anchorage-dependent and independent transformants which were capable of growing as C-lines *in vivo*, the proportion of the I-type relative to the C-type would be the same in these two experiments. As the proportion of I-type varies, it seems that most transformants which express the C phenotype *in vivo* are anchorage-independent and most anchorage-dependent transformants are of the I phenotype.

Because no tumours were anchorage-dependent, any anchorage-dependent transformants must be at a stage on the pathway to cancer that allows the *in vivo* selection of anchorage-independent variants at a high frequency.

Second, the proportion of I- and C-type cells depends on the chemical carcinogen used to transform the N-line. When transformants were selected for anchorage independence before testing in mice, they were induced with various chemical carcinogens. All of the transformants tested directly in mice were induced with BPE. If BPE influences the tumorigenic phenotype of the transformants, then it must induce more I-type transformants than several other carcinogens that were analysed collectively.

The existence of the I phenotype shows that at least some transforming events result in cells which are sensitive to a tumour surveillance mechanism operating in normal but not ATxFL mice. ATxFL mice are severely depressed with respect to both immunological (cell-mediated and humoral immunity) and non-immunological (natural killer (NK) and natural cytotoxic (NC) cells) host protective mechanisms. Although it is possible that one (or both) of these host protective mechanisms is responsible for the destruction of I-lines by normal mice, we cannot ascribe the surveillance measured here to either mechanism. Regardless of the mechanisms of surveillance, the frequency with which transformation by a chemical carcinogen results in the I phenotype is at least three times that of the C phenotype. This is important because the efficacy of any surveillance mechanism is a function of the proportion of newly transformed cells which are susceptible to surveillance.

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A transforming gene present in human sarcoma cell lines

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Morphological transformation of NIH/3T3 cells by transfection with DNA has been used to identify transforming sequences in human tumours[1,2]. Transforming activity has been reported for DNAs isolated from bladder, mammary, colon and lung carcinomas, neuroblastoma, lymphoid and myeloid tumours[1–6]. Each of these tissues seems to contain different transforming sequences except for the colon and lung tumours where the same sequence seems to be involved[5]. We now report that in two different human sarcoma cell lines, a fibrosarcoma and an embryonal rhabdomyosarcoma, the DNAs have transforming activity. The transforming gene is the same in both sarcomas but differs from the activated sequences detected in other tumours. We have also found that the transforming gene has no detectable homology to eight retrovirus oncogenes tested.

High molecular weight DNA was prepared from three different types of human sarcoma cell lines and assayed for transforming activity on NIH/3T3 cells using the calcium phosphate co-precipitation technique[7]. Table 1 shows that DNA from embryonal rhabdomyosarcoma cell line RD (ref. 8) and from one of two fibrosarcomas, HT1080 (ref. 9), was able to transform NIH/3T3 cells. DNA extracted from a second fibrosarcoma cell line, HS10CL7, and from an osteogenic sarcoma, MG63 (ref. 10), as well as normal human DNA did not cause morphological transformation. The transforming efficiencies of RD and HT1080 DNAs were similar, though lower than that of the T24 bladder carcinoma cell line. Individual cells in sarcoma DNA-induced foci were morphologically similar to those produced by the bladder line, but the foci themselves were smaller. This suggests that the transfectants produced using sarcoma DNA grow more slowly than those derived using T24 DNA. The morphologically altered cells were found to initiate rapidly growing tumours with short latent periods (10 days) when injected into nude mice whereas the clone of untransformed 3T3 cells did not produce tumours over a 2-month observation period (Table 1). The transfectants differed from the NIH/3T3 cell line in being able to grow in low-serum and in anchorage-independent conditions. Furthermore, DNA isolated from primary transfectants was able to transform NIH/3T3 cells, demonstrating that the transforming activity could be passaged serially (Table 1).

