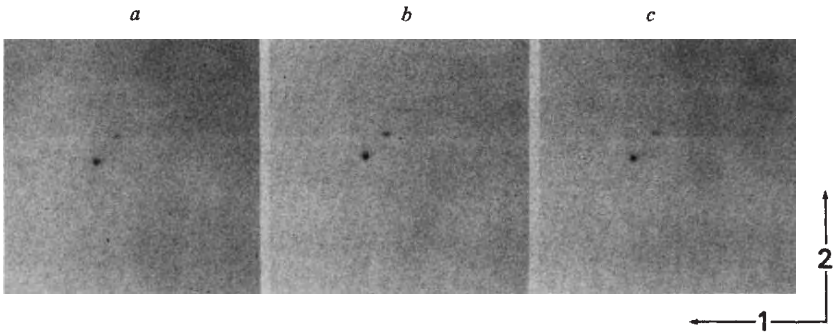

Fig. 5 Fingerprints of tryptic peptides. A 1-ml aliquot of SVβ^{maj}-3-infected cell extract was immunoprecipitated as described in Fig. 4 legend, eluted in SDS sample buffer, mixed with 5 μg of unlabelled mouse globin and electrophoresed through a 20% polyacrylamide-SDS gel. A sample of authentic ^{35}S-methionine-labelled mouse β^{maj}-globin from induced Friend cells was electrophoresed in a separate track. The globin bands were identified by staining, excised, washed with 10% methanol, then digested *in situ* with trypsin-TPCK (Worthington)[21]. The supernatants were lyophilised, resuspended in 25 μl of H_2O and analysed by two-dimensional ascending chromatography on 20×20 cm cellulose thin-layer plates (Brinkman). The first solvent was isoamyl alcohol/pyridine/H_2O (90:120:105)[22]. The second solvent was butanol/pyridine/acetic acid/H_2O (32.5:25:5:20) containing 7% (w/v) 2,5-diphenyloxazole[21]. The autoradiographs were exposed for 8 d at −70 °C. *a*, Globin from SVβ^{maj}-3-infected cells; *b*, authentic mouse β^{maj}-globin; *c*, mixture of globin from SVβ^{maj}-infected cells and authentic mouse β^{maj} globin (one-half the amounts used in *a* and *b*).

can be inserted into the late region of the recombinant if it is to be encapsidated into virions. This obstacle might be overcome by propagating the hybrid genome as an episome in persistently infected monkey cells[9], thus overcoming the requirement for encapsidation. Furthermore, it is clear that, although we have concentrated on the globin system, the approach should also be applicable to a variety of other genes.

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Rabbit β-globin mRNA production in mouse L cells transformed with cloned rabbit β-globin chromosomal DNA

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Mouse thymidine kinase-negative L cells were transformed with a cloned rabbit chromosomal β-globin gene linked to the cloned thymidine kinase gene of herpes simplex virus type 1. Most thymidine kinase-positive cell lines contained one or more copies of rabbit β-globin DNA and produced up to 2,000 copies of rabbit β-globin RNA per cell indistinguishable from its authentic counterpart. No mouse β-globin mRNA was detected.

RECOMBINANT DNA technology has made it possible to isolate, amplify and determine the primary structure of any eukaryotic DNA segment of interest. To analyse the function of a significance of particular regions of a genome, it is desirable to generate localised modifications in the nucleic acid and then evaluate the resulting changes in the biological properties of the genome, *in vitro* or *in vivo* (see ref. 1). The reintroduction of defined nucleic acid segments, in particular of cloned DNA, into eukaryotic cells has been accomplished by various methods. Mechanical microinjection of RNA or DNA into oocytes[2,3] or into somatic cells[4] delivers a large dose of nucleic acid to a relatively small number of identifiable cells. Biophysical and biochemical approaches use liposomes[5,6] or erythrocytes[7] loaded with RNA which are fused with the target cells, or direct treatment of target cells with nucleic acid in the presence of facilitating agents[8–11]. Efficient delivery of DNA to a large population of cells is attained by the use of DNA incorporated

When a high proportion of the treated cells take up and retain the administered DNA the experiment can be evaluated by assaying the entire cell sample; this is the case when oocytes are individually injected, or when cells are infected with viral particles containing a hybrid SV40 genome. Where the foreign DNA transforms only a minute proportion of the cells, as is the case in most procedures involving administration of exogenous naked DNA, a selection procedure must be used to enrich for transformed cells.

