VIROCRINE TRANSFORMATION: The Intersection Between Viral Transforming Proteins and Cellular Signal Transduction Pathways

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ABSTRACT

This review describes a mechanism of viral transformation involving activation of cellular signaling pathways. We focus on four viral oncoproteins: the E5 protein of bovine papillomavirus, which activates the platelet-derived growth factor β receptor; gp55 of spleen focus forming virus, which activates the erythropoietin receptor; polyoma virus middle T antigen, which resembles an activated receptor tyrosine kinase; and LMP-1 of Epstein-Barr virus, which mimics an activated tumor necrosis factor receptor. These examples indicate that diverse viruses induce cell transformation by activating cellular signal transduction pathways. Study of this mechanism of viral transformation will provide new insights into viral tumorigenesis and cellular signal transduction.

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INTRODUCTION

Many tumor viruses stimulate the proliferation of their host cells. The analysis
of viral transforming proteins has revealed several strategies by which viruses
achieve this end. In some cases, viruses have transduced cellular genes involved
in the control of cell growth and differentiation. Other viruses encode proteins
that induce the synthesis of cellular DNA replication proteins, thereby mobi-
lizing the cellular replication machinery so that it can support replication of the
viral DNA. Activation of the replication machinery in this manner frequently
stimulates cellular as well as viral DNA replication. Viruses have evolved two
general strategies to induce this replicative state. Several groups of DNA viruses
encode proteins that inactivate cellular tumor suppressor proteins such as p53
and $p105^{RB}$ that inhibit cell proliferation. This releases the brakes on the cell cy-
cle, thereby resulting in increased proliferation. Viruses also can activate cellu-
lar pathways that normally stimulate cell proliferation. Several RNA and DNA
viruses encode homologues of cellular growth factors, growth factor receptors,
or downstream components of growth factor signaling pathways, which act in
a similar manner to their cellular counterparts. This review describes another
class of viral transforming proteins that activate growth factor signaling path-
ways, even though these viral proteins bear no obvious resemblance to cellular
proteins. These viral proteins either activate components of the signaling path-
way or mimic the structure of an activated component. Here, we focus on viral
proteins that either activate a particular growth factor receptor in the absence of
its normal ligand or mimic an activated receptor. We propose the term "virocrine
transformation" to encompass this mechanism of viral transformation (36).

A BRIEF INTRODUCTION TO GROWTH FACTOR
RECEPTOR FUNCTION

Peptide growth factors or cytokines are often required for cellular survival and
proliferation. These proteins are usually soluble and initiate signaling by bind-
ing to specific receptors at the cell surface, resulting in receptor activation.
In general, receptors are composed of an extracellular ligand binding domain,
a transmembrane segment, and a cytoplasmic domain required for signaling.
Signaling is initiated by ligand binding. For most receptor tyrosine kinases,
including the platelet-derived growth factor (PDGF) receptor, ligand binding
induces receptor dimerization (Figure 1) (56). This stimulates the intrinsic ty-
rosine kinase activity of the receptor and induces receptor autophosphorylation
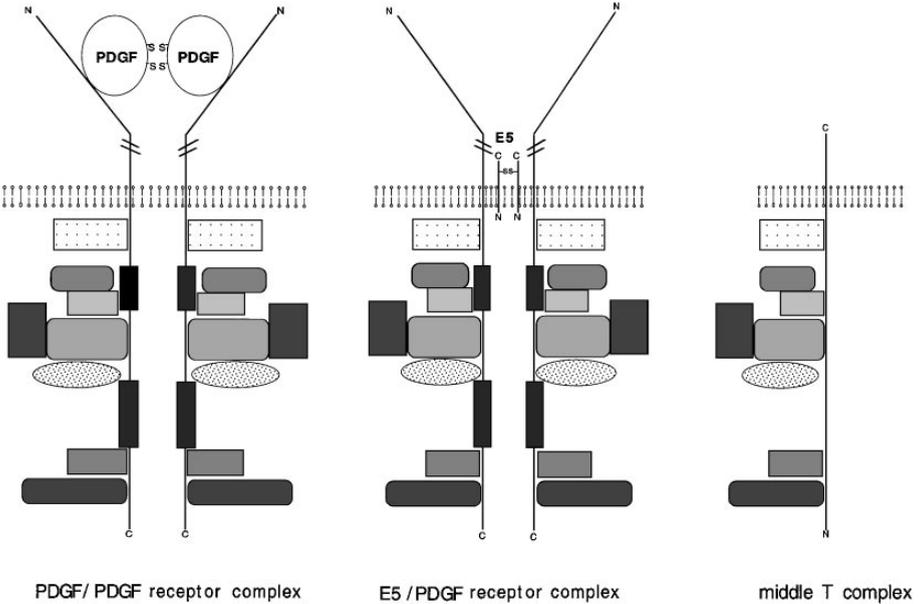
Figure 1 Schematic diagram of activated receptor tyrosine kinase complexes and the related middle T complex. Left, a complex containing dimeric PDGF β receptor activated by PDGF bound to the extracellular domain of the receptor. Middle, a complex containing dimeric PDGF β receptor activated by the BPV E5 protein bound to the transmembrane region of the receptor. Right, a complex containing polyomavirus middle T antigen. The cell membrane is shown with the cytoplasmic domain of the receptor or mT extending down from the membrane, bound to various cellular signaling proteins represented by *shaded boxes*. The split kinase domain of the PDGF β receptor is depicted as *black boxes*. The spectrum of cellular proteins bound under these various situations is not fully defined and may not be the same in all three cases. [Modified from (36).]

