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Expression of the chromosomal mouse β^{maj}-globin gene cloned in SV40

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The complete chromosomal mouse β^{maj}-globin gene, including its intervening and flanking sequences, has been cloned in the monkey virus SV40. The mouse gene is transcribed, processed and translated in infected monkey kidney cells to yield mouse β^{maj}-globin.

WE have identified several conserved sequences in the chromosomal DNA of cloned mouse α- and β-globin genes that may have an important role in their ordered expression and in the processing of their initial transcripts[1–5]. Despite the fact that these sequences have been preserved during several hundred million years of separate evolutionary development, we cannot be certain of their function or importance until they, or mutants derived from them, have been tested in an appropriate biological system. Our first approach to the development of such tests has involved the use of animal viruses as vectors of chromosomal genetic information. Here, we have inserted the complete genomic mouse β^{maj}-globin gene into the monkey virus SV40 and used the resulting recombinant to infect cultured monkey kidney cells. Despite the species and cell-type differences, the mouse globin signals for RNA splicing, polyadenylation and translation are recognised in this host, and substantial quantities of mouse β^{maj}-globin are produced. Thus, the SV40–monkey cell system seems to provide a useful and logical extension of the recombinant DNA technology, returning the cloned gene to a cell in which its function can be tested.

The present experiment was based on our previous experience with SV40 hybrids carrying both prokaryotic and eukaryotic DNA fragments. Initially, we used an *Escherichia coli* suppressor tRNA gene to develop the SV40 late region deletion vehicle system. We found that such vehicles could be used to propagate the foreign DNA either as virus in producer cells or as cloned reverse transcript, into two different sites in the SV40 late region[10]. As anticipated, expression of the cDNA gene into a stable, translatable mRNA required the retention of the appropriate signals for mRNA processing (and possibly translation) in the viral vector. A similar conclusion was reached by Mulligan *et al.*[11], who inserted the rabbit sequence at a different site in the viral late region. Most recently, we constructed viruses carrying a portion of the mouse β^{maj}-globin gene, and provided evidence for the use of a globin splice junction and polyadenylation site when the sense strand of the gene was transcribed from the viral late region promoter[12]. However, because of the limitations of our biochemical analyses, we could not be certain that the processing reactions were occurring at exactly the appropriate sites. This encouraged us to insert the complete chromosomal gene into SV40, thereby providing a more stringent test for functional mRNA synthesis—translation into authentic globin.

Construction of an SV40 recombinant carrying the complete chromosomal mouse β^{maj}-globin gene

In the recombinant virus described here, the complete chromosomal mouse β^{maj}-globin gene, including its intervening and adjacent flanking sequences, replaces the viral late gene region. The gene is inserted in the same orientation as the SV40 late protein coding sequences. Therefore, readthrough transcription from the strong viral late region promoter, which is preserved in the vector, generates the globin coding sequence rather than its complement. The construction of this recombinant, depicted in Fig. 1, involved four steps: preparation of the appropriate globin and viral vector DNA segments; ligation *in vitro* of these segments to form linear recombinant molecules with the appropriate orientation; cyclisation of the recombinant

