The octamer-binding proteins Oct-1 and Oct-2 repress the HIV long terminal repeat promoter and its transactivation by Tat

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Although the HIV-1 long terminal repeat (LTR) contains four potential binding sites for the octamer-binding protein, Oct-1, which is known to interact with the HIV-1 Tat protein, the effect of the Oct-1 factor on HIV LTR-driven gene expression has not previously been reported. We show here that both Oct-1, and to a lesser extent the related Oct-2 protein, can repress both the basal activity of the HIV-1 LTR and its transactivation by Tat. These effects are still observed with an HIV LTR construct containing only a single octamer-binding site located between the TATA box and the transcriptional start site. The stronger inhibitory effect of Oct-1 on both these promoters is dependent upon its C-terminal region which cannot be effectively replaced by the equivalent region of Oct-2. These effects are discussed in terms of the regulation of HIV LTR activity in different cell types and in response to T-cell activation.

INTRODUCTION

The HIV-1 LTR (long terminal repeat) promoter contains consensus binding sites for a number of different cellular transcription factors (for reviews see [1–3]). Several such factors, such as stimulating protein 1 (SP1) and nuclear factor κB (NF-κB), bind within the first 100 bp immediately upstream of the transcriptional start site and play an essential role in stimulating the activity of the HIV-1 LTR and its response to stimuli such as T-cell activation [4–6] (Figure 1). In contrast, a region of the LTR further upstream from the transcriptional start site constitutes a negative regulatory element whose binding of cellular transcription factors reduces the activity of the promoter [7].

As well as such regulation by cellular factors, the HIV-1 LTR is also strongly transactivated by the viral Tat protein which binds to the HIV-1 RNA at the transactivation response element (TAR) region (+19 to +42 relative to the transcriptional start site) and produces a large increase in transcriptional initiation as well as overcoming a block to transcriptional elongation (for reviews see [8,9]). Interestingly, the ability of Tat to transactivate via the TAR region appears to be dependent upon its interactions with a number of cellular RNA-binding proteins which can bind to the TAR region [10,11]. Hence, Tat can apparently interact with cellular transcription factors. Moreover, such interactions appear to be involved in the ability of Tat to transactivate in a TAR-independent manner in both glial cells [12,13] and activated T-cells [14]. In both these cases the Tat response element in the HIV-1 promoter has been mapped to the location of the NF-κB sites, suggesting that Tat interacts with NF-κB-binding proteins in glial cells and T-lymphocytes and is thereby recruited to the HIV-1 promoter.

This idea is supported by the finding that NF-κB can be purified on an affinity column to which Tat has been bound [15]. Indeed these studies suggested that Tat could also interact with the SP1 with very high affinity, as well as binding somewhat more weakly to the Oct-1 transcription factor [15]. Thus Tat can interact with both NFκB and SP1, both of which bind to the HIV-1 LTR directly and regulate its expression. In contrast however, no specific role for Oct-1 in regulating the expression of the HIV-1 LTR has previously been reported; however, its interaction with Tat suggested the possibility that this might occur. Indeed inspection of the HIV-1 LTR sequence (for review see [1,2]) revealed four sequences with a good match [6 or 7 bases out of 8] to the consensus octamer-binding sequence ATGCAAAT, which acts as the target site for octamer-binding proteins such as Oct-1 (for review see [16]). These sequences are located at −375 to −368, −229 to −222, and −175 to −168 within the negative regulatory element, and at −14 to −7 between the TATA box and the transcriptional start site (Figure 1). We have therefore investigated the response of the HIV-1 LTR to the overexpression of Oct-1, as well as of the related protein Oct-2 which binds to the same binding site as Oct-1.

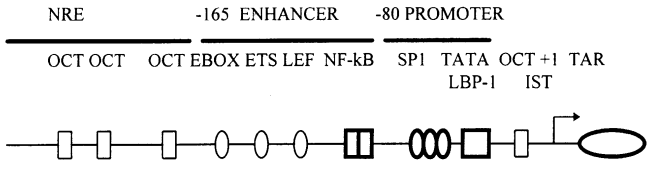

Figure 1 Binding sites for cellular transcription factors within the HIV-1 LTR

The four putative binding sites for octamer-binding proteins (OCT) are indicated. Abbreviations: IST, initiator of short transcripts; LBP-1, leader-binding protein.

