Common Features of the *yes* and *src* Gene Products Defined by Peptide-Specific Antibodies

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Anti-peptide antibodies generated against a hydrophilic domain of pp60^{src} comprising amino acid residues 498 through 512 were shown to be cross-reactive with the corresponding region in the *yes* transforming proteins encoded by Yamaguchi 73 and Esh sarcoma viruses. This cross-reactivity was demonstrated by immunoblot and immunoprecipitation analyses, and the identity of the proteins was verified by partial proteolytic mapping. By utilizing a combination of immunofluorescence and interference-reflection microscopy, these cross-reactive anti-peptide antibodies were shown to produce an immunofluorescence staining pattern in Yamaguchi 73 and Esh sarcoma virus-transformed chicken embryo fibroblasts remarkably similar to that of pp60^{src} in Rous sarcoma virus-infected chicken cells. Like the *src* gene products, the *yes* transformation-specific polypeptides were found to be concentrated within adhesion plaque structures and needle-like interdigitating cell-cell junctions. This analogous subcellular distribution suggests that these *onc* proteins are functionally related and may share common intracellular targets.

Yamaguchi 73 sarcoma virus (Y73) and Esh sarcoma virus (ESV) are replication-defective transforming retroviruses that were independently isolated from spontaneous sarcomas in chickens (25, 46). Both viruses induce sarcomas in vivo and are able to transform fibroblastic cells in vitro (19, 26). Recent biological and biochemical studies have shown that the transforming properties of these viruses are directed by genetic information that is of cellular origin (43, 49). By the criteria of molecular DNA hybridization, these cell-derived transformation-specific sequences appear to be unrelated to other retroviral *onc* gene sequences (43, 49) and have been termed *yes* in accordance with the established nomenclature for *onc* genes (8).

The *yes* transforming genes of Y73 and ESV encode 90,000 (P90^{gag-yes})- and 80,000 (P80^{gag-yes})-*M_r* *gag*-linked phosphoproteins, respectively (18, 19, 26). Initial studies concerning the structures of the *gag-yes* gene products indicated that they were unrelated to other *onc* transforming proteins, including pp60^{src} (1, 18, 19). These findings were consistent with the lack of homology observed between these *onc*-specific sequences as detected by nucleic acid hybridization studies (43, 49). However, recently, the nucleotide sequence of the Y73 genome was determined (27), and, although the sequence of the *yes* gene is only partially related to *src*, the amino acid sequences of the predicted protein products are closely related, exhibiting a strikingly high degree of homology. The greatest extent of homology exists in the carboxy-terminal domains of *yes* and *src* where more than 85% of the amino acid residues are identical.

In addition to structural relatedness, several functional similarities regarding the *yes* and *src* gene products have been reported. First, these oncogenic proteins exhibit associated kinase activities specific for tyrosine residues of substrate proteins (18, 19, 22, 26, 42) and display similar metal-ion requirements and pH-dependent profiles (15, 18, 34). Second, the newly synthesized forms of these polypeptides in transformed cells are associated in a ternary complex with the same 90,000- and 50,000-*M_r* host cell proteins (3, 29,

31, 32). Finally, the *src* and *yes* gene products induce the phosphorylation of a similar set of cellular proteins in vivo (9), including a 36,000-*M_r* membrane-associated polypeptide (9, 10, 13, 20, 33) and vinculin (41), an adhesion plaque protein associated with the cytoskeletal framework of the cell (6, 16).

In this report we have identified yet another functional similarity between the *src* and *yes* transformation-specific polypeptides. By using anti-peptide antibodies generated against a carboxy-terminal region of pp60^{src} (17), the *src*-encoded protein product, which were cross-reactive with the *yes* gene products, we have been able to demonstrate that the *gag-yes* proteins concentrate within transformed fibroblasts at intracellular locations remarkably similar to those occupied by pp60^{src} in Rous sarcoma virus (RSV)-transformed cells. These findings imply that the mechanisms of cellular transformation evoked by *src* and *yes* may be closely related.

