or a macrophage, but not into any other kind of blood cell; the decision to become a granulocyte or a macrophage seems to depend on the concentration of a glycoprotein signalling molecule, granulocyte/macrophage colony-stimulating factor (GM-CSF)[34]. As neural progenitor cells in developing optic nerve seem not to differentiate into neurones or ependymal cells *in vivo*, and as we have found no evidence that they can develop into neurofilament-containing neurones or type 1 astrocytes *in vitro*[5], our results are consistent with the hypothesis that the A2B5$^+$ glial progenitor cell in 7-day optic nerve is committed to becoming either an oligodendrocyte or a type 2 (fibrous) astrocyte.

But what determines which of these differentiation pathways such a glial progenitor cell will follow? While it is likely that signals from axons normally have an important influence on the decision *in vivo*[35], it is clear that these cells can be induced to develop into either fibrous astrocytes or oligodendrocytes *in vitro* in the absence of recognizable neurones. Perhaps an intimate association with axons permits a cell to become an oligodendrocyte by protecting it from a soluble signal (such as the one in perinatal serum) that would otherwise induce it to become an astrocyte. Or perhaps degenerating axons (resulting from the death of retinal ganglion cells that occurs during normal development[36,37]) induce neighbouring progenitor cells to become fibrous astrocytes rather than oligodendrocytes. Since the decision can be controlled in culture it should be possible to determine the cellular and molecular mechanisms that underlie it.

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1. Jacobson, M. *Developmental Neurobiology* 2nd edn, 27–55 (Plenum, New York, 1978).
2. Bignami, A., Eng, L. F., Dahl, D. & Uyeda, C. T. *Brain Res.* **43**, 429–435 (1972).
3. Schachner, M. *et al. J. Cell Biol.* **75**, 67–73 (1977).
4. Raff, M. C. *et al. Brain Res.* **174**, 283–308 (1979).
5. Raff, M. C. *et al. J. Neurosci.* (in the press).
6. Van Heyningen, W. E. *J. gen. Microbiol.* **31**, 375–387 (1963).
7. Eisenbarth, G. S., Walsh, F. S. & Nirenberg, M. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4913–4917 (1979).
8. Dimpfel, W., Huang, R. T. C. & Habermann, E. *J. Neurochem.* **29**, 329–334 (1977).
9. Mirsky, R. *et al. Brain Res.* **148**, 251–259 (1978).
10. Bottenstein, J. E. & Sato, G. H. *Proc. natn. Acad. Sci. U.S.A.* **76**, 514–517 (1979).
11. Raff, M. C. *et al. Nature* **274**, 813–816 (1978).
12. Ranscht, B. *et al. Proc. natn. Acad. Sci. U.S.A.* **79**, 2709–2713 (1982).
13. Abney, E., Williams, B. & Raff, M. C. *Devl Biol.* (submitted).
14. Mirsky, R. in *Neuroimmunology* (ed. Brockes, J.) 141–181 (Plenum, New York, 1982).
15. Schachner, M. in *Neuroimmunology* (ed. Brockes, J.) 215–250 (Plenum, New York, 1982).
16. Pruss, R. *Nature* **280**, 688–690 (1979).
17. Dahl, D. & Bignami, A. *Brain Res.* **116**, 150–157 (1976).
18. Sommer, I. & Schachner, M. *Devl Biol.* **83**, 311–327 (1981).
19. Vaughn, J. *Z. Zellforsch.* **94**, 293–324 (1969).
20. Skoff, R. P., Price, D. L. & Stocks, A. *J. comp. Neurol.* **169**, 291–312 (1976).
21. Skoff, R. P., Price, D. L. & Stocks, A. *J. comp. Neurol.* **169**, 313–333 (1976).
22. Peters, A., Palay, S. L. & Webster, H. de F. in *The Fine Structure of the Nervous System: The Neurons and Supporting Cells* 2nd edn, 233–244 (Saunders, Philadelphia, 1976).
23. Norenberg, M. D. & Martinez-Hernandez, A. *Brain Res.* **161**, 303–310 (1979).
24. Riepe, R. E. & Norenberg, M. D. *Nature* **268**, 654–655 (1977).
25. Moscona, A. A., Mayerson, P., Linser, P. & Moscona, M. in *Tissue Culture in Neurobiology* (eds Giacobini, E., Vernadakis, A. & Shahar, A.) 111–127 (Raven, New York, 1980).
26. Sarthy, P. V. & Lam, D. M. K. *J. Cell Biol.* **78**, 675–684 (1978).
27. Russell, D. S. & Rubinstein, L. J. in *Pathology of Tumours of the Nervous System*, 127–131 (Arnold, London, 1959).
28. Turner, J. E. & Singer, M. *J. comp. Neurol.* **156**, 1–18 (1974).
29. Schonbach, C. *J. comp. Neurol.* **135**, 93–119 (1969).
30. Patterson, P. H. *A. Rev. Neurosci.* **1**, 1–17 (1978).
31. Furshpan, E. J., MacLeish, P. R., O'Lague, P. H. & Potter, D. D. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4225–4229 (1976).
32. Landis, S. C. & Patterson, P. H. *Trends Neurosci.* **4**, 172–175 (1981).
33. Metcalf, D. & Moore, M. A. S. *Haemopoietic Cells* (North-Holland, Amsterdam, 1971).
34. Metcalf, D. in *Cellular Controls in Differentiation* (eds Lloyd, C. W. & Rees, D. A.) 125–144 (Academic, London, 1981).
35. Privat, A., Valat, J. & Fulcrand, J. *J. Neuropath. exp. Neurol.* **40**, 46–60 (1981).
36. Potts, R., Dreher, B. & Bennett, M. R. *Devl Brain Res.* **3**, 481–486 (1982).
37. Lam, K., Sefton, A. J. & Bennett, M. R. *Devl Brain Res.* **3**, 487–491 (1982).
38. Cham, B. E. & Knowles, B. R. *J. Lipid Res.* **17**, 176–181 (1976).
39. Engvall, E. & Ruoslahti, E. *Int. J. Cancer* **20**, 1–5 (1977).

