are not defined biochemically, linked restriction polymorphisms could be used to screen for diseases such as Duchenne muscular dystrophy (DMD), Huntington's chorea or cystic fibrosis[25,26]. Polymorphic probes showing linkage to DMD have recently been described[26,27] and have proved important in confirming the localization of the Duchenne locus in the Xp21 region, and for analysing X-linked myopathies having variant clinical features[28]. Their diagnostic value is limited, however, as they are only loosely linked to the disease locus (≈17 centimorgans). Other potentially useful linkages are those of G6PD with haemophilia A[17,29], adenoleukodistrophy[30] and the fragile X syndrome[10]. Especially in the latter case, the distance between the marker and the disease locus needs to be estimated more precisely. Furthermore, no polymorphism has yet been reported for the cloned G6PD sequences[31] (at the protein level, this marker is only polymorphic in certain populations). At present, the factor IX marker seems to offer the best prospects for use in screening of the fragile X syndrome, and we have already demonstrated in two cases a carrier status which had been overlooked on the basis of the cytogenetic analysis. More studies are needed to refine the linkage data and to find additional polymorphisms associated with the factor IX probe. In conjunction with an informative marker on the other side of the fragile X locus, this would provide a reliable and generally usable screening test.

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A yeast gene encoding a protein homologous to the human c-*has*/*bas* proto-oncogene product

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Organisms amenable to easy genetic analysis should prove helpful in assessing the function of at least those proto-oncogene products which are highly conserved in different eukaryotic cells. One obvious possibility is to pursue the matter in *Drosophila melanogaster* DNA, which has sequences homologous to several vertebrate oncogenes[1–3]. Another is to turn to the yeast *Saccharomyces cerevisiae*, if it contains proto-oncogene sequences. Here we report the identification of a gene in *S. cerevisiae* which codes for a 206 amino acid protein (YP2) that exhibits striking homology to the p21 products of the human c-*has*/*bas* proto-oncogenes[4,5] and the transforming p21 proteins of the Harvey (v-*ras*^{H})[6] and Kirsten (v-*ras*^{K})[7] murine sarcoma viral oncogenes. The YP2 gene is located between the actin[8,9] and the tubulin gene[10] on chromosome VI[11] and is expressed in growing cells. The protein it encodes might share the nucleotide-binding capacity of p21 proteins[22–24].

Previously we have isolated two recombinant plasmids (pYA102 and pYA208) containing overlapping DNA fragments which harbour the yeast actin gene[8,9]. In studying the region 5′ to the actin gene we detected another gene whose transcription product is a polysomal RNA coding for a protein of ~20,000 molecular weight (MW)[8]. This protein, provisionally termed 'protein 2', will be subsequently referred to as YP2 protein.

The location of the YP2 coding region was mapped with neighbouring DNA fragments which were ^{32}P-nick-translated and used to identify the YP2 mRNA on RNA blots. A 2.18-kilobase (kb) *Hin*dIII–*Pst*I fragment spanning the region from the actin gene codon 260 to nucleotide 960 upstream from the actin mRNA cap site[9,12] (fragment A in Fig. 1) and a 0.79-kb *Xho*I–*Hin*cII fragment originating in the actin gene intron and ending 577 base pairs (bp) 5′ to the actin mRNA start site (fragment C in Fig. 1), hybridized to the 1,430-nucleotide actin mRNA and to the 830-nucleotide YP2 mRNA (as shown in Fig. 2). A 1.72-kb *Hin*dIII–*Pst*I fragment with the *Hin*dIII restriction site residing in codon 391 of the β-tubulin gene[10] hybridized, in addition to the tubulin gene transcript of about 1,600 nucleotides, also to the YP2 mRNA (Fig. 2). These results established that the unique *Pst*I and *Hin*cII restriction sites must reside within the YP2 gene.

The nucleotide sequence of the YP2 gene region was determined by the chemical sequencing method of Maxam and Gilbert[13] and is presented in Fig. 3. Screening for possible reading frames on both strands led to the identification of only one open frame for a protein of about 20,000 MW. The second longest continuous stretch not interrupted by stop codons could code for a protein of only 104 amino acids. Because we found no indication for the presence of an intervening sequence within the sequenced region, neither by searching for an unspliced transcript in the temperature-sensitive splice-defective yeast *rna-2* mutant[14,15] nor by the existence of a TACTAACA sequence which is a common structural feature of introns in all yeast polymerase II-transcribed genes[16], these results established that the protein YP2 is 206 amino acid residues long (including the initiating methionine) and has a MW of 23,213.

