sequence, metamorphosed at its base in the greenschist or the amphibolite facies depending on its thickness and be invaded by sills and plugs of kilometre-sized mafic intrusions. The immediately underlying anomalous mantle ($V_p \simeq 7.5$ km s^{-1}) would be composed of hot peridotites and/or of partially serpentinized peridotites. The latter interpretation agrees with the one proposed by Hess[37], in which a lower oceanic crust is formed by serpentinized peridotites. The formation of this special crust would require conditions in which the creation of a normal oceanic crust would be impossible. Such conditions could occur when the transition from continental to oceanic lithosphere occurs in a trough filled with a thick pile of young sediments, a situation opposed to that of a starved rift[1]. The basaltic magma released by the underlying mantle diapir into the immature sediments does not meet conditions to create a volcanic cap below which a plutonic section can fractionate. On the contrary, as already proposed by Saunders *et al.*[6], the magma reacts with these sediments and is frozen in them as sills and plugs, thus contributing to the metamorphism of the sediment. Dolerite and gabbro sills material and metamorphosed sediments have been recovered by DSDP drilling in the Gaymas Basin of the Gulf of California[6] which, as mentioned above, seems to correspond to an appropriate environment.

It may be of interest to contrast the situation on the Arabian edge of the Red Sea and in the trough itself (Fig. 2). On the edge, the disruption of the continental crust with a little and old sedimentary cover leads directly to the creation of typically ophiolitic segments of oceanic crust, whereas in the trough where young and wet sediments have accumulated, the geophysical record does not show the existence of such oceanic crust but suggests a direct contact between sediments and serpentinized mantle.

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Heritable formation of pancreatic β-cell tumours in transgenic mice expressing recombinant insulin/simian virus 40 oncogenes

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Following the transfer into fertilized mouse eggs of recombinant genes composed of the upstream region of the rat insulin II gene linked to sequences coding for the large-T antigen of simian virus 40, large-T antigen is detected exclusively in the β-cells of the endocrine pancreas of transgenic mice. The α- and δ-cells normally found in the islets of Langerhans are rare and disordered. Well-vascularized β-cell tumours arise in mice harbouring and inheriting these hybrid oncogenes.

INSULIN, a polypeptide hormone involved in the control of carbohydrate metabolism, is synthesized and stored in the β-cells of the endocrine pancreas, from which it is released into the bloodstream in response to various signals. The endocrine pancreas is comprised of isolated islands or islets, which are dispersed throughout the larger mass of the exocrine pancreas; the islets consist of four major cell types, α, β, δ and PP, which synthesize the hormones glucagon, insulin, somatostatin and pancreatic polypeptide, respectively[1,2]. Rat and human insulin genes have been cloned and analysed[3-13]. There are two non-allelic rat insulin genes that differ in nucleotide sequence and in the number of introns; the rat insulin I and II genes have one and two introns, respectively[5,6]. The two genes share a region of considerable sequence homology in the 5′ flanking region, extending ~300 base pairs (bp) upstream of the point of transcriptional initiation[12]; the human gene is 75% homologous over the first 240 bp of this region[8]. Expression of hybrid genes using the 5′ flanking regions of both the human insulin and rat insulin I genes occurs when they are introduced transiently into cultured cells derived from an insulinoma[11].

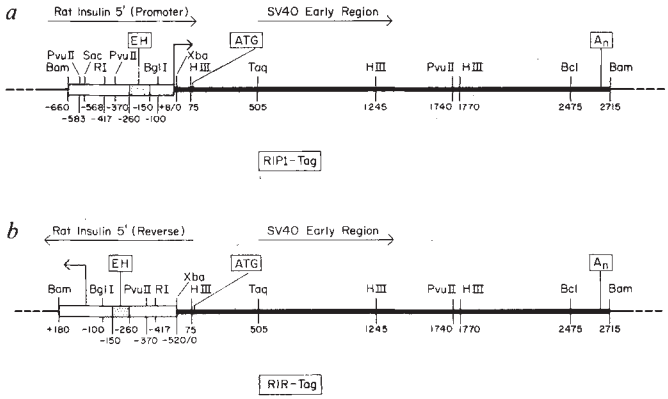

Fig. 1 Structure of recombinant insulin/large-T antigen genes. The plasmids pRIP1-Tag (*a*) and pRIR-Tag (*b*) consist of the coding information of the SV40 early region fused to sequences derived from the 5′ flanking region of the rat insulin II gene. Boxed regions denote insulin gene flanking DNA and the thick solid line refers to the SV40 early region. The cap site where insulin gene transcription initiates is indicated by an L-shaped arrow, and the associated transcriptional enhancer element[11] (EH) is shown (the insulin gene promoter, which lies between them, is not indicated). The points of SV40 large-T antigen translation initiation and transcription termination are indicated by boxed ATG and A_n respectively. The *BglI* site in SV40 was converted to an *XbaI* site using an oligonucleotide linker, and the *Xba/BglI* to *Bam* fragment comprising the SV40 early region[24] was linked to two different orientations of the insulin gene flanking region. This fragment of the SV40 early region includes the early mRNA cap sites, but lacks all known components of the SV40 early promoter. For *RIP1-Tag*, an *Xba* linker was inserted into the *DdeI* site at +8 bp relative to the point of initiation of insulin gene transcription, and the *Bam* to *Dde/Xba* fragment from −660 to +8 was combined with the *Xba/Bam* fragment of the SV40 early region and inserted as a *Bam* linear into a derivative of pBR322 that lacks the R1 site, and includes a *lacUV5* promoter in place of the R1 to *Bam* fragment of pBR. For the plasmid pRIR-Tag, which carries the insulin flanking region in the opposite orientation, a *Dde* site at −520 was converted to an *Xba* site, and the *Bam/Xba* fragment extending from +180 nucleotides past the cap to −520 bp upstream was combined with the structural gene for large-T antigen and inserted into the same plasmid vector. The two hybrid genes were similarly prepared for microinjection, except that for *RIR-Tag*, the *Bam* insert was first purified from the plasmid vector by gel electrophoresis and electroelution, followed by passage over a DEAE-Sephacel column and pRIP1-Tag was linearized using *SalI*. The DNAs were extracted with phenol, chloroform, precipitated in 70% ethanol, resuspended in 10 mM NaCl, 5 mM Tris *p*H 7.4, 0.1 mM EDTA and passed through a 1-ml spin column of Sepharose CL6B, after which the DNA was diluted to concentrations of ~75 copies per pl for pRIP1-Tag/*Sal* or ~20 copies per pl for the *RIR-Tag/Bam* insert. The DNAs were injected into fertilized one-cell embryos essentially as described[14,16], except that the injected embryos were cultured overnight to the two-cell stage before being re-implanted into the oviducts of pseudopregnant females. The F_2 embryos were derived from matings of B6D2F1 (C57BL/6J × DBA/2J) males and females, obtained from the Jackson laboratory.