Thymidine kinase-negative cells acquire thymidine kinase activity after transformation with herpes virus DNA, or even better, with an appropriate, purified restriction fragment[9]. As

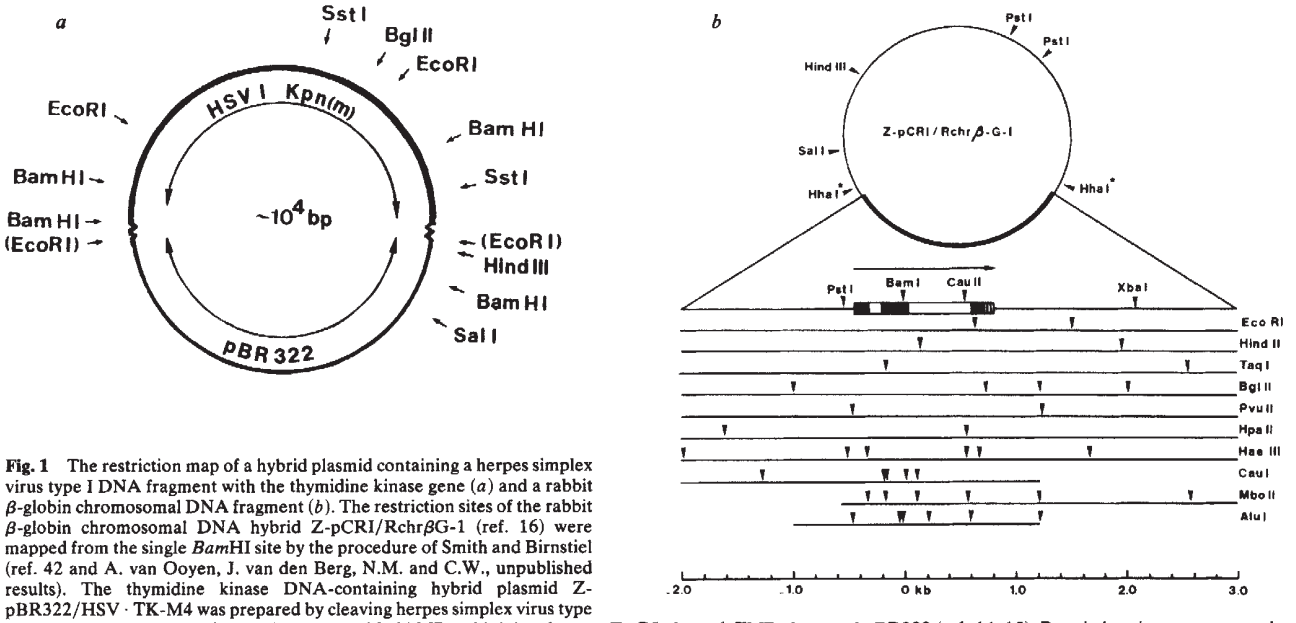

Fig. 1 The restriction map of a hybrid plasmid containing a herpes simplex virus type I DNA fragment with the thymidine kinase gene (*a*) and a rabbit β-globin chromosomal DNA fragment (*b*). The restriction sites of the rabbit β-globin chromosomal DNA hybrid Z-pCRI/RchrβG-1 (ref. 16) were mapped from the single *Bam*HI site by the procedure of Smith and Birnstiel (ref. 42 and A. van Ooyen, J. van den Berg, N.M. and C.W., unpublished results). The thymidine kinase DNA-containing hybrid plasmid Z-pBR322/HSV · TK-M4 was prepared by cleaving herpes simplex virus type 1 DNA with *Kpn*I, elongating the fragments with dAMP and joining them to *Eco*RI-cleaved, TMP-elongated pBR322 (refs 14, 15). Restriction sites were mapped from the single *Bgl*II site by the procedure of Smith and Birnstiel[41] and by total cleavage. The *Eco*RI sites in parentheses are obliterated.

Table 1 Rabbit β-globin DNA and β-globin mRNA content of transformed mouse L cell lines

DNA used for transfection: β-globin	DNA used for transfection: Thymidine kinase	No. transformants: No. dishes	Cell line	Approximate no. rabbit β-globin DNA copies per cell*	Approximate no. rabbit β-globin RNA transcripts per cell†: Expt 1	Expt 2
—	TK-P1	83/2	P1/S-1	0	<20	
—			P1/S-2	0	<20	
—	TK-M2	82/2	M2/S-1	NT	<20	
			M2/S-2	0	<20	
—	TK-M4	78/2	M4/S-1	NT	<20	
			M4/S-2	0	<20	
RβG	TK-P1	71/4	P1/R-1	0	NT	
			P1/R-2	1	NT	
			P1/R-3	3	300	600
			P1/R-4	8	300	50
			P1/R-5	1	50	400
			P1/R-6	2	<20	
			P1/R-7	6	<20	
			P1/R-8	1	100	
RβG	TK-M2	59/5	M2/R-1	20	2,000	2,000
			M2/R-2	0.25	NT	
			M2/R-3	1	300	
			M2/R-4	8	<20	
			M2/R-5	0.25	200	
			M2/R-6	0.25	200	
			M2/R-7	3	200	
			M2/R-8	3	<20	
RβG	TK-M4	14/4	M4/R-1	1	150	
			M4/R-3	8	200	
			M4/R-5	0	<20	
			M4/R-6	0.25	NT	
			M4/R-7	1	400	

