between the Y chromosomes of *M.m. domesticus* and *M.m. musculus*. Surprisingly, the Y chromosome carried by the laboratory reference strain C57BL/6 showed the characteristic patterns of *M.m. musculus* on both the *Msp*I and *Hin*dIII digests. This finding was unexpected as it is generally assumed (and mitochondrial data support this assumption) that the laboratory strains were derived from *M.m. domesticus*.

To determine whether C57BL/6 is exceptional in this respect, we probed both *Hin*dIII and *Msp*I genomic blots of nine commonly used established inbred laboratory mouse strains with pY353/B. The polymorphic patterns obtained (Fig. 3*a*,*b*) show that the Y carried by SJL is of the *M.m. domesticus* type whereas all the other strains tested (A/J, BALB/c, C57BL/6, CBA/HeJ, C3H, DBA/2, 129/sv and 163H) are clearly *M.m. musculus*-derived. The latter strains of mice must therefore be considered as genetic hybrids between two semispecies, with the maternal contribution derived from *M.m. domesticus* and the paternal genetic information derived from *M.m. musculus*. Further genetic studies (which are in progress) should reveal the extent of the hybrid characteristics and show whether they are restricted to the Y chromosome or not. These data suggest that the Y-chromosome polymorphism involving SJL reported recently[12] may result from the semispecies origin of the Y and not strict intraspecies polymorphism. In fact, no Y-chromosome RFLPs were detected in the new wild-derived *M.m. domesticus* and *M.m. musculus* lines; this could be related to the nature of the probe as it has been shown that the highly repeated cloned Bkm probe[13] is extensively polymorphic both in wild and laboratory mouse strains (K. Jones, personal communication).

We consistently found that the intensity of hybridization of pY353/B to the A/J and DBA/2 inbred strains was 2–4 times less than that to the other strains, despite the blots containing approximately equal amounts of DNA from each mouse. As the hybridizations were performed under stringent washing conditions (0.1 × SSC, 68 °C), this difference probably represents a copy-number polymorphism rather than sequence divergence. Preliminary data using DNA from a mouse carrying a partially deleted Y chromosome (supplied by P. Burgoyne and E. Simpson) suggest that large blocks of DNA carrying this sequence are dispersed on the mouse Y chromosome (C.E.B., unpublished observations). It remains to be determined whether these copy-number polymorphisms arose during the establishment of the strains or whether they were present in the males which started the lines.

Historically, most of the laboratory mouse strains studied here (with the notable exception of SJL) are known to have originated from domesticated mice of North European and North American pet dealers and mouse fanciers. These mice were a mixture of locally caught North European (presumed *M.m. domesticus* origin) mice, subsequently carried across the Atlantic to North America, and imported 'fancy' Chinese and Japanese mice[14,15]. If the *M.m. musculus* type Y chromosome described here originated from the European mice it would imply that some *M.m. domesticus* wild mice carry the *M.m. musculus* Y chromosome. Although we have been unable to show any such exchanges in the wild mice studied so far, they mainly originate from South-East Europe where such exchanges might not occur. It is known that *M.m. domesticus* and *M.m. musculus* mice hybridize in Europe (Fig. 1) and that in Scandinavia and Denmark there is a considerable one-way mitochrondrial gene flow northeastwards from *M.m. domesticus* into *M.m. musculus*[2]. In the southeastern part of the hybrid zone this flow is reversed, occurring in the *M.m. musculus* to *M.m. domesticus* direction[4]. We are at present surveying a very large European mouse population to determine whether there is a Y-chromosome gene flow between the semispecies.

Alternatively, the *M.m. musculus* type Y could have been introduced through the Chinese or Japanese mice. In this regard, Bonhomme *et al.* have recently shown, using numerous biochemical markers, that the Asiatic subspecies *Mus musculus molossinus* (found in China and Japan) is closely related to *M.m. musculus*[5]. Mitochondrial DNA polymorphisms also support this view[6,16]. If this similarity extended to the Y chromosome, it could provide a simple explanation for the presence of a *M.m. musculus* type Y in the laboratory mouse strains, that is, being derived from the subspecies *M.m. molossinus*.