and tyrosine phosphorylation of other proteins. Autophosphorylation of the receptor on tyrosine residues plays an essential role in receptor signaling by generating specific binding sites for cellular signaling proteins containing *src* homology 2 (SH2) or phosphotyrosine binding (PTB) domains (22). Cellular proteins that stably bind receptors in such a phosphotyrosine-dependent fashion include *src* family tyrosine kinases, phospholipase Cγ (PLCγ), phosphoinositol 3′ kinase (PI3K), and Shc (22). Once recruited to the tyrosine-phosphorylated receptor, these cellular proteins are activated by a variety of mechanisms, including conformational changes, direct phosphorylation by the receptor, and subcellular relocalization. The signal initiated by ligand binding and receptor activation is then propagated through a cascade of cytoplasmic and nuclear signal transducers, including the *ras*-MAP kinase pathway (22).

Unlike receptor tyrosine kinases, many cytokine receptors, such as the erythropoietin receptor, do not possess intrinsic tyrosine kinase activity. Nevertheless, these receptors utilize a related mechanism of signaling (63, 133). Upon ligand binding, cytokine receptors dimerize and form complexes with cytoplasmic tyrosine kinases, such as the JAK family kinases (63, 96). This results in activation of these kinases, which in turn catalyze tyrosine phosphorylation of the receptor and recruitment of other proteins to the receptor, thereby activating the downstream signaling cascade.

The tumor necrosis factor (TNF) receptor can also signal cell proliferation in some situations. Like cytokine receptors, the TNF receptor is not an enzyme and seems to function by mobilizing intracellular proteins. TNF and related proteins bind to TNF receptors and induce aggregation of the receptor. This results in the aggregation of proteins that bind TNF receptors, including tumor necrosis factor receptor-associated factors (TRAFs) that are constitutively bound to the cytoplasmic tail of the TNF receptor 2 (112, 128). TRAF aggregation in some cell types including B lymphocytes is believed to activate cytoplasmic serine/threonine kinases, which in turn activate the transcription factor NFκB and stimulate proliferation (12). The activated TNF receptor 1 binds directly to other proteins such as TNF receptor-associated death domain protein (TRADD), which can itself bind TRAF2, leading to NFκB activation and proliferation (61a, 128). Alternatively, TRADD can initiate apoptosis by activating a different signaling cascade.

Viral proteins interact with each of the cellular signaling pathways outlined above. Table 1 lists the viral proteins described in this review and indicates the points at which these viral proteins intersect with cellular signaling pathways.

Table 1   Viral proteins and their actions

Viral proteins that activate cellular receptors	
Bovine papillomavirus E5 protein	PDGF β receptor
Spleen focus forming virus gp55	Erythropoietin receptor
Viral proteins that mimic cellular receptors	
Polyomavirus middle T antigen	Receptor tyrosine kinase
Epstein-Barr virus LMP-1	TNF receptor 1 and 2
Viral proteins that engage downstream signaling components	
Herpesvirus saimiri Tip	pp56$^{c\text{-}lck}$
Herpesvirus saimiri STP-C488	p21$^{c\text{-}ras}$
Herpesvirus saimiri STP-A11	*src* family tyrosine kinases
Epstein-Barr virus LMP-2	*c-lyn* and *c-syk* tyrosine kinases
Hepatitis B virus HBx	pp60$^{c\text{-}src}$

DIRECT ACTIVATION OF GROWTH FACTOR RECEPTORS BY VIRAL PROTEINS THAT DO NOT RESEMBLE NORMAL LIGANDS

Some oncogenic retroviruses and DNA tumor viruses encode proteins that closely resemble the normal cellular ligands of growth factor receptors (see 10, 35, 132). These proteins evidently bind the receptors and initiate signaling by the same mechanisms that are utilized by their cellular counterparts. In many cases, these viral proteins can act in an autocrine fashion, i.e. they are synthesized in the same cell as the receptor. Recent studies of viral transformation have revealed a related but distinct mechanism of receptor activation.

The BPV E5 Protein and the PDGF β Receptor

The E5 gene of bovine papillomavirus type 1 (BPV) can cause stable and acute transformation of cultured fibroblasts. This gene encodes the E5 protein, a very unusual hydrophobic transforming protein of only 44 amino acids that is primarily localized to the membranes of the endoplasmic reticulum (ER) and Golgi apparatus in transformed cells (19, 20, 117). The E5 protein is thought to be a type II transmembrane protein with its carboxyl-terminal third in the lumen of the ER and Golgi apparatus (19). The predominant form of the E5 protein in transformed cells is a disulfide-linked homodimer, but monomeric E5 protein is also present (17).

In transformed fibroblasts, the BPV E5 protein is present in a stable complex with mature and immature intracellular forms of the endogenous PDGF β receptor (105, 106) (Figure 1). Strikingly, the PDGF β receptor in cells expressing the E5 protein is constitutively activated as assessed by several criteria: It displays increased tyrosine-kinase activity, exhibits increased levels of tyrosine phosphorylation, and is constitutively bound to several cellular signal transduction proteins (37, 106, 107). Receptor activation occurs rapidly and in a dose-dependent fashion upon acute expression of the E5 protein (107). These findings suggest the simple model that the E5 protein directly induces activation of the PDGF β receptor, and that the activated receptor then initiates a sustained mitogenic signal similar to that induced by the normal ligand, resulting in growth transformation.