MATERIALS AND METHODS

Cells and viruses. Chicken embryo cells (CEC) of phenotype C/E and negative for the expression of chicken helper factor were cultured from 10-day-old White leghorn chicken embryos (H and N Laboratories, Redmond, Wash.). Primary cultures were infected with virus and passaged by trypsinization at least four times before experimental use. Cells were grown in Ham's F10 medium supplemented with 10% tryptose phosphate broth, 5% calf serum, 1% chicken serum, and 1% dimethyl sulfoxide.

Virus stocks of Y73 and ESV originating from chicken cells transformed by these viruses were generously supplied by K. Beemon, Johns Hopkins University, and P. K. Vogt, University of Southern California, respectively. The isolation and properties of RD10-4A1L, a Schmidt-Ruppin strain of RSV rescued from transformed rat cells, have been described previously (39). The Prague C strain of RSV was from a laboratory stock.

Antisera. The production of antiserum against the synthetic peptide Cys-Trp-Arg-Lys-Asp-Pro-Glu-Glu-Arg-Pro-Thr-Phe-Lys-Thr-Leu, corresponding in sequence to residues 498 through 512 of Prague C pp60^{src}, and the purification of peptide-specific immunoglobulin G fraction by affinity chro-

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FIG. 1. Immunoblot analysis of total cellular proteins resolved by SDS-PAGE. Whole cell extracts prepared by lysing unlabeled intact cells in boiling electrophoresis sample buffer were separated on a denaturing discontinuous 7.5% acrylamide gel (17, 28). Proteins were then electrophoretically transferred to unmodified nitrocellulose, and the nitrocellulose strip was incubated with affinity-purified anti-peptide 1 antibodies as described previously (17). Immunocomplexes were detected by subsequent reaction with ^{125}I -labeled protein A, followed by autoradiography. (A) Uninfected CEC; (B) Y73-transformed CEC; (C) ESV-transformed CEC; (D) RD10-transformed CEC. Numbers at the left of the figure denote the molecular masses of proteins identified by the anti-peptide 1 antibodies as determined by comparison with nitrocellulose sheets containing transferred marker proteins.

matography on peptide-bound Sepharose have been detailed previously (17). Serum from tumor-bearing rabbits was obtained by the procedure of Brugge and Erikson (4) as described by Rohrschneider (35). Antiserum specific for p19^{src} was kindly provided by D. Bolognesi, Duke University.

Immunoblot analysis. Confluent cultures of uninfected and infected CEC were scraped into cold phosphate-buffered saline with a rubber policeman, and the cells were collected by low-speed centrifugation. The cells were then lysed in boiling electrophoresis sample buffer (17, 28), and samples of the total cellular extracts were electrophoresed on 7.5% polyacrylamide gels containing sodium dodecyl sulfate (SDS). After electrophoresis, proteins were transferred to nitrocellulose strips, and these strips were incubated sequentially with antibody and ^{125}I -labeled protein A as described previously (17). Immunocomplexes were visualized by autoradiography.

Radiolabeling of viral proteins and immunoprecipitation analysis. Confluent cultures of infected CEC were rinsed once in Eagle minimum essential medium containing 5% calf serum and only 5% of the normal level of methionine and then incubated for 18 h in this medium supplemented with 100 μCi of [^{35}S]methionine per ml. Cells were subsequently washed in cold phosphate-buffered saline and frozen at -70°C . The [^{35}S]methionine-labeled cultures were lysed in RIPA buffer containing 5 mM EDTA, and immunoprecipitations were performed as described previously (17). The resulting immunocomplexes were then subjected to SDS-polyacrylamide gel electrophoresis (PAGE) on 7.5% acrylamide gel (28). After electrophoresis, the gel was treated with En^3Hance (New England Nuclear Corp., Boston, Mass.), dried, and autoradiographed.

