Selective Activation of T Cell Kinase p56\textsuperscript{ck} by Herpesvirus saimiri Protein Tip*

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Infection with Herpesvirus saimiri, a T lymphotropic virus of non-human primates, immortalizes human T cells in vitro. The cells show a mature activated phenotype and retain their antigen specificity. We have previously shown that in H. saimiri transformed cells a viral gene product termed tyrosine kinase interacting protein (Tip) associates with the T cell-specific tyrosine kinase p56\textsuperscript{ck} and becomes phosphorylated by the enzyme on tyrosine residues. Here we show that p56\textsuperscript{ck} is activated by recombinant and native Tip in cell-free systems. A dramatic increase of Lck activity was also observed in T cell lines transfected with Tip. p60\textsuperscript{lyn} and p53/56\textsuperscript{ terminates the other Src-related kinases expressed in H. saimiri transformed T cells, did not phosphorylate Tip, and were not activated by the protein. The selective activation of p56\textsuperscript{ck} by Tip could contribute to the transformed phenotype of H. saimiri infected cells, and it might explain the T cell selectivity of the transformation event.

Certain strains of Herpesvirus saimiri readily transform human T cells to continuous growth in cell culture (1). Remarkably, the antigen-specific response of the immortalized T cells as well as their mature and activated phenotype are very stable. The T cell receptor-CD3 complex, the CD4 or CD8 coreceptors, CD2, and the IL-2\textsuperscript{1} receptors remain present and functionally competent over many months in culture (2–6), suggesting that the signaling apparatus of mature T cells may be required for the viral transformation. The viral genome harbors approximately 75 open reading frames. Many have been tested for transcription in transformed human cells but, so far, only ORF 1 and ORF 2 (7) encoded on a single bicistronic mRNA have been found in the permanently growing cells. We have previously shown, that the gene product of ORF 1, tyrosine kinase interacting protein (Tip) is expressed in transformed cells. Tip associates with the protein tyrosine kinase p56\textsuperscript{ck} and can be phosphorylated by this enzyme in vitro (8). p56\textsuperscript{ck} is a non-receptor tyrosine kinase of the Src family which is selectively expressed in thymocytes and mature T cells. The kinase tightly associates with the coreceptor molecules CD4 or CD8 and becomes activated after cross-linking of these receptors on the cell surface. One of the substrates of p56\textsuperscript{ck} is the \(\gamma\)-chain of the T cell receptor, and T cells deficient in p56\textsuperscript{ck} fail to respond to T cell receptor mediated signals. This attributes a crucial role to the enzyme in the antigen-specific response of T cells (9, 10). The ORF 2-encoded saimiri transformation-associated protein (StpC) is also expressed in H. saimiri transformed human T cells and might complement the action of Tip. StpC is a strong oncoprotein in rodent fibroblasts and epithelial cells of transgenic mice, but not in T cells (11, 12). The mechanism of oncogenesis by StpC is not known. Transformation of T cells probably requires the action of Tip on the T cell-specific kinase p56\textsuperscript{ck} in addition to StpC. Here we further characterize this interaction and show that Tip selectively interacts with p56\textsuperscript{ck}, but not with the other Src-related kinases p60\textsuperscript{lyn} or p53/56\textsuperscript{lyn} which are expressed in human T cells immortalized by H. saimiri. Furthermore, this interaction leads to a dramatic activation of the enzyme. This may explain why many human cell types can be infected by H. saimiri but only T cells are transformed to permanent growth.

EXPERIMENTAL PROCEDURES

Cells and Cell Culture—The human CD4+ T cell clones 61/39, 61/48, 68/4, and 68/5 as well as their H. saimiri immortalized derivative T cell lines 39-HVS, 48-HVS, 68/4-HVS, and 68/5-HVS have been described previously (2, 8). CB15 is a CD4+ T cell line derived from cord blood (1), 3C a CD8+ T cell line derived from peripheral blood by infection with H. saimiri. Nontransformed T cell clones were cultured in complete medium (CM, RPMI 1640, 4 mM L-glutamine, 50 \(\mu\)g/ml gentamicin, and 10% screened fetal bovine serum) supplemented with 100 units/ml recombinant human IL-2 (Eurocetus, Amsterdam, Netherlands) at 37 °C, 5% CO\textsubscript{2} in humidified atmosphere. They were restimulated every 14 days with 0.5 \(\mu\)g/ml phytohemagglutinin in the presence of irradiated peripheral blood mononuclear cells. After transformation with H. saimiri, human T cell lines were kept in 50% CM medium (Vitromex, Vilshofen, Germany) and 50% CM in the presence of 100 units/ml IL-2. Jurkat cells were derived from the original culture (13). Raji and BJ AB are Burkitt’s Lymphoma lines. BW clones, a murine thymoma line stably transfected with the murine CD3-\(\gamma\)-chain and the human \(\gamma\)-chain (14), was a gift of B. Malissen. Raji, BJ AB, and BW clones were grown in complete medium.