Several studies indicate that the PDGF β receptor can indeed mediate transformation by the E5 protein. Variant fibroblast cell lines with decreased ability to respond to PDGF are relatively resistant to E5 transformation (111). In addition, E5 mutants unable to bind and activate the PDGF receptor are transformation-defective (61, 91, 101, 119). [There is some controversy about this point, but the most recent and complete experiments examining this issue revealed an excellent correlation between the abilities of various E5 mutants to bind and activate

the receptor and to transform C127 cells (101, 121; O Klein, GW Polack, D DiMaio, unpublished observations).] The strongest evidence for an essential role for the PDGF β receptor in E5 transformation is provided by gene transfer experiments in which cells that do not express endogenous PDGF β receptor become susceptible to E5-induced proliferation or tumorigenesis only when an exogenous PDGF β receptor is expressed (37, 51, 102, 123). In these heterologous systems, transformation is accompanied by E5/PDGF β receptor complex formation and by E5-induced tyrosine phosphorylation of the receptor. Other receptor tyrosine kinases, including the closely related PDGF α receptor and the epidermal growth factor (EGF) receptor, are unable to mediate E5-induced transformation (51, 102, 123).

Further insight into the role of the PDGF β receptor in transformation by the E5 protein is provided by studies in which PDGF β receptor mutants have been analyzed for their ability to bind the E5 protein and support E5 transformation. The intrinsic tyrosine-kinase activity of the receptor is required for mitogenic signaling, suggesting that E5 transformation occurs through activation of the receptor signal transduction pathway (37). This interpretation is supported by the finding that treatment of E5-transformed cells with specific inhibitors of PDGF receptor tyrosine kinase activity reverses receptor tyrosine phosphorylation and the transformed phenotype (O Klein, P Irusta, D DiMaio, unpublished observations). In contrast, deletion of most of the extracellular ligand binding domain of the receptor prevented receptor activation by PDGF but had no effect on complex formation with the E5 protein and did not impair E5 induced receptor activation and mitogenic activity (37, 123). Therefore, E5 transformation proceeds in the absence of activation of the receptor by PDGF, indicating that it is ligand-independent.
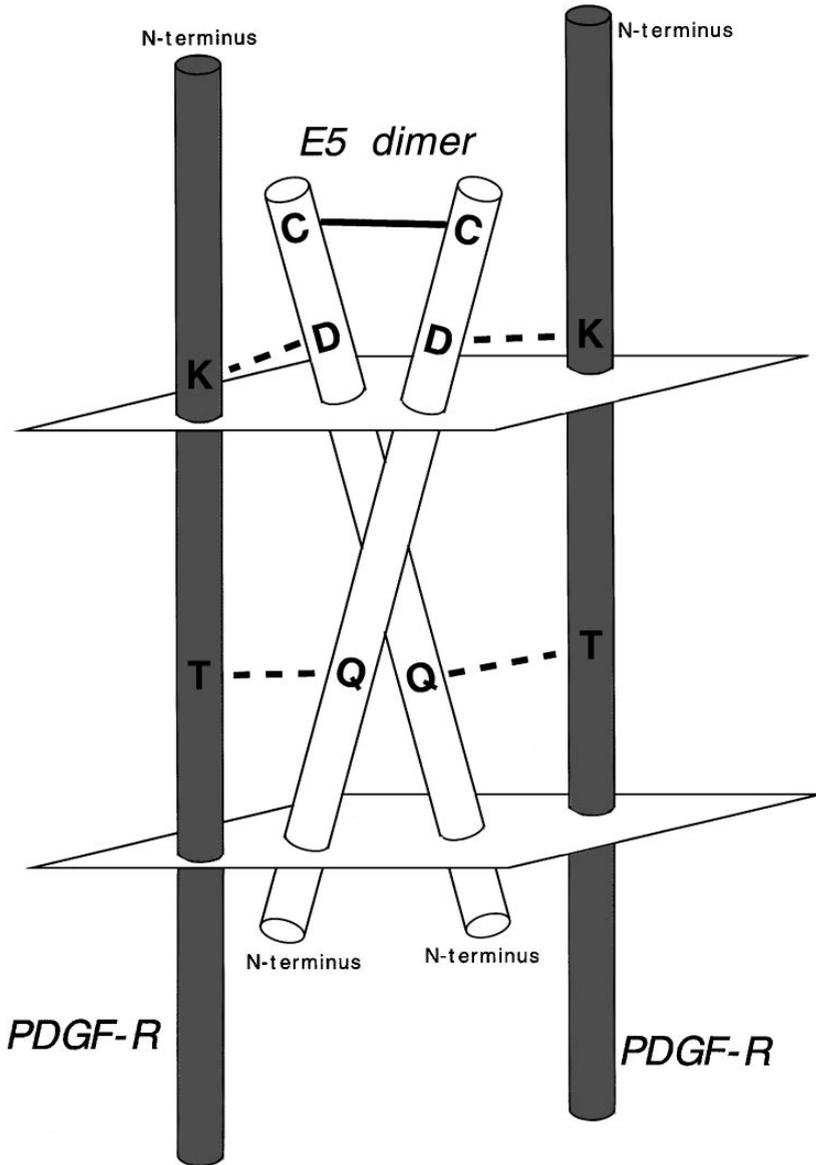
PDGF is soluble, hydrophilic, and much larger than the E5 protein, and it binds the extracellular domain of the receptor. Thus, the biochemical mechanism of PDGF β receptor activation by PDGF is likely to differ from that utilized by the E5 protein, which is very small, hydrophobic, and does not interact with the extracellular domain of the receptor. The molecular interactions responsible for complex formation and receptor activation are still under investigation, but currently available evidence favors the model that the interaction between these two proteins is direct and not mediated by other cellular proteins (101, 108, 121). Analysis of PDGF receptor mutants and chimeras indicates that the transmembrane region of the PDGF receptor is essential for complex formation and activation, suggesting that the transmembrane domains of these two proteins may line up with one another in the cell membrane (25, 52, 108, 123). In fact, specific amino acids in the transmembrane and juxtamembrane domains of both proteins have been identified that are required for complex formation and receptor activation (101, 108). The ability of the E5

protein to distinguish between the related PDGF α and β receptors also maps to the transmembrane/juxtamembrane region of the receptor (123). Strikingly, the specific residues of the PDGF β receptor required for complex formation with the E5 protein are absent from the α receptor.