Partial proteolytic peptide mapping. [^{35}S]methionine-

labeled proteins were located on dried polyacrylamide gels by autoradiography, and the radioactive protein bands were excised. After rehydration, the proteins were extracted from the gel slices as follows. The swollen gel pieces were homogenized in small glass tissue grinders in the presence of 0.05 M Tris-hydrochloride (pH 8.0) and transferred to screw cap conical centrifuge tubes. After the concentration of SDS and 2-mercaptoethanol were brought to 1% (wt/vol) and 0.1% (vol/vol), respectively, the tubes were tightly capped and heated to 95°C for 10 min. The capped tubes were then rotated overnight at 37°C . The gel pieces were centrifuged, and the supernatants were collected. The homogenized gel chunks were then resuspended in another sample of extraction buffer containing SDS and 2-mercaptoethanol, heated to 95°C for 5 min, and rotated for 2 h at 37°C . Supernatants were collected and combined with the initial extracts. At this point ca. 1.5 ml of buffered protein solution was obtained. Rabbit immunoglobulin G (25 μg) was then added as a carrier, and the proteins were precipitated by the addition of 3 ml of methanol:acetic acid (100:1) (39). Precipitation was carried out at -70°C overnight. After thawing, the tubes were spun at $200,000 \times g$ for 1 h at 4°C . Overall recovery of radioactivity from the gel pieces ranged from 65 to 80%.

The precipitated proteins were solubilized in 150 μl of digestion buffer (0.125 M Tris-hydrochloride [pH 6.8], 1 mM EDTA, 1 mM 2-mercaptoethanol, 15% [vol/vol] glycerol, 0.15% [wt/vol] SDS), and 50- μl samples were incubated with 0, 10 ng, or 1 μg of *Staphylococcus aureus* V8 protease (Miles Laboratories, Inc., Elkhart, Ind.) for 1 h at room temperature. Laemmli sample buffer (3 $\times$) was added, and the samples were heated to 95°C and electrophoresed on an SDS-10 to 20% polyacrylamide gradient gel. Protein and peptide fragments were identified by fluorography after treatment with En^3Hance .

Immunofluorescence and interference-reflection microscopy. Uninfected or infected CEC were plated on ethanol-sterilized glass cover slips in 60-mm tissue culture dishes at ca. 2×10^5 cells per ml and grown for at least 40 h before use. Cells were then gently rinsed in phosphate-buffered saline, fixed in methanol, and stained for indirect immunofluorescence as described previously (17, 44). For viewing, cover slips were mounted in Elvanol (Monsanto Co., St. Louis, Mo.) or in phosphate-buffered saline above 3-mm glass chambers. The latter mounting procedure allowed for simultaneous viewing of both immunofluorescence and interference-reflection images. Mounted cells were viewed with a Zeiss Photomicroscope III equipped with epi-illumination. Interference-reflection microscopy was performed according to previously published procedures (12, 36, 44).

RESULTS

Anti-peptide antibodies generated toward pp60^{src} identify the *yes* gene products of Y73 and ESV. In a previous report (17) the characterization of site-specific anti-peptide antibodies prepared against a region of pp60^{src} comprising amino residues 498 through 512 was described. This region is located near the carboxy terminus of the *src* molecule and, according to a mathematical prediction model (21), represents the most hydrophilic domain of the *src* gene product. The amino acid sequence of this region is shown below along with the sequence of the corresponding region in the Y73 *yes* transforming protein:

RSV Prague *src* (40) C-W-R-K-D-P-E-E-R-P-T-F-K-Y-L

Y73 *yes* (25) C-W-K-K-D-P-D-E-R-P-T-F-E-Y-I

Clearly, this hydrophilic domain of the *src* gene product is homologous to a similar region contained in the *yes* protein. Only 4 of the total 15 amino acid residues comprising this region differ between the two transforming gene products (underlined amino acids). Moreover, of the four differences which are identified, only one amino acid replacement appears to be significantly different (double-underlined amino acid). This replacement represents a change from lysine to glutamic acid. The other substitutions in the Y73 transforming protein represent conservative changes, indicating the highly conserved nature of this region.

Since this hydrophilic domain is similarly contained in the *src* and *yes* transforming gene products, we tested the ability of anti-peptide antibodies directed against this region of pp60^{src} (termed anti-peptide 1) to cross-react with the *yes* gene products of Y73- and ESV-transformed cells. We utilized two immunochemical techniques to demonstrate the cross-reactivity of these anti-peptide antibodies: immunoblot and immunoprecipitation analyses.

