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ONCOGENE RELATED SEQUENCES IN FUNGI: LINKAGE OF SOME TO ACTIN

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SUMMARY We used v-ras^{Ki}, v-bas, Ras-1, v-mos and v-abl DNA fragments as probes to detect homologous sequences in genomic DNA from a variety of fungi. Cellular homologs were identified in most of them and the number of related fragments detected varied with the probe used. In addition, we found that some onc gene homologs were linked to actin-related sequences.

Cellular homologs of various viral oncogenes have been isolated and characterized from several vertebrates including man (1-5). More recently, ras oncogene related sequences were found in yeasts (6-9) and Drosophila (10). With the objective of cloning such sequences and studying their role in lower eukaryotes, we screened a number of yeast and filamentous fungi with ras probes including for the first time v-mos and v-abl sequences. Since a yeast gene coding for a protein homologous to the p21 product of the human has/bas proto-oncogene has been shown to be linked to actin and β-tubulin genes (9), we used an actin gene probe to examine possible linkage with the homologs detected with the v-onc probes.

MATERIALS AND METHODS

DNA (≃50 kbp) was isolated from mid-log vegetatively growing yeast (11) or mycelia (12). Samples were digested with the appropriate restriction endonucleases and electrophoresed in 0.8% agarose gels and transferred to nitrocellulose filters by the Southern method (13). The filters were prehybridized and hybridized under relaxed conditions and washed as described earlier (5). The v-onc sequences used as probes were v-bas from Balb-murine sarcoma virus, v-mos from Moloney murine sarcoma virus, v-abl from Abelson murine leukemia virus, v-ras^{Ki} derived from Kirsten murine sarcoma virus and Ras-1 from Saccharomyces cerevisiae. The v-ras^{Ki} fragment was purchased from Oncor, Inc. (Gaithersburg, Maryland). The actin sequence was from Dictylostelium discoideum (14). All probes were released from their plasmid vectors by digestion with appropriate endonucleases, purified and nick translated (15) to about 10^8 CPM/μg.

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RESULTS

v-onc gene related sequences: A v-ras^{Ki} oncogene sequence was first used to probe filters carrying HindIII and EcoRI digests of DNA from several yeasts and filamentous fungi. As shown in Fig. 1 and Table I, homologous sequences were present in almost all DNAs tested. Some species (e.g. A. niger, C. ulmi, lanes a,c) gave complex hybridization patterns consisting of several bands with variably high strength signals. Other species (e.g. A. oryzae, P. tannophilus, S. castellii, S. occidentalis, S. pombe and S. commune, lanes b,e,h,i, k,l,n) gave simple patterns of one to three bands with weak signals. In yet others (e.g. C. ishiwadae, P. stipidis, S. alluvius, S. cerevisiae and S. commune, lanes d,f,g,j,m), the pattern was simple with relatively strong signals. The use of similar DNA loads should have eliminated major differences in signal strength and complexity. The experiments with other DNA probes support this view. In at least one case, the signal strength was enhanced by use of an alternative restriction site (cf. lanes k,l and m,n). Since the target size of the v-ras^{Ki} probe is small (0.6 kbp) and contains neither HindIII nor EcoRI sites, it appears that the bulk of the fragments detected here may be-

