eventually cover. In the absolute reference frame the ridges must move relative to their immediately underlying asthenospheric sources[19].

What is not clear is why the past positions of the ridges in the approximate absolute reference frame provided by South America seem to match the areas of uplift so well. One possibility is that because asthenospheric sources are probably vertically extensive[38,39] and have horizontal velocities much less than the overlying plates[38–40], the asthenospheric zone extending from vertically above the deep source of a new ridge to the present position of the ridge may all have become hotter than it was before ridge creation. If so, this increased temperature could provide the heat needed for the phase change.

In detail, this model must be modified. For example, the uplift of southern Africa has been treated as a uniform vertical uplift simultaneously affecting all of high Africa, yet it is quite clear from Fig. 1*c*, *d* that we would expect a wave of uplift to have moved across high Africa starting in the east, and taking a geologically significant time to reach the west. This problem will not be discussed here in detail because part of its solution depends on considering still earlier phases of uplift probably resulting from the breakup of Gondwanaland. Nevertheless, it is worth noting that there is a distinct structural and topographic asymmetry to southern Africa. The Cenozoic Kalahari basin lies in the western half of southern Africa, while the eastern half consists primarily of Precambrian rocks: during much of Cenozoic time, the eastern half of southern Africa has been structurally raised relative to the western half. In addition, the topography is markedly asymmetric: the highest parts of southern Africa are in Lesotho, very close to the eastern margin. Here, and nowhere else, the topography rises to well over 3 km. This structural and topographic asymmetry is what would be expected from a more detailed application of the model. The same mechanism is envisaged for the uplift of south-east Australia, where the expected topographic asymmetry is quite clear.

All these speculations can be tested by better data from the uplift of land surfaces, high-pressure petrology and three-dimensional mantle flow models.

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Isolation and preliminary characterization of a human transforming gene from T24 bladder carcinoma cells

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DNA from T24, a cell line derived from a human bladder carcinoma, can induce the morphological transformation of NIH 3T3 cells. Using techniques of gene rescue to clone the gene responsible for this transformation, we have found that it is human in origin, <5 kilobase pairs in size and is homologous to a 1,100-base polyadenylated RNA species found in T24 and HeLa cells. Blot analysis indicates extensive restriction endonuclease polymorphism near this gene in human DNAs.

THE progression of a cell lineage from normalcy to malignancy may involve the mutation or activation of one or more genes. The genomes of retroviruses contain candidates for such 'oncogenes'. Certain retroviruses capable of inducing neoplasia *in vivo* and cell transformation *in vitro* contain transduced cellular genes which entirely encode the oncogenic proteins of these viruses[1,2]. If these or other oncogenes are expressed in tumours of viral or nonviral origin, the introduction of these genes into cultured cells might transform the recipients and render them tumorigenic. Indeed, DNA from some chemically transformed mouse cells can morphologically transform NIH 3T3 mouse fibroblasts following DNA-mediated gene transfer[3]. More recently, it has been reported that DNA from certain human tumour cell lines can also morphologically transform NIH 3T3 cells[4,5]. We have detected transforming activity in DNA from 5 of 21 human tumour cell lines[6]; the resulting

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NIH 3T3 transformed cells are tumorigenic in nude mice[4–6]. Human DNA from one colon and two lung carcinoma cell lines appear to contain the same or highly related transforming elements, while DNA from a bladder carcinoma and from a neuroblastoma cell line each contain different transforming elements[6]. These transforming genes may have played a critical part in the origins of the tumours from which these cell lines derive. Here, we report the molecular cloning and preliminary characterization of the transforming gene from the human bladder carcinoma cell line, T24 (ref. 7).

Strategy of gene isolation

In principle, a gene can be isolated whenever its transfer into an appropriate host results in the stable acquisition of a selectable phenotype, using either 'search' or 'rescue' strategies. In the former, a complete genomic library is constructed in plasmids, phage or other cloning vehicles, and individual members of the library are screened by DNA-mediated gene transfer. This approach has been used successfully to isolate genes from lower organisms[8]. The rescue strategy entails cleaving DNA from cells containing the gene with a restriction endonuclease and then ligating this DNA to a defined 'marker' sequence. The ligated DNA is then used in gene transfer experiments, ultimately yielding a cell bearing the transferred gene linked to the marker sequence. The gene is isolated by one of several means on the basis of its association with the marker. The chicken thymidine kinase (tk) and hamster adenine phos-

Table 1 Transfection of NIH 3T3 cells with uncleaved and restriction endonuclease-cleaved DNAs from T24 and T24-a2 cell lines and recombinant bacteriophage and plasmid

a	Suppressor rescuant	Foci per 0.25 μg DNA
	λsup2.9	86
	λsup1.12	0
	λsup5.3	0
	λsup5.4	0

b	λsup2.9 (Foci per 0.25 μg)	pTB-1 (Foci per 0.05 μg)
Undigested	86 (1.5×10^{-8})*	372 (1×10^{-7})*
*Bam*HI	35	NT
*Bgl*II	71	NT
*Eco*RI	176	NT
*Hin*dIII	91	NT
*Xba*I	0	NT

	T24 (Foci per 70 μg)	T24-a2 (Foci per 20 μg)
Undigested	11 (5×10^{-7})*	6 (9×10^{-7})*
*Bam*HI	15	8
*Bgl*II	7	4
*Eco*RI	7	34
*Hin*dIII	13	10
*Xba*I	0	0