MATERIALS AND METHODS

Plasmid DNA

The HIV-1 LTR-based plasmids have previously been described [17]. Tat and its mutant derivatives were expressed under the control of the simian virus 40 promoter. The expression vectors encoding Oct-1 and Oct-2 [18], or chimaeras containing different regions of each molecule [19], have previously been described.

Abbreviations used: CAT, chloramphenicol acetyltransferase; LTR, long terminal repeat; NF-κB, nuclear factor κB; Oct-1/2, octamer-binding protein 1/2; SP1, stimulating protein 1; TAR, transactivation response element.

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DNA transfection and chloramphenicol acetyltransferase (CAT) assays

HeLa and BHK-21 (clone 13 [20]) cells were transfected according to the calcium phosphate procedure of Gorman [21]. Routinely, 10 μg of the reporter plasmid, 10 μg of the Oct-1 or Oct-2 plasmids and 2 μg (HeLa) or 5 μg (BHK) of the Tat expression vector were added to 2×10^6 cells on a 90 mm plate. Assays of CAT activity in the transfected cells were carried out according to Gorman [21] with extracts which had previously been equalized for protein content as described by Bradford [22]. In all cases values obtained in the CAT assays were equalized for differences in plasmid uptake between samples, based on the results of dot blot hybridization of an aliquot of the transfected cell extract with a DNA probe derived from the ampicillin resistance gene [23].

RESULTS AND DISCUSSION

In initial experiments we transfected HeLa cells with a reporter construct (LTR HIV–CAT) in which the full-length HIV LTR drives expression of the readily assayable CAT gene, [17] together with an expression vector containing the Oct-1 coding region under the control of the cytomegalovirus immediate early promoter in the vector PJ7 by calcium phosphate-mediated transfection [21]. In these experiments (Table 1) the Oct-1 expression vector was able to produce a significant fall in the activity of the HIV promoter, reducing it to less than one sixth of its normal level. Moreover, although the inclusion of a Tat expression vector resulted in an almost 200-fold activation of the HIV promoter, this activation was strongly inhibited by Oct-1. Hence the ubiquitously expressed Oct-1 factor can repress the HIV promoter when overexpressed in HeLa cells. This effect was not due to a non-specific inhibitory effect of Oct-1 on all promoters, since we did not observe such reduced activity on a variety of other promoters of both viral and cellular origin.

In order to extend these results, we wished to test whether the inhibitory effect could also be observed with the closely related Oct-2 factor, which is expressed only in B-lymphocytes [24,25] and in neuronal cells [26,27]. As this factor is expressed in a variety of different, alternatively spliced forms [18], we used expression vectors encoding both the predominant B-cell form, Oct-2.1, and the predominant neuronal form, Oct-2.5 [28]. In these experiments (Table 1) both the Oct-2.1 and 2.5 expression vectors were able to repress the HIV LTR and its activation by Tat, although this effect was somewhat weaker than that observed with Oct-1. These results indicate therefore that both Oct-1 and Oct-2 can repress the HIV promoter and its activation by Tat when co-transfected into HeLa cells. A similar repression of both basal LTR activity and Tat transactivation by both Oct-1 and Oct-2 was also observed in BHK fibroblast cells which are of rodent origin [20], indicating that these effects are not unique to human cells.

Table 1 Effect of Oct-1 or Oct-2 on the activity of the HIV LTR in the presence or absence of Tat

CAT activity in HeLa cells transfected with the full-length LTR HIV CAT plasmid together with an expression vector lacking any insert or the same vector containing cDNA inserts encoding Oct-1, Oct-2.1 and Oct-2.5. Transfections were carried out either in the absence (−) or presence (+) of a plasmid encoding the HIV Tat protein. In all cases the values obtained have been normalized to the level of activity obtained upon transfection of the LTR HIV CAT plasmid with the empty expression vector in either the absence or presence of Tat. Note that the presence of Tat resulted in a 250-fold increase in the activity of the HIV promoter. Values are the average of three determinations ± S.D.

