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Interaction of Nuclear Protein p140 with Human Immunodeficiency Virus Type 1 TAR RNA in Mitogen-Activated Primary Human T Lymphocytes

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Several lines of evidence suggest that cellular proteins play a role during human immunodeficiency virus type 1 (HIV-1) Tat-mediated *trans* activation. A recent report from this laboratory has shown that a 140-kDa HeLa nuclear protein (p140) binds specifically to the lower stem region of the Tat response element, TAR RNA. Since HIV-1 *trans* activation is most efficient in proliferating T cells, we investigated the binding of p140 to TAR RNA in unstimulated and mitogen-activated, G_1-phase primary T lymphocytes. TAR RNA/protein-binding activity was low in resting cells but increased significantly within 2 h of activation and remained elevated for at least 48 h. Corresponding increases in p140 protein levels were observed with most but not all donors, suggesting that an additional nuclear factor(s) may be required for efficient binding of this protein to TAR RNA in activated T cells.

Although quiescent $CD4^+$ T helper (T_H) cells can be infected by the human immunodeficiency virus type 1 (HIV-1), productive HIV replication is dependent upon T-lymphocyte activation (8, 11, 13, 26, 38, 44, 45). Other viruses, such as DNA viruses and oncoretroviruses, require their host cells to undergo specific cell cycle events (e.g., DNA replication and mitosis, respectively) (5, 22). It has been reported, however, that highly efficient HIV-1 reverse transcription, integration, and replication can occur in growth-arrested HeLa-T4 cells and in S-phase-arrested T-cell lines (23, 24). Therefore, HIV replication would appear to depend mostly on T-cell activation events (i.e., the transition from G_0 to G_1) rather than T-cell proliferative events (i.e., S phase or mitosis) (24).

T-cell activation begins when the T-cell receptor-CD4 complex interacts with a major histocompatibility complex (MHC) type II protein, presenting processed antigen (MHC/PEP), on the surface of an antigen-presenting cell (reviewed in references 19, 31, and 42). The production of interleukin-2 and its receptor, following activation, subsequently allows the T cell to progress through the cell cycle and divide (1). Plant lectins such as phytohemagglutinin (PHA), Ca^{2+} ionophores, and phorbol esters are examples of mitogens commonly used to activate T cells in vitro (40, 41). These mitogens mimic the events of T-cell activation (27, 30).

The HIV-1 processes which appear to require lymphocyte activation include complete reverse transcription, integration, effective proviral activation, and efficient Tat-mediated *trans* activation (6, 8, 18, 28, 38, 39, 44). To date, the mechanism by which Tat-mediated *trans* activation occurs is not precisely understood. The HIV-1 Tat RNA response element TAR (nucleotides +1 to +80) is a stable stem-loop structure present within the 5′ nontranslated region of all HIV messages (13). The direct interaction of Tat with TAR results in a potent increase in the production of full-length viral transcripts (4, 34). In this manner, HIV is able to upregulate or maximize its own transcriptional efficiency (2). The Tat-TAR interaction may function by increasing transcriptional initiation, elongation efficiency, or both (10, 21). Interestingly, the binding of Tat to TAR RNA mutants which lack the loop or have altered loop sequences is not sufficient to support *trans* activation in vivo (34). This observation suggests that a cellular protein(s), which also interacts with TAR, is necessary for *trans* activation. Furthermore, the efficiency of Tat-mediated *trans* activation varies greatly depending upon the cell type (3), implying that cell type-specific proteins play a role during the *trans* activation process. Indeed, several human, cellular TAR-binding proteins have been identified (12, 25, 35, 43). We have shown that a 140-kDa HeLa nuclear protein (p140) binds specifically to the lower stem region of TAR RNA (33). Since efficient HIV-1 transcription and Tat-mediated *trans* activation require T-cell activation, we investigated whether the interaction between p140 and HIV TAR RNA was similarly regulated in mitogen-activated primary human T lymphocytes. Human peripheral blood T cells were derived from normal, adult donors. These donors were not permitted to be on medication of any kind and were given blood analyses prior to donation. All donors were found to be negative for HIV and hepatitis A, B, and C viruses.

Cell cycle status of activated T lymphocytes. Primary human T lymphocytes (90 to 95% pure by countercurrent centrifugal elutriation) were maintained at 37°C and 5% carbon dioxide in RPMI 1640 medium containing 10% fetal bovine serum (heat inactivated for 1 h at 56°C), 1.5% penicillin-streptomycin, 1%

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glutamine, and 0.15% fungizone. The cells (2×10^6/ml) were activated by treatment with PHA (Murex Diagnostics, Research Triangle Park, N.C.) (1 μg/ml) or a combination of PHA and phorbol myristate acetate (PMA; Sigma Chemical Company, St. Louis, Mo.) (5 ng/ml) for 24 to 48 h.