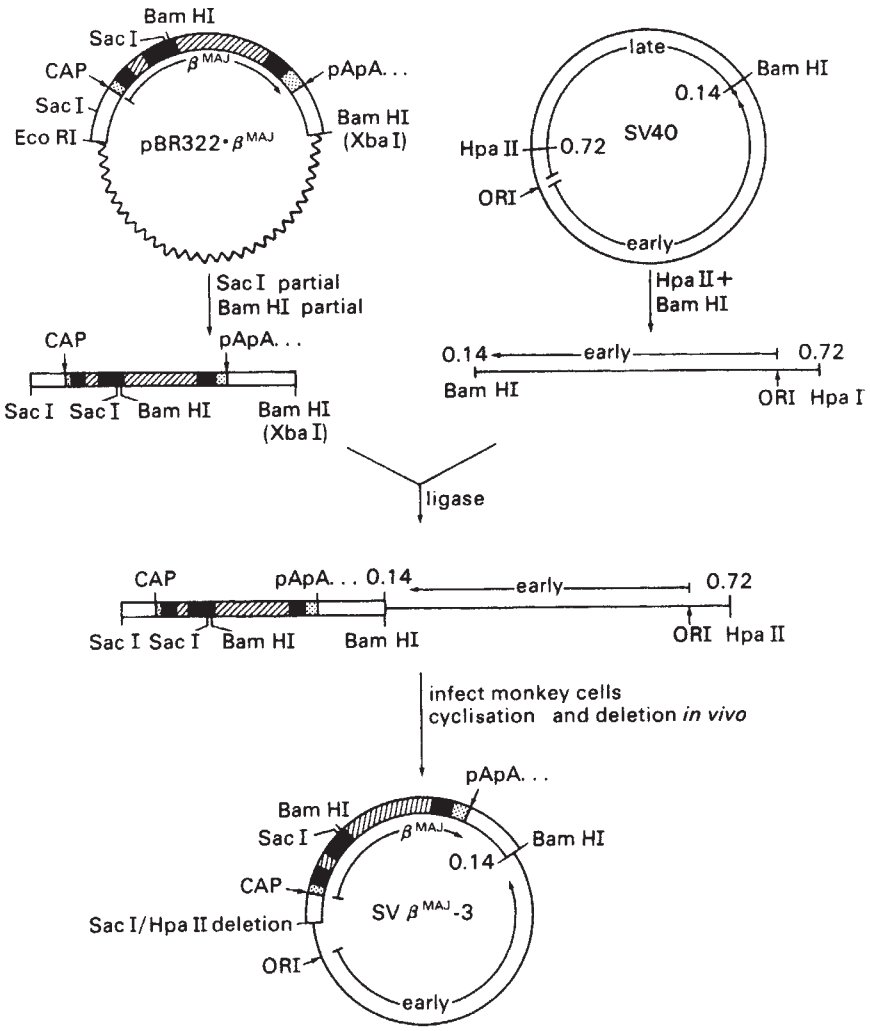


Fig. 1 Construction and propagation of the SV40–mouse β^{maj}-globin gene recombinant. Open bars: mouse β^{maj}-globin gene flanking sequences; stippled bars: globin transcribed, untranslated sequences; solid bars: globin coding sequences; hatched bars: globin intervening sequences; wavy lines: plasmid sequences; solid lines: SV40 sequences. The methods used for DNA preparation, restriction endonuclease cleavage, isolation of fragments on gels, ligation, DNA infection of African green-monkey kidney cells and preparation of virus stocks have been described elsewhere[6,10,12]. **The chromosomal β^{maj}-globin gene fragment:** The upper left diagram shows the structure of pBR322·β^{maj}, which contains the mouse chromosomal β^{maj}-globin DNA sequences from an *Eco*RI site located 1,200 base pairs upstream from the globin cap site to an *Xba*I site located 600 base pairs downstream from the poly(A) addition site. This plasmid was derived from the original recombinant pMB9·β^{maj} (ref. 2) by linking the *Xba*I site to a synthetic dodecamer containing a *Bam*HI site, cloning the resulting *Bam*HI–*Bam*HI fragment in pBR322 (ref. 26), then reconstituting the gene by insertion of the 5′-*Eco*RI–*Bam*HI globin gene fragment. This plasmid was partially digested with *Sac*I and *Bam*HI to generate the 2,400-base pair fragment that was cloned in SV40. **The SV40 vector:** The upper right diagram shows the structure of wild-type SV40 DNA including the direction of transcription of the late and early mRNAs, the origin of viral DNA replication (ORI), and the cleavage sites for *Bam*HI and *Hpa*II relative to the *Eco*RI site at 0.00/1.00 map units[27,28]. The DNA was cleaved with *Bam*HI and *Hpa*II to generate the 3,000-base pair vector fragment. This fragment retains the entire early region, the origin of replication and the sequences corresponding to the 5′- and 3′-termini of the viral late mRNAs; it lacks the coding sequences for the late viral proteins VP1, VP2 and VP3. **The recombinant molecule:** A mixture containing 24 μg of the globin gene fragment and 18 μg of the SV40 vector fragment was treated with DNA ligase, then electrophoresed through a 1% agarose gel to purify the 5,400-base pair linear recombinant genome. This molecule, formed by joining the two *Bam*HI termini, contains the globin coding sequences on the same strand normally occupied by the viral late gene coding sequences. **Propagation:** Approximately 10 ng of the purified recombinant DNA mixed with 70 ng of DNA from the temperature-sensitive early gene mutant helper tsA_{239} and used in a DNA plaque infection of monkey kidney cells at 41 °C. Fifty complementation plaques were picked and used to prepare virus stocks. DNAs from these stocks were immobilised on nitrocellulose filters and tested for hybridisation to a probe containing globin gene 5′-flanking sequences (see Fig. 2 legend). Of three isolates showing unequivocal hybridisation, one was kept for further analysis and designated SVβ^{maj}-3.

