Molecular cloning of the actin gene from yeast *Saccharomyces cerevisiae*

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ABSTRACT

Two overlapping DNA fragments from yeast *Saccharomyces cerevisiae* containing the actin gene have been inserted into pBR322 and cloned in *E.coli*. Clones were identified by hybridization to complementary RNA from a plasmid containing a copy of *Dictyostelium* actin mRNA. One recombinant plasmid obtained (pYA102) contains a 3.93-kb HindIII fragment, the other (pYA208) a 5.1-kb PstI fragment, both share a common 2.2-kb fragment harboring part of the actin gene. Cloned yeast actin DNA was identified by R-loop formation and translation of the hybridized actin mRNA and by DNA sequence analysis. Cytoplasmic actin mRNA has been estimated to be about 1250 nucleotides long. There is only one type of the actin gene in *S.cerevisiae*.

INTRODUCTION

Actin is an abundant protein not only in muscle but also in nonmuscle cells. It is the major structural protein of microfilaments found in the cytoplasm of all eukaryotic cells. In the cytoplasm of mammalian cells two forms of actin, β - and γ -actin, are found which can be separated from each other and from skeletal muscle α -actin by isoelectric focusing (1-3). Protein sequence analyses have shown that although there are certain amino acid exchanges between actins from different species, actin is an evolutionary rather stable protein (4-6). This is impressively illustrated by the primary sequence of the only actin of the lower eukaryote, *Physarum polycephalum*, which differs from mammalian cytoplasmic actin by only 17 out of 375 amino acid residues (7). The constraint to conserve the primary structure of actins during evolution obviously follows from the function these proteins fulfil within the cell. Actin seems to be involved in all forms of cell motility, transport of macromo-

lecules within the cell, phagocytosis, chromosome movement and cytokinesis (for reviews see 8,9) and interacts with a number of other proteins.

One approach to elucidate the function of actin in mitosis and cytokinesis would be to compare structure and expression of actin genes in mutants defective in these processes. For these studies yeast seems particularly suited since several temperature sensitive mutants are available. Actin was recently isolated from yeast by Koteliansky et al. (10) and ourselves (Gallwitz and Seidel, unpublished). Actin genes from the slime mold *Dictyostelium discoideum* have been isolated and shown to be repeated 15-20 times in the genome (11-13). Because of the stability of the primary structure of actin during evolution it seems not surprising that actin mRNA from *Dictyostelium* cross-hybridizes with actin DNA from different species.

We report here on the isolation by molecular cloning and the characterization of the actin gene from yeast *S.cerevisiae*. We also present evidence to show that there is very likely only one type and one copy of the actin gene in *S.cerevisiae*.

MATERIALS AND METHODS

1. Bacterial and yeast strains and plasmids

The ampicillin (Ap) and tetracyclin (Tc) resistant plasmid pBR322 (14) was grown in a derivative of *E.coli* K12, RR1. The bacterial strain used for cloning was the lysogenic *E.coli* 1398 (ED8767 lysogenized by λ L512) (15) and was kindly provided by P.Kourilsky, Paris. The recombinant plasmid pcDd actin B containing 430 bp of *Dictyostelium* actin cDNA was generously provided by R.Firtel, San Diego. The haploid yeast strain A364A was obtained from the Yeast Stock Center, Berkeley.

2. Enzymes

Restriction endonucleases AluI, HindIII, PstI and EcoRI were obtained from Boehringer, Mannheim; endonuclease HaeII was from Miles. T4 polynucleotide kinase, *E.coli* DNA polymerase I and calf intestine alkaline phosphatase were purchased from Boehringer. *E.coli* RNA polymerase and T4 DNA ligase were gifts from K.Geider, Heidelberg. β -Glucuronidase was obtained from Sigma.

3. Preparation of yeast DNA

DNA was obtained from *S.cerevisiae* strain A364A following the procedure of Cryer et al. (16). Spheroblasts were prepared using β -glucuronidase at a concentration of 600 units / 10 g cells.