The E5 protein, like PDGF, induces receptor dimerization (CC Lai, C Henningson, D DiMaio, unpublished information). Because the E5 protein exists in a dimeric form in transformed cells and dimerization-defective E5 mutants do not activate the receptor nor induce cell transformation (61, 91, 101), complex formation between two molecules of the PDGF β receptor and a dimeric E5 protein can in principle be sufficient to cause dimerization of the receptor, which in turn activates the receptor tyrosine kinase. On the basis of computational searches and other considerations, we have recently proposed a model in which an E5 monomer is unable to bind the PDGF β receptor, because the site for receptor binding is generated by dimerization of the E5 protein (T Surti, O Klein, K Ascheim, D DiMaio, SO Smith, submitted for publication). According to this model, one receptor molecule binds to one face of the E5 dimer and a second receptor molecule binds to the other face of the dimer (Figure 2). The finding that dimerization-defective E5 mutants are unable to bind the PDGF β receptor is consistent with this model (101). However, it should be noted that the stoichiometry of the E5 protein and the PDGF receptor in the complex has not been established, and there is no high-resolution structural information available.

The results summarized above provide compelling evidence that the E5 protein activates the PDGF β receptor by means of biochemical interactions that are distinct from those utilized by the normal ligand, and that receptor activation drives cell transformation. However, cellular proteins other than the PDGF β receptor can bind to the E5 protein. These proteins include the 16-kd pore-forming subunit of vacuolar H^+-ATPase, a 125-kd protein related to α-adaptin, and the EGF receptor (36). The role of these proteins in E5-induced transformation of fibroblasts remains unclear (121), although it is possible that these interactions are important for other biological activities of the BPV E5 protein, such as NFκB induction, modulation of EGF receptor stability, and as-yet-poorly-defined effects in epithelial cells (20, 75, 90).

The E5 proteins of other papillomaviruses, including the high-risk human papillomaviruses (HPV), also appear to modulate signal transduction pathways. Early experiments suggested that growth factor signaling was enhanced by the HPV16 E5 protein (79, 109). This may be due, at least in part, to effects on recycling of the EGF receptor (124). More recently, it has been shown that the E5 proteins of HPV16 and the related rhesus monkey papillomavirus can activate *ras*, MAP kinase, and PI3K pathways in cells (48, 53). However, unlike the case for the BPV E5 protein, the mechanisms involved are far from

clear. Although HPV E5 proteins can form stable complexes with growth factor receptors in cell systems that overexpress the viral protein and the receptor, such complexes have not been observed with endogenous growth factor receptors in epithelial cells, the normal host cells for HPV replication and tumorigenesis (26, 62).

Friend Erythroleukemia Virus gp55 and the Erythropoietin Receptor

The spleen focus forming virus (SFFV) is a replication-defective retrovirus that induces erythroleukemia in infected mice. The viral protein gp55 is responsible for the initial proliferative stage of erythroleukemia (2, 3, 24, 31, 115). Most acutely transforming retroviruses carry a transduced cellular oncogene. In contrast, the SFFV gp55 gene is an intrinsic viral gene that encodes a dimeric transmembrane viral envelope glycoprotein (31, 50, 136).

Normal erythroblast proliferation results from the interaction between erythropoietin (EPO) and its cell surface receptor, a member of the cytokine receptor superfamily (133). EPO binding induces homodimerization of the EPO receptor, activation of cytoplasmic tyrosine kinases, and recruitment and activation of cellular signal transducing proteins. Leukemic erythroblasts isolated from SFFV-infected mice can proliferate in an EPO-independent manner, and similar effects can be elicited by SFFV infection of erythroblast cell lines, suggesting that gp55 might activate the EPO receptor signaling pathway (59, 114, 133). This hypothesis was confirmed in cell lines that normally require interleukin-3 (IL-3) for continued survival and proliferation. Coexpression of gp55 and murine EPO receptor in these cells allows sustained proliferation in the absence of the IL-3, whereas expression of neither protein alone supports proliferation in the absence of growth factors (59, 83). The ability of gp55 plus EPO receptor to substitute for IL-3 suggests that gp55 expression results in constitutive activation of the EPO receptor, which provides the necessary survival and proliferative signals (31, 133). This interpretation is substantiated by the

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Figure 2 Model for the interaction between the BPV E5 protein and the PDGF β receptor. A complex between a dimer of the E5 protein (*open rods*) and the transmembrane and juxtamembrane domains of two PDGF receptor molecules (*grey rods*) is shown. The space bounded by the planes represents the cell membrane, with the cytoplasm at the bottom. The transmembrane domains of the E5 protein and the PDGF receptor are oriented in an antiparallel fashion relative to each other. *Solid line*, a disulfide bond between two carboxy-terminal cysteines in the E5 protein; *dotted lines*, putative non-covalent bonds between residues that have been shown by mutational analysis to be important for complex formation. A central feature of this model is that each PDGF receptor molecule forms contacts with both monomers of the E5 protein in the E5 dimer.

observation that a dominant-negative EPO receptor blocks gp55 transformation, and by the correlation between pathogenicity of various gp55 mutants and their ability to confer EPO resistance (5, 45, 130a). In addition, in cells expressing gp55, JAK kinases and other proteins downstream of the EPO receptor appear constitutively activated (84a, 102a, 120, 137).