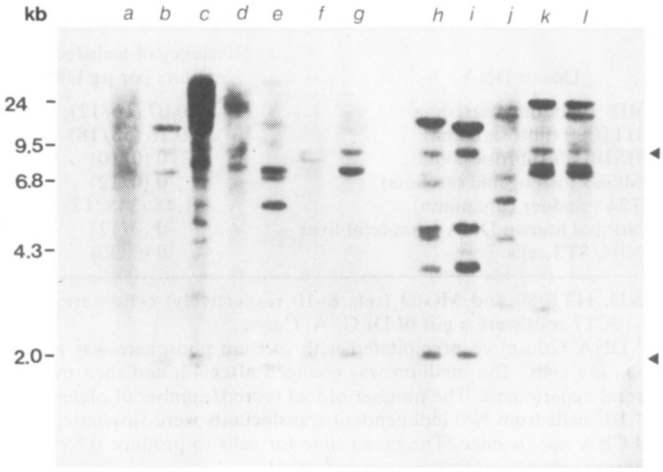

Fig. 1 Presence of human repetitive sequences in secondary transformants derived from RD and HT1080. DNAs (20 μg) were digested with *Eco*RI, phenol-extracted and loaded on a 1-cm thick, 0.8% agarose gel. After electrophoresis the DNA was transferred to nitrocellulose and the blot was hybridized essentially as described by Wahl *et al.*[20] except that the formamide concentration was 30%, the hybridization temperature 60 °C and mouse DNA was used as carrier. The probe used was fetal liver DNA (previously sonicated to an average length of 4 kb) nick-translated to a specific activity of 2×10^8 c.p.m. per μg. The filter was washed in 0.4 × SSC/0.1% SDS for 30 min at 60 °C, and subjected to autoradiography with an intensifying screen at −70 °C for 5 days. Lanes *a*, NIH/3T3; *b*, *c*, secondaries derived from HT1080 primary 125; *d*, *e*, secondaries derived from HT1080 primary 144; *f*, *g*, secondaries derived from HT1080 primary 120; *h*, *i*, secondaries derived from RD primary 145; *j*, secondary from RD primary 149; *k*, *l*, secondaries derived from RD primary 148. Arrows show the position of the 8.8 kb band conserved in all transfectants and the 1.8 kb band present in some transfectants. (In lane *e* of the blot shown the 8.8 kb band is poorly visible, however in other blots of this DNA the band is present though faint.)

To determine whether the transforming activity of the human sarcoma cell lines was related to sequences previously identified in other tumour types or to known viral oncogenes the following experiments were performed.

First, DNAs from the sarcoma cell lines, or from primary transfectants, were digested with various restriction endonucleases before transfection of NIH/3T3 cells. Enzymes having sites in the transforming sequence should destroy the transforming activity of the DNA. Digestion of DNA from primary transfectants with six enzymes that cut DNA on average every 3–7 kilobases (kb) completely destroyed the transforming

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Table 1 Transfection of NIH/3T3 cells with DNAs from human sarcoma cell lines

Donor DNA	Efficiency of transfection* (no. foci per μg DNA)	Tumorigenicity of transfectants†	Efficiency of secondary transfection*‡ (no. foci per μg DNA)
RD (rhabdomyosarcoma)	0.07 (16/12)	2/2, 2/2 (10 days)	0.14 (11/4), 0.16 (13/4), 0.13 (10/4)
HT1080 (fibrosarcoma)	0.16 (58/18)	3/3, 3/3 (10 days)	0.13 (10/4), 0.28 (22/4), 0.73 (44/3)
HS10C17 (fibrosarcoma)	0 (0/10)	—	—
MG63 (osteogenic sarcoma)	0 (0/12)	—	—
T24 (bladder carcinoma)	1.45 (348/12)	3/3, 3/3 (10–12 days)	6
Normal human DNA from fetal liver	0 (0/12)	—	—
NIH/3T3 cells	0 (0/30)	0/8 (8 weeks)	—

RD, HT1080 and MG63 (refs 8–10 respectively) cells were obtained from the American Type Culture Collection via Flow Laboratories. HS10C17 cells were a gift of Dr G. A. Currie.