Whatever the origins of the Y chromosome, these data are of particular importance in studies involving hybrid genomes. Eicher *et al.* have reported that C57BL/6 differs from *M.m. domesticus* at an autosomal locus involved in gonadal determination and development (*tda*-1) as well as at the Y-localized testis-determining locus (*Tdy*)[17]. On the basis of the findings reported here, it seems probable that both *Tdy* and *tda*-1 in C57BL/6 have co-evolved from *M.m. musculus*. The partial or complete sex reversal reported can be interpreted as a result of the inability of a *M.m. domesticus* Y chromosome to interact with the *M.m. musculus tda*-1 locus. If this were true it would predict that the introduction of a Y from *M.m. musculus* into the C57BL/6 genome would not result in sex reversal, but the introduction of the SJL Y into the C57BL/6 genome would. Experiments are in progress to test this.

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Simian virus 40 enhancer increases number of RNA polymerase II molecules on linked DNA

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Since the discovery of transcription enhancer sequences in the genomes of the DNA viruses simian virus 40 (SV40)[1–3] and polyoma virus[4], these elements have been shown to play an important part in the control of both viral and cellular gene expression[5–10]. Enhancer elements act in *cis* to increase the amount of RNA produced from linked genes in a manner largely independent of distance and orientation[1–3,11,12]. The mechanisms by which enhancers act are not understood; in particular, it is not known whether the enhancer-dependent increase in the level of stable RNA reflects an increase in the rate of transcription. To address this question, we have used an *in vitro* nuclear transcription assay[13,14] to examine the effect of the SV40 enhancer on transcription of cloned human β-globin genes transiently introduced into HeLa cells[15]. We show here that the SV40 enhancer acts at least in part to increase the number of RNA polymerase II molecules transcribing the linked gene.

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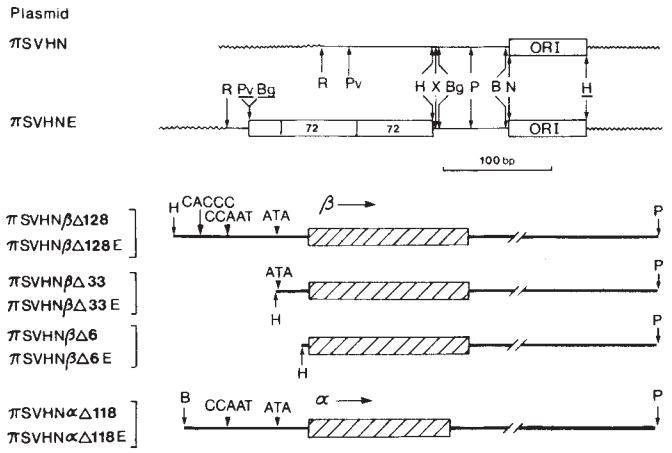

Fig. 1 Maps of the α- and β-globin gene plasmids used in HeLa cell transfection studies. Plasmids were constructed by standard procedures using the πVX miniplasmid as the parental plasmid[16,25]. The two miniplasmids πSVHN (no enhancer) and πSVHNE (with enhancer) are shown at the top of the figure. Open boxes represent SV40 sequences, with the origin of DNA replication (ORI) and the enhancer sequence (72-bp repeat) indicated. The enhancer fragment contains SV40 nucleotides 272 (*Pvu*II site converted by *Bgl*II linker) to 100 (*Xho*I linker converted to *Hin*dIII). The πVX plasmid vector sequences are shown as wavy lines, the polylinker as thin lines. The human β- and α-globin genes used are shown at the bottom, with the exons indicated by the shaded boxes, and introns and flanking sequences by thick lines. The β-globin promoter elements CACCC, CCAAT and ATA[18,19] are indicated. The β-globin plasmids lacking the SV40 enhancer, πSVHN$\beta\Delta$128, πSVHN$\beta\Delta$33 and πSVHN$\beta\Delta$6, and the enhancer-containing β-globin plasmids πSVHN$\beta\Delta$128E, πSVHN$\beta\Delta$33E and πSVHN$\beta\Delta$6E contain the β-globin genes on a *Hin*dIII–*Pst*I fragment inserted between the corresponding sites in the polylinker sequences of πSVHN and πSVHNE. The β-globin deletion mutants were constructed as described previously[22,26], and contain 128, 33 and 6 bp of 5′ flanking sequence of the β-globin gene. The α-globin plasmids πSVHN$\alpha\Delta$118 and πSVHN$\alpha\Delta$118E were constructed by inserting the *Bam*HI (artificially introduced at position −118) to *Pst*I fragment of the human α_1-globin gene between the *Bgl*II and *Pst*I sites of the two miniplasmids. The locations of relevant restriction enzyme cleavage sites are shown: R, *Eco*RI; Pv, *Pvu*II; H, *Hin*dIII; X, *Xba*I; Bg, *Bgl*II; P, *Pst*I; B, *Bam*HI; N, *Nco*I; sites underlined were destroyed during plasmid construction.