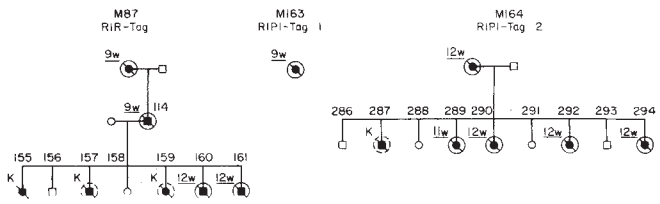

Fig. 2 An early pedigree of insulin/large-T antigen transgenic mice. A partial pedigree analysis is presented, to examine the heritability of the phenotype of premature death and islet hyperplasia. Females are indicated by open circles, males by open squares and transgenic mice by solid circles and squares. The circled slash denotes premature death, with the age at death shown in weeks (w). A slash alone with a superscript K indicates the animal was killed and the dotted circle around a killed mouse denotes evidence of islet hyperplasia following histopathology. (M155 was killed but not examined for hyperplasia.) Each original transgenic mouse is assigned a genotype consisting of the name of the gene that was injected and acquired followed by a number which distinguishes it from the other independent mice carrying the same gene. Therefore, progeny of a particular mouse can be readily identified as such (for example, all mice of genotype *RIP1-Tag2* are progeny of the original transgenic mouse 164).

Fig. 3 (Opposite) Histology and pathology of normal and insulin/T-antigen transgenic mice pancreas. *A*, Cresol violet-stained sections of a normal pancreas and a pancreas derived from a transgenic mouse bearing solid tumours. *a*, Normal (non-transgenic) pancreas with a single islet in the centre and an adjacent blood vessel (×50). *b*, Part of a solid tumour (one of those pictured below) near two normal-sized islets (at the top) in M384 of the *RIP1-Tag2* line (×50). *c*, A normal-sized islet from a non-transgenic mouse (×200). *d*, A normal-sized transgenic islet (from *b*) (×200). In all cases, exocrine cells show purple cytoplasmic staining with somewhat deeper-coloured nuclei, whereas endocrine cell nuclei stain dark violet with minimal cytoplasmic staining. Note the denser packing of nuclei in all sizes of islets derived from the transgenic mouse relative to the normal mouse islet. Compare the normal islet in *a* with the two small islets near the top of *b* and the higher magnifications in *c* and *d*. The extent of cytoplasmic staining observed in the exocrine tissue varies with the length of time the sections were stored before treatment with cresol violet. *B*, Comparison of a whole pancreas from a normal mouse (*a*) with that of M384 (*b*), showing several vascularized solid tumours in the transgenic pancreas (×25).

Methods. Sections (14 μm) were taken on a cryostat from pancreas flash frozen in liquid nitrogen, stored at −70 °C and then mounted in tissue Tek OCT embedding medium and sectioned at −20 °C on to gelatinized microscope slides. The sections were fixed for 10 min in 4% paraformaldehyde in phosphate-buffered saline (PBS), washed in PBS and air dried. Sections were then rehydrated in PBS, treated in filtered cresol violet for 2 min, washed for 5 min in two changes of 70% ethanol, then washed twice for 2 min each in 90% ethanol, 100% ethanol and xylene and then mounted under coverslips in Gurr Fluromount. The cresol violet solution consists of 0.25% (w/v) thianin that had been dissolved by heating in 200 mM hydroxyacetate plus 26 mM NaOH and stored at 50 °C.

Here I seek to address aspects of insulin gene expression by the introduction of recombinant insulin genes into the mouse germ line, using microinjection of DNA into fertilized mouse embryos. This technique has been used previously to examine the control and consequences of expression of a number of genes[14-23], including two oncogenes. The complete simian virus 40 (SV40) early region, including the viral transcriptional enhancer and promoter, is not expressed at detectable levels in normal tissues of transgenic mice harbouring it, but choroid plexus tumours heritably arise, and these tumours produce high levels of large-T antigen[19]. The cellular proto-oncogene c-*myc*, when linked to the long terminal repeat of mouse mammary tumour virus, is expressed in a number of tissues, including mammary cells, and two lines of mice heritably develop breast tumours[20].

The recombinant oncogenes used here consist of regulatory information associated with the rat insulin II gene linked to protein coding information for the oncogene SV40 large-T antigen (Tag). The rationale for using such a hybrid is threefold: (1) to examine the ability of the insulin sequences to mediate correct tissue- and cell-type specific expression in the β-cells of the endocrine pancreas, using a readily identified and distinguishable marker protein (a viral antigen); (2) to examine the consequences to the organism of tissue-specific expression of an oncogene, in particular the possibility that SV40 large-T antigen might induce β-cell proliferation and consequent oncogenesis; and (3) to assess the prospects that oncogenes can be used to facilitate the establishment of stable cell lines from rare cell types specifically expressing such fusion genes. This report describes analyses pertaining to the first two of these questions.

