1294–1300 *Nucleic Acids Research, 1998, Vol. 26, No. 5*

Transcriptional interference perturbs the binding of Sp1 to the HIV-1 promoter

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Received October 24, 1997; Revised and Accepted January 8, 1998

ABSTRACT

Transcriptional interference between adjacent genes has been demonstrated in a variety of biological systems. To study this process in RNA polymerase II (pol II) transcribed genes we have analysed the effect of transcription on tandem HIV-1 promoters integrated into the genome of HeLa cells. We show that transcriptional activation at the upstream promoter reduces transcription from the downstream promoter, as compared with basal transcription conditions (in the absence of an activator). Furthermore, insertion of a strong transcriptional termination element between the two promoters alleviates this transcriptional interference, resulting in elevated levels of transcription from the downstream promoter. Actual protein interactions with the downstream (occluded) promoter were analysed by *in vivo* footprinting. Binding of Sp1 transcription factors to the occluded promoter was reduced, when compared with the footprint pattern of the promoter protected by the terminator. This suggests that promoter occlusion is due to disruption of certain transcription factors and that it can be blocked by an intervening transcriptional terminator. Chromatin mapping with DNase I indicates that a factor binds to the termination element under both basal and induced conditions.

INTRODUCTION

Termination of transcription may be defined as arrest of the elongating polymerase followed by release of the transcription complex and nascent RNA from the DNA template. It is known that in eukaryotes functional poly(A) signals are required for efficient transcriptional termination by RNA polymerase II (pol II), since mutations which disrupt polyadenylation also result in the loss of termination (1–3). Furthermore, the strength of the poly(A) signal correlates with termination efficiency, since a stronger processing signal is more effective in directing termination (4). Actual termination sites remain ill defined and in most cases studied so far transcription continues well beyond the poly(A) site (5) until a downstream element is encountered which forces the elongating polymerase to pause or arrest (6,7). Pause sites can take various forms, such as DNA-bound protein complexes or intrinsic DNA sequence elements, either of which are capable of causing poly(A) site-dependent termination of transcription (6,8–11).

It has been reported that inefficient transcriptional termination can cause interference with DNA replication and with transcription of an adjacent, downstream gene, leading to impairment of these central cellular processes (12–16). This emphasizes the need for an efficient termination process in some cases. Transcriptional interference (promoter occlusion) has been observed in various eukaryotic systems, including genes transcribed by pol I and II, in prokaryotic operons and in certain viruses (12). *In vitro* experiments in the pol I system have suggested a mechanism for promoter occlusion where transcription through the promoter may cause displacement of a bound transcription factor. Interference is prevented by terminator elements which reside upstream of each pol I promoter (17,18). In contrast to the less complex pol I promoter, transcription by pol II depends on multiple protein–protein and protein–DNA interactions in the promoter and enhancer regions, which form stable initiation complexes (19).

In the present work we have studied tandem pol II promoters, derived from the HIV-1 long terminal repeat (LTR), stably integrated into the chromosome. We investigated the importance of an intervening transcriptional terminator in this context and furthermore have also investigated DNA–protein interactions on the downstream promoter. Analysis of cytoplasmic RNA revealed that upon transactivation with the viral Tat protein transcription from the downstream promoter is reduced (as compared with already high basal transcriptional conditions). A transcriptional terminator placed between the two LTR promoters allowed increased transcription from both promoters upon transactivation by Tat. Actual DNA–protein interactions on the downstream promoter were analysed by DMS *in vivo* footprinting. While we consistently observed footprints when the promoter was preceded by the terminator, these were reduced when the protective intervening terminator was absent, suggesting reduced binding of transcription factors to the occluded promoter. The footprint pattern changed mainly across the binding sites for transcription factor Sp1. Furthermore, DNase I footprinting *in vivo* indicated binding of a protein to the 5′-end of the pause site, corresponding to the position where the termination factor MAZ has been shown to bind *in vitro* (11). This binding activity is stable under induced conditions. Taken together, our results demonstrate that a pol II termination element located between strong pol II promoters plays a critical role in preventing transcriptional interference in a

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chromosomal context. We suggest that transcriptional interference may result in destabilization of specific transcription factors on the occluded promoter.

MATERIALS AND METHODS

Plasmid constructs

The interference constructs (L1αL2α and L1α-SPAC-L2α) have been previously described (20). For stable transfection into HeLa cells a 2404 bp *Sfi*I–*Bam*HI fragment, containing the SV40 early promoter driving the *neo* gene, was inserted between *Sfi*I and *Eag*I sites of these constructs to generate CI and CII.

