Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 308–312, January 1984
Biochemistry

Stimulation of *in vitro* transcription from heterologous promoters by the simian virus 40 enhancer

[S100 extract/whole cell extract/72-base-pair repeat/RNA polymerase B (II)/eukaryotic promoter]

PAOLO SASSONE-CORSI, JOSEPH P. DOUGHERTY, BODHAN WASYLYK, AND PIERRE CHAMBON*

Laboratoire de Génétique Moléculaire des Eucaryotes du Centre National de la Recherche Scientifique, Unité 184 de Biologie Moléculaire et de Génie Génétique de l'Institut National de la Sante et de la Recherche Médicale, Institut de Chimie Biologique, Faculté de Médecine, Strasbourg 67085 Cédex, France

Communicated by Peter Reichard, September 15, 1983

ABSTRACT **Insertion of the simian virus 40 enhancer upstream from the +33 to −34 adenovirus major late promoter element or the +62 to −102 conalbumin promoter region causes a 10-fold stimulation of specific transcription using a whole cell extract but not an S100 extract. Many of the *in vivo* effects of the enhancer were mimicked *in vitro*. This stimulation occurred only in *cis*, with either orientation of the 72-base-pair repeat, and was markedly decreased by deletions known to diminish the enhancer activity *in vivo*. However, *in vitro*, the 72-base-pair repeat did not stimulate at a distance or in the presence of a strong upstream promoter element.**

The structure of promoters for RNA polymerase B (II) is complex (for reviews and refs., see refs. 1–12). The "TATA" box, located about 30 base pairs (bp) upstream from the cap site, is required for selective and efficient transcription *in vivo* and *in vitro* and may interact with a general initiation factor (13, 14). Further upstream elements (to about −110) are required *in vivo* and *in vitro* for efficient transcription and could interact with species-specific factor(s) (6, 15–17). Enhancers, first identified in simian virus 40 (SV40) (the 72-bp repeat; refs. 18 and 19) and in other viruses (for reviews, see refs. 20 and 21), and recently in cellular genes (22–26), are indispensable for efficient transcription *in vivo* and appear to exhibit some species-, tissue-, or cell-specificity (20–26), which suggests an interaction with specific regulatory molecules and a role in control of gene expression during differentiation. The SV40 enhancer is a *cis*-acting bidirectional potentiator of initiation from homologous or heterologous potential promoter elements (10, 27–29). Moreover, it activates preferentially proximal potential promoter elements (10, 30), although it can act over considerable distances [several kilobase pairs (kbp)] with a decreased efficiency (10, 28). We have suggested (10, 28) that the SV40 enhancer functions, at least in part, as an entry site for some elements of the transcription machinery. *In vitro* studies with the purified components involved in transcription will ultimately be required to verify such a model. We report here that the SV40 72-bp repeat stimulates specific transcription *in vitro* from heterologous promoter elements.

MATERIALS AND METHODS

The *in vitro* transcription assay and the preparation of HeLa cell S100 extract (31) or whole cell extract (WCE) (32) have already been described (1, 6, 33). The DNA template for run-off assays was a complete *Taq* I digest. The *Taq* I fragment containing the adenovirus serotype 2 major late promoter (Ad2MLP) or the conalbumin promoter in the pSVCT series extends from position 23 in pBR322 to 4739 (BBB system; ref. 34) in the SV40 early region, whereas in the pTCT series the conalbumin promoter-containing fragment extends from position 651 in pBR322 to 4739 in SV40 (see Fig. 1 for the construction of recombinants). The run-off RNAs were analyzed on 5% acrylamide/8.3 M urea gels (35). The experiments in this paper were reproduced repeatedly using several different preparations of plasmid DNA and cellular extracts.