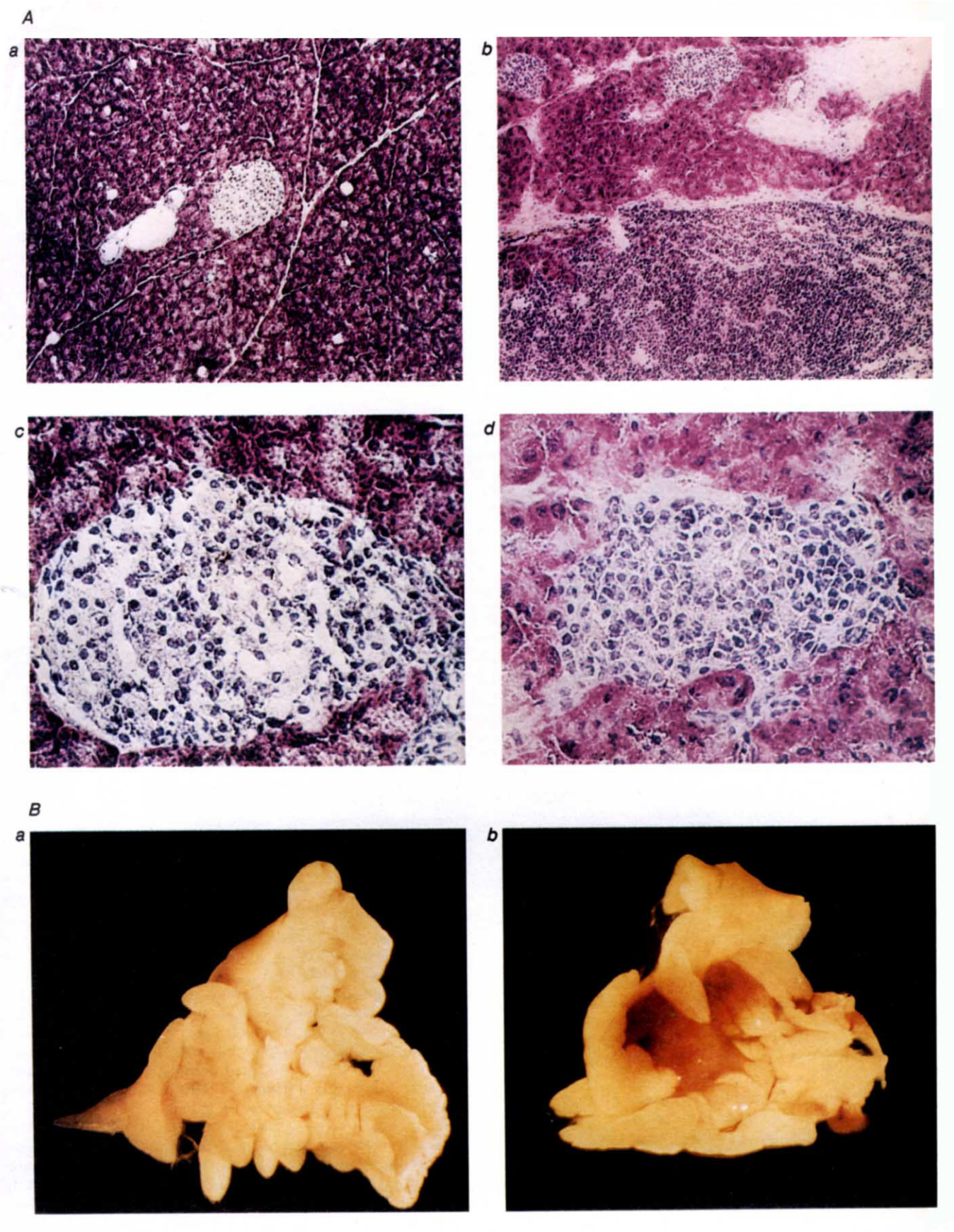

Fig. 3

Hybrid gene constructs

Two hybrid genes were constructed to combine DNA 5′ to the coding sequences of rat insulin II gene with the large-T antigen structural information. This 5′ flanking region includes the insulin gene promoter as well as a transcriptional enhancer element that stimulates initiation of transcription in cultured insulinoma cells but not in fibroblasts[11]. The first configuration, denoted *RIP1* (rat insulin promoter 1) carries the insulin control region aligned to promote transcription of the large-T antigen gene (Fig. 1). The two genes were linked together in regions corresponding to the 5′ untranslated portion of each messenger RNA. The resulting hybrid gene, *RIP1-Tag*, thus comprises a complete transcription unit with 660 bp of sequence located 5′ to the point of transcriptional initiation of the insulin gene linked to protein coding and termination information from the early region of SV40.

The second configuration, denoted *RIR-Tag* (rat insulin reverse), includes 520 bp of DNA 5′ to the insulin gene and the first 180 bp of the transcribed portion of the gene. In this hybrid, the promoter element for insulin gene transcription is inverted with respect to the coding information for SV40 large-T antigen, and, therefore, does not comprise a fusion in the 5′ untranslated regions of the two genes, but rather includes an inverted promoter and enhancer region linked to the *Tag* structural gene, as shown in Fig. 1. Both hybrid genes lack the SV40 early promoter and transcriptional enhancer element, and each includes protein coding information for SV40 small-T antigen, as well as for large-T, because the two proteins are derived from the primary transcript of the SV40 early region by differential splicing of overlapping introns[24].

The two fusion genes were injected into fertilized one-cell mouse embryos, which were then inserted into the oviducts of pseudopregnant female mice and allowed to develop. Four transgenic mice were produced from the *RIP1-Tag* injections and one from the *RIR-Tag* injections. The apparent heterozygous copy number (per diploid genome) of the acquired DNA ranged from ~1 for mouse 87, which carries *RIR-Tag*, to ~5 for mouse 163, which carries and is genotyped as *RIP1-Tag1*, and 5 for mouse 164 (*RIP1-Tag2*). Two of the founder mice (165 and 168) were mosaic for the acquired transgene, using the criteria of infrequent transmission to progeny and lower apparent copy number in the founder than in its progeny. The non-mosaic progeny of M165 (*RIP1-Tag3*) have copy numbers of ~25, whereas those of M168 (*RIP1-Tag4*) have ~12 copies per diploid genome. In each case of multiple copies, genetic and biochemical analysis indicates that the genes are heterozygous and integrated in a single location (data not shown).

Phenotype of transgenic mice

During the initial breeding analysis of the insulin large-T antigen transgenic mice, a phenotype of premature death emerged. The three original non-mosaic mice died at 9–12 weeks of age. Two of these transmitted the transgene to offspring before dying, whereas the third (M163) did not. Surprisingly, this phenotype appeared with the *RIR-Tag* gene, which has the insulin promoter inverted with respect to the large-T antigen coding region, as well as with the *RIP1-Tag* gene, in which the insulin promoter is aligned to transcribe the large-T antigen gene.

