1496–1499 Nucleic Acids Research, 1994, Vol. 22, No. 8 © 1994 Oxford University Press

The activation region of the Tat protein of human immunodeficiency virus type-1 functions in yeast

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Received November 2, 1993; Revised and Accepted March 9, 1994

ABSTRACT

The N-terminal 48 amino acids of the Tat protein of human immunodeficiency virus type (HIV)-1 constitute its activation region. This region can autonomously activate transcription when targeted to the HIV-1 long terminal repeat or certain heterologous promoters either through DNA binding sites located upstream of the transcription initiation site or via downstream RNA binding sites in mammalian cells. To determine whether the Tat activation region can function in yeast, we have assayed the effect of a chimeric gene (*GAL – Tat48*) expressing the DNA binding domain of the yeast transcription factor Gal4 (residues 1 – 147) and the activation region of Tat on *GAL1* promoter-directed expression of the *lacZ* reporter gene in *Saccharomyces cerevisiae*. Our results indicate that the Gal – Tat48 fusion protein can induce significant activation of the *GAL1* promoter. Analysis of a number of Tat mutants located within the activation region indicate that the amino acid residues of Tat essential for trans-activation in mammalian cells are also required for transactivation in yeast. Our results suggest that Tat-mediated transcriptional activation may involve a mechanism conserved among yeast and mammalian cells.

INTRODUCTION

The Tat protein coded by HIV-1 is a potent activator of transcription from the viral long terminal repeat (1, 2). The mechanism of Tat-mediated transcriptional activation remains poorly understood. Unlike conventional transcriptional activators which function through direct or indirect interactions with DNA promoter elements, the Tat protein interacts with a nascent RNA target termed TAR (3, 4). Since TAR sequences may be substituted with target sequences of other RNA binding proteins it appears that the primary function of TAR is to target Tat to the HIV LTR (5, 6). Although the requirement for an RNA target makes Tat unique among various eukaryotic transcriptional activators, it is becoming evident that Tat may ultimately function in a manner similar to other transcriptional activators which function through interactions with a DNA target. This was inferred from our studies where we showed that Tat can trans-activate a synthetic promoter devoid of HIV sequences when targeted to the promoter by upstream Gal4 DNA binding sequences. Similarly, Tat fusion proteins containing heterologous DNA binding domains were also shown to trans-activate the HIV-1 LTR, human actin promoter and HSV-1 TK promoter when targeted to these promoters *via* cognate *cis*-acting DNA binding sites (7–11). Thus, studies with various Tat fusion proteins and their activity on heterologous promoters suggest that Tat-mediated trans-activation is not unique to the HIV LTR. Domain substitution studies also indicate that Tat has a modular structure like other eukaryotic transcriptional activators. The N-terminal 48 amino acid region can efficiently activate transcription in a manner similar to the native Tat protein when targeted to the promoter via upstream DNA binding sites or downstream RNA binding sequences (5, 6, 12, 13).

Detailed mutational analyses of sequence requirements for Tat trans-activation of various promoters indicate that Tat-mediated trans-activation is dependent on the presence of DNA motifs required for binding of the cellular transcription factor Sp1 (7, 10, 14–16). It appears that other transcription factors such as USF, ATF and Ap1 can partially substitute for Sp1 (8,17,18). In addition, Tat also appears to have a preference for TATA motifs similar to those present in the HIV LTR (10, 14, 18). Further understanding of the mechanism of Tat-mediated transcriptional activation would be greatly facilitated by the use of model systems such as yeast since the ease of genetic manipulation could be exploited to dissect the mechanism of Tat trans-activation. We, therefore, investigated whether HIV-1 Tat protein can function as a transcriptional activator in yeast *Saccharomyces cerevisiae*. Here, we report that the activation region of HIV-1 Tat protein can efficiently activate transcription from the yeast *GAL1* promoter. Our results suggest that Tat-mediated trans-activation involves a mechanism conserved among yeast and mammalian cells.

MATERIALS AND METHODS

Plasmids and yeast

The yeast indicator strain GGY1::171 expressing the *E.coli lacZ* reporter gene under the transcriptional control of GAL1 promoter has been described (19). The yeast shuttle vector pMA424 (20)

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which expresses the N-terminal 147 amino acid DNA binding region of the yeast transcription factor Gal4 was a gift from Dr S.Fields and Dr P.Bartel. Plasmid pMA-GAL4 expressing the entire GAL4 gene was constructed by ligating the XhoI−BamHI restriction fragment of pG525 (21) to the XhoI−BamHI digested vector pMA424. GAL4−Tat chimeric plasmids were constructed by cloning the Tat-coding sequences of wt and mutant tat genes generated by PCR between the EcoRI and BamHI sites of pMA424 vector.