RESULTS

Insertion of the SV40 72-bp Repeat Upstream from Ad2MLP and Conalbumin Promoter Elements *cis*-Activates *in Vitro* Specific Transcription from Linear and Circular Templates with WCE, but Not with S100 Extract. Insertion of the SV40 72-bp repeat upstream from Ad2MLP and conalbumin promoter elements markedly stimulates transcription from these promoters after short-term transfections in HeLa cells (6, 10). For instance, no RNA could be detected after transfection with pSVA34 [which possesses the Ad2MLP sequence from +33 to −34, but not the upstream sequence to −97 (see ref. 6); Fig. 1*A*] or with pTCT (which contains the conalbumin promoter sequence from +62 to −102; Fig. 1*C*). Insertion of the 72-bp repeat (coordinates 113 to 270) in both orientations in close apposition to the Ad2MLP (pSVBA34 and pSVBIA34; Fig. 1*A*) or the conalbumin promoter (pTCTB and pTCTBI; Fig. 1*C*) elements resulted in a dramatic increase, at least 100-fold, of RNA synthesis (6, 10). *Taq* I digests of the same recombinants were used as templates with a WCE transcription system. Comparison of the run-off transcripts obtained with pSVA34 and pSVBA34 indicates that the 72-bp repeat markedly stimulated (about 10-fold) the synthesis of the specific run-off RNA (Fig. 2). A similar stimulation of specific transcription was observed when the conalbumin recombinants, pTCT and pTCTB, were used with a WCE (Fig. 3*A*). pSVCT, a conalbumin promoter recombinant in which the SV40 region containing the 72-bp repeat extends further toward the late genes (Fig. 1*D*), gave the same result as pTCTB. For both adenovirus serotype 2 (Ad2) and conalbumin recombinants, the strongest stimulation was observed for DNA concentrations <400 ng per 25 μl of reaction mixture, whereas there was very little stimulation with >600 ng of DNA (data not shown). However, with both Ad2 (S100 extract, pSVA34, pSVBA34; Fig. 2) and conalbumin (pTCT, pTCTB; Fig. 3*B*) recombinants, very little (not more than 2-fold) or no stimulation by the 72-bp repeat was observed using a number of different S100 extracts.

Because the conformation of the template could affect *in vitro* transcription (6, 38), we compared the amount of RNA synthesized from linear and circular pSVA34 and pSVBA34 DNA using a WCE and nuclease S1 mapping. The extent of stimulation of specific transcription by the 72-bp repeat was

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: SV40, simian virus 40; Ad2 and Ad5, adenovirus serotypes 2 and 5; Ad2MLP, Ad2 major late promoter; WCE, whole cell extract; bp, base pair(s); kbp, kilobase pair(s).

*To whom reprint requests should be addressed.