Identification of transforming gene in two human sarcoma cell lines as a new member of the *ras* gene family located on chromosome 1

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A molecular clone containing part of the transforming gene from two human sarcoma cell lines, HT1080 and RD, has been obtained and shown to represent a new member of the human ras *gene family. The transforming gene has undergone no major rearrangements and has not been amplified in either sarcoma cell line. The major transcript from the gene is 2,200 nucleotides long and is present at the same levels in both normal fibroblasts and tumour cells. The same gene is also activated in HL60, a promyelocytic leukaemia line and in SK-N-SH, a neuroblastoma line. The gene, N-*ras, *is located on chromosome* 1.

DNA TRANSFECTION studies using NIH/3T3 cells as recipients have led to the identification of different transforming genes present in a variety of human tumour cell lines[1–5]. In the case of the human bladder tumour line, EJ, the transforming gene has been cloned and shown to be an activated form of the c-Ha-*ras*1 gene[6–10], which is one of two distinct cellular sequences[11] that have been shown to share homology with v-Ha-*ras*, the oncogene carried by Harvey murine sarcoma virus[12]. The activation of this gene is the result of an alteration in the coding sequence of the cellular p21 protein as a consequence of a single base mutation[13–15]. Another *ras* transforming gene has been identified in a wide variety of different tumour cell lines (for example, colon, lung, pancreas, bladder and a rhabdomysarcoma)[5,10], and has been shown to be an activated form of c-Ki-*ras*2 (one of the two known cellular homologues[11] of v-Ki-*ras*, the viral oncogene carried by Kirsten sarcoma virus[16]), though in these cases the mechanism of activation is not known[17]. It has now been shown that more than two-thirds of all solid human tumours or cell lines, except lymphocyte neoplasms[3], having a detectable transforming gene contain an

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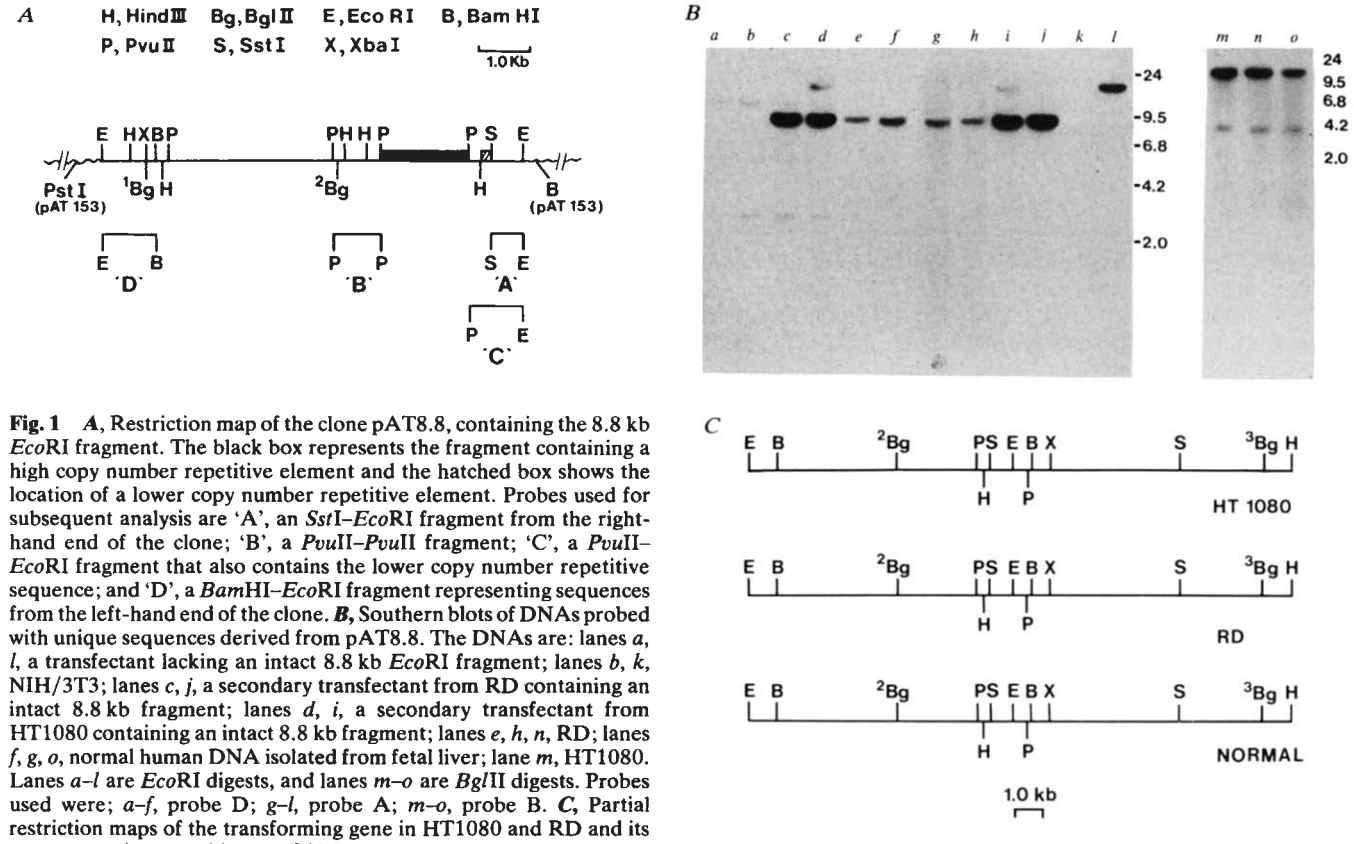

