Effect of Dimerization on Signal Transduction and Biological Function of Oncogenic Ros, Insulin, and Insulin-like Growth Factor I Receptors*

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The avian sarcoma virus UR2 codes for an oncogenic Gag-Ros fusion protein-tyrosine kinase (PTK). We have previously derived two retroviruses, T6 and NM1, coding for oncogenic Gag-insulin receptor and Gag-insulin-like growth factor I receptor (IGFR) fusion proteins, respectively. The Gag-IGFR fusion protein dimerizes, whereas Gag-Ros does not. To identify sequences affecting dimerization and the effect of dimerization on signaling and biological functions, we generated recombinants exchanging the extracellular and transmembrane sequences among the three fusion receptors. The presence of multiple cysteines in the Gag sequence appears to preclude dimerization, since deletion of the 3′ cysteine residue allows for dimerization. Most of the chimeric receptors retain high PTK activity and induce transformation regardless of their configuration on the cell surface. UT, a UR2/T6 chimera, retained mitogenic activity but has a markedly reduced transforming ability, while UN7, a UR2/NM1 recombinant, which also harbors Y950F and F951S mutations in IGFR, exhibits dramatic reductions in both activities. All of the fusion receptors can phosphorylate insulin receptor substrate 1 and activate PI 3-kinase. UT protein induces Shc phosphorylation, whereas UN7 protein does not, but both are unable to activate mitogen-activated protein kinase. Our results show that overexpressed oncogenic Gag fusion receptors do not require dimerization for their signaling and transforming functions and that the extracellular and transmembrane sequences of a receptor PTK can affect its specific substrate interactions.

Growth factors elicit their proliferative or differentiative signals by binding to their respective receptors, which generally have intrinsic protein-tyrosine kinase (PTK) activity (1, 2). Typically, these receptors are characterized by an extracellular ligand binding domain, a transmembrane (TM) domain, and a cytoplasmic domain containing the PTK catalytic sequence. Ligand binding results in receptor dimerization, autophosphorylation, kinase activation, and phosphorylation of cellular substrates leading to gene activation and ultimately proliferation or differentiation of cells. For most receptor PTKs the immediate event upon ligand binding is the dimerization of the receptors, which are predominantly monomeric in their inactive state. It is thought that receptor dimerization allows for cross-phosphorylation and activation of the PTK activity (2–4).

It has been well established that the epidermal growth factor receptor (EGFR) and the platelet-derived growth factor receptor undergo ligand induced dimerization, which is a necessary step for receptor autophosphorylation and kinase activation (5–8). Studies with a truncated EGFR lacking the extracellular domain have shown that it is capable of forming heterodimers with the full-length receptor (9) as well as homodimers (10), indicating that the extracellular domain is not necessary for dimerization. It has also been shown that ligand-independent dimerization of EGFR is sufficient for the activation of its kinase activity (11, 12). In addition, studies of the oncogenic ErbB2, an EGFR-related receptor PTK, have shown that a point mutation, Glu664 to Val664, in the TM domain results in the stabilization of homodimers and constitutive activation of the receptor (13, 14). Coexpression of several short ErbB2 transmembrane polypeptides with the native receptor were shown to inhibit dimerization and function of the full-length receptor, suggesting that the TM domain of ErbB2 is involved in its dimerization (15). Certain sequences in the TM domains of ErbB2 and a variety of other receptor PTKs have been implicated in mediating dimerization (16, 17).

The c-Ros, insulin receptor (IR) and insulin-like growth factor 1 receptor (IGFR) are three closely related receptor PTKs. In its inactive state, c-Ros, like most receptor PTKs, is monomeric, but it is unknown in which form the activated receptor exists. The avian sarcoma virus UR2 codes for the oncogenic v-Ros, which is a fusion protein of viral Gag and truncated c-Ros (18, 19). The Gag-Ros fusion receptor is a 68-kDa monomeric protein with the Gag portion protruding extracellularly (20). IR and IGFR, however, in the absence of their ligands exist as a heterotetrameric receptor complex consisting of two α and two β subunits linked by disulfide bonds. It is thought that binding of ligand to the α-subunit leads to intermolecular phosphorylation of the opposite β-subunit and activation of its kinase activity (21).