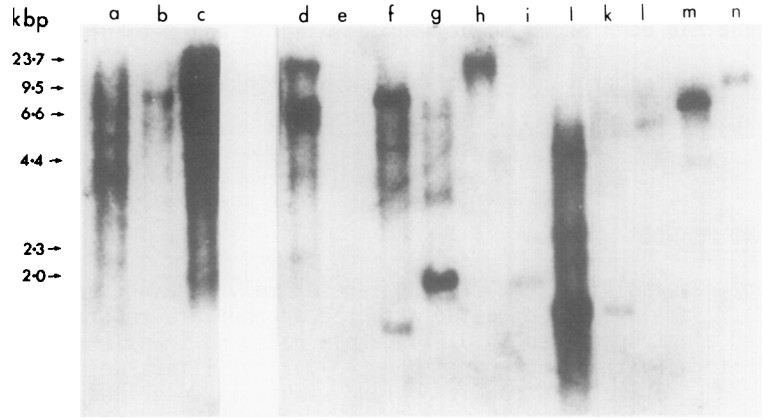

Fig. 1. v-ras^{Ki} related sequences in fungi.
A ^{32}P-labelled 0.6 kbp Sst II-Hinc II fragment containing the onc sequence was hybridized at low stringency (5) to 10 µg of Hind III (a-j,l,n) or EcoR I (k,m) DNA from a) Aspergillus niger, b) Aspergillus oryzae, c) Ceratocystis ulmi, d) Candida ishiwadae, e) Pachysolen tannophilus, f) Pichia stipidis, g) Schwanniomyces alluvius, h) Schwanniomyces castellii, i) Schwanniomyces occidentalis, j) Saccharomyces cerevisiae, k,l) Schizosaccharomyces pombe, m,n) Schizophyllum commune. → indicates position of λDNA Hind III fragments used as markers.

Table I. Summary of oncogene and actin related DNA sequences in yeast

DNA Source	DNA fragments (kbp) identified with gene probes of					
	v-ras^{Ki}	v-bas	Ras-1	Actin	v-mos	v-abl
S. cerevisiae	5.2,2.8, 2.2,1.8	10,6.7,5.7, 4.5,2.8, 2.4,1.8	6.6,2.4, 1.8	3.8,2.4	7.4,4.6	6.4,3.8, 3.3,3.1
S. alluvius	6.8,3.5, 2.1	14,11,5.5	9,8,2.1	≈14	4.7	8.8,7.2, 5.8
C. ishiwadae	22,7.8,6	≈22,8	10.5,7	10.5,5.6	4.8	11,8.2,7, 5.7
S. pombe	6.8,(1.6*)	13	7.6,1.6	7.6	5.0	13

Data based on HindIII DNA digests in Figs. 1-4 (*) EcoR-1 digested DNA.
kbp = kilobase pairs.

long to a family of related genes as reported in Drosophila, where different small onc gene probes were used (16).

Focusing mainly on yeasts, the v-bas probe gave strong signals with S. alluvius, S. cerevisiae and C. ishiwadae (Fig. 2A, lanes a-c, Table I). The

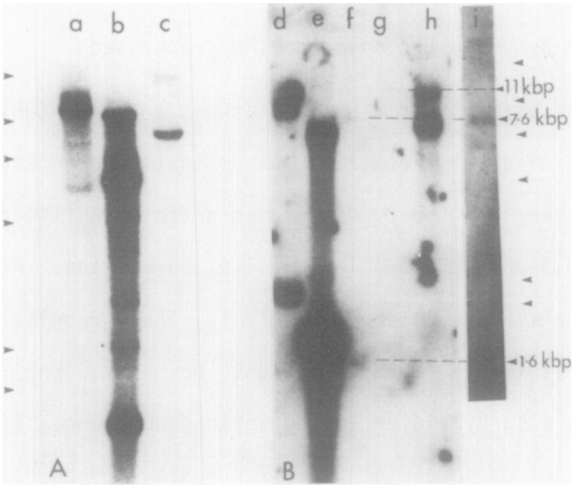

Fig. 2. Fungal DNA homologous to v-bas (A) and Ras-1 (B).
^{32}P-labelled fragments containing v-bas (0.67 kbp HindIII-BamHI) and Ras-1 (1.7 kbp HindIII-HindIII) were used to probe HindIII DNA of a,d) S. alluvius, b,e) S. cerevisiae, c,h) C. ishiwadae. f.i) S. pombe, g) S. commune. Lane i, a longer exposure of f. →, λDNA HindIII fragments.

largest number of fragments were detected in S. cerevisiae. We note that S. pombe gave a single weak band at 13 kbp. A band of similar intensity was also detected at 23 kbp for S. commune (data not shown). Results with the Ras-1 gene are shown in Fig. 2B, lanes d-i and Table I. As expected (6), S. cerevisiae gave two strong bands (6.6 kbp and 1.8 kbp). A weaker band at 2.4 kbp, not reported earlier may be a partial digestion product. Both S. alluvius and C. ishiwadae gave bands of similar intensities to those of S. cerevisiae but substantially greater than the 7.6 kbp and 1.6 kbp bands of S. pombe (lanes f and i). An even weaker band of 11-12 kbp was found in S. commune (lane g).