Fig. 1 ***A***, Restriction map of the clone pAT8.8, containing the 8.8 kb *Eco*RI fragment. The black box represents the fragment containing a high copy number repetitive element and the hatched box shows the location of a lower copy number repetitive element. Probes used for subsequent analysis are 'A', an *Sst*I–*Eco*RI fragment from the right-hand end of the clone; 'B', a *Pvu*II–*Pvu*II fragment; 'C', a *Pvu*II–*Eco*RI fragment that also contains the lower copy number repetitive sequence; and 'D', a *Bam*HI–*Eco*RI fragment representing sequences from the left-hand end of the clone. ***B***, Southern blots of DNAs probed with unique sequences derived from pAT8.8. The DNAs are: lanes *a*, *l*, a transfectant lacking an intact 8.8 kb *Eco*RI fragment; lanes *b*, *k*, NIH/3T3; lanes *c*, *j*, a secondary transfectant from RD containing an intact 8.8 kb fragment; lanes *d*, *i*, a secondary transfectant from HT1080 containing an intact 8.8 kb fragment; lanes *e*, *h*, *n*, RD; lanes *f*, *g*, *o*, normal human DNA isolated from fetal liver; lane *m*, HT1080. Lanes *a–l* are *Eco*RI digests, and lanes *m–o* are *Bgl*II digests. Probes used were; *a–f*, probe D; *g–l*, probe A; *m–o*, probe B. ***C***, Partial restriction maps of the transforming gene in HT1080 and RD and its counterpart in normal human DNA.

Methods: ***A***, DNA was isolated from an HT1080 secondary transfectant that contained only three *Eco*RI fragments (one of them being the 8.8 kb fragment) hybridizing to a nick-translated total human DNA probe. The DNA (200 μg) was digested with *Eco*RI, size-fractionated on a 5–20% potassium acetate gradient, and the fractions containing the 8.8 kb fragment were identified after analysing a small aliquot from each on a Southern blot. DNA from enriched fractions was ligated with the arms of *Eco*RI digested Charon 4A phage DNA and the mixture was packaged *in vitro*. A library containing 2×10^5 recombinants was screened using a total human DNA probe and four recombinants were identified that contained a human repetitive sequence. Analysis of one of these clones confirmed the presence of an 8.8 kb *Eco*RI fragment. The recombinant phage DNA was digested with *Eco*RI and the insert was subcloned into pAT153. A clone was obtained, pAT8.8, which was found to contain an 8.8 kb insert and a restriction map was obtained using standard procedures with single, double and partial enzyme digests. In ***B***, transfectants lacking an 8.8 kb *Eco*RI fragment were derived as follows: DNA (600 μg) was isolated from a primary transfectant, partially digested with *Sau*3A, and centrifuged through a 5–20% potassium acetate gradient. Fractions were pooled as follows: pool 1, 5–10 kb; pool 2, 10–16 kb; and pool 3, 16–22 kb. These pooled DNAs (60 μg of each) were used to transfect NIH/3T3 cells and only pools 2 and 3 yielded transformed foci. It was found that transfectants derived using pool 2 DNA contained only the 8.8 kb *Eco*RI fragment harbouring a human repetitive sequence whilst pool 3 transfectants had both an 8.8 kb and a 7.8 kb *Eco*RI fragment. DNA isolated from the latter was then used in a third round of transfection yielding foci that still had the 7.8 kb fragment but no longer contained an intact 8.8 kb species. In ***C***, to map restriction sites in RD and normal DNA corresponding to those in the cloned fragment, DNA from all three sources (RD, HT1080, and normal) was digested with the relevant restriction enzymes (or combination of restriction enzymes) and analysed using Southern blots probed with a number of fragments derived from the cloned sequence. To map restriction sites outside of and to the right of the cloned sequence, DNA from HT1080, RD and normal DNA was digested with the relevant enzyme and probed on blots with probe A. This only allows identification of the nearest restriction site to the cloned fragment.

activated form of c-Ki-*ras*2[5]. Both the Kirsten and Harvey viral oncogenes show extensive homology at the protein and DNA level[18–20], and it is also apparent that c-Ha-*ras*1 and c-Ki-*ras*2, along with c-Ha-*ras*2 and c-Ki-*ras*1, are four members of a human *ras* gene family[11].