Biochemical studies demonstrate that gp55 and the EPO receptor exist in the cells as a stable complex with at least a fraction of these complexes at the cell surface (45, 81, 83, 141). However, as is the case with BPV E5 and PDGF, gp55 bears no amino acid sequence similarity to EPO. Thus, the nature of the interaction between gp55 and EPO receptor is quite different from the inter- action between EPO and the EPO receptor. The biochemical mechanism of EPO receptor activation by gp55 has not been established, and it has not been clearly shown that gp55 and EPO receptor contact one another directly, nor that gp55 induces dimerization of the EPO receptor (76, 127). Nevertheless, genetic studies have identified the domains required for the productive interac- tion between these two proteins. Both the extracellular ligand binding domain and transmembrane domain of the EPO receptor are required for signaling by gp55 (31, 142). Similarly, the transmembrane and extracytoplasmic domains of gp55 are required for complex formation with the EPO receptor and signaling (31, 119a, 122, 133), and biological studies show that these same two domains are required for leukemogenicity (24, 123, 131).

The nature of the interaction between the EPO receptor and gp55 has been probed in more detail by using two different variants of gp55, gp55A and gp55P. Both proteins induce leukemia and bind the EPO receptor, but only gp55P is able to cooperate with the EPO receptor to confer EPO-independent proliferation of hematopoietic cell lines (115). This difference between gp55A and gp55P, as well as differences in their pathogenicity in animals, maps to their transmembrane domains, again highlighting the importance of this portion of gp55 in mediating its interaction with EPO receptor (24, 115). It has recently been shown that gp55A is able to support erythroid differentiation in murine fetal liver cells, a process that is normally mediated by EPO (27). Strikingly, gp55A had no such effect on cells isolated from EPO receptor knock-out mice, establishing that this effect is mediated by the EPO receptor (27). Thus, gp55A as well as gp55P can activate the EPO receptor, although the details of the cellular response depend on the particular system used. Taken together, analysis of gp55 demonstrates that it mimics EPO by binding and activating the EPO receptor, which in turn delivers a proliferative signal.

Other cytokine receptors may also serve as the targets of retroviral envelope proteins. The gp70 proteins of some mink cell focus forming (MCF) viruses associate with the interleukin 2 (IL-2) receptor, resulting in receptor activation (82). A small hydrophobic protein encoded by human T cell leukemia virus 1

(HTLV-1), p12^I also appears to interact with the IL-2 receptor (99). Thus, activation of cytokine receptors by retroviral proteins may be a strategy commonly employed by these viruses to induce cell proliferation.

VIRAL PROTEINS THAT MIMIC ACTIVATED GROWTH FACTOR RECEPTORS

The analysis of BPV E5 and gp55 summarized in the preceding section revealed that viruses can activate growth factor receptors and transform cells without synthesizing proteins that resemble the normal ligands of the receptor. As summarized in this section, viruses have evolved another mechanism to activate growth factor signaling pathways, namely the synthesis of proteins that resemble not a growth factor, but an activated receptor itself.

Polyomavirus Middle T Antigen

The middle T antigen (mT) of mouse polyomavirus and related viruses can cause growth transformation of established lines of rodent fibroblasts and is required for virus-induced tumorigenicity in rodents (14, 34). mT is a 421-amino acid protein anchored to the plasma membrane by a carboxyl-terminal segment of hydrophobic amino acids, with most of the protein extending into the cytoplasm (Figure 1). Although mT has no known intrinsic catalytic activity, in transformed cells it forms a stable complex with pp60$^{c\text{-}src}$, the product of the *c-src* cellular proto-oncogene, and other kinases of the *src* family including *c-fyn* and *c-yes* (29, 60). The association of *c-fyn* with hamster polyomavirus mT appears to be mediated by the ability of the *c-fyn* SH2 domain to recognize a specific phosphotyrosine on mT, but SH2/phosphotyrosine interactions do not mediate the association of mouse polyomavirus mT with *src* family members (13, 40, 41). Complex formation with mT stimulates the tyrosine kinase activity of pp60$^{c\text{-}src}$ and related kinases (11, 28). The mechanism of kinase activation by mT is unclear, but it may involve removal of an inhibitory phosphate group from a carboxyl-terminal tyrosine in pp60$^{c\text{-}src}$ and stabilization of the enzyme in an active conformation.

The importance of the mT/*src* interaction has been assessed by using a variety of approaches. Studies of mT mutants indicate that the association of mT with *src* family members is necessary for transformation, a conclusion supported by the inhibitory effects of dominant negative alleles of *src* family members on mT transformation (14, 15, 34, 49). However, activation of *src* family members is not sufficient for transformation, because some transformation-deficient mT mutants still display elevated levels of associated tyrosine kinase activity (84). Studies in animals unable to express specific *src* family members and in cells derived from such animals indicate that no individual *src* family member is

required for mT activity (73, 74, 126). Rather, these studies indicate that mT induced tumorigenesis and cell transformation is a complex process involving redundant and/or alternative cellular signaling pathways. In the case of mouse mT-induced hemangioma formation, *c-yes* appears to play a more important role than the other *src* family members tested (73).