Expression vector	−Tat	+Tat
Vector	100	100
Oct-1	15 ± 3	12 ± 2
Oct-2.1	40 ± 6	32 ± 6
Oct 2.5	36 ± 16	21 ± 10

Table 2 Effect of Oct-1 or Oct-2 on the activity of a truncated HIV LTR construct

Assay of CAT activity in HeLa cells transfected with an HIV reporter construct containing only the region downstream of −83 relative to the transcriptional start site together with octamer-binding protein and Tat expression vectors indicated. All values have been normalized to the level of CAT activity obtained upon co-transfection of the reporter with an expression vector lacking any insert either in the absence (−) or presence (+) of the Tat expression vector. In this experiment the average transactivation of the reporter by the Tat construct was approximately 77-fold. Values are the average of two determinations ± S.D.

Expression vector	−Tat	+Tat
Vector	100	100
Oct-1	10 ± 1	5 ± 1
Oct-2.4	24 ± 1	30 ± 2
Oct-2.5	16 ± 5	3 ± 1

As noted above, the HIV LTR contains four sequences related to the octamer motif, three of which are located in a region several hundred nucleotides upstream of the transcriptional start site within the negative regulatory element, whereas the other is located between the TATA box and the transcription start site. To determine whether the octamer-related sequences within the negative regulatory element were involved in the ability of Oct-1 and Oct-2 to repress the HIV promoter, we repeated our transfection experiments using an HIV promoter construct lacking sequences upstream of −83 relative to the transcriptional start site and thus lacking the negative regulatory element and enhancer region of the LTR. As illustrated in Table 2, this promoter construct was repressed by Oct-1 and Oct-2.1 or 2.5 even more strongly than was the full-length HIV LTR. Moreover, although this construct was transactivated approx. 70-fold by the inclusion of a Tat expression vector in the co-transfection experiments, this activation was effectively prevented by both Oct-1 and the different forms of Oct-2. Hence, the upstream octamer-like sequences are unnecessary for the effect of octamer-binding proteins on the HIV LTR promoter, which can be achieved using only a minimal HIV promoter containing sequences downstream of −83, but still including the octamer-like motif located adjacent to the TATA box.

Although our experiments establish that both Oct-1 and Oct-2 can repress the HIV promoter, in all these experiments Oct-1 was able to produce a much stronger effect compared with Oct-2. In order to map the region of Oct-1 which allows it to do this, we used a series of expression vectors encoding chimaeric proteins with different regions derived from Oct-1 and Oct-2.1 [19]. Each of these vectors is denoted by a three-letter code representing the factor from which the N-terminus, POU domain and C-terminus are derived. As illustrated in Table 3, in these experiments consistently stronger repression was observed with the chimaeric factors in which the C-terminus was derived from Oct-1 compared with those in which it was derived from Oct-2.1. This effect was observed both for the inhibition of basal activity of the HIV-LTR in the absence of Tat as well as for the blockage of Tat-mediated transactivation. Similar effects were observed on both

Table 3 Effect of Oct-1/Oct-2 chimaeras on different HIV LTR constructs

Assay of CAT activity in HeLa cells transfected with either the full length HIV reporter or the −83 reporter together with expression vectors encoding chimaeric proteins containing the N-terminal (N), POU domain (P) and C-terminal (C) regions derived from either Oct-1 (1) or Oct-2 (2) as indicated. In all cases the level of CAT activity obtained was normalized to the level obtained with each reporter in the absence or presence of Tat when co-transfected with expression vector lacking any insert (0 in columns N, P and C). Values are the average of two determinations ± S.D.

Expression vector			Full-length HIV LTR		−83 HIV LTR	
			−Tat	+Tat	−Tat	+Tat
N	P	C				
0	0	0	100	100	100	100
1	1	1	15 ± 3	13 ± 2	10 ± 1	5 ± 1
1	2	1	19 ± 1	26 ± 1	17 ± 7	10 ± 2
2	2	1	11 ± 1	7 ± 1	11 ± 3	2 ± 1
2	1	2	58 ± 6	46 ± 16	40 ± 3	46 ± 4
2	2	2	40 ± 5	35 ± 3	23 ± 2	30 ± 2

the full-length HIV promoter and on the promoter containing the region downstream of −83.

