Mutations of Ros Differentially Effecting Signal Transduction Pathways Leading to Cell Growth Versus Transformation*

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The signaling functions of the oncogenic protein-tyrosine kinase v-Ros were studied by systematically mutating the tyrosine residues in its cytoplasmic domain. The carboxyl mutation of Tyr-564 produces the most pronounced inhibitory effect on v-Ros autophosphorylation and interaction with phospholipase Cγ. A cluster of 3 tyrosine residues, Tyr-414, Tyr-418, and Tyr-419, within the PTK domain of v-Ros plays an important role in modulating its kinase activity. The mutant F419 and the mutant DI, deleting 6-amino acids near the catalytic loop, retain wild type protein tyrosine kinase and mitogenic activities, but have dramatically reduced oncogenicity. Both mutant proteins are able to phosphorylate or activate components in the Ras/microtubule-associated protein kinase signaling pathway. However, F419 mutant protein is unable to phosphorylate insulin receptor substrate 1 (IRS-1) or promote association of IRS-1 with phosphatidylinositol 3-kinase. This tyrosine residue in the context of the NDY motif may define a novel recognition site for IRS-1. Both F419 and DI mutants display impaired ability to induce tyrosine phosphorylation of a series of cytoskeletal and cell-cell interacting proteins. Thus the F419 and DI mutations define v-Ros sequences important for cytoskeleton signaling, the impairment of which correlates with the reduced cell transforming ability.

Autophosphorylation of receptor protein-tyrosine kinase (RPTKs) is one of the earliest detectable events in response to binding of their cognate ligand. This step has two pivotal roles, one is to activate the intrinsic PTK activity of the receptor and the other is to create site(s) for substrate protein interaction, both of which are essential in initiating pathways of signal transduction mediated by the receptor PTKs (1).

The oncogene v-ras encoded by the avian sarcoma virus UR2 is a truncated receptor-like PTK (2). The proto-oncogene c-ras codes for a receptor PTK with an extended extracellular domain (3–5). v-ras differs from c-ras in that all but 21 nucleotides that code for the extracellular domain of c-ras are truncated and the remaining gene is fused in frame at its 5′ end to viral gag sequences. As a result, v-ras codes for a gag-Ros transmembrane fusion protein of 68 kilodaltons called P68 gag-ras that is constitutively active (6). In addition, there are minor alterations in the transmembrane (TM) domain and carboxyl region of v-Ros in comparison with c-Ras (7). The kinase domain of Ros is highly homologous with the kinase domains of insulin receptor (IR) and insulin like growth factor I receptor (IGFR) (4). The PTK domain of Ros has two distinct sequence features in comparison with members of the Src family and other receptor PTKs (4). One is a cluster of 3 tyrosine residues consisting of a single tyrosine followed by twin tyrosines 4 residues downstream. The other is a 6-amino acid insertion 3 amino acids downstream of the predicted PTK catalytic loop defined from the crystal structure of the kinase domain of IR (8). These characteristic sequence features of the v-Ros PTK domain are also shared by its closely related RPTKs, IR, and IGFR (4).

Mutation of the twin tyrosines or all 3 tyrosines in IR or IGFR resulted in reduced tyrosine phosphorylation of IRS-1 and activation of PI3 kinase (9–12). The importance of these tyrosine residues was further suggested by the crystal structure of the PTK domain of IR (8). These characteristic sequence features of the v-Ros PTK domain are also shared by its closely related RPTKs, IR, and IGFR (4). Mutation of the twin tyrosines or all 3 tyrosines in IR or IGFR resulted in reduced tyrosine phosphorylation of IRS-1 and activation of PI3 kinase (9–12). The importance of these tyrosine residues was further suggested by the crystal structure of the PTK domain of IR (8). These characteristic sequence features of the v-Ros PTK domain are also shared by its closely related RPTKs, IR, and IGFR (4).