The primary biochemical role of the *src* kinases in mT transformation appears to be the phosphorylation of mT itself on tyrosine residues. Tyrosine phosphorylation of mT generates binding sites for a variety of other cellular signaling proteins including PI3K, PLC$_\gamma$, and Shc (21, 33, 125, 134, 139). As is the case for the association between these same signaling proteins and growth factor receptors, the association with mT appears to be mediated by the ability of the SH2 and PTB domains of these proteins to recognize specific phosphotyrosine residues on the viral protein (21, 33, 139). Once Shc and PLC$_\gamma$ are recruited to the phosphorylated mT complex, they also undergo tyrosine phosphorylation, an event that is also probably catalyzed by *src* kinases (9, 21, 33). Phosphorylated Shc bound to mT recruits Grb2 to the mT complex, which in turn activates the *ras* protooncogene product and eventually the MAP kinase signaling pathway (9, 21, 22). In addition, association between mT and PI3K results in increased PI3K enzymatic activity and the elevation of PI3K products in the cells (84). Thus, the interactions between cellular signaling molecules and mT mobilize signaling pathways that are similar to those elicited by growth factor treatment. Indeed, analysis of mT mutants indicates that efficient transformation depends on the assembly of these complexes and the consequent activation of the cellular proteins (9, 33, 49, 134). Similarly, a dominant negative mutant of Shc blocked mT transformation (9), and *ras* activity is essential for mT transformation (15, 110). In animals, the efficiency of tumor formation or the spectrum of tumors formed is affected by mutations in mT that inhibit association with the cellular signaling proteins (17, 138). In addition to proteins known to be involved in propagating mitogenic signals, several other interesting cell proteins are present in the mT complex and may play roles in transformation. The 14-3-3 proteins also form a complex with mT and may activate the raf kinase (44, 46, 64, 103). mT also forms a complex with phosphoprotein phosphatase 2A, an interaction proposed to be important for the assembly or the correct subcellular distribution of the mT signaling complex (13, 49, 104, 129).

Thus, the structure and activity of the complex of mT and its associated cellular proteins closely resemble that of the transmembrane and cytoplasmic domains of an activated growth factor receptor bound to proteins involved in signal transduction (34). However, unlike ligand-activated growth factor receptors, mT appears to exist in activated kinase complexes as a monomer, and dimerization of mT is not required for cell transformation (23, 118). Because mT is devoid of intrinsic tyrosine kinase activity but associates with and

activates *src* kinases, mT is most similar to activated cytokine receptors that are tyrosine phosphorylated by the action of associated cytoplasmic kinases such as JAK kinases. These results indicate that the mT protein mimics an activated growth factor receptor and thereby delivers a constitutive mitogenic signal to cells.

Epstein-Barr Virus LMP-1

Epstein-Barr Virus (EBV) encodes latent membrane protein 1 (LMP-1), a 62 kDa integral membrane protein with six transmembrane domains that is constitutively aggregated in the plasma membrane (57). LMP-1 is expressed in B lymphocytes latently infected with EBV, and it is required for B cell transformation by the virus (57, 71). In addition, LMP-1 is sufficient to induce stable growth transformation of cultured rodent fibroblasts (4, 130). In B cells, LMP-1 induces the expression of a variety of cellular genes including B cell activation markers and Bcl-2 (85, 88, 113). LMP-1 expression also results in activation of the transcription factor NFκB (55, 58, 95).

The structure of LMP-1 is somewhat reminiscent of G-protein coupled receptors, leading to the suggestion that LMP-1 resembles a cell surface receptor (89). This notion has been confirmed by biochemical and genetic studies. To identify cellular proteins that might mediate LMP-1 transformation, Mosialis et al (98) used the yeast two-hybrid system to identify B lymphocyte proteins able to bind to a membrane-proximal segment of the cytoplasmic domain of LMP-1 required for transformation of primary B lymphocytes (72). In both yeast and B cells, it was found that this segment of LMP-1 binds to TRAFs, the factors that associate with activated tumor necrosis factor (TNF) receptor 2 and mediate its proliferative signal (98, 112). By binding various TRAFs, LMP-1 induces their constitutive aggregation in the absence of ligand and therefore appears to mimic the structure of the ligand-activated TNF receptor 2 complex (Figure 3) (98). In B-lymphocytes, the primary TNF receptor is CD40, which like LMP-1 engages multiple TRAFs and induces NFκB activation. Dominant negative mutants of TRAF2 and TRAF3 partially inhibit LMP-1-induced activation of NFκB, thus providing a genetic link between the viral protein and activation of the transcription factor (32, 70). Genetic results also suggest that the ability of LMP-1 to aggregate and bind TRAFs is responsible for NFκB activation, the initial proliferative stages of B-lymphocyte transformation, and fibroblast transformation (32, 65, 70, 95, 97, 116). The ability of LMP-1 to engage TRAFs also appears responsible for its effects in epithelial cells, including activation of NFκB, synthesis of interleukin-6, and induction of EGF receptor expression (42, 43, 94).