We have previously identified a transforming gene in two human sarcoma cell lines, HT1080 (a fibrosarcoma line) and RD (a rhabdomyosarcoma line) and have shown that this gene is neither c-Ha-*ras*1 nor c-Ki-*ras*2[4]. Here we report that this transforming gene is a new member of the human *ras* gene family located on chromosome one.

Molecular cloning

DNAs isolated from second-round NIH/3T3 transfectants derived from both HT1080 or RD contain an 8.8 kilobase (kb) *Eco*RI fragment that carries a human repetitive sequence and we have concluded that this fragment is closely linked to the transforming gene[4]. Accordingly a partial gene library was constructed in Charon 4A, using DNA isolated from a secondary HT1080 transfectant that had been digested with *Eco*RI and enriched for the 8.8 kb fragment by size fractionation. Recombinant phage carrying a human repetitive sequence were identified after screening the library with nick-translated total human DNA. One recombinant was further characterized and found to contain an 8.8 kb fragment. This was subcloned into pAT153[21] and a restriction map of the recombinant plasmid, pAT8.8, is shown in Fig. 1*A*. The clone contains two repetitive elements, as judged by hybridization to a total human DNA probe; one (contained within the black box, Fig. 1*A*) being present at around 10^5 copies and the other (within the hatched box, Fig. 1*A*) at around 10^4 copies, in the human genome. Unique sequences derived from the clone hybridized to an 8.8 kb *Eco*RI fragment in transfectants derived from both HT1080 and RD. Isoenzyme analysis has shown that these two human cell lines are different (S. Povey, personal communication), thereby demonstrating that the transforming gene in these two different cell lines is the same.

Boundary mapping

To determine which sequences, if any, within the cloned fragment are not required for biological activity, we used transfectants (derived from DNA that had been partially digested with *Sau*3A before transfection) which no longer show an intact 8.8 kb *Eco*RI fragment when hybridized to a total human DNA probe. *Eco*RI digests of DNA isolated from these transfectants were probed on Southern blots with sequences derived from the left-hand end (probe D, Fig. 1*A*) or the right-hand end (probe A, Fig. 1*A*) of the cloned fragment. The results obtained with one such transfectant are shown in Fig. 1*B*. As expected, both probes hybridize to an 8.8 kb *Eco*RI fragment in normal (lanes *f*, *g*) and RD (lanes *e*, *h*) DNA, and in HT1080 (lanes *d*, *i*) and RD (lanes *c*, *j*) transfectants that contain an intact 8.8 kb sequence. However, although probe D does cross-hybridize with some mouse sequences (lane *b*), no human sequences are contained in the transfectant lacking an intact 8.8 kb fragment (lane *a*). Probe A, on the other hand, clearly hybridizes to human sequences in this transfectant (lane *l*). The biologically active transforming gene must, therefore, be to the right of the *Bam*HI site in the cloned sequence. All transfectants so far analysed maintain the *Bgl*II site 2 (Fig. 1*A*).

Using probe A it was also possible by Southern blot analysis to map several restriction sites in HT1080 DNA located to the right of the cloned fragment. The results are shown in Fig. 1*C*; in particular there is a *Hin*dIII site, a *Bgl*II site and an *Sst*I site, 9.0, 8.0 and 5.5 kb, respectively, to the right of the cloned fragment. This same probe A was then used to screen all NIH/3T3 transfectants derived from HT1080 and RD. All maintained the *Pvu*II site mapped at approximately 0.5 kb into the uncloned segment (Fig. 1*C*), but three transfectants no longer had the *Sst*I site 5.5 kb away from the cloned sequence. We conclude, therefore, that the oncogene must be to the left of this *Sst*I site and is contained within a 13 kb stretch of DNA, 7.8 kb of which is carried by clone pAT8.8.

Comparison with its normal allele

Using a variety of probes derived from pAT8.8 it was possible to compare the restriction map of the transforming gene in HT1080 with that of RD and of DNA isolated from normal human fetal liver (Fig. 1*C*). No differences could be detected amongst any of the three loci. Furthermore, the intensity of hybridization of cloned probes with HT1080, RD and normal DNA (Fig. 1*B*, lanes *m*, *n*, *o*) show that there has been no detectable gene amplification in the tumour cell lines. Gene amplification does seem to have occurred in some (Fig. 1*B*, lanes *i*, *j*) transfectants, though we have not been able to correlate gene copy number with any altered biological features of the transfected cells.

RNA from the transforming gene

Figure 2 shows the result of a Northern blot of poly(A) selected mRNA hybridized with probe B (Fig. 1*A*). Three major species of RNA can be seen, with sizes of 5,800, 2,200 and 1,500 nucleotides, in HT1080 cells (lane *c*), and in both HT1080 (land *d*) and RD (lane *e*) transfectants. Probe C (Fig. 1*A*) also gave a pattern of hybridization on Northern blots similar to probe B except that a very dark background was apparent throughout the tracks containing RNA from the human cell lines (data not shown). This is presumably a consequence of the repetitive element present in this clone. Probe A showed no hybridization to the three major RNA species on Northern blots and also failed to cross-hybridize with sequences in mouse DNA (Fig. 1*B*, lane *k*). Both these observations are consistent with the fragment being part of an intron; such a sequence would not be present in mRNA and is likely to have diverged considerably between mouse and man.