DNA (total concentration 30 μg ml^{-1}) was precipitated with calcium phosphate and 1 ml of suspension was applied to 10^6 NIH 3T3 cells in a 10-cm culture dish, as previously described[12]. Restriction endonuclease-digested T24 and T24-a2 DNAs were mixed in a 1:2 ratio with uncleaved NIH 3T3 DNA before precipitation; samples of phage and plasmid DNAs were added to 30 μg NIH 3T3 DNA before precipitation. Cellular DNAs were prepared by SDS–proteinase K lysis and phenol/chloroform extraction[6]. Phage DNAs were prepared from CsCl gradient-purified virions[18] and plasmid DNA was purified from saturated culture of bacteria[19]. *a* Shows the NIH 3T3 focus-forming activity of DNAs from four independent suppressor-containing phages; *b* compares the focus-forming activity of λsup2.9 DNA and pBR322 subclone pTB-1 DNA, and compares the restriction endonuclease sensitivity profile of the activity in λsup2.9 DNA with the previously reported[6] activities in T24 and T24-a2 cellular DNAs. NT, not tested.

* Value in parentheses is the calculated frequency of focus induction per transforming gene equivalent. In these calculations, we have taken the molecular weight of the human haploid genome to be 1.5×10^{12}.

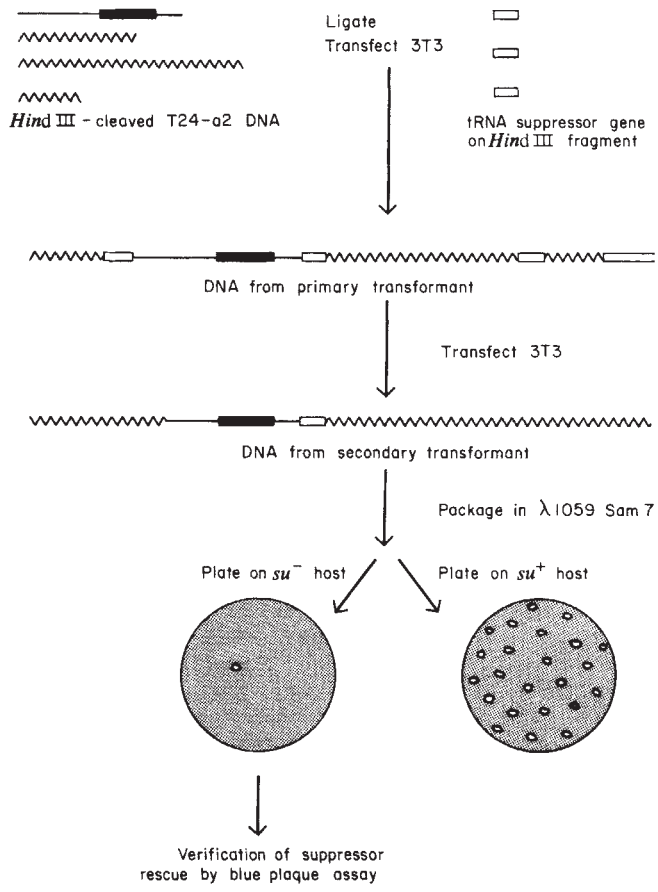

Fig. 1 Suppressor rescue of the T24 transforming activity. A recombinant DNA plasmid, pK-7, containing a *supF* tRNA amber suppressor gene was given by Dr U. RajBhandary. The suppressor gene was further subcloned into pBR322 plasmid (K.S. *et al.*, in preparation). This subclone, pK-5, was cleaved with restriction endonuclease *Hin*dIII, and the 1.1-kbp suppressor DNA fragment was purified by gel electrophoresis and binding to glass powder[20]. T24-a2 is a transformed NIH 3T3 cell line derived from transfection of NIH 3T3 cells with T24 DNA. *Hin*dIII-cleaved T24-a2 DNA was ligated in fourfold mass excess to suppressor fragment DNA with T4 DNA ligase (New England Biolabs) at a total DNA concentration of 250 μg ml^{-1}. Ligated DNA was mixed with a twofold mass excess of NIH 3T3 DNA and transfected to NIH 3T3 cells by methods described elsewhere[12]. This ligated DNA induced foci at a threefold lower efficiency than native T24-a2 DNA in the absence of suppressor. Resulting transformed cells were cultured from individually picked foci. DNA from these cells should contain some *supF* sequences closely linked to the transforming element, but most *supF* genes, introduced by co-transfer, will not be closely linked. These DNAs were transfected again to NIH 3T3 cells, yielding secondary foci bearing only closely linked *supF* sequences. DNA from six secondary foci were partially digested with restriction endonuclease *Sau*3A, and 10–20-kbp fragments were purified by centrifuging 150 μg DNA through a 15–40% (w/v) sucrose gradient (in 100 mM NaCl, 10 mM Tris *p*H 7.4, 1 mM EDTA) at 23,000 r.p.m. in a Beckman SW27 rotor for 24 h at 20 °C. From the six individual size-selected DNA preparations, a total of 5.3 μg DNA fragments (250 μg ml^{-1}) were ligated to a twofold mass excess of gel-purified phage DNA arms generated by cleavage of λ1059Sam7 DNA with endonuclease *Bam*HI. λ1059Sam7 was derived from *in vivo* recombination between λ1059 and λSam7 (K.S. *et al.*, in preparation). Chimaeric phage DNA molecules were packaged into virions by a standard procedure[15]. A total of 8.4×10^6 phage particles were generated by packaging, as measured by titration on *E. coli* BNN45 *supEsupF* cells. These phages were plated onto *E. coli* KS624 *sup*^{0}(r$^-$, m$^+$) cells (K.S. *et al.*, in preparation). Thirty-four individual plaques which arose were picked and the phages screened for the presence of amber suppressor by spotting on to lawns of *lacZ*^{am} *sup*^{0} cells in the presence of *lacZ* inducer isopropyl thiogalactoside and indicator substrate, Xgal (5-bromo-4-chloro-3-indoyl-β-D-galactoside; ref. 21). Suppressor-bearing phage suppressed the *lacZ*^{am} mutation, generating a blue plaque by β-galactosidase cleavage of Xgal. By this procedure, we isolated four suppressor-bearing phages. In the upper part of the figure, — denotes human DNA sequences; ∿, mouse DNA sequences; ▬, T24 transforming gene; and ▭, tRNA *supF* gene.