Yeast transformation and β-galactosidase assay

Yeast strain GGY1::171 was transformed with various plasmid derivatives of pMA424 by the LiAc method of Shiestl and Giest (22). Transformed cells were plated on synthetic dropout medium (SD) lacking histidine. After 4 days at 30°C, colonies were lifted onto nitrocellulose filters and permeablized by freezing the filters for 5−10 sec on aluminium foil floats placed over liquid nitrogen. Filters were overlaid on Whatman filters saturated with Z buffer containing 1 mg/ml X-Gal and incubated at 30°C (23). Colonies which turned blue within a period of 1−6 h were picked and patched onto fresh plates lacking histidine. Yeast cells from these patches were used for quantitation of β-galactosidase expression. The levels of β-galactosidase expression were quantitated from four independent transformants grown in liquid selective media using the ONPG (O-nitophenyl-β-D-galactopyranoside)-based assay (24).

RESULTS AND DISCUSSION

Activation of *GAL1* promoter

We have previously shown that a chimeric gene expressing the DNA binding domain of the yeast trans-activator Gal4 and the N-terminal 48 (Gal−Tat48) or 58 (Gal−Tat58) amino acids of Tat protein can trans-activate a synthetic promoter containing the E1B TATA motif, three Sp1 binding sites and five Gal4 binding sites (7). In these assays, the Gal−Tat48 protein was found to be more efficient than the Gal−Tat58 protein. Here, we tested the activity of Gal4 fusion proteins containing 101 (Gal−Tat 101), 82 (Gal−Tat 82), 67 (Gal−Tat 67), 58 (Gal−Tat 58) and 48 (Gal−Tat 48) amino acids of the HIV-1 Tat (strain SF2) protein on the *GAL1* promoter of *Saccharomyces cerevisiae*. A yeast strain GGY1::171 containing the *E.coli lacZ* reporter gene fused to the *GAL1* promoter was transformed with the multicopy vector plasmid pMA424 which expresses the N-terminal 147 amino acid DNA binding domain of Gal4 or pMA-GAL4 expressing the entire 881 amino acids of the Gal4 protein or derivatives of pMA424 expressing the various Gal−Tat fusion proteins. Transformed cells were selected by *HIS3* prototrophy and the expression of β-galactosidase (*lacZ*) was examined by a filter-based X-Gal assay. These assays indicated (Fig. 1) that cells transformed with pMA424 did not turn blue while cells transformed with pMA-GAL4 turned blue within 10 minutes, indicating efficient trans-activation of the *GAL1* promoter by the Gal4 protein. Cells transformed with Gal−Tat101 did not show any detectable β-galactosidase expression even after overnight incubation (Fig. 1). Similarly cells transformed with Gal−Tat82, 67 and 58 also did not turn blue (results not shown). In contrast, cells transformed with Gal−Tat48 showed intense blue color within 20−60 minutes of incubation with X-gal indicating trans-activation of the *GAL1-lacZ* reporter gene by the N-terminal 48 amino acid region of Tat. The intensity of the blue color induced by Gal−Tat48 was weaker compared to that induced by the native Gal4 trans-activator. This is expected considering that Gal4 protein contains two activation domains (20) and is the cognate trans-activator of the *GAL1* promoter in yeast.

The relative levels of trans-activation in cells transformed with the *GAL−Tat* chimeric genes were determined from the level of β-galactosidase activity (24). As expected, Gal−Tat48 transformed cells induced readily detectable β-galactosidase activity, whereas Gal−Tat101, 82, 67, 58 and pMA424 did not induce significant activity (Fig 2). In both of our assay systems (qualitative and quantitative), Gal−Tat101, 82, 67 and 58 fusions did not induce detectable β-galactosidase expression. This is somewhat surprising since these fusion proteins contain an intact activation region. To rule out the possibility that Gal−Tat101 is unable to trans-activate the *GAL1* promoter due to lack of stable fusion protein synthesis in yeast, we carried out immunoprecipitation analysis using antibodies specifc for Tat. These results indiate that yeast cells transformed with the Gal−Tat101 plasmid synthesize significant amounts of the Gal−Tat101 fusion protein of about 28 kD (Fig 3A). The level of expression of Gal−Tat101 is more or less comparable to the level of expression of Gal−Tat48 (Fig. 3B). Similar results were also obtained by a protein blot analysis (not shown). It is possible