4. Cloning of yeast DNA

500 ng of HindIII- or PstI-cleaved and phosphatase-treated pBR322 (10 μ g of linearized plasmid DNA treated with 5 units of calf intestine alkaline phosphatase in 200 μ l of 50 mM Tris-HCl, pH 8, for 30 min at 37°C, heated at 68°C for 15 min and extracted with phenol) and 250 ng of HindIII- or PstI-digested yeast DNA were ligated for 12 h at 4°C with 5 units of T4 DNA ligase. Thermosensitive *E.coli* strain 1398 was transformed with the recombinant plasmid DNA as described by Cami and Kourilsky (15). Between $3-5 \times 10^5$ Ap- or Tc-resistant transformants were obtained per microgram of pBR322 DNA.

5. Identification of clones containing yeast actin DNA

To identify recombinant clones containing actin DNA bacterial colonies were partially lysed at 42°C for 4 h, DNA was then transferred to nitrocellulose filters and filters were alkali-treated and heated as described (15). Hybridization was performed either with nick-translated (17) probes or with complementary RNA (cRNA) transcribed from specific DNA fragments with *E.coli* RNA polymerase. The *Dictyostelium* actin cDNA clone used as a heterologous hybridization probe (pcDd actin Bx) contained a DNA fragment about 1150 bp long and was obtained by screening a collection of cDNA clones using nick-translated *Dictyostelium* cDNA of clone pcDd actin B (kindly provided by R.Firtel) which contained a 430 bp DNA fragment representing only part of the complete actin cDNA.

³²P-labeled cRNA (about 2×10^7 cpm / μ g) was prepared from purified *Dictyostelium* DNA excised with PstI from the cloning vehicle pBR322. Ap-resistant clones containing yeast DNA inserted into the HindIII site of pBR322 were hybridized in 50% deionized formamide, 0.6 M NaCl, 30 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.25% SDS, 200 μ g/ml *E.coli* tRNA, 5 μ g/ml denatured *E.coli* DNA and $5 \times 10^5 - 1 \times 10^6$ cpm per ml at 41°C for 26 h. Ten filters (ϕ 85 mm) were hybridized together in 25 ml of this

solution in a plastic petri dish which was tightly closed with parafilm and sealed in a plastic bag. After hybridization filters were washed 4 times in 1 l 50% formamide, 5 x SSC, 0.25% SDS at 37°C for 1 h each. They were then washed in 2 x SSC at room temperature for 2-4 h, treated with pancreatic RNAase A (50 µg/ml) in 2 x SSC for 30 min and finally washed for 2 h in 1 l 2 x SSC with 2 changes. The cloned HindIII fragment containing yeast actin DNA was nick-translated and used as probe to screen for clones containing the PstI fragment harboring the yeast actin gene. Hybridization of DNA replicas from lysed bacterial colonies (see above) was performed in 2 x SSC, 1 mM EDTA, 0.25% SDS, 2 x Denhardt's solution (18), 25 mM sodium phosphate, pH 7.2, 5 µg/ml denatured E.coli DNA, 5 µg/ml poly(A) and 2×10^6 cpm/ml ($6-8 \times 10^7$ cpm/µg DNA) for 24 h at 68°C. Filters were washed 3 times in 25 mM sodium phosphate, pH 7.2, 2 x SSC, 1 mM EDTA, 0.25% SDS, 1 x Denhardt's solution at 68°C over a period of 3 h and then in 2 x SSC as described above for RNA-DNA hybridizations (omitting the RNAase digestion step). Filters were exposed to Kodak RP X-Omat (XR-5) film at -70°C for 1-5 days.

6. Isolation of plasmid DNA

Plasmid DNA was isolated from bacteria using the cleared lysate method of Clewell and Helinski (19). DNA was further purified by equilibrium centrifugation in ethidium bromide-CsCl gradients and finally on a 5-23% linear sucrose gradient in 50 mM Tris-HCl, pH 7.6, 1 mM EDTA (20,000 rpm, 16 h, SW27 rotor).