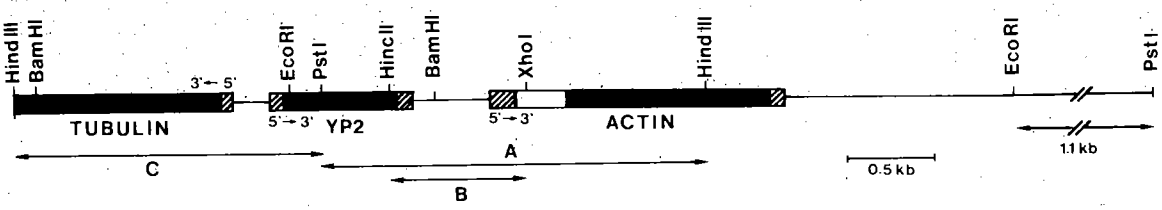

Fig. 1 Location of the YP2 gene between the actin and the β-tubulin gene on two overlapping DNA fragments cloned in the recombinant plasmids pYA102 and pYA208[8]. The direction of transcription of the three genes is shown by small arrows. The fragments (A, B and C) used to locate the YP2 gene (see Fig. 2) are indicated. Only those restriction endonuclease cutting sites mentioned in the text are shown. Solid blocks, exon; open blocks, intron; hatched blocks, untranslated regions.

Visual inspection of the YP2 amino acid sequence revealed a striking homology with the p21 proteins encoded by the human c-*has/bas* proto-oncogene[4,5] and their viral relatives, v-*ras*^{H} (ref. 6) and v-*ras*^{K} (ref. 7). A computer-assisted comparison of the 206-residue YP2 and the 189-residue p21 proteins established that a homology of 38% exists between residues 9–171 of the YP2 and residues 4–165 of the human p21 protein with only three one-residue gaps. The homology was statistically highly significant by alignment matrix techniques[17,18]. Sequence alignment showed (Fig. 5) four blocks of five to eight residues which match perfectly. In addition, many changes between the two sequences are conservative with respect to the chemical properties of the amino acid side chains and to the observed evolutionary amino acid replacements. The striking homology does not extend much further than the glutamine residue in positions 171 and 165 of the YP2 and the p21 protein, respectively. Interestingly, it is this residue which separates the nearly identical 165-residue segment of the transforming p21 proteins encoded by v-*ras*^{H} (ref. 6) and v-*ras*^{K} (ref. 7) viral oncogenes from their more divergent carboxyl terminal region of 24 amino acids.

The amino acid sequence homology near positions where single point mutations are responsible for the transforming ability of altered c-*ras* genes[6,7,19,20] is particularly strong. Gly 12 of the normal human c-*has/bas* proto-oncogene product corresponds to Ser 17 of YP2; p21 v-*ras*^{K} also has Ser, v-*ras*^{H} has Arg and the activated c-*has/bas* gene products of T24 and EJ bladder carcinoma have Val in this position. Gln 61 of the normal c-*has/bas* proto-oncogene-encoded protein is preserved in YP2 as Gln 67 where Leu 61 is responsible for the activation of the Hs242 oncogene in a human lung carcinoma-derived cell line[21]. Thr 59 which is phosphorylated in p21 v-*ras*^{H} and v-*ras*^{K} (refs 22–24) is Ala 65 in YP2, similar to Ala 59 of the normal c-*has/bas* product.

The 5′ and 3′ ends of the YP2 mRNA were mapped by S_1 nuclease protection experiments[25,26] (data not shown) and it was found that there are two major cap sites, 34 and 84 nucleotides upstream from the AUG initiation codon, and seven termination sites all of which are A residues (as indicated in Fig. 3). The termination sites are preceded by three putative polyadenylation signal sequences, a situation reminiscent of the *S. cerevisiae* actin gene[12].