We originally cloned the β^{maj}-globin gene in bacteriophage λ as part of a 7,000-base pair fragment of chromosomal DNA[1]. Complete nucleotide sequence analysis of this gene revealed that it is divided into three discontinuous coding segments by two intervening sequences of DNA[5]. The shorter intervening sequence is 116 base pairs long and interrupts the gene between amino acid codons 30 and 31, whereas the longer is 646 base pairs and interrupts between amino acid codons 104 and 105. Because the maximum amount of DNA which can be inserted in the SV40 late region is only 2,500 base pairs, it was not possible to use the whole 7,000-base pair globin fragment. Instead, we prepared a 2,400-base pair subfragment extending from a *Sac*I site, located 430 base pairs upstream from the 5′-end of the globin mRNA sequence, to an *Xba*I site, located 600 base pairs downstream from the 3′-end of the mRNA sequence. This fragment contains, in addition to the entire globin coding sequence, the sequences adjacent to the sites at which the cap is added to the 5′-end and poly(A) to the 3′-end of the globin mRNA. It also contains two RNA splice junctions and their corresponding intervening sequences. To facilitate the preparation of this fragment and its cloning in SV40, we converted

The viral vector segment, which was prepared by cleavage of wild-type SV40 DNA with *Bam*HI at map position 0.14 and *Hpa*II at map position 0.72, retains the viral origin of replication, the entire early gene region and the sequences corresponding to the extreme 5′- and 3′-termini of the late 16S and 19S mRNAs. It lacks the coding sequences for the viral late proteins VP1, VP2 and VP3 and the splice junctions for their corresponding mRNAs[13–16]. We have previously demonstrated that various foreign DNA fragments inserted in this vector are efficiently transcribed from the SV40 late promoter, but the resulting transcripts are not appropriately processed and are rapidly degraded in the cell[10]. This might be because the viral late mRNA splice junctions are absent from the hybrid virus. Recombinants formed with this vector lack the viral late genes and are therefore defective. However, because the recombinants retain a functional early gene region, they can be propagated by co-infection with a temperature-sensitive early gene mutant virus as helper[6,7,34]. When such mixed infections are carried out at the non-permissive temperature, progeny virus is produced only by those cells infected with both the recombinant, which provides functional early gene product, and

ligase to form a linear recombinant molecule that was purified by electrophoresis in an agarose gel. As shown in Fig. 1, the globin gene could join the vector in only one orientation, such that the globin coding sequence is on the strand normally occupied by the viral late protein coding sequences. The resultant recombinant molecule is about 150 base pairs longer than wild-type DNA and has non-complementary termini. However, previous experiments indicated that such molecules could cyclise *in vivo*, and that this is frequently accompanied by the generation of deletions around the junction between the viral and foreign DNAs (D.H.H., unpublished results). Accordingly, the recombinant DNA preparation was mixed with DNA from a temperature-sensitive early gene mutant as helper, and used in a DNA infection of cultured kidney cells. As anticipated, this mixed infection yielded a substantial number of complementation plaques, whereas infection with equivalent amounts of the recombinant or helper DNAs alone did not.

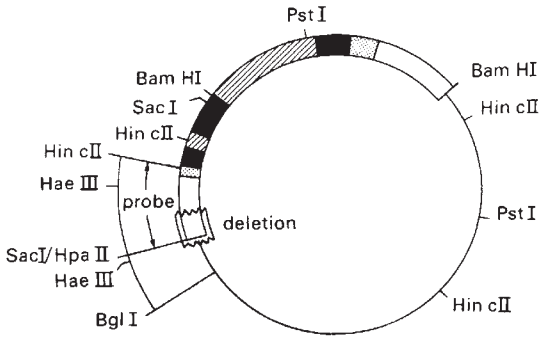
To identify a clone of virus having the desired structure, we prepared virus stocks from these plaques and tested their DNA for hybridisation to a globin probe containing the globin mRNA cap site and the 5′-genomic flanking sequences (see Fig. 2). Of the first 50 plaques tested, three showed unequivocal hybridisation. Presumably, the others had either suffered deletions extending past the 5′-end of the globin gene, or contained only low levels of recombinant virus. One of the positive clones, designated SVβ^{maj}-3, was kept for further analysis. This hybrid multiplies quite efficiently in monkey kidney cells. In our standard conditions of infection, the ratio of intracellular recombinant to helper genome is about 1 : 1.

An analysis of SVβ^{maj}-3 by restriction endonuclease cleavage and gel blot hybridisation (Fig. 2) confirmed that it had the structure expected from its manner of construction and selection. Although the recombinant has the same length as the helper, we were able to purify its DNA because it contains no *Eco*RI sites and one *Sac*I site, whereas the helper contains one *Eco*RI site and no *Sac*I sites. By digestion with the appropriate endonucleases, we showed that the recombinant has deleted 150 base pairs from the 5′-end of the original globin fragment, leaving 280 base pairs of genomic flanking sequence 5′ to the mRNA cap site. Less than 14 base pairs are deleted from the SV40 vector, leaving the predominant viral late mRNA cap sites intact. Thus, SVβ^{maj}-3 contains the complete mouse β^{maj}-globin gene in the sense orientation relative to the viral late region promoter.