7. Gel electrophoresis

DNA fragments were separated for analytical purposes on 0.9% agarose slab gels (12 x 11 x 0.24 cm) in the Tris-borate-EDTA buffer system (20). For preparative isolation of DNA fragments either 0.7 cm thick agarose or 6% polyacrylamide slab gels were used. DNA fragments were stained with ethidium bromide and photographed under UV light. DNA was recovered from gel slices by electrophoresis into a dialysis tubing in 10 mM Tris-borate buffer containing 0.2 mM EDTA

8. Transfer of nucleic acids to nitrocellulose filters and ABM-paper

DNA fragments were transferred to nitrocellulose filters

by the procedure of Southern (21). RNA was denatured by glyoxylation (22) and transferred to ABM-paper according to Alwine et al. (23). Hybridization with nick-translated yeast actin DNA was performed in 45% formamide, 4 x SSC, 1 x Denhardt's solution, 20 mM sodium phosphate, pH 6.8, 0.1% SDS, 10% dextran sulfate (24), 50 µg/ml denatured E.coli DNA, 20 µg/ml poly (A) and 10^6 cpm/ml (8×10^7 cpm/µg DNA) at 42°C for 18 h.

9. Preparation and fractionation of yeast RNA

Total cytoplasmic or polyribosomal RNA was isolated from logarithmically growing yeast following the procedure of Hereford and Rosbash (25). 25 mg of ethanol-precipitated RNA was chromatographed on a 2 ml oligo (dT)-cellulose column in 0.4 M NaCl, 10 mM Tris-HCl, pH 7.5, 0.1% SDS at room temperature. The column was then washed with 0.1 M NaCl in the same buffer and poly(A)(+) RNA was eluted with 10 mM Tris-HCl, pH 7.5, 0.1% SDS. For some experiments polyribosomal RNA was fractionated on 15-30% linear sucrose gradients as described (28).

10. R-loop formation and mRNA translation

For R-loop formation the recombinant plasmid pYA208 was cleaved with PstI. In a total volume of 100 µl 30 µg of DNA was hybridized with 30 µg of oligo(dT)-cellulose-fractionated yeast RNA as described (26). R-loops were allowed to form between 58°C and 40°C during a 6 h incubation time in which the temperature was lowered at 1°C/20 min. R-looped RNA and plasmid DNA were separated from unreacted RNA by chromatography on a BioGel-A15Q column (26). After ethanol precipitation and heat denaturation R-looped RNA was translated in the nuclease-treated reticulocyte lysate (27) using 35 S-methionine as label. Labeled proteins were separated on a 10% polyacrylamide-SDS gel and identified by autoradiography as described previously (28).

11. DNA sequence analysis

DNA fragments were labeled at their 5'-ends with (γ - 32 P)ATP and polynucleotide kinase and sequencing was performed according to Maxam and Gilbert (29,30).

12. Biohazard considerations

Cloning experiments and growth of recombinant DNA plasmids were carried out under L2-B1 conditions following the rules of

the Bundesminister für Forschung und Technologie of the Federal Republic of Germany.

RESULTS AND DISCUSSION

1. Cloning of yeast actin DNA

Yeast DNA from the haploid strain A364A (31) was digested with various restriction endonucleases, DNA fragments were separated on 0.9% agarose gels and transferred to nitrocellulose filters by the method of Southern (21). Hybridization was performed with labeled cRNA from cloned *Dictyostelium* DNA complementary to about 1150 nucleotides of the actin mRNA. Complete digests of yeast DNA with restriction endonucleases PstI, EcoRI, HindIII or HindIII/PstI each gave a single hybridization band (Fig. 1). The length of these hybridizing fragments was approximately 5.1 kb, 4.2 kb and 3.9 kb for endonucleases PstI, EcoRI and HindIII, respectively. DNA digested with PstI and HindIII simultaneously resulted in the formation of one hybridizing fragment about 2.2 kb long. Using nick-translated cloned *Dictyostelium* actin cDNA the same hybridizing DNA fragments were found.