The *Tat* gene (derived from a *Tat* expression plasmid; 21) was cloned into pBabe hygro, a plasmid containing the hygromycin resistance gene driven by the SV40 early promoter (22).

Transfections and cell culture

HeLa cells were transfected by the calcium phosphate precipitation technique with 5 µg plasmid. The medium was changed 12 h after transfection and selective medium [MEM (Gibco), containing 400 µg/ml G418 (Gibco), 300 µg/ml hygromycin (Boehringer Mannheim), 10% fetal bovine calf serum] added 48 h after transfection. Single colonies were picked and expanded; cell pools consisted of ~100 individual colonies. Cells were maintained in medium containing 200 µg/ml G418. *Tat*-expressing HeLa cells were established by transfecting CI and CII clonally derived cell lines with a *Tat*-expressing plasmid and propagated in the same selective medium containing additionally 200 µg/ml hygromycin.

RNase protection analysis

RNA extraction and RNase protections were carried out as described previously (20). Riboprobe L is complementary to the *Pvu*II–*Sma*I region of L2 (Figs 2 and 3; 20). Riboprobe S, for the internal standard, was constructed by inserting a restriction fragment (*Ava*I–*Hin*dIII from CI) spanning the *neo* gene into *Ava*I/*Hin*dIII-cut pGEM-3 (Promega). The plasmid was linearized with *Nco*I for *in vitro* transcription. Riboprobes were purified on 5% sequencing gels and 5×10^5 c.p.m. probe were hybridized with 10 µg cytoplasmic RNA. Gels were scanned in a phosphorimager and quantified using appropriate software (Molecular Dynamics).

DMS *in vivo* footprinting

HeLa cells (10^6) grown in 10 cm dishes were incubated with DMS (1 µl/ml; Aldrich) for 5 min. DNA extraction and linker-mediated PCR reactions were performed as described in *Current Protocols* (1996). All primers were purified on 20% sequencing gels.

DNase I *in vivo* footprinting

HeLa cells (up to 2×10^6) were trypsinized, washed in PBS, then in physiological buffer (23), before being taken up in 200 µl physiological buffer (+1% NP-40) and treated with 175 U DNase I (Boehringer Mannheim) for 5 min at 4°C. After DNase I treatment 5 ml lysis buffer (50 mM Tris–HCl, pH 8, 20 mM EDTA, 1% SDS) were added and the DNA phenol extracted and analysed by linker-mediated PCR (24).

RESULTS

It has previously been shown that a strong transcriptional termination signal can block interference between two HIV-1 LTR promoters on plasmids transiently transfected into HeLa cells (20). To study promoter interference in a chromosomal context we stably integrated these tandem promoter constructs into HeLa cells. Two constructs (CI and CII; Fig. 1) were transfected, one containing an intervening termination signal [SPAC, consisting of the strong poly(A) site SPA and the downstream *C2* pause element] between the two LTRs (CII; 25) and the other without this intervening termination signal (CI). Individual cell clones containing the CI and CII transgenes respectively were expanded and maintained in selective (G418-containing) medium. Since the HIV-1 promoter is inducible by the viral transactivator Tat, we were able to detect transcriptional interference by enhancing transcription with Tat. This was achieved by stably transfecting these clonally derived CI and CII cells lines with a *Tat*-expressing vector, allowing a direct comparison of basal and Tat *trans*-activated transcription of one particular clone. *Tat* expression was monitored at the RNA level by S1 nuclease mapping of the *Tat* transcript (data not shown). In the