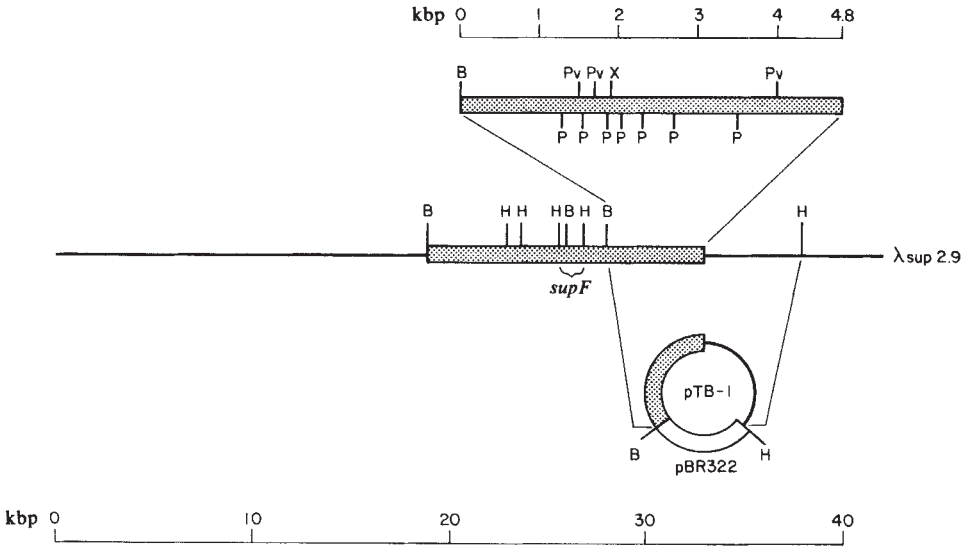

Fig. 2 Restriction endonuclease cleavage map of λsup2.9 and pTB-1. The figure shows the restriction map of DNA from the recombinant bacteriophage λsup2.9 which was active in transformation assays. Phage arms are denoted by a thin line, and the 14-kbp DNA insert by a shaded box. The suppressor DNA fragment is marked *supF*. Below is shown the circular map of pTB-1, with the *Bam*HI/*Hin*dIII fragment of λsup2.9 cloned into pBR322, denoted by an open box. Above is shown a more detailed restricted map of the cloned T24 bladder carcinoma transforming sequences. The cluster of *Pst*I sites were mapped by the method of Smith and Birnsteil[22]. There are no *Eco*RI, *Hin*dIII or *Bgl*II sites in this sequence. B, *Bam*HI; H, *Hin*dIII; P, *Pst*I; Pv, *Pvu*II.

phoribosyl transferase genes were isolated using the *Escherichia coli* plasmid pBR322 as the marker sequence[9,10].

In this study, we have adopted a modified rescue strategy using an *E. coli* tRNA amber suppressor gene, *supF*, as marker. Details of this method will be published elsewhere (K.S. *et al.*, in preparation) but the scheme is outlined in Fig. 1. From pK-7, a plasmid which contains *supF*, we constructed a new plasmid from which the *supF* gene could be recovered as a 1.1-kilobase pair (kbp) DNA fragment following cleavage with *Hin*dIII. This suppressor DNA fragment was ligated in equimolar ratio to *Hin*dIII-cleaved DNA from T24-a2, an NIH 3T3 transformant containing the T24 transforming gene. *Hin*dIII cleavage does not affect the transforming activity of either T24 or T24-a2 DNA (see Table 1). We used T24-a2 DNA as our gene source because it has a higher specific activity of transformation than T24 DNA. Ligated DNA was mixed with a twofold mass excess of NIH 3T3 cellular DNA and added as a calcium phosphate precipitate to NIH 3T3 cells[11,12]. Morphologically transformed foci were picked 2 weeks later and grown into mass culture. Blot hybridization analysis indicated that cells from these foci contained multiple copies of the bacterial tRNA gene. Presumably, at least one of these copies directly flanked the transforming gene while the others did not. Therefore, DNA from these transformants was again used for gene transfer to generate new foci. DNA from a few of the resulting foci retained one or two copies of the tRNA gene, and these DNAs were used in subsequent experiments.