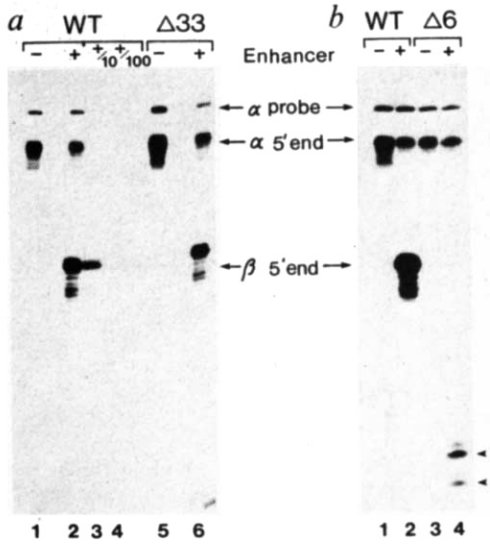

Fig. 2 Effect of the SV40 enhancer on the amount of accurately transcribed β-globin RNA in transfected HeLa cells. HeLa cells were transfected with 20 μg of plasmid DNA per 9-cm dish and the RNA was analysed by S_1 nuclease mapping procedures[27]. Each transfection included an equal mass of a human α-globin gene plasmid to serve as an internal reference. Unless otherwise stated, 40 μg of total cell RNA was used for each assay. The positions of nuclease-resistant DNA fragments mapping the 5′ ends of correctly initiated globin transcripts are indicated; − or + indicates the absence or presence, respectively, of the SV40 enhancer on the β-globin plasmid. The plasmids used contain β-globin genes with 128 (WT), 33 (Δ33) and 6 (Δ6) bp of 5′ flanking sequence. ***a***, Effect of the SV40 enhancer on the $\beta\Delta$128 (intact promoter) and Δ33 (deleted upstream element) β-globin genes. The α-globin plasmid πSVHSα1 (ref. 15), which does not contain an SV40 enhancer, was used as a reference. Lane 1, plasmid πSVHN$\beta\Delta$128; lane 2, plasmid πSVHN$\beta\Delta$128E; lane 3, plasmid πSVHN$\beta\Delta$128E, but the hybridization included only 4 μg of RNA; lane 4, 1/10 of the S_1 nuclease-resistant counts loaded in lane 3; lane 5, plasmid πSVHN$\beta\Delta$33; lane 6, plasmid πSVHN$\beta\Delta$33E. In lanes 3 and 5, the α-globin probe was omitted from the hybridization reaction. ***b***, Effect of the SV40 enhancer on the $\beta\Delta$128 (intact promoter) and $\beta\Delta$6 (no promoter) globin genes. The α-globin gene plasmid πSVHPα2 (ref. 26), which contains an SV40 enhancer, was used as an internal reference. Lane 1, plasmid πSVHN$\beta\Delta$128; lane 2, plasmid πSVHN$\beta\Delta$128E; lane 3, plasmid πSVHN$\beta\Delta$6; lane 4, plasmid πSVHN$\beta\Delta$6E. RNA which apparently initiates within the 5′-noncoding region of the gene is indicated by the arrows to the right of lane 4. Cell culture, DNA transfection and S_1 nuclease mapping procedures have been described previously[15,28]. The single-stranded [5′-^{32}P]-labelled probes were: β-globin, *Hae*III–*Mst*II*, nucleotides −76 to +70; α-globin, *Hha*I (artificially constructed; R.T., unpublished) to *Bss*HII*, nucleotides −11 to +97.