Thymidine kinase-negative mouse L cells (LMTK$^-$) were cultured in Dulbecco's modified Eagle's medium (Flow), 10% calf serum (Eurobio) and 30 μg ml^{-1} bromodeoxyuridine (BUdR). Before transfection the cells were cultured for three doublings in the absence of BUdR, then plated at 4×10^5 cells per 9-cm dish 24 h before DNA addition. TK-gene-containing plasmid DNA was prepared in P-2 containment conditions. To prepare concatenated DNA for transfection, 1.25 μg TK-gene plasmid (Z-pBR322/HSV · TK-P1, -M2 or -M4 (refs 14, 15) and 6.25 μg Z-pCR1/RchrβG-1 (ref. 16) were digested with *Sal*I (New England Biolabs), heated for 10 min at 65°C to inactivate the enzyme, and mixed into a final volume of 0.1 ml with 70 mM Tris-HCl (*p*H 7.2), 10 mM $MgCl_2$, 10 mM dithiothreitol and 1 mM ATP. Ligation was with T_4 DNA ligase for 3 h at 16 °C; no monomeric DNA was detectable by agarose gel electrophoresis. After phenol extraction, ether extraction and ethanol precipitation, the DNA was mixed with 80 μg salmon sperm DNA and used for transfection of five dishes of LMTK$^-$ cells according to the protocol of Wigler *et al.*[17]. Control cells were transfected with *Sal*I-cleaved TK-gene plasmid, without ligation. After 19 days in modified HAT[13] medium (15 μg ml^{-1} hypoxanthine, 1 μg ml^{-1} aminopterin, 5 μg ml^{-1} thymidine in Dulbecco's modified Eagle's medium), colonies were picked with cloning rings and grown up for analysis (see Figs 2, 4 and 5).

* The β-globin DNA content of 10–30 μg of total cell DNA was estimated visually from the autoradiogram of Fig. 2 and additional similar experiments.

only cells possessing thymidine kinase activity can survive and grow in a medium containing hypoxanthine, aminopterin and thymidine (HAT medium)[13], it is possible to select from among 10^6–10^7 cells the few tens or hundreds of cells which were transformed by DNA containing the thymidine kinase gene[9]. We have used this system to select mouse cells transformed with cloned rabbit chromosomal β-globin DNA linked to a cloned herpes simplex virus type 1 (HSVI) DNA fragment containing the thymidine kinase (TK) gene. We find that 19 of 21 cell lines selected in HAT medium contain rabbit β-globin DNA, and 12 of 16 such lines synthesise rabbit β-globin-specific RNA which is indistinguishable from natural β-globin mRNA in all aspects tested.

Transformation of TK⁻ mouse cells with rabbit β-globin DNA linked to HSVI–TK DNA

We have prepared hybrid plasmids consisting of pBR322 linked to a fragment of HSVI DNA containing the TK gene, either a *Bam*HI (Z-pBR322/HSV · TK-P1) or a *Kpn*I fragment (Z-pBR322/HSV · TK-M2 and Z-pBR322/HSV · TK-M4)[14,15]. Figure 1 shows the restriction maps of the hybrid plasmid Z-pBR322/HSV · TK-M4 (*a*) and of the hybrid Z-pCRI/Rchrβ G-1 (*b*, abbreviated to RβG), which consists of pCRI linked to a β-globin chromosomal rabbit DNA fragment[16]. The plasmids containing β-globin and thymidine kinase DNA were cleaved at their single *Sal*I sites and joined at a molar ratio of 2.5:1 by T_4 DNA ligase to form concatenates. TK⁻ L cells were transformed with the concatenates (1.5 μg total DNA per 8×10^5 cells) using the calcium phosphate method[8] as described by Wigler *et al.*[17]. Cells transformed in parallel with a *Sal*I-cleaved thymidine kinase plasmid alone served as a control. After 19 days in HAT-containing medium 8–47 colonies per dish were recovered (Table 1). Each of 27 colonies was expanded to about 6×10^7 cells, and DNA and RNA were isolated.