Our previous studies have shown that N-terminal truncation of IR or IGFR and fusion of the truncated receptors to retroviral Gag sequences activates their cell-transforming and tumorigenic potential. The retrovirus coding for the highly oncogenic Gag-IR and Gag-IGFR fusion receptors were named T6 and NM1, respectively (22, 23). Both receptors, essentially, have Gag fused to the extracellular boundary of the TM domain of IR and IGFR. Unlike UR2 Gag-Ros, NM1-encoded Gag-IGFR exists as a dimerized transmembrane receptor like the native IGFR. The physical status of T6-encoded Gag-IR is...
unclear. One of the major differences between the dimeric Gag-IGFR and monomeric Gag-Ros is the length of the Gag sequence fused to the respective receptors. The UR2 Gag-Ros contains 150 amino acids of Gag fused to the seventh amino acid upstream from the TM domain of v-Ros (18). The NM1 Gag-IGFR, on the other hand, has only 48 amino acids of Gag fused to the extracellular boundary of the IGFR TM domain (23). This raises a possibility that the Gag sequences in conjunction with the linked respective TM domains may determine the dimeric or monomeric status of the fusion receptors.

Whereas the requirement of dimerization for the function of a native RPTK is well established, such a requirement for their oncogenic counterparts remain elusive. Oncogenic Met, in which the Tpr (translocated promoter region) sequence has been directly fused to the Met kinase domain, dimerizes through a leucine zipper motif in the Tpr. Mutation of the leucine zipper motif in the Tpr abolishes dimerization and the oncogenic activity of Met (24). Oncogenic Fms and ErbB2 forms of these receptors have been reported. To determine the oncogenic activity of Met (24). Oncogenic Fms and ErbB2 fusion receptors, chimera among the three have been generated in which the Gag and TM domains are exchanged among them. In addition, deletions of the 3' Gag of two Gag-IR-derived receptors, so as to render their extracellular sequences similar to that of nM1 Gag-IGFR, were engineered to assess the effect of the Gag sequence on dimerization. Our study has identified the sequences affecting dimerization, signal transduction, and biological function of those oncogenic fusion receptors.

EXPERIMENTAL PROCEDURES

Cells and Viruses—The preparation and maintenance of primary chicken embryo fibroblasts (CEFs) and colony-forming assays were done as described previously (30).

Construction of Chimeric Receptors—The construction of the UR2/NM1 recombinant was done using two DNA fragments obtained by polymerase chain reaction (PCR). Using pUR2 (31) as a template, a 5' oligonucleotide containing a BglII site and a 3' oligonucleotide coding for the C terminus of the UR2 TM domain containing a HpaI site were used to clone the Gag and TM domains of UR2 Gag-Ros. To obtain the cytoplasmic sequence of NM1 Gag-IGFR, a 5' oligonucleotide coding for a HpaI site and a 3' oligonucleotide containing a SpHl site were used with pNM1 (23) as a template. Those two fragments were digested with HpaI and then ligated to generate the UR2/NM1 chimeric DNA. This chimeric fragment was used as the template in a second PCR with the 5' BglII and 3' SpH1 terminal oligonucleotides for amplification. The chimeric DNA was cloned back into pNM1 using the BglII and SpHl sites replacing the corresponding fragment. The recombinant receptor plasmid was named pUN. The other recombinants were generated in a similar manner using oligonucleotides designed for each specific chimera. The T6/UR2 and NM1/UR2 chimeric DNAs were individually cloned back into pUR2 using XmaI and EcoRI to give rise to pTU and pNU, respectively. The UR2/T6 and NM1/T6 chimeric DNAs were individually cloned into pTU (22) using XmaI and XhoI to generate pUT and pNT, respectively. To delete the 3' region of Gag, pTU and pTU were each digested with BanHI to free a 315-base pair fragment, and the remaining plasmid DNAs were recircularized using T4 DNA ligase. The PCR-derived DNAs for all of the constructs were sequenced to confirm the parental sequences and to detect possible PCR errors.

Antibodies—For preparation of the rabbit polyclonal anti-IR serum, a polypeptide including the entire catalytic domain of human IR produced in baculovirus and obtained from Ron Kohanski (Mount Sinai School of Medicine) was used. Rabbit polyclonal anti-IGFR and anti-Ros sera have previously been described (32, 33). Polyclonal anti-Shc and recombinant anti-phosphotyrosine antibody conjugated to alkaline phosphatase (RC23) were obtained from Transduction Laboratories. Rabbit polyclonal anti-MAP kinase (TR10) was a gift from Michael Weber (University of Virginia).