The YP2 gene has the same orientation as the actin gene whereas the β-tubulin gene, which was recently sequenced by Neff *et al.*[10], is transcribed in the opposite direction to the YP2 gene. According to our S_1 nuclease mapping experiments, the tubulin mRNA, like the YP2 mRNA, has two major cap sites and only 195 bp separate the most distant 5′ start sites of the two mRNAs (Fig. 3). An interesting feature of the YP2–tubulin

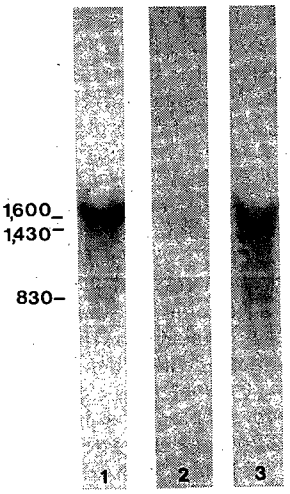

Fig. 2 Mapping of the YP2 gene through the identification of YP2 mRNA on Northern blots. The DNA fragments A, B and C (shown in Fig. 1) were nick-translated with [α-^{32}P]dCTP and hybridized to total cellular RNA after it had been glyoxylated, separated on 1.5% agarose gels and transferred to nitrocellulose filters as described previously[16]. Hybridization was to fragment C (lane 1), fragment B (lane 2) and fragment A (lane 3). The lengths (in nucleotides) of the hybridizing RNAs are indicated on the left margin.

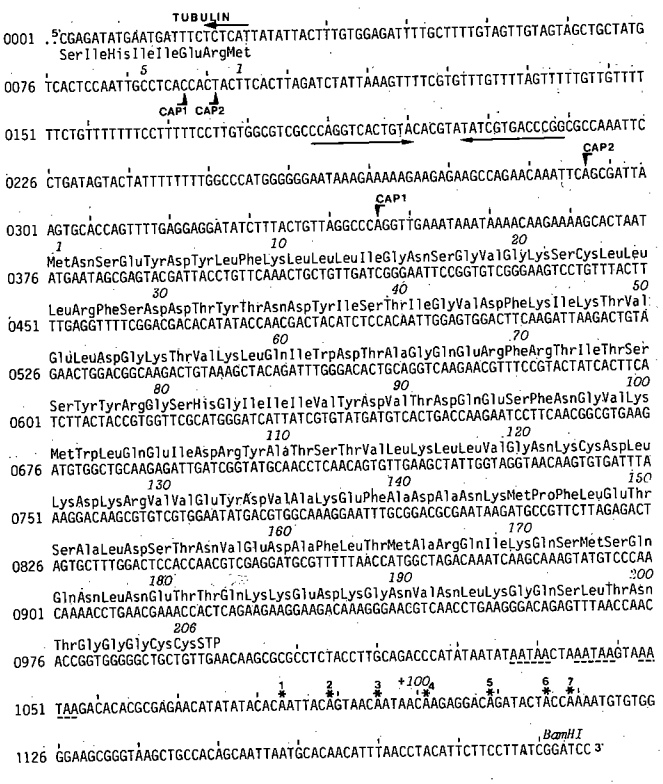

Fig. 3 Nucleotide sequence of the YP2 gene and the YP2–tubulin inter-gene region. The two major cap sites of the YP2 and the tubulin mRNAs are indicated by arrowheads. Note the opposite orientation of the YP2 and tubulin gene. The multiple termination sites of the YP2 mRNA are indicated with asterisks; the presumptive polyadenylation signal sequences are underlined. The inverted repeat is shown by arrows.

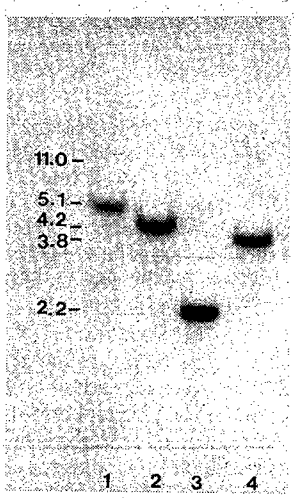

Fig. 4 Southern blot analysis to determine the YP2 gene copy number. DNA from the haploid yeast strain A364A[14], which was also used to isolate the YP2 gene[8], was digested to completion with the restriction endonucleases *Pst*I (lane 1), *Eco*RI (lane 2), *Bam*HI (lane 3) or *Hin*dIII (lane 4) and 10 μg of DNA were separated on a 0.9% agarose gel in 89 mM Tris-borate, 1 mM EDTA, *p*H 8.3 (ref. 31). The fragments were transferred bidirectionally to nitrocellulose filters[32]. Hybridization was performed at 68 °C in 3×SSC, 50 mM sodium phosphate *p*H 6.8, 2 mM EDTA, 2×Denhardt's solution, 50 μg ml^{-1} sonicated, denatured calf thymus DNA and 4×10^5 c.p.m. ml^{-1} of denatured, ^{32}P-nick-translated 526 bp *Eco*RI/*Hin*cII fragment (specific activity 10^8 d.p.m. μg^{-1}) from the YP2-coding region (see Fig. 1). Filters washed at 65 °C and either 1×SSC or 0.1×SSC gave essentially the same labelled fragments. The lengths (in kb) of the hybridizing fragments are shown on the left margin.