Identification of rabbit β-globin DNA in thymidine kinase-transformed mouse L cells

The DNA from the thymidine kinase-transformed cell lines was cleaved with *Eco*RI and *Pst*I, denatured, subjected to agarose gel electrophoresis and transferred to nitrocellulose filters by the procedure of Southern[18]. As reference, the chromosomal β-globin DNA hybrid RβG, cleaved with the same enzymes, was run in parallel. The filter was hybridised with a ^{32}P-labelled, cloned rabbit β-globin cDNA probe devoid of plasmid sequences[19]. As shown in the restriction map of Fig. 1, cleavage of rabbit chromosomal DNA by *Pst*I and *Eco*RI is expected to yield two β-globin-specific fragments of 1,200 and 900 base pairs, respectively. The autoradiogram (Fig. 2) shows that the four DNA preparations from cells transformed with the rabbit β-globin–TK DNA concatenates had two bands with the same mobilities and the same relative intensities as the 900- and 1,200-base pair bands derived from the β-globin chromosomal plasmid. Altogether, 19 of 21 TK-positive cell lines contained β-globin DNA by this analysis. The control, DNA from cells transformed with a TK plasmid only, did not show these characteristic bands. From the intensities of the bands of *Eco*RI- and *Pst*I-cleaved cell DNA relative to those of known amounts of similarly treated cloned chromosomal DNA run in parallel, we estimate visually that the cell lines P1/R-3, P1/R-4, P1/R-5 and M2/R-1 contain about 3, 8, 1 and 20 copies of rabbit β-globin DNA per cell (Table 1).

Identification and characterisation of rabbit β-globin-specific transcripts from transformed mouse L cells

In vivo transcription of the extraneous rabbit β-globin gene was

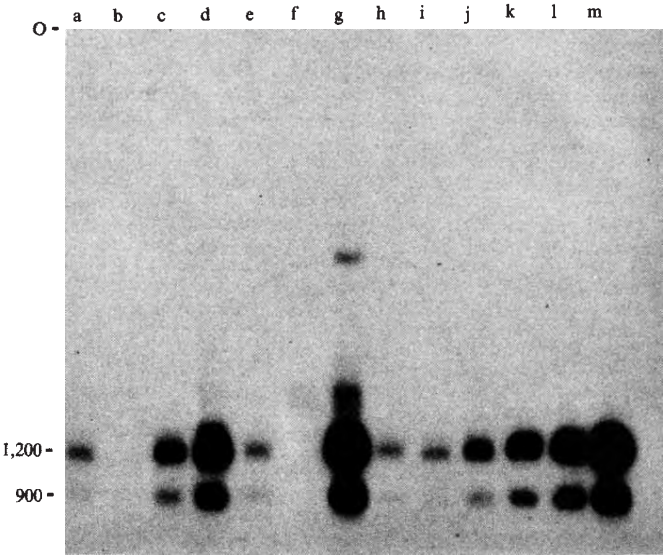

Fig. 2 Detection of chromosomal rabbit β-globin sequences in DNA of transformed mouse L cell lines. Mouse L cell lines transformed with the rabbit β-globin hybrid DNA linked to a thymidine kinase plasmid, or with a thymidine kinase plasmid only, were obtained as described in Table 1 legend. 1.5×10^7 cells were lysed with 1.1% SDS in 0.15 M NaCl and 0.1 M EDTA (*p*H 8), extracted with $CHCl_3$ and isoamyl alcohol (24:1), and the DNA spooled during addition of ethanol to 66%. After treatment with pancreatic RNase, 20 μg ml^{-1} in 10 mM NaCl, 0.5 mM EDTA and 5 mM Tris-HCl (*p*H 7.5) for 8 h at 37 °C, overnight incubation at 37 °C with 100 μg ml^{-1} pronase in 100 mM NaCl, 5 mM EDTA and 50 mM Tris-HCl (*p*H 7.5) and phenol extraction, the DNA was collected by spooling as above. About 300–600 μg of DNA were recovered. Cell DNA (10 μg) was digested with *Pst*I (MRC, Porton) and *Eco*RI (MRC, Porton), denatured and subjected to electrophoresis on a 1.4% agarose gel as described by Jeffreys and Flavell[43]. The DNA was transferred to a 15×17 cm Millipore membrane[18] and hybridised to a ^{32}P-labelled rabbit β-globin probe (specific activity, 8×10^7 c.p.m. per μg; about 325 ng in 25 ml) as described[43]. The probe was obtained as follows. The rabbit β-globin cDNA-containing hybrid plasmid PβG[44] was cleaved with nuclease S_1 in formamide[45], joined to *Hin*dIII linkers[46] and recloned in the *Hin*dIII site of pBR322 (ref. 19). A hybrid plasmid containing the β-globin sequence present in PβG was identified by nucleotide sequence analysis (Z-pBR322 · H3/RcβG-4.13) and prepared in quantity. After cleaving with *Hin*dIII, the β-globin-specific fragment was purified by agarose gel electrophoresis and nick-translated[47] using [α-^{32}P]-labelled dATP and dCTP (400 Ci mmol^{-1} each; NEN, Dreieich). Autoradiography of the filter was for 30 days. Lanes b and f, DNA from cell lines P1/S-2 and M2/S-2, transformed only with a *Sal*I-cleaved TK-gene plasmid. Lanes c, d, e and g, DNA from cell lines P1/R-3, P1/R-4, P1/R-5 and M2/R-1, transformed with the rabbit β-globin containing hybrid RβG, linked to a TK-gene plasmid. Lanes a and h–m contain as reference RβG digested with *Pst*I and *Eco*RI: 1.6, 1.6, 1.6, 3.2, 6.4, 12.8 and 32 pg of DNA hybridisable to the probe (1.8 pg is the equivalent of one β-globin gene). Lanes a, h and j–m also contain 10 μg of DNA from cell line P1/S-2 digested with *Pst*I and *Eco*RI as carrier. The mobility of the 900- and 1,200-base pair bands is about 1% less in the presence of 10 μg total DNA per slot (compare lanes h and i) than in its absence. O, origin. Ordinate in base pairs.