To clone the tRNA suppressor gene and its flanking sequences, we used a biological selection based on the ability of the cloned tRNA gene to suppress amber mutations when incorporated into a mutant of bacterophage λ. We introduced by molecular recombination an amber mutation (Sam7)[13] into the lysis gene of λ1059, a cloning vehicle which accepts 7–18-kbp DNA fragments having GATC3′OH cohesive ends[14]. The resulting phage, λ1059Sam7, required a *supF* host to complete a lytic cycle. DNA from transformed animal cells containing one to two copies of *supF* were partially digested with restriction endonuclease *Sau*3A and ligated to purified arms of λ1059Sam7 DNA generated by *Bam*HI cleavage. The ligated DNAs were packaged into phage particles[15] and plated onto sup^0 and *supF* hosts. Phages which grew on sup^0 hosts were further identified as *supF*-containing by their ability to convert a $lacZ^{am}$ sup^0 host to $lacZ^+$. From such a screening process, we obtained four independent *supF* phage recombinants.

Cloned sequences transform NIH 3T3 cells

DNA from the four *supF* phages were mixed with NIH 3T3 carrier DNA and assayed for the ability to transform NIH 3T3 cells. As shown in Table 1, λsup2.9 DNA induced transformed foci at an efficiency of ~300 foci per μg. The three other phage DNAs were inactive in transformation. As a first step in verifying whether the λsup2.9 transforming activity is the same as that in T24 DNA, the activity of λsup2.9 cleaved with restriction endonucleases was compared with similarly cleaved T24 DNA. Table 1 shows that the transforming activity of λsup2.9 DNA is destroyed by cleavage with *Xba*I but not with *Bam*HI, *Bgl*II, *Eco*RI or *Hin*dIII. The same is true for T24 DNA.

To localize the transforming sequences of λsup2.9, we subcloned restriction fragments of λsup2.9 into pBR322. Cleavage

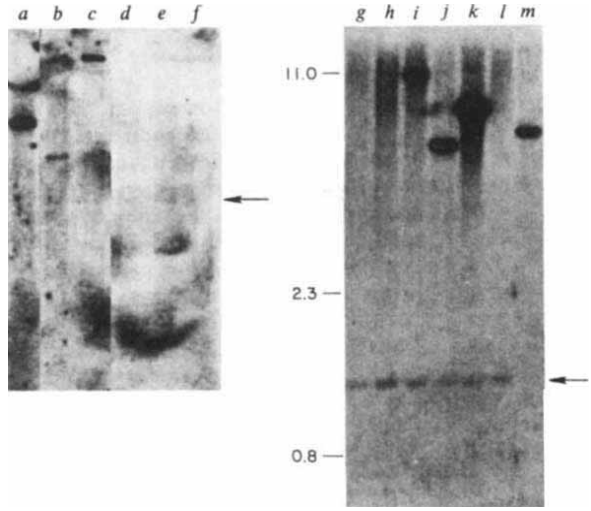

Fig. 3 Hybridization of pTB-1 DNA and one of its restriction endonuclease fragments to filter-blotted DNA from transformed NIH 3T3 cells. DNAs from NIH 3T3 cells, transformed NIH 3T3 cells[6] and T24 cells were cleaved with either *Hin*dIII or *Bam*HI restriction endonuclease. Digested DNAs (6 μg of each) were electrophoresed through 1.0% agarose gels and transferred to nitrocellulose filters as previously described[16]. Blotted DNAs were hybridized with nick-translated ^{32}P-DNA from either pTB-1 or the 800-bp *Pst*I fragment of pTB-1. Hybridizations were performed at 74 °C for 16 h in 6× SSC, 1× Denhardt's solution and 20 μg ml^{-1} denatured salmon sperm DNA[23]. *Hin*dIII-cleaved cellular DNAs probed with pTB-1 ^{32}P-DNA (20 ng ml^{-1}) (*a–f*); *Bam*HI-cleaved DNAs probed with the *Pst*I fragment ^{32}P-DNA (2 ng ml^{-1}) (*g–m*). *d*, *l*, DNA from NIH 3T3. Secondary transformants of NIH 3T3 transformed with T24 DNA: T24-a1-3 (*c*, *k*); T24-a2-1 (*a*, *j*); T24-a5-4 (*b*, *i*). *e*, *h*, Secondary transformant of NIH 3T3 transformed with DNA from Calu-1, a human lung carcinoma cell line. *f*, *g*, Primary transformant of NIH 3T3 transformed with DNA from SK-N-SH, a human neuroblastoma cell line. *m*, T24. Hybridization of probe to *Hin*dIII-cleaved λsup2.9 DNA and *Pvu*II- or *Pst*I-cleaved pTB-1 DNA provided molecular weight standards as indicated (in kilobase pairs).