Thus, more effective repression is observed when the C-terminus of Oct-1 is present compared with when the equivalent region of Oct-2.1 is present. Such a finding is of interest, since the C-terminus of Oct-2.1 contains a strong activation domain which is important for its ability to activate a variety of octamer-containing promoters [29,30]. In contrast, the equivalent region of Oct-1 does not function as an activation domain for such conventional RNA polymerase II promoters, resulting in it having only a weak ability to activate most octamer-containing promoters in the absence of specific co-activators, such as the herpes simplex VP16 protein [31] or the B-cell specific co-factor OCAB [32]. Similarly, Oct-2.5, which has a C-terminal region more similar to Oct-1 than Oct-2.1, exerted a stronger inhibitory effect than Oct-2.1 (Tables 1 and 2).

Indeed the Oct-1 factor has previously been shown to repress the human papilloma virus type 16 promoter [33–35] by competing with the strong activator nuclear factor 1, one of whose binding sites in the HIV promoter overlaps that of Oct-1. In this case, therefore, the binding of a very weak or inactive activator prevents the binding of a strong transactivator and therefore reduces promoter activity. In contrast, in the HIV promoter the ability of Oct-1 to repress a minimal HIV promoter, which contains an octamer-binding site between the TATA box and the transcriptional start site, suggests a different mechanism. Thus a number of viral genes have been shown to be repressed by the binding of cellular or viral factors to a region between the TATA box and the transcriptional start site. Examples of such repression, which include the binding of leader binding protein-1 to a site overlapping the HIV-1 TATA box [36] as well as the binding of the ICP4 protein to the herpes simplex virus immediate early promoters [37], have been shown to involve inhibition of TBP binding to the TATA box [38] or inhibition of the binding of RNA polymerase II to the pre-initiation complex [39].

It is likely therefore that the inhibitory effects that we have observed, similarly involve the binding of Oct-1 to the octamer-like motif in this region, preventing the binding of either TBP to the TATA box or the subsequent entry of RNA polymerase II. In this model, the weaker effect of Oct-2 on this process would be accounted for by some activation being produced by its strong C-terminal activation domain, although this would be a much smaller effect than the strong inhibition produced by binding to this region of the promoter. Alternatively, the inhibitory effect of Oct-1 and Oct-2 may not require DNA binding but could result from a squelching effect in which Oct-1/Oct-2 competed for a co-activating molecule essential for transcriptional activation.

Whatever their mechanism, the effects we have observed on the HIV promoter are common to both Oct-1 and Oct-2 and do not appear to involve a 40-amino acid region within the N-terminus of Oct-2, which we have previously shown to mediate its ability to repress some promoters in neuronal cells [28,40]. Thus these inhibitory effects are specific to Oct-2 and cannot be reproduced with the corresponding region of Oct-1. Indeed, in our experiments, no enhancement of the inhibitory effect on the HIV promoter was observed when the N-terminus of Oct-2 was substituted for the equivalent region of Oct-1 (see Table 3). Interestingly, however, we have previously documented an interaction between the inhibitory domain of the Oct-2 promoter and the HIV Tat protein [41]. It is likely that this effect represents a distinct phenomenon which is not involved in the effects observed here.

Thus the effect of Oct-2 merely mimics the somewhat stronger effect of Oct-1, with the effect of Oct-2 being weaker due to its C-terminal activation domain. If the effects we have observed are of biological significance they are therefore likely to involve the ubiquitously expressed Oct-1 protein rather than the Oct-2 protein, which is specifically expressed only in B-lymphocytes and neuronal cells. The presence of Oct-1 in a wide variety of cells, including T-lymphocytes, suggests that it may play a role in inhibiting the HIV promoter in specific situations. This could account for the much weaker activity of the HIV LTR compared with other retroviral LTRs, such as those of Rous Sarcoma virus or Moloney Murine Leukaemia virus [42], even though it has binding sites for several positively acting factors such as NF-κB or SP1. Interestingly, in activated T-lymphocytes the Oct-1 protein forms a complex with another cellular factor and is involved in the activation of the interleukin-2 receptor gene promoter in response to T-cell activation [43]. It will evidently be of interest to examine whether this association of Oct-1 with a cellular factor also abolishes its ability to inhibit the HIV LTR. Such studies, as well as mutagenesis of the octamer-like sequence in the HIV LTR, which permit the analysis of its role in non-stimulated and stimulated cells, should allow an understanding of the role of Oct-1 in the regulation of the HIV life cycle.

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