Figure 1 shows the results of an immunoblot analysis of total cellular proteins from uninfected CEC and CEC transformed by Y73, ESV, and a Schmidt-Ruppin D isolate of RSV termed RD10 (39). Antibodies to this *src*-derived synthetic peptide, in addition to readily recognizing pp60^{src} in RSV-transformed CEC (lane D), also identified proteins of *M_r* 90,000 and 80,000 in Y73- and ESV-transformed CEC, respectively (lanes B and C) (Fig. 1). Under these exposure conditions the anti-peptide 1 antibodies failed to detect any

FIG. 2. SDS-PAGE analysis of immunoprecipitates prepared from extracts of [³⁵S]methionine-labeled cells. RIPA buffer extracts of metabolically labeled RD10-, Y73-, and ESV-transformed CEC were prepared, and labeled proteins were immunoprecipitated with 3 μ l of tumor-bearing rabbit serum preabsorbed with an excess of virion proteins (A), 3 μ l of anti-p19^{gag} serum (a), 20 μ g of anti-peptide 1 (b), or 20 μ g of anti-peptide 1 preabsorbed with 100 μ g of excess peptide 1 (c). Immunocomplexes were collected, washed, heated in boiling electrophoresis sample buffer, and electrophoresed on 7.5% polyacrylamide gels as described previously (17). [³⁵S]methionine-labeled proteins were detected by fluorography. The low-molecular-weight polypeptides detected in Y73- and ESV-transformed cells by anti-p19^{gag} serum may represent p19-containing breakdown products of the *gag-yes* proteins.

FIG. 3. Partial proteolytic peptide mapping of the *gag-yes* gene products immunoprecipitated by anti-P19^{gag} serum or anti-peptide 1 antibodies. Proteins were immunoprecipitated from extracts of [³⁵S]methionine-labeled cells and resolved by SDS-PAGE. Radioactive protein bands were located by autoradiography, the area of the dried gel was excised, and the protein was isolated from the gel slice as described in the text. Proteins were dissolved in digestion buffer and then treated with 0 (a), 10 ng (b), or 1 μ g (c) of *S. aureus* V8 protease for 1 h. After digestion, peptide fragments were resolved by SDS-PAGE on 10 to 20% acrylamide gradient gels and fluorographed. Numbers at the right of the figure denote the molecular masses of marker proteins in kilodaltons (k).

specific proteins from the control uninfected CEC (Fig. 1, lane A). However, upon overexposure of the autoradiograph, low levels of a 60,000-*M_r* protein were observed (data not shown). This protein probably represents the endogenous cellular analog of viral pp60^{src}, as these anti-peptide antibodies have been shown to identify this protein in normal cells (17). The recognition of the 90,000- and 80,000-*M_r* proteins in Y73- and ESV-infected cells, like the identification of pp60^{src} in RSV-transformed CEC, could be eliminated by prior incubation of the anti-peptide antibody with an excess of soluble synthetic peptide (data not shown). The molecular masses of the polypeptides identified by immunoblotting were consistent with the masses predicted for P90^{gag-yes} of Y73 and P80^{gag-yes} of ESV.

The antibodies were next tested for their ability to immunoprecipitate metabolically labeled proteins from extracts of cultured cells (Fig. 2). Uninfected or virally infected CEC were grown to confluency and labeled with [³⁵S]methionine, and detergent extracts were prepared for immunoprecipitation analysis. Control immunoprecipitations were used to identify the authentic *onc* gene products. For RSV-transformed CEC, serum from tumor-bearing rabbits was used (RD10; Fig. 2, lane A). Since the transforming proteins of Y73 and ESV represent fusion proteins between *yes* sequences and p19^{gag} sequences (18, 19, 26), antibodies directed against the p19^{gag} determinant were used to identify these proteins (Y73 and ESV; Fig. 2, lanes a). Adjacent to