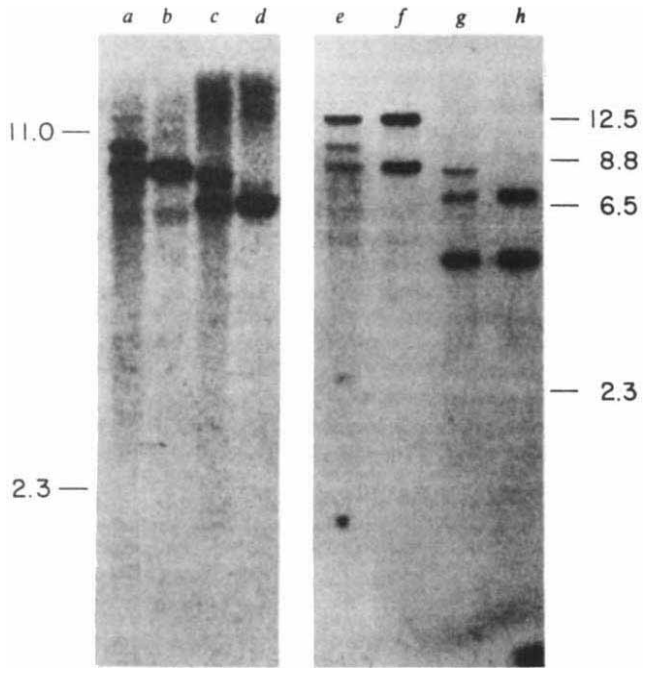

Fig. 4 Hybridization of pTB-1 DNA and its 800-bp *Pst*I fragment to filter-blotted DNA from T24 cells and human placenta. Human placental DNA (given by J. Fiddes) and T24 DNA were cleaved with restriction endonucleases, electrophoresed through 1.0% agarose gels and transferred to nitrocellulose filters. Blotted DNAs were hybridized with 20 ng ml^{-1} pTB-1 ^{32}P-DNA (*e–h*) or 2 ng ml^{-1} gel-purified, ^{32}P-labelled 800-bp *Pst*I pTB-1 DNA fragment (*a–d*). *Bgl*II-cleaved placenta DNA (*a*, *e*); *Bgl*II T24 (*b*, *f*); *Bam*HI placenta (*c*, *g*); *Bam*HI T24 (*d*, *h*). Hybridization of probes to *Xba*I- or *Hin*dIII-cleaved λsup2.9 DNA and *Pvu*II-cleaved pTB-1 DNA provided molecular weight standards (in kilobase pairs).

of λsup2.9 DNA with *Bam*H1 and *Hin*dIII generates seven restriction fragments, five of which contain heterogeneous ends (Fig. 2). These were then inserted between the *Bam*HI and *Hin*dIII sites of pBR322 DNA. When individual plasmid subclones were tested for their ability to transform NIH 3T3 cells, only subclone pTB-1 had transforming activity. pTB-1 DNA contains a 9.8-kbp inserted sequence spanning the rightmost *Bam*HI site in λsup2.9 to the *Hin*dIII site within the right arm of the phage DNA. As this region contains 5.0 kbp of λ DNA sequences, the transforming activity in pTB-1 is encoded in a transforming sequence no larger than 4.8 kbp. A more detailed restriction endonuclease map of this 4.8-kbp region is shown at the top of Fig. 2. The specific transforming activity of pTB-1 was higher than that of λsup2.9, but was consistently lower in a series of experiments than the specific activity calculated for the chromosomal gene in T24, assuming the gene is present in T24 as a single copy (for example, see Table 1). This agrees with our previous results for clones of the chicken tk gene which transfer at reduced efficiencies compared with the chromosomal gene[9]

pTB-1 contains transforming sequences of T24 bladder carcinoma cells

Hybridization studies provide further evidence that we have cloned transforming sequences from the T24 bladder carcinoma cell line. DNAs from NIH 3T3 cells, NIH 3T3 cells transformed with T24 DNA and NIH 3T3 cells transformed with DNA from other human tumour cell lines[6] were digested with *Hin*dIII, electrophoresed through agarose gels, and transferred to nitrocellulose filters by the procedure of Southern[16]. These studies used DNAs from secondary NIH 3T3 transformants resulting from two cycles of transfection of tumour cell DNA through NIH 3T3 cells. The filters were hybridized with nick-translated ^{32}P-labelled pTB-1 DNA. Figure 3 shows that pTB-1 sequences hybridize weakly to a specific *Hin*dIII fragment in DNA from NIH 3T3 cells and all its transformants (indicated by arrow at right of lane *f*). pTB-1 also hybridizes weakly to specific restriction fragments of normal rat cellular DNA (data not shown). In addition to these interspecies homologies, pTB-1 hybridizes strongly to a single *Hin*dIII fragment in each of three independent secondary transformants of T24 (Fig. 3, lanes *a–c*), while NIH 3T3 DNA shows no additional hybridization (lane *d*). We conclude that pTB-1 contains the transforming sequences present in T24 DNA. DNA from an NIH 3T3 secondary transformant derived from human lung carcinoma, Calu-1 DNA (Fig. 3, lane *e*) and DNA from an NIH 3T3 primary transformant derived from human neuroblastoma, SK-N-SH DNA (lane *f*), also lack additional sequences homologous to pTB-1, demonstrating that the transforming element in T24 differs from that in Calu-1 and SK-N-SH cells. This conclusion is consistent with our previous findings[6].

Human placental DNA contains sequences homologous to the T24 transforming gene

If the transforming activity in T24 DNA arose by the acquisition of exogenous (for example, viral) genetic information, then