Two pieces of information strongly indicate that the 2,200 nucleotide RNA species is the major transcript from the human transforming gene: (1) it is the only transcript absent from NIH/3T3 cells (lane *f*) and present in transfectants (lanes *d*, *e*); and (2) it is known that *Bgl*II, *Bam*HI[4] and *Sst*I (unpublished data) all destroy the transforming activity of HT1080 DNA. However, we have already shown that *Bgl*II sites 1 and 3 (Figs 1*A*, *C*) lie outside the transforming gene and therefore only *Bgl*II site 2, which is within probe B, can be located in the gene. Similarly, the *Sst*I site in the cloned fragment (Fig. 1*A*) and the *Bam*HI site in the uncloned segment of DNA (Fig. 1*C*) must lie within the transforming gene. The 2,200 nucleotide mRNA detected by probe B must, therefore, be derived from the transforming gene.

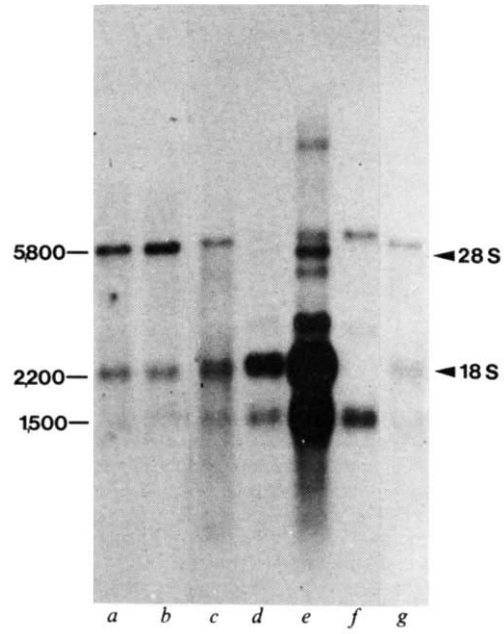

Fig. 2 Northern blots using probe B to identify the transforming gene transcript. Lanes are as follows: *a*, B6FS, a human fibrosarcoma cell line lacking a detectable transforming gene; *b*, normal human fibroblasts (FLOW 2002); *c*, HT1080; *d*, an HT1080 transfectant; *e*, an RD transfectant; *f*, NIH/3T3; g, EJ, a human bladder tumour line.

Methods: Cells were lysed in 10 mM Tris, *p*H 7.5, 0.1 M NaCl, 0.1 mM EDTA and 1% SDS, followed by sonication, phenol extraction and treatment with RNase-free DNase. Total RNA was passed once through an oligo(dT) cellulose column and poly(A) containing mRNA was eluted with water. mRNA (3 μg) samples were heated at 60 °C for 5 min in 50% de-ionized formamide, 2.2 M formaldehyde and 20 mM sodium phosphate *p*H 7.0 and then electrophoresed at 2 V cm^{-1} overnight through an 0.8% agarose gel containing 20 mM sodium phosphate, *p*H 7.0 and 2.2 M formaldehyde. The gel was blotted to nitrocellulose as described by Thomas[33] and the blot was hybridized with 50% formamide, 3×SSC and 10% dextran sulphate containing 10^6 c.p.m. ml^{-1} of nick-translated probe B at 42 °C for 20 h followed by washing with 0.1×SSC at 60 °C.

Finally, the amount of the 2,200 nucleotide transcript in normal human fibroblasts (Fig. 2, lane *b*) is similar to that in HT1080 cells (lane *c*) or in RD cells (data not shown). It is therefore unlikely that enhanced gene expression is the mechanism of activation of this oncogene. We have also detected this transcript in a human fibrosarcoma cell line, B6FS (lane *a*), which does not have a detectable transforming gene (unpublished results) and in EJ (lane *g*) which contains a different transforming gene, c-Ha-*ras*1.

Homology with *ras* gene family

We have shown that the transforming sequence present in the two human sarcoma cell lines does not have detectable homology to 15 cloned viral oncogenes (ref. 4 and unpublished results). Recently, however, Chang *et al.*[11] have identified, by probing human gene libraries with v-Ha-*as* and v-Ki-*ras* probes, four distinct members of a human *ras* gene family and in the conditions we used to analyse Southern blots of transfectants we would have detected only two of these—c-Ha-*ras*1