Antiserum to the unique regions of Src-related kinases were raised by immunizing rabbits with fusion proteins containing the unique regions of murine p56\textsuperscript{ck}, p60\textsuperscript{lyn}, p53/56\textsuperscript{ terminates, and p62/63- and glutathione S-transferase. Antiserum to the Src-kinases p56\textsuperscript{ck}, p59\textsuperscript{ck}, and p58\textsuperscript{lyn} were generated by immunization of rabbits with synthetic peptides corresponding to the unique regions (15). A monoclonal antibody reactive with p60\textsuperscript{lyn} has been described (15). The anti-phosphotyrosine antibody PY-20 was purchased from Transduction Laboratories (Lexington, KY), a rabbit antiserum to phosphotyrosine was obtained from UBI (Lake Placid, NY). Antiserum against Tip were a gift of H. Fickenscher, Erlangen, Germany, and have been used in immunoblot analysis. The nucleotide sequence(s) reported in this paper has been submitted to the GenBank\textsuperscript{1} EMBL Data Bank with accession number M55264.

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1 The abbreviations used are: IL-2, interleukin-2; GST, glutathione S-transferase; HTLV-I, human T lymphotropic virus type I; PAGE, polyacrylamide gel electrophoresis; Stp, saimiri transformation-associated protein; Tip, tyrosine kinase interacting protein; MOPS, 4-morpholinopropanesulfonic acid.
described (8). The monoclonal anti-Tip antibody BN12 was generated from spleen cells of Balb/c mice immunized with a β-Gal-Tip fusion protein which has been described (8). BN12 reacts with β-Gal-Tip in enzyme-linked immunosorbent assay and immunoblot sytems, and it stains a 40-kDa band in cell lines transfected with full-length Tip (see below) but not in nontransformed cells. Rabbit anti-mouse polyclonal serum was purchased from Dianova (Hamburg, Germany).

Immunoprecipitations—Cells (1.5 × 10⁶/1 ml in serum-free medium) were lysed in TNE buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 2 mM EDTA, and 1% Nonidet P-40) supplemented with 1 mM sodium orthovanadate (Na3VO4), 5 mM NaF, and 10 μg/ml each of aprotinin and leupeptin (Sigma) for 20 min on ice. Lysates were cleared at 14,000 g for 30 min, and the protein concentration in the supernatants was determined. 5 μl of antiseraum of protein were added for at least 1 h at 4 ºC to precipitate Src-related kinases. This was followed by incubation with 50 μl of a (⅓/v/v) suspension of Staphylococcus aureus particles (Pansorbin, Calbiochem). In the case of murine antibodies, the S. aureus particles were preincubated with 50 μg/ml rabbit anti-mouse antibodies and washed. The immunoprecipitates were washed five times in TNE buffer. For reprecipitation after the in vitro phosphotransferase reaction (see below), the phosphotransferase reaction was stopped by boiling samples in 1% SDS, 10 mM Tris, and 1 mM sodium orthovanadate. This dissociated the immune complexes. This detergent was then added at least 110/100 with 1% Triton, 10 mM Tris, 50 mM NaCl, 5 mM EDTA and the solution was precleared from the remaining antibody-antigen complex with 50 μl of S. aureus suspension followed by centrifugation. The supernatants were incubated with the second antibody for 1 h at 4 ºC and precipitated with S. aureus as described above.