FIG. 4. Immunofluorescent localization of P90^{src-yes} in Y73-transformed CEC. Antibodies specific for the peptide 1 region of pp60^{src} which were cross-reactive with the P90^{src-yes} protein of Y73 were utilized to examine the intracellular distribution of the *yes* gene product in Y73-transformed CEC. (A) Fluorescence staining pattern of P90^{src-yes}; (B) interference reflection image of the adhesion plaques under the same cells. The fluorescence patches observed in A coincide with the adhesion plaques identified in B (arrows). The fluorescent images in C and D have been enlarged to show more detail. Note the fluorescent staining of adhesion structures toward the periphery of the cell in C and the needle-like cell-cell junctions in D. In D the focal plane was slightly above the ventral substratum. Essentially all of the fluorescence except a nonspecific nucleolar stain could be eliminated by preincubation of the antibody with excess peptide 1 before immunolabeling (E). Bar in E, 20 μ m.

each control lane are the proteins immunoprecipitated by the anti-peptide antibody (Fig. 2, lanes b). Immunoprecipitations with anti-peptide antibody were also performed in the presence of excess soluble synthetic peptide, to demonstrate that the proteins were identified via authentic peptide-specific interactions (Fig. 2, lanes c).

The antibodies against this *src*-derived peptide identified proteins in the virus-infected cells which appeared to comigrate with the authentic *onc* proteins. The proteins were not present in uninfected CEC (CEC; lane b) or in immunopreci-

pitates prepared in the presence of excess peptide (lanes c) (Fig. 2). The anti-peptide antibody strongly recognized proteins of M_r 80,000 and M_r 60,000 present in ESV- and RD10-transformed CEC, respectively. However, in Y73-infected cells, only a weak band corresponding to the 90,000- M_r protein was detected. Later bleedings of the same peptide-immunized rabbit did yield antibodies which were somewhat better at identifying the 90,000- M_r protein, but the immunoprecipitations were never as efficient as those for the 80,000- and 60,000- M_r polypeptides found in ESV- and RD10-

infected CEC. This weaker association of anti-peptide antibody for the 90,000- M_r protein of Y73-infected CEC also appeared to parallel the immunoblotting experiments (Fig. 1).

To demonstrate that the proteins identified by the anti-peptide 1 antibodies were in fact the authentic *gag-yes* gene products, partial proteolytic peptide maps were compared. The comigrating [35]methionine-labeled proteins immunoprecipitated by anti-p19^{gag} serum or anti-peptide 1 antibodies were extracted from the dried polyacrylamide gel and treated with *S. aureus* V8 protease. Peptide fragments were

subsequently resolved by SDS-PAGE on gradient acrylamide gels. The results (Fig. 3) show a comparison of these peptides produced from the 90,000- and 80,000- M_r proteins detected with anti-p19^{gag} and anti-peptide 1 antibodies. Clearly, all of the major peptide fragments generated from the 90,000- M_r proteins are identical, and likewise all fragments from the 80,000- M_r proteins are of identical size. Therefore, antibodies to this *src*-derived synthetic peptide recognize the same *gag-yes* gene products of Y73- and ESV-infected CEC detectable with anti-p19^{gag} antibodies.

A comparison of the peptide fragments generated from

FIG. 5. Indirect immunofluorescence distribution of P80^{gag-yes} in CEC transformed by ESV. ESV-infected CEC were grown on ethanol-sterilized glass cover slips, fixed in methanol, and stained by indirect immunofluorescence, using antibodies specific for the peptide 1 region of pp60^{src} (17). A and B show the same field of cells examined by immunofluorescence and interference-reflection microscopy, respectively. Note the coincident distribution of fluorescence within adhesion plaque structures (thick arrows). The long, thin arrows point to a scroll-shaped, close-contact area which was observed in a few of the transformed cells. C and D present a more extensive view of the distribution of P80^{gag-yes} in these cells. Again all fluorescence except a nonspecific nucleolar stain could be abolished by prior incubation with excess peptide (E). Bar in E, 20 μ m.

