A functional genetic approach suggests a novel interaction between the human immunodeficiency virus type 1 (HIV-1) Tat protein and HIV-1 TAR RNA in vivo

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Human immunodeficiency virus type 1 (HIV-1) Tat and human Cyclin T1 form a complex and together recognize the viral TAR RNA element with specificity. Using HIV-1/equine infectious anaemia virus TAR chimeras, we show that in addition to the well-characterized interaction with the bulge, Tat recognizes the distal stem and the loop of TAR. These data support previously proposed, but unproven, molecular models.

In order to analyse functional interactions between hTat and hTAR, we constructed TAR elements chimeric between HIV-1 and EIAV and tested their activity in canine D17 cells, which are permissive for both hTat (via hTAR) and eTat (via eTAR) trans-activation (see Fig. 2 for the predicted structures of the RNA chimeras). We observed that the TAR loops were not interchangeable and that sequential addition of TAR motifs added to function. Our data are most consistent with the existence of at least two interactions between hTat and hTAR in vivo, each of which partially adds to hTat to hTAR binding.

Canine D17 cells support Tat-activation of both HIV-1 and EIAV. It has been shown previously that overexpression of eTat in D17 cells squelches the ability of hTat to activate hTAR (Carroll et al., 1992). We observed that hTat, but not the inactive hTat mutant K41A, when overexpressed in D17 cells squelches the ability of eTat to activate eTAR (data not shown). These findings suggested that hTat and eTat interact with the same cofactor in D17 cells. Indeed we (Albrecht et al., 2000) and others (Taube et al., 2000) cloned canine Cyclin T1 (cCycT1) from D17 cells and demonstrated its ability to rescue EIAV Tat-activation in human cells, strongly suggesting that cCycT1 is a cofactor for eTat and hTat in D17 cells.

The plasmid phTAR, containing the chloramphenicol acetyltransferase (CAT) reporter gene driven by the HIV-1 LTR, was modified by replacing the hTAR loop with the eTAR loop (hTAR-e1), or with the eTAR U•G base pairs and loop (hTAR-e2) (see diagrams in Fig. 2). The size of the distal stem + loop was kept constant by adding or removing C•G base pairs. Canine D17 cells were transfected, using Lipofectin (GibcoBRL), in duplicate with 250 ng of the

The human immunodeficiency virus type 1 (HIV-1) Tat protein (hTat) trans-activates transcription by binding human Cyclin T1 (hCycT1) and the viral trans-activation response (hTAR) RNA element (Cullen, 1998). hTAR forms a 59 nucleotide stem–bulge–loop hairpin structure at the 5' end of all viral transcripts (Colvin & Garcia-Blanco, 1992). Both the sequence and secondary structure of hTAR are essential for Tat-activated transcription. While the U-rich hTAR bulge is sufficient for hTat binding in vitro (see Churcher et al., 1993, and references therein), the CUGGGA loop and the C•G base pair that closes it are necessary for trans-activation in vivo (Feng & Holland, 1988) and binding of the hCycT1–hTat complex to TAR (Wei et al., 1998; Bieniasz et al., 1998).

Like HIV-1, equine infectious anaemia virus (EIAV) trans-activates transcription via a Tat protein (eTat), TAR RNA (eTAR) and the equine homologue of Cyclin T1 (eCycT1) (Bieniasz et al., 1999; Taube et al., 2000). In the 25 nucleotide stem–loop EIAV TAR, the CUGC loop sequence, the two U•G base pairs proximal to the loop and the overall secondary structure are required for function (Carvalho & Derse, 1991). Importantly, eTat binds eTAR only in the presence of eCycT1 (Bieniasz et al., 1999). Biochemical and 2-D NMR studies have demonstrated conservation of structure between the hTAR and eTAR terminal loops, despite dissimilar sequence (Colvin et al., 1993; Hoffman et al., 1993), and the 3-D NMR structure of eTAR reveals an exposed guanosine extending from the loop, similar to that in hTAR (Hoffman & White, 1995).
indicated TAR chimera, with or without the indicated Tat, as well as 50 ng luciferase DNA transfection control and pBlueScript and/or pRSPA-s DNA to bring total DNA to 1.15 μg. Constant protein amounts of cell extracts were assayed for CAT activity and the CAT activities of untransfected wells were subtracted. CAT activity and transactivation were measured as described previously (Suñe & Garcia-Blanco, 1999; Albrecht et al., 2000). As expected, hTat activated transcription of hTAR but eTat did not. Replacing the hTAR loop with the eTAR loop (hTAR-e1) partially rescued eTat activity while impairing hTat activity (Fig. 1). Replacing the hTAR loop and distal C-G base pair with the eTAR loop and U-G base pairs (hTAR-e2) fully rescued eTat and further impaired hTat function (Fig. 1). As expected, neither K41A nor R43G (eTat mutant) activated hTAR-e2 (data not shown). We observed that eTat, but not hTat or R43G, activated transcription via eTAR (data not shown), confirming the ability of D17 cells to support both HIV-1 and EIAV Tat-activation and the inability of hTat to activate eTAR.