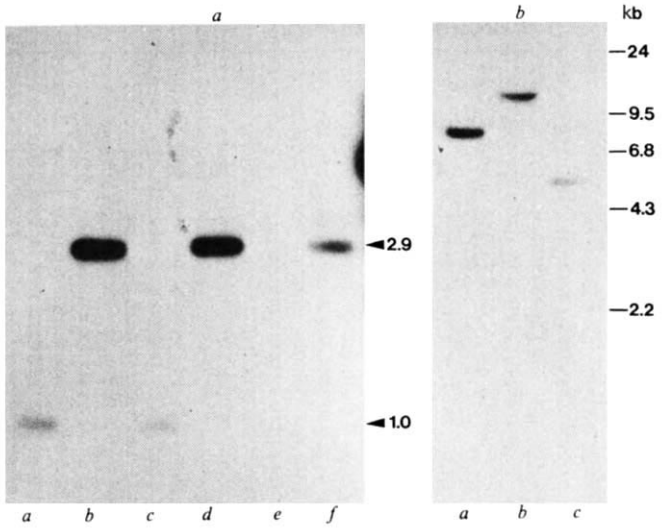

Fig. 3 ***A***, Detection of homology with the cloned v-Ki-*ras* and the human c-Ha-*ras*1 genes. Lanes *a*, *c*, *e*, 0.2 μg of *Eco*RI digested HiHi-3[20] (the v-Ki-*ras* oncogene is located on a 1.0 kb fragment); lanes *b*, *d*, *f*, 0.2 μg of *Sst*I digested pHa-1[11] (the c-Ha-*ras*1 gene is located on a 2.9 kb *Sst*I fragment). The blots were hybridized in 50% formamide, 3×SSC, 10% dextran sulphate and 10^6 c.p.m. ml^{-1} of nick-translated probe B at 42 °C for 20 h and washed as follows: lanes *a* and *b*, 0.6×SSC, 50 °C; lanes *c* and *d*, 0.6×SSC, 55 °C; lanes *e* and *f*, 0.6×SSC, 60 °C. ***B***, Analysis of the transforming gene present in HL60 and SK-N-SH cell lines. *Eco*RI digests of DNA derived from NIH/3T3 cell transformed by HT1080 (lane *a*), SK-N-SH (lane *b*) and HL60 (lane *c*) DNAs were electrophoresed through an 0.8% agarose gel and blotted onto nitrocellulose. The Southern blot was hybridized at 42 °C for 20 h with 50% formamide, 3×SSC, 10% dextran sulphate and 10^6 c.p.m. ml^{-1} of nick-translated probe A and then washed in 0.1×SSC at 60 °C.

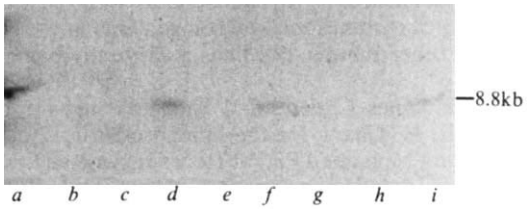

Fig. 4 Detection of the human N-*ras* gene in mouse: human cell hybrids. Southern blot of DNA from mouse:human hybrids hybridized with probe A. DNAs are as follows: lane *a*, human; *b*, mouse; *c*, CTP41P2; *d*, SIR19A; *e*, FIR5R3; *f*, CTP34B4; *g*, DUR4.3; *h*, 3W4C110; *i*, SIR7411. Hybridization conditions were as described in the legend to Fig. 3*B*.

and c-Ki-*ras*2. It was therefore important, now that a cloned segment of the sarcoma transforming gene had been obtained, to test again for homology with cloned *ras* probes. Figure 3*A* shows that in conditions of low stringency of washing, homology could be detected between probe B (Fig. 1*A*) and both v-Ki-*ras* (lanes *a*, *c*, *e*) and human c-Ha-*ras*1 (lanes *b*, *d*, *f*). After comparing the restriction maps of the sarcoma transforming gene with those published for the four known human *ras* genes[11] it is clear that the sarcoma transforming gene must be a novel member of the *ras* gene family, not previously identified. Furthermore, Southern blot analysis using various stringencies of washing reveals that the T_m for probe B: c-Ha-*ras*1 hybrids is about 60 °C at 0.09 M Na^+, and we estimate that the sarcoma transforming gene and the c-Ha-*ras*1 gene have diverged at roughly 15% of their nucleotide sequences. (For comparison, it is known from sequence data[18,19] that v-Ki-*ras* and v-Ha-*ras* differ at some 23% of their nucleotide protein-coding sequences.) Our failure to detect this transforming gene on Southern blots of DNA from HT1080 or RD transfectants using either v-Ha-*ras* or v-Ki-*ras* probes[4] can be ascribed to this divergence in DNA sequence. It is interesting that in addition to the transforming gene transcript two other species of RNA of 5,800 and 1,500 nucleotides are detected in both mouse and human cells (Fig. 2). These transcripts are almost identical in size to those observed by others using c-Ha-*ras*1 gene probes in human cells[6,8] and v-Ki-*ras* probes[22] in mouse cells. It is not clear which species are the authentic messages corresponding to the p21 proteins.

Recently a transforming gene has been cloned from HL60, a promyelocytic leukaemia line (J. M. Cunningham, M. J. Murray and R. A. Weinberg, personal communication) and from SK-N-SH, a human neuroblastoma line[23]. On the basis of *Alu* blots[1-4] these genes were previously thought to be distinct from each other and from the transforming gene described here. However, after exchanging clones with Cunningham, Murray and Weinberg, we and they have shown that the restriction maps of part of their HL60 clone and of our HT1080 clone are identical. Furthermore, probe A (Fig. 1*A*), which is probably an intron sequence and which does not cross-hybridize with any other sequence in mouse or human DNA, hybridizes with DNA isolated from HT1080, SK-N-SH or HL60 transfectants (Fig. 3*B*, lanes *a*, *b*, *c*, respectively). We conclude that the same transforming gene is activated in the four cell lines HT1080, RD, HL60 and SK-N-SH and after consultation with Wigler and with Weinberg it has been proposed that this gene be called N-*ras*.