The interaction between an activated receptor PTK and some of its substrates is mediated by a family of src-homology domain 2 or 3 (SH2/SH3)-containing proteins (13). Although RPTKs in general do not contain SH2 or SH3 motifs, most of their downstream signaling proteins thus far identified contain the SH2 and/or SH3 domains (1, 13, 14). Their association with RPTKs is facilitated by the tyrosine phosphorylation of specific sites on the receptors in response to the binding of ligands to receptors’ extracellular domains. For example, phosphorylation of specific tyrosine residues on platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) receptors is required for binding of several of their downstream signaling molecules including GAP, P13 kinase, and PLCγ (reviewed in Cantley (1)). In contrast, IR and IGFR bind IRS-1 and Shc at an NPXY motif in the receptors’ juxtamembrane domains (15–17). The Shc- and Grb2-mediated activation of Ras/MAP kinase pathway is involved in the mitogenic signaling by growth factors and cytokines (1, 18). Other signaling pathways involving PLCγ, IRS-1, and P13 kinase have also begun to be elucidated. IRS-1 serves as the major adaptor for recruiting other signaling molecules upon stimulation of IR by insulin and most likely of IGFR by IGF-1 (19). Tyrosine-phosphorylated IRS-1 is capable of binding to and activating P13 kinase (20). Mutation of the IRS-1 recognition motif NPXY diminishes insulin-induced tyrosine phosphorylation of IRS-1 and activation of P13 kinase (17).

Reorganization of cytoskeletal structure and alteration of...
membrane properties represents an important component of the process of malignant cell transformation. However, the processes involved in cytoskeletal signaling remained unclear. Ever increasing attention has been directed toward understanding the transmission of signals from the interaction of integrins and their extracellular matrix ligands, particularly at adhesion plaques (21). The focal adhesion protein-tyrosine kinase, pp125 FAK, colocalizes with the integrin receptor in cellular focal adhesions and is activated upon engagement of the integrin receptor with its ligand or upon Src transformation (22, 23). Cadherin and its associated catenins constitute other important molecules in cytoskeleton-mediated signaling. Cadherins mediate Ca\(^{2+}\)-dependent cell-cell adhesion via homophilic interaction of these cell surface molecules (24).

If specific tyrosine residues of an oncogenic RPTK interact with different intermediate substrates leading to distinctive signal transduction pathways, it may, therefore, be possible to impair specific pathways responsible for different biological effects by mutating those substrates interacting tyrosine residues. To explore the functional role of specific tyrosine residues of the oncogenic v-Ros, particularly with respect to their roles in mitogenic versus transforming activity, we have systematically mutated all of the tyrosine residues in the cytoplasmic domain of v-Ros. In addition, we have also removed the 6-amino acid insertion in the v-Ros catalytic domain. These mutants allowed us to identify the tyrosine residues of v-Ros important for regulating PTK activity and interaction with specific substrates. They also allow the differentiation of signaling pathways leading to mitogenicity versus morphological transformation and anchorage-independent growth.

**EXPERIMENTAL PROCEDURES**

**Cells and Viruses**—Chicken embryo fibroblasts (CEF) were prepared from 11-day-old embryos and maintained according to the previously published procedure (6, 7, 25). The CEF were maintained as monolayer culture except in the colony formation assay, where they were suspended in the agar medium. Unless otherwise specified, CEF were maintained in F10 medium containing 5% calf serum and 1% chick serum (6, 7, 25). Molecurally cloned avian sarcoma virus UR2 and its helper virus UR2AV have been described (2, 7).

**Biological Assay**—Cell transformation was monitored by morphological change and anchorage-independent growth as described previously (7, 11, 25, 26). For colony formation in methyl cellulose, medium containing 1.3% pure methyl cellulose was used for the top layer on the same platform of bottom layer agar.

**Antibodies**—Anti-Ros and anti-IRS-1 antibodies were made in our laboratory and have been described (6, 11). Anti-MAP kinase polyclonal antibody TR10 was a gift from M. Weber. Anti-annexin II polyclonal antibody was a gift from T. Hunter. Monoclonal antibodies for cortactin, tensin, and CAS were gifts from T. Parsons and A. Bouton. Anti-p190 Rho/GAP polyclonal antibody was a gift from S. Parsons. Anti-Grb2 polyclonal antibody was a gift from B. Mayer. Antibodies for FAK, β- and γ-catenin and an alkaline phosphatase-coupled anti-phosphotyrosine (Tyr(P)) antibody RC20 were purchased from Transduction Lab. Antibodies for PLC\(_\gamma\) and PI3 kinase were purchased from Upstate Biotechnology Inc. Anti-β1-integrin and anti-N-cadherin were purchased from Sigma and Zymed Laboratories Inc., respectively.