Transcription and processing of globin mRNA

Monkey cells infected with SVβ^{maj}-3 efficiently synthesise a stable globin mRNA that is spliced and polyadenylated at the appropriate globin gene sites. However, most of this RNA is initiated at the SV40 late promoter region rather than the globin promoter. Evidence for this is derived from gel transfer hybridisation experiments and the translation studies described below.

To characterise the globin RNA made in infected monkey cells, poly(A)-containing RNA was purified[13,14], denatured[15], electrophoresed through an agarose gel, transferred and covalently attached to diazobenzyloxymethyl paper[16], then hybridised with various ^{32}P-labelled DNA probes to reveal the positions of the separated RNAs (Fig. 3). Hybridisation with total SV40 DNA as probe yielded two bands corresponding to



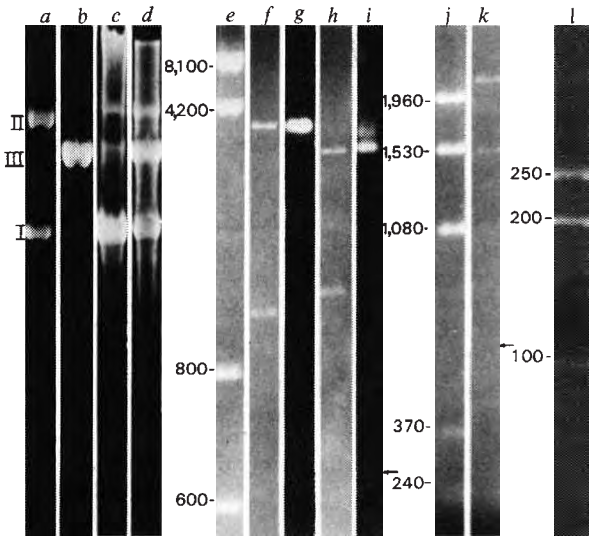


Fig. 2 Structural analysis of the SV40-mouse β^{maj}-globin gene recombinant. The upper diagram is a restriction endonuclease cleavage map of the recombinant virus SVβ^{maj}-3. Solid lines: SV40 sequences; open bars: globin gene flanking sequences; stippled bars: globin gene transcribed, untranslated sequences; solid bars: globin gene coding sequences; hatched bars: globin gene intervening sequences. The bottom panels show an analysis of the recombinant virus by restriction endonuclease cleavage and gel transfer hybridisation. A mixture of SVβ^{maj}-3 recombinant plus tsA_{239} helper DNA was prepared by the method of Hirt[29] from monkey kidney cells that had been infected with the recombinant plus helper stock for 48 h at 40 °C. To prepare pure SVβ^{maj}-3 DNA, this mixture was digested with *Eco*RI, which has one site in the helper but none in the recombinant, and the resistant recombinant genomes were recovered as covalently closed circles as shown in track *d*. This preparation was digested with *Sac*I, which has one site in the recombinant but none in the helper, and the linear recombinant molecules were further purified by electrophoresis through a 1% agarose gel. A fragment containing the deleted junction between the globin and SV40 fragments was prepared by digestion with *Hinc*II plus *Bgl*I as shown in the diagram and electrophoresis of the resulting fragments through a 2% agarose gel. Tracks *a–d* were electrophoresed through 1% agarose gels, *e–i* through 1.4% agarose gels, *j*, *k* through 2% agarose gels, and *l* through an 8% acrylamide gel. Tracks *a–f*, *h* and *j–l* are photographs of ethidium bromide-stained gels. Tracks *g* and *i* are autoradiographs of nitrocellulose transfer strips that were hybridised to a pMB9 · βG2/*Sac*I–*Hinc*II probe containing flanking and transcribed, untranslated sequences from the 5′-portion of the globin gene (see upper diagram). Each track represents the following: *a*, undigested wild-type SV40 DNA. The positions of covalently closed circular (form I), nicked circular (form II) and linear (form III) SV40 are shown to the left; *b*, *Eco*RI digest of wild-type SV40 DNA gives linear molecules; *c*, undigested SVβ^{maj}-3 plus tsA_{239} DNA; *d*, *Eco*RI digest of SVβ^{maj}-3 plus tsA_{239} DNA gives about 50% resistant form I recombinant molecules and 50% sensitive form III helper molecules; *e*, *Sac*I plus *Pst*I digest of pMB9 · βG2 DNA gives marker fragments with lengths (in base pairs) shown to the left; *f*, *Bam*HI digest of SVβ^{maj}-3/*Sac*I DNA gives 3,700- and 1,500-base pair fragments; *g*, transfer of track *f* showing hybridisation to 3,700-base pair fragment; *h*, *Pst*I digest of SVβ^{maj}-3/*Sac*I DNA gives 3,000-, 1,570- and 630-base pair fragments. The position of the 630-base pair fragment, which can be seen only faintly in this reproduction, is indicated by the arrow to the right; *i*, transfer of track *h* showing hybridisation to 3,000-base pair fragment; *j*, *Hinc*II digest of wild-type SV40 DNA gives marker fragments with lengths (in base pairs) shown to the left; *k*, *Hinc*II digest of SVβ^{maj}-3/*Sac*I DNA gives fragments of 2,100, 1,530, 1,080 and 580 base pairs. The position of the 580-base pair fragment, which can be seen only faintly in this reproduction, is indicated by the arrow to the right; *l*, *Hae*III digest of the SVβ^{maj}-3/*Hinc*II–*Bgl*II junction fragment gives three fragments with the lengths (in base pairs) shown to the left. The lengths of these fragments were estimated by their mobility relative to several sequenced SV40 and globin gene fragments (not shown). There are single *Hae*III sites in the globin gene sequence between the *Hinc*II site and the deleted *Sac*I site and in the SV40