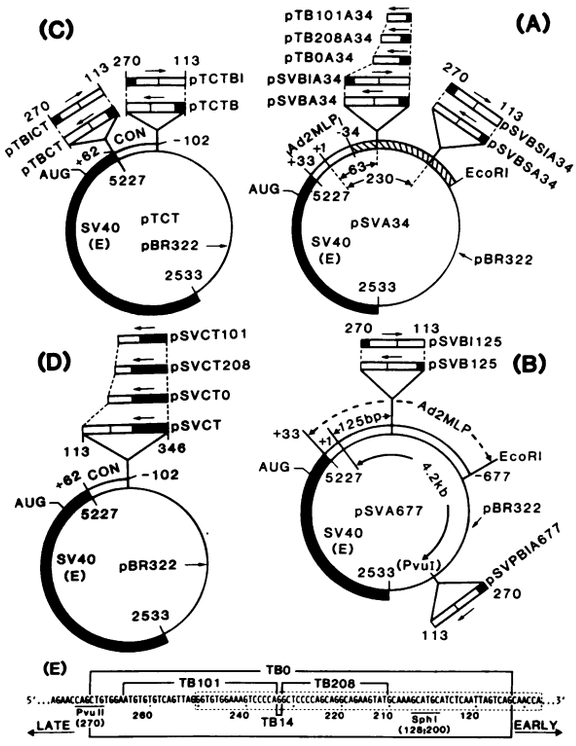

FIG. 1. Structures of chimeric recombinants containing the wild-type or mutated SV40 72-bp repeat and elements of either the Ad2MLP or the conalbumin promoter. (*A*) pSVA34, pSVBA34, pSVBIA34, pTB208A34, and pTB101A34 have been described (6). pSVA34 contains the SV40 early coding region [heavy line, SV40 (E), coordinates 5227–2533] downstream from the Ad2MLP region between +33 and −34, which includes the cap site (+1) and the complete TATA box (double line, the replacing Ad2 sequences upstream from −34 are hatched). In pSVBA34 and pSVBIA34, the wild-type (coordinates 113–272) segment containing the 72-bp repeat (open boxes) was inserted in both orientations [the arrows indicate the natural orientation with respect to the SV40 (E) sequence] in an *Sst* I site 63 bp upstream from the Ad2MLP cap site. pTB0A34, pTB14A34 (not shown), pTB208A34, and pTB101A34 contain the fraction of the 72-bp repeat present in the deletion mutants TB0 (one exact 72-bp sequence deleted between the *Sph* I sites at SV40 coordinates 128 and 200, shown in *E*), TB14, TB208, and TB101 as described in *E* and refs. 28 and 36. In pSVBSA34 and pSVBSIA34, the 72-bp repeat region was inserted in both orientations in an *Sac* II site located 230 bp upstream from the Ad2MLP cap site. (*B*) pSVA677 and pSVPBIA677 are the previous recombinants pSVA500 and pSVPBIA500 (6), renamed since sequence analysis (37) has shown that they contain the Ad2MLP region from +33 to −677. pSVB125 and pSVBI125 were derived from pSVPBIA677. The 72-bp repeat was inserted in both orientations in an *Sma* I site that was created in pSVPBIA677 by site-directed mutagenesis at position −125 with respect to the Ad2MLP capsite. (*C*) pTCT, pTCTB, pTCTBI, pTBCT, and pTBICT have been described (10, 28). pTCT contains the conalbumin promoter region from +62 to −102 (double line) in front of the SV40 early coding region. In pTCTB and pTCTBI, the 72-bp repeat was inserted in both orientations immediately upstream from the conalbumin promoter, while it was inserted immediately downstream from it in pTBCT and pTBICT. (*D*) pSVCT has been described (10). It is similar to pTCTB, but the 72-bp repeat region extends further toward the late region to coordinate 346 (*Hpa* II site). pSVCT0, pSVCT208, and pSVCT101 were derived from pSVCT. The *Sph* I fragment of pSVCT extending from 128 (SV40) to 561 (pBR322) was replaced with the corresponding *Sph* I fragments from the previously described (28, 36) deletion mutants TB0, TB208, and TB101, respectively (shown in *E*). Therefore, replacing the *Sph* I fragment of pSVCT with the *Sph* I fragment of TB0, TB208, or TB101 resulted in recombinants (pSVCT0, pSVCT208, pSVCT101) in which the TB0, TB208, and TB101 deletions of the 72-bp repeat are adjacent to the conalbumin promoter element. (*E*) Sequence of the 72-bp repeat region in pTB0A34, pTB14A34, pTB208A34, pT101A34, pSVCT0, pSVCT208, and pSVCT101. The boxed sequence corresponds to the 72-bp repeat region (with exactly one 72-bp sequence deleted) inserted in pTB0A34 (the 72-bp sequence is boxed with a dashed line). The corresponding sequence in pSVCT0 extends further in the late region from the *Pvu* II site to the *Hpa* II site (coordinate 346). The sequences deleted in the TB14, TB208, and TB101 mutants are bracketed. The recombinants shown in *A*, *B*, and *D* contain the whole pBR322 DNA; those shown in *C* contain the large *Bam*HI/*Eco*RI fragment only.

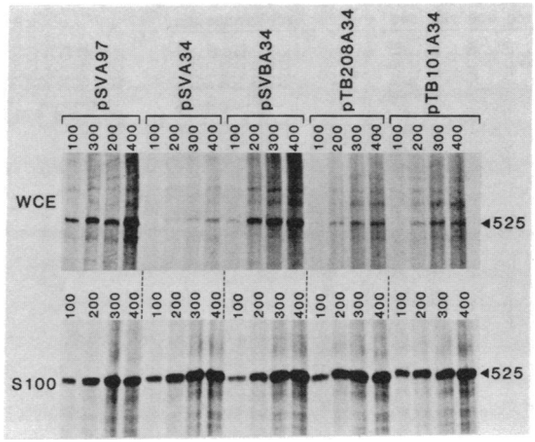

FIG. 2. Effect of the 72-bp repeat on transcription from the +33 to −34 Ad2MLP element using a WCE or an S100 extract. pSVBA34, pTB208A34, and pTB101A34 are described in Fig. 1 *A* and *B*. pSVA97 (see *Results* and ref. 6) is analogous to pSVA34 but contains the Ad2MLP sequences from +33 to −97. Complete *Taq* I digests of 100, 200, 300, or 400 ng were used, as indicated, in run-off transcription assays with WCE or S100 extract. The size of the run-off RNA initiated at the Ad2MLP cap site is 525 nucleotides.

the same regardless of the conformation of the template (Fig. 4, lanes 1–4).

As previously observed by others (see, for instance, figure 1 in ref. 39), we have often noticed an increase in background and even in nonspecific bands that paralleled the increase in intensity of the specific transcripts (see, for instance, Figs. 2 and 3). The origin of this variable nonspecific labeling is complex (RNA aggregation, labeling of DNA, nonspecific polymerase B transcripts, polymerase C transcripts; unpublished observations; see also ref. 39), and to date we have no satisfactory explanation for this increase of nonspecific labeling. To show that stimulation of specific transcription by the 72-bp repeat was in fact a *cis*-effect, a linear template containing the adenovirus serotype 5 (Ad5) E3 promoter was added as an internal control (Fig. 5 *A* and *B*). No change in the extent of specific transcription from the Ad5 E3 template was observed under conditions where the stimulation for pSVBA34 was evident. Other experiments have shown that the E3 promoter can be stimulated in *cis* by the 72-bp repeat (unpublished results). The *cis*-effect of the 72-bp repeat was further supported by the results of transcription of pSVPBIA34, which is analogous to pSVA34, except that the 72-bp repeat is now inserted 4.1 kbp from the Ad2MLP capsite (see ref. 6), so that after digestion with *Taq* I, the 72-bp repeat and the Ad2MLP element are on different fragments. As shown in Fig. 6 (lanes 1 and 2), the extent of transcription from the Ad2MLP was the same whether the template was pSVA34 or pSVPBIA34.