developed by Weaver *et al.*[21], as exemplified in Fig. 3. The hybrid plasmid RβG contains a *Bgl*II site immediately following the 3′-end of the β-globin coding sequence. The hybrid was cleaved with *Bgl*II and labelled with ^{32}P at the 5′-termini. After digestion with *Pst*I, the *Pst*I–*Bgl*II (1,291/1,299) fragment, labelled at the *Bgl*II site (on the (−)strand), was isolated. The labelled DNA was denatured, hybridised with the RNA sample in 80% formamide, treated with nuclease S_1 and analysed by polyacrylamide gel electrophoresis in 7 M urea. Only if the labelled 5′-terminus of the DNA is protected by RNA complementary to it is a radioactive fragment recovered. As the probe is labelled on the (−)strand, only (+)strand RNA is detected. Mature rabbit β-globin mRNA protects a fragment about 134 nucleotides long (from the *Bgl*II site to the 3′-end of the large intervening sequence (intron)), whereas a precursor containing both introns would protect about 1,200 nucleotides and a precursor containing only the large intron would protect 929 nucleotides.

The probe discriminates between mouse and rabbit globin

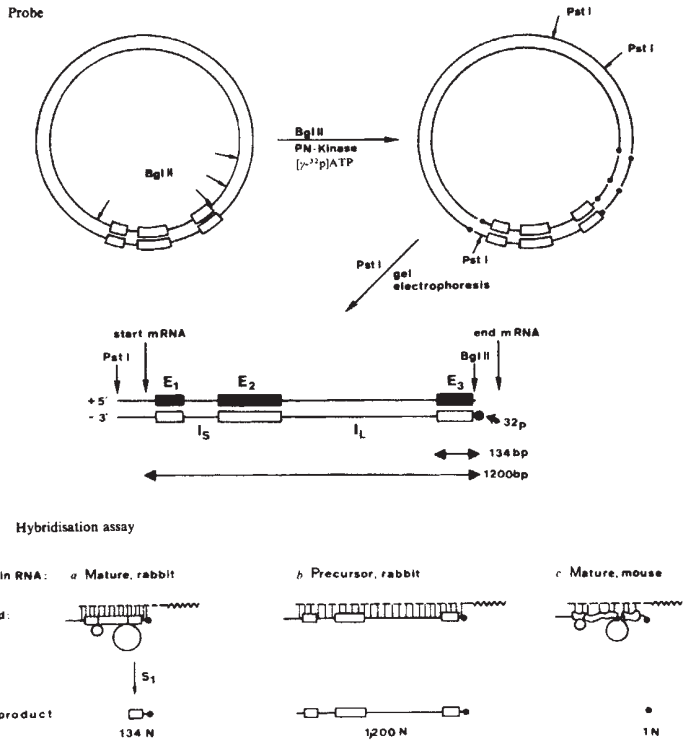

Fig. 3 Application of the Weaver–Berk–Sharp method to distinguish mature and precursor rabbit β-globin mRNA in the possible presence of mouse β-globin mRNA. The procedure is a modification of the method of Berk and Sharp[20]. The probe is a DNA restriction fragment ^{32}P-labelled at one 5′-terminus (rather than being uniformly labelled). The denatured DNA is hybridised to the RNA sample in 80% formamide, digested with S_1 nuclease, denatured and analysed by polyacrylamide gel electrophoresis in 7 M urea. Only if the 5′-terminus of the probe was hybridised to RNA is a labelled DNA fragment recovered. The *Pst–Bgl*II ((+)strand 1,291/(−)strand 1,299 bases) fragment derived from the rabbit chromosomal β-globin plasmid (see Fig. 1), labelled at the *Bgl*II site, is diagnostic for (+)strand β-globin sequences and allows the distinction between rabbit precursor RNA, rabbit mature RNA and mouse RNA. E, exon; I_s and I_L, small and large intron, respectively. PN·Kinase, polynucleotide kinase.