DNA Transfections—Transfections were done using the calcium phosphate co-precipitation method (31). Primary CEFs were plated at a density of 1 X 10^5 cells/6-cm dish and incubated for 18–20 h at 37 °C. The medium was then replaced with fresh medium containing 10% calf serum. 50 μl of 2.5 M CaCl2 was added to 450 μl of sterile H2O containing 10 μg each of transforming viral plasmid DNA and SacI-digested UR2AV helper viral DNA (31). This solution was slowly added to 500 μl of 2 × Heptes-buffered saline (50 mM Hepes, pH 7.2, 280 mM NaCl, 10 mM KCl, 1.5 mM Na2HPO4, and 12 mM dextrose) that was being aerated. This solution was allowed to set for 15 min at room temperature and then added to the cells along with chloroquine at a final concentration of 100 μM. The cells were incubated at 37 °C for 4 h, after which they were washed three times with phosphate-buffered saline and replenished with fresh medium. At confluence, the cells were transferred to 10-cm dishes and overlayed with soft agar the next day to select for transformed cells. Culture media were harvested 7–21 days post-transfection as virus stocks.

Protein Analysis—[35S]Metionine metabolic labeling, in vitro kinase, and PI 3-kinase assays were done as described previously (23). Tunicamycin treatment and assessment of glycosylation followed those described previously (32). Western analyses were done as before (22) with the following modifications. After SDS-PAGE gel electrophoresis, the gel was placed directly onto an electroblotting apparatus and transferred without pretreatment of the gel with transfer buffer (25 mM Tris base, 192 mM glycine, and 20% methanol). The gel was transferred at 100 V for 1 h with an ice block as a cooling chamber. After blocking with 3% bovine serum albumin in TBS-Tween (10 mM Tris, pH 7.5, 50 mM NaCl, and 1% Tween 20), the filters were probed with antibody in blocking solution for 2 h at room temperature or overnight at 4 °C. The filters were washed in TBS-Tween three times for 20 min at room temperature. If a secondary antibody was required, blocking and binding was repeated as above. After extensive washing to remove nonspecific bound antibodies, the filter was developed with nitro blue tetrazo- 

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RESULTS

Construction of Chimeric Receptors—Earlier recombinants that dimerize on the cellular membrane, oncogenic RPTKs, various recombinants among the three receptors were constructed (Fig. 1). In those recombinants the Gag and TM domains among the receptors have been swapped. Those chimeras were named UN (UR2/NM1), UT (UR2/T6), NU (NM1/UR2), NT (NM1/T6), and TU (T6/UR2). UN6 and UN7 are

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parallel clones of the UN recombinant; however, subsequent sequencing of the PCR-derived DNA fragments revealed Y950F and F951S mutations in the IGFR juxtamembrane region in UN7. Comparisons of the extracellular sequences of UR2 with those of UR2 differentially affects the morphological appearance of the transformed cells in monolayer culture (data not shown) and their ability to form colonies in soft agar (Fig. 2).

The exceptions were UN7 and UT, which were essentially unable to promote colony formation, and the infected cells exhibited a flat spindle morphology as opposed to an elongated fusiform morphology characteristic of the cells transformed by their parental viruses, NM1 and T6. UN7 harbors the Y950F and F951S mutations, which may account for its impaired transforming ability. While UN7-infected cells behaved like normal CEF, cells infected with UT, despite their inability to form colonies in soft agar, appeared to grow much faster than normal CEF. A comparison of the growth rates among normal CEF and UT- and T6-infected cells revealed that in F10 medium containing 5% serum, UT- and T6-infected cells grew equally well and about 60% faster than CEF; in 0.5% serum, UT- and T6-infected cells grew 4 and 6 times faster than CEF, respectively (Fig. 3). Therefore, replacing the Gag and TM sequences of T6 with those of UR2 differentially affects the mitogenic versus transforming activity of the Gag-IR fusion receptor. This result implicates strongly the role of the TM domain in the function of a RPTK, since UT differs from T6 essentially in only the swapping of the TM domain.