We first evaluated the effect of the SV40 enhancer on the accumulation of β-globin RNA using the S_1 nuclease mapping procedure. HeLa cells were transfected with plasmids carrying a human β-globin gene[15,16] with or without the SV40 enhancer (Fig. 1), and stable RNA was analysed 48 h after transfection. The DNA fragment containing the enhancer does not include sequences sufficient for SV40 early or late promoter function. As an internal reference, a plasmid containing the human α-globin gene was also included in the transfection mix. Figure 2*a* shows that the presence of the SV40 enhancer next to the intact β-globin promoter increases the amount of correctly initiated RNA produced by two to three orders of magnitude[1,16] (compare lane 1 with lanes 2–4).

We next isolated nuclei from transfected cells and incubated them with ^{32}P-labelled ribonucleoside triphosphates under conditions in which only limited elongation of previously initiated RNA chains occurs[13] (see Fig. 3 legend). The amount of *in vitro*-synthesized, labelled RNA specific for the α- or β-globin genes or vector DNA was determined by hybridization of partially fragmented RNA to a large excess of double-stranded DNA fragments immobilized on nitrocellulose[14,17]. The amount of radioactive RNA that hybridizes to a given fragment reflects the number of previously initiated RNA polymerase molecules present on the corresponding region of the transfected plasmid DNA[14]. The results of such a nuclear transcription experiment are shown in Fig. 3; in this experiment HeLa cells were transfected with β-globin plasmids that differ by the presence or absence of the SV40 enhancer sequence, together with a plasmid containing the human α-globin gene as an internal reference. Both globin and vector-specific RNAs were readily detectable after *in vitro* elongation of preinitiated RNA, and a substantial increase in the amount of β-globin specific RNA was observed when the gene was linked to the enhancer (Fig. 3*a*, compare lanes 1 and 2). A similar effect was observed with the α-globin gene using the β-globin plasmid as an internal reference (Fig. 3*a*, compare lanes 2 and 3). Examination of stable RNA showed that in the absence of a linked enhancer, the α-globin gene produced readily detectable levels of correctly initiated RNA, and that the presence of the enhancer at the 5′ side of the gene resulted in a 30–50-fold increase in the amount of RNA produced (data not shown). When 2 μg ml^{-1} α-amanitin was included in the nuclear transcription reaction, no globin- or vector-specific transcripts were synthesized (Fig. 3*b*, compare

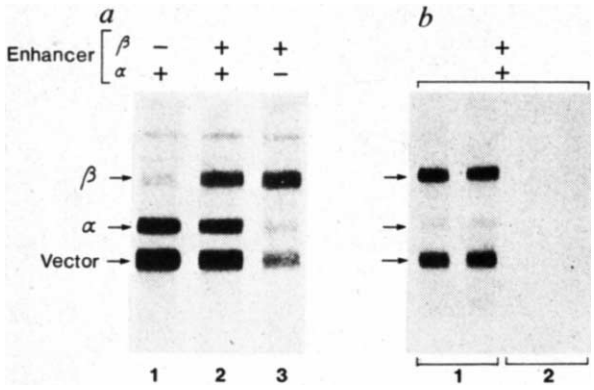

Fig. 3 Effect of the SV40 enhancer on nuclear transcription of transfected α- and β-globin plasmids. Each 'lane' is an autoradiogram of a nitrocellulose strip carrying fragments of β-globin, α-globin or vector DNA, after hybridization to RNA synthesized *in vitro*. The identities of the fragments and the presence (+) or absence (−) of the SV40 enhancer on each of the transfected plasmids are indicated. *a*, Effect of the SV40 enhancer on α- and β-globin nuclear transcription. HeLa cells were transfected with the following plasmids: lane 1, πSVHN$\beta\Delta$128 + πSVHN$\alpha\Delta$118E; lane 2, πSVHN$\beta\Delta$128E + πSVHN$\alpha\Delta$118E; lane 3, πSVHN$\beta\Delta$128E + πSVHN$\alpha\Delta$118. *b*, Effect of α-amanitin on nuclear transcription of transfected plasmids. HeLa cells were transfected with πSVHN$\beta\Delta$128E and πSVHPα2 (ref. 26), and nuclear transcription reactions performed either in the absence (lane 1) or presence (lane 2) of 2 μg ml^{-1} α-amanitin. Duplicate strips are shown.