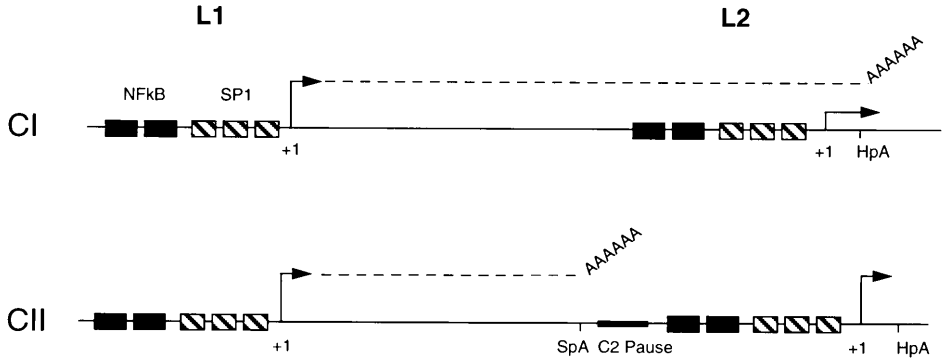

Figure 1. Schematic diagram of the two constructs (CI and CII) used in this study. Both contain two minimal HIV-1 LTR promoters in tandem (L1 and L2). CII contains a termination element placed between the LTR promoters [the SpA poly(A) site followed by the *C2* pause site], allowing termination of L1 transcripts upstream of the L2 promoter. L1 transcripts derived from the CI construct have to read through the L2 promoter to use the HIV poly(A) site (HpA). The transcriptional start sites are indicated by arrows, transcripts initiated at L1 are indicated by dashed lines and the major upstream promoter elements are shown as boxes (NF-κB in black, SP1 white).

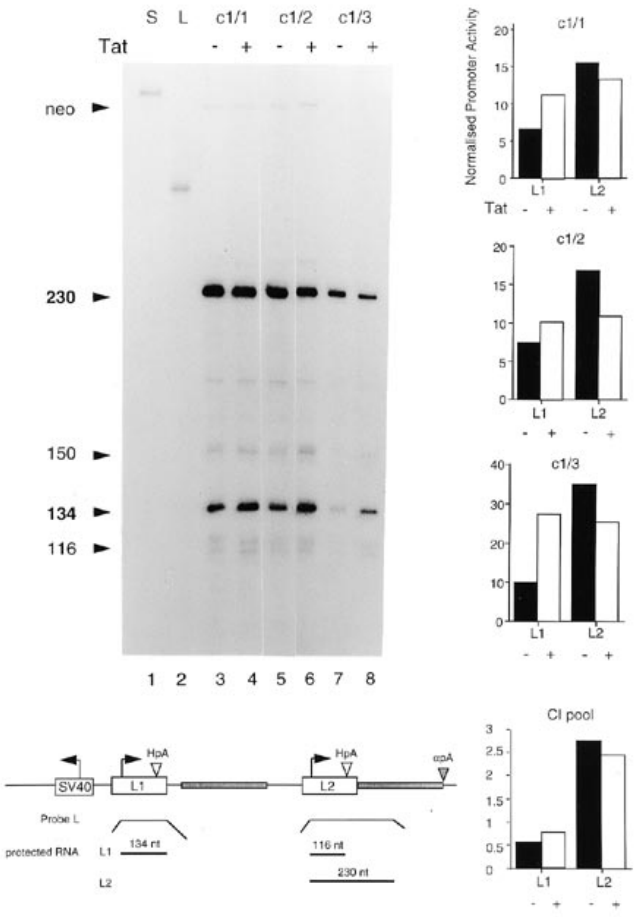

Figure 2. *Trans*-activation by Tat leads to reduced transcription from the L2 promoter in CI cell lines. RNase protection assay of cytoplasmic RNA from HeLa cells stably transfected with CI. Lanes 1 and 2, undigested riboprobes S and L; lanes 3, 5 and 7, three different CI clones under basal transcription conditions; lanes 4, 6 and 8, the same clones transactivated by Tat. The gel was quantified in a phosphorimager. Bars represent RNA levels from the L1 and L2 promoters under basal (–Tat) and *trans*-activated conditions (+Tat) and were normalized to the *neo* control. Also shown are values for a complex CI cell pool (note the lower scale). The diagram at the bottom shows the origin of the protected RNA species. The tandem promoters L1 and L2 (white boxes) driving the α2-globin reporter fragments (grey bars) and the SV40 promoter (which is driving the *neo* gene) are shown, as are the HIV-1 poly(A) site at +80 (relative to the start site at +1; empty triangles), the α2-globin poly(A) site (grey triangle) and the LTR-specific antisense riboprobe (L) which protects transcripts initiated at both LTR promoters simultaneously.