Dimerization Assays—To assess the physical configuration of those fusion receptors, [35S]methionine-labeled proteins were immunoprecipitated with their respective antisera and denatured with or without β-mercaptoethanol in the sample buffer to detect the existence of intermolecular disulfide linkages (Fig. 4A). Chimeric receptors containing the Gag and TM domains of NM1, namely NT and NU, were able to form dimers, while their parental, T6 and UR2 proteins, were unable to. Conversely when the UR2 Gag and TM domains were used to replace those of NM1, the resulting UN receptors were not able to dimerize. Swapping the Gag and TM domains between T6 and UR2, generating TU and UT recombinants, had no effect on their monomeric status. However, when 3′ Gag sequence was deleted from T6 and TU chimeric receptors, they were able to form dimers. These results strongly suggest that the 3′ Gag containing a cysteine residue prevents the formation of intermolecular disulfide bonds.

Since β-mercaptoethanol will only affect disulfide-linked dimerization and may not necessarily disrupt other possible molecular interactions, an alternative method of assessing dimerization was also used (Fig. 4B). Cells were labeled as before, and the surface proteins were chemically cross-linked in

Fig. 2. Anchorage-independent growth of virus-infected CEFs.

CEF were infected with the various virus stocks and overlaid with soft agar the next day. Four days after infection, colony assays were set up using 1.5 × 10⁵ cells/6-cm dish and maintained at 42°C. Photographs were taken 14 days after the assay was started. Plates were treated with 40% INT solution (Sigma catalog number 940–5) to stain the colonies 2 days before photography.
either NM1-derived extracellular and TM sequences or those described above; namely, only NM1 and chimeras with UR2 and T6 fusion receptors and some of the recombinant due to low cross-linking efficiency.

dimerization. A plausible explanation is that the cysteine 111 forms an intramolecular disulfide bond with either cysteine 12 or 16, thereby precluding the intermolecular disulfide bond linkage not only because it occupies the S-S linkage but because it may also interfere sterically with the association between monomers.

**Glycosylation of the Chimeric Receptors**—One of the post-translational modifications of receptor PTKs is glycosylation, which may affect the function and stability of the protein. The UR2 and NM1 Gag fusion receptors are not glycosylated (23, 34), whereas preliminary data indicate that the T6 Gag-IR is. However, a UR2 mutant TM1, deleting the 3-amino acid insertion in the TM domain of the UR2 protein, is glycosylated, indicating that the TM domain may affect glycosylation (34). To determine if this was the case in our recombinant chimeras, virus-infected cells were labeled in the presence or absence of tunicamycin, and immunoprecipitated receptors were resolved in SDS-PAGE gels. None of the chimeric receptors except those encoded by UT and its parental T6 were glycosylated (Fig. 5).

The TU protein appeared as multiple forms on SDS-PAGE; they were, however, not due to glycosylation. Although there appeared to be a decrease in the higher molecular weight forms of the TU protein, this was apparently due to an overall lower level of protein in the tunicamycin-treated cells. Repeated experiments showed that tunicamycin had no effect on the TU receptor protein. The results with T6, UR2, TU, and UT indicate that the cytoplasmic domain of the T6 protein dictates the glycosylation, provided that sites are available in the extracellular Gag sequence, since only those receptors with the T6 cytoplasmic domain are glycosylated. NT and ΔT6 receptors were not glycosylated, most likely due to their lack of the 3' Gag sequence containing the glycosylation site(s).

**Receptor Kinase Activity and Intracellular Phosphorylation**—To assess the kinase activity of the receptors, their ability to autophosphorylate in *vitro* and intracellularly was determined (Fig. 6). All of the receptors exhibited high autophosphorylation activity both *in vitro* and *in vivo*, including the UN7 and UT mutants, despite their greatly reduced transforming ability. Although all of the recombinants exhibited a high autophosphorylation level, the dimeric NT and ΔT6 proteins have a significantly higher level of *in vitro* and *in vivo* activity than
the phosphorylation of those high molecular mass proteins by cells (Fig. 7) and that a 250-kDa protein in particular was more efficiently tyrosine-phosphorylated in the UT-infected cells (data not shown).

The UN7 and UT proteins, despite the fact that they all have the same IR cytoplasmic domain, including the PTK region. This suggests that although the monomeric Gag-IR is capable of autophosphorylation, the dimeric forms are more efficient. This phenomenon was not observed for the IGFR- or Ros-derived recombinants. However, the difference in autophosphorylation does not appear to affect their ability to phosphorylate cellular substrates (see below) or their transforming ability (Fig. 2). The UT and UN7 proteins, the two nontransforming chimeras, are expressed at a similar level and are as stable as the others (as judged by metabolic labeling and steady state level (Figs. 4–6)). Labeling of intact cell surface proteins demonstrated that UT and UN7 fusion receptors were localized on the cell surface as abundantly as the T6 and UR2 proteins (data not shown).