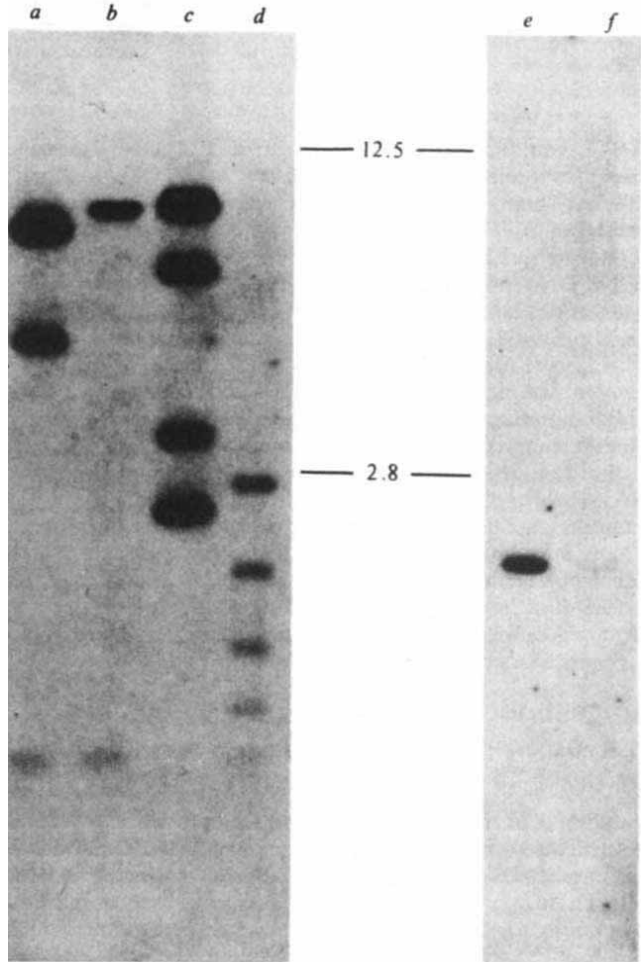

Fig. 5 Hybridization of pTB-1 DNA to filter-blotted T24 and pTB-1 DNAs cleaved with restriction endonucleases. T24 cellular DNA (6 μg) and pTB-1 (50 pg) were cleaved with restriction endonucleases, electrophoresed through a 1.0% agarose gel, then transferred to a nitrocellulose filter. pTB-1 DNA was cleaved with *Pst*I (*a*) and *Pvu*II (*c*); T24 DNA was cleaved with *Pst*I (*b*) and *Pvu*II (*d*). These blotted DNAs were hybridized to total pTB-1 ^{32}P-DNA (20 ng ml^{-1}). 20 pg of the 1.9-kb purified fragment of *Bam*HI/*Xba*I-cleaved pTB-1 (*e*) and T24 DNA cleaved with *Bam*HI/*Xba*I (*f*) were hybridized to the ^{32}P-labelled 1.9-kbp *Bam*HI/*Xba*I pTB-1 fragment (5 ng ml^{-1}). Hybridization of probe to *Xba*I-cleaved λsup2.9 DNA provided molecular weight standards (in kilobase pairs).

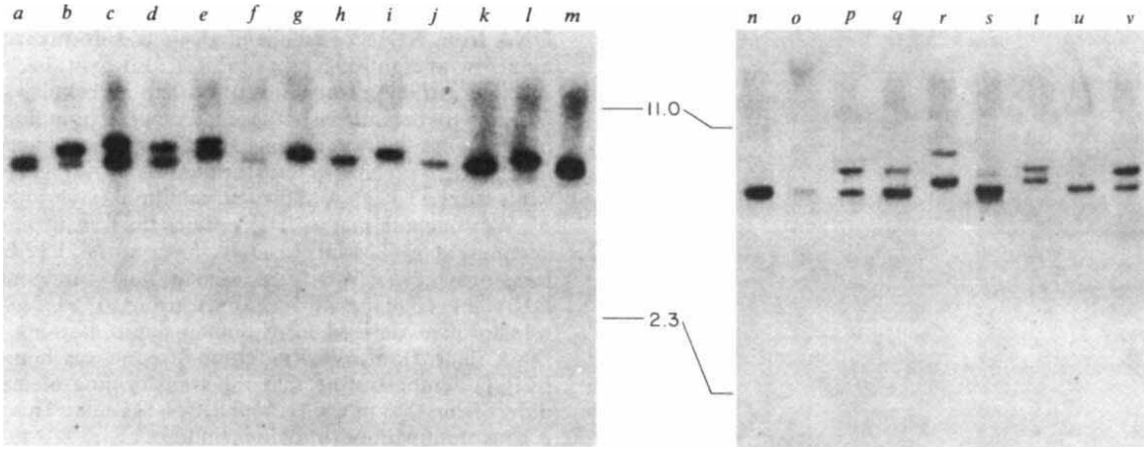

Fig. 6 Hybridization of the 800-bp *Pst*I fragment of pTB-1 DNA to filter-blotted DNAs from human tumours and tumour cell lines. Human tumour cell lines were given by J. Fogh. Human tumours were obtained from the NCI Tumor Repository. DNAs from human tumours and tumour cell lines were cleaved with *Bam*HI restriction endonuclease, electrophoresed through 1% agarose gels and then transferred to nitrocellulose filters. Blotted DNAs were hybridized to 2 ng ml^{-1} gel-purified ^{32}P-labelled 800-bp *Pst*I pTB-1 DNA fragment. DNAs from tumour cell lines: *a*, epidermis RPMI 2650; *b*, colon HT-29; *c*, cervix C4II; *d*, glial cell T98; *e*, osteosarcoma 8387; *f*, breast MCF-7; *g*, cervix HeLa; *h*, bladder T24; *i*, melanoma RPMI 4445; *j*, fibrosarcoma SW594; *k*, bladder 575A; *l*, bladder 496P; *m*, bladder TCCsup; *u*, pancreas Capan-1; and *v*, bladder VM-CUB-2. DNAs from human tumours: *n*, colon 031-10687; *o*, lung 031-9337; *p*, lung 031-11971; *q*, colon 76-640; *r*, lung 031-13137; *s*, colon 021-769; and *t*, lung 76-126. Hybridization of the probe to *Hin*dIII-cleaved λsup2.9 DNA and *Pvu*II-cleaved pTB-1 DNA provided molecular weight standards (indicated in kilobase pairs).