To confirm that the cells had exited G_0 following mitogen treatment, we assayed for the presence of a G_1-phase-specific protein, cyclin E, at 24 h poststimulation. Cyclin E and its kinase partner, CDK2, are responsible for regulating the transition from G_1 into S (36). Cyclin E RNA and protein levels typically appear during late G_1, and cyclin E/CDK2 kinase activity will peak at the G_1/S-phase border (36). Cyclin E protein levels were assayed by Western (immunoblot) analysis by the Amersham enhanced chemiluminescence protocol. Polyclonal anti-human cyclin E antiserum (kindly provided by Jim Roberts, Fred Hutchinson Cancer Research Center, Seattle, Wash.) was used at a 1:3,000 dilution, and monoclonal anti-human cyclin E antiserum (Santa Cruz Biotechnology, Inc., Santa Cruz, Calif.) was used at a 1:2,000 dilution. Cyclin E-dependent kinase activity was monitored by immunoprecipitation (IP) of cyclin E from T-cell nuclear extracts (see below), followed by in vitro histone H1 kinase assays. A modified IP assay was derived from that of Hamada and Tsuruo (14). After combining 10 μg of nuclear protein with polyclonal anti-cyclin E antiserum (Upstate Biotechnology, Inc., Lake Placid, N.Y.) (5 μg per reaction), the IP reaction mixes were pelleted by microcentrifugation and washed three times with RIPA buffer (modified from that of Courtneidge and Smith [7]) and once with kinase buffer (50 mM HEPES [*N*-2-hydroxyethylpiperazine-N_1-2-ethanesulfonic acid, pH 7.9], 10 mM $MgCl_2$, 6 mM EGTA [ethylene glycol tetraacetic acid], 2.5 mM dithiothreitol [DTT], 10 μM ATP). The pellets were then resuspended in 10 μl of kinase buffer, and 5 μCi of [γ-^{32}P]ATP (>3,000 Ci/mmol; Amersham) and histone H1 (type III-S; Sigma) (10 μg) were added to each sample. Phosphorylated H1 was resolved on sodium dodecyl sulfate (SDS)–10% polyacrylamide gels as described previously (33), and autoradiography was performed. Figure 1A shows typical results obtained from unstimulated and PHA-activated T-cell nuclear extracts. Both cyclin E protein levels and H1 kinase activity rose significantly by 24 h of mitogen activation, indicating that, at this time, a proportion of the T cells were in late G_1.

[^{3}H]thymidine incorporation assays and/or flow cytometry was performed to monitor the progression of activated T cells beyond the G_1 phase of the cell cycle. Thymidine incorporation assays were performed as follows. Cells (10^6) were pulsed with 20 μCi of [^{3}H]thymidine (25 Ci/mmol; Amersham, Arlington Heights, Ill.) for 1 h at 37°C. After lysis with a 0.1% Nonidet P-40 buffer (0.1% Nonidet P-40, 10 mM Tris [pH 7.5], 125 mM NaCl), the samples were spotted onto Whatman glass microfiber filters and washed consecutively with 20% trichloroacetic acid, 5% trichloroacetic acid, and 80% ethanol, and radioactivity was counted in a Beckman LS 6000IC liquid scintillation counter. Cell cycle analysis was performed as follows. Cells (5×10^6) were fixed in 70% ice-cold ethanol overnight, washed once with cold phosphate-buffered saline (PBS), resuspended in 20 U of DNase-free RNase (Pharmacia) in PBS, and incubated at 37°C for 30 min. The lymphocytes were then stained with propidium iodide (Sigma). Cell cycle analysis was performed on a Becton-Dickinson FACScan cytometer, and the data were interpreted by using the SOBR model according to the manufacturer's directions. Each analysis was based on a minimum of 10,000 cells with three to five S-phase peaks to fit the model.

Consistent with the results obtained following incorporation of [^{3}H]thymidine (Fig. 1B), cytometric analysis also indicated that significant numbers of activated lymphocytes entered S phase approximately 48 h following stimulation with PHA (Figure 1C). Results similar to those above were observed with all donor lymphocytes treated with PHA or PHA plus PMA (data not shown).

TAR RNA binding in nuclear extracts from mitogen-activated T cells. Because of the small packed cell volumes and fragile nature of primary human T lymphocytes, the original Dignam and Roeder protocol (9) was modified for the preparation of nuclear extracts from these cells. In brief, the lymphocytes were kept on ice in 5 packed cell volumes of buffer A (10 mM HEPES [pH 7.9], 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT) for no more than 2 min, and if necessary, the cells were homogenized in 1 ml of buffer A with only two to five strokes of a Dounce homogenizer with a B pestle. The nuclear extract was microdialyzed against a 1,000-fold excess of buffer D on Millipore (Bedford, Md.) type VS membranes (pore size, 0.025 μm) for 1.5 to 2.0 h. Nuclear extracts made from unstimulated and mitogen-activated primary T cells were used in RNase protection gel mobility shift assays to analyze ribonucleoprotein (RNP) complex formation. The RNase protection assays were performed essentially as described by Rounseville and Kumar (33). Each gel shift reaction mix included 3×10^5 cpm of ^{32}P-labeled wild-type (wt) TAR RNA (nucleotides +1 to +57), 66.7% (vol/vol) Dignam buffer D containing 20 μg of nuclear protein, and 2 mM $MgCl_2$. Following a 20-min room temperature incubation, the reaction mixes were treated with 2 μl of an RNase stock solution (RNases A [1 mg/ml] and T_1 [5 U/ml]) for an additional 15 min. The samples were then analyzed on a 4% polyacrylamide (80:1, acrylamide-bisacrylamide) native gel (33), and autoradiography was performed. Relative changes in RNP formation were determined by densitometric analysis of gel shift autoradiographs. All densitometry was performed on a Hoefer Scientific (San Francisco, Calif.) GS 300 transmittance/reflectance scanning densitometer linked to a Perkin-Elmer (Norwalk, Conn.) LCI-100 Laboratory Computing Integrator.