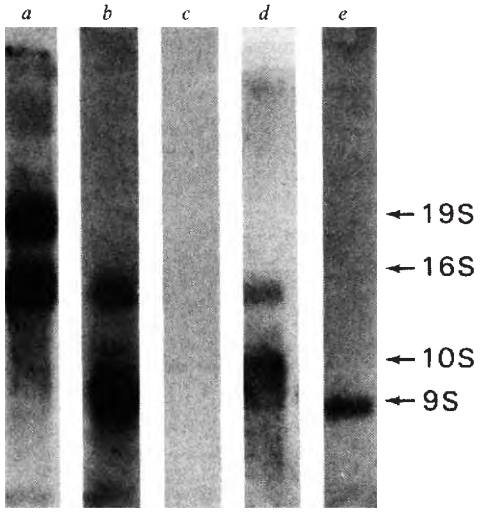


Fig. 3 Gel transfer hybridisation analysis of the globin RNA made in monkey cells infected with the SV40–mouse β^{maj}-globin gene recombinant. **RNAs and hybridisation:** A confluent monolayer of about 2×10^7 monkey kidney cells was infected with 5 ml of the SVβ^{maj}-3 plus $\underline{tsA}_{239}$ stock and incubated for 48 h at 40 °C. Total cell poly(A)-containing RNA was prepared by extraction with hot phenol[13] and oligo(dT)–cellulose chromatography[14], denatured with glyoxal[15], electrophoresed through a 1.5% agarose gel and transferred to diazobenzyloxymethyl paper[16]. Each lane contains 10% of the total sample. The resulting imprints were preincubated, hybridised with approximately 0.1 ng of ^{32}P-labelled, nick-translated DNA probe (25–50 c.p.m. per pg), washed and autoradiographed[10,16]. Globin mRNA was prepared from reticulocytes obtained from anaemic mice. **Probes** (see maps, Figs 1, 2): The globin probes were prepared by cleavage of the plasmid pMB9 · MβG2, which contains the entire 7,000-base pair chromosomal mouse β^{maj}-globin fragment[2]. The globin coding sequence probe contained 900 base pairs extending from the *Pst*I site 75 base pairs upstream from the 3′-coding segment to the *Xba*I site 600 base pairs downstream from the poly(A) addition site. The globin intervening sequence probe contained 600 base pairs extending from the *Bam*HI site 18 base pairs upstream from the large intervening sequence to the *Pst*I site 75 base pairs upstream from the 3′-coding segment. Control experiments with globin mRNA show that hybridisation of the 18 base pairs of coding sequence included in this intervening sequence fragment would not be detected in our conditions of hybridisation, washing and autoradiography. Wild-type SV40 DNA was prepared as described previously[6]. The SV40 late mRNA leader probe contained 250 base pairs extending clockwise from the *Bgl*I site at 0.67 map units in the *Hpa*II site at 0.72 map units. Each track represents the following: *a*, SVβ^{maj}-3 RNA hybridised with total SV40 DNA probe; *b*, SVβ^{maj}-3 RNA hybridised with globin coding sequence probe; *c*, SVβ^{maj}-3 RNA hybridised with globin intervening sequence probe; *d*, SVβ^{maj}-3 RNA hybridised with SV40 late mRNA leader sequence probe; *e*, globin mRNA (10 ng) hybridised with globin coding sequence probe.