**Phosphorylation of Cellular Substrates**—The pattern of phosphorylation of cellular proteins reflects a receptor PTK activity as well as its affinity and specificity toward substrates in vivo. To determine any quantitative or qualitative effect of receptor dimerization on the phosphorylation of cellular substrates, total cellular lysates from various virus-infected cells were analyzed for protein tyrosine phosphorylation (Fig. 7). All the chimeras induced dramatically higher tyrosine phosphorylation of cellular proteins over normal CEF. No major difference of substrate patterns were noticed among them except for variation in the extent of receptor protein autophosphorylation. The UN7 and UT proteins, despite their greatly reduced transforming capability, were able to phosphorylate cellular proteins as efficiently as their transforming counterparts. This is consistent with the observation of their in vitro and in vivo autophosphorylation activity (Fig. 6). It was also noticed that several high molecular mass proteins of more than 200 kDa were more efficiently tyrosine-phosphorylated in the UT-infected cells (Fig. 7) and that a 250-kDa protein in particular was specifically phosphorylated in UN7 cells. The significance of the phosphorylation of those high molecular mass proteins by the non-transforming UN7 and UT proteins is unclear. Our results imply that the UT and UN7 proteins most likely fail to recognize certain specific cellular substrates, resulting in their reduced transforming ability.

**IRS1 Phosphorylation and PI 3-Kinase Activity**—IRS1 is a major substrate of IR and IGFR and is believed to be responsible for the recruitment of other signaling molecules such as Grb2-Sos complexes, Syp phosphatase, and PI 3-kinase (35–37). It is known that the association of IRS1 with p85 activates the catalytic activity of PI 3-kinase (37). IRS1 is also a substrate of the UR2 Gag-Ros (34). Therefore, phosphorylation of IRS1 and the association of PI 3-kinase activity with IRS1 or with the fusion receptor PTKs themselves were compared (Fig. 8). All of the chimeric and parental fusion receptors, including UT and UN7 fusion receptors, were capable of phosphorylating IRS1 well above the basal level of normal CEF. UN7, in which the putative IRS1 binding site had been mutated, was also able to phosphorylate IRS1. This confirms our previous observation that mutation of Tyr950 in the NM1 protein did not affect its ability to phosphorylate IRS1 or transform cells (38).

Consistent with the phosphorylation of IRS1, all of the fusion receptors promoted increased IRS1-associated PI 3-kinase. There also existed a significant level of receptor-associated PI 3-kinase activity, although it was much less than that of IRS1. There appears to be significant variation among the receptors in their ability to associate with PI 3-kinase or to promote its association with IRS1. The TM domain of UR2 seems to be able to confer a somewhat higher IRS1-associated PI 3-kinase activity, as seen in UN and UT recombinants in comparison with NM1 and T6 proteins. The converse is also apparent, namely that there is a decrease in the IRS1-associated PI 3-kinase activity when the TM domain of UR2 is replaced by that of T6 or NM1 as seen in the TU and NU recombinants.

**Src Phosphorylation and MAP Kinase Activity**—Growth factor receptors activate the Ras/MAP kinase pathway by phosphorylating Shc and recruiting Grb2-Sos complexes to membranes via the SH2 domain of Grb2 (39–41). We therefore assessed the ability of the fusion receptors to phosphorylate Shc and activate MAP kinase (Fig. 9). All of the chimeras were able to phosphorylate Shc with the exception of UN7, which
harbored the Y950F and F951S mutations. Tyr950, in addition to being the IRS1 binding site, also interacts with Shc (42). Activation of the MAP kinase activity by UN7 was also decreased as compared with NM1, as would be expected given its failure to phosphorylate Shc. Surprisingly, UT also exhibited a decreased ability to activate MAP kinase as well, despite its capability to induce elevated Shc phosphorylation. This result suggests that an additional event(s) independent of Shc phosphorylation is required to activate MAP kinase.