**Construction of pBUR2**—The plasmid pUR2H1 contains the full-length UR2 genome cloned at the HindIII site of pBR322 (2, 7). The UR2 genome in this plasmid is permuted with respect to the HindIII site at the 3' region of the UR2 genome. To facilitate DNA transfection and expression of viral genes, a nonpermuted UR2 plasmid was reconstructed. This was done by isolating the 2.3-kilobase pair PstI to NruI fragment containing the entire gag-Ros coding sequence from pUR2H1 and using it to replace the gag-IGFII sequence in a nonpermuted viral plasmid pHUG1FII constructed previously (26). The resulting plasmid was named pUR2 which served as the parental plasmid for mutant construction.

**Construction of Mutants**—The mutants were engineered by a M13-mediated mutagenesis kit (Promega) described previously (11) or polymerase chain reaction using oligonucleotides containing specific base changes. The sites of mutagenesis was sequenced to confirm the mutation.

**Construction of Mutants of the UR2 v-Ros**—The UR2 encoded P68\(^{\text{gag-ros}}\) is shown with different structural domains indicated. The numbers correspond to the amino acid positions (2). TM1 deletes a 3-amino acid insertion in the TM domain of UR2 P68 and has been described (25). The kinase activity and relative mitogenic and cell transforming activities of each mutant are indicated. The + means a decrease of activity to different extents as detailed in the text. The kinase activity of DI protein is inactive in vitro, but is active in vivo.

![Fig. 1. Mutants of the UR2 v-Ros.](image)

**RESULTS**

**Construction of v-Ros Site-specific Mutants**—All the tyrosine residues in the cytoplasmic domain of P68\(^{\text{gag-ros}}\) were converted individually, or in combination to phenylalanines (Fig. 1). The viruses encoding the mutant v-Ros proteins were named according to the positions of the mutated tyrosine residues (2) with the exception of double and triple mutants F2, F3, and F4. In addition, a unique 6-amino acid insertion located 3 amino acids downstream of the predicted catalytic loop was deleted to generate the mutant DI.

**Biological Properties of the v-Ros Mutants**—Two or three independent clones of each mutant v-Ros expression plasmid were co-transfected with UR2AV helper virus DNA into CEF to assess their biological activity. Most mutants showed both mitogenic and transforming activity indistinguishable from that of parental UR2 (Fig. 1). However, the mutant F2 containing the Y418F and Y419F double mutation had only a residual transforming activity. The triple mutant F3 had undetectable mitogenic and transforming activity. Mutants F419 and DI
shown dramatically reduced transforming ability as reflected in morphological alteration of the transfected cells (data not shown) and ability of the cells to form colonies in soft agar (Fig. 2). However, both mutants displayed wild-type mitogenic activity when infected cells were maintained as monolayer culture in either 5% or 0.5% serum-containing medium (Fig. 2).

The double mutant F4 displayed a further reduced transforming capability in comparison with F419 (data not shown) despite the fact that a single mutation of Tyr-564 produced no detectable difference from UR2. This result indicates that the effect of the Tyr-564 mutation, which impairs the interaction of v-Ros with PLCγ (see below), can only be detected in the background of a weak transforming protein such as F419.

Tumorigenicity of F419, DI, and the parental UR2 was compared. The result (Table I) shows that both mutants have dramatically reduced tumorigenicity. In fact, none of the chicks succumbed to the tumors induced by the mutants during the 1-month observation period (Table I) and even in an extended period of 2 months in a separate experiment (data not shown). This result is consistent with the impaired ability of the two mutants in promoting anchorage independent growth, but does not correspond to their mitogenic activity in monolayer cultures.