Cells assayed from seven of the eight donors showed relatively low levels of TAR binding activity in unstimulated extracts. In these samples, RNP complex formation was significantly increased an average of 2.5-fold (raw densitometric data not shown) upon activation with PHA or PHA plus PMA for 24 h (Fig. 2, upper panels). Interestingly, cells from donor 2 (Fig. 2, upper panels, lane 3) demonstrated high-level TAR binding activity in unstimulated extracts, with only a 1.6- to 1.7-fold increase in protein binding upon mitogen activation. In cells from another individual (donor 11), treatment with PHA alone caused a 2.4-fold increase in RNP formation. However, stronger TAR-binding activity (i.e., a 3.4-fold increase) was observed when these cells were treated with the combination of PHA and PMA (Fig. 2, lanes 13, 14, and 15). These data demonstrate that activated primary T cells, in G_1, possess higher TAR RNA/protein-binding activity than resting or G_0-phase cells. Pronounced differences in binding upon cellular activation may not, however, exist in all individuals, and in others, treatment with more than one mitogen may be required to produce the most effective protein-TAR RNA interactions.

To determine the kinetics of TAR-RNP complex formation, primary lymphocytes from one donor were treated with PHA plus PMA, and nuclear extracts were prepared at 2, 6, 24, and 48 h of stimulation. The results are shown in Fig. 3A. Increased RNP complex formation was observed within 2 h of mitogen stimulation and was sustained throughout the 48-h treatment. The nature of the complex, however, changed with time, becoming a faster-moving species and finally a distinct doublet as the cells progressed through G_1 into S (Fig. 3A, lanes 2 to 5).