FIG. 6. Localization by indirect immunofluorescence of pp60^{src} in RD10- and Prague C-transformed CEC. Cells grown on ethanol-sterilized glass cover slips were fixed in methanol and reacted with antibodies directed to the peptide 1 region of pp60^{src}, followed by reaction with rhodamine-conjugated goat anti-rabbit immunoglob-

ulin G. (A) RD10-transformed CEC; (B) Prague C-infected CEC; (C) uninfected CEC. Preincubation of antibody with excess peptide before immunolabeling abolishes the fluorescence staining patterns observed (not shown). Bar in C, 20 μ m.

digestion of P90^{gag-yes} and P80^{gag-yes} confirms earlier results that the *yes* gene products encoded by Y73 and ESV are related and display only a few differences (18). Upon *S. aureus* V8 protease digestion, each transforming protein produces several common characteristic peptides ranging in size from M_r 14,000 to M_r 40,000. The major differences occur in the high-molecular-mass peptide fragments (M_r 60,000 to M_r 70,000). Since the p19^{gag} moieties of these proteins show little variation (18), the majority of the differences probably occur in the *yes*-specific portions. These results are consistent with tryptic peptide analysis of the *gag-yes* proteins from Y73- and ESV-infected cells which have also shown differences in the *yes*-specific domains of these proteins (18).

The anti-peptide 1 antibodies, in addition to identifying the *yes* and *src* gene products upon immune precipitation analysis, also identified other prominent polypeptides in the M_r 50,000 to M_r 55,000 range (Fig. 2). These proteins were found equally in normal or transformed CEC. The identity of these proteins is unknown at this time, but they appear to be peptide specific and therefore may represent related normal gene products. Alternatively, their recognition may be the consequence of fortuitous cross-reactions commonly observed when anti-peptide antibodies are used (17, 24, 30, 47). Whatever their nature, they were not detected by immune blotting techniques (Fig. 1) or by indirect immunofluorescence microscopy (see Fig. 6C).

Immunofluorescence localization of the *gag-yes* gene products of Y73- and ESV-infected CEC. Having shown that the anti-peptide antibodies identified the authentic *gag-yes* proteins, we next tested for the intracellular expression of the *yes* gene products by indirect immunofluorescence microscopy. Y73- and ESV-transformed CEC were thus fixed in methanol and stained sequentially with the cross-reactive anti-peptide antibody and rhodamine-labeled goat anti-rabbit immunoglobulin G. Figures 4 and 5 show the results of these experiments. The peptide antibodies stained both Y73- and ESV-transformed cells. A rather uniform fluorescence was seen in what appeared to be the cytoplasm of the cells, and a more dramatic punctate stain was seen at the ventral cell surface. Interference-reflection microscopy indicated that these fluorescent patches on the ventral surface corresponded precisely to the adhesion plaques or focal-contact areas of the cell (A and B of Fig. 4 and 5). In addition, numerous interlacing patches of fluorescence appeared between cells and probably represent complex networks of cell-cell junctions. The Y73-transformed cells showed that most clearly (Fig. 4D). Junctions which stained in the ESV-transformed CEC were morphologically dissimilar, displaying an almost fiber-like appearance (Fig. 5C and D). These fluorescence patterns were not observed in uninfected CEC stained with the anti-peptide antibody (Fig. 6C) and were completely abolished (except for a nonspecific nucleolar stain) in Y73- and ESV-transformed cells by incubation of the antibody with excess peptide before the immunolabeling (E of Fig. 4 and 5).

The patterns of fluorescence observed in the Y73- and ESV-infected cells are very similar to those produced when RSV-transformed cells are stained with pp60^{src}-specific antibodies (17, 36, 37, 39). To illustrate this point, we have

included the fluorescence patterns obtained when the Schmidt-Ruppin D and Prague C strains of RSV-infected CEC were stained with the same anti-peptide antibodies specific for the *src* protein (Fig. 6A and B). The slight differences observed in the adhesion structures between these infected cells appear to be strain specific and probably are a consequence of the morphological changes induced by each virus. Note that the locations of the *src* proteins at adhesion plaques and cell-cell junctions are remarkably similar to the locations of the *gag-yes* gene products found in Y73- and ESV-transformed CEC. These results demonstrate that the *yes* proteins accumulate at adhesion plaques and cell-cell junctions of Y73- and ESV-transformed cells just as pp60^{src} does in RSV-transformed cells.