Chromosomal location of N-*ras*

To determine the chromosomal location of the N-*ras* gene we made use of a series of mouse:human hybrid cell lines (Table 1), containing different human chromosomes. DNA was isolated from each cell line, digested with *Eco*RI and analysed an Southern blots. Probe A (Fig. 1*A*) was used as this was known not to cross-hybridize with mouse sequences. Figure 4 shows the results obtained with DNA from some of the hybrid cell lines. Out of 17 hybrid cell lines analysed, 7 contained chromosome 1 and hybridized with the N-*ras* probe (Table 1). Ten hybrids did not contain chromosome 1, and none of these hybridized with the probe. It is clear, therefore, that N-*ras* is located on chromosome 1. This is in contrast to the other members of the *ras* family which are located on human chromosomes 11 (c-Ha-*ras*1)[24], X (c-Ha-*ras*2)[25], 6 (c-Ki-*ras*1)[25] and 12 (c-Ki-*ras*2)[25].

Discussion

We have isolated a molecular clone containing part of the transforming gene previously identified by us in two human sarcoma cell lines, and shown it to be a novel member of the *ras* gene family. Various unique sequence probes derived from this clone have been used to construct a partial restriction map of the whole of the transforming gene, and its surrounding sequences, in HT1080, RD and in DNA isolated from normal cells. From these restriction patterns it is clear that the gene is not grossly rearranged in the tumour cell lines, and futhermore it appears that gene amplification is not required for its transforming activity. We have identified the major transcript of the transforming gene as being a 2,200 nucleotide species that is present at similar levels in the two sarcoma cell lines, in normal fibroblasts and in various other tumours either lacking a detectable transforming gene or containing a different transforming gene. Enhanced levels of transcription from this gene do not appear to be responsible for its transforming activity. From these results we would tentatively expect that the mechanism of activation of this gene will be an alteration in the nucleotide sequence leading to a change in the amino acid sequence of the gene product. Since we have also been able to show that this is a new member of the *ras* gene family (now called N-*ras*) the mechanism of activation described above is also suggested by analogy with the known mechanism of activation of another *ras* gene, c-Ha-*ras*1, in the human bladder tumour line EJ[13,14]. In the case of c-Ha-*ras*1 activation, a glycine residue at position 12 of the normal p21 protein has been altered to a valine

Table 1 Hybridization of an N-*ras* specific probe to DNA from mouse:human cell hybrids

Hybrid	Presence of 8.8 kb *Eco*RI fragment	Human chromosomes present in hybrid cell lines																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
CTP41p2	−			+			+	+							+		+	+			+			+
SIR19A	+	+	+	+		+		+					+		+	+			+	+		+		+
CTP34B4	+	+	+			+	+	+					tr	+	+		+	tr	+					+
DUR 4.3	−			+		+					+	+	+	+	+	+		+	+		+	+	+	+
3W4C110	−	tr									+	+	+		+							+		+
SIR7411	+	+	+	tr									+		+				tr			+	tr	+
C10b	−			+						+	+				+							+	+	
FIR5R3	−														+				+					
F4SC13C112	+	+								+					+									+
F4SC13C119	+	+													+									+
HORLI	−															+								
HORL9D2R1	−											+												
HORL9X	−																							+
Mog 13/9	−																							+
Mog 13/10	+	+	+												+							+	+	+
Mog 13/17	−																					+	+	+
Mog 13/22	+	+																				+	+	+

Most of the hybrids used in this study have been described by Heisterkamp *et al.*[34]. Exceptions are the Mog hybrids[35], CTP34B4[36], 3W4C110[37] and the SIR hybrids[38]. The human chromosome content of the hybrids was deduced by karyotypic[39,40], antigenic[41] and enzymatic analyses[42]. + Indicates the presence of a particular human chromosome; tr, the presence of part of a chromosome as a translocation.

residue. Furthermore, the first 36 amino acid residues of v-Ha-*ras*[18] and v-Ki-*ras*[19] are identical to those of the human c-Ha-*ras*1 protein[14] except that once again the amino acid at position 12 has been substituted—to arginine, in the case of v-Ha-*ras*, and to serine in v-Ki-*ras*. It remains to be seen whether the activation of N-*ras* in both HT1080 and in RD involves a similar alteration.

From these studies using transfection and those of others screening human libraries with v-Ki-*ras* and v-Ha-*ras* probes, five members of the human *ras* gene family have now been identified. c-Ha-*ras*1 and c-Ki-*ras*2 both encode p21 proteins[20], but it is not yet known whether the other members of the *ras* family also encode p21 proteins. The results of Northern blotting presented here and elsewhere[6] clearly indicate that N-*ras* and c-Ha-*ras*1 are expressed not only in normal fibroblasts but in many tumour cells. It appears that both gene products are required for normal cell growth and disturbances to either may lead to malignant transformation. It is interesting that although other members of the *ras* gene family which have been activated in human tumours are also found incorporated into animal retroviruses[12,16,26], no transforming retrovirus containing N-*ras* has yet been described.