Activation of *in Vitro* Specific Transcription by the 72-bp Repeat Is Independent of Its Orientation and Abolished by Deletion Mutations Known to Drastically Affect the Enhancer Activity *in Vivo*. In pSVBIA34 and pTCTBI, the 72-bp repeat is inserted in the inverted orientation with respect to pSVBA34 and pTCTB (Fig. 1 *A* and *C*). Nevertheless, the

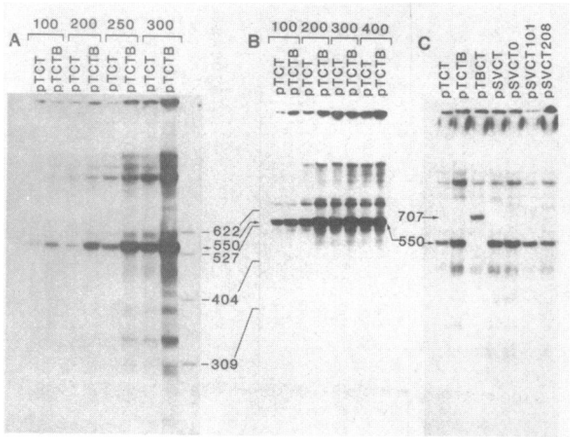

FIG. 3. Effect of the 72-bp repeat on transcription from the +62 to −102 conalbumin promoter region. The chimeric recombinants pTCT, pTCTB, pTBCT, pSVCT, pSVCT0, pSVCT208, and pSVCT101 are described in Fig. 1 *C* and *D*. (*A* and *B*) Run-off transcription with *Taq* I digests of pTCT and pTCTB using a WCE and an S100 extract, respectively. DNA concentrations (100, 200, 250, 300, or 400 ng per assay) were as indicated. The length of the specific run-off transcript initiated at the conalbumin cap site is 550 nucleotides. (*C*) Comparison of run-off transcription from a number of chimeric recombinants using a WCE. Each reaction mixture contained 200 ng of *Taq* I-digested recombinant as indicated. Arrows point to the specific run-off RNAs. The size of the specific run-off for pTBCT is 707 nucleotides, because in this recombinant the 72-bp repeat region is located downstream from the conalbumin promoter region, which results in adding 157 nucleotides to the RNA initiated at the conalbumin capsite.

same extent of stimulation was obtained with pSVBA34 and pSVBIA34 (Fig. 4*A*) and with pTCTB and pTCTBI (results not shown).

It has been reported that deletion of exactly one 72-bp repeated sequence does not significantly decrease the activity of the SV40 enhancer (TB0 series, Fig. 1*E*; see also refs. 10, 28, and 36). The Ad2MLP and conalbumin promoter recombinants, pTB0A34 (Fig. 1*A*) and pSVCT0 (Fig. 1*D*), with only one 72-bp sequence were constructed from pSVBA34 and pSVCT, respectively. No significant decrease in the intensity of the specific run-off was observed when they were transcribed *in vitro* (compare pSVBA34 and pTB0A34 with pSVA34 in Fig. 5*A* and pSVCT and pSVCT0 with pTCT in Fig. 3*C*). The effect of the TB208 and TB101 deletions, located within the 72-bp repeat region (see Fig. 1*E*) and known to almost abolish the enhancer activity *in vivo* (6, 28, 36), was then studied using the Ad2MLP and conalbumin promoter recombinants pTB208A34 and pTB101A34 (Fig. 1*A*), and pSVCT208 and pSVCT101 (Fig. 1*D*), which contain the 208 or 101 deletions. In all cases, no significant stimulation of specific transcription was observed by the remaining part of the 72-bp repeat (compare pSVA34, pSVBA34, pTB208A34,