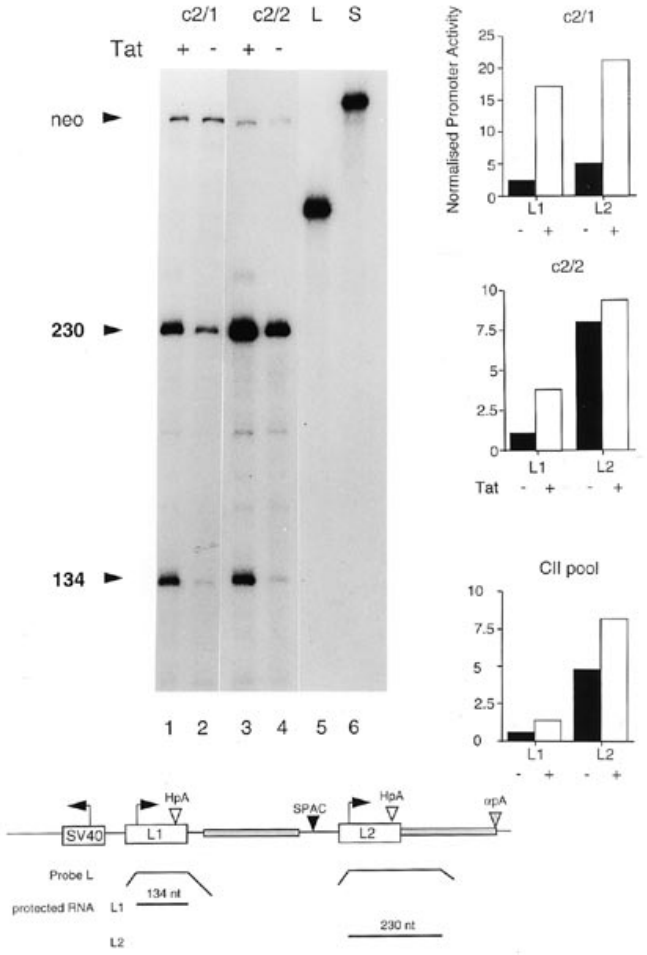

Figure 3. The intervening terminator (SPAC) allows increased transcription from L2 promoters in the presence of Tat. RNase protection assay of cytoplasmic RNA from HeLa cells stably transfected with CII. Lanes 1 and 3, two different CII clones under *trans*-activated conditions (+Tat); lanes 2 and 4, the same clones under basal conditions (–Tat); lanes 5 and 6, undigested riboprobes L and S. *Trans*-activation allows an increase in transcription from both promoters, L1 and L2, in the presence of the terminator. Values were normalized to the internal *neo* standard. Also shown are values for a complex CII cell pool. Diagram as in Figure 2, except that the intervening terminator, SPAC, containing the synthetic poly(A) site is shown (filled triangle).

experiments described below we analysed individual clones derived from these stable transfections which were checked for their integrity and copy number by Southern blotting (data not shown). Clones containing single copy integrants were employed. Furthermore, to obtain position-independent values we also analysed complex cell pools consisiting of >100 positive clones.

It should be noted that the CI and CII constructs both contain an adjacent SV40 promoter–enhancer element which is used to drive expression of the *neo* gene. Consequently, the HIV LTR promoters are significantly active in the absence of Tat, presumably due to the proximity of the SV40 enhancer (26,27). Even so, the presence of Tat further activated the LTR promoters and allowed detection of transcriptional interference effects between the adjacent LTR promoters.

Transcriptional activity from the two promoters (L1 and L2) was assessed by RNase protection assays (Figs 2 and 3). Cytoplasmic RNA, extracted from various individual clones and pools, was analysed using a mixture of two riboprobes: probe L, an LTR-specific riboprobe which recognizes both LTR transcripts simultaneously and distinguishes between L1 read-through and L2 initiating transcripts (derived as a *Pvu*II–*Sma*I fragment from L2); probe S hybridizes to transcripts initiated at the SV40 promoter (which drives transcription of the *neo* gene contained within the transfected plasmid). Probe S was included as an internal control in each assay which allowed direct comparison of

a single clone under basal and *trans*-activated conditions, when normalized to the internal standard.

Transcriptional interference occurs between stably integrated tandem HIV-1 LTR promoters

As shown in Figure 2, RNase protection analysis of transcripts derived from three CI clones yields two major products of 134 and 230 nt, corresponding to transcripts initiating on the L1 and L2 promoters respectively. Figure 2 also shows quantitative analysis of this data as well as comparable data for a CI pool. These results demonstrate a relatively small change in L1 and L2 promoter activity following *trans*-activation by Tat, presumably due to their already high basal levels. Using the SV40 RNA as an internal standard (which is not responsive to Tat), a direct assessment of the change in LTR promoter activity under induced conditions was determined. Under basal conditions (–Tat) the L2/L1 ratio was calculated as ~3 (varying from 2.4 to 3.5 between the three clonal and pool RNAs, taking the number of labeled residues into account). However, in the presence of Tat, while the activity of L1 increased, L2 activity actually decreased (relative to basal transcription conditions), indicating that the *trans*-activated upstream promoter may be reducing transcription from the downstream promoter. The LTR fragment comprising L2 contains an additional 20 bp from the LTR at its 3′-end, which might explain the consistently higher L2 promoter activity. As mentioned above, using these chromosomally integrated constructs transcription from the HIV-1 promoters is rather high, even in the absence of Tat. In contrast, transcription from the same constructs (lacking the SV40 driven *neo* gene) could hardly be detected without Tat in transient expression assays (20). The most likely explanation for this difference is the presence of the SV40 regulatory region containing the enhancer (380 bp from *Sfi*I to *Pvu*II), which has been shown to influence expression from the HIV-1 and human globin gene promoters (28). The proximity of this potent enhancer could affect basal transcription rates, thereby leading to the relatively muted Tat enhancement (27,28). However, since these tandem promoters are differently affected by Tat, we infer that the activated L1 promoter (+Tat) reduces transcription from the downstream L2 promoter.