pTB-1 would probably hybridize to a T24 DNA sequence absent from human placenta DNA. To examine this possibility, T24 and human placenta DNAs were cleaved with either *Bam*HI or *Bgl*II restriction endonuclease, and digested DNAs were analysed by filter blot hybridization, using ^{32}P-labelled pTB-1 DNA as probe. Figure 4 shows that pTB-1 hybridizes to 8- and 12-kbp *Bgl*II fragments (lane *f*) and to 5.0- and 6.8-kbp *Bam*HI fragments (lane *h*) from T24 DNA. The probe hybridizes to *Bgl*II (lane *e*) and *Bam*HI fragments (lane *g*) of identical size in human placental DNA. We also observed that T24 and human placental DNAs have similarly sized *Eco*RI or *Hin*dIII fragments homologous to pTB-1 (data not shown). As all the T24 DNA restriction fragments homologous to pTB-1 have similarly sized counterparts in placental DNA, we conclude that the T24 transforming gene originated from human genetic information without the gross rearrangements of 'normal' DNA sequences. The fact that additional 9.5-kbp *Bgl*II and 7.9-kbp *Bam*HI fragments homologous to pTB-1 are present in placental DNA but absent from T24 DNA, we attribute to genetic polymorphism (see below).

Subgenomic fragments of pTB-1 suitable as hybridization probes for the T24 transforming gene

We wished to use pTB-1 as a hybridization probe to analyse the structure of the transforming gene in T24 and other human cell lines, and to monitor transcription of the gene in these cells. These applications require the human sequences in pTB-1 to be contiguous and not rearranged with respect to the chromosomal gene. Such rearrangements frequently occur after DNA-mediated transfer into animal cells[17]. We therefore compared the restriction endonuclease map of the human sequences in pTB-1 with that of the homologous sequences in T24 DNA. Filter hybridization of T24 DNA cleaved with *Bam*HI and *Xba*I to the ^{32}P-labelled 1.9-kbp pTB-1 *Bam*HI/*Xba*I fragment revealed a single homologous DNA fragment (Fig. 5*f*) co-migrating with the 1.9-kbp pTB-1 fragment (lane *e*), proving that the region extending from the *Bam*HI site to the *Xba*I site within the transforming gene is not rearranged in pTB-1. The 800-base pair (bp) *Pst*I fragment of pTB-1 (Fig. 5*a*) mapping between 2.7 and 3.5 kbp from the *Bam*HI site (see Fig. 2) can also be found in *Pst*I-cleaved T24 DNA (Fig. 5*b*), and this *Pst*I fragment hybridizes to DNA restriction fragments unique to NIH 3T3 secondary transformants of T24 (Fig. 3*i–k*). These results demonstrate that the 800-bp *Pst*I fragment of pTB-1 is an unaltered component of the transforming gene or its flanking DNA sequences. However, the 2.3-kbp *Pvu*II fragment of pTB-1 (Fig. 5*c*) spanning 1.7 to 4.0 kbp from the *Bam*HI site is not present in *Pvu*II-cleaved T24 DNA (Fig. 5*d*), indicating that human DNA sequences more than 3.5 kbp from the *Bam*HI site in pTB-1 are rearranged. Thus, the 1.9-kbp *Bam*HI/*Xba*I and 0.8-kbp *Pst*I pTB-1 fragments were used as hybridization probes to study the structure and expression of the transforming gene.

Human DNAs are highly polymorphic near the T24 transforming gene

The restriction fragments of T24 and human placental DNAs homologous to the transforming gene were again compared, using the 800-bp *Pst*I fragment of pTB-1 as hybridization probe. The *Pst*I fragment hybridized only to the 8-kbp *Bgl*II fragment (Fig. 4*b*) and the 6.8-kbp *Bam*HI fragment (land *d*) from T24 DNA. The probe detected identically sized *Bgl*II and *Bam*HI fragments from human placenta DNA (Fig. 4*a,c*), although the cleaved placenta DNA additionally contains a larger homologous fragment in each case. The presence of these larger fragments indicates the existence of genetic polymorphism near the transforming gene.

To measure the extent of this polymorphism, DNAs from 40 human tumours and tumour cell lines were cleaved with restriction endonuclease *Bam*HI and then analysed by gel electrophoresis and Southern blot hybridization, using the 800-bp *Pst*I fragment of pTB-1 as probe. The data for 21 of these DNAs are shown in Fig. 6. Sequences homologous to the probe were found in DNAs from all human sources, but the number and sizes of the homologous restriction fragments differed among the DNA samples. *Bam*HI-cleaved DNA from each human source contained one, two, or in one case, three discretely sized fragments ranging in molecular weight (MW) from 6 to 9 kbp. Among these DNAs, at least six different homologous *Bam*HI fragments were evident. We have also detected the same extent of restriction endonuclease polymorphism among normal placenta DNAs from different individuals (data not shown).