* DNA (20 μg) co-precipitated with calcium phosphate was applied to a 60-mm culture dish seeded 24 h previously with 3×10^5 NIH/3T3 clone D4 cells. The medium was changed after 4 h and then every other day and foci were scored after 16–17 days. Values are the mean of several experiments. The number of foci scored/number of plates examined is given in parentheses.

† 10^6 cells from two independent transfectants were suspended in 0.2 ml of medium and injected subcutaneously into two or three 8–12 week old CBA *nu/nu* mice. The mean time for cells to produce 0.5 cm diameter tumours was also recorded. Each value represents the takes from a different independent primary transfectant.

‡ Each set of values represents the transfection efficiencies for a different primary transfectant.

activity (Table 2). Digestion of DNA from a primary transfectant induced by the bladder carcinoma line T24 showed a different sensitivity to digestion, so the sarcoma transforming sequence is not identical to that of the bladder carcinoma[11]. Similarly, comparison with published data shows that the transforming sequence of the sarcoma DNAs is not the same as that present in mammary[3] or lymphocyte[6] neoplasms. In addition, none of four enzymes (*Xho*I, *Cla*I, *Sal*I and *Sma*I) having restriction sites that contain the rare dinucleotide CpG and which therefore cut human DNA relatively occasionally inactivated the sarcoma transforming activity. The restriction enzyme sensitivity profiles of RD and HT1080 DNAs are identical for 10 enzymes, suggesting that the transforming activity is associated with the same sequence in both tumours (Table 2).

To characterize the transforming sequence further, we analysed the DNA of secondary transfectants for the presence of human repetitive sequences. After two consecutive rounds of transfection only a small amount of human DNA remains in the transformed cells; any repetitive DNA sequences that are conserved in all secondary transfectants would therefore be expected to be closely linked to or contained in the transforming

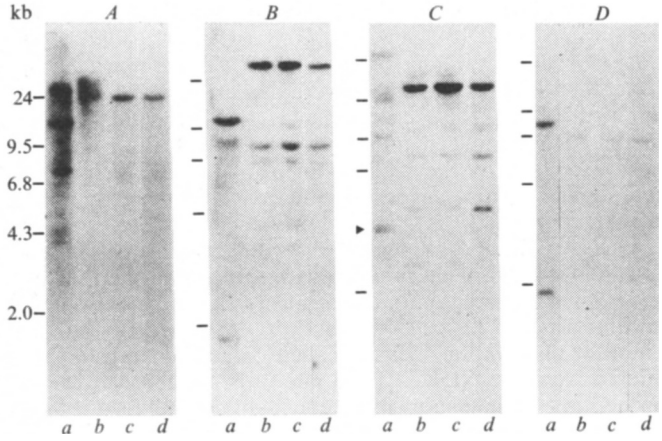

Fig. 2 Southern blots of *Eco*RI (*A–C*) or *Bam*HI (*D*) digestions of the following DNAs: *a*, human fetal liver; *b*, NIH/3T3; *c*, a primary HT1080 transfectant; and *d*, a primary RD transfectant. The blots were hybridized with the following ^{32}P-labelled viral oncogene probes: *A*, a 1.5 kb *Pst*I fragment containing *v-myc*; *B*, a 2.5 kb *Pvu*II fragment containing *v-erb-A* and *v-erb-B*; *C*, a 1.8 kb *Pvu*II fragment containing *v-Ki-ras* and flanking sequences (the arrow indicates the position of the human 3 kb *Eco*RI fragment homologous to the viral oncogene and identified in NIH/3T3 cells transfected with human lung carcinoma DNA[16]; *D*, a 0.9 kb *Pst*I/*Xba*I fragment containing *v-sis*. All four blots were hybridized in 50% formamide, 5 × SSC at 42 °C except *D* which was at 50 °C. The filters were washed as follows: *A*, 0.3 × SSC, 65 °C; *B*, 0.1 × SSC, 45 °C; *C*, 0.3 × SSC, 60 °C; and *D*, 0.6 × SSC, 50 °C.