Having observed that specific yeast DNA fragments cross-hybridized with *Dictyostelium* actin cDNA or a cRNA derived from it we decided to isolate the HindIII fragment by cloning it in *E. coli*. In a shotgun experiment HindIII-generated yeast DNA fragments were ligated to HindIII-cleaved pBR322 with T4 DNA ligase and introduced into the lysogenic *E. coli* strain 1398 (15). The linearized cloning vehicle was treated with alkaline phosphatase to reduce self-ligation of the plasmid (32). After selection of transformants by Ap-resistance, recombinants (600-1000 colonies per 85 mm plate) were screened for yeast actin DNA using ³²P-labeled cRNA complementary to *Dictyostelium* actin cDNA. One positive clone (pYA102) had the expected 3.9-kb yeast DNA insert which hybridized to the *Dictyostelium* probe.

Digestion of the cloned 3.9-kb yeast DNA segment with PstI generated two fragments (A and B) 2.2 kb and 1.73 kb long (Fig 2, lane b). Only the 2.2-kb fragment A hybridized to the ³²P-labeled *Dictyostelium* probe. This finding was in accord with the result presented in Fig. 1 showing that a double digest of yeast DNA with endonucleases PstI and HindIII generated a frag-

Fig. 1: Identification of yeast actin DNA in restriction endonuclease-treated total yeast DNA. Ethidium bromide stained DNA fragments (right) and fragments hybridizing to ^{32}P -cRNA from cloned *Dictyostelium* actin cDNA (left). Lanes a-e: EcoRI-, HindIII/PstI-, PstI- and HindIII-digested DNA; lane e: plasmid pYA102 DNA digested with PstI. Ten μg of DNA was run in each track.

ment of the same length which hybridized to the probe from *Dictyostelium*.

Since cleavage of yeast DNA with PstI generated one hybridizing fragment 5.1 kb long (Fig. 1) it was easily predictable that in the genome the next PstI cleavage site was about 3 kb from the HindIII site of fragment A. The hybridizing HindIII and PstI fragments of yeast DNA therefore should have the same 2.2-kb segment in common. The 5.1-kb PstI fragment was then cloned using the same protocol described above, but as hybridization probe nick-translated fragment A from the recombinant plasmid pYA102 was used. Out of 22 positive clones 20 had the expect-

Fig. 2: Southern analysis of endonuclease -digested cloned HindIII/PstI fragment A. Ethidium bromide stained gel (right), DNA fragments hybridizing to nick-translated cloned *Dictyostelium actin* cDNA (left). Lane a: fragment A; b: HindIII/PstI-digested pYA102; c, d: partial digests of fragment A with AluI/BamHI and AluI; e-g: HaeIII-, HaeIII/BamHI-, BamHI-digest of fragment A; h: pBR322-AluI (partial). Film exposure 22 h at -70°C.

ted 5.1-kb yeast DNA insert. The clone pYA208 was selected for further studies. Cleavage of the cloned PstI fragment with HindIII generated two fragments (A and C) 2.2 kb and 2.9 kb long. Whereas, as predicted, the 2.2-kb segment A hybridized to *Dictyostelium actin* cDNA, the 2.9-kb fragment C did not. This was suggestive for fragment A harboring the actin gene, or part of it.