the viral late 16S and 19S mRNAs, presumably due to transcription of the helper virus. When a fragment containing globin coding sequences was used as the probe, we detected a strong globin RNA band at approximately 10S, indicating molecules up to 300 bases longer than authentic globin mRNA (800 bases). This RNA did not hybridise with a probe derived from the large globin intervening sequence, indicating that it has been eliminated and that the second globin splice junction functioned properly in the infected cells. Because the globin RNA is efficiently translated into mouse β^{maj}-globin (see below), it is clear that the first splice junction is also functional and that both splicing reactions are accurate. To determine whether this RNA was initiated at the globin gene promoter or at the upstream viral promoter, we used as probe an SV40 fragment containing the viral late mRNA leader sequences. This probe hybridised strongly to the ~10S globin RNA band, indicating that most of these molecules arise from transcription initiation at the viral promoter. This may also explain the length heterogeneity of the globin RNA as the 5′-viral leader segments are known to be quite variable in length[17,18]. The SV40 leader probe also detected a 16S band and a very faintly hybridising 19S band (not visible in the reproduction of Fig. 3, lane *d*) which probably comprising about 15% of the total globin-specific RNA. This RNA is at least partially spliced as shown by its failure to hybridise to the globin large intervening sequence probe. We suspect, but have not proved, that this species results from readthrough past the globin polyadenylation site to the downstream viral polyadenylation site. A similar situation was encountered in our more detailed studies of a recombinant carrying a segment from the 3′-portion of the mouse β^{maj} gene[12].

Judging from the intensities of the bands shown in Fig. 3, we estimate that the steady-state level of the mouse globin RNA produced in monkey cells compares favourably with that of viral late mRNA. Assuming that the globin and late viral mRNAs are initiated at the same rates from the SV40 late promoter, this indicates that the globin RNA is as stable as late viral mRNA. We estimate that approximately 5% of the total cell poly(A)-containing RNA is globin RNA, representing roughly 200,000 molecules per cell.

Mouse β^{maj}-globin is synthesised by infected monkey cells

Monkey kidney cells infected with SVβ^{maj}-3 synthesise a protein which we have identified as mouse β^{maj}-globin by immunoprecipitation, two-dimensional electrophoresis and tryptic fingerprint analysis. These tests indicate that the mouse globin mRNA is accurately spliced in monkey cells, and that the mouse gene translational signals are functional.

Figure 4 (lanes *a–h*) shows an experiment in which monkey cells were infected with either the SVβ^{maj}-3 plus helper stock or with no virus or wild-type SV40 as controls, incubated until late in the lytic cycle, then labelled for 1 h with ^{35}S-methionine. Sonic extracts were prepared, and a portion of each was electrophoresed through a polyacrylamide-SDS gel, which separates proteins according to their molecular weight[19]. As might have been anticipated from the fact that SV40 does not shut down host cell protein synthesis, the resulting autoradiograms showed a complex variety of proteins in which the globin region of the gel was obscured by host bands. However, when the cell proteins were first immunoprecipitated with purified goat anti-mouse β-globin antibody, we detected a band with the same mobility as authentic mouse globin in the SVβ^{maj}-3-infected cell extract but not in the mock or wild-type SV40-infected cell extracts. (The additional bands seen in these lanes were also seen using anti-SV40 T-antigen serum, and apparently result from artefactual trapping during the immunoprecipitation procedure.) The exact co-migration of this band with authentic mouse β^{maj}-globin was confirmed by a mixing experiment (Fig. 4, lane *h*).

The identity of this protein as mouse β^{maj}-globin is supported by a two-dimensional electrophoresis experiment (Fig. 4, lanes *i–m*). Extracts were prepared and immunoprecipitated as described above, unlabelled mouse globin was added to the eluted proteins and the mixtures were submitted to non-equilibrium *p*H gradient gel electrophoresis, which separates proteins according to their isoelectric points[20]. When the proteins co-migrating with mouse β^{maj}-globin, identified by staining, were eluted and electrophoresed through a polyacrylamide-SDS gel, we detected a strong globin band in the SVβ^{maj}-3-infected preparation but not in the mock or wild-type SV40-infected preparations. A mixing experiment (Fig. 4, lane *m*) confirmed the co-migration of this band with authentic globin. Note that the non-equilibrium *p*H gradient gels used in this experiment are capable of separating proteins that differ by only a single charge unit[20].