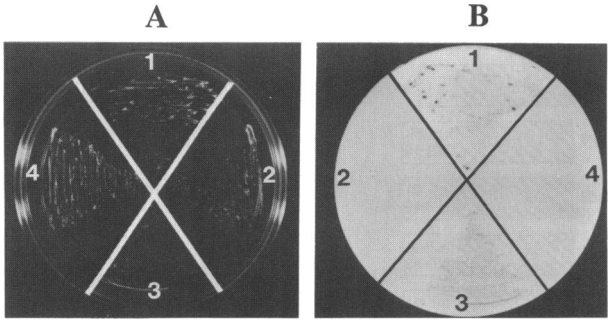

Figure 1. X-gal staining of transformed yeast cells. **A.** Transformed yeast cells grown on plates containing selective media lacking histidine. **B.** X-gal staining of filter lift. The numbers designate cells transformed with pMA-Tat48 (1), pMA-Tat101 (2), pMA-GAL4 (3) or pMA-424 vector (4).

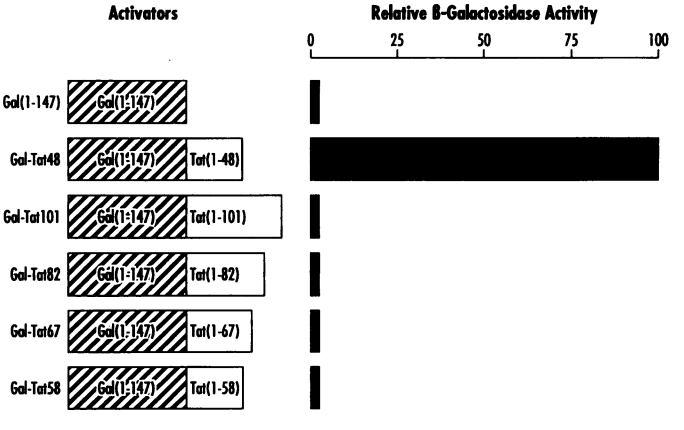

Figure 2. Activation of *GAL1-lacZ* expression in yeast cells by Tat activation region. Three to four independent colonies from cells transformed with various plasmids were grown in liquid selective media lacking histidine and the level of β-galactosidase expression was determined using the ONPG-based assay. The averages of these estimations are illustrated in the figure.

that the presence of additional sequences such as the Arg-rich basic region in the fusion protein is inhibitory to the activity of the Gal−Tat fusion proteins. It should be noted that we have also observed a reduced level of trans-activation in HeLa cells with Gal−Tat58 compared to Gal−Tat48 (7). Similarly, Gal4 fusion proteins of other transcriptional activators such as p53 (25) have been shown to be more active in mammalian cells when the fusion protein contains only the activation domain than rather the entire coding region of the trans-activator. Alternatively, since Tat101 contains the TAR RNA-binding domain, it is possible that the activation domain may not undergo suitable conformational change for lack of TAR RNA.

Effect of Tat mutations

To localize the sequences of Tat activation region required for trans-activation in yeast, we first tested Gal4−Tat fusions expressing different segments within the N-terminal 48 amino acid region (Fig. 4). These studies indicate that Gal4−Tat fusion proteins containing either the N-terminal 31 amino acids of Tat (Gal−Tat31) or amino acids 32 to 48 of Tat (Gal−Tat32−48) did not induce significant trans-activation. In contrast, a Gal−Tat fusion protein containing Tat residues 8 to 48 induced about one half of the activity induced by Gal−Tat48. Although we have reported earlier (26) that a Tat mutant lacking the N-terminal five amino acid (2 to 6) residues was severely defective in trans-activation in HeLa cells, we have observed that Tat mutants lacking the N-terminal region (residues 1 to 8) show significant activity in canine D18 cells (our unpublished observation). Thus, the very N-terminal region of Tat may be important for Tat activity in certain mammalian cells and may not be absolutely required in other cell types. Similarly, other investigators have also reported varying effects of the N-terminal mutants of HIV-1 Tat (27). Our present results indicate that the Tat region encompasing residues 8 to 48 is sufficient for trans-activation in yeast and thus similar to what is observed in certain mammalian cells.

In addition, the effects of several single amino acid substitution mutants on trans-activation were also tested and the results are shown in Fig. 4. Among these substitution mutants Tat(22)C→G, Tat(30)C→G, and Tat(37)C→G were found to be negative for trans-activation indicating that the conserved Cys residues are important for trans-activation in yeast as in mammalian cells (26, 28−30). Mutant Tat(41)K→T showed about 40% activity of the Gal−Tat48 indicating that the Lys(41) residue is not absolutely essential for trans-activation in yeast. Lys(41) is conserved among the various lentivirus Tat proteins (31) and has been shown to be essential for trans-activation in HeLa cells (26, 30). In contrast this mutation appears to result only in partial reduction of Tat activity in certain human T4 cell lines (32) and in canine (D18) cells (our unpublished results). These results indicate that the conserved Tat sequences (residues 8−40) encompassing the Cys-rich and core domains are required for trans-activation in yeast in a manner similar to certain mammalian cells.