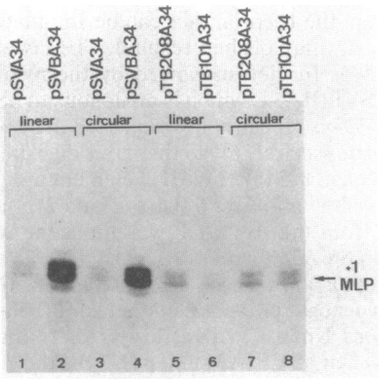

FIG. 4. The stimulatory effect of the 72-bp repeat on *in vitro* transcription is independent of the template conformation. Quantitative nuclease S1 mapping of *in vitro* RNA transcripts obtained using WCE and linear (*Taq* I digests) or circular (at least 70% of the molecules were superhelical) templates (as indicated on the figure) was carried out as described (figure 4 of ref. 6). Nuclease S1 digestion was carried out using 2,000 units of nuclease S1 (Miles) in the presence of 30 mM sodium acetate, pH 4.5/3 mM $ZnCl_2$/400 mM NaCl for 2 hr at 25°C. The nuclease S1-resistant material was analyzed on 8% acrylamide/8.3 M urea gel (35). MLP +1 indicates the nuclease S1-resistant fragments corresponding to specific transcription from the Ad2MLP.

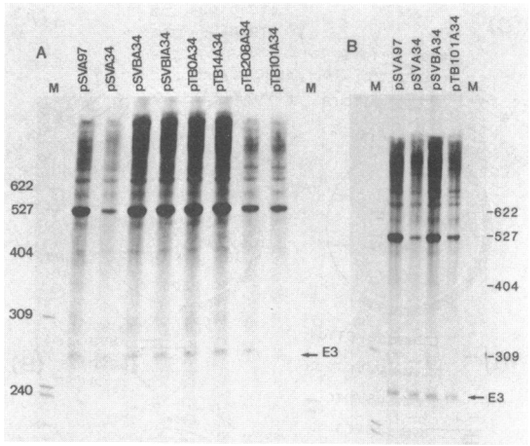

FIG. 5. Effect of deletions within the SV40 72-bp repeat and of its inversion on stimulation of transcription from the +33 to −34 Ad2MLP element. The run-off RNA transcripts were synthesized using a WCE. *A* and *B* are duplicate experiments. The length of the specific Ad2MLP run-off is 525 nucleotides. Each reaction mixture contained 300 ng of *Taq* I-digested recombinant (see Fig. 1 *A* and *B*) as indicated in the figure. As an internal transcription control, 40 ng per reaction of an Ad5 E3 template was added [a *Bgl* I linearized M13mp8 clone containing the E3 promoter region (*Eco*RI/*Pst* I fragment) and giving a 268-nucleotide run-off RNA]. Lanes M, size markers (^{32}P-end labeled *Msp* I fragments of pBR322).

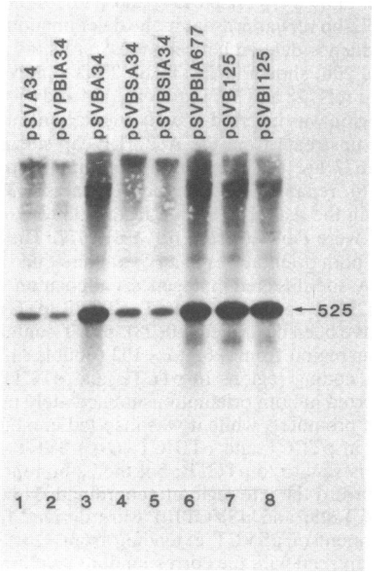

FIG. 6. Effect of the upstream Ad2MLP element and distance on the stimulatory effect of the 72-bp repeat on *in vitro* transcription. Each assay mixture contained 300 ng of *Taq* I-digested recombinant. The run-off transcripts were synthesized with a WCE. The length of the specific run-off RNA initiated at the Ad2MLP cap site is 525 nucleotides.

and pTB101A34 in Fig. 5, and pTCT, pSVCT, pSVCT101, and pSVCT208 in Fig. 3*C*). The strong down-effect of the TB101 and TB208 deletions was identical, whether the template was linear or circular (Fig. 4). On the other hand, the single base deletion in the 72-bp sequence of the Ad2MLP recombinant pTB14A34 (see legend to Fig. 1), which does not affect the enhancer activity *in vivo* (unpublished results), does not change the *in vitro* stimulation of transcription (Fig. 5*A*). Specific transcription from the internal control E3 template remained unchanged in all cases (Fig. 5), supporting the conclusion that the 72-bp repeat is *cis*-acting.

The *in vivo* effect of the SV40 enhancer is drastically decreased when it is inserted immediately downstream from the conalbumin promoter element (10)—i.e., in recombinants pTBCT and pTBICT (Fig. 1*C*). A similar drastic decrease in the stimulation of specific transcription was also obtained *in vitro* [compare pTCT, pTCTB and pTBCT in Fig. 3*C*; in other experiments pTBCT and pTBICT gave identical results (not shown)]. Additional experiments are required to establish whether this lack of *in vitro* stimulation of transcription of the conalbumin promoter element in pTBCT and pTBICT is only apparent and in fact related to early termination of transcription within the 72-bp repeat, as it has been suggested to account for the *in vivo* results (10).