Table 2 Effect of restriction endonuclease digestion on the transforming activity of sarcoma DNAs

Enzyme	No. of foci per μg DNA HT1080-f125	RD-f145	T24-f106
No enzyme	0.2	0.28	1.0
*Eco*RI	0	0	1.0
*Bam*HI	0	0	ND
*Hin*dIII	0	0	0.51
*Bgl*II	0	0	0.4
*Pvu*II	0	0	0
*Pst*I	0	0	0
*Xho*I	0.1	0.2	ND
*Sal*I	0.22	0.18	ND
*Cla*I	0.32	0.18	ND
*Sma*I	0.13	0.07	ND

DNA (100–200 μg ml^{-1}) was digested according to the manufacturers' instructions but using a 10-fold excess of enzyme. After addition of enzyme an aliquot containing 1 μg of DNA was removed and added to 1 μg of λ DNA. Complete digestion of λ DNA was taken to correspond to complete digestion of cellular DNA. 60 μg of digested DNA were added to 120 μg of high molecular weight mouse DNA and calcium phosphate co-precipitates were divided between six plates of NIH/3T3 cells. For all cell lines, DNAs of primary transfectants were used for restriction digests because their transforming activity was higher than that of the original cell lines. In confirmatory experiments, digests of DNA from the original human cell lines gave similar results.

sequence[4,5]. A Southern blot[12] profile of such conserved repetitive sequences can be used to distinguish different transforming sequences[4,5]. Accordingly, DNAs were isolated from secondary transfectants derived from RD and HT1080 DNAs, digested with *Eco*RI and probed on Southern blots with nick-translated total human DNA[13]. In the conditions of hybridization used, only human repetitive sequences can be detected. Although several fragments are present in each transfectant, only a single 8.8 kilobase pair (kbp) *Eco*RI fragment is conserved in all secondary transfectants (Fig. 1). Identical Southern blots probed with the plasmid BLUR-8 (ref. 14), which contains a cloned repetitive sequence from the human *Alu* family, show a similar pattern of hybridization (data not shown). However, the band corresponding to the 8.8 kbp *Eco*RI fragment is less intense than the other bands. We assume that the repetitive sequence present in this fragment is only distantly related to the *Alu* repetitive element contained in BLUR-8. Many of the secondary transfectants also contain a second fragment of 1.8 kbp (Fig. 1). However, as this fragment is not conserved in all secondary transfectants, it is probably not contained in the transforming sequence but may be linked to it. The observation that an 8.8 kbp *Eco*RI fragment is conserved in all secondary transfectants derived from both RD and HT1080 DNAs again strongly suggests that the transforming sequence in the two

DNAs is the same. Furthermore, the size of the *Eco*RI fragment is different from the fragments found by other investigators in secondary transfectants from DNAs of colon, lung, neuroblastoma and promyelocytic leukaemia[4,5].

Recently it has been shown that the transforming sequence activated in bladder carcinoma cell lines is a human cellular homologue of *v-Ha-ras*, the oncogene of Harvey sarcoma virus[15]. The transforming sequence of a lung carcinoma cell line is homologous to *v-Ki-ras*, the oncogene of Kirsten sarcoma virus[16]. Furthermore, Eva *et al.*[17] have reported that human sarcoma cells contain high levels of RNA transcribed from the cellular homologue of *v-sis*, the oncogene of simian sarcoma virus. It was therefore important to determine whether the sarcoma transforming sequence is related to these or other retrovirus oncogenes. We have probed Southern blots of transfectants to see whether they contain human sequences homologous to *v-erb-A*, *v-erb-B*, *v-Ki-ras*, *v-myc*, *v-sis* (Fig. 2) and to *v-abl*, *v-Ha-ras* and *v-src* (data not shown). None of these probes detected any human sequences in the transfectants although the conditions used detected the mouse homologues in the transfectants and the human homologues in human DNA.