Mutational analyses have indicated that trans-activation mediated by the native Tat (14, 33, 34) or the Gal−Tat fusion proteins (7, 17) is strongly dependent on the mammalian transcription factor Sp1. Since yeast cells do not contain Sp1, it is possible that the basal *GAL1* promoter can be activated by Tat in the absence of upstream factors. As the requirement for Sp1 can be partially substituted by other transcription factors such as ATF, USF and Ap1 (8, 17), it is possible that yet uncharacterized yeast upstream factors may play a role in the activation of the basal *GAL1* promoter by Tat. The *GAL1* promoter region consists of primarily the TATA region and the Gal4 binding upstream activating sequences (UAS_G) The sequences located between UAS_G and the TATA region have been shown to interact with a cellular factor involved in glucose-mediated repression of *GAL1* transcription (35, 36). The role of yeast upstream sequences, if any, in Tat trans-activation remains to be investigated. Our results suggest that the activation domain of Tat activates transcription in yeast and in mammalian cells by similar mechanisms. The well-developed yeast genetic system could now be exploited to investigate the mechanism of Tat trans-activation.

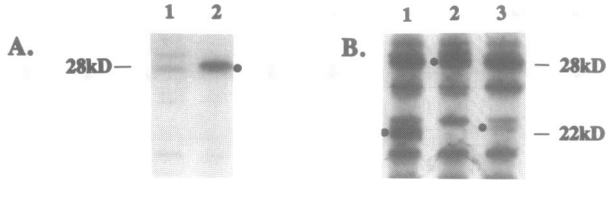

Figure 3. Immunoprecipitation of Gal−Tat fusion proteins. Yeast cells transformed with various plasmids were grown in liquid selective media lacking histidine. About 10^6 cells were labeled with ^{35}S-mix (NEN) for 3 hours in selective media lacking methionine, cysteine and histidine and immunoprecipitated using rabbit polyclonal antibody specific for the N-terminal 17 amino acids of the Tat protein. **A.** Expression of Gal−Tat101. Immunoprecipiates were analyzed in a 12 % polyacrylamide gel. 1, preimmune rabbit serum; 2, Tat antiserum. **B.** expression of Gal−Tat101, 48 and 37. Immunoprecitates (Tat antibodies) were analyzed in a 15 % polyacrylamide gel and the autoradiogram was exposed for a longer time than in A. 1, Gal−Tat48; 2, Gal−Tat101; 3, Gal−Tat37.

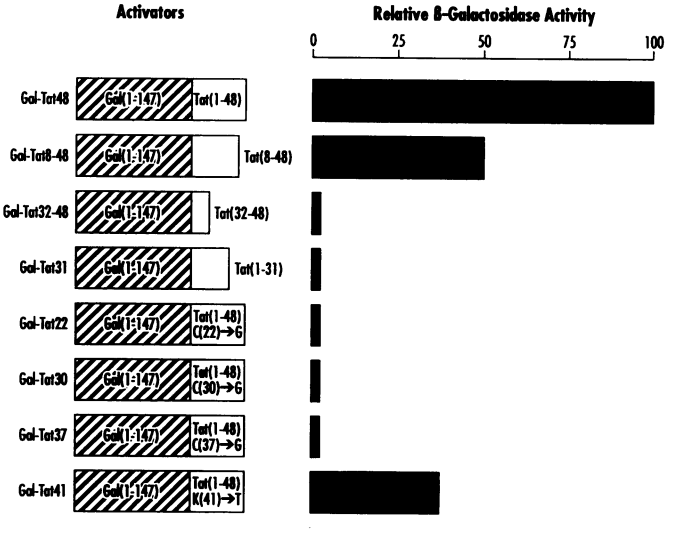

Figure 4. Effect of Tat mutations on activation of *GAL1-lacZ* expression. The levels of β-galactosidase expression in yeast cells transformed with various Tat mutants were determined as described in Fig. 2.

ACKNOWLEDGEMENTS

We thank Drs Stanley Fields and Paul Bartel for the generous gift of yeast strain GGY1::171 and plasmid pMA424. This research was supported by research grants from the National Institute of Allergy and Infectious Diseases to G.C.

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