The phenotype of premature death proved to be heritable and co-segregated with the acquired hybrid gene; the partial pedigree in Fig. 2 illustrates this observation. The penetrance is virtually complete in the early generations. The analysis that follows deals primarily with the progeny of the two original non-mosaic mice that transmitted—M164 (*RIP1-Tag2*) and M87 (*RIR-Tag*). Progeny of each were killed and subjected to pathological and biochemical analysis. The only obvious abnormal pathology consisted of hyperplasia of the islets of Langerhans, determined following standard histochemical staining of tissue sections. Solid tumours were observed in a few of these mice. Figure 3*a* shows representative pancreas sections of a normal mouse and of a transgenic mouse (M384, *RIP1-Tag2*) that carried visible tumours; both were ~12 weeks old. Sections were stained with cresol violet, which distinguishes the endocrine islets from the surrounding exocrine tissue. Sections from M384 showed normal-sized islets, enlarged (hyperplastic) islets and solid tumours. All stained with similar characteristics, and were identifiable as islet-like regions by comparison with normal islets. Note, however, that all islet-like areas in the transgenic mouse appear more densely packed with cells than do the normal mouse islets, demonstrated by comparison of Fig. 3*A c* and *d*.

Analysis of the mice listed in Fig. 2 shows that all transgenic mice in these two lineages have the phenotype of islet hyperplasia (if killed) and/or premature death. Before their sudden death, the mice seem normal in appearance and activity. The pedigree stops at the generation during which the mice were placed on a high sugar diet to counteract indications that they were hypoglycaemic, which is a probable consequence of islet hyperplasia, and a possible cause of sudden premature death. Subsequent generations, maintained on this diet, now live somewhat longer and heritably develop solid tumours of the pancreas between 10 and 20 weeks of age. An example of a pancreas containing several tumours is shown in Fig. 3*B*. These tumours eventually comprise a significant fraction of the mass of the pancreas and are highly vascularized. Individual tumours can reach a size of 5 mm in diameter, with up to five or six separate tumours visible in a pancreas. There is no indication that the tumours metastasize other organs.

Cell-type specificity

To evaluate the observed hyperplasia of the islets of Langerhans, I examined normal and transgenic mouse pancreas for expression of both SV40 large-T antigen and polypeptide hormones characteristic for the major cell types. Thin sections were prepared from fresh frozen pancreas obtained from two transgenic mice (M384, *RIP1-Tag2*, and M306, *RIR-Tag*) carrying visible pancreatic tumours as well as from a normal mouse. The sections were analysed by immunostaining using antisera to glucagon, insulin and somatostatin and each visualized with rhodamine-conjugated second antibody to identify the α-, β- and δ-cell types of the islets. Large-T antigen was identified using both a polyclonal antiserum and monoclonal antibodies, each visualized by horseradish peroxidase-conjugated second antibody. Figure 4*a* shows representative immunostains of normal (non-transgenic) islets for the presence of insulin, large-T antigen, glucagon and somatostatin. The islets are comprised predominantly of α- and β-cells with fewer δ-cells; no cells react with antibodies to large-T antigen. Controls using second antibodies alone show no specific staining (not shown).

The islet tumours (Fig. 4*b*, *c*) in contrast, seem to consist of solely of insulin-producing cells, identifiable as β-cells by immuno- and histochemical staining. Glucagon- and somatostatin-producing cells are rare if not completely absent from the tumours. Characteristic localization of large-T antigen to the nucleus is observed in the islet tumour cells; it is clearly absent from adjacent exocrine cells. Evidence from both polyclonal serum (shown) and monoclonal antibodies (not shown) indicates the same pattern of large-T antigen expression. By these criteria, the islet tumours are essentially pure populations of proliferating β-cells. Occasional clusters of α-cells, perhaps remnants of an islet, can be detected on the edges of a tumour. Thus, both hybrid oncogenes specifically induce proliferation of the β-cells of the islets of Langerhans, eventually resulting in the appearance of pure β-cell tumours. In comparison, naturally occurring insulinomas are characteristically a mixed population of both β- and δ-cells, producing insulin and somatostatin, respectively[25,26].

Tissue-specific expression

The ability of the insulin control region to mediate tissue-specific expression of the SV40 large-T antigen following integration of the hybrid genes into the mouse germ line was assessed by