Tryptic fingerprint analysis provided further evidence that the protein encoded by SVβ^{maj}-3 is mouse β^{maj}-globin. The globin band from SVβ^{maj}-3-infected cells was purified by immunoprecipitation and polyacrylamide-SDS gel electrophoresis, digested with trypsin and submitted to two-dimensional

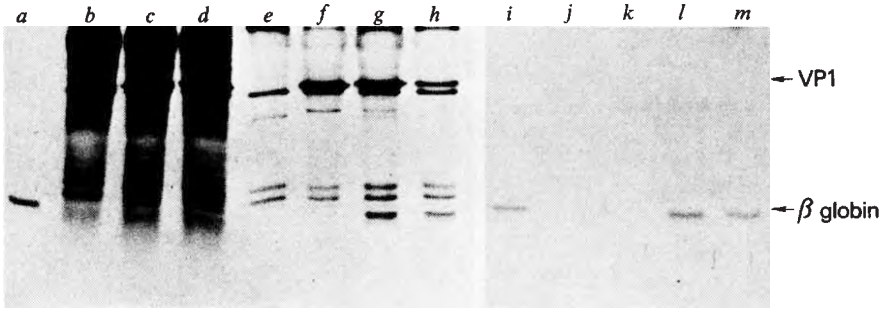


Fig. 4 Production of mouse β^{maj}-globin in monkey cells infected with the SV40-mouse β^{maj}-globin gene recombinant. **Proteins:** Confluent monolayers of about 2×10^7 monkey cells were treated with 1 ml of medium, wild-type SV40 or the SVβ^{maj}-3 plus tsA_{239} stock and incubated for 48 h at 40 °C. The cells were washed three times with methionine-free medium, then labelled for 1 h with 200 μCi of ^{35}S-methionine (516 Ci mmol^{-1}, NEN) in 1 ml of methionine-free media. After washing twice in ice-cold phosphate-buffered saline (PBS), the cells were scraped off the plates in PBS, collected by centrifugation, resuspended in 0.8 ml of PBS containing 1% Triton X-100 and 0.5% sodium deoxycholate, sonicated for 1 min at full power in a Heat Systems sonicator, and centrifuged at 12,000*g* for 30 min. The supernatants were recovered and analysed as described below. To prepare authentic mouse β^{maj}-globin, Friend erythroleukaemia cells were induced with 1% dimethyl sulphoxide for 3 d, labelled with 25 μCI ml^{-1} ^{35}S-methionine for 12 h, then lysed by freeze-thawing. Haemoglobin was purified on a carboxymethyl cellulose column and converted to globin by precipitation with 2% HCl in acetone[30]. Pure β^{maj}-globin chains were prepared by carboxymethyl cellulose column chromatography in the presence of 8 M urea[31]. **Immunoprecipitation:** 10 μl of heparin (10 mg ml^{-1}), 10 μl of normal goat serum and 50 μl of a 10% suspension of heat-killed formalin-fixed *Staphylococcus aureus* Cowen I bacteria were added to 500 μl of cell extract[32]. Following 30 min on ice, the bacteria were removed by centrifugation, the supernatant was added to a predetermined excess of purified goat anti-mouse β-globin antibody and incubated 12 h at 4 °C. Then, 50 μl of *S. aureus* suspension was added, the mixtures were kept at 4 °C for 1 h and the bacteria collected by centrifugation. The pellet was washed three times in PBS containing 1% Triton X-100 and 0.5% deoxycholate. A 20% aliquot of each sample was resuspended in 25 μl of SDS sample buffer[19], boiled for 5 min, centrifuged to remove the bacteria, then analysed by polyacrylamide-SDS gel electrophoresis. The remainder of each sample was resuspended in 100 μl of non-equilibrium *p*H gradient electrophoresis gel sample buffer[20], mixed with 5 μg of unlabelled mouse globins, incubated for 1 h at room temperature, centrifuged to remove the bacteria, then analysed by two-dimensional electrophoresis. **Gel electrophoresis:** 20% polyacrylamide-SDS gels were prepared, run and fluorographed as described elsewhere[19,33]. Non-equilibrium *p*H gradient gels[20] were prepared in 0.6 × 10 cm tubes and electrophoresed at 400 V (constant voltage) for 3 h. The gels were soaked in 50% methanol for 24 h, stained in 0.01% Coomassie brilliant blue, 50% methanol and 12.5% acetic acid for 15 min, then destained in 7% methanol and 1.25% acetic acid for 18 h. The region of the gel containing mouse β^{maj}-globin (well separated from β^{min}- and α-globin) was excised and the proteins eluted by electrophoresis, precipitated with 2% HCl in acetone, resuspended in 50 μl of SDS sample buffer, boiled for 5 min and electrophoresed through a polyacrylamide-SDS gel. **Quantification of globin synthesis:** 0.67% of the trichloroacetic acid-precipitable radioactivity in the extract from SVβ^{maj}-3-infected cells was recovered in the immunoprecipitate. Of this, 15% was in the globin band as determined by densitometry of the autoradiogram (track *g*). Thus, 0.67% × 5% = 0.1% of the ^{35}S-methionine incorporated during the 1-h pulse was found in globin. Tracks *a–h* are one-dimensional, polyacrylamide-SDS gel analyses of total cell extracts and immunoprecipitates. Tracks *i–m* are the second dimension from the two-dimensional analyses (non-equilibrium *p*H gradient gel electrophoresis followed by polyacrylamide-SDS gel electrophoresis) and were run on a separate polyacrylamide-SDS gel. Each track represents the following *a*, mouse β^{maj}-globin; *b*, total mock-infected cell extract; *c*, total wild-type SV40-infected cell extract; *d*, total SVβ^{maj}-3-infected cell extract; *e*, immunoprecipitate of mock-infected cell extract; *f*, immunoprecipitate of wild-type SV40-infected cell extract; *g*, immunoprecipitate of SVβ^{maj}-3-infected cell extract; *h*, mixture of mouse β^{maj}-globin and immunoprecipitate of SVβ^{maj}-3-infected cell extract (one-half the amounts applied to tracks *a* and *g* were mixed after immunoprecipitation and elution); *i*, mouse β^{maj}-globin; *j*, mock-infected; *k*, wild-type SV40-infected; *l*, SVβ^{maj}-3-infected; *m*, mixture of mouse β^{maj}-globin and SVβ^{maj}-3-infected samples (one-half the amounts applied to tracks *i* and *l* were mixed after immunoprecipitation elution).