To locate the actin gene more precisely fragment A was isolated by preparative agarose gel electrophoresis and subjected to digestion with various restriction endonucleases. The fragments generated were separated on 1.6% agarose gels, transferred to nitrocellulose filters and hybridized to nick-translated cloned *Dictyostelium actin* cDNA. As can be seen in Fig. 2, lane e, HaeIII digestion gave two fragments which were 1130 and 1070 bp

long, only the smaller of the two hybridized. BamHI digestion generated two fragments approximately 1570 and 630 bp long (Fig. 2, lane g) the larger of which hybridized to the *Dictyostelium* probe. A double digest with HaeIII and BamHI resulted in the formation of three fragments (Fig. 2, lane f) with lengths of 1070 bp, 620 bp and 510 bp. The ³²P-labeled probe hybridized only to the largest fragment. When fragment A was digested with AluI five cleavage sites were found, the location of the different cutting sites is indicated in Fig. 3. In Fig. 2, lanes c and d, partial digests with AluI and AluI/BamHI are shown. Only the 540-bp and the adjacent 150-bp AluI fragments hybridized (the AluI fragments larger than 540 bp that hybridized in the Southern blot presented in Fig. 2 resulted from incomplete digestion; longer film exposure clearly showed that in addition to the 540-bp fragment only the 150-bp segment hybridized). This result

Fig. 3: Recombinant plasmids containing yeast actin DNA. Orientation of the DNA inserts and restriction map. Black region in the map of fragment A shows coding segment (amino acids 5-260, verified by sequence analysis). Arrows with wavy lines indicate AluI cutting sites.

showed that only part of the DNA complementary to Dictyostelium actin mRNA (which is about 1150 bp long and should have almost full length (11)) cross-hybridizes to the cloned yeast DNA.

A summary of different restriction endonuclease cleavage sites of the two recombinant plasmids containing yeast actin DNA is presented in Fig. 3. In addition, this figure shows the polarity of the yeast DNA inserts in both plasmids. The polarity was determined by cleaving pYA102 with PstI and pYA208 with HindIII and Southern blotting of the fragments generated. Since only the yeast DNA fragment A hybridized to the cRNA made from cloned Dictyostelium actin cDNA one of the two cleavage products obtained with each recombinant plasmid exhibited a strong hybridization signal. This is shown in Fig. 1, lane e, for the plasmid pYA102.

11. Identification of actin mRNA by R-looping and hybridization

For further characterization of the cloned yeast DNA the recombinant plasmid pYA208 was linearized with PstI and hybridized under R-loop conditions (26) to cytoplasmic RNA fractions from *S.cerevisiae*. The hybridized RNA was translated in a nuclease-treated rabbit reticulocyte lysate. As can be seen in Fig.4, a protein comigrating with authentic actin was synthesized under the direction of R-looped RNA. In addition, an other protein (protein 2) with an approximate molecular weight of 18-20,000 daltons was synthesized in vitro under the direction of RNA hybridizing to the cloned yeast DNA. The presumptive actin mRNA as well as the mRNA coding for protein 2 is found in cytoplasmic RNA fractions adsorbed and not adsorbed to oligo (dT)-cellulose.

To identify yeast actin mRNA and to determine its length cytoplasmic RNA was denatured by glyoxylation (22), separated on a 1.5% agarose gel and transferred to ABM-paper (23). Hybridization probe was nick-translated cloned HindIII/PstI fragment A. Two hybridization bands were observed (Fig. 5). Using 25 S, 18 S and 5 S ribosomal RNA as length markers (33) the two hybridizing RNAs were calculated to be about 1250 and 660 nucleotides long. To code for actin, a protein with 375 amino acids, the coding portion of the mRNA would require 1125 nucleotides. The larger of the two hybridizing RNAs seen in Fig. 5 is therefore likely

Fig. 4: Autoradiogram of in vitro synthesized proteins. RNA used to prime the reticulocyte lysate was I. polyribosomal RNA: a, RNA not adsorbed to oligo(dT)-cellulose; b, RNA eluted from oligo(dT) cellulose with 0.1 M NaCl; c, poly(A)(+)RNA. II. R-looped RNA: d, f, same as b; e, g, same as c; R-loops in d and e were not de-natured before addition to the cell-free system. h, no RNA added. Stained skeletal muscle α -actin served as marker.

to be the actin mRNA and it can be concluded that yeast actin mRNA has only few untranslated nucleotides at the 5'-and (or) the 3'-end. The length of the other RNA which hybridized to nick-translated fragment A would well fit for an mRNA coding for protein 2 which has a molecular weight of about 18-20,000 daltons (see Fig. 4). The cloned yeast DNA fragment A therefore harbors at least part of a second gene which codes for protein 2. It should be noted that actin mRNA can also be detected in total yeast RNA (Fig. 5, lane e).