In Vitro Phosphotransferase Assay—Immunoprecipitates were washed once in kinase buffer (20 mM MOPS, pH 7.0, and 5 mM MgCl2). The pellets were then incubated for 5 min at room temperature in 25 μl of kinase assay mixture containing 1 μM ATP (Boehringer Mannheim), 10 μl of [γ-32P]ATP (Amersham) and 2 μg of rabbit muscle enolase (Sigma) in kinase buffer. The phosphotransferase reaction was stopped with sample buffer (62.5 mM Tris, pH 6.8, 2% SDS, 10% glycerol, and 5% β-mercaptoethanol). Samples were then incubated for 30 min at room temperature and microfuged. Supernatants were boiled for 5 min before separation by 8% SDS-PAGE. Gels were dried and autoradiographed. For the experiments described in Fig. 4D, 0.1 μg of recombinant p56lck was incubated in 50 μl of kinase buffer in the presence of different amounts of β-Gal or β-Gal-Tip as indicated. The kinase reaction was started by the addition of 25 μCi of [γ-32P]ATP and carried out for 5 min at room temperature. It was stopped with sample buffer, and samples were separated by 8% SDS-PAGE. Gels were dried and exposed to Kodak XAR5 film. Quantitative analysis was performed on a PhosphorImager (Molecular Dynamics).

Immunoblots—For immunoblotting, cell lysates or immunoprecipitates were separated by 8% or 12% SDS-PAGE and transferred to nitrocellulose membranes. Blots were incubated for 1 h at room temperature in blocking buffer (phosphate-buffered saline, pH 7.4, 5% nonfat dry milk, and 0.05% Tween 20) at room temperature in blocking buffer (phosphate-buffered saline, pH 7.4, 5% nonfat dry milk, and 0.05% Tween 20) followed by incubation with antisera diluted in blocking buffer. After thorough washing in phosphate-buffered saline containing 0.1% Tween 20, blots were incubated for 1 h at room temperature. It was stopped with sample buffer, and samples were separated by 8% SDS-PAGE. Gels were dried and exposed to Kodak XAR5 film. Quantitative analysis was performed on a PhosphorImager (Molecular Dynamics). For the experiment described in Fig. 6B, the blot was incubated with the mouse monoclonal antibody BN12. Bands were visualized by incubation with goat anti-mouse Ig coupled to horseradish peroxidase followed by enhanced chemiluminescence (Amerham) according to the manufacturer’s instructions.

Recombinant Proteins and Cell Transfection—A fusion protein of p56lck-phosphotransferase (GST-Lck) was produced in a baculovirus expression system and purified by affinity chromatography on glutathione-Sepharose columns (16). For the experiment described in Fig. 4D, GST-Lck was cleaved with thrombin (ICN, Meckenheim, Germany), and uncleaved GST-Lck as well as GST were precipitated with glutathione-Agarose (Sigma). The supernatant contained p56lck. A fusion protein of aminoterminus tagged Tip and β-galactosidase as well as β-galactosidase alone were gifts of B. Biesinger, Erlangen, Germany, and have been described (8). Tip-encoding sequences on the plasmid pAB/8 (gift of B. Biesinger, '87, EMBL accession number M55264) were amplified by polymerase chain reaction with the following primers: forward, CGCCGGCTCGAGATGGCAATAAGAGGAGAAGA; reverse, CGGCCGCGCCGCTACTTTTCCATTATGG. This inserted a Xho restriction site 3’ of the coding regions which were used to clone the gene into the eukaryotic expression vector BCMGSNeo (gift of H. Kara-
we transfected the mouse T cell line BW\textsubscript{\textgreek{a}}\textsubscript{\textgreek{c}}. BW\textsubscript{\textgreek{a}}\textsubscript{\textgreek{c}}-Tip expresses about 1 \mu g of Tip/mg of cell protein as determined by comparison with \beta-Gal-Tip on immunoblots (Fig. 3A and data not shown), but about 200-fold less p56\textsuperscript{ck} than the human T cell line Jurkat which is representative of the other T cell lines used in our experiments (Fig. 3B). Immune complex phosphorylation assays performed with p56\textsuperscript{ck} and p60\textsuperscript{yn} immunoprecipitated directly from BW\textsubscript{\textgreek{a}}\textsubscript{\textgreek{c}}-Tip again showed selective association and phosphorylation of Tip by p56\textsuperscript{ck} (Fig. 3A and data not shown). Finally, we used recombinant GST-Lck, GST-Fyn, and GST-Lyn as well as Lck, Fyn, and Lyn precipitated from non-infected human T cells and B cells as above to co-immunoprecipitate Tip from BW\textsubscript{\textgreek{a}}\textsubscript{\textgreek{c}}-Tip lysates which had been depleted of endogenous Lck. The results confirmed that p60\textsuperscript{yn} and p53/56\textsuperscript{yn} are not able to bind and phosphorylate H. saimiri Tip (data not shown).