Methods. Nuclei were isolated by hypotonic lysis using a Dounce homogenizer (type A pestle) as described previously[13]. Cell lysis was evaluated by Trypan blue staining. Nuclei from two 9-cm plates of transfected cells were used for each reaction. Isolated nuclei were resuspended at 0 °C in a total volume of 100 μl, comprising 50 μl 2×transcription buffer[13] (50 mM dithiothreitol, 180 mM KCl, 10 mM $MgCl_2$, 20 mM Tris-HCl, 50% glycerol, *p*H 7.8), brought to 0.25 mM ATP, 0.125 mM GTP, 0.125 mM CTP. 100 μCi [α-^{32}P]UTP (410 Ci mmol^{-1}; Amersham) was added to each reaction. The nuclei were incubated at 37 °C for 5 min; no incorporation occurred after this time. Under these conditions, pre-initiated RNA chains are elongated by 300–500 nucleotides[13]. RNA was isolated by protease K treatment, followed by two extractions with phenol/chloroform as described previously[27]; residual DNA was removed by treatment with RNase-free DNase. Approximately 5×10^6 to 10^7 c.p.m. RNA were routinely obtained. Fragmentation of RNA by partial alkali hydrolysis was as described elsewhere[29]. For analysis of the *in vitro*-synthesized RNA by strip blot hybridization[14], plasmids πSVHN$\beta\Delta$128 and πSVHN$\alpha\Delta$118 (25 μg each) were digested with *Xba*I and *Pst*I and the fragments resolved on a 10 cm-wide 1.2% agarose gel, then transferred to nitrocellulose[30]. Duplicate 1 cm-wide strips of nitrocellulose were then hybridized to equal numbers of counts as described[40], washed, and exposed to Kodak X-AR5 film.

lanes 1 and 2), indicating that the *in vitro* transcription is due to RNA polymerase II. We also verified that reinitiation does not occur in the system by performing nuclear incubations in the presence of 0.5% sarkosyl or 1 mg ml^{-1} heparin. Although these additions slightly diminished the total *in vitro* RNA synthesis, little effect on *in vitro* synthesis of α- and β-globin specific RNA was observed (data not shown).

These results demonstrate that the SV40 enhancer causes a substantial increase in the number of RNA polymerase II molecules present on linked DNA. However, the experiment does not distinguish between *in vitro* elongation of transcripts initiated at the β-globin promoter and the elongation of nascent transcripts initiated at other sites in the plasmid DNA. To address this problem, we examined the effect of promoter deletions on β-globin gene specific transcription in isolated nuclei. Plasmids were constructed in which the enhancer is located at the 5′ side of truncated β-globin genes containing either 33 base pairs (bp) of 5′ flanking sequence (which includes the ATA box, but lacks the upstream promoter elements CACCC and CCAAT[18,19]), (plasmids πSVHN$\beta\Delta$33 and πSVHN$\beta\Delta$33E; Fig. 1) or only 6 bp of 5′ flanking sequence (which lacks all promoter elements), (plasmids πSVHN$\beta\Delta$6 and πSVHN$\beta\Delta$6E; Fig. 1). The transcription of these plasmids in transfected HeLa cells was then analysed by S_1 nuclease mapping and by the nuclear transcription assay. The S_1 analysis (Fig. 2) shows that (1) neither of the truncated β-globin genes directs the synthesis of detectable levels of correctly initiated β-globin RNA in the absence of a linked SV40 enhancer sequence (Fig. 2*a*, lane 5; Fig. 2*b*, lane 1); (2) deletion of sequences 5′ to the ATA box has essentially no effect on the level of correctly initiated RNA produced when the enhancer is in close proximity to the ATA box, indicating that the enhancer can compensate for the deletion of upstream promoter sequences as reported previously for other promoters[20,21] (Fig. 2*a*, lane 6). (We note, however, that when the SV40 enhancer is located farther upstream from the $\beta\Delta$33 gene, or on its 3′ side, virtually no transcription is observed[22].) (3) Deletion of all promoter elements results in the loss of correctly initiated β-globin RNA and the concomitant appearance of a small amount of RNA that is apparently initiated at, or spliced to, sites in the 5′-untranslated region of the gene (Fig. 2*b*, lane 4).

