−	−

from a recent retroviral transduction of c-*fps* and could not be an FSV contaminant. Although the *fps* peptide sequences of NCP98 and 16L P142$^{gag\text{-}fps}$ and UR1 P150$^{gag\text{-}fps}$ are indistinguishable by this limited immunological test, the *fps* peptide sequence of FSV P140$^{gag\text{-}fps}$ has apparently diverged from NCP98, since it has acquired an epitope for 88AG antibody which is not present in NCP98. If so, the mutation(s) in FSV *fps* that led to the formation of the antigenic determinant for 88AG antibody must have occurred in an early FSV stock, and it is apparently stable, since proteins of all FSV variants are recognized by 88AG. It is also possible that the coding sequence of FSV contains cellularly-derived information not present in c-*fps*. A further, if unlikely, possibility is that the coding sequence of c-*fps* is polymorphic. It is not clear whether the structural change in FSV P140$^{gag\text{-}fps}$ represented by the 88AG antibody binding site is of functional significance, but it cannot be essential for transformation.

The binding of both 88AG and p26C to FSV P140$^{gag\text{-}fps}$ is inhibited by denaturing agents such as sodium dodecyl sulfate, suggesting that the integrity of their respective antigenic determinants requires the protein to be in a native conformation. It is not yet possible to exactly define the sequences recognized by each antibody, but several circumstantial lines of evidence suggest that 88AG and p26C anti-*fps* antibodies may both be directed towards epitopes outside the C-terminal *fps* kinase domain of FSV P140$^{gag\text{-}fps}$. Neither antibody inhibits the P140$^{gag\text{-}fps}$ kinase activity or recognizes RSV p60^{src} or Yamaguchi-73 p90$^{gag\text{-}yes}$ (data not shown) which share C-terminal sequences with FSV P140$^{gag\text{-}fps}$. In addition, although we have shown that ST-FeSV P85$^{gag\text{-}fes}$ and FSV P140$^{gag\text{-}fps}$ (which are related over their C-terminal 475 amino acids [11]) are both recognized by some anti-FST antisera, ST-FeSV P85$^{gag\text{-}fes}$ is not bound by either monoclonal antibody; PRCII P105$^{gag\text{-}fps}$, which has sustained a deletion in its N-terminal *fps* region, is not efficiently precipitated by p26C (or 88AG). C-terminal proteolytic fragments of P140$^{gag\text{-}fps}$ are not precipitated by either monoclonal antibody (data not shown). Although none of these arguments is conclusive in itself, since conformational changes within the protein might affect the antigenic sites without actual loss of the corresponding amino acids, they are all consistent with the notion that the monoclonal antibodies bind sites outside of the catalytic domain. We have recently constructed a set of in-phase linker insertion mutants throughout the FSV genome (J. C. Stone, T. Atkinson, M. Smith, and T. Pawson, Cell, in press), and it will be of interest to determine whether the proteins encoded by these mutants are recognized by either monoclonal antibody.

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