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# A single intronless actin gene in the fission yeast *Schizosaccharomyces pombe*: nucleotide sequence and transcripts formed in homologous and heterologous yeast

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## ABSTRACT

The actin gene of the fission yeast *Schizosaccharomyces pombe* has been isolated by using as a hybridization probe cloned actin DNA from the budding yeast *Saccharomyces cerevisiae*. In contrast to most actin genes studied from diverse eukaryotic species, the *S. pombe* gene is not interrupted by introns. The protein sequence deduced from the nucleotide sequence of the gene shows that the *S. pombe* actin is more closely related to the mammalian γ-actin than to the actin of *S. cerevisiae*. Three transcripts of 1240, 1650 and 1850 nucleotides having the same 5'end but differing in the length of their 3'untranslated region are generated in the fission yeast. Only one messenger RNA of 1330 nucleotides is formed from the *S. pombe* actin gene in *S. cerevisiae*. Contrary to the observation made with other *S. pombe* genes transcribed in the budding yeast, the heterologous actin gene transcript is initiated 39 nucleotides upstream of the initiation start site used in the homologous yeast. The mRNA termination (or 3'processing) mechanism in the two ascomycetes also differs as the 3'end of the *S. pombe* actin gene transcript in *S. cerevisiae* does not coincide with either of the three 3'ends mapped in the fission yeast.

## INTRODUCTION

In contrast to all other eukaryotic species examined so far, the unicellular yeast *Saccharomyces cerevisiae* contains only one actin gene copy per haploid genome (1-4). The protein-coding sequence of the *S. cerevisiae* actin gene is discontinuous, an intervening sequence of 309 bp interrupts the codon of amino acid residue four (2-4). This intron position is unique, it has not been detected in any other actin gene of the different eukaryotic organisms studied.

For reasons of an evolutionary comparison and to study the expression and function of actin in a unicellular organism dividing by medial fission, we have now isolated and sequenced the actin gene from another ascomycete, the fission yeast *Schizosaccharomyces pombe*. We show in this report that *S. pombe* likewise has only one actin gene copy per haploid genome, but in contrast to *S. cerevisiae* this gene is not split. We have also investigated the transcription of the *S. pombe* actin gene in *S. pombe* and *S. cerevisiae* and found that the transcription start as well as the termination sites differ in the two ascomycetes.

## MATERIALS AND METHODS

### Cloning and sequencing procedures

DNA from *S. pombe* strain L975 (kindly provided by Dr. Gutz, Braunschweig) was isolated, cut with different restriction enzymes and subjected to a Southern blot analysis using cloned *S. cerevisiae* actin DNA, the 935 bp *ClaI/KpnI* region harbouring codons 4 to 302 (2), as hybridization probe. Hybridization at 67°C and 2 x SSC identified a hybridizing *Hind*III DNA fragment of about 7 kb which was cloned in *E. coli* using *Hind*III-cut and phosphatase-treated pBR322. The sequence of the actin gene from the recombinant plasmid pSPA2 contained in a 7.3 kb *Hind*III fragment was determined according to Maxam and Gilbert (5). The *S. pombe* actin gene on a 4.8 kb *Bgl*II DNA fragment, inserted into the *BamH*I restriction site of the yeast/*E. coli* shuttle vector YRp70 (6,7), was used to transform the *trp1*⁻ *S. cerevisiae* strain YNN27 to tryptophan prototrophy. Yeast transformations were performed with lithium acetate (8).

### RNA analyses

RNA was isolated from transformed and untransformed yeast cells and analyzed on Northern blots as previously described (7,9). Transcription start sites and mRNA 3'ends were determined by S1 nuclease protection (10). As shown in Figure 1, a 256 bp *NcoI/HhaI* fragment, labelled at the *Nco*I site, was used for 5'end mapping and either a 420 bp *BamHI/Hae*III fragment or a 2.2 kb *BamHI/Bgl*II fragment, 3'end-labelled at the *BamH*I site, was used for mapping the 3'ends of the *S. pombe* actin gene transcripts in *S. pombe* and *S. cerevisiae*. In all cases 50 µg total cellular RNA was used to generate the hybrids with the labelled fragments and S1 nuclease digestion with 10 to 100 units/ml was performed at 37°C for 3 hours.

## RESULTS

### Cloning and sequence determination of the *S. pombe* actin gene

In a Southern blot analysis, cloned *S. cerevisiae* actin DNA crosshybridized to an *S. pombe Hind*III DNA fragment of about 7 kb under moderately stringent hybridization conditions. *Hind*III-digested *S. pombe* DNA was therefore cloned in pBR322 and one out of 12000 clones tested, pSPA2, gave a positive signal with the heterologous hybridization probe. The recombinant plasmid carried a 7.3 kb insert that hybridized back to the *S. cerevisiae* DNA probe.