Increase of p56\textsuperscript{ck}. Enzymatic Activity by Tip—p56\textsuperscript{ck} was immunoprecipitated from Jurkat cell lysates and the enzymatic activity was determined by a phosphotransferase assay in the presence of rabbit muscle enolase as exogenous substrate. Addition of increasing concentrations of \beta-Gal-Tip fusion protein augmented the enzymatic activity of Lck by a factor of 3.4 while \beta-Gal alone showed a negative effect if any (Fig. 4, A and B). Similarly, lysates of BW\textsubscript{\textgreek{a}}\textsubscript{\textgreek{c}}-Tip depleted of endogenous p56\textsuperscript{ck} enhanced the phosphorylation of enolase by Lck about 6-fold in this system, while lysates of the parent cell line did not affect Lck activity (Fig. 4C). Recombinant Lck could be used instead of the Lck immune complexes in the latter experiments with comparable results (data not shown). Neither \beta-Gal-Tip nor native Tip precipitated from Lck-free cell lysates ever showed kinase activity in these systems (data not shown). Roughly equal amounts of both recombinant (Fig. 4A) and cell-derived (Fig. 4C) Tip were needed to activate Lck; the amount of p40\textsuperscript{tip} present in 1 mg of BW\textsubscript{\textgreek{a}}\textsubscript{\textgreek{c}}-Tip cell protein (i.e., 1 \mu g) is equivalent to 2 \mu g of \beta-Gal-Tip (M<sub>r</sub> = 90,000). At very high Tip concentrations, phosphorylation of enolase decreased again due to substrate competition (data not shown). This precludes accurate measurement of the maximal activation of Lck by Tip. The factor of about 3–6 is, therefore, a conservative estimate of the enzymatic activation of p56\textsuperscript{ck} by Tip. To exclude that activation of p56\textsuperscript{ck} by viral Tip is mediated by other T cell-derived molecules which might have coprecipitated with either Lck or Tip in the experiments shown in Fig. 4, A–C, Lck was cleaved from GST-Lck by thrombin and incubated with \beta-Gal-Tip. Again, \beta-Gal-Tip but not the fusion partner \beta-Gal enhanced the enzymatic activity of this recombinant p56\textsuperscript{ck} preparation as shown by an increase of Lck autophosphorylation (Fig. 4D). Re-evaluation of an effect of Tip on p60\textsuperscript{yn} or p53/56\textsuperscript{yn} in the presence of enolase never showed activation of these enzymes (data not shown).

Enzymatic Activity of Lck is Increased in T Cells Transfected with Tip—Measurement of the specific activity of Lck in BW\textsubscript{\textgreek{a}}\textsubscript{\textgreek{c}} and BW\textsubscript{\textgreek{a}}\textsubscript{\textgreek{c}}-Tip showed a dramatic increase in the Tip transfected cell line (Fig. 5A). BW\textsubscript{\textgreek{a}}\textsubscript{\textgreek{c}} cells express extremely low quantities of Lck (Fig. 3B) precluding measurement of its activity in untransfected cells and necessitating precipitation from large protein amounts prior to detection on immunoblots (Fig. 5B). In contrast, specific activation of Lck could not be
demonstrated reproducibly in H. saimiri infected T cells. Tip is expressed in very low amounts in these cells and can be detected only by the sensitive phosphotransferase assay but not on immunoblots. Any effect of Tip on Lck, which is expressed in very low amounts in these cells and can be detected on immunoblots, would be masked by the background activity on immunoblots. Any effect of Tip on Lck, which is very abundant only by the sensitive phosphotransferase assay but not expressed in very low amounts in these cells and can be detected on immunoblots, might not be masked by excess of inactive enzyme. Expectedly, the factor of 3–6 observed in our system is, therefore, much higher than what is usually observed in T cells. The positions of Lck, enolase, and Tip are shown on the right, molecular mass standards are on the left.