III. Identification of yeast actin DNA by nucleotide sequence analysis

Ultimate proof that the cloned yeast DNA contains indeed

Fig. 5: Identification of actin mRNA. Glyoxylated RNA was separated on a 1.5% agarose gel and transferred to ABM-paper; hybridization probe was nick-translated HindIII/PstI fragment A. a, fraction of total cytoplasmic RNA eluting from oligo(dT)-cellulose with 0.1 M NaCl; b-d, 5-16 S polyribosomal RNA fractionated on oligo(dT)-cellulose: b, RNA not adsorbed; c, 0.1 M NaCl-eluted RNA; d, poly(A)(+) RNA; e, total yeast RNA. Arrows indicate position of 25 S and 18 S ribosomal RNA (33).

the actin gene is its identification by nucleotide sequence analysis. Since the 540-bp AluI fragment of the cloned segment A hybridized strongly to nick-translated cloned actin cDNA from *Dictyostelium* (see Fig. 2) this DNA fragment was chosen for sequence analysis. It was isolated by preparative polyacrylamide gel electrophoresis, labeled at its 5'-ends with (γ - 32 P)ATP and polynucleotide kinase and, after strand separation, was sequenced according to Maxam and Gilbert (29,30). A partial nucleotide sequence of the 540-bp AluI fragment (black area of this fragment in Fig. 3) identified this part of the cloned yeast DNA as segment of the actin gene coding for the NH₂-terminal region (amino acid 5 - 57) of the protein (Fig. 6).

A comparison of the amino acid composition of yeast actin deduced from the nucleotide sequence with the corresponding re-

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5' ..GTT GCT GCT TTG GTT ATT GAT AAC GGT TCT GGT ATG TGT AAA
   ..CAA CGA CGA AAC CAA TAA CTA TTG CCA AGA CCA TAC ACA TTT
      val ala ala leu val ile asp asn gly ser gly met cys lys
          5          10          15

GCC GGT TTT GCC GGT GAC GAC GCT CCT CGT GCT GTC TTC CCA
CGG CCA AAA CGG CCA CTG CTG CGA GGA GCA CGA CAG AAG GGT
ala gly phe ala gly asp asp ala pro arg ala val phe pro
      20          25          30

TCT ATC GTC GGT AGA CCA AGA CAC CAA GGT ATC ATG GTC GGT
AGA TAG CAG CCA TCT GGT TCT GTG GTT CCA TAG TAC CAG CCA
ser ile val gly arg pro arg his gln gly ile met val gly
      35          40          45

ATG GGT CAA AAA GAC TCC TAC GTT GGT GAT GAA..3'
TAC CCA GTT TTT CTG AGG ATG CAA CCA CTA CTT..
met gly gln lys asp ser tyr val gly asp glu
      50          55

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Fig. 6: Partial DNA sequence of the 540-bp *AluI* fragment derived from cloned *HindIII*/*PstI* fragment A (see Fig. 3). Amino acids underlined differ from the corresponding residue in *Physarum actin* (7).

gion of the actin from *Physarum polycephalum* (7) shows a high degree of homology. There are only three amino acid differences in position 6, 41 and 43 between actin from these two species and it is interesting to note that the amino acids in position 6 and 41 of yeast actin are the same as in the cytoplasmic actins of mammals (7).