The location of the *S. pombe* actin gene on the cloned DNA fragment was mapped using standard methods and the nucleotide sequence was determined according to the method of Maxam and Gilbert (5) following the strategy described in Figure 1. As can be seen in Figure 2, the coding part of the gene is continuous. From mRNA mapping studies discussed below there is also no evidence for an intron located in the 5'untranslated region.

### One actin gene and three transcripts in *S. pombe*

To determine the number of actin genes in the genome of *S. pombe*, chromosomal DNA

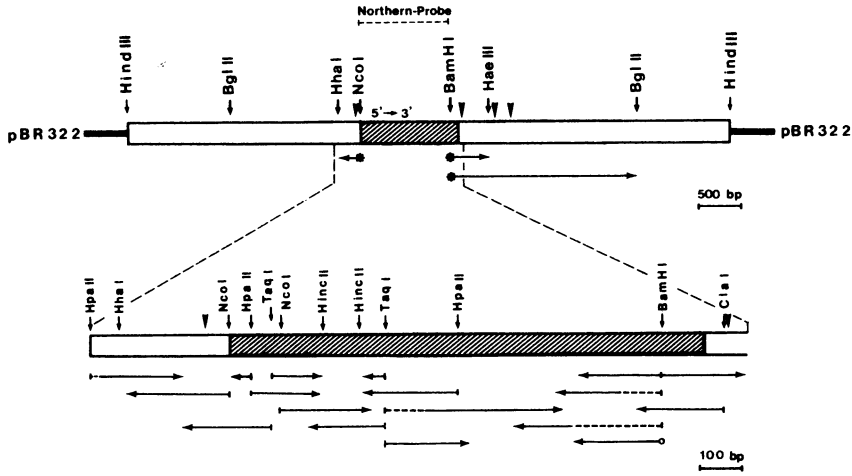


**Figure 1.** Cloned 7.3 kb *S. pombe Hind*III DNA fragment carrying the actin gene (*act1*). In the upper part of the figure, restriction sites are shown that were used either for subcloning fragments or for generating fragments to map the 5'and 3'ends of the actin mRNAs. Thick arrows indicate the transcription initiation and termination sites. Horizontal arrows represent the fragments used for S1 nuclease protection experiments, asterisks indicate the end-labelled sides. Also shown is the *NcoI/BamHI* fragment used for the Southern and Northern blot analyses of Figures 3 and 4. The sequencing strategy is shown in the lower part of the figure, with the horizontal arrows indicating the length of the sequenced regions. The shaded area represents the protein-coding region.

was digested with the restriction endonucleases *Hind*III, *BamH*I, *Bgl*II, *Hind*III/*BamH*I and *Bgl*II/*BamH*I which, according to the known location of restriction sites on the cloned 7.3 kb fragment, led to actin DNA-containing fragments of predictable length (see Figure 1). In a Southern blot analysis (Figure 3), using as hybridization probe a $^{32}$P-nick-translated 906 bp *NcoI/BamHI* homologous actin gene fragment (codon 41 to codon 342), only the anticipated fragments gave a positive signal. Since different actin genes in one species, because of their extensive homologies, are expected to crosshybridize efficiently, we conclude from this result that, similar to the situation in *S. cerevisiae*, there is only one actin gene copy in the genome of the fission yeast *S. pombe*.

In a search for the borders of the actin gene we first determined the size of the actin mRNA. Total cellular or polyadenylated RNA was denatured by glyoxylation, separated electrophoretically and transferred to nitrocellulose filters for hybridization to cloned actin DNA. As a surprising result we observed three mRNA species of about 1240, 1650 and 1850 nucleotides which hybridized with comparable intensity to the labelled probe. The three actin mRNAs were found in rather equal amounts in logarithmically and stationary cells (Figure 4).

S1 nuclease protection experiments were then performed to exactly locate the 5'and 3'ends of the different mRNAs. Using a 256 bp *NcoI/Hha*I fragment, 5'end-labelled at its *Nco*I