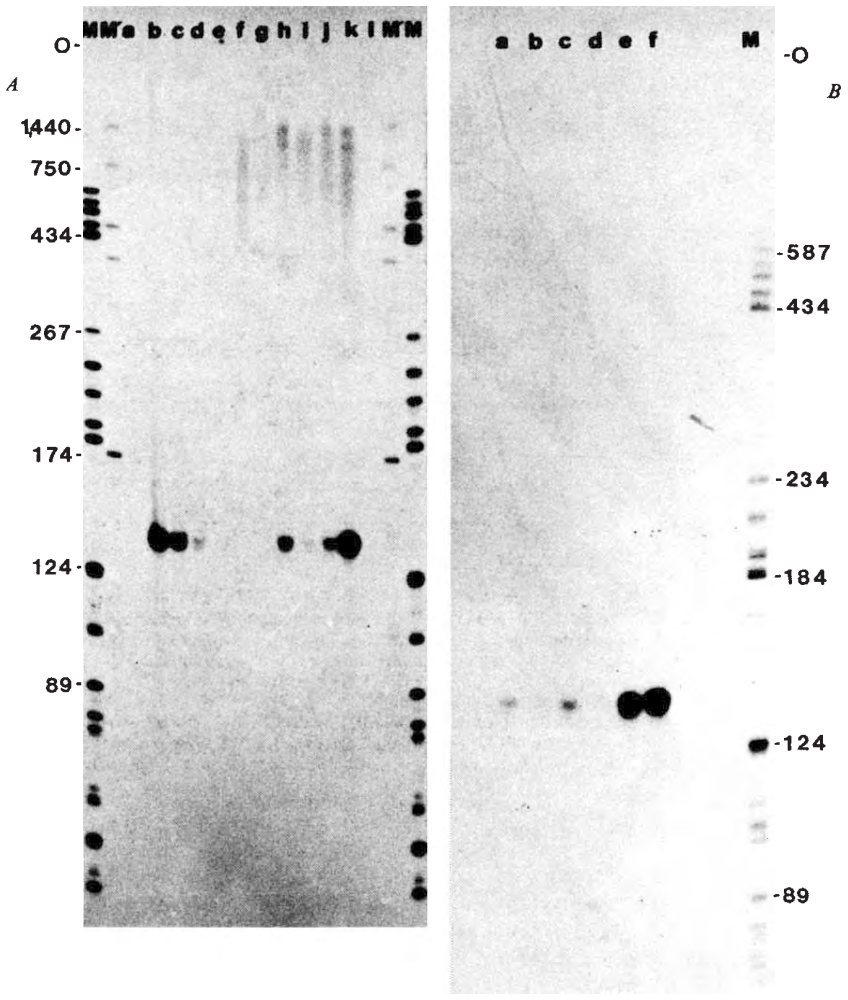

Fig. 4 Detection and characterisation of rabbit β-globin RNA in transformed mouse L cells by the Weaver–Berk–Sharp method. Total cell RNA was prepared from about 10^8 cells as described elsewhere[29]. The yield was about 1.6 mg per 10^8 cells. The ^{32}P-labelled probe (described in Fig. 3) was prepared by cleaving 20 μg Z-pCRI/RchrβG-1 with *Bgl*II (New England Biolabs), treating with bacterial alkaline phosphatase (BAPF, Worthington) and labelling with polynucleotide kinase (P-L Biochemicals) and [γ-^{32}P]ATP (specific activity 3,300 Ci mmol^{-1}, NEN) as described elsewhere[48]. After purification on a Sephadex G-50 column, the DNA was cleaved with *Pst*I (MRC, Porton), and the labelled 1,291–1,299-base pair fragment isolated after electrophoresis through a 1.5% agarose gel and passed through a Chelex column. About 25 ng of probe (13,000 c.p.m.) were denatured by heating in 80% formamide for 15 min at 100 °C and mixed with 50 μg RNA. Hybridisation was carried out in 10 μl of 0.4 M NaCl, 0.04 M PIPES (*p*H 6.4), 0.001 M EDTA and 80% formamide, at 57 °C for 16 h under paraffin oil. The sample was diluted with 100 μl 0.25 M NaCl, 0.03 M sodium acetate (*p*H 4.5) and 0.002 M $ZnSO_4$ and digested with 50 units of S_1 nuclease (purified by the procedure of Wiegand *et al.*[49]). After $CHCl_3$ extraction to remove the paraffin oil, and precipitation with ethanol, the samples were dissolved in 5 μl 90% formamide, 0.05% bromphenol blue and 0.05% xylene cyanol FF, heated for 10 min at 100°C, and analysed by electrophoresis through a 0.1 × 28 × 40 cm 5% polyacrylamide gel (acrylamide : bis-acrylamide, 30 : 1) in 0.089 M Tris base, 0.089 M boric acid, 2.5 mM EDTA and 7 M urea. Autoradiography was carried out with use of an Ilford Fast Tungstate intensifier screen at −70 °C for 2–5 days. *A*, Unfractionated RNA samples. Lanes a–e, 0, 3, 1, 0.3 and 0.1 ng rabbit globin mRNA ($\alpha + \beta$). Lanes f, g, RNA from cell lines P1/S-1 and P1/S-2, which had been transformed only with a *Sal*I-cleaved TK-gene plasmid. Lanes h-k, RNA from cell lines P1/R-3, P1/R-4, P1/R-5 and M2/R-1, transformed with the rabbit β-globin DNA-containing hybrid linked to a TK-gene plasmid. Lane l, 50 ng mouse globin mRNA. Lanes M, M′, [5′-^{32}P]-labelled fragments of plasmid pBR322 as size markers[50]. *B*, Poly(A)$^+$ and poly(A)$^-$ fractions of RNA samples. RNA (50 μg) from cell line M2/R-1 or rabbit globin mRNA (3 ng) was passed through a 0.1 ml oligo(dT) cellulose (type 7, P-L Biochemicals) column in 0.2 M NaCl, 0.01 M EDTA, 0.1% SDS and 0.1 M Tris-HCl (*p*H 7.5)[51]. The column was washed with the same buffer and eluted with H_2O. The RNA in the flow-through and wash (poly(A)$^-$) and in the H_2O eluate (poly(A)$^+$) was precipitated with ethanol. Lanes a, b,