Additional minor bands visible in Figure 2 derive from L1 transcripts reading into the L2 region of the construct. In particular, the L1 transcript is polyadenylated at the downstream L2 HIV poly(A) site (HpA; Fig. 1), giving rise to a 116 nt 3′ doublet. A small fraction of transcripts read through this poly(A) site, generating bands of ~150 and 248 nt. The 248 nt species arises from read-through of Hp(A) in L2 to the 5′ splice site of the α-globin reporter gene. The origin of the 150 nt species may derive from a cryptic splice site. Both of these L1 species, as well as the major 134 nt fragment, increase upon *trans*-activation. The constant band of ~200 nt does not change upon *trans*-activation and derives from incompletely digested riboprobe. Overall the 3′-end products of transcription initiated at the L1 promoter are clearly present at much lower levels than the 134 nt 5′ product using probe L. Presumably a significant fraction of L1 transcripts is spliced out in the L2 region. Identification of these various minor L1-derived bands was confirmed by S1 nuclease mapping (data not shown).

An intervening transcriptional terminator alleviates interference and restores L2 promoter activity upon Tat transactivation

To examine the role of a strong transcriptional termination element placed between two pol II promoters in a chromosomal context, cytoplasmic RNA extracted from clones and pools stably transfected with the CII construct was analysed by RNase protection (Fig. 3). CII contains a strong synthetic poly(A) site (SPA) followed by the *C2* transcriptional pause site (11,25,29) between the two LTR promoters. As mentioned above, transcriptional termination signals have previously been shown to relieve interference in transient transfection assays (30). Since promoters may be regulated differently when part of higher order chromatin we wished to examine the role of this termination element between the LTR promoters in stable integrants. As shown in Figure 3, no read-in transcripts were detected from the L1 promoter, suggesting 100% SPA use. Secondly, whereas *trans*-activaton led to a relative decrease of L2 in CI clones, we consistently observed an increase for both promoters, L1 and L2, in CII when transcription was enhanced by Tat. These results suggest that transcription from the downstream promoter, upon *trans*-activation, can only increase when preceded by the termination element.

The data presented in Figures 2 and 3 indicate that interference occurs between stably integrated tandem LTR promoter constructs which was detected upon transcriptional enhancement by Tat. Inhibition of the downstream promoter can be overcome by the presence of the intervening termination signal.

In vivo footprinting indicates reduced binding of transcription factors to the occluded L2 promoter

Promoter occlusion by the incoming polymerase complex may be caused by impeding transcription factor access to the promoter or alternatively by actual displacement of factors already bound to the promoter (17). We therefore analysed actual DNA–protein interactions on the downstream L2 promoter by *in vivo* footprinting (31). It should be noted that neither restricted access nor displacement of factors is likely to be an ‘all-or-nothing effect’ and its resolution *in vivo* is therefore limited. Footprinting was carried out using dimethylsulfate (DMS) as the modifying agent. DMS methylates freely accessible guanosine residues of DNA in living cells, unless they are protected by DNA-bound factors. Cells derived from both lines (CI and CII) were incubated with DMS for 5 min (qualitatively similar results were obtained with 2 and 10 min incubations), genomic DNA was purified, cleaved with piperidine and analysed by linker-mediated PCR on 6% sequencing gels. As a control phenol-extracted chromosomal DNA was treated with DMS *in vitro* and analysed in parallel.