The study of the mechanism of LMP-1–induced lymphocyte transformation is complicated by the ability of a segment of LMP-1 downstream of the
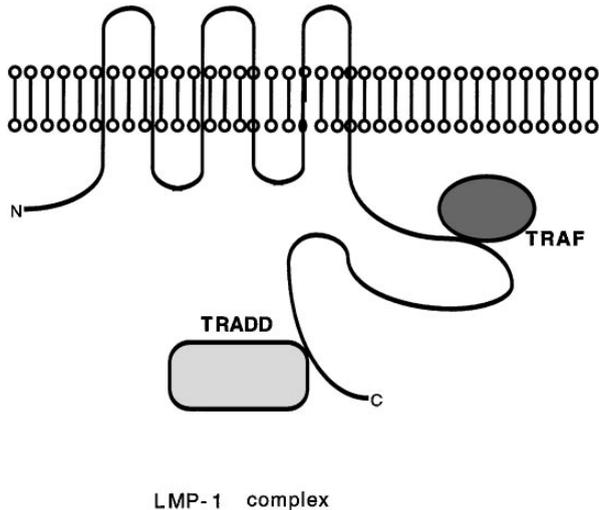
Figure 3 A model for the EBV LMP-1 signaling complex. LMP-1 is present in the plasma membrane with six membrane-spanning domains. The membrane proximal segment of the cytoplasmic carboxy-terminal tail associates with TRAFs, and the membrane-distal segment of the tail associates with TRADD. Although for simplicity only a single LMP-1 molecule is shown, LMP-1 exists in membranes in a constitutively aggregated form, so the associations represented here result in aggregation of the cellular signaling molecules.

TRAF binding site to activate NFκB by a second, independent mechanism (16, 32, 70, 116). Recently, this segment of LMP-1 has been shown to associate with another TNF receptor-associated protein, TRADD, which is involved in mediating TNF-induced NFκB activation and apoptosis (66). Mutations in the carboxyl-terminal tail of LMP-1 that block TRADD binding also impair B cell transformation and NFκB activation (66). Thus, the LMP-1/TRADD interaction appears important for the ability of LMP-1 to induce high level NFκB activation and efficient long-term outgrowth of lymphoblastoid cells. The ability of LMP-1 to engage productively two different classes of TNF receptor-associated signaling molecules, and the strikingly similar response of B lymphocytes to LMP-1 expression and TNF treatment, provides compelling reason to regard LMP-1 as a constitutively activated mimic of the TNF receptor (12, 98). It is interesting to note that upon TNF addition, TNF receptor 1 directly engages TRADD but not TRAFs, whereas TNF receptor 2 engages TRAFs but not TRADD (128). Therefore, LMP-1, which directly engages both TRADD and TRAFs, may deliver a composite signal different from that delivered by either TNF receptor 1 or TNF receptor 2 alone.

VARIATIONS ON A THEME, AND IMPLICATIONS

Growth factors are powerful mitogenic stimuli, and cells have evolved elaborate signaling cascades to transmit proliferative signals from the cell surface to the nucleus. The examples reviewed here illustrate that viral activation of these cellular growth factor signaling pathways can result in cell transformation. These examples differ in some regards from the classically described mechanisms of growth factor receptor activation, which involve circuits composed of authentic growth factors and growth factor receptors or structurally related molecules. We suggest the term "virocrine transformation" to capture the central features of the viral mechanism described here (36). This term emphasizes the viral origin of the transforming protein and indicates a mechanism of action involving activation of signal transduction pathways. The viral protein acts either by activating a cellular signaling molecule such as a growth factor receptor, as in the case of BPV E5 or SFFV gp55, or through mimicry of an activated cellular signaling molecule, as in the case of polyomavirus mT or EBV LMP-1. Importantly, viral proteins that activate signaling pathways do not necessarily bear any obvious sequence similarity to cellular signaling molecules. In fact, studies on BPV E5 and gp55 have established the principle that growth factor receptors can be activated by proteins that do not resemble natural ligands. In those cases where the viral proteins deviate markedly from the structure of the normal ligands, the nature of the interactions that drive receptor binding may be very different from the interactions responsible for binding of the normal ligand to the receptor. Virocrine transforming proteins could even activate a signaling pathway by using biochemical mechanisms that are entirely different from those utilized by the normal ligand. It is also possible that receptors and pathways activated by different means may deliver qualitatively different signals to cells. This is suggested by the finding that EPO receptor activated by EPO or by gp55 elicits different biological responses and displays differential association with cellular proteins (2, 127, 137).

The virocrine mechanism of transformation differs in some crucial aspects from the other strategy commonly employed by DNA tumor viruses, namely inactivation of cellular tumor suppressors. Tumor-suppressor proteins and the cell cycle machinery they control are widely expressed in many cell types, whereas there is considerable specificity to the signal transduction pathways that are present in various cells. Therefore, a virus utilizing a virocrine strategy can target its proliferative stimulus to a specific cell type that expresses a particular receptor or set of downstream signaling molecules. Because growth factors can promote survival or differentiation as well as induce proliferation, viral proteins that signal through these pathways can influence many aspects of cell behavior. Finally, transforming proteins that activate positive signaling pathways function

in a dominant fashion since they do not have to overcome inhibitory signals. As a consequence, low concentrations of a positive factor may be sufficient to drive proliferation. For example, it appears that a relatively small fraction of the PDGF receptor is in a complex with BPV E5, and mT binds only a small fraction of pp60$^{c\text{-}src}$ (C Henningson, D DiMaio, unpublished observations; 23). This is in contrast to the situation of viral proteins that inactivate tumor suppressor proteins, because in that case the viral protein must neutralize a substantial fraction of its cellular target to release the brakes on cell growth.