Figure 4 shows a nuclear transcription analysis of the β-globin promoter deletions. The RNA synthesized *in vitro* was hybridized to the separate strands of the β- and α-globin genes cloned into M13mp8. The linkage of the SV40 enhancer to the intact β-globin gene increases specifically the amount of transcription of the coding (or 'anti-sense') DNA strand (Fig. 4*a*, compare strips 1, 2 with 3, 4). A small amount of transcription of the noncoding strands of both β- and α-globin genes was detected, even in the absence of a linked SV40 enhancer. We also observed a decrease in the number of RNA polymerase molecules on the α-globin reference gene when the test β-globin gene was linked

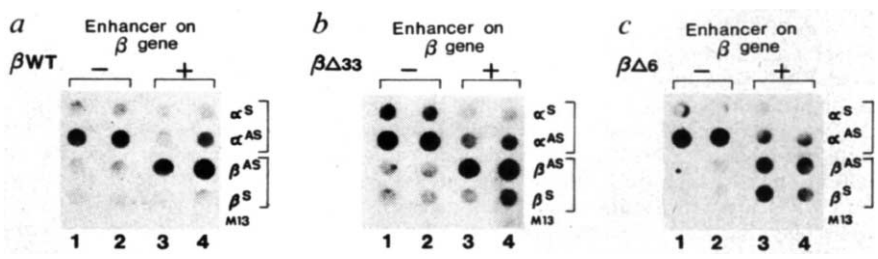

Fig. 4 Effect of the SV40 enhancer on nuclear transcription of β-globin promoter deletion mutants. Each panel contains pairs of duplicate strips of dot blots on which are spotted recombinant M13 phage carrying the sense (S) and anti-sense (AS) strands of the α- and β-globin genes. Bona fide transcripts hybridize with the anti-sense strand. HeLa cells were transfected with πSVHPα2 (ref. 26) and *a*, plasmid πSVHN$\beta\Delta$128 (strips 1, 2) or πSVHN$\beta\Delta$128E (strips 3, 4); *b*, πSVHN$\beta\Delta$33 (strips 1, 2) or πSVHN$\beta\Delta$33E (strips 3, 4); *c*, πSVHN$\beta\Delta$6 (strips 1, 2) or πSVHN$\beta\Delta$6E (strips 3, 4).

Methods. Nuclear transcriptions, RNA preparation and hybridization conditions were as described in Fig. 3 legend. Recombinant M13 phage were constructed by insertion of the 1.5-kb *Pst*I fragment of the human α_1-globin gene, or a 2.2-kb *Pst*I fragment of the human β-globin gene (from a *Pst*I linker at the endpoint of the −33 deletion to the *Pst*I site 500 bp on the 3′ side of the gene) into M13mp8 in either orientation, by standard procedures. The M13 DNAs (5 μg) were spotted onto nitrocellulose in 10×SSC with a 'Manifold' apparatus (Schleicher & Schuell).

to the enhancer, suggesting that co-transfected enhancers can compete for limiting factors in the *in vitro* transcription assay. We do not observe this effect at the level of stable RNA under our conditions, indicating that factors that may be limiting *in vitro* are not limiting *in vivo*. Others have reported enhancer competition *in vivo* using different transcription units and transfection conditions[23]. In agreement with the observation that deletion of the β-globin upstream elements in plasmid πSVHNβΔ33E does not affect the accumulation of stable RNA, we find that the behaviour of this plasmid in the nuclear transcription assay is identical to that observed with the plasmid carrying the intact β-globin gene (Fig. 4, compare *a* and *b*). A strikingly different result was obtained when all the β-globin promoter elements were removed (Fig. 4*c*). Again, a small amount of *in vitro* transcription of both strands of the truncated gene was detected even in the absence of a linked enhancer sequence. However, linkage of the SV40 enhancer sequence to the gene lacking promoter elements resulted in a small increase in transcription of both DNA strands (Fig. 4*c*, compare strips 1, 2 with 3, 4); this increase may result from the activation of normally silent 'cryptic' start sites such as that observed in the 5′-untranslated region of the β-globin gene (Fig. 2*b*, lane 4). We conclude that enhancer effects seen in the nuclear transcription assays do depend on known promoter elements of the β-globin gene, and therefore relate to the production of bona fide β-globin RNA.