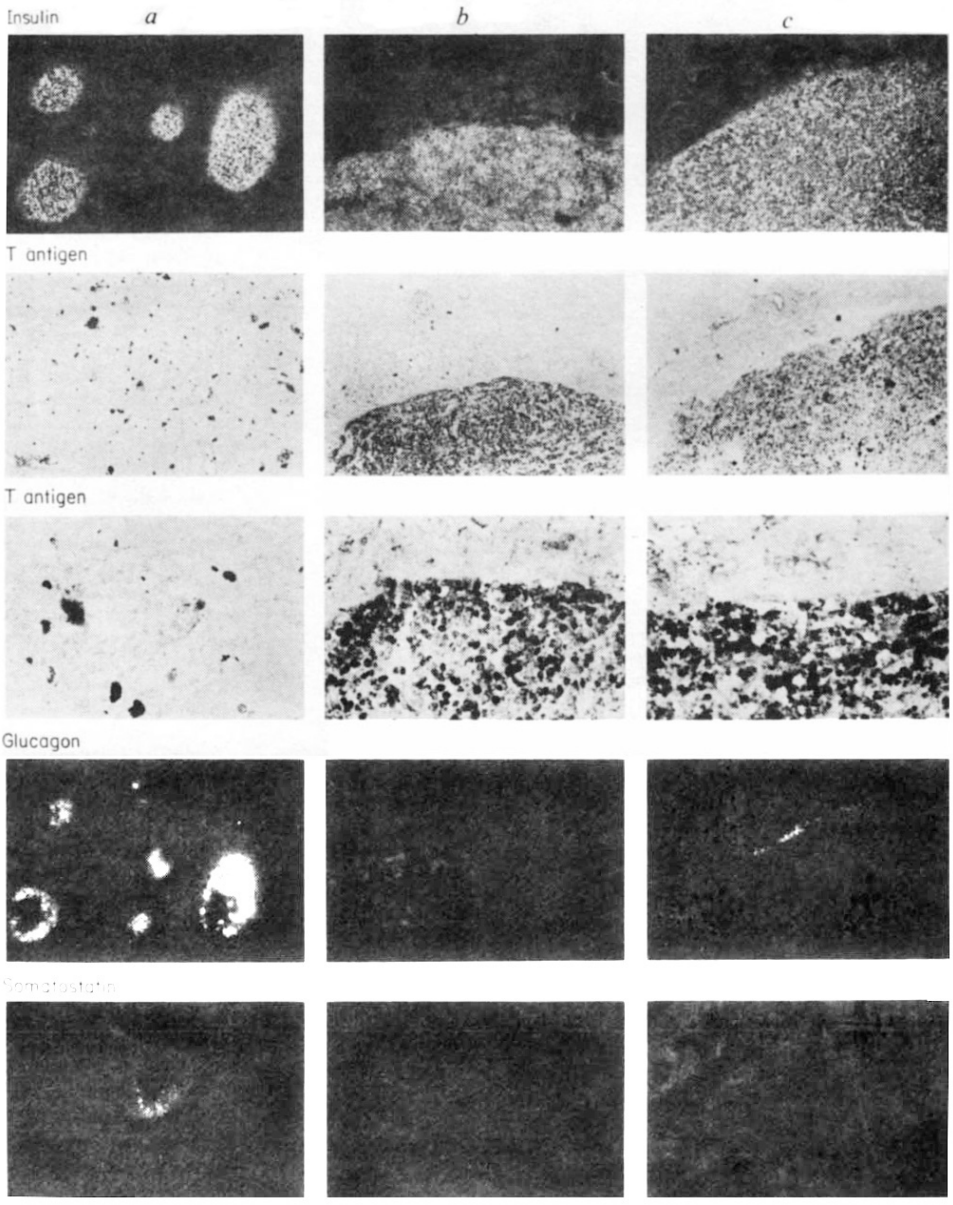

Fig. 4 Analysis of islet cell hormone and large-T antigen expression in a normal mouse and two tumour-bearing transgenic mice. Each column shows an analysis of one mouse: *a*, normal (non-transgenic); *b*, 384 from the *RIP1-Tag2* line; *c*, 306 from the *RIR-Tag* line. In each case, near (but not necessarily adjacent) thin sections were analysed for the presence of the specified antigen by using an antiserum to that antigen followed by a conjugated second antibody to visualize the first. The first row examines insulin, the second and third rows large-T antigen, the fourth row glucagon and the fifth row somatostatin. Magnification is ×50 except for the third row, which is ×200. The use of second antibodies alone gives no specific staining. In *a*, insulin and glucagon plates are of the same cluster of islets, whereas the somatostatin and large-T antigen are of other islets, because that cluster was not present in those sections. The immunostains for the hormones were visualized with rhodamine-conjugated second antibody and epifluroscent illumination, whereas the immunostain for large-T antigen used peroxidase-conjugated second antibody, and was viewed under bright-field illumination. *b*, *c*, Orientation of all the plates is the same. The islet tumour is on the bottom, with adjacent exocrine tissue above it. Column *b* is aligned so the tumour is a centred arc, whereas the plates in *c* have the tumour ascending to the right. The glucagon and somatostatin plates of *b* and *c* are of sections near those used for insulin and large-T antigen; the outline of the exocrine/tumour boundary can be seen in each. The ×200 plates of large-T antigen also show an exocrine/tumour boundary, in which peroxidase staining occurs in nuclei in the tumour tissue but not in the exocrine tissue.

Methods. 14-μm sections were taken as described in Fig. 3 legend, except that for the large-T antigen immunostaining the sections were not fixed, but rather air dried on gelatinized slides. The hormone immunostains were performed on paraformaldehyde-fixed sections as in Fig. 3. Sections were rehydrated in Net-Gel (150 mM NaCl, 5 mM EDTA, 50 mM Tris *p*H 7.4, 0.25% gelatin, 0.02% NaN_3, 0.05% NP40), then treated for 30 min in blocking solution (45% Net-Gel, 45% Dulbecco's modified Eagle's medium, 10% fetal bovine serum, 1% bovine serum albumin (BSA), 0.5% NP40). The sections were then incubated overnight at room temperature in the first antibody, in a binding solution (blocking solution without BSA), washed several times for 30 min in Net-Gel, incubated with second antibody in binding solution for 3 h at room temperature, and again washed in several changes Net-Gel. The sections treated with rhodamine-conjugated second antibody were taken through graded alcohol into xylene and mounted under coverslips in Gurr Fluromount+2% diazabicyclooctane. The large-T antigen sections were visualized with horseradish peroxidase-conjugated second antibody, which was developed by treatment for 10 min in 0.25 mg ml^{-1} diaminobenzidine, 3 mg ml^{-1} nickel sulphate, 0.003% H_2O_2. The slides were then washed several times in Net-Gel and mounted. The antisera, sources and dilutions are as follows: guinea pig anti-porcine insulin (gift of R. Santerre) 1:5,000; rabbit anti-large-T antigen (a gift of D. Lane) 1:2,000; rabbit anti-human glucagon, Dako A565, 1:1,000; rabbit anti-human somatostatin, Dako A566, 1:1,000; horseradish peroxidase-conjugated swine anti-rabbit IgG, Dako P217, 1:200; rhodamine-conjugated goat anti-guinea pig IgG, Capel 2207-0081, 1:200; rhodamine-conjugated sheep anti-rabbit IgG, Capel 2212-0084, 1:200.

protein blotting analyses, using monoclonal antibodies that specifically recognize large-T antigen. Figure 5*a* presents a comparison of pancreas from several mice, including one normal (non-transgenic) mouse, several insulin/large-T antigen transgenic mice, and one carrying a collagen/large-T antigen fusion gene (M410) which therefore lacks the insulin gene sequences (D.H., unpublished results). M287 and M289 are siblings in the *RIP1-Tag2* lineage (see Fig. 2); M287 had large solid tumours whereas M289 did not, which illustrates the difference in levels of expression observed in mice of the same age and genotype with and without solid tumours. M277 contains immunoprecipitable large-T antigen, demonstrating that the *RIP1-Tag3* lineage derived from the founder M165 also expresses the fusion gene. Large-T antigen is not detectable in a normal (F_1) pancreas or in the transgenic mouse (M410) that lacks the insulin gene regulatory region. The *RIR-Tag* transgenic mouse 155 does not show expression in the blot demonstrated here, but other analyses show a low level of large-T antigen. The *RIP1-Tag4* lineage, exemplified here by M274, does not show detectable expression of large-T antigen by protein blotting. However, pancreatic tumours occasionally arise in this line, which indicates that the *RIP1-Tag4* insertion can express.