Recently there has been great interest in the role of chromosomal rearrangements in activating cellular oncogenes, particularly c-*myc*[27–30] and c-*abl*[31]. We have shown that N-*ras* is located on chromosome 1. Alterations in chromosome 1, particularly trisomy, are frequently found in human tumours[32]; indeed HT1080 itself has a translocation involving chromosome 1 (B. Reeves, personal communication). Our results comparing restriction maps of N-*ras* in normal and in HT1080 DNA, however, show no rearrangements within 1 kb either side of this gene. Nevertheless, it is possible that certain mechanisms of activation of cellular oncogenes, for instance chromosomal rearrangements, may have led to genetic changes in tumours that are not detectable by transfection into NIH/3T3 cells. The possible role of chromosomal rearrangements in the activation of N-*ras* in other tumour cell lines is currently being investigated.

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Note added in proof: We have recently cloned the sequence downstream of the 8.8 kb *Eco*RI fragment of HT1080. *In vitro* ligation of cloned sequences stretching from the *Bam*HI site in pAT8.8 through to the *Sst*I site in the previously uncloned segment (Fig. 1*C*) efficiently transform NIH/3T3 cells as predicted in this article.

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1. Murray, M. J. *et al. Cell* **25,** 355–361 (1981).
2. Perucho, M. *et al. Cell* **27,** 467–476 (1981).
3. Lane, M. A., Sainten, A. & Cooper, G. M. *Cell* **28,** 873–880 (1982).
4. Marshall, C. J., Hall, A. & Weiss, R. A. *Nature* **299,** 171–173 (1982).
5. Pulciani, S. *et al. Nature* **300,** 539–542 (1982).
6. Goldfarb, M., Shimizu, K., Perucho, M. & Wigler, M. *Nature* **296,** 404-409 (1982).
7. Shih, C. & Weinberg, R. A. *Cell* **29,** 161–169 (1982).
8. Santos, E. *et al. Nature* **298,** 343–347 (1982).
9. Parada, L. F., Tabin, C. J., Shih, C. & Weinberg, R. A. *Nature* **297,** 474–478 (1982).
10. Der, C. J., Krontiris, T. E. & Cooper, G. M. *Proc. natn. Acad. Sci. U.S.A.* **79,** 3637–3640 (1982).
11. Chang, E. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 4848–4852 (1982).
12. Ellis, R. W. *et al. J. Virol.* **36,** 408–420 (1980).
13. Tabin, C. J. *et al. Nature* **300,** 143–149 (1982).
14. Reddy, E. P., Reynold, R. K., Santos, E. & Barbacid, M. *Nature* **300,** 149–152 (1982).
15. Taparowsky, E. *et al. Nature* **300,** 762–765 (1982).
16. Kirsten, W. H. & Myer, L. A. *J. natn. Cancer Inst.* **39,** 311–335 (1967).
17. Der, C. J. & Cooper, G. M. *Cell* **32,** 201–208 (1983).
18. Dhar, R. *et al. Science* **217,** 934–937 (1982).
19. Tsuchida, N., Ryder, T. & Ohtsubo, E. *Science* **217,** 937–939 (1982).
20. Ellis, R. W. *et al. Nature* **292,** 506–511 (1981).
21. Twigg, A. J. & Sherratt, D. *Nature* **283,** 216–218 (1980).
22. Ellis, R. W., Defeo, D., Furth, M. E. & Scolnick, E. M. *Molec. Cell Biol.* **2,** 1339–1345 (1982).
23. Shimizu, K., Goldfarb, M., Perucho, M. & Wigler, M. *Proc. natn. Acad. Sci. U.S.A.* **80,** 383–387 (1983).
24. McBride, O. W. *et al. Nature* **300,** 773–774 (1982).
25. O'Brien, S. J., Nash, W. G., Goodwin, G. L., Lowy, D. R. & Chang, E. *Nature* **302,** 839–842 (1983).
26. Anderson, P. R. *et al. Cell* **26,** 129–134 (1981).
27. Taub, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 7837–7841 (1982).
28. Dalla-Favera, R. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 7824–7827 (1982).
29. Crews, S. *et al. Science* **218,** 1319–1321 (1982).
30. Sheng-Ong, G. L. C., Keath, E. J., Piccoli, S. P. & Cole, M. D. *Cell* **31,** 443–452 (1982).
31. DeKlein, A. *et al. Nature* **300,** 765–767 (1982).
32. Rowley, J. D. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5729–5733 (1977).
33. Thomas, P. S. *Proc. natn. Acad. Sci. U.S.A.* **77,** 5201–5205 (1980).
34. Heisterkamp, N. *et al. Nature* **299,** 747–749 (1982).
35. Povey, S. *et al. Ann. hum. Genet.* **43,** 241–248 (1980).
36. Jones, E. A. *et al. Somatic Cell Genet.* **2,** 483–496 (1976).
37. Nabholz, M., Miggiano, V. & Bodmer, W. F. *Nature* **223,** 358–363 (1969).
38. Whitehead, A. S. *et al. Proc. natn. Acad. Sci. U.S.A.* **79,** 5021–5025 (1982).
39. Bobrow, M. & Cross, J. *Nature* **251,** 74–79 (1974).
40. Casperson, T., Lomakka, G. & Zech, L. *Hereditas* **67,** 89–102 (1971).
41. Goodfellow, P. N. & Solomon, E. in *Monoclonal Antibodies in Clinical Medicine* (eds McMichael, A. & Folve, J.) 365–393 (Academic, London, 1982).
42. Harris, H. & Hopkinson, D. A. *Handbook of Enzyme Electrophoresis in Human Genetics* (North-Holland, Amsterdam, 1976).