Some of the *in Vivo* Effects of the 72-bp Repeat Enhancer Are Not Seen *in Vitro*. The SV40 enhancer potentiates transcription *in vivo* not only when located in close apposition to a potential promoter element, but also when located at a distance from it (27, 28). However, this potentiator effect decreases sharply as the distance increases, perhaps because of the presence of interposed potential promoter-like elements (6, 10). To analyze the effect of distance on stimulation of *in vitro*-specific transcription by the 72-bp repeat, pSVBSA34 and pSVBSIA34 were constructed with the enhancer in both orientations 230 bp from the Ad2MLP cap site (Fig. 1*A*). *In vivo* (transient expression assays), RNA synthesized from pSVBSA34 and pSVBSIA34 was about 5% of that obtained after transfection with pSVBA34, under conditions where synthesis from pSVA34 was undetectable (data not shown; see also ref. 6). No significant stimulation of specific transcription was found with these recombinants when compared with pSVA34 and pSVBA34 (Fig. 6, lanes 1, 3, 4, and 5). This lack of stimulation of transcription may not be too surprising, because, in contrast to the *in vivo* situation, transcription from pSVA34 is readily detectable *in vitro* and the extent of stimulation is only 10-fold with pSVBA34. This would result in an unnoticed 1.5-fold stimulation with pSVBSA34, should the *in vitro* situation reflect the *in vivo* one (5% RNA synthesized from pSVBSA34 compared with pSVBA34 taken as 100%; see above).

In all of the recombinants used up to now, there was no functional upstream promoter sequence. The pSVA34 series lacks the Ad2MLP upstream sequences between the TATA box and position −97, which is important for efficient transcription both *in vivo* and *in vitro* with a WCE, but not an S100 extract, transcription system (ref. 6; see also pSVA97 in Fig. 2). The conalbumin promoter region from +62 to −102 may contain some upstream promoter element between −44 and −102, but this element functions poorly in a HeLa WCE *in vitro*, causing at most a 2-fold stimulation (data not shown). It is noteworthy that the extent of stimulation of *in vitro* transcription, which can be obtained with a WCE by inserting the 72-bp repeat upstream from the +33 to −34 Ad2MLP element (pSVBA34 vs. pSVA34), is identical to that obtained with an Ad2MLP recombinant that contains both the +33 to −34 and the upstream promoter elements, but not the 72-bp repeat (Fig. 2, pSVA97 in which the Ad2MLP sequences are extended to position −97; see ref. 6). This observation prompted us to to test whether the stimulation of *in vitro* transcription by the 72-bp repeat could be seen even in the presence of functional upstream promoter elements.

pSVA677 contains the Ad2MLP region from +33 to −677 (Fig. 1*B*). The extent of *in vitro* transcription with a WCE is identical to that of pSVA97, but both are transcribed *in vivo* at an undetectable rate unless the SV40 enhancer is added (ref. 6; unpublished results). pSVPBIA677 is derived from pSVA677 by inserting the 72-bp repeat about 4.2 kbp upstream from the Ad2MLP cap site. It is transcribed *in vivo* with an efficiency ≈5–10% that of pSVBA34 (unpublished results), and it is transcribed *in vitro* with the same efficiency as pSVA97 and pSVBA34 (Fig. 6, lanes 3 and 6; other results not shown). pSVPBIA677 was used to construct pSVB125 and pSVBI125 in which the 72-bp repeat was inserted in both orientations in close apposition to the Ad2MLP upstream element 125 bp from the Ad2MLP capsite (Fig. 1*B*). The amount of RNA transcribed *in vivo* from pSVB125 after transfection of HeLa cells was similar to that obtained with pSVBA34 (data not shown), supporting our previous conclusion (6) that insertion of the 72-bp repeat within the Ad2MLP region can mask the effect of deletions of promoter elements. No stimulation of *in vitro* transcription by the 72-bp repeat was observed with either pSVB125 or pSVBI125 when compared with pSVPBIA677 (Fig. 6, lanes 6–8; the 72-bp repeat present 4.2 kbp from the cap site in these three recombinants is not associated with the fragment containing the adenovirus template after *Taq* I digestion for run-off assay).

DISCUSSION

A 5- to 12-fold stimulation of *in vitro* transcription by the SV40 enhancer from Ad2MLP and conalbumin promoter elements was reproducibly observed with a number of different plasmid preparations and WCEs using either linear or circular templates. The *in vitro* stimulation is in keeping with many of the known properties of the SV40 enhancer *in vivo* (see Introduction). The 72-bp repeat *in vitro* acts both in *cis* and bidirectionally. Deletion of just one 72-bp sequence has no effect, but deletion mutations that lead to a strong decrease of the enhancer activity *in vivo* also abolish the *in vitro* stimulation.