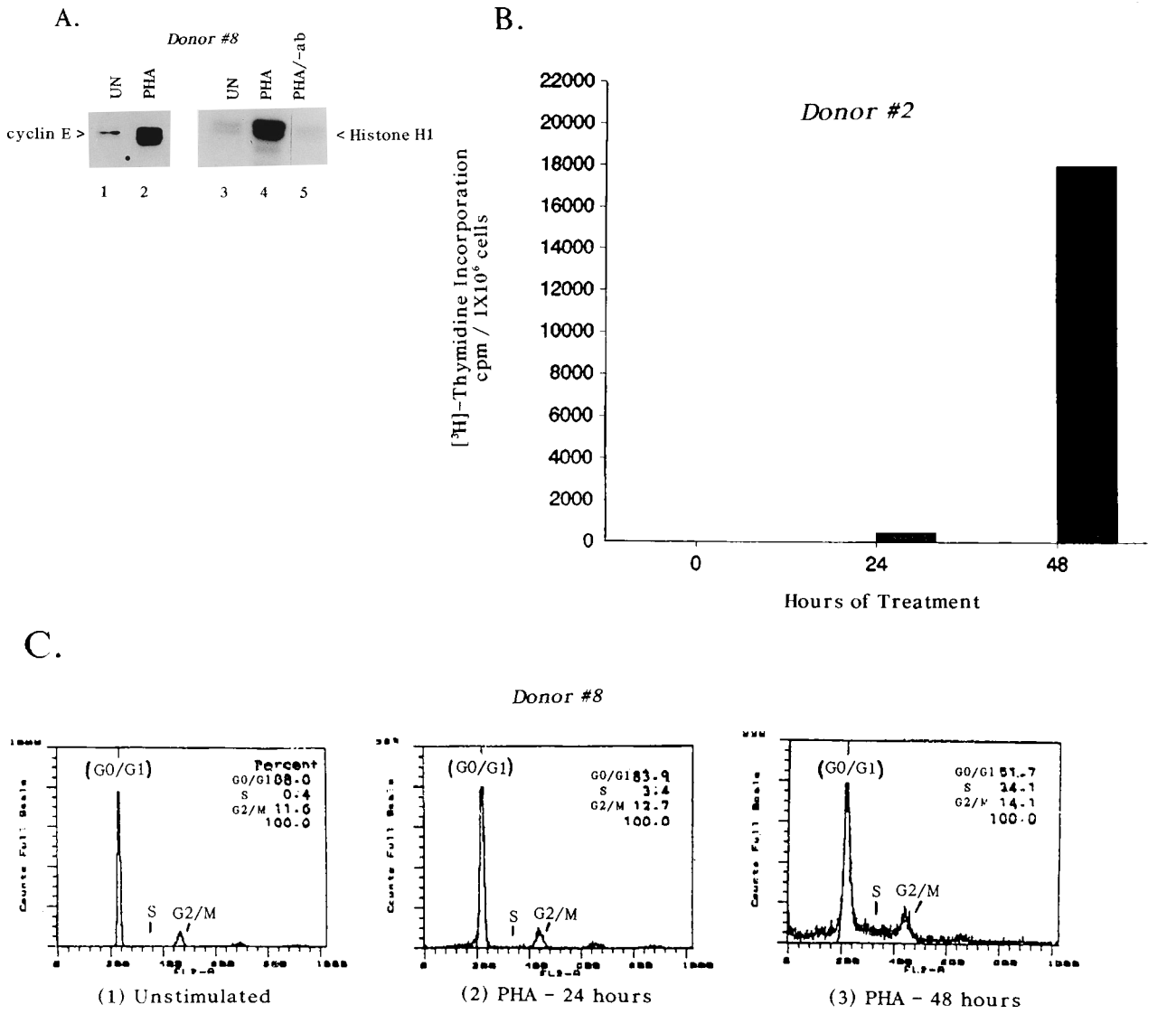

FIG. 1. (A) Anti-cyclin E Western blot and IP-histone H1 kinase assays. Cyclin E-dependent protein levels and H1 kinase activity were significantly increased in the 24-h, PHA-stimulated T-cell nuclear extract (NE) (lanes 2 and 4) versus the unstimulated NE (lanes 1 and 3). Lane 5 represents a control IP reaction performed without the cyclin E antiserum. (B) [^{3}H]thymidine incorporation assay. PHA-treated T cells began to significantly replicate their DNA sometime between 24 and 48 h of activation. Hatched bars, unstimulated; solid bars, PHA treated. (C) Flow cytometry. A very small percentage of the lymphocytes were in S phase at 24 h of stimulation (panel 2); however, significantly more cells were in S at 48 h of stimulation (panel 3). These cells typically did not enter S phase synchronously (panel 3).

These results show, then, that the increased interaction of nuclear proteins with HIV TAR RNA can occur during the early stages of T-cell activation. In addition, the nature of the RNP complex may vary as cells progress through the cell cycle, due either to qualitative, quantitative, or posttranslational changes in the protein(s) associated with TAR RNA.

To ensure that the changes in the binding of protein to TAR RNA observed during T-cell activation were specific and did not represent a general change in nucleic acid protein interaction, the binding efficiency of nuclear extracts from stimulated and unstimulated T lymphocytes to the SP1 consensus binding site was determined. An oligonucleotide containing the consensus binding site for SP1 was designed and synthesized on an ABI DNA synthesizer as described previously (17, 37). Sense and antisense SP1 oligonucleotides were annealed, and the double-stranded oligonucleotide was end labeled in a kinase reaction by polynucleotide kinase with [γ-^{32}P]ATP (6,000 Ci/mmol). After removal of the unincorporated nucleotides by gel filtration, labeled oligonucleotide probe (5×10^5 cpm) was incubated with 20 μg of nuclear extract protein from unstimulated and activated T lymphocytes in the presence of 2 μg of poly(dI dC) and 5$\times$ binding buffer (75 mM HEPES [pH 7.9], 25% glycerol, 250 mM KCl, 2.5 mM EDTA, 2.5 mM DTT) at room temperature for 20 min. After mixing with a 10$\times$ loading dye (90% glycerol containing bromophenol blue), samples were resolved in a 4% native polyacrylamide gel (60:1, acrylamide-bisacrylamide).

Nuclear extracts of stimulated and unstimulated T lymphocytes from three donors were tested for their ability to bind to the SP1 oligonucleotide. A representative result from one donor is shown in Fig. 4. Both unstimulated and stimulated T cells showed a slower-migrating and a faster-migrating complex, as has been reported before (37). In contrast to TAR binding, however, nuclear extracts from both the stimulated

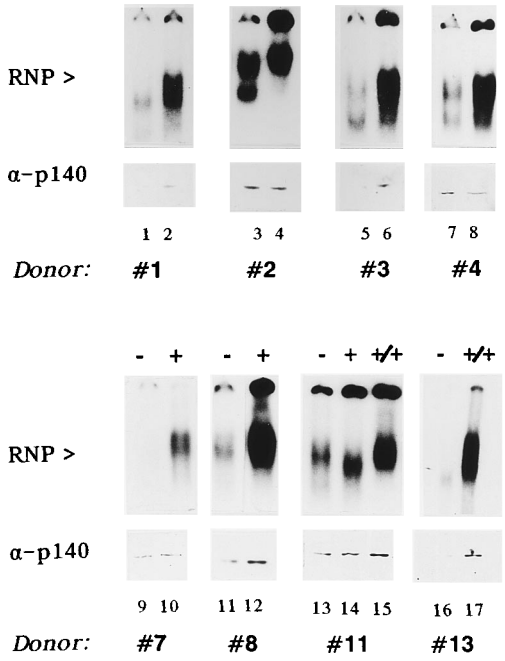

FIG. 2. (Upper panels) RNase protection gel mobility shift assays; (Lower panels) anti-p140 Western blot analysis. Lanes: −, unstimulated; +, stimulation with PHA; +/+, stimulation with PHA and PMA. Cells from all but one of the eight donors demonstrated poor TAR-RNP complex formation in unstimulated nuclear extracts (NEs) (lanes 1, 5, 7, 9, 11, 13, and 16). TAR RNA binding activity was significantly increased, however, in the 24-h mitogen-activated NEs (lanes 2, 6, 8, 10, 12, 15, and 17). Cells from donor 2 had approximately equal binding activity in both the unstimulated and activated NEs (lanes 3 and 4). Cells from donor 11 showed a significant increase in protein binding upon stimulation with both PHA and PMA (lanes 13, 14, and 15). Cells from five of the seven donors that showed increases in RNP complex formation upon mitogen activation also showed corresponding increases in p140 protein levels (lanes 2, 6, 12, 15, and 17). Those from donors 4 and 7, however, did not (lanes 7 and 8 and 9 and 10).