DISCUSSION

In this report, we have shown that antibodies specific for a synthetic peptide comprising amino acid residues 498 through 512 of the Prague C *src* protein cross-react with the corresponding regions of the *gag-yes* gene products of Y73 and ESV. This cross-reactivity was demonstrated by immune blotting techniques and by immune precipitation analysis. The identity of the immune-precipitated proteins as *yes* gene products was confirmed by partial proteolytic peptide mapping. Studies with this site-specific antibody indicate that when immunocomplexed with the *gag-yes* gene products the ability of this transforming protein to phosphorylate substrate peptides at tyrosine residues was diminished significantly (unpublished data). However, the inhibition was not as extensive as that observed when pp60^{src} was assayed in a similar manner (17).

The availability of antibodies cross-reactive with the *yes* gene products afforded us a unique opportunity to examine the intracellular distribution of these proteins in Y73- and ESV-infected CEC. Immunofluorescence analysis with the anti-peptide antibodies revealed that the *yes* transformation-specific polypeptides were located at several cytoplasmic sites. A detectable amount appeared to reside in the cytoplasm of the cell, producing a diffuse cellular fluorescence. However, the *gag-yes* polypeptides were also detected within adhesion plaques and cell-cell junctions of Y73- and ESV-transformed CEC.

The cellular locations of the *gag-yes* transforming proteins are remarkably similar to those found for pp60^{src} in RSV-transformed cells (5, 11, 36, 37) and for p120^{gag-*abl*} in Abelson murine leukemia virus-transformed fibroblasts (38). At the immunofluorescence level, these transforming proteins show qualitatively similar locations within adhesion plaques (36, 37). Cell fractionation studies have also revealed major similarities, demonstrating that these gene products are found in membrane as well as cytoskeleton fractions (2, 5, 11, 48; unpublished data). These characteristic locations would tend to suggest that *src*, *yes*, and *abl* belong to a common group of transforming proteins that are functionally related and may effect cellular transformation by similar mechanisms.

An important question regarding the adhesion plaque location of these *onc* proteins concerns the functional significance of this localization. As with any of the other cellular locations, it is not entirely clear what the *onc* proteins are doing at those sites and how that relates to transformation. Since cellular transformation by the *src*, *yes*, and *abl* gene products most likely proceeds through phosphorylation of host cell proteins on tyrosine amino acids (22, 42), it is possible that substrates for the transformation process reside within the adhesion plaques. Indeed, vinculin, a cytoskele-

tal-associated adhesion plaque protein (6, 16), has been identified as a substrate for the tyrosine kinases encoded by *src*, *yes*, and *abl* (41). Although the significance of this phosphorylation event is not understood, this substrate relationship is consistent with the location of these transforming proteins, as other *onc*-encoded tyrosine kinases not associated with the adhesion plaques fail to phosphorylate vinculin (37, 41). In addition, these *onc* gene products may function as cytostructural elements within the adhesion plaques disrupting some aspect of normal cellular function.

Regardless of the nature of these events, it is clear that an adhesion plaque localization is not essential for two important markers of transformation: anchorage independent growth and tumor formation. Several RSV mutants have been described that lack adhesion plaque pp60^{src} yet induce tumors in animals and cause cells to grow in soft agar (39). Also, the related *fps* and *fes* gene products not found in adhesion plaques are competent transforming proteins (14, 37). Studies with partial transformation mutants of RSV have suggested, however, that the localization of pp60^{src} in adhesion plaques is correlated with the decreased expression of fibronectin in the extracellular matrix (39). It is not known how these events are molecularly related; however, fibronectin is associated with both stress fibers and adhesion plaques and exhibits a close transmembrane relationship with adhesion plaque proteins (7, 23, 45). Presumably then, an *onc* protein in the adhesion plaques could affect fibronectin expression, and we are currently exploring this possibility.

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