sequence[22] whereas mouse mRNA does not (ref. 23 and N.M., unpublished results), and therefore does not protect the labelled 5′-terminus of the probe. Figure 4*A* shows that as little as 0.1 ng rabbit β-globin mRNA (lane e) gave the expected 134-nucleotide fragment whereas 50 ng mouse globin mRNA (lane l) gave no such band. The RNA from transformed cell lines P1/R-3, P1/R-4, P1/R-5 and M2/R-1 (lanes h–k) yielded as major product the 134-nucleotide fragment. Thus, the majority of globin-specific transcripts are partly or entirely devoid of the large intron. Some weak bands corresponding to fragments of 750–1,200 nucleotides may be due to unprocessed or incompletely processed precursor molecules. RNA from cells transformed with only a thymidine kinase plasmid (lines P1/S-1 and P1/S-2, lanes f and g) gave no detectable bands. From the amount of cell RNA added to the assay (50 μg), the amount of RNA per cell (about 25 pg)[24] and the fact that the intensity of the 134-nucleotide band is similar to that given by 3 ng of total rabbit globin mRNA (about half of which is β-specific), we estimate that cell line M2/R-1 contains about 2,000 rabbit β-globin RNA molecules per cell. By the same criteria, P1/R-3, P1/R-4 and P1/R-5 contain about 600, 50 and 400 β-globin transcripts per cell. As relative band intensities were estimated visually, these values as well as those in Table 1 are only approximate. Altogether, 12 of 16 globin DNA-containing clones had β-globin-specific transcripts (see Table 1).

To determine whether the small intron was present, RNA was hybridised to RβG DNA which had been digested with *Bam*HI, labelled at the 5′-termini and cleaved again with *Bgl*II. After S_1 cleavage and denaturation, polyacrylamide gel electrophoresis revealed a 212-nucleotide fragment, in the case of both authentic rabbit β-globin mRNA and RNA from samples P1/R-3, P1/R-4, P1/R-5 and M2/R-1 (Fig. 5). This shows that the small intron was not present in the transcripts, because otherwise a 480-nucleotide fragment would have appeared.

The 5′-terminus of the β-globin-specific transcripts was mapped by the same procedure as before, using as probe a restriction fragment extending from the *Mbo*II cleavage site at position 129/128 to the *Pst*I site at position −95/−99, [5′-^{32}P]-labelled at the *Mbo*II-cleaved end. As shown in Fig. 6, natural rabbit β-globin mRNA gave rise to a labelled fragment 128 nucleotides long; RNA from the cell line M2/R-1 gave rise to a strong and P1/R-3 to a weaker band in the same position as the β-globin mRNA; in the case of RNA from cell lines P1/R-4 and P1/R-5, only very weak bands were discernible on the original films (but not on the photograph), perhaps because of the relatively low content of β-globin-specific RNA in these cell lines (Table 1). This experiment showed that the only detectable 5′-terminus of the β-globin transcripts mapped in the same position as that of authentic β-globin mRNA; however, we cannot exclude the possibility that a certain proportion of molecules may have a variety of 5′-termini which escaped detection because of a broad distribution of the resulting bands.

The presence of poly(A) on the β-globin-specific transcripts from transformed L cells was demonstrated by fractionating RNA from M2/R-1 on an oligo(dT) column and assaying separately the flow-through fraction [poly(A)$^-$] and the fraction retained at high salt [poly(A)$^+$], using as probe the *Pst*I–*Bgl*II (1,291/1,299) fragment 5′-labelled at the *Bgl*II terminus. About 80% of the β-globin transcript was detected in the poly(A)$^+$ fraction (Fig. 4B).