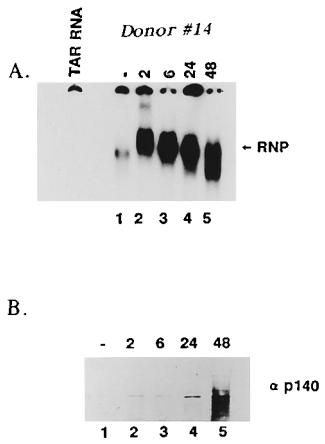

FIG. 3. (A) Time course analysis of RNP complex formation. TAR binding activity was significantly increased by 2 h poststimulation, and protein binding remained strong for up to 48 h (lanes 2 to 5). (B) Anti-p140 Western blot. Protein levels of p140 were elevated at 2 and 6 h of stimulation (lanes 2 and 3). The amount of p140 was significantly increased at 24 and 48 h of stimulation (lanes 4 and 5). Donor 14 is the same individual as donor 13; however, the blood was donated on two separate occasions.

and unstimulated cells showed the formation of the upper complex with similar intensity. A higher intensity of the faster-moving complex was observed in the unstimulated cells. These results therefore demonstrate that the patterns of protein-TAR interactions observed in stimulated and unstimulated T lymphocytes are specific.

Identification of p140-TAR RNA binding in T lymphocytes. We have previously shown that specific RNP complex formation requires stem regions I and II, the unpaired A, stem region III, and the UCU bulge of HIV TAR RNA (see Fig. 5A) (33). Furthermore, by UV cross-linking of HeLa nuclear proteins to ^{32}P-labeled TAR, a 140-kDa protein was observed to bind specifically and directly to this stem-loop structure (33). It is important to note that although p140 binds directly to TAR RNA and is essential for RNP nucleation (33), other protein-protein or protein-RNA interactions may also contribute to TAR-RNP complex formation.

To ensure that we were observing p140 binding in the present studies, UV cross-linking and RNase protection mobility shift assays were performed with specific TAR RNA mutants (TMs). These mutants were selected based on their relative abilities to form RNP complexes and bind p140 in HeLa cell nuclear extracts. TM31 has stem region IV and the loop deleted (Fig. 5B) (33) but still binds p140 and forms a wt-like RNP complex. Conversely, TM37, containing a single base substitution (C18→A18), which markedly alters the stem III structure, fails to form RNP complexes or bind p140 (Fig. 5B) (33). Both the unstimulated (Fig. 6, odd-numbered lanes) and mitogen-activated (Fig. 6, even-numbered lanes) T-cell extracts were capable of binding TM31, albeit less efficiently in unstimulated cells; however, there was no complex formation with mutant TM37 TAR RNA.

To perform UV cross-linking assays, gel shift reactions were carried out as previously described (33). Following RNase digestion, the samples were irradiated (approximately 6 to 6.5 cm from the source) with short-wave UV light at 8 mW/cm^2 for 10 min (33). An equal volume of 2× SDS gel loading buffer was

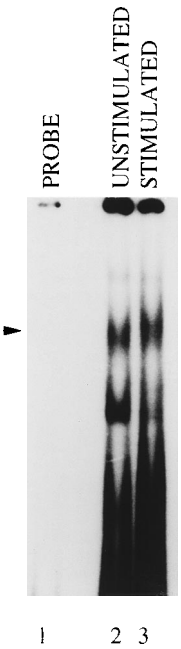

FIG. 4. SP1 binding in stimulated and unstimulated T lymphocytes. Nuclear extract from stimulated and unstimulated T cells was incubated with an SP1 oligonucleotide as described in the text. The arrowhead points to the nucleoprotein complex. Lane 1, probe alone; lane 2, unstimulated cells; lane 3, stimulated cells.