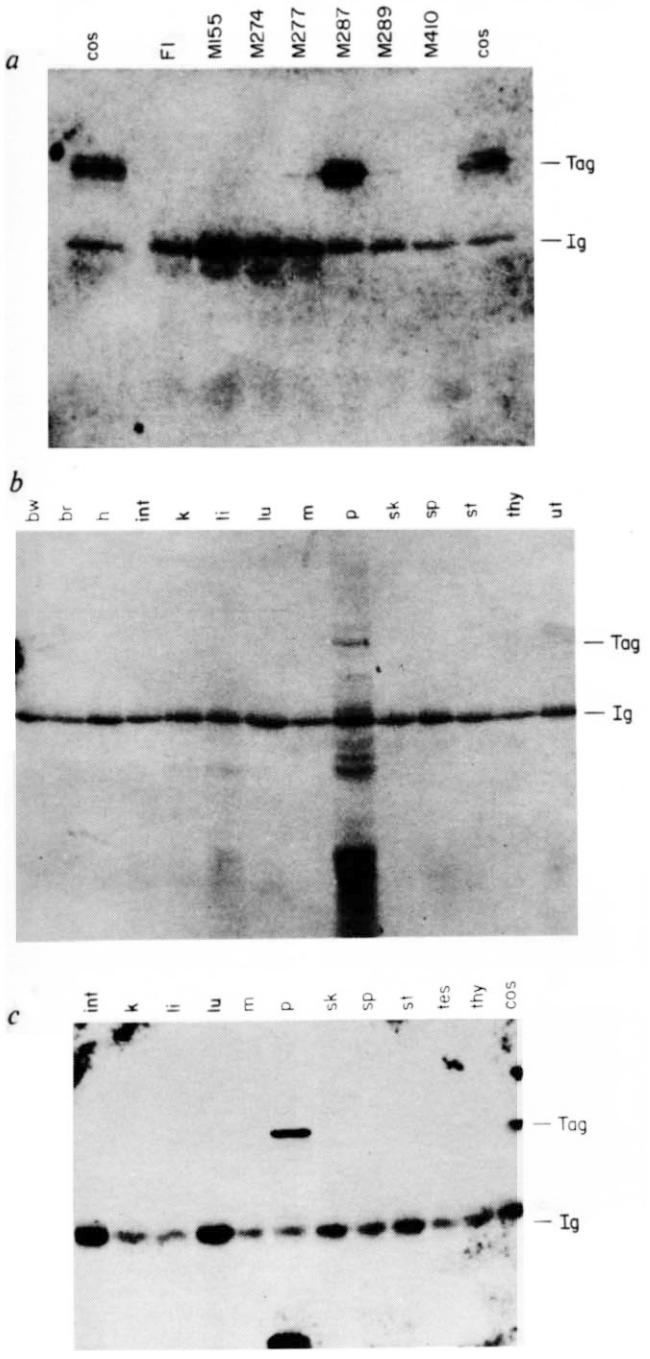

Fig. 5 Tissue specificity of transgene expression. The presence of SV40 large-T antigen in tissues was evaluated by protein blotting[39,40], in which tissue homogenates were immunoprecipitated with a monoclonal antibody (PAb 419) (ref. 41) to T antigen, fractionated on a 10% SDS-polyacrylamide gel, transferred to nitrocellulose by electroblotting and visualized with a second radiolabelled monoclonal antibody (PAb 416), which recognizes a different epitope on the protein[41]. *a*, Pancreas from several mice are compared. F_1 is a non-transgenic mouse, M155 an *RIR-Tag* transgenic, M274 is *RIP1-Tag4*, M277 is *RIP1-Tag3*, M287 and M289 are from siblings from the *RIP1-Tag2* lineage, with M287 having visible tumours and M289 only hyperplastic islets, and M410 is a mouse harbouring a type I collagen/SV40 large-T antigen fusion gene. To provide a control of authentic large-T antigen, total protein from a lysate of the SV40-transformed monkey cell line cos A2 (ref. 42) was immunoprecipipitated and similarly treated, except that only 5% as much protein was used compared with the amount of pancreas protein. *b*, *c*, Tissue specificity of large-T antigen expression in the *RIP1-Tag2* (*c*) and *RIP1-Tag3* (*b*) lineages. Equal concentrations of each tissue were examined: bw, body wall; br, brain; h, heart; int, intestine; k, kidney; li, liver; lu, lung; m, muscle, p, pancreas; sk, skin, st, stomach; tes, testes; thy; thymus; ut, uterus. A separate analysis indicates that no large-T antigen can be detected in body wall, brain or heart of M287 (not shown). The radiolabelled antibody PAb416 sticks to the vast excess of immunoglobulin protein used in the immunoprecipitation, thus generating a band of relative molecular mass 55,000 in all samples. This band is further identifiable as IgG using either radiolabelled protein A or rabbit anti-mouse IgG.

Methods. Tissues were flash frozen in liquid nitrogen, stored at −70 °C, and then homogenized in 50 mM Tris *p*H 7.2, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM dithiothreitol, 1% NP40, 100 μg ml^{-1} DNase I, 50 μg ml^{-1} RNase A, 75 μg ml^{-1} phenylmethylsulphonyl fluoride. Protein concentrations were determined using the Bio-Rad protein assay reagent. 500 μg of tissue protein or 25 μg of cos cell protein was incubated overnight at 4 °C with 100 μl of tissue culture supernatant containing the monoclonal antibody PAb419, and then precipitated with 100 μl of 3% protein A–Sepharose (Pharmacia) for several hours. The immunoprecipitate was fractionated on a 10% SDS–polyacrylamide gel, transferred to nitrocellulose by electroblotting overnight, incubated first in a blocking solution (Fig. 4) for 12 h and then in a binding solution (Fig. 4) containing 10^5 c.p.m. ml^{-1} of PAb416, which had been radiolabelled with ^{125}I using the chloramine-T method. The blots were washed in Net-Gel and exposed with intensifying screens onto Kodak XAR film.