DISCUSSION

Previously we have suggested that two factors cooperate in the transformation of human T cells by H. saimiri with StpC acting as the basic oncoprotein complemented by the T cell-specific action of Tip (8). The results of our analysis of the Tip/Lck interaction presented here lend support to our hypothesis. Tip binds to and directly activates the Src-related kinase p56\(^{1ck}\), which is expressed in large amounts in T cells and thymocytes. Because of substrate competition at high concentrations of Tip, the maximal enzymatic activation of Lck could not be determined. The factor of 3–6 observed in our system is, therefore, a conservative estimate. Besides Lck, which is by far the most abundant Src-related kinase in human T cells, we observed significant activities of p60\(^{fyn}\) and, unexpectedly, also of p53\(^{m}\) in transformed T cells. However, neither p60\(^{m}\) nor p53\(^{m}\) was affected by Tip. Expression of p53\(^{m}\) in T cells is unusual. It has been observed previously only after infection of T cells by the retrovirus HTLV-I (18). This could be partially explained by transactivation of the Lyn promoter by the HTLV-I-encoded transcription factor p40\(^{tax}\). However, p40\(^{tax}\) is not related to the H. saimiri encoded p40\(^{tip}\) (19). Furthermore, the appearance of Lyn in HTLV-I-infected T cells was accompanied by a gradual loss of Lck activity, which correlated with dedifferentiation of these cells to IL-2-independent growth (18). Neither decrease of Lck abundance and activity nor IL-2 independence was ob-

\(2\) J. B. Bolen, unpublished observations.
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FIG. 6. Tyrosine phosphorylation analysis. A, transformation by H. saimiri increases the cellular content of phosphotyrosine. Phosphotyrosine-containing proteins were precipitated from 2 mg of protein of T cell lines transformed in H. saimiri, from their nontransformed parental clones and from phytohemagglutinin-stimulated T cell blasts using the monoclonal antibody PY-20. For control, only PY-20 but no cell lysate was added. After separation by 12% SDS-PAGE and transfer to a nitrocellulose membrane, bands containing phosphotyrosine were revealed with a polyclonal antiserum and \textsuperscript{125}I-protein A followed by autoradiography. B, Tip is phosphorylated on tyrosine residues in vivo. Phosphotyrosine-containing proteins were precipitated from cell lysates (200 \mu g of protein) as described above (lanes 1–3) and separated by 8% SDS-PAGE. 50 \mu g of whole cell lysate from the same cell lines was loaded in lanes 4–6 of the same gel. Proteins were transferred to nitrocellulose, and the blot was probed with the anti-Tip monoclonal antibody BN12. Bands were visualized by enhanced chemiluminescence. Tip expression in H. saimiri transformed cells (e.g. 39-HVS) regularly escaped detection by immunoblotting. The positions of Tip and the Ig heavy and light chains are shown on the right, the molecular mass markers are on the left.

What might be the consequences of the Tip-mediated activation of Lck, an enzyme which plays a pivotal role in T cell signaling? It is well documented that p56\textsuperscript{lck} which is constitutively active by virtue of mutation or deletion of its regulatory tyrosine 505 acts as an oncprotein in fibroblasts. In these cells, wild-type p56\textsuperscript{lck} was not effective even when overexpressed (20–22). In thymocytes of p56\textsuperscript{lck} transgenic mice, increased expression of both wild-type and active mutants of Lck induced the formation of thymomas (23). Therefore, activation of p56\textsuperscript{lck} by Tip may contribute to the transformed phenotype of H. saimiri infected T cells. On the other hand, transfection of Tip into fibroblasts did not result in transformation (11). However, fibroblasts do not express Lck, and our data show that enzymatic activation by Tip is highly selective for this Src family member. In agreement with this, no kinase activity was associated with Tip in transfected murine fibroblasts.\textsuperscript{3}