PTK Activity of the Mutant v-Ros Proteins—The kinase activities of mutant v-Ros proteins were analyzed by in vitro auto- and trans-phosphorylation, in vivo tyrosine phosphorylation of the Ros proteins, as well as their ability to phosphorylate cellular proteins (Fig. 3). Mutation of both Tyr-418 and Tyr-419 (F2) resulted in a greatly reduced kinase activity, particularly its in vivo autophosphorylation and ability to phosphorylate cellular substrates. Only a residual in vitro kinase activity and no detectable in vivo kinase activity was detected for the F3 protein containing the triple mutation of Y414F, Y418F, and Y419F. These results suggest that these residues, particularly Tyr-418 and Tyr-419, play a major role in modulating the PTK activity of v-Ros. Surprisingly, despite a lack of detectable activity in the in vitro autophosphorylation (Fig. 3A) and phosphorylation of the exogenously added substrate, enolase (Fig. 3B), the DI protein, appears to have wild-type kinase activity intracellularly as reflected in its in vivo autophosphorylation (Fig. 3A) and phosphorylation of cellular proteins (Fig. 3C). The apparent paradox of the in vitro and in vivo kinase activity of the DI protein will be discussed. As expected, the DI protein exhibited a slightly faster mobility in the SDS-gel when detected with anti-Ros antibody (Fig. 3, A and B, bottom panels). The F564 protein containing the carboxyl-terminal tyrosine to phenylalanine mutation appears to be underphosphorylated and to have a faster mobility when detected in Western blotting with anti-Tyr(P) antibody (Fig. 3 A and C). However, F564 protein has no detectable decrease in in vitro kinase activity, although its phosphorylated products also appear to be down-shifted in gel mobility in comparison with those of the parental v-Ros (Fig. 3A, top panel and other data not shown). The mobility downshifting of in vivo phosphorylated protein was also apparent for the F4 protein containing the Y419F and Y564F double mutation (Fig. 3A). Again, no detectable difference of in vitro or in vivo kinase activity was observed for the F4 protein. The expression levels of the various mutant v-Ros proteins in transfected cells was comparable, with the exception of F2 and F3, where the expression levels were 4–5-fold lower. More protein lysates from F2- and F3-infected cells were needed in order to normalize the Ros protein in the experiments shown in Fig. 3. Except for the mutants described above, no effect on the in vitro or in vivo PTK activity was observed for the rest of mutants.

Phosphorylation and Activation of Specific Signaling Proteins—To identify the tyrosine site(s) required for interaction of v-Ros with specific substrates, the mutant proteins were compared for their ability to phosphorylate or activate various signaling molecules. Our results (Fig. 4) show that with the exception of the kinase-defective mutants, F2 and F3, all the mutants were capable of inducing tyrosine phosphorylation of Shc and 5C2, an 88-kDa cellular protein we previously identified to be a prominent substrate of v-Ros (25). In addition to F2 and F3, the F564 protein was also unable to cause tyrosine phosphorylation of PLCγ despite its wild-type kinase activity level. As expected this is also true for F4. Therefore, Tyr-564 may serve as PLCγ recognition site directly or be critical for the formation of a PLCγ site elsewhere in the protein. Our obser-
vation that F564 has a wild-type transforming activity level indicates that phosphorylation of PLCγ is not essential for this activity. However, since mutation of Tyr-564 in the background of F419 further reduces its transforming ability, it is likely that phosphorylation of PLCγ may enhance cellular transformation by v-Ros.

**Activation of MAP Kinase**—To further explore the biochemical basis for the reduced transforming activity of F419 and DI, we examined the ability of these mutants to activate MAP kinase, a downstream effector of the Ras signaling pathway. Consistent with their ability to induce tyrosine phosphorylation of Shc, both the F419 and DI proteins are able to promote association of Grb2 with three distinct tyrosine phosphorylated proteins with gel mobilities corresponding to those of Shc proteins (46, 52, and 66 kDa). The mutants also activate MAP kinase as efficiently as the wild type v-Ros (Fig. 5). This is consistent with the observed mitogenic activity of F419 and DI mutants.

**IRS-1:Phosphorylation and Association with PI3 Kinase**—We next examined the signaling molecules IRS-1 and PI3 kinase, which were previously shown to be phosphorylated and activated by v-Ros (25). Our result shows that mutation of Tyr-419 specifically decreases the ability of v-Ros to cause tyrosine phosphorylation of IRS-1 (Fig. 6A). No such effect was observed for any of the other mutants, with the exception of the kinase inactive ones (data not shown). Consistent with the reduced phosphorylation of IRS-1, F419 protein also failed to promote association of PI3 kinase with IRS-1 as reflected in the in vitro PI3 kinase assay (Fig. 6B). This observation was confirmed by reciprocal immunoprecipitation and Western blot using anti-p85 and anti-IRS-1 antibodies to detect their physical interaction. Association of IRS-1 with the 85 kDa subunit of PI3 kinase was observed in DI- and UR2-, but not in F419-infected cells (data not shown). These results indicate that phosphorylation of IRS-1 and activation of PI3 kinase are not essential for promoting the growth of cells on monolayer culture.