The tissue specificity of fusion gene expression was evaluated further by comparing a number of different tissues from two mice (Fig. 5*b*, *c*), progeny of the two founders M164 and M165. Both show tissue-specific expression of SV40 large-T antigen, as only pancreas is producing immunoprecipitable protein that co-migrates with authentic large-T antigen. Some degradation of the antigen in pancreatic tissue is observed routinely; it is not clear whether this represents a normal situation in the β-cells or is simply a consequence of the protein isolation procedure. Analysis of a mouse from the *RIR-Tag* lineage also shows expression only in the pancreas, as does a similar examination of the tissues of mouse 163 (*RIP1-Tag1*) (not shown). In a normal pancreas, insulin-producing β-cells comprise only ~1-2% of the total cell mass[1,2]. Therefore, a lower limit can be established for inappropriate expression: either <~1% of the cells in another tissue could be expressing at levels comparable with those in β-cells, or all the cells in an inappropriate tissue could be expressing at levels of <1% of those observed in the islet cells. Within these limits, the expression of both insulin/large-T antigen hybrid genes is tissue specific to the pancreas. Taken together with the immunohistochemical analysis, the transferred insulin sequences are specifying correct expression in the β-cells of the endocrine pancreas.

Penetrance and the onset of oncogenesis

The relationship between large-T antigen expression and the development of β-cell tumours bears on possible mechanisms for oncogenesis. The two previous examples of oncogene expression in transgenic mice give contrasting results[27]. An apparently dormant SV40 early region is activated to express at high levels, and frequently amplified, in the development of choroid plexus tumours[19]. In contrast, a murine mammary tumour virus *myc* fusion gene is expressed in all mammary glands and in several other tissues before oncogenesis, which occurred in only 1 of the 10 mammary glands, implying that a secondary event is required to elicit tumour development[20]. In the insulin/large-T antigen transgenic mice examined to date, a similar pattern emerges. No more than 4 or 5 of the ~100 islets in a pancreas develop into solid β-cell tumours, which are highly vascularized and readily distinguishable from normal pancreatic tissue (Fig. 3*B*). Yet most islets show hyperplasia and all islets examined have increased β-cell density. Furthermore, the β-cells seem to be expressing the hybrid genes well before the development of hyperplasia and tumours, as is demonstrated in an examination of the pancreas from an 8-week-old mouse (M633) in the *RIP1-Tag2* lineage. This mouse exhibited normal pathology and histology, in particular showing no hyperplasia or visible tumours in the pancreas. Thin sections were prepared from the pancreas and stained with antibodies

for insulin and large-T antigen; a representative analysis of a pair of islets is shown in Fig. 6. Large-T antigen is expressing in both of these islets, as well as in all other islets examined in this pancreas. Thus, large-T antigen is expressing in the β-cells of a young mouse before emergence of obvious hyperplasia and solid tumours.

The interpretation of these observations is that the hybrid insulin/large-T antigen genes are expressed in all β-cells, thereby inducing their prolifieration, which leads to initial hyperplasia followed by oncogenic transformation. The development of solid tumours occurs in only a few of the islets, which indicates that additional events are probably necessary to mediate the rapid proliferation and extensive vascularization characteristic of the β-cell tumours. It will be of interest to distinguish between two possible classes of transformation: (1) an islet-specific change which affects most of the β-cells in an individual islet; and (2) a rare cell alteration that then produces a clonal tumour arising from that one altered cell.

Cell–cell interaction

Examination of the distribution of cell types in islets of these transgenic mice shows that islets of every size class (small, normal, enlarged, tumour) are densely packed with β-cells. Furthermore, the α- and δ-cells are disordered and rare. Comparison of the glucagon immunostains of normal islets (Fig. 1) and islets of the young transgenic mouse 633 (Fig. 6) shows that the characteristic peripheral distribution of α-cells has been disrupted and that their total number is reduced. Similarly, the normal polar organization of δ-cells is disordered and the δ-cell number severely retarded. This applies to every islet examined in this pancreas, in analyses that included sections taken to include different anatomical regions. These results suggest that during biogenesis of the islets, the α- and δ-cells are either sterically excluded or specifically suppressed by the altered β-cells.

Conclusions

The results presented here demonstrate that DNA within 520 bp upstream of the rat insulin II gene is sufficient to mediate qualitatively correct tissue- and cell-type-specific expression of hybrid insulin/SV40 large-T antigen genes. Protein blotting analysis of four mice harbouring independent insertions of such genes demonstrates expression only in the pancreas among the major tissues examined. The *in situ* analyses of thin sections from the pancreas demonstrates that this expression is cell-type specific, as large-T antigen is only detected in the insulin-producing β-cells of the islets of Langerhans.

The specification of tissue- and cell-type expression by the insulin gene 5′ flanking region occurs in both possible orientations when it is located upstream of the large-T antigen structural gene. Both tissue specificity and bidirectionality are qualities previously associated with transcriptional enhancer elements using transfection of cultured cells[28,29]. It is not clear whether this flanking region carries an additional real or cryptic promoter element oriented away from the insulin gene, or whether non-specific initiation of transcription is occurring in the *RIR-Tag* hybrid gene. RNA analyses will be necessary to address these possibilities.

There are three phases to the phenotype elicited by these hybrid oncogenes: the development of disordered islets, the subsequent enlargement of those islets into obvious hyperplasia and the formation of solid tumours. In young mice, the islets are of normal size, but densely packed with β-cells. The organization of both α- and δ-cells is disrupted and numbers of each severely reduced, δ-cells being particularly rare. These results suggest that islet cells interact to maintain a proper balance, and the transgenic β-cells effectively reduce α- and δ-cell number and organization by retarding their normal cell development. This possibility motivates a similar examination of the distribution of PP cells in the islets and islet tumours of these mice, because PP cells, like δ-cells, are often found at significant levels in naturally occurring insulinomas.

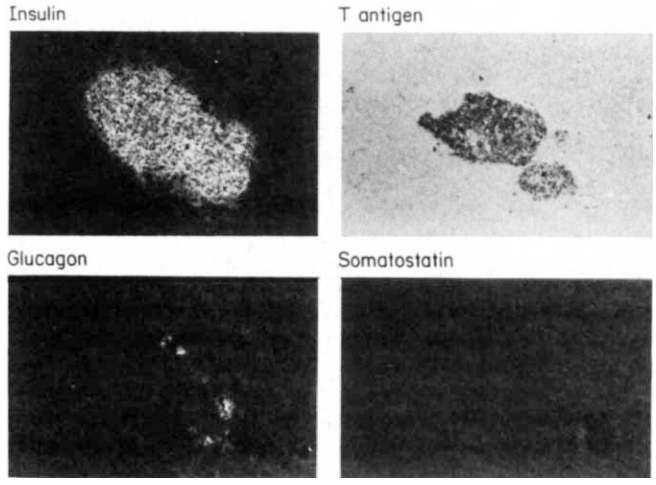

Fig. 6 Immunohistology of islets in the pancreas of a young transgenic mouse. Mouse 633, from the *RIP1-Tag2* lineage, was killed at 8 weeks of age and its pancreas flash frozen in liquid nitrogen, sectioned in a cryostat and analysed for the expression of islet cell hormones and large-T antigen as described in Fig. 4. legend. The two islets shown are representative of all islets in these and other thin sections from this pancreas. The sections used for insulin on large-T antigen are near but not adjacent and the islet sizes have changed somewhat across that distance (×50).