hTat binds hCycT1 and the hTat:hCycT1 complex binds hTAR in a bulge–loop-specific manner. Whereas hTat has shown to bind the TAR bulge in vitro, additional specific contacts in the hTat:hTAR:hCycT1 complex are expected (Zhang et al., 2000). In addition to the well-characterized hTat binding to the hTAR bulge and hCycT1 binding to the hTAR loop, which has been inferred by others (Garber et al., 1998a), we propose that hTat binds the distal stem and loop of hTAR in vivo (Fig. 2). Specifically, we believe that hTat recognizes the distal G-C base pairs in the stem but will productively interact with G-U base pairs, whereas the reverse is true for eTat. Additionally hTat, we posit, interacts with the TAR loop, very likely the 3′-end of the loop, which is not conserved between HIV-1 and EIAV (Fig 2). We propose that CycT1 recognizes the CUG in the loop, particularly the G that has been proposed to have similar architecture in the HIV-1 and EIAV TAR loops (R. A. Colvin & M. A. Garcia-Blanco, unpublished; Hoffman & White, 1995). We propose that changing hTAR to hTAR-e1 and hTAR-e2 progressively weakened an interaction between hTat and the distal stem and terminal loop of hTAR (Fig. 2, top row). Conversely, changing hTAR to hTAR-e1 and hTAR-e2 strengthened an interaction between eTat and eTAR (Fig. 2, bottom row). The alternative explanation that a special geometry between cCycT1–hTat–hTAR was disrupted does not readily explain differences between hTAR-e1 and hTAR-e2.

Numerous in vitro observations also support this model. The hTat RNA-binding domain binds the hTAR bulge in vitro, but the affinity and specificity of this hTat–hTAR interaction are enhanced by amino acid residues in the activation domain (Churcher et al., 1993). Tat from both HIV-1 and HIV-2 binds weakly to HIV-2 TAR RNA in a loop-dependent manner in the absence of hCycT1, and the HIV-2 TAR loop sequence specificity of hTat is lost on truncation of the hTat activation domain (Garber et al., 1998b). The structure of the bovine immunodeficiency virus (BIV) Tat–TAR complex reveals Tat in close proximity to the loop (Puglisi et al., 1995). Using circular dichroism and molecular modelling, amino acids 59–72 of hTat have been suggested to interact with UGG at positions 31–33 in the hTAR loop in vitro (Loret et al., 1992). Footprinting experiments suggested that hTat bound the bulge and A-35 of the loop simultaneously, consistent with these residues being on the same face of the upper stem in hTAR (Colvin & Garcia-Blanco, 1992). Furthermore, hTat has been cross-linked to U31 (Wang et al., 1999) and to U35 (Farrow et al., 1998) of the hTAR loop and site-specific RNA cleavage has demonstrated that hTat is located in the proximity of both the bulge and loop of hTAR (Huq & Rana, 1997). Taken together, these data suggest at least three RNA–protein interactions in the hCycT1–hTat–hTAR complex. In addition to hCycT1 binding to the hTAR loop, we propose at least two interactions between hTat and hTAR, rather than the single interaction of hTat with the hTAR bulge (Fig. 2). While our results do not definitively prove the existence of this RNA–protein interaction(s), we offer this model as the most parsimonious explanation of the available data.

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