**Effect of v-Ros Mutations on Cytoskeleton-associated Proteins**—Reorganization of cytoskeletal structure is intimately related to morphological transformation. The effect of cytoskeletal alteration on cell-to-cell and cell-to-matrix interactions could play an important role in the growth of cells in agar. We compared v-Ros and its mutant proteins for their ability to cause tyrosine phosphorylation and interaction of a series of cytoskeletal proteins involved in the formation of focal adhesion plaques and cell-cell interactions. No difference was observed among F419, DI, and UR2 PTKs in causing tyrosine phosphorylation of FAK, cortactin, paxillin, CAS (21), a Crk-associated protein, and annexin II (27), a cytoskeleton-associated Ca²⁺-dependent phospholipid binding protein (Fig. 7).
Greatly increased tyrosine phosphorylation of annexin II, cortactin, and paxillin was observed in cells infected with wild-type v-Ros and the mutants. In contrast, the increase in tyrosine phosphorylation of FAK and CAS is only about 2-fold above the control CEF. We observed a tyrosine-phosphorylated 190-kDa protein associated with β1 integrin in UR2-, but much less in F419- and DI-infected cells. However, no significant tyrosine phosphorylation of integrin was detected (Fig. 8). In addition, tensin was more abundantly phosphorylated in UR2- than in the mutant-infected cells. Similarly, the p190 Rho/GAP was more highly phosphorylated in UR2- than in the mutant-infected cells, particularly in comparison with the F419 cells.

For the proteins involved in cell-cell interaction, we observed significantly more abundant tyrosine phosphorylation of β- and γ-catenin, as well as more association between cadherin and β-catenin, in UR2-, than in F419- and DI-infected cells (Fig. 9). Association of β-catenin with cadherin was confirmed by Western blotting of the anti-cadherin immunoprecipitates with an anti-β-catenin antibody (data not shown). Neither α-catenin nor cadherin was significantly tyrosine phosphorylated by any of our v-Ros proteins. These results indicate that F419 and DI proteins are either incapable or less effective in promoting tyrosine phosphorylation and interaction between various proteins involved in the formation of focal adhesion plaques and cell-cell interactions.

**DISCUSSION**

This study identifies several sequences in v-Ros that play important roles in regulating PTK activity and cell transform-
ing functions. The kinase positive and transformation-negative (K+T-) or attenuated (K+T) mutants are useful in that they may allow identification of the signaling components essential for cell growth and transformation. We previously generated a mutant called TM1 by deleting 3 amino acids in the TM domain of v-Ros (Fig. 1) (25). This mutation has no effect on the kinase activity, but impairs both the mitogenic and transforming activities of v-Ros. The kinase-positive, mitogenicity positive but transformation-defective mutants (K+M+T-), represented by F419 and DI in this study, are thus particularly useful since they are selectively impaired in signaling pathways leading to distinct biological properties. Our results with F419 and DI indicate that activation of the Ras/MAP kinase pathway is not sufficient for cell transformation. Our data also suggest that the cytoskeletal protein-mediated signaling may be more closely related to morphological transformation and anchorage independent growth of cells. Numerous site-specific deletion mutants within the N-terminal region of v-Src have been reported to affect its ability to induce morphological transformation and promote colony formation in soft agar (28). However, it is not clear how those mutants affect the growth of cells on monolayer culture. Our F419 and DI mutants resemble a recently reported tyrosine 807 mutant in v-fms, which retains mitogenic but not morphological transforming activity (29).

Our data show that all the tyrosine residues in the cytoplasmic domain of gag-Ros, except for Tyr-419 and Tyr-564 are not individually required for Ros's biochemical and biological properties. Our finding of the effect of the triple tyrosine mutation cluster on v-Ros PTK activity is consistent with those of other RPTKs including IR (9) and IGFR (10, 11), which also contain such a tyrosine cluster. However, mutation of Tyr-418 of v-Ros, which corresponds to Tyr-1162 of IR, suggested to be the “gate-keeper” of its catalytic site (8), did not yield any detectable biochemical or biological effect on v-Ros. Instead, mutation of the third tyrosine Tyr-419 in the cluster resulted in the impairment of v-Ros-transforming ability and substrate specificity. This result is consistent with our previous observation on the mutation of the corresponding tyrosine residue Tyr-1136 of an oncogenic gag-IGFR fusion PTK except that the mutation in that case resulted in dramatic decrease of both mitogenic and transforming activity (11). Deletion of the 6-amino acid insertion near the catalytic loop of v-Ros resulted in the loss of in vivo kinase activity, but produces little effect on the in vitro tyrosine phosphorylation of the mutant DI protein. Moreover, the DI protein is able to induce tyrosine phosphorylation of the array of cellular substrates with a pattern indistinguishable from that of the wild-type v-Ros. The simplest explanation for this observation is that the deletion results in an enzyme whose conformation is relatively unstable and is easier to inactivated during cellular protein extraction and in vitro processing. However, the possibility that the mutant DI protein is phosphorylated by other endogenous PTK(s) and becomes activated in vivo cannot be ruled out. If so, the active DI protein is apparently inactivated again during the protein extraction and processing since neither auto- nor trans-phosphorylation activity could be detected in vitro.