The prospect of disruption in islet-cell interactions is intriguing in view of observations that each of the three hormones influences secretion of the other two by their respective cell types. Somatostatin inhibits the release of both insulin and glucagon, insulin inhibits the release of glucagon and somatostatin, whereas glucagon stimulates secretion of insulin and somatostatin (see, for example, ref. 30). The organization of the endocrine pancreas into separate islands has led to suggestions that the islet cells arise from a common stem cell[31,32]. The character of the alterations in islet composition produced by the insulin/large-T fusion gene should allow more detailed examination of islet biogenesis. If islet cell types are interacting through growth factors, the results presented here suggest that they are probably suppressors of cell growth rather than inducers. Insulin itself is one candidate for such a suppressive growth factor.

The dense packing and disorganization of islets is followed by their expansion (hyperplasia), which seems to be a direct consequence of the expression of large-T antigen. Preliminary analysis indicates that serum insulin levels are elevated (two- to ten-fold), but not in proportion to the increased number of insulin-producing cells (>100-fold), which implies that either normal regulation of insulin secretion remains in effect, or that the β-cell structure, organization and/or vascularization is inappropriate for effective secretion. Isolation of β-cell lines from these transgenic mice should be facilitated by the ability of large-T antigen to establish (or immortalize) primary cells to growth in culture, with large-T antigen synthesis here maintained by the co-expression of the insulin and insulin/large-T antigen genes in β-cells. The availability of such β-cell lines will allow more detailed examination of the characteristics of altered β-cells. We may now examine how islet disorganization is correlated with changes in glucagon, somatostatin and insulin serum levels over a detailed timescale. It seems likely that the mice described in this study suffer from serious alterations in regulation of carbohydrate metabolism and that the explanation of their premature death does not simply result from excessive insulin in their serum, but also involves other factors.

Solid β- cell tumours eventually develop from a few of the hyperplastic islets. Tumour development occurs between 10 and 20 weeks of age, is heritable and has occurred in all progeny of the two lines (*RIP1-Tag2* and *RIR-Tag*) in which mice have lived to this age, and is occurring now in the *RIP1-Tag3* line. The solid tumours are frequent in the sense they arise in every

mouse harbouring these genes, although they occur only in a fraction of the islets. The characteristics of tumour formation suggest that synthesis of large-T antigen is necessary but insufficient to produce that condition in every islet. Large-T antigen is of a class of oncogenes that can both immortalize primary cells and release (or transform) cultured cells from their normal growth controls, such as contact inhibition and dependence on serum growth factors[24]. Yet, *in vivo* SV40 virus will produce tumours in mice only after a long latent period; oncogenesis seems to be related to the efficacy of the immune response and the presence of large-T antigen on the cell surface as well as in the nucleus[33-35]. There is no evidence of an immune response to hyperplastic islets, nor of large-T antigen on the surface of β-cells; it remains to be established whether the frequency of solid tumour formation is associated with an escape from immune surveillance. A similar hybrid gene, composed of the 5′ flanking region of the rat elastase gene linked to the coding information for SV40 large-T antigen, produces tumours of the exocrine pancreas in transgenic mice (R. Brinster and R. Palmiter, personal communication), which further indicates that T-antigen is oncogenic in mice. Given that large-T antigen synthesis is the primary event, there are other potential secondary alterations that may be necessary to induce the development of solid β-cell tumours. These include activation or inactivation of growth factors or receptors, or the cooperation of another oncogene[36,37]. It is well established that nascent tumours cannot exceed certain size constraints without becoming vascularized; thus the induction of angiogenesis[38] is probably one (if not the only) secondary event necessary for solid tumour development. Biochemical and further histochemical comparisons of the islets before and after the predictable development of tumours may provide insight into these possibilities.

The ability to examine the pre- and postnatal development of the endocrine pancreas as well as heritable oncogenesis, using as markers the hormone gene products of these cell types and the SV40 tumour antigen, will provide a means to examine the consequences of cell-specific oncogene expression and the ontogeny of the effects reported here, as well as to address further aspects of the control of insulin-gene expression. It will be of interest to compare different recombinant insulin/oncogenes (such as *myc* and *ras*) for their effects on islet-cell biogenesis and cell–cell interaction, and on secondary events necessary for the development of β-cell tumours.

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LETTERS TO NATURE

Colour, albedo and nucleus size of Halley's comet

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Halley's comet (1982 i) is now bright enough for broadband photometry by large infrared telescopes[1–3]. We report here on photometry of the comet in the *B* (0.44 μm), *J* (0.55 μm), *V* (1.25 μm) and *K* (2.2 μm) broadband filters during a time when the coma was very weak and presumed to contribute negligibly to the broadband photometry. The *V* − *J* and *J* − *K* colours suggest that the colour of the nucleus of Halley's comet is similar to that of the D-type asteroids, which in turn suggests that the surface of the nucleus is of albedo < 0.1.

The first detection of Halley's comet in the near-infrared was made on 20 December 1984, when the comet was 5.39 AU from the Sun[1]. Our new observations at *J* and *K* were made on February 18.3 and 20.3, 1985 with the 3-metre NASA Infrared Telescope Facility (IRTF) at Mauna Kea, Hawaii, and measure reflected sunlight, not thermal emission from the comet itself. Simultaneously, we obtained *B* and *V* filter data with the 2.2-metre University of Hawaii telescope, also on Mauna Kea. All data were corrected for extinction and system response by comparison with standard stars. Table 1 gives the results of the observations, together with the comet's distances from the Earth and the Sun, and the solar phase angles. The *B* and *V* photometry was done with a 9.5-arc s diameter aperture, with sky measurements of the same field taken after the comet had moved completely away. With the IRTF we used an 8-arc s aperture and a 20-arc s amplitude oscillation of the telescope's secondary mirror to correct for the sky radiation. Both telescopes were set to follow the apparent motion of the comet across the sky. We