As it was evident that sequences homologous to the ras group of oncogenes were well conserved in various fungi, we wanted to examine whether these DNAs contained v-mos and v-abl related homologs. As shown in Fig. 3 (lanes a-d), v-mos related sequences exist in some species as a relatively unique band between 4.5 and 5.0 kbp in size. However, some yeasts (S. cerevisiae and C. ishiwadae) also have at least an additional weak band at 7.4 kbp. Utilizing the v-abl probe and the same DNA, multiple bands were detected in most yeast (Fig 3, e-h, Table I) with varying intensities. The data obtained with these two new onc probes again establish that the species examined here contain different gene families since different number and sets of hybridizing sequences are detected depending on the probe used.

onc gene homologs and actin: Actin has been shown to be a highly conserved protein in a variety of eukaryotes (23). As demonstrated in Fig. 4 (lane a), the actin probe derived from the D. discoideum actin mRNA (14) detected two related fragments (2.4 and 3.8 kbp) in DNA of S. cerevisiae. A summary of the other hybridizing fragments is given in Table I. These results show that the DNA fragments of C. ishiwadae (10.5 kbp) and S. pombe (7.6 kbp), which contain Ras-1 homology, also hybridize to the actin gene probe. Since under the same relaxed conditions of annealing the 1.7 kbp Ras-1 probe does not hybridize to the actin cDNA probe, these results allow us to infer that some ras related sequences in C. ishiwadae and S. pombe are linked to an actin-like sequence.

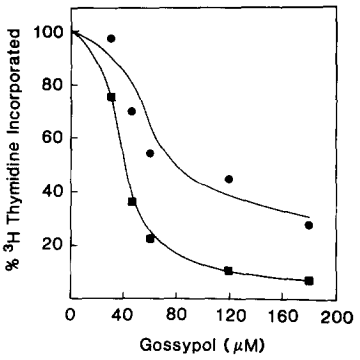

Figure 5: Percent of [^{3}H] thymidine incorporated into DNA in the presence of various concentrations of gossypol. Wild type mouse L cells (■) and ribonucleotide reductase overproducing mutant mouse L cells (●). The [^{3}H] thymidine labelling was performed as described in Materials and Methods.

ribonucleotide reductase activity DNA synthesis has been shown to be more resistant to inhibition by hydroxyurea than it is in wild type counterparts (13,19). The results of experiments showing the effect of gossypol on DNA synthesis in wild type mouse L cells and in the ribonucleotide reductase overproducing mutant cell line is shown in Figure 5. Clearly, DNA synthesis in the mutant cell line is more resistant to gossypol inhibition, a result which supports the view that ribonucleotide reductase is an important intracellular target of gossypol. In addition to this, preliminary results using rat myoblast cells selected for resistance to hydroxyurea (22) showed increases in ribonucleotide reductase activity and cross-resistance to the cytotoxic effects of gossypol (data not shown).

Attempts have been made to exploit the role of ribonucleotide reductase in the proliferation of neoplastic cells by developing antitumor drugs that selectively inhibit the reductase activity (eg. hydroxyurea and guanazole). Potent inhibition of ribonucleotide reductase activity by gossypol is consistent with the antiproliferative properties associated with this drug (4) and support the further investigation of gossypol as an antineoplastic agent. Furthermore, some of the effects exerted by this drug on spermatogenesis may be attributable to the inhibition of ribonucleotide reductase activity, necessary for duplication of the genetic material before recombination and reductive cell division. Since the prolonged use of antineoplastic agents in

results with the actin probe further support the notion that some ras-related sequences are linked to actin-related sequences. In an earlier study, a gene coding for a protein similar to the c-has/bas proto-oncogene product was mapped between actin and tubulin genes in S. cerevisiae (9). However, in S. pombe no tubulin-ras linkage was found (24) and actin-ras linkage was not tested. Our results indicate a 7.6 kbp fragment may carry both actin and ras related sequences. It is tempting to speculate that actin and the ras homolog function as a single genetic unit where they are found together. In fact, the v-fgr oncogene from Gardner-Rasheed feline sarcoma virus (GR-FeSV), an acute transforming retrovirus, appears to have arisen by recombination of two cellular genes, one coding for the structural protein actin and the other for a protein kinase (25). At present, attempts to clone various v-onc homologs from some of the fungi studied here are in progress.

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