β^{maj}-globin prepared from induced Friend erythroleukaemia cells. The identity of the peptides from the SVβ^{maj}-3 protein with authentic β^{maj}-globin was confirmed by fingerprinting a mixture containing equal amounts of radioactivity from both samples. Presumably, the two spots detected represent the two internal methionine-containing tryptic peptides of β^{maj}-globin[23]. The globin gene also encodes an N-terminal methionine which is removed in globin-producing cells. Because only the expected two spots were observed in the SVβ^{maj}-3 protein digest, we conclude that the monkey cells have appropriately removed the N-terminal methionine. Note also that one of the methionine-containing peptides (Leu 105–Lys 121) lies within the third coding sequence immediately after the second splice junction (Arg 104–Leu 105). This provides further evidence for accurate splicing of the mouse globin mRNA in monkey cells.

We estimate that the mouse β^{maj}-globin accounts for approximately 0.1% of the ^{35}S-methionine incorporated during a 1-h pulse (see Fig. 4 legend). This is close to the synthesis rates for the viral proteins VP2 and VP3. Thus, the globin mRNA seems to be translated about as efficiently as viral mRNA in infected monkey kidney cells.

Distribution of RNA processing enzymes

Although the ubiquity of the enzymes and factors involved in translation has long been recognised, relatively little is known about the species and cell-type distribution of RNA processing enzymes. Our previous data[12] and experiments presented here establish that monkey kidney cells can both splice and polyadenylate mouse globin RNA. Although we have not characterised the polyadenylation reaction in detail, we believe that the splicing reaction is both specific and efficient. This is indicated by the translation of the resulting mRNA into β^{maj}-globin, and by the failure to observe accumulation of unspliced

lineage, we suspect that the RNA splicing and polyadenylation signals are widely recognised among mammalian species and cell types. Our findings are inconsistent with the notion that the differentiation of a globin-producing cell requires the production of enzymes that specifically process globin and other erythroid mRNAs. On the other hand, the results support the validity of comparing the sequences of globin genes from divergent organisms so as to define the nucleotide sequence specificity of RNA processing enzymes.

SV40 transducing viruses as genetic tools

We have established that a mammalian chromosomal gene can be expressed in a foreign cell after cloning in SV40. The chief difference between this and earlier experiments by Mulligan *et al.*[11] and ourselves[10] using cDNA recombinants is that here expression of the globin gene depends on the mouse genomic signals for RNA splicing and polyadenylation rather than on the corresponding viral signals. Hence, it seems to be a useful substrate for determining the effects of nucleotide sequence alterations on RNA processing. Further, although we have used the SV40 late promoter in this recombinant, it should be possible to bring the globin gene under the control of its own promoter by inverting the mouse fragment relative to the viral vector.

Although cell transformation techniques may also be useful for globin gene transfer experiments (refs 24, 25 and R. Axel, personal communication), there are several advantages of SV40 as a vector for this purpose. First, its small size simplifies localised biochemical mutagenesis. Second, it replicates efficiently, reaching levels up to 100,000 copies per cell late in infection, thus simplifying the detection of gene products encoded by the transduced DNA. Finally, by placing the genomic fragment under the optional control of the strong viral

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