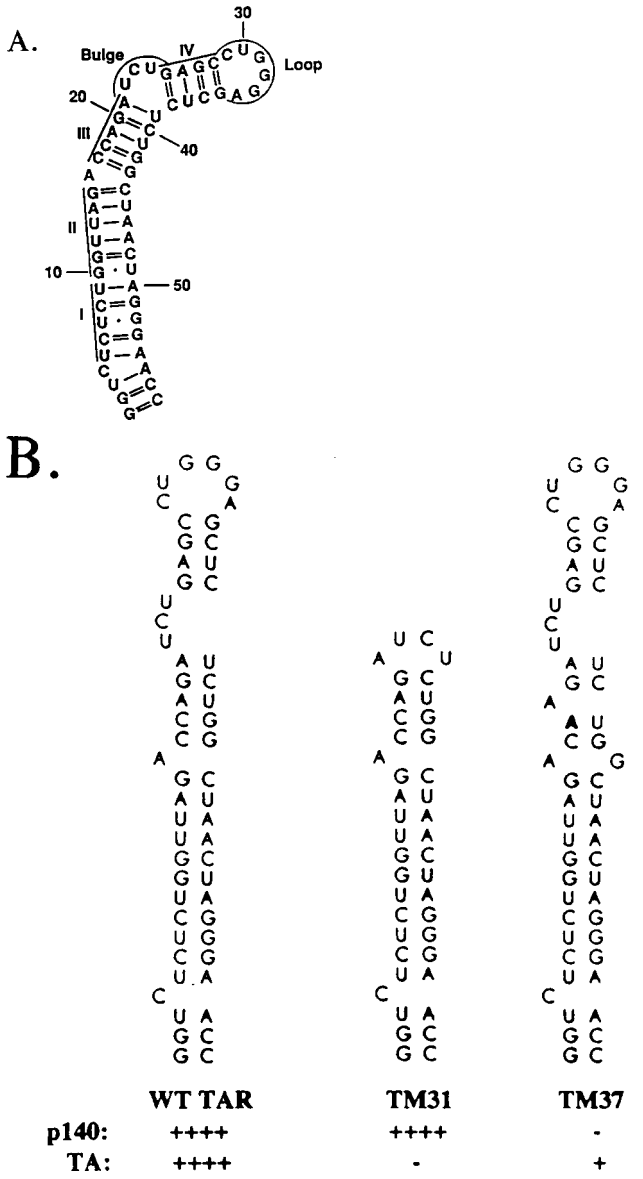

FIG. 5. (A) Structure of TAR RNA (nucleotides +1 to +57) (taken from Rounseville and Kumar [32]). TAR can be divided into stem regions I and II, the unpaired A, stem region III, the UCU bulge, stem IV, and the loop. (B) Structures of TAR mutants TM31 and TM37 (modified from Rounseville and Kumar [32]). TM31 is a deletion mutant lacking stem IV and the loop. TM37 is a base substitution mutant, in which C18 has been converted to A18, causing a disruption of stem region III. TA, relative Tat-mediated *trans* activation efficiency.

then added to each reaction mix, and the samples were resolved by SDS-polyacrylamide gel electrophoresis (PAGE) (33). These assays confirmed that p140 from donor nuclear extracts bound to wt TAR and TM31 but not to TM37 (Fig. 7, donor 2, lanes 1 to 6; donors 3 and 4, lanes 1 to 4). A distinct UV-cross-linked p140 band was not observed in some unstimulated extracts, probably because of their poor binding abilities and the inefficiency of the UV cross-linking procedure. These results demonstrate that this 140-kDa TAR-binding protein is present in primary T cells and that its binding characteristics are consistent with those observed in HeLa cell nuclear extracts.

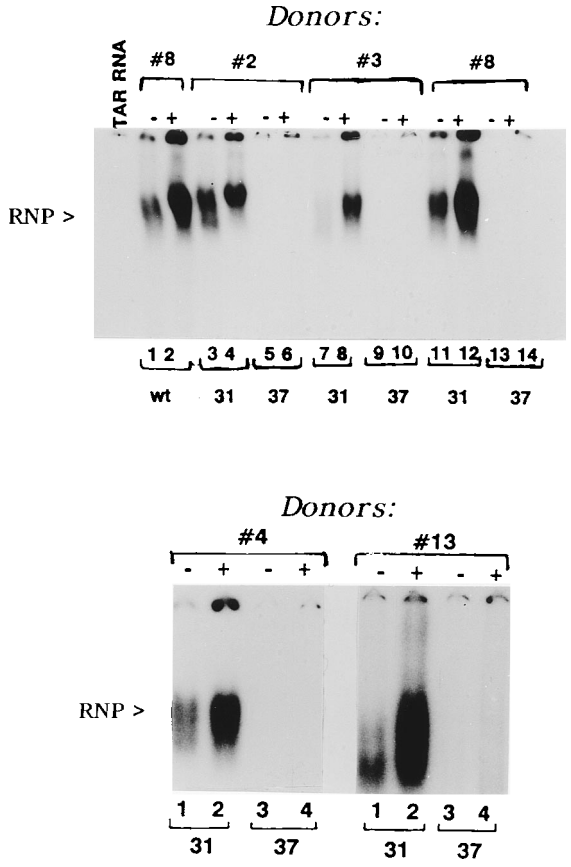

FIG. 6. Gel mobility shift assays with TM31 and TM37. Lanes: −, unstimulated; +, mitogen activated. Both unstimulated (odd-numbered lanes) and mitogen-activated (even-numbered lanes) nuclear extracts formed specific RNP complexes (arrow) with wt TAR RNA and TM31 but not TM37.

Regulation of p140 levels during T-cell stimulation. To determine if changes in p140 protein levels correlated with the changes in RNP complex formation observed in the activated T cells, the amount of p140 in the nuclear extracts was assayed by Western blot analysis with an anti-p140 polyclonal antiserum (final dilution, 1:500 or 1:1,000) (Fig. 2, lower panels, and 3B).