The IRS-1 and Shc recognition site on IR has been identified as the NPXY motif in the juxtamembrane region of IR, in which the N, P, and Y residues are important for the interaction (16). v-Ros is capable of inducing tyrosine phosphorylation of IRS-1 and Shc (25). However, there is no corresponding NPXY sequence in v-Ros. The Y419F mutation specifically decreases the tyrosine phosphorylation of IRS-1, but not of Shc. Therefore, the NDYY sequence of v-Ros likely defines an alternative recognition site for IRS-1. Shc may interact with v-Ros at another site. Alternatively, the presence of either of the twin tyrosines in NDYY may be sufficient for Shc recognition.

The Tyr-564 is the only residue that upon single mutation results in a pronounced reduction of intracellular autophosphorylation of v-Ros. Tryptic mapping of the in vitro autophosphorylated v-Ros proteins of UR2, F2 (Y418F/Y419F), and F564 revealed that several tryptic spots were missing in F564, but not in F2, protein in comparison with those of UR2 v-Ros protein (data not shown). These observations suggest that Tyr-564 is the major phosphorylation site of v-Ros in vitro and in vivo. Mutation of Tyr-564 also indicates that it is important for recognition of PLCγ. The interaction site for PLCγ maps to the carboxyl tyrosine residues of a number of RPTKs including EGF receptor (30), PDGF receptor (31), IGFR (11), and Met (32). Activation or overexpression of PLCγ has been implicated in stimulating DNA synthesis and promoting cell transformation by EGF and PDGF receptors (33). Some other reports, however, concluded that PLCγ was not important for PDGF-induced DNA synthesis (34). Our results shows that PLCγ plays only a minor role in v-Ros-mediated transformation of CEF.

Activation of PI3 kinase has been implicated in diverse functions including mitogenesis (35, 36), GLUT4 translocation/glucose transport (35, 37), membrane ruffling (38), and activation of p70 S6 kinase that is involved in stimulating protein synthesis (35). Our result with F419 and DI suggests that PI3 kinase could play a significant role in v-Ros induced cell transformation, but its activation is insufficient for morphological transformation and anchorage-independent growth and is not important for growth in monolayer cultures.

Our data suggest that signaling involving cytoskeletal proteins and cell-cell interactions may play an important role in morphological transformation and anchorage independent growth. The Rho family of GTP-binding/GTPase proteins including, Rho, Rac, and CDC42 are key players in regulating the cytoskeletal structure and membrane properties and are also important in mediating Ras-induced cell transformation (39, 1505).
40). In this regard, it is intriguing that p190 Rho/GAP, a regulator of Rho, is underphosphorylated in the F419- and DI-infected, in comparison with the UR2-infected cells (Fig. 8). EGF-dependent actin cytoskeleton disassembly is modulated by expression of c-Src and correlates with increased tyrosine phosphorylation of p190 Rho/GAP (41). This phenomenon could be explained by increased activity of Rho/GAP resulting in diminished abundance of Rho/GTP needed to promote the formation of actin stress fibers.

Our observation of the increased tyrosine phosphorylation of tensin and a β1 integrin-associated 190-kDa protein, as well as cadherin-catenin complex involved in cell-cell interaction in UR2, but not in F419- or DI-infected cells is also intriguing. It raises a possibility that those proteins are involved in mediating morphological transformation and anchorage-independent growth of UR2. Further work is required to elucidate the identity of the 190-kDa protein which we know is not p190Rho/GAP.

The cytoplasmic region of cadherin interacts with α-, β- and γ-catenins, which may serve as the bridge for interaction with actin, as well as signaling effectors (24). In v-Src-transformed cells, β-catenin is tyrosine-phosphorylated, and although cadherins are expressed on the cell surface, they are functionally inactive (42). The homology between β-catenin and a segment polarity gene in Drosophila called armadillo raises the possibility that β-catenin has a similar role in developmental signaling (43). Thus, catenins may play a dual role in cell-cell interaction and in signaling. The increased tyrosine phosphorylation of catenins and their enhanced association with cadherin in v-Ros transformed cells may not only affect the function of cadherin, but also modulate the cytoplasmic pool of the catenin involved in signaling.

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