We have demonstrated here that the SV40 enhancer causes an increase in the number of RNA polymerase II molecules engaged in the transcription of linked DNA. The increase is dependent on known components of the β-globin gene promoter[18,19]. It was reported previously that deletion of the adenovirus 2 E1A enhancer causes a reduction in synthesis of E1A RNA measured in pulse labelling experiments[24]. Our observations exclude the possibility that the enhancer acts solely to allow stabilization of nascent transcripts. The magnitude of the enhancer effect observed in the nuclear transcription assay appears to be smaller than the two to three orders of magnitude effect on the level of stable RNA (compare Figs 2 and 3). Moreover, the 10-20-fold difference in the effect of the SV40 enhancer on the production of stable α- and β-globin RNA[15] is apparently not observed in the nuclear transcription assay. However, accurate quantitation of enhancer effects in the *in vitro* nuclear transcription assay is difficult. At least some of the low level of α-globin nuclear transcription observed in the absence of a linked enhancer sequence (Fig. 3*a*) must be due to elongation of correctly initiated RNA, as this plasmid does produce easily detectable levels of stable α-globin RNA (data not shown; see ref. 15). It is possible, however, that the low level of β-globin nuclear transcription observed in the absence of a linked enhancer does not represent elongation of RNA initiated at the correct β-globin cap site. This possibility is suggested by the observation that nuclear transcription in the absence of the enhancer is not significantly dependent on the β-globin promoter (Fig. 4, compare *a* and *c*). The magnitude of the effect we observe therefore provides a minimum estimate of the actual effect of the enhancer on polymerase loading. We cannot exclude the possibility that the enhancer also acts at levels other than transcription initiation to increase the production of stable RNA.

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Simian virus 40 enhancer increases RNA polymerase density within the linked gene

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Enhancers are regulatory DNA elements, usually about 200 base pairs (bp) long, which are able to stimulate transcription of linked genes in eukaryotic cells[1,2]. This activation can be exerted over large distances, and from a position 5′ or 3′ to the gene[1]. Enhancers have been identified in viral genomes[1-9] and cellular genes[10-15]. Using a transient expression assay, we have analysed transcription of the rabbit β-globin gene and the thymidine kinase gene from herpes simplex virus with and without a simian virus 40 (SV40) enhancer. S_1 nuclease mapping shows a high level of specific transcripts when the genes are linked to the enhancer. To determine whether this increased number of transcripts is due to a higher transcription rate, or perhaps to a shift from nonspecific to specific initiation, we have performed run-on transcription assays with isolated nuclei. Our results, presented here, demonstrate that the SV40 enhancer increases the RNA polymerase density within the linked gene. Therefore, enhancers apparently increase the rate of transcription initiation without influencing the specificity of initiation.

To study the enhancer effect on polymerase II transcription units, we constructed clones containing the rabbit β-globin gene and the herpes simplex virus (HSV) thymidine kinase (*tk*) gene with and without the SV40 enhancer (see Fig. 1).

The rabbit β-globin and the HSV *tk* genes, which were previously shown to respond to an enhancer[1,8], were cloned as 2-kilobase (kb) fragments into the plasmid pUC9 (ref. 16). The SV40 72-bp repeat enhancer was cloned as a 195-bp fragment[17,18] ~1.5 kb downstream of the respective promoters.

To demonstrate enhancer-dependent expression of the two genes, we transfected the clones into human HeLa cells, using the calcium phosphate co-precipitation method[18,19]. RNA was extracted after 2 days and analysed by S_1 nuclease mapping. The S_1 analysis clearly shows that the enhancer stimulates the level of transcription of the β-globin and *tk* genes ~40-fold and 15-fold, respectively (Fig. 2). The effect on β-globin transcription, however, is less pronounced than in previous experiments in which stimulation by the SV40 enhancer was ~200-fold[1]. This difference results mainly from an increased level of transcripts in the absence of an enhancer when using the pUC vector instead of the previously used pBR322 (data not shown), and was not analysed further.

An analysis of both the 5′ and 3′ ends of the rabbit β-globin transcripts demonstrates correct initiation, 3′ processing and