Cells from five of the eight donors did demonstrate increases in the amount of nuclear p140 upon mitogen activation (Fig. 2, lanes 2, 6, 12, 15, and 17). Among these five, the average increase in the level of p140 was 3.4-fold. Furthermore, Fig. 3A and B demonstrate that increases in TAR-binding activity were paralleled by increases in p140 protein levels. Densitometric analysis demonstrated 2.9-, 4.2-, 4.0-, and 2.8-fold increases in RNP complex formation and 3.9-, 2.2-, 12.0-, and 17.6-fold increases in p140 levels at 2, 6, 24, and 48 h of mitogen activation, respectively. In cells from donors 4 and 7, however, nuclear p140 protein levels did not increase despite the fact that TAR binding was elevated in their activated extracts (Fig. 2, lanes 8 and 10). These latter results suggest that other nuclear proteins and/or modifications may also contribute to TAR-RNP complex formation when T lymphocytes enter the cell cycle upon mitogen activation.

Interaction of HIV-1 Tat with p140. To determine if the presence of Tat would influence the interaction of p140 with TAR RNA, recombinant Tat protein expressed in *Escherichia coli* (kindly provided by John Brady, National Cancer Institute, Bethesda, Md.) was added to the RNase protection gel retar-

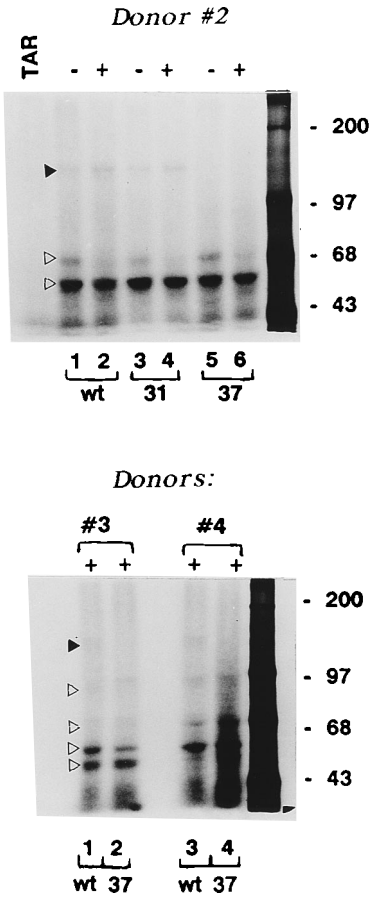

FIG. 7. UV cross-linking assays with TM31 and TM37. Lanes: −, unstimulated; +, mitogen activated. p140 (solid arrowhead) bound directly and specifically to wt TAR and TM31 but not to TM37. Open arrowheads designate nonspecific RNA-binding proteins. Sizes are shown at the right (in kilodaltons).

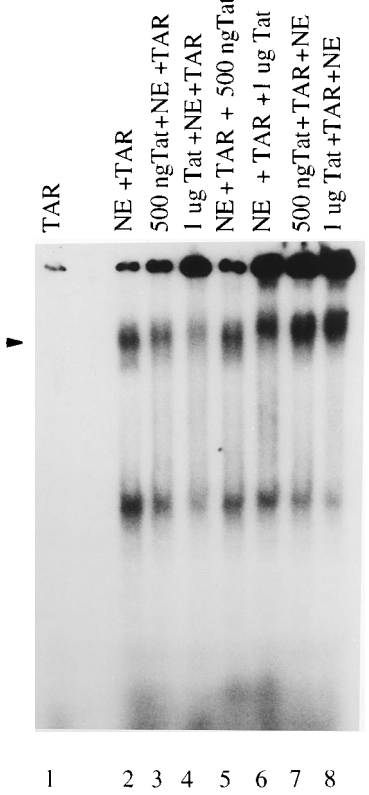

FIG. 8. Tat, TAR, and p140 interaction in RNase protection gel retardation assays. T-lymphocyte nuclear extracts (NEs), competent in RNP complex formation, were incubated with Tat and TAR in different combinations, RNase digested, and analyzed by native PAGE as described in the text. The arrowhead indicates the RNP complex. Lane 1, TAR RNA alone; lane 2, control binding reaction; lanes 3 and 4, 500 ng and 1 μg of Tat incubated with nuclear extract before the addition of TAR, respectively; lanes 5 and 6, 500 ng and 1 μg of Tat added to the nuclear extract-TAR reaction mix, respectively; lanes 7 and 8, 500 ng and 1 μg of Tat incubated with TAR before the addition of nuclear extract, respectively.

dation assays. Nuclear extract from T lymphocytes was incubated with TAR RNA as described earlier. In one set of experiments, 500 ng or 1 μg of Tat was incubated with nuclear extract before the addition of radioactive TAR RNA (Fig. 8, lanes 3 and 4, respectively). In another reaction, nuclear extract and TAR RNA were incubated before Tat protein was added (Fig. 8, lanes 5 and 6, respectively). In a third set, Tat protein and TAR RNA were incubated before the addition of nuclear extract (Fig. 8, lanes 7 and 8). Irrespective of the order of addition, Tat decreased the mobility of the TAR p140-RNP complex. Addition of 1 μg of Tat caused a higher supershift than did 500 ng of Tat (Fig. 8, lanes 5 and 7 compared with lanes 6 and 8). These results indicate that both Tat and p140 are present in the same RNP complex and they do not compete with each other for the same binding site in TAR. On the other hand, when Tat was incubated with nuclear extract prior to addition of TAR, although it resulted in a supershift, the intensity of the RNP complex was reduced (Fig. 8, lanes 3 and 4) compared to the intensity in the reaction without Tat (Fig. 8, lane 2). The latter result could indicate a sequestration of Tat cofactors, which may be required for p140 TAR interactions. These results indicate that Tat and p140, in an RNase protection gel shift assay, are part of the same RNP complex and do not compete for the same binding sites on the TAR RNA.