Transfection with constitutively active p56\textsuperscript{lck} resulted in increased responsiveness to T cell receptor-mediated signals in a murine T cell hybridoma, a model for mature T cells (24). Enhanced tyrosine phosphorylation in response to TcR triggering in T cells transformed by H. saimiri has been documented earlier (3). To investigate whether this could be the result of Lck activation by Tip, we first attempted to demonstrate an increase of the specific enzymatic activity of Lck in T cells transformed by the virus. While a dramatic increase of p56\textsuperscript{lck} activity was readily observed in T cells overexpressing Tip after transfection, we could not clearly show it in the T cell lines transformed by wild-type virus. This may be due to the very low level of Tip expression in these cells. Activation of a small fraction of Lck by the associated Tip would then be masked by the excess of nonactivated enzyme. However, the small fraction of Tip-activated Lck might be non-randomly distributed in the cells and still have a significant effect on the phosphorylation of selected substrates. We have, therefore, tested the basal level of tyrosine phosphorylation in T cells without any further stimulation. This most clearly reflects the influence of H. saimiri. Three bands with molecular masses of around 70, 55, and 30 kDa were more strongly phosphorylated in the virally transformed T cells. The prominent 55-kDa band, which faintly also appears in the nontransformed T cells, very likely corresponds to p56\textsuperscript{lck}, which is by far the most abundant and most active Src family kinase in all our T cell lines. Lyn is much less active than Lck in H. saimiri transformed T cells, and it is not expressed in the nontransformed parental cell lines. Increased phosphorylation of p56\textsuperscript{lck} is probably a direct effect of its activation by Tip. However, it cannot be excluded that p56\textsuperscript{lck} or one of the other prominent bands on the phosphotyrosine immunoblot might also be a substrate of p53\textsuperscript{56}/p56\textsuperscript{m}. Other nonidentified kinases or phosphatases may also play a role.

One of the most striking changes in the behavior of human T cells transformed by H. saimiri is their hyper-reactivity to ligation of CD2. Because the ligand for CD2, LFA-3, is also expressed on these cells, cell contact leads to ligation of CD2, which results in autostimulation, IL-2 production, and autocrine growth (6). Blockade of the CD2/LFA-3 interaction can halt the growth of H. saimiri transformed T cells, so that this autocrine loop appears to be essential for the transformed phenotype (6).\textsuperscript{4} p56\textsuperscript{lck} has been found in complex with CD2, and the enzyme becomes activated after CD2 cross-linking (25, 26). Therefore, activation of p56\textsuperscript{lck} by Tip could enhance the T cell responses to CD2. However, the dissection of CD2-mediated signaling in H. saimiri transformed T cells requires further investigation.

The mechanism by which Tip activates p56\textsuperscript{lck} is not understood. It is likely that the hydrophobic stretch at the carboxy terminus of Tip inserts into the cell membrane and brings the molecule into proximity of Lck. In fact, the Tip homologues of a related H. saimiri strain is located in the outer cell membrane (27). The Tip sequence contains a 10-amino acid stretch with strong similarity to a sequence in the carboxy terminus of Src family kinases as well as a type II SH3-domain binding motif (8). These are necessary and sufficient for efficient binding of Tip to Lck (28). Analysis of the crystal structure of the regulatory domains of Lck revealed dimerization of the SH2/SH3 domain structure and binding of the regulatory carboxyl-terminal peptide containing tyrosine 505 to the contact area of the two Lck molecules (29). At the cell membrane, such a closed conformation could block the catalytic domains of Lck and inactivate the enzyme. The authors suggest that ligand binding to the SH2 or SH3 domain of Lck might induce the open, enzymatically active state (29). We have shown that Tip is

\textsuperscript{3} N. Wiese and B. M. Bröker, unpublished observations.

\textsuperscript{4} B. M. Bröker, unpublished observation.
phosphorylated on tyrosine residues in transfected T cells, and that it can be phosphorylated by Lck on tyrosine residues in cell-free systems (8). But phosphotyrosine does not interfere with the association of Tip with Lck making involvement of the SH2 domain of Lck unlikely. However, interaction of the class II SH3 binding motif with the SH3 domain of Lck could induce the active open conformation of the enzyme.

Polyoma virus middle T antigen is another viral protein which binds and activates Src family kinases, namely p60src, p62src, and p59fyn (30). Besides Src family kinases, middle T binds a multitude of cellular proteins involved in signal transduction, the function of which seems to converge in the activation of the mitogen-activated protein kinase pathway (30). Whether there are other signaling molecules associated with Tip remains to be discovered. It could be that the oncprotein StpC complements Tip so that the two factors together achieve a function comparable to that of polyoma middle T antigen.

The relevance of Tip-induced activation of p56lck for the transformation of human T cells by H. saimiri remains to be finally proven. Our data support the notion that in the presence of the oncprotein StpC the action of Tip on the T cell kinase p56lck could be a decisive factor. This would explain the T cell transformation by which binds and activates Src family kinases, namely p60c-src, the active open conformation of the enzyme. IISH3 binding motif with the SH3 domain of Lck could induce SH2 domain of Lck unlikely. However, interaction of the class II SH3 binding motif with the SH3 domain of Lck could induce the active open conformation of the enzyme.