On the other hand, some of the effects of the SV40 enhancer *in vivo* are not seen *in vitro*. First, the extent of the *in vitro* stimulation is lower than that *in vivo* by at least one order of magnitude. Second, the 72-bp repeat is effective *in vitro* only if located in close apposition to the stimulated promoter element, with no effect at distance. Third, the *in vitro* stimulation is seen only in the absence of a functional upstream region, whether it is deleted (the Ad2 pSVA34 series) or nonfunctional in a HeLa WCE (the conalbumin promoter recombinants). In this respect, we note that not more than a 2-fold stimulation by the 72-bp repeat has been observed on the SV40 early promoter, which has a strong upstream element (the 21-bp repeat region; see ref. 11) active in HeLa cell extracts (unpublished results). This discrepancy between the *in vivo* and *in vitro* effects of the SV40 enhancer may mean that, in spite of the similarities stressed above, the stimulation *in vitro* is in fact not related to the enhancer function, but rather to the effect of some as yet unidentified upstream-like element(s) present in the 72-bp repeat. Extensive site-directed mutagenesis should establish whether the same sequences are responsible for the *in vivo* and *in vitro* effects of the enhancer.

In any case, it is questionable whether one should expect to readily mimic *in vitro* all of the *in vivo* properties of the SV40 enhancer, as established by transfecting HeLa cells. Clearly, there is a major discrepancy between the *in vivo* and *in vitro* requirements for efficient transcription; transcripts from the chimeric conalbumin or Ad2 recombinants pTCT (10), pSVA97 (6), and pSVA677 cannot be detected *in vivo* without addition of the SV40 enhancer. It may be relevant

that the situation is markedly different *in vivo* where the same recombinants can replicate, leading to a much higher number of template copies. For instance, transcription from pTCT becomes readily detected when, after addition of the SV40 replication origin, it is allowed to replicate in COS cells (ref. 40; unpublished observations). Therefore, the *in vitro* situation may resemble that *in vivo* where there is a large number of template copies: the decreased affinity of the transcription machinery for the template in the absence of the 72-bp repeat is compensated by an increase in the number of available template copies. Consequently, the effect of the 72-bp repeat would be seen *in vitro* only when it acts on a weak promoter element—e.g., in the absence of a functional upstream region (pSVA34 and pTCT)—and provided the template concentration is not too high. Along the same lines, the observed weaker transcription of pSVA34 and pTCT in WCE compared to S100 extracts (Figs. 2 and 3) may also help in revealing the effect of the 72-bp repeat *in vitro*. Finally, *in vivo* the DNA may have a chromatin structure that is inaccessible for transcription unless the SV40 72-bp repeat is present to "open" it (for refs., see ref. 41 and 42). On the contrary, there is no evidence that a chromatin structure is reconstructed in the *in vitro* WCE system (see discussion in ref. 6) and the template is likely to be much more accessible than *in vivo*.

Whatever mechanism is ultimately responsible for the *in vitro* stimulatory effect of the 72-bp repeat, this effect is observed using a WCE and not an S100 extract system. Taken together with other studies, which indicate that enhancers exhibit some species and cell specificities *in vivo*, this observation suggests the involvement of a factor(s) that, in S100 extracts, is either not present or is in an inappropriate ratio to other factors modulating transcription *in vitro*. Purification studies will establish whether such a putative factor(s) exists and, if so, whether indeed the SV40 enhancer functions, at least in part, as an entry site for some elements of the transcription machinery (10, 28).

We are grateful to M. Wintzerith and R. Hen for a gift of the *Sma* I mutated pSVPBIA677 plasmid. We thank C. Kédinger, C. Hauss, J. M. Egly, N. Miyamoto, and V. Moncollin for gifts of materials. J.P.D. is supported by a post-doctoral fellowship from the American Cancer Society. This investigation was supported by grants from Centre National de la Recherche Scientifique (ATP 3582), Institut National de la Sante et de la Recherche Médicale (PRC 124026), Ministère de l'Industrie et de la Recherche (82 V 1283), the Fondation pour la Recherche Médicale, the Association pour le Développement de la Recherche sur le Cancer, and the Fondation Simone and Cino del Duca.