Several HIV-1 processes, including Tat-mediated *trans* activation, require T-cell activation for maximum efficiency. In addition, effective Tat *trans* activation also appears to require the presence of cell type-specific proteins. Rodent cells, for example, demonstrate inefficient *trans* activation (16, 29). However, rodent-human somatic cell hybrids containing human chromosome 12 exhibit significantly increased levels of Tat-dependent *trans* activation (16, 29). Although several groups have identified cellular TAR RNA-binding proteins, some of which have been cloned and/or purified, the precise role(s) of these cellular factors during Tat-dependent *trans* activation remains unclear. Interestingly, activated protein kinase C has recently been shown to be necessary for efficient Tat *trans* activation in Jurkat cells (18). Mitogen activation of T cells may therefore be required at both the level of basal transcription, or proviral activation, and Tat-dependent *trans* activation (18). We have found that the efficient binding of p140 to TAR RNA also requires protein kinase C activity (15).

In this report, we describe the binding of a 140-kDa nuclear protein to HIV-1 TAR RNA in primary human T lymphocytes. Upon mitogen activation of these cells, p140 binding to TAR RNA was increased. Increased RNP complex formation was correlated with increases in nuclear p140 protein levels. However, this was not always the case (e.g., donors 4 and 7), suggesting that other nuclear proteins and/or post-translational modifications of these proteins also may contribute to the nucleation of the RNP complex.

The experiment describing RNP complex formation at various time points after T-cell activation further corraborates this contention and would explain the varied nature of the RNP complexes observed between the early and late stages of T-cell activation (Fig. 3A, lanes 2 to 5). Subsequent to the T-cell receptor/MHC/PEP interaction, a number of proteins within the T cell are activated or synthesized, including signal transduction mediators and transcription factors (reviewed in reference 39) as well as cell cycle regulatory proteins (reviewed in reference 34). These proteins will regulate the progression of the lymphocytes through the different phases of the cell cycle (e.g., G_0 to G_1, S, G_2, and M). As this dynamic population of nuclear proteins interacts with TAR RNA, the overall conformation of the complex and net charge-to-mass ratio (*q*/*m* ratio) are likely to change, producing the varied mobility shifts observed throughout the G_1 and early S phase of T-cell activation. In support of the hypothesis that other cellular proteins interact with TAR RNA upon activation of T lymphocytes, we have found that a 50-kDa phosphoprotein/kinase specifically associates with the HIV-1 Tat/wt TAR RNA complex in activated T cells (unpublished data).

Of the cells from eight donors assayed, those from one (donor 2) had a significant amount of p140 and TAR RNA binding in unstimulated extracts. These lymphocytes appeared to be quiescent, based on [^{3}H]thymidine, flow cytometry, and cyclin E Western blotting and kinase assays (Fig. 1B and data not shown). It is important to note, however, that since cyclin E is only a mid-late G_1-phase protein, we cannot eliminate the possibility that this donor's lymphocytes were already in early G_1 rather than in resting G_0 phase. A portion of these cells could have been activated by an unknown antigenic event or during the cell purification process.

Alternatively, these results may imply that there exists an endogenous genetic component which dictates the expression and/or efficiency of nuclear p140 binding to TAR RNA. Previous studies have used cell lines to examine TAR RNA-protein interactions and are therefore unable to address this particular issue. It is known that some individuals progress more rapidly into the late stage of HIV-1 infection, AIDS-related complex/AIDS, than others (2). Factors which may contribute to the accelerated or decelerated pace of HIV disease progression include genetic characteristics which render an individual more or less susceptible to HIV-1 replication, exposure to other antigenic agents, and the frequent or continual activation of the immune system in general (2). If p140 is found to play a role during Tat *trans* activation, the fact that some individuals have more of this protein and stronger p140/TAR binding in their unstimulated lymphocytes may cause an increased sensitivity or resistance to high-level HIV-1 replication.

An important issue is to decipher the role of p140 in viral replication and Tat *trans* activation. Our experiments demonstrate that Tat and p140 associate with TAR RNA in the same RNP complex and that p140 and Tat may associate with each other via protein-protein interactions, since incubation of Tat with nuclear extract resulted in partial loss of RNP complex formation (Fig. 8). Furthermore, our preliminary experiments have demonstrated that infectious HIV-1 proviral clones containing altered TAR RNA lower stem regions, which destabilize the p140 binding domain, replicate inefficiently in viral growth assays (32). Importantly, a complementary mutation which restores the wt TAR secondary structure restored normal viral growth in some T-cell lines (A3.01 and MT4 cells), while in one T-cell line (J6.1 cells), viral growth was restored only partially. Similar observations, emphasizing an essential role of the lower stem region of TAR in viral replication and Tat *trans* activation, were reported by another group (20).

Even though a precise role for the TAR RNA stem-binding protein in viral replication is still unclear, it is clear that the lower stem domain of TAR, perhaps by its interaction with a specific host protein(s), forms an essential component in regulation of viral activation. This report supports the hypothesis that agents which activate resting T cells may contribute, at least in part, to the overall pace of HIV-1 disease progression.

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