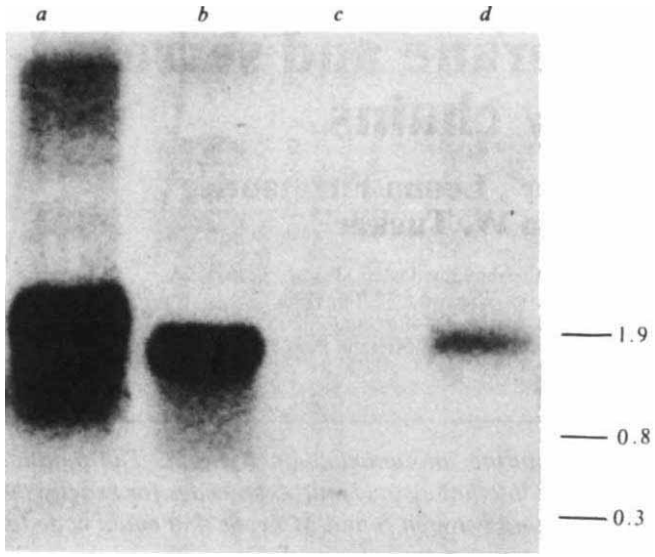

Fig. 7 Hybridization of restriction fragments of pTB-1 DNA to filter-blotted RNA. Total cellular RNAs were prepared by lysis of cells in 4 M guanidine thiocyanate[24] and removal of DNA and protein by extraction with hot phenol[25]. Polyadenylated RNA was selected by oligo(dT)-cellulose chromatography; 5 μg of each poly(A)$^+$ RNA were denatured in formaldehyde/formamide and electrophoresed through a 1.2% agarose gel containing 20 mM MOPS *p*H 7.0, 5 mM Na acetate, 1 mM EDTA, 2.2 M formaldehyde, and the RNA was transferred to a nitrocellulose filter, with 20× SSC as transfer buffer, as described by Thomas[26]. The filter was hybridized at 42 °C in 5× SSC, 50% formamide, 10% dextran sulphate with 2 ng ml^{-1} 800-bp *Pst*I pTB-1 ^{32}P-DNA fragment and 3 ng ml^{-1} 1.9-kb *Bam*HI/*Xba*I pTB-1 ^{32}P-DNA fragment. Poly(A)$^+$ RNA from 3T3 secondary transformant T24-a2-1 (*a*), T24 (*b*), NIH 3T3 (*c*), HeLa (*d*). *Bam*HI/*Xba*I-cleaved and *Pst*I-cleaved pTB-1 DNA provide molecular weight standards (in kilobases).

The hybridization intensities of the homologous *Bam*HI DNA fragments varied among the cleaved DNAs from different tumour sources. This variation is readily explained neither by inaccuracies of DNA concentration measurements nor by incomplete endonuclease digestions among the DNA samples analysed. We therefore suspect that the copy number of DNA sequences homologous to the T24 transforming gene varies about fivefold among the tumours and tumour cell lines.

Expression of pTB-1 sequences in transformed cells

To determine whether the transforming sequences are expressed in T24 cells and in NIH 3T3 cells transformed with T24 DNA, poly(A)$^+$ total cellular RNA was prepared from T24 cells, NIH 3T3 cells, a T24 DNA-induced NIH 3T3 transformant and HeLa cells. The RNAs were electrophoresed in formaldehyde/agarose gels, transferred to nitrocellulose filters and hybridized with a ^{32}P-labelled mixture of the 1.9-kbp *Bam*HI/*Xba*I fragment and the 0.8-kbp *Pst*I fragment of pTB-1 DNA. The results are given in Fig. 7. NIH 3T3 cells transformed by T24 DNA contain heterogeneously sized RNA species (1,500–1,800 bases) homologous to the pTB-1 fragments (Fig. 7*a*), while untransformed NIH 3T3 cells show no homology (lane *c*). A homologous RNA of discrete size (1,100–1,200 bases) is found in T24 cells (lane *b*).

The altered size of the transcripts found in NIH 3T3 transformants suggests that certain RNA processing signals in the transforming sequences were lost during gene transfer or are not recognized in the mouse cell host. A homologous RNA of the same size as that in T24 RNA is also found in the HeLa cells (Fig. 7*d*) but at reduced concentration. HeLa DNA does not transform NIH 3T3 in transfection assays. No homologous RNA species has been found in human placenta (data not shown). These results indicate that the transforming sequences are transcribed, that expression of these sequences is not restricted to T24 cells and that there is differential expression in cells from different sources. We estimate that transcripts of the T24 bladder carcinoma gene comprise 0.01% of poly(A)$^+$ RNA from T24 cells.

Discussion

Previous studies using DNA-mediated gene transfer showed that DNA from T24 bladder carcinoma cells can efficiently induce foci of morphologically transformed cells in NIH 3T3 recipients[6]. In this study we have cloned from T24 DNA biologically active sequences which are responsible for transformation. Hybridization studies indicate that the transforming sequences contained in T24 are of human origin and closely homologous sequences are present in DNA from each of 40 different human sources that we have examined.

Of the 21 human DNAs screened, only 5 can efficiently transform NIH 3T3 cells[6]. T24 DNA transfers a different transforming element from the other four active donors; we conclude that the transforming sequences of T24 differ in some critical respect from the homologous sequences found in DNAs from other human sources. The transforming gene of T24 probably did not result from the gross rearrangement of normal sequences, as cleavage of T24 and human placental DNAs with *Bgl*II, *Bam*HI, *Hin*dIII or *Eco*RI yields similarly sized fragments that are homologous to the transforming gene. However, the extent of restriction endonuclease polymorphism at this locus in human DNA limits the strength of this conclusion. Further studies should elucidate the origins of the T24 transforming gene and determine whether its biological properties derive from the expression of an altered protein product or from an altered pattern of gene expression.

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