We conclude from these experiments that the same transforming sequence is present in cell lines derived from the two different types of sarcoma, a rhabdomyosarcoma and a fibrosarcoma. (Recently other workers[18] noted that HT1080 DNA has transforming activity.) We have shown that this sarcoma sequence is different from transforming sequences identified in other human tumour lines and in eight RNA tumour viruses. These data therefore support the idea that different transforming sequences are activated in different tumour types and that these sequences may have a role in both the normal physiology and the neoplastic transformation of those tissues[3-6,19]. Further characterization of the transforming sequence and the elucidation of its mode of action will depend on the isolation of molecular clones of the transforming gene and its normal allele.

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The production of membrane or secretory forms of immunoglobulins is regulated by C-gene-specific signals

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Selective patterns of antibody isotypes are produced in response to thymus-dependent and thymus-independent antigens and mitogens[1-5]. Together with information on the organization of immunoglobulin C_H genes in myelomas[6,7], various models on the control of C-gene expression and switch in normal B cells have been proposed but only secreted products or secretory cells have been considered. We report here a dissociation between expression of membrane-bound immunoglobulins and secretion of the same isotype, and in this case describe C-gene specific signals which regulate the production of membrane versus secretory forms of immunoglobulin. These results indicate that regulation of isotypic patterns operate at levels other than immunoglobulin gene structure, and suggest that the secretory phenotype alone is inadequate as the measure of C-gene expression.

Spleen cells were exposed to either the T-independent mitogen lipopolysaccharide (LPS)[8] or helper T cells which specifically activate a large set of B cells[9]. Activation of B cells under these two conditions results in two distinct patterns of γ-isotype secretion although activation of μ secretion is comparable in both cases. T-independent induction results in predominant secretion of γ2b and γ3, while helper cells induce primarily γ1 and γ2a, but here we have also studied the expression of membrane-bound immunoglobulins. As shown in Table 1, following T-independent induction, γ1 is the predominant membrane isotype, indicating a clear discrepancy between the expression and the secretion of this particular isotype. The dissociation between expression of membrane and secretory form of γ1 cannot be explained by different kinetics, as high frequencies of γ1-bearing blasts are observed from days 3 to 7 of culture. Passive adsorption of γ1 on LPS blasts is unlikely because the concentration of this isotype in culture supernatants remains low (A.C. and L.F., in preparation), and therefore would require the presence of Fc receptors with exquisite specificity and high affinity for γ1, which is not the case for B lymphocytes[10]. In contrast, no dissociation of membrane and secretory isotypes was observed after exposure to T helper cells.

We addressed the question of the fate of such LPS-induced, γ1-bearing cells, and of the mechanisms responsible for the dissociation between expression and secretion observed in LPS cultures. We attempted to correct the secretory defect of LPS-activated populations by exposure to specific T-cell help. LPS-activated blasts re-exposed to either LPS or to helper cells produce 3 days later quite distinct isotypes (Table 2). The T-dependent re-stimulation resulted in: the appearance of a high number of γ_1 secretors which are missing in LPS culture (the number of these is very close to the one of γ1-bearing cells in the starting population); decrease of γ3 and γ2b secretors (although at values still higher than in helper cultures) and a significant increase of α plaque-forming cells (PFC). Note that in both cases a comparably high fraction (40%) of all recovered cells are high rate secretors and, of these, a similar proportion (57% and 56%) secrete γ; both stimuli seem therefore to be equally competent in inducing switch and terminal maturation. The differences are isotype specific and relate to the quality of the stimulus controlling terminal maturation in the same population of activated cells.

To define further the specificity of the maturation signals, we analysed similar cultures both for cells expressing membrane-bound immunoglobulins and PFC. Table 3*a* shows that LPS is fully competent to induce γ3 secretion, and ineffective with regards to γ1 secretion. On the other hand, helper activity does induce γ1 secretion. As, however, it also doubles the number of γ1-bearing blasts, LPS-activated, γ1-bearing blasts may remain nonsecretory in the presence of helper cells, which

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