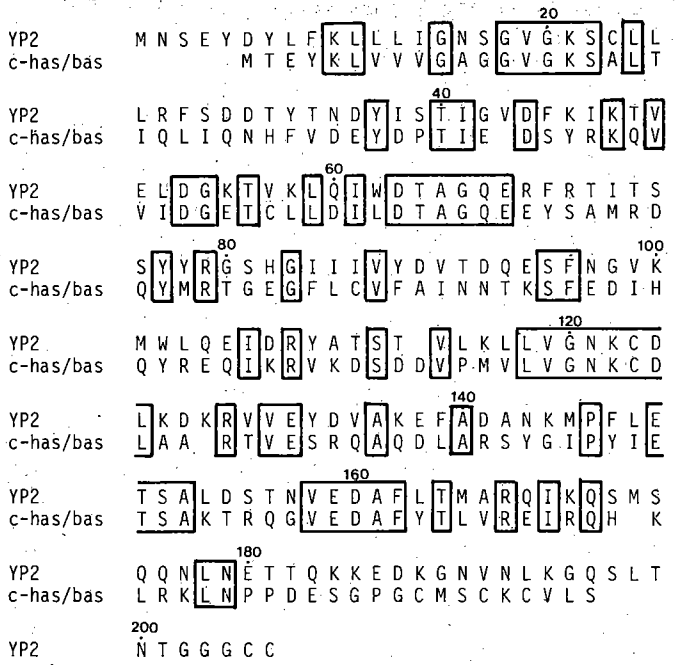

Fig. 5 Alignment of the amino acid sequences of the YP2 and the p21 protein as deduced from the nucleotide sequences of the respective genes. The human c-*has/bas* sequence is from refs 4 and 5. Identical residues are boxed. The residues of the YP2 protein are numbered beginning with the initiating methionine (see Fig. 3). Sequences are given in the one letter code.

intergene region is an almost perfect inverted repeat of 13 nucleotides within the centre of this 195-bp segment (Fig. 3). The repeat could be folded into an energetically stable stem and loop structure ($\Delta G = -10.6$ kcal mol^{-1})[27] and it is tempting to speculate that this hairpin structure might be involved in a bidirectional transcription regulation of the YP2 and the tubulin gene.

The YP2 gene copy number was determined with a ^{32}P-nick-translated 526-bp *Eco*RI/*Hin*cII fragment (including codons 16–190) which was hybridized to restriction endonuclease-digested *S. cerevisiae* DNA on Southern blots. As shown in Fig. 4, only one hybridizing fragment was detected with either *Bam*HI-, *Hin*dIII-, *Eco*RI- or *Pst*I-digested DNA. The lengths of these fragments were those predicted from the known location of restriction sites within or adjacent to the YP2 gene (see Fig. 1). As the *Pst*I cutting site lies in the coding segment used as hybridization probe, two hybridizing *Pst*I fragments were expected. Only the known 5.1-kb fragment cloned in the plasmid pYA208 (see Fig. 1) displayed a significant hybridization signal, but longer exposure of the autoradiogram shown in Fig. 4 gave a second hybridizing fragment of about 11 kb (from which only 145 bp were contained in the hybridization probe). As the same Southern blot results were obtained in stringent and less stringent filter washing conditions (65 °C and either 0.1×SSC or 1×SSC), we conclude that there is only one copy of the YP2 gene per haploid genome.