DISCUSSION

Our results indicate that overexpressed and constitutively activated IR, IGFR, and Ros receptor PTKs do not require dimerization for their signaling and cell-transforming function. This is contrary to the well-established model that ligand-induced dimerization of a RPTK is required for its cross-phosphorylation and activation of the kinase activity (2–4). In the cases of native IR and IGFR, which exist as heterotetramers on the cell surface, ligand binding is thought to induce conformational changes that allow cross phosphorylation and activation of the kinase activity (4, 43). The ligand of c-Ros is unknown; therefore, it is not clear whether c-Ros requires dimerization for its activation. However, we have previously constructed EGFR-Ros chimeras and shown that epidermal growth factor induces their dimerization and activation (44). Presumably, c-Ros may undergo ligand-induced dimerization and activation similar to other RPTKs. Our current results indicate that the oncogenic Gag fusion receptors can abrogate the requirement of dimerization for their biochemical and biological functions. Two issues arise; namely, do they require autophosphorylation for activating their PTK activity? If so, do they autophosphorylate intramolecularly or intermolecularly? Site-specific mutation of tyrosine residues of the Gag-IGFR, Gag-IR, and Gag-Ros indicate that phosphorylation of the tyrosine residues known to modulate the kinase activity of IR, IGFR, and Ros is important for the PTK activity of these receptors (38). The next question is then how those tyrosine residues are phosphorylated. Our results are consistent with the possibility of intramolecular cis-phosphorylation and activation. This is in

FIG. 8. Tyrosine phosphorylation of IRS1 and association of PI 3-kinase activity with IRS1 or fusion receptors. Cells were lysed with Nonidet P-40 extraction buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM Na3VO4, 1% Nonidet P-40), and 600 μg of total lysates was immunoprecipitated with anti-IRS1. The immunoprecipitated proteins were resolved on a 10% SDS-PAGE gel, transferred to nitrocellulose, and blotted with anti-phosphotyrosine antibody (A). From the remaining lysates, 200 and 400 μg of total protein were immunoprecipitated, respectively, with anti-IRS1 or appropriate anti-receptor serum. The immunoprecipitates were subjected to an in vitro PI 3-kinase assay (B). The results of three separate PI 3-kinase assays were quantitated using ImageQuant version 3.3 (Molecular Dynamics), and the average values were plotted (C). Values were normalized against the average value for CEF.

FIG. 9. Tyrosine phosphorylation of Shc and activation of MAP kinase. Cells were lysed with RIPA, and equivalent protein amounts of total cell lysates were immunoprecipitated with anti-Shc antibody. The immunoprecipitated proteins were resolved by SDS-PAGE electrophoresis in duplicate gels, transferred, and blotted (A) with either anti-phosphotyrosine antibody (upper part) or anti-Shc (lower part). A parallel culture was starved overnight in serum-free F10 medium and then extracted with RIPA. Equivalent protein amounts of lysates were immunoprecipitated with anti-MAP kinase (TR10) and subjected to an in vitro MAP kinase assay with myelin basic protein (MBP) as an exogenous substrate. Proteins were then resolved on a 15% SDS-PAGE gel, dried, and visualized by autoradiography (B). The results of two experiments were quantitated, and the results were plotted (C). Values were normalized against the average value for CEF.

2 Q. Xiong and L.-H. Wang, unpublished observations.
3 C. S. Zong, J. L.-K. Chan, and L.-H. Wang, unpublished observations.
agreement with a report that IR is capable of undergoing intramolecular phosphorylation (45). The trypsin-truncated IR was shown to undergo autophosphorylation at similar tyrosine residues as the native receptor, and this autophosphorylation is independent of the concentration of the receptor (45). However, we cannot rule out the possibility that trans-phosphorylation may have occurred, given that the oncogenic receptors are overexpressed such that local concentration of the receptor is exceedingly high to allow such interaction.

Specific interaction between a RPTK and its substrates is generally thought to be mediated by specific sequence motifs and conformation of the receptor and substrate molecules. Numerous studies with chimeric receptors indicate that the specificity of substrate recognition is dictated by the cytoplasmic domains of the chimeras (46, 47). Our previous studies of a TM domain mutant of v-Ros (34) and EGFR-Ros chimera (44) indicate that the TM sequence could play an important role in receptor recognition and interaction of substrates. Our current study further strengthens this notion. The extracellular sequence of T6 Gag-IR is essentially the same as that of UT Gag-IR; the only difference is the additional five linker-derived amino acids between the TM domain of T6 and the retroviral Gag p19 in the former fusion receptor. The only other difference between UT and its parental T6 proteins lies in the TM domain; UT contains the UR2-derived TM domain. The results between UT and its parental T6 proteins lie in the TM domain and are somewhat reduced mitogenic activity, the biochemical basis for its greatly reduced transforming ability remains to be identified.

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