The mobility of the rabbit β-globin-specific RNA present in the cell line M2/R-1 was compared with that of natural rabbit globin mRNA using the 'Northern transfer' procedure of Alwine *et al.*[25]. The RNAs were subjected to electrophoresis on an agarose gel in denaturing conditions, transferred to diazobenzyloxymethyl paper and hybridised to ^{32}P-labelled rabbit β-globin cDNA[19]. As shown in Fig. 7, poly(A)$^+$ RNA derived from 150 μg of M2/R-1 RNA gave an autoradiographic band with the same mobility and the same intensity as about 8 ng of authentic rabbit globin mRNA; no band was found with the poly(A)$^+$ RNA from 150 μg of RNA from cells transformed

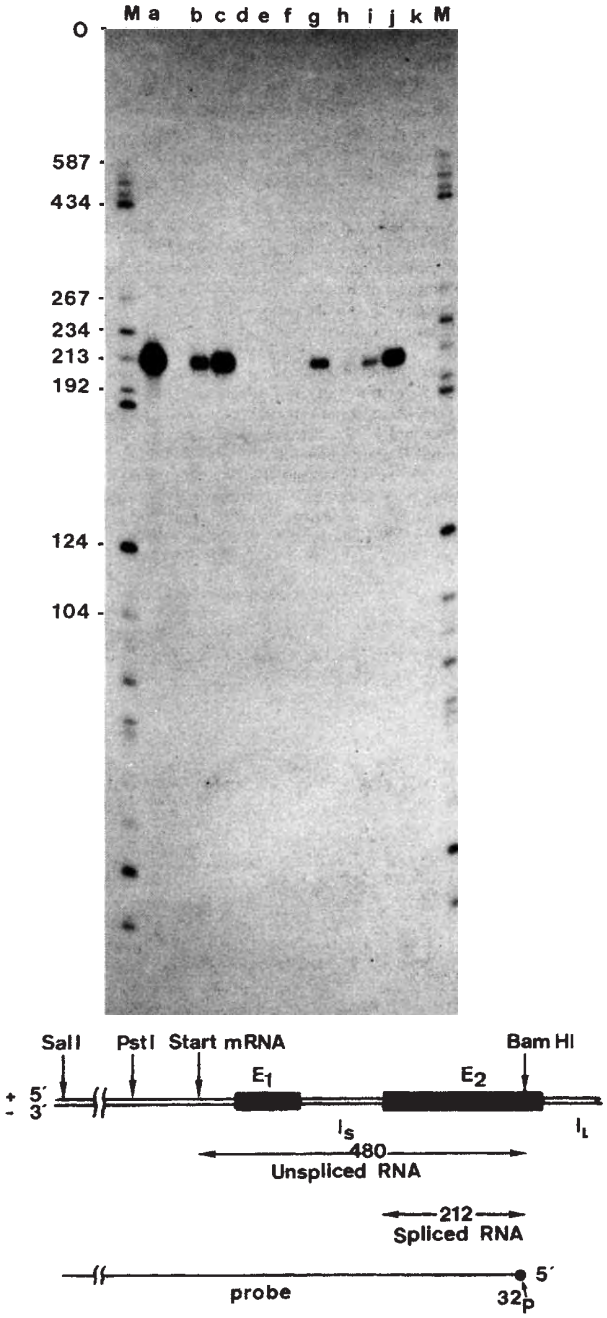

Fig. 5 Assay for the presence of the small intron in rabbit β-globin transcripts from transformed mouse cells. The RNA preparations are those described in Fig. 4 legend. The probe was prepared by digesting Z-pCRI/RchrβG-1 with *Bam*HI, labelling the 5′-terminus with ^{32}P-phosphate (specific activity 2,100 Ci mmole^{-1}) and cleaving with *Bgl*II. Lanes a–c, 3, 0.3 and 1.1 ng rabbit globin RNA (α + β). Lane d, 50 ng mouse globin RNA. Lanes e, f, RNA from cell lines P1/S-1 and P1/S-2, transformed only with a *Sal*I-cleaved TK-gene plasmid. Lanes g–j, RNA from cell lines P1/R-3, P1/R-4, P1/R-5 and M2/R-1, transformed with the rabbit β-globin DNA-containing hybrid linked to a TK-gene plasmid. Lane k, no RNA. M, Size marker: pBR322 digested with *Bsp*I and [5′-^{32}P]-labelled.

Finally, we determined whether transformed L cells produce mouse β-globin mRNA, in particular when they are synthesising rabbit β-globin mRNA. We again used the Weaver–Berk–Sharp assay, with the [5′-^{32}P]-labelled 123-nucleotide *Bsp–Bsp* (−) strand fragment of cloned mouse β-globin cDNA[26] as probe. This (−)strand fragment, which corresponds to the mouse β-globin mRNA segment extending from nucleotide 222 to 344

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