Since any two proteins with more than, say, 30% sequence homology over more than 100 residues have essentially equal secondary and tertiary structure[33], we propose that the YP2 protein of yeast and the c-*ras* and v-*ras* p21 proteins of vertebrates have similar function, but perhaps altered specificity. The functions of p21 proteins are not known, but it has been established that they bind GTP[22–24]. Since YP2 is strongly conserved relative to the *ras* gene products in the region predicted to contribute to nucleotide binding[28,29], YP2 is also likely to bind GTP. However, binding motifs of YP2 are far from perfectly matched to the consensus sequence. For example, there is sequence agreement with the predicted nucleotide-binding site of bovine ATPase (β-subunit), but it is essentially limited to Lys-Leu-X-Leu-X-Gly-X-X-Gly-Val-Gly-Lys-Ser where Val/Leu and Ser/Thr are counted as identities. The best working hypothesis for mutational tests of nucleotide binding remains, however, the region around Gly 12 (p21 *ras*)/Ser 17 (YP2). Gln 61 (p21 *ras*)/Gln 67 (YP2) may also be near the nucleotide-binding site in the tertiary structure, consistent with the observed point mutation of the activated Hs242 c-*has/bas*[21].

Focusing on the two transformational hotspots of p21, it is interesting that YP2 protein shares a glutamine 61 with human proto-oncogene derived p21 but shares a serine 12 with the transforming v-*ras*^{K} p21, suggesting that normal cellular function is possible in spite of the Gly→Ser replacement, contrary to the prediction of Wierenga and Hol[29]. Alternatively, the functional specificity of the YP2 protein in fast-growing yeast cells may be like that of the viral oncogene-derived protein in transformed vertebrate cells.

As mentioned above, the inverted repeat structure found about 80 bp in front of the 5′ start sites of the YP2 and the tubulin mRNAs might be involved in a bidirectionally acting common regulation of their two genes. Taking this as a working hypothesis (which is testable by mutating or deleting this sequence segment), one function of the YP2 could be viewed as a GTP-binder interacting with tubulin in the tubulin assembly process which is GTP-dependent (for review see ref. 30). Alternatively, it could function as a phosphokinase or as part of a ATPase or GTPase, as suggested by viral p21s on the basis of sequence homology with *Escherichia coli* ATPase β-subunit[28]. In this context it is worth mentioning that viral p21s autophorphorylate the threonine in position 59[22].

Whatever the biological function of this protein, its conservation from yeast to man points to it having an elementary physiological role in all eukaryotic cells. Since there is only one copy of the YP2 gene in the yeast genome which lends itself to relatively easy genetic manipulation, there is hope that the biochemical function of the protein can be elucidated in this unicellular organism.

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ras-Related gene sequences identified and isolated from *Saccharomyces cerevisiae*

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The oncogenes of Harvey and Kirsten murine sarcoma viruses (v-*ras*^{H} and v-*ras*^{K}) and their cellular homologues (c-*ras*^{H} and c-*ras*^{K}) constitute two members of the *ras* gene family. Each functional member of the *ras* gene family encodes a 21,000 molecular weight protein (p21^{ras})[1]. *ras* genes have been detected in a wide variety of vertebrate species[2], including *Xenopus laevis* (R. E. Steele, personal communication), and in *Drosophila melanogaster*[3]. We report here the detection of *ras*-related genes in the yeast *Saccharomyces cerevisiae*, and the isolation of two *ras*-related molecular clones, c-*ras*^{sc}-1 and c-*ras*^{sc}-2, from the DNA of *Saccharomyces*. Both c-*ras*^{sc}-1 and c-*ras*^{sc}-2 hybridize specifically to probes prepared from mammalian *ras* DNA. Sequencing of c-*ras*^{sc}-1 reveals extensive amino acid homology between the protein encoded by c-*ras*^{sc}-1 and the p21 encoded by c-*ras*^{H}. Our studies suggest that these clones can be used to elucidate the normal cellular functions of *ras*-related genes in this relatively simple eukaryotic organism.

The transforming genes of Harvey and Kirsten murine sarcoma viruses have been named *ras* oncogenes because these two oncogenic retroviruses were originally isolated by passage of murine leukaemia viruses in rats[1]. The *ras* oncogene of Harvey murine sarcoma virus has been named v-*ras*^{H} and that of Kirsten murine sarcoma virus has been named v-*ras*^{K} (ref. 4). Recently, mutated forms of c-*ras*^{H} and c-*ras*^{K} have been identified in certain human cancers and in cell lines derived from these cancers[5]. An additional member of the *ras* gene family, called n-*ras*, has also been found as an activated oncogene in a human neuroblastoma[6].