Although the examples cited in this review involve activation of the initial steps of signaling pathways, viral proteins also interact with and activate more downstream components. For example, the STP-C488 oncoprotein of herpesvirus saimiri (HVS) subgroup C forms a complex with cellular p21^{ras}, resulting in ras activation (67). Point mutations in STP that interfere with *ras* binding and activation also inhibit cell transformation, suggesting that STP induces cell transformation by activating *ras* (67). In contrast, the weak transforming protein STP A11 from HVS subgroup A forms a stable complex with pp60$^{c\text{-}src}$ (78). Although this association does not result in increased *src* activity, STP-A11-tyrosine phosphorylated by pp60$^{c\text{-}src}$ is able to bind to *lck* and *fyn*, which are *src* family members specifically expressed in T lymphocytes, the natural host cell for HVS transformation. These interactions appear mediated by the SH2 domain of *src* family members recognizing a phosphotyrosine on STP-A11. These findings raise the possibility that STP-A11 may transform cells by binding to pp60$^{c\text{-}src}$ which catalyzes tyrosine phosphorylation of the viral protein, thereby allowing it to interact with and activate other *src* family members (78). *Src* family members may also be the crucial cellular targets of the HBx protein of hepatitis B virus. This protein activates the *ras-raf*-MAP kinase signaling pathway in mammalian cells, resulting in cell cycling (6, 7, 30, 100). Recent evidence indicates that HBx activates *src* family members, which are then responsible for activation of the downstream signaling pathway (77). The mechanism of *src* activation by HBx has not been established.

There are also viral proteins that bind cellular signaling proteins and inhibit their activity, thereby modulating the response of cells to the normal ligands. An attractive mechanism for such an effect is that a viral protein may act in a dominant negative fashion by interacting with a subset of signaling proteins that is not sufficient to initiate a signal or by interacting with the entire complement of signaling molecules without being able to induce the next step in the signaling cascade. This may be the case for LMP-2, an EBV-encoded membrane protein that is tyrosine-phosphorylated and forms a complex with the *src* family protein tyrosine kinases *c-lyn* and *c-syk* in B lymphocytes transformed by EBV (18, 86). However, unlike polyoma mT, which activates *src* family members, LMP-2 inhibits them (86, 92). Specifically, LMP-2 inhibits the effects

of surface immunoglobulin cross-linking on tyrosine kinase activity, activation of downstream signaling events such as substrate tyrosine phosphorylation and calcium mobilization, and reactivation of latent EBV (92, 93). Considering that the normal B-lymphocyte response to immunoglobulin cross-linking is activation of *src* kinases, LMP-2 may be acting as a dominant negative decoy by binding to these signaling proteins without having the ability to activate them. In fact, the specific short amino-acid sequences on LMP-2 required for kinase association and inhibition are the same motifs used by normal B-cell signaling proteins to assemble active kinase complexes (47). Thus, LMP-2 appears to be a negative regulator of the *src* family kinases in B lymphocytes, thereby playing a role in the maintenance of the latent state.

Another viral protein that regulates tyrosine kinase activity in lymphocytes is the Tip protein of various strains of herpesvirus saimiri, a protein that cooperates with STP in inducing T-lymphocyte transformation (38). In transformed cells, Tip is tyrosine-phosphorylated and in a complex with the T cell specific *src* family kinase $p56^{c-lck}$ (8, 68, 87). $p56^{c-lck}$ can tyrosine phosphorylate Tip in vitro (8), but the biological consequences of Tip/$p56^{c-lck}$ complex formation are unclear. Some reports maintain that this interaction results in stimulation of *lck* tyrosine kinase activity, but other evidence indicates that Tip downregulates the activity of $p56^{c-lck}$ and inhibits *lck*-mediated signal transduction in T cells (39, 54, 69, 135). HVS encoding a Tip mutant unable to bind *lck* causes more severe neoplastic disease in animals than does wild type HVS, lending further support to the conclusion that the Tip-*lck* interaction is not essential for oncogenicity and indeed may inhibit transformation (39). If Tip, in fact, inhibits tyrosine kinase signaling in T cells, it would seem quite analogous to EBV LMP-2, which has similar effects in B cells. A major role of Tip, as appears to be the case for LMP-2, may be in establishing or maintaining the latent viral state. Tip also interacts with another cellular protein, Tap, and this interaction appears to affect NFκB activity and other aspects of the T cell response to Tip (140).

Small viral proteins such as BPV E5 may also serve as models for the rational design of small proteins and other molecules that can influence cellular signaling pathways. The tendency of membrane spanning regions to adopt an α-helical conformation and the availability of powerful computational methods to explore the energetics of potential interhelical interactions suggests that the transmembrane domains of growth factor receptors may be particularly suitable targets for such an approach (1, 80). Proteins that modulate growth factor signaling pathways may have important research and clinical uses. For example, the ability to activate growth factor receptors in the central nervous system or to inactivate the erbB2 growth factor receptor in breast carcinoma may have great clinical utility. The targeted expression of such proteins has an advantage over systemic delivery of drugs that modulate receptor activity, because the effects

of these proteins may be restricted to the cell in which they are expressed, thereby providing a mechanism to fine-tune the response of particular cells to a circulating growth factor. The specificity of the BPV E5 protein suggests that it may even be possible to design molecules that display greater selectivity than do the natural ligands of the receptor. Similarly, the ability of LMP-1 to bind directly both TRAFs and TRADD suggests that proteins with novel signaling properties can be constructed.

As the mechanisms by which viral proteins transform cells continue to be elucidated, additional examples of virocrine transformation will be identified. There is a great diversity of signaling molecules and pathways in cells. Therefore, it is likely that viral transforming proteins will show a comparable diversity in their mode of action. In fact, it is possible that study of such viral proteins will identify novel cellular signaling proteins and pathways, much as study of SV40 large T antigen resulted in the identification of p53. Thus, we can be confident that studies on viral transforming proteins will provide new insights into cellular signaling pathways and may suggest novel approaches for the manipulation of these pathways.

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