Detection of the v-abl Gene Product at Cell-Substratum Contact Sites in Abelson Murine Leukemia Virus-Transformed Fibroblasts

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Monoclonal antibodies to the p15 and p12 gag proteins were used to detect the P120\textsuperscript{gag-abl} transforming protein of Abelson murine leukemia virus in nonproductively transformed normal rat kidney fibroblasts. The results demonstrate that, in addition to the prominent plasma membrane location, P120\textsuperscript{gag-abl} was associated with points of adhesion between the cell and the substratum. The localization of P120\textsuperscript{gag-abl} was qualitatively similar to that reported for pp60\textsuperscript{src} in the same normal rat kidney fibroblast cells and suggests that these transforming proteins may share some common transformation features.

Abelson murine leukemia virus (A-MuLV) is a defective oncornavirus that arose through the recombination of a nondefective MuLV with cellular sequences of a prednisolone-treated BALB/c mouse (7, 19). The acquired cellular sequences encode a transforming function that permits A-MuLV to transform both fibroblast and lymphoid cells. This transforming gene is termed v-abl and directs the synthesis of a 120,000-dalton phosphoprotein containing MuLV gag proteins (p15, p12, and a portion of p30) at the amino-terminal end (14, 23–25). The P120\textsuperscript{gag-abl} also contains a tyrosine-specific kinase activity (18, 21, 23) that has been mapped to a 1.2-kilobase segment of the v-abl gene. This region is essential for fibroblast transformation and shows a high degree of homology with the kinase domain of pp60\textsuperscript{src} (13, 14). The v-abl transforming protein, P120\textsuperscript{gag-abl}, has been detected in the plasma membrane of A-MuLV-transformed lymphoid cells (24). The majority of the sequence probably resides on the cytoplasmic side of the membrane; however, portions of the gag sequence are detectable on the cell surface (L. Schiff-Maker and N. Rosenberg, personal communication). The cell plasma membrane P120\textsuperscript{gag-abl} is also associated with the detergent insoluble matrix (1). This overall localization is analogous to that reported for pp60\textsuperscript{src} in Rous sarcoma virus (RSV)-transformed fibroblasts, except that the RSV transforming protein is probably not exposed on the cell surface (15).

These data suggest that, although the ontogeny of A-MuLV and RSV differ, similarities may exist in their structure and mechanism of fibroblast transformation. In addition to the localizations described for P120\textsuperscript{gag-abl}, pp60\textsuperscript{src} is also found at specialized regions of the ventral cell membrane called adhesion plaques and close-contact areas (15). These regions serve as anchor points between the cell and the substratum and represent the termini of microfilament bundles at the cell membrane in untransformed cells (8, 9, 22). To determine whether the similarities between the v-abl gene product and the v-src gene product localization included the substratum contact sites, we examined by indirect immunofluorescence the cellular distribution of P120\textsuperscript{gag-abl} in normal rat kidney (NRK) cells nonproductively transformed with A-MuLV (Ab-NRK) cells (12, 25). For these studies we used a mixture of monoclonal antibodies to the MuLV p15 and p12 gag proteins (from B. Chesebro, Rocky Mountain Laboratories, Hamilton, Mont.). Both of these determinants are contained in the P120\textsuperscript{gag-abl} protein. The ability of these antibodies to immunoprecipitate P120\textsuperscript{gag-abl} from the Ab-NRK cells is shown in Fig. 1. A single prominent band of 120 kilodaltons is recognized by these antibodies. Other protein bands are also precipitated by the monoclonal antibodies, but these are nonspecific and appear with the control immune precipitation as well (Fig. 1, lane B). These results demonstrate the specificity of the monoclonal antibodies for P120\textsuperscript{gag-abl} and confirm that the Ab-NRK cells are, in fact, nonproductively transformed and lack detectable p15- or p12-containing gag proteins other than P120\textsuperscript{gag-abl}.

The immune precipitation results indicate that the P120\textsuperscript{gag-abl} protein will be the principal (if not only) protein detected in immunofluorescence assays in which the p15 and p12 monoclonal antibodies are used. The affinity-purified rhodamine-labeled secondary antibody used in these assays did not stain the Ab-NRK cells when tested alone. The results of this assay on the Ab-NRK cells are presented in Fig. 2 and 3. In general, the strongest fluorescence was seen at the cell plasma membrane (Fig. 2A and 2B). This was evident by the very intense fluorescence that outlined the cellular periphery at each focal plane. Cell-cell junctions also stained very strongly with the anti p15 and anti-p12 monoclonal antibodies. These monoclonal antibodies did not stain uninfected NRK cells (data not shown). The plasma membrane fluorescence appeared rather uniform (Fig. 2B), except at the ventral cell surface at which a distinct nonrandom distribution was seen (Fig. 2C). The fluorescence at the ventral cell surface was organized into circular patches that matched the form and location of substratum contact areas detected by interference-reflection microscopy in the same cells (Fig. 2D). The relative darkness of these patches suggests that they represent close-contact areas (9).

Not all close-contact areas of the cell contained fluorescence. The results in Fig. 3A and B demonstrate that the Ab-NRK cells contained many close-contact areas and only a subset, usually in a well-defined circular configuration, exhibited strong fluorescence. Some close-contact regions,

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in fact, contained slightly less fluorescence than the surrounding areas. A few Ab-NRK cells contained more scattered punctate fluorescence rather than the circular patches at the ventral cell surface (Fig. 3C and D). These also corresponded to close-contact regions. Some fluorescence was evident in focal contact sites which appear to be darkest by interference-reflection microscopy; however, very few focal contacts were seen between the Ab-NRK cells and the substrate. These fluorescence patterns at the ventral cell surface are somewhat different from those seen for pp60
c in RSV-transformed chicken fibroblasts; however, in RSV transformed NRK cells very similar patterns of pp60
c-containing close-contact areas have been reported (2, 11). Therefore, both P120
ab and pp60
c localize to similar substratum-contact structures in NRK cells transformed by A-MuLV and RSV, respectively.