1. Corden, J., Wasylyk, B., Buchwalder, A., Sassone-Corsi, P., Kédinger, C. & Chambon, P. (1980) *Science* **209**, 1406–1414.
2. Breathnach, R. & Chambon, P. (1981) *Annu. Rev. Biochem.* **50**, 349–383.
3. Hentschel, C. C. & Birnstiel, M. L. (1981) *Cell* **25**, 301–313.
4. Shenk, T. (1981) *Curr. Top. Microbiol. Immunol.* **93**, 25–46.
5. Mellon, P., Parker, V., Gluzman, Y. & Maniatis, T. (1981) *Cell* **27**, 279–288.
6. Hen, R., Sassone-Corsi, P., Corden, J., Gaub, M. P. & Chambon, P. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7132–7136.
7. Pelham, H. R. B. (1982) *Cell* **30**, 517–528.
8. Fromm, M. & Berg, P. (1982) *J. Mol. App. Genet.* **1**, 457–481.
9. McKnight, S. L. & Kingsbury, R. (1982) *Science* **217**, 316–324.
10. Wasylyk, B., Wasylyk, C., Augereau, P. & Chambon, P. (1983) *Cell* **32**, 503–514.
11. Everett, R. D., Baty, D. & Chambon, P. (1983) *Nucleic Acids Res.* **11**, 2447–2464.
12. Dierks, P., Van Ooyen, A., Cochran, M. D., Dobkin, C., Reiser, J. and Weissmann, C. (1983) *Cell* **32**, 695–706.
13. Davison, B. L., Egly, J. M., Mulvihill, E. R. & Chambon, P. (1983) *Nature (London)* **301**, 680–686.
14. Travers, A. (1983) *Nature (London)* **303**, 755.
15. Tsuda, M. & Suzuki, Y. (1981) *Cell* **27**, 175–182.
16. Notake, M., Kurosaki, T., Yamamoto, T., Handa, H., Mishina, M. & Numa, S. (1983) *Eur. J. Biochem.* **133**, 599–605.
17. Dynan, W. S. & Tjian, R. (1983) *Cell* **32**, 669–680.
18. Benoist, C. & Chambon, P. (1981) *Nature (London)* **290**, 304–310.
19. Gruss, P., Dhar, R. & Khoury, G. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 943–947.
20. Yaniv, M. (1982) *Nature (London)* **297**, 17–18.
21. Khoury, G. & Gruss, P. (1983) *Cell* **33**, 313–314.
22. Boss, M. A. (1983) *Nature (London)* **303**, 281–282.
23. Banerji, J., Olson, L. & Schaffner, W. (1983) *Cell* **33**, 729–740.
24. Gillies, S. D., Morrison, S. L., Oi, V. T. & Tonegawa, S. (1983) *Cell* **33**, 717–728.
25. Queen, C. & Baltimore, D. (1983) *Cell* **33**, 741–748.
26. Chandler, V. L., Maler, B. A. & Yamamoto, K. R. (1983) *Cell* **33**, 489–499.
27. Banerji, J., Rusconi, S. & Schaffner, W. (1981) *Cell* **27**, 299–308.
28. Moreau, P., Hen, R., Wasylyk, B., Everett, R. D., Gaub, M. P. & Chambon, P. (1981) *Nucleic Acids Res.* **9**, 6047–6068.
29. Fromm, M. & Berg, P. (1983) *J. Mol. App. Genet.* **2**, 127–135.
30. de Villiers, J., Olson, L., Banerji, J. & Schaffner, W. (1982) *Cold Spring Harbor Symp. Quant. Biol.* **47**, 911–919.
31. Weil, P. A., Segall, J., Harris, B., Ng, S.-Y. & Roeder, R. G. (1979) *J. Biol. Chem.* **254**, 6163–6173.
32. Manley, J. L., Fire, A., Cano, A., Sharp, P. A. & Gefter, M. L. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3855–3859.
33. Sassone-Corsi, P., Corden, J., Kédinger, C. & Chambon, P. (1981) *Nucleic Acids Res.* **9**, 3941–3958.
34. Tooze, J., ed. (1982) *DNA Tumor Viruses* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
35. Maxam, A. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–580.
36. Wasylyk, B., Wasylyk, C., Matthes, H., Wintzerith, M. & Chambon, P. (1983) *EMBO J.* **2**, 1605–1611.
37. Gingeras, T. R., Sciaky, D., Gelinas, R. E., Parsons, B. L., O'Neil, K. E. & Roberts, R. J. (1982) *J. Biol. Chem.* **257**, 13475–13491.
38. Grosschedl, R. & Birnstiel, M. L. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 297–301.
39. Rio, D. C. & Tjian, R. (1983) *Cell* **32**, 1227–1240.
40. Gluzman, Y. (1981) *Cell* **23**, 175–182.
41. Jongstra, J., Reudelhuber, T., Oudet, P. & Chambon, P. (1983) in *Gene Expression*, UCLA Symposia and Cellular Biology, eds. Hamer, D. & Rosenberg, M. (Liss, New York), Vol. 8, in press.
42. Fromm, M. & Berg, P. (1983) *Mol. Cell. Biol.* **3**, 991–999.