A typical analysis is shown in Figure 4, where panel CII represents samples from a CII clone and pool. Binding sites for transcription factor Sp1 are clearly visible as compared with the control (co, lane 7). As expected, these sites are engaged in protein interactions on an active HIV-1 promoter (32). In detail, the CII profiles compared with the control DNA (lane 7) indicate a partial protection of G residues in Sp1 sites 1 and 2, in particular at positions –54 and –55 with respect to the transcriptional start site, a hypersensitive site between the proximal Sp1 sites 1 and 2, at position –60, strong protections in Sp1 site 3 and a hypersensitive site at –79. The distal Sp1 site 3 is known to have the highest affinity for Sp1 *in vitro* (33). Whereas these footprints are clearly

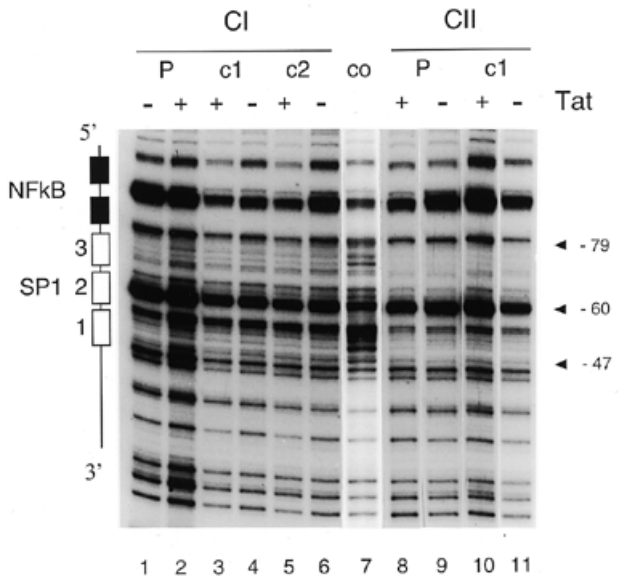

Figure 4. DMS *in vivo* footprinting across the L2 promoter. Footprinting was performed on the coding strand of various clones and pools. The promoter region from –15 (relative to the start site at +1) to the distal NF-κB sites is shown. The left half of the figure (CI) shows the reduced footprints across the upstream elements (indicated on the left side) of CI integrants. Lanes 1 and 2, DNA from a CI cell pool; lanes 3–6, DNA from two CI clones (+Tat and –Tat); lane 7, *in vitro*-treated control DNA (co), the right half showing footprints over the upstream elements; lanes 8 and 9, DNA from a CII cell pool; lanes 10 and 11, DNA from a CII clone. Three G residues are indicated at the right side (filled arrowheads, –47, –60 and –79 relative to the start site at +1)

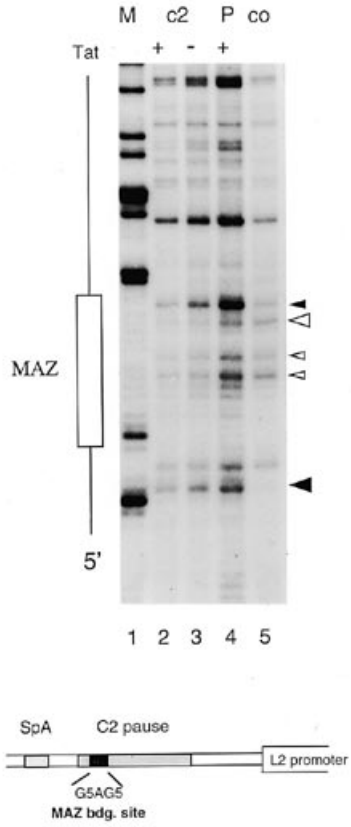

Figure 5. DNase I *in vivo* footprinting of the *C2* pause site. The 5′-end of the *C2* pause site, containing the MAZ binding site, is shown. All samples (lanes 1–5) were probed with the same set of primers. Lane 1, G ladder (CII DNA treated with DMS and piperidine as a marker); lanes 2 and 3, DNA from a CII clone (+Tat and –Tat); lane 4, DNA from a CII pool (+Tat); lane 5, naked DNA treated with DNase I. The white box on the left hand side corresponds to the *in vitro* MAZ binding site. Arrowheads show protected (empty) and hypersensitive sites (filled) with the degree being indicated by size of the arrowhead. The diagram at the bottom shows the position of the pause site and the MAZ binding site.

apparent in CII clones (and pools), they are significantly reduced in CI clones (and pools) lacking the intervening terminator. The clearest differences are seen over the distal, high affinity Sp1 site 3, where all G residues are less protected in CI integrants. Also, the G residues at –54 and –55 are markedly less protected in the CI clones (G residues in SP1 site 1 are also slightly less protected). A reduction in hypersensity over the NF-κB sites was also noted (mainly in CI clones), although with less consistency. Overall these results indicate that protein interactions on the downstream promoter, especially over the Sp1 sites, in CI clones are reduced when compared with those on the corresponding promoter preceded by the terminator. We also observed minor differences in the protein interactions in CI clones when *trans*-activated by Tat as compared with basal conditions. In particular, the two NF-κB sites and position –79 evident in c1/1 and c1/2, which are less hypersensitive in the presence of Tat, indicate perturbed protein interactions under *trans*-activated conditions.

Taken together, the genomic footprinting data presented in Figure 4 reveal reduced binding of Sp1 (and to a lesser extent NF-κB) to the downstream promoter in the absence of an upstream terminator, indicating an effect of interference on protein interactions at the promoter. Since Sp1 activates transcription synergistically (34) and both proteins, Sp1 and NF-κB, are known to contact components of the basal transcription factor TFIID (35), displacement of these factors would explain reduced transcription from an occluded promoter.

A factor binds to the *C2* pause site *in vivo*

The *C2* pause element originates from the 420 bp intergenic region of the human complement genes *C2* and *factor B*, located in the MHC class III region. A region of 156 bp downstream of the *C2* poly(A) site, which contains a protein binding site towards its 5′-end, has been implicated in pol II termination (Fig. 5; 11,29). The zinc finger protein MAZ (Myc-associated zinc finger protein), which was originally identified as a factor that binds to the c-*myc* P2 promoter (36), correlated with the termination efficiency of the pause sites in various assays (29).

To see whether a protein binds to the MAZ site in our integrated construct *in vivo* we analysed the chromatin structure of this region by DNase I *in vivo* footprinting. Footprinting was carried out in isolated nuclei, which were taken up in physiological buffer (23) and partially digested with DNase I (24). The cleavage pattern of the non-coding strand was analysed by linker-mediated PCR and compared with naked DNA, partially digested *in vitro* (no specific primers could be designed to analyse the coding strand). Figure 5 shows a hypersensitive site at the 5′-border of the protein binding site, slight protection in the centre (compare lane

2 and 5), a strongly protected residue towards the 3′-end and a hypersensitive site at the 3′-border. The *in vivo* footprinting pattern is in agreement with previous *in vitro* footprinting data, where a 27 bp region at the same position was protected from DNase I using HeLa cell extract (29). The data also indicated that the pattern did not change upon Tat induction (Fig. 5, lanes 2 and 3), suggesting stable binding of the protein to this site under basal and induced conditions. The same pattern was obtained when analysing DNA from a cell pool in the presence of Tat (lane 4). These results indicate that a protein binds to the *C2* pause site *in vivo* and that binding is not abolished by Tat-actived transcription from upstream. It would therefore seem likely that the binding of this factor to the terminator sequence is not disrupted by transcriptional interference.

DISCUSSION

Transcriptional interference between adjacent transcription units has been observed in various systems. Initially experiments in *Escherichia coli* showed that transcription from the strong prophage λ promoter Pl inhibits the adjacent *gal* promoter (12). Interference between promoters has also been detected on plasmids transiently transfected into mammalian cells (30). *In vitro* experiments in pol I systems led to the suggestion of a possible mechanism for this phenomenon. Template competition experiments in the mouse pol I system indicated actual displacement of transcription factor D by the incoming polymerase, which allowed transcription of competitor templates by the displaced factor (17). A similar observation was made on the pol I promoter in the lower eukaryote *Acanthamoeba*, where a single initiation factor (TIF, the *Acanthamoeba* analogue of mouse factor D) is required for pre-initiation complex assembly and polymerase recruitment to the promoter. *In vitro* footprinting analysis revealed displacement of TIF from a dimeric pol I promoter construct by pol I passage through the promoter (18).

Earlier work has emphasized a role for transcriptional termination elements to relieve interference between transcription units on plasmids transiently transfected into mammalian cells. To examine the importance of terminators between transcription units in the genome we used HIV-1 LTR driven reporter constructs stably integrated into HeLa cells. Since the HIV promoter is *trans*-activated by Tat we could directly compare individual clones under basal and *trans*-activated conditions and the effect of a *trans*-activated promoter on the downstream transcription unit. As mentioned above, the high basic transcription rates of the transgenes were ascribed to the proximity of the SV40 regulatory region with its strong enhancer. Therefore, the relative increase in transcription in the presence of Tat in this system was not drastic (27). Nevertheless, upon *trans*-activation, while transcription from the upstream promoter L1 increased up to 5-fold, transcription from the downstream L2 promoter increased only when preceded by the terminator. Mapping the 3′-end of the upstream L1 transcript revealed its extension into the L2 promoter in CI (–terminator) cell lines, whereas in CII lines it was processed at the intervening terminator. The same terminator consisting of the strong synthetic poly(A) site SPA linked to the *C2* pause site (SPAC) has been shown to prevent promoter interference between the MLV LTR promoter and the *keratin 10* gene in a recent study (22). Stable cell lines transfected with an MLV expression vector containing the whole *keratin 10* gene downstream of the MLV promoter followed by a hygromycin B resistance gene were selected and tested for keratin expression by western blotting. No keratin 10 was detected in these clones unless SPAC was positioned between the MLV and *keratin* promoters. Eighteen of 22 hygromycin B-resistant clones synthesized keratin 10 when SPAC was present (22).

Since interference might be caused by disruption of transcription factor binding to the promoter by an upstream elongation-competent polymerase complex, we mapped DNA–protein interactions on the L2 promoter *in vivo*. Comparison of occluded versus protected L2 promoter by DMS *in vivo* footprinting revealed reduced footprints at the occluded promoter. Differences in the protein binding pattern in CI and CII were mainly evident across the three Sp1 binding sites and to a lesser degree at the tandem NF-κB binding sites. Reduction in Sp1 binding was consistent in several experiments and ~3-fold, whereas changes at the NF-κB sites varied. Since Sp1 activates transcription synergistically (34) and partly by interacting with TAF 110, a component of the basal transcription factor TFIID (37), loss of Sp1 would be expected to result in a reduction in transcriptional activity. Interestingly, Sp1 sites were shown to have no effect on transcriptional termination (10), whereas a bound CAAT box binding protein was effective in termination. It seems possible that, depending on the nature of the DNA–protein interaction, binding of some factors is disrupted by transcription, whereas others remain stably bound. The ability of DNA-bound proteins to impede polymerase elongation has previously been demonstrated (38).

DNase I *in vivo* footprinting across the *C2* pause site of the terminator indicated binding of a factor to the 5′-part of the of the pause element. This binding pattern did not change after Tat *trans*-activation, suggesting a stable contact despite increased transcription from upstream. This region of the pause site, in particular the sequence G_5AG_5 has been shown to bind the zinc finger protein MAZ *in vitro*, which has been implicated in termination in various assays. Insertion of the *C2* pause site between two poly(A) sites greatly increased use of the weak upstream site, probably by pausing the polymerase. Binding of this factor to the pause site might be necessary to halt the polymerase (Fig. 6). The role of MAZ in the termination process is currently being investigated *in vitro*.

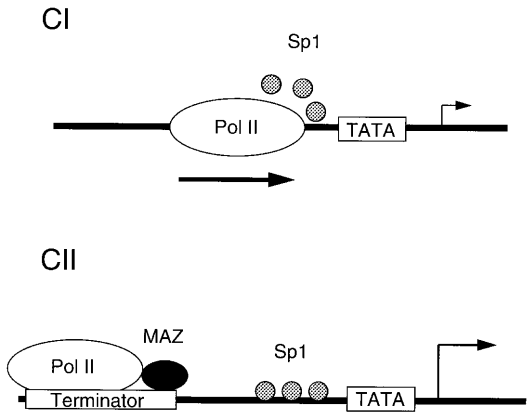

Figure 6. Model for interference between stably integrated HIV-1 promoters. Shown are the L2 promoter regions of both the CI and CII constructs. Sp1 transcription factors (filled grey circles) are displaced by the incoming polymerase (pol II) in CI. In CII the polymerase is stalled at the terminator, possibly by MAZ (black oval) bound to the pause site of the terminator, allowing stable binding of Sp1 to the downstream promoter.

Our data suggest that transcriptional termination elements play an important role in regulation of gene expression, to allow individual expression of adjacent transcription units. The absence of a terminator can lead to reduction or impairment of adjacent gene expression, probably resulting from a disruption of DNA-bound regulatory proteins by a processive polymerase complex. We are currently testing our model for transcriptional interference (Fig. 6) in yeast, where transcription units are densely packed and therefore efficient transcriptional termination of more significance.

ACKNOWLEDGEMENTS

We thank C.Birse and S.Murphy for critical reading of the manuscript and S.Murphy, M.Ashe and B.Lee for helpful suggestions. We also thank T.Grange for providing us with the DNase I footprinting protocol. I.G.H. was supported by the Oesterreichisches Bundesministerium fuer Wissenschaft und Forschung. This work was supported by a Wellcome Trust Programme Grant no. 032773 to N.J.P.

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