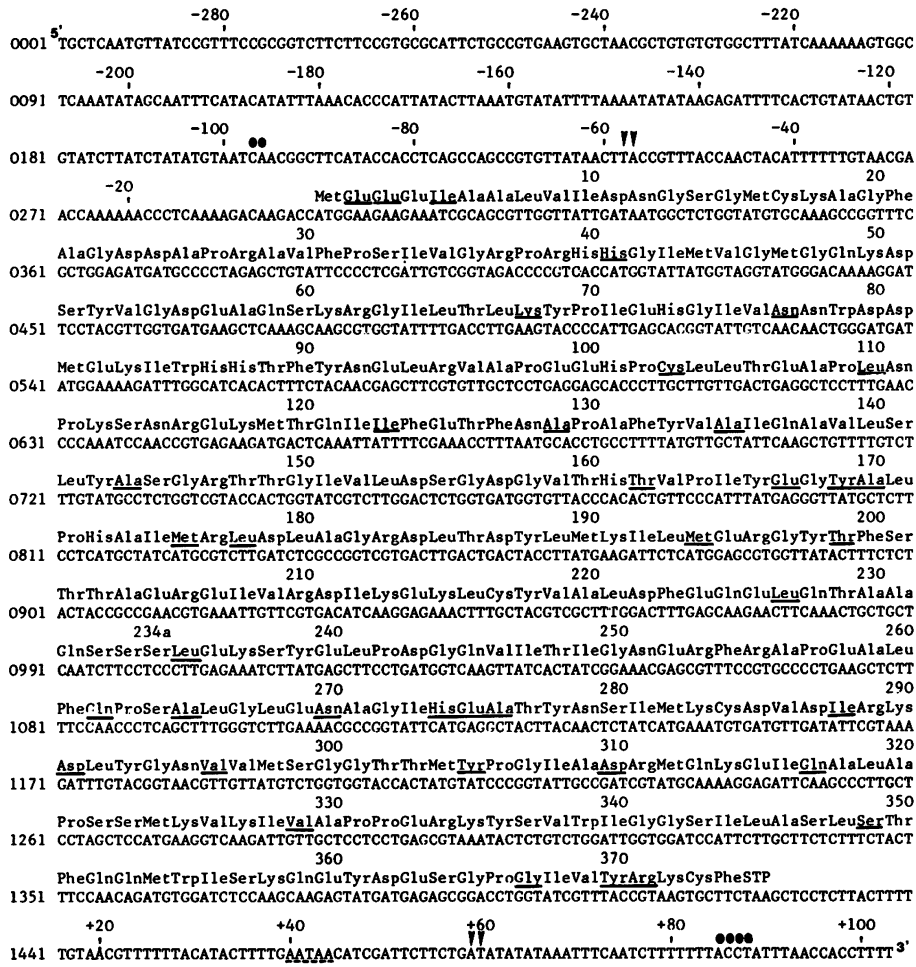

**Figure 2.** DNA sequence of the *S. pombe* actin gene (*act1*) and primary structure of actin deduced from the nucleotide sequence. Amino acid residues not identical with those of the *S. cerevisiae* actin (2) are underlined. Initiation sites and 3'ends of the transcripts formed in *S. pombe* and *S. cerevisiae* are marked with arrows and filled circles, respectively. A possible polyadenylation signal sequence is indicated by a broken line.

site (located within the ATG translation initiation codon), only one S1 nuclease-protected fragment was observed which identified the A (or T) residue 57 (or 58) nucleotides upstream from the ATG codon as the mRNA cap site (Figure 5). As there is neither an AG dinucleotide nor a CTPuAPy sequence immediately upstream of the 5'end mapped with S1 nuclease, both of which are conserved sequences within the 3'end of all *S. pombe* introns (9), this experimental finding precludes the existence of an intervening sequence in the 5'untranslated region of the gene.

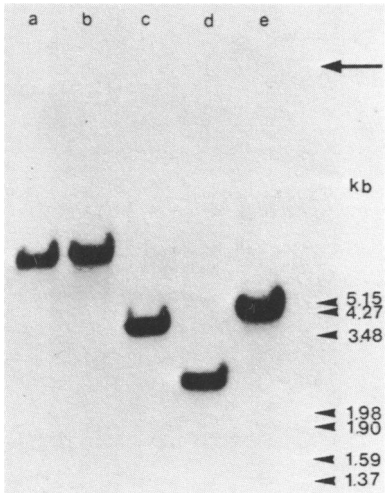


**Figure 3.** Southern blot analysis to determine the number of actin genes in *S. pombe*. Cellular DNA was digested with restriction endonucleases *Hind*III (lane a), *BamH*I (lane b), *Hind*III/*BamH*I (lane c), *BamH*I/*Bgl*II (lane d) or *Bgl*II (lane e), separated on a 0.9 % agarose gel in Tris-borate buffer and transferred to nitrocellulose. The filter was hybridized with a cloned, $^{32}$P-nick-translated *Nco*I/*BamH*I fragment of the protein-coding region (see Figure 1). Only the fragments predicted from known restriction sites (Figure 1) with a length of 7.3 kb, 8.4 kb, 3.9 kb, 2.6 kb and 4.8 kb (lanes a to e) gave a hybridization signal. Arrows and numbers to the right indicate the position and lengths (in kb) of *Eco*RI/*Hind*III λ DNA fragments used as size