A moleculary cloned 1.0-kilobase pair (kbp) v-*ras*^{H}-specific DNA probe was used in relaxed hybridization conditions to detect *ras*-related sequences by Southern blot analyses of *Eco*RI- and *Xba*I-digested yeast genomic DNA (Fig. 1*a*, lanes 1, 2). Two major bands of approximately 8.0 and 3.8 kbp were detected in the *Eco*RI-digested DNA. A minor band of approximately 1.8 kbp was also detected. However, the hybridization of v-*ras*^{H} to this minor band has not been consistently reproducible.

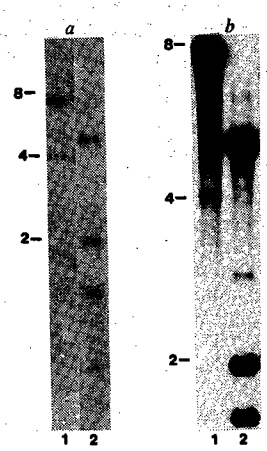

Fig. 1 Southern blot analysis of restriction endonuclease-digested high molecular weight DNA prepared from the yeast *S. cerevisiae*[12]. Haploid genomic DNA (20 μg) was digested with the enzymes *Eco*RI (lane 1) and *Xba*I (lane 2), electrophoresed in agarose gels, transferred to nitrocellulose filters and hybridized to ^{32}P-labelled nick-translated probes prepared from either v-*ras*^{H}-specific cloned DNA (plasmid clone I4)[13] (*a*) or the yeast-derived *ras*^{H}-reaction clone c-*ras*^{sc}-1 (*b*) (this clone was isolated as described in Fig. 2 legend). The autoradiogram in *a* represents an 18-h exposure. Approximate fragment sizes are indicated in kbp.
Methods: Hybridizations were performed at 42 °C for 24–36 h in a solution containing 40% formamide; 6×SSC (1×SSC = 0.015 M sodium citrate, 0.15 M sodium chloride), 4×Denhardt's containing 0.8% each bovine serum albumin, Ficoll and polyvinylpyrrolidine; 0.1% SDS; and 1 mg ml^{-1} low molecular weight salmon tests DNA. The filters were washed sequentially, three times each for 20 min, in 2×SSC, 1×SSC and 0.5×SSC with 0.1% SDS at 22 °C and 42 °C, respectively.

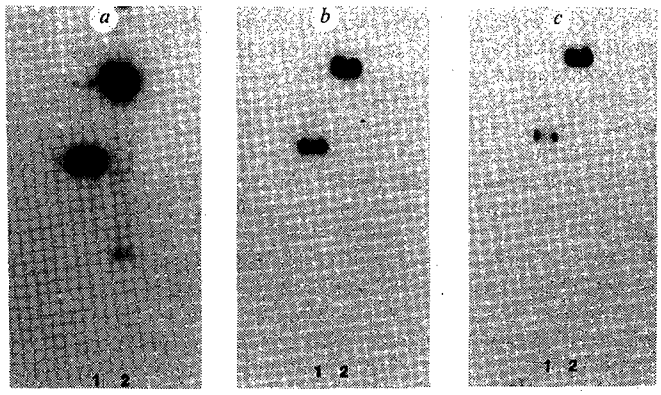

Fig. 2 Effect of hybridization conditions on homology between v-*ras*^{H} and yeast clones c-*ras*^{sc}-2 and c-*ras*^{sc}-1. The two *ras*^{H}-reactive yeast clones, c-*ras*^{sc}-2 (lane 1) and c-*ras*^{sc}-1 (lane 2), were isolated from a partial *Eco*RI library of yeast DNA cloned into the bacteriophage vector λgtwesλB (gift of Dr L. Schultz). *a*, 55 °C hybridization, 60 °C washes. *b*, 60 °C hybridization, 60 °C washes. *c*, 60 °C hybridization, 65 °C washes. **Methods:** Phage DNAs were prepared according to published procedures[14], digested with the restriction endonuclease *Eco*RI to release the inserts, electrophoresed on agarose gels, transferred to nitrocellulose filters and hybridized to ^{32}P-labelled v-*ras*^{H}-specific cloned DNA. The hybridization solution (4×SSC, 1×Denhardt's, 0.1% SDS and 1 mg ml^{-1} low molecular weight DNA) and wash solutions (1×SSC, 0.1% SDS and 0.1×SSC and 0.1% SDS, respectively) used in each case were the same; however, the temperature of hybridization and/or washes was elevated in order to increase the stringency conditions.

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