From the location of the sequenced actin DNA the direction of transcription can be deduced (see Fig. 3). It becomes also clear that the actin gene extends into the *HindIII*/*PstI* fragment C of the cloned DNA in pYA208. This has recently been verified by nucleotide sequence analysis (Gallwitz and Sures, in preparation).

IV. Number of actin genes in *S.cerevisiae*

It was mentioned above and shown in Fig. 1 that using as hybridization probe either cloned actin cDNA from *Dictyostelium* or cRNA transcribed from it, only one particular hybridization band was detected in a Southern blot with either *EcoRI*-, *PstI*-, *HindIII*- or *HindIII*/*PstI*-digested yeast DNA. This was already suggestive for the existence of only one type of actin gene in *S.cerevisiae*. To see whether the actin gene or its flanking se-

quences are repeated elsewhere in the yeast genome a Southern analysis with endonuclease-cleaved yeast DNA was performed using nick-translated cloned 3.93-kb DNA segment from pYA102 (see Fig. 3) as hybridization probe. Fig. 7 shows the ethidium bromide stained along with the hybridizing DNA fragments after transfer to nitrocellulose filters. The PstI/HindIII-digested pYA102 DNA showed, as expected, strong hybridization of the two fragments A (2.2 kb) and B (1.73 kb). (Hybridization of the 3.58-kb pBR322 fragment resulted from a slight contamination with pBR322 of the yeast DNA used for nick-translation). A double digest of total yeast DNA with PstI and HindIII gave the same two hybridization bands (Fig. 7, lane c) whereas of the HindIII-cleaved yeast DNA only the expected 3.93-kb fragment hybridized (lane d). PstI-digested DNA showed hybridization only to the expected 5.1-kb fragment and to a larger faintly labelled

Fig. 7: Hybridization of nick-translated 3.93-kb HindIII fragment (from clone pYA102) to restriction endonuclease-digested total yeast DNA. Ethidium bromide stained gel (right), hybridizing DNA fragments after Southern transfer (left). a-d, EcoRI-, PstI-, HindIII/PstI- and HindIII-digest; e, EcoRI-cleaved DNA; f, HindIII/PstI-digested pYA102 DNA (3.58-kb and 0.8-kb fragments are derived from pBR322 DNA; small arrows point to yeast DNA fragments A and B). Ten μ g of DNA was run in each track. Film exposure 24 h at -70°C .

fragment. EcoRI-digestion resulted in the formation of two hybridizing DNA segments which were 4.15 kb and 1.68 kb long (lane a). The larger of these two is the fragment which also hybridized to the *Dictyostelium* actin cDNA (see Fig. 1) and spans the region between the EcoRI sites in the cloned DNA fragments B and C (see Fig. 3). The 4.15-kb EcoRI fragment contains the total actin gene (Gallwitz and Sures, in preparation). From the length of the second hybridizing EcoRI fragment it can be concluded that the next EcoRI cutting site in the genome must be very close to the HindIII site of segment B.

The result of the experiment described indicates that neither actin coding sequences (region coding for amino acids 1 to 260, black region shown in restriction map of Fig. 3) nor the 5'-flanking region of the gene seems to be repeated elsewhere in the genome. Because of the conserved primary structure of actins from species as distant as *Physarum* (7), *Dictyostelium* (K.Weber, personal communication) and yeast (this paper) on one side and mammals on the other (4,7,34), we tend to conclude that there is only one type of actin gene in *S.cerevisiae*. Especially noteworthy is the fact that the N-terminal third of the primary structure of all actins sequenced thus far (including the yeast sequence presented here) shows only very few amino acid differences (except in the variable N-terminal region comprising the first seven amino acids). We therefore believe it to be highly unlikely that an other type of actin gene would have been overlooked with the homologous hybridization probe which contained the coding region for amino acids 1 to 260 (Gallwitz and Sures, in preparation). The Southern analysis presented in Fig. 7 makes a tandem arrangement of several copies of the same gene equally unlikely.

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