Another indication that pp60
 and P120
 may share intracellular locations comes from double-label immunofluorescence experiments comparing the location of P120
 with the cytoskeletal protein vinculin. Guinea pig antibodies were used to localize vinculin (16, 20), and the specificity of that antiserum for vinculin is demonstrated by the results of an immune precipitation experiment presented in Fig. 1. The immunofluorescence results in Fig. 4 demonstrate that again, like pp60
 (16, 20), P120
ab colocalizes with the cytoskeletal protein vinculin. The strong plasma membrane fluorescence of P120
ab was not matched by an equivalent concentration of vinculin in the membrane (compare Fig. 4A with B); however, junctions between cells did contain both P120
ab and vinculin (Fig. 4C and D). Likewise, the circular close-contact patches on the ventral cell surface all showed a coincidence of fluorescence for both P120
ab and vinculin. These results indicate that the RSV and A-MuLV-transforming proteins reside at similar cellular locations and each colocalize with the cytoskeletal protein vinculin within cell-cell junctions and cell-substratum contact sites. This is consistent with the observation that each transforming protein is known to cause the increased phosphorylation of vinculin on a tyrosine residue (17); however, the exact function of that phosphorylation event is yet unknown.

In addition to pp60
 and P120
ab, at least one other viral onc gene product has been detected at cell-substratum contact sites, and that is the v-yes gene product (4). Both Esh sarcoma virus and Yamaguchi 73 sarcoma virus contain the yes-transforming gene and express the protein as an amino-terminal gag-linked product (5, 6). The protein product is termed p80
 for Esh sarcoma virus and p90
 for Yamaguchi 73 sarcoma virus, and each of these transforming proteins is related to pp60
 (10) and has been detected in cell-substratum adhesion areas of the transformed cells (4). Cell fractionation studies indicated that the intracellular localization was analogous to that seen for pp60
 in RSV-transformed cells (L. Gentry and L. Rohrschneider, manuscript in preparation). Therefore, the intracellular localization studies define a class of transforming proteins found principally on the cytoplasmic face of the plasma membrane and concentrated at specialized regions of the membrane known as adhesion plaques and close-contact areas. Viral gene products synthesized from v-src, v-abl, and v-yes belong to this class of transforming proteins and probably effect at least some parameters of fibroblast transformation by very similar mechanisms.

Any class of transforming proteins is defined both by common characteristics among members of that class and also by differences with other nonmembers. In this regard it is important that the v-onc gene products from v-fps and v-fes have not been detected in adhesion areas of transformed cells, even though each contains a tyrosine kinase activity and has shown at least some plasma membrane affiliation (3, 15). In addition, many other distinct onc gene products have been characterized, but no others have been detected in cell-substratum adhesion areas. This argues that the cell-substratum classification defines a unique group of transforming proteins.

A-MuLV unlike RSV or Yamaguchi 73 sarcoma virus, transforms lymphoid cells in addition to fibroblasts, yet the lymphoid cells lack adhesion plaques. This suggests that A-MuLV transforms these two cell types by different mechanisms, and this view is supported by the finding that some sequences of the gag-abl gene product are necessary for lymphoid but not for fibroblast transformation (13).

Within fibroblasts, however, the location of three distinct viral transforming proteins in related intracellular target sites suggests that they may be performing some common task at these locations. The exact function of these onc gene products at these sites is not well understood, but results with partial transformation mutants in the src gene of RSV suggest that the localization of pp60
 in adhesion plaques may be related to the decreased expression of fibronectin in the extracellular matrix (16). The identification of abl and yes as members of this same class of transforming proteins will allow the further testing of the relationship between adhesion plaques and fibronectin expression in these viral systems.
FIG. 2. Localization of the Abelson virus transforming protein (P120<sup>env-abl</sup>) by indirect immunofluorescence. A mixture of monoclonal antibodies to the p15 and p12 gag proteins (each diluted 1:100) of MuLV were used to detect P120<sup>env-abl</sup> in NRK cells nonproductively transformed with A-MuLV. Cells were fixed in 4% formaldehyde as described previously (20), and the secondary rhodamine antibody was affinity purified. The uniform plasma membrane fluorescence is shown in panels A and B. Panels C and D show the same field of cells as in panel B, but in C the focal plane is near the cell-substratum junction and in D an interference-reflection image of these cells is shown. The darker close-contact areas (arrows) match the location of the P120<sup>env-abl</sup>-specific fluorescence at the ventral surface. Bar, 20 μm.
FIG. 3. Distribution of P120gag-abl-containing close-contact areas in Ab-NRK cells. Indirect immunofluorescence was used to stain the cells with monoclonal anti-p15 and anti-p12 antibodies, and panels A and C show the various fluorescence patterns on the ventral cell surface. The corresponding substratum attachment sites are shown in the respective interference-reflection images in panels B and D. Bar, 20 μm.
FIG. 4. Double fluorescence showing the location of P120 in Ab-NRK cells with respect to vinculin. Ab-NRK cells were fixed in 4% formaldehyde and stained with mouse monoclonal anti-p15/p12 antibodies (diluted 1:100), followed by an affinity-purified rhodamine-labeled second antibody (panels A, C, E, G, and I). Guinea pig antibodies to vinculin (diluted 1:20) were visualized after staining with an affinity-purified fluorescein-labeled second antibody (20) (panels B, D, F, H, and J.) Bar, 20 μm.
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