Spacing between the enhancer and promoter of the long terminal repeat of a murine leukaemia retrovirus is required for transcriptional activation in T cells

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In T cells, transcriptional activation by the long terminal repeat (LTR) of the mink cell focus-forming murine leukaemia virus requires some spacing between the enhancer and promoter. A large size-range of intervening sequences (11–93 bp) is able to activate transcription efficiently. Neither a specific nucleotide sequence nor stereospecific alignment of the spacer is important.

Cell-type-specific transcriptional activation by a retroviral long terminal repeat (LTR) region is a major determinant of viral pathogenicity (Hallberg et al., 1991; LoSardo et al., 1990; Speck et al., 1990). It has previously been demonstrated that sequences in the LTR of the MCF13 murine leukaemia virus that regulate transcription specifically in T cells also contribute to the ability of this retrovirus to induce T-cell lymphoma (Tupper et al., 1992). Some of these sequences are located in the region between the enhancer and promoter, DEN (for downstream of enhancer). An important function of DEN which has recently been identified is that it is the only region in the MCF13 LTR that regulates transcription in activated T cells (Chen & Yoshimura, 1994). This study demonstrates an additional function of DEN in transcriptional regulation: the maintenance of proper spacing between the enhancer and promoter. Our data indicate that the spacing provided by DEN is important for transcriptional activation specifically in T cells.

For this study an LTR reporter gene construct containing a single enhancer repeat was used (M3, Fig. 1) (Tupper et al., 1992). It was observed that deletion of the DEN region from M3 (DEN, Fig. 1) resulted in a 4- to 15-fold decrease in transcriptional activity in different cell types (Table 1). The greatest decrease in activity was detectable in lymphocytes. Because previous work on other promoter regions has shown that the maintenance of proper spacing between cis-regulatory elements is important for transcriptional activation (Ach & Weiner, 1991; Gaston et al., 1990; Kliwer et al., 1992; Naar et al., 1991; Tansey et al., 1993), we tested whether this also could be one of the functions of the DEN region in the MCF13 LTR.

The DEN region of M3 was initially replaced with pUC19 plasmid sequences corresponding to the same number of nucleotides which constitute DEN (ΔDEN + 93, Fig. 1). Plasmid sequences were inserted between the Rsal and Drai sites of the ΔDEN clone. The reporter gene in all the LTR constructs that were made for this study was the chloramphenicol acetyltransferase (CAT) gene (Gorman et al., 1982). To confirm this construct and the other constructs described below, the nucleotide sequences of the altered sites were determined by the dideoxynucleotide method (Sequenase, United States Biochemical). The ΔDEN + 93 plasmid DNA was transfected into different cell types by lipofection (Dorman & Yang, 1989) and CAT enzymatic activity in cellular extracts was measured 48 h post-transfection, as described by Tupper et al. (1992). As shown in Table 1, the 93 bp plasmid insert increased the transcriptional activity of the ΔDEN clone to wild-type (M3) levels in T cells, but not in B cells or fibroblasts. These results indicated that the insertion of non-LTR sequences between the enhancer and promoter could activate transcription from the viral promoter in a cell-type-dependent manner.

The role of spacing between the enhancer and promoter in T cells was further examined by determining whether the exact length of the distance between these two regulatory elements was important for transcriptional activation. To do this, 11, 33 or 500 bp were inserted between the enhancer and promoter of the ΔDEN construct (Fig. 1). The ΔDEN + 11 and ΔDEN + 33 clones were constructed by inserting oligonucleotides containing random sequences of 11 or 33 bp in length between the Rsal and Drai sites of the ΔDEN clone. For the 500 bp insertion, pUC19 sequences were amplified by PCR and this DNA fragment was inserted into the same restriction endonuclease sites.

The ΔDEN + 11, ΔDEN + 33 and ΔDEN + 500 plasmid DNAs were transfected into the L691 and Jurkat T-cell lines, and the CAT activity in cellular extracts was measured. The 11- and 33 bp insertions resulted in transcriptional activities in T cells that were comparable to the wild-type M3 clone (Table
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Fig. 1. MCF13 murine leukaemia virus LTR constructs. M3 is the MCF13 LTR that contains a single enhancer repeat (indicated by En box) ligated to the CAT reporter gene. The DEN region is indicated as well as the CAT (C) and TATA (T) boxes in the promoter region. Numbering is relative to the start site of transcription, which is shown by the arrow. ∆DEN is the LTR construct that is missing the DEN region between the RsaI and DraI sites. LTR constructs with different lengths of nucleotide insertions in place of the DEN region are designated as ∆DEN plus the size of the respective insertion.

Table 1. Separation of the MCF13 enhancer and promoter activates transcription in a cell-type-specific manner

CAT values are expressed as a percentage of the activity of M3, which has been assigned the arbitrary value of 100. Each value represents the mean of two to ten independent transfections performed in at least two different experiments. At least two plasmid preparations were used for each construct tested. Standard errors of the mean are included. Nonchromatographic CAT activity was determined by measuring counts per minute (c.p.m.) of [14C]acetylated chloramphenicol. The range of c.p.m. was $5 \times 10^3$ to $1 \times 10^5$ for the CAT assays. The values were corrected for background (less than $10^3$ c.p.m.).

<table>
<thead>
<tr>
<th>LTR construct</th>
<th>Percentage CAT activity ± SEM</th>
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<tbody>
<tr>
<td></td>
<td>T cells</td>
</tr>
<tr>
<td></td>
<td>L691</td>
</tr>
<tr>
<td>∆DEN</td>
<td>15.0 ± 5.8</td>
</tr>
<tr>
<td>∆DEN + 93</td>
<td>86.0 ± 5.9</td>
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</table>

2). However, transcriptional activation significantly decreased when the size of the insertion was 500 bp, indicating that there was a limit to the distance by which the enhancer and promoter could be separated. Thus the data showed that as few as 11 and as many as nearly 100 nucleotides could provide sufficient spacing between the enhancer and promoter for effective transcriptional activation.

Is stereospecific alignment important for the spacing effect of the DEN region? It has been observed that in addition to proper spacing between transcription elements, stereospecific alignment of these elements may be required for transcriptional activation (Ach & Weiner, 1991; Naar et al., 1991; Takahashi et al., 1986; Vilen et al., 1991). Therefore we examined whether interactions between the MCF13 enhancer and promoter required a specific alignment. Clones with insertions corresponding to whole helical turns were ∆DEN + 11, ∆DEN + 33 and ∆DEN + 93 based on the value of 10.5 bp per helical turn of DNA (Rhodes & Klug, 1980; Tullius & Dombroski, 1985). On the other hand, the 500 bp insertion placed the enhancer and promoter on opposite sides of the DNA double helix. The decrease in transcriptional activity observed for the ∆DEN + 500 clone, as discussed above, may have been the result of the placement of the enhancer and promoter on opposite sides of the DNA helix. To test this
 possibility a second clone (ΔDEN + 38) was constructed, which also contained an insertion producing an uneven number (3·5) of helical turns. Because the ΔDEN + 38 clone had transcriptional activity comparable to wild-type, it is concluded that stereospecific alignment is not required for interactions between the enhancer and promoter for transcriptional activation in T cells.

This study demonstrates that transcriptional activation by the MCF13 enhancer in T cells requires that it be separated from the promoter by as few as 11 bp and as many as 93 bp, which is the size of the normally intervening DEN region. The present results do not address the possibility that distances smaller than 11 bp, or between 100 and 500 bp, would also be effective. It was also observed that large distances, such as 500 bp, between the enhancer and promoter interfered with the efficient interaction of these transcriptional elements. The data indicate that stereospecific alignment of the promoter and enhancer by the intervening sequences is not required for transcriptional activation.

Promoter elements can interact with each other in a manner that is independent of or dependent on stereospecific alignment (Amin et al., 1994; Gaston et al., 1990; Giniger & Ptashne, 1988; Sowden et al., 1989; Takahashi et al., 1986). The present results indicate that the promoter and enhancer regions of the MCF13 LTR can interact effectively with each other in a manner that is independent of stereospecificity. Although this study concludes that a function of the DEN region is to provide spacing between the enhancer and promoter, this is not its sole function; we have demonstrated that DEN is the only region of the MCF13 LTR that is able to potentiate transcription in activated T cells (Chen & Yoshimura, 1994), an activity which is dependent on the binding of specific transcription factors (Yoshimura et al., 1997). It appears, therefore, that there are multiple mechanisms by which the DEN region can regulate transcription in T cells, and the state of activation of these cells may determine which mechanism is used. We are presently testing whether one or both of these mechanisms are important for viral lymphomagenesis. The observation that separation of the enhancer and promoter is not sufficient for transcriptional activation in B lymphocytes and fibroblasts suggests that other functions of the DEN region are required for these cell types. These functions may include the binding of transcription factors or the formation of transcriptionally active chromatin. We have recently observed that the DEN region contains an NF-κB binding site, which regulates transcription in activated T cells (Yoshimura et al., 1997). Because NF-κB is known also to regulate transcription in B cells (Baeuerle & Henkel, 1994; Grilli et al., 1993), it is possible that binding of this factor to DEN sequences results in transcriptional activation in this cell type. Our studies have demonstrated that the same enhancer and promoter utilize different mechanisms by which they interact with each other to regulate cell-type-specific transcription.

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### Table 2. Effect of distance variation and sterealignment on transcriptional activation in T cells

Experimental details were as in Table 1.

<table>
<thead>
<tr>
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<tr>
<td></td>
<td>L691</td>
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<tr>
<td>ΔDEN</td>
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<td>ΔDEN + 11</td>
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<td>ΔDEN + 93</td>
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<td>ΔDEN + 500</td>
<td>36·4 ± 3·5</td>
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References


Kliwer, S. A., Umesono, K., Heyman, R. A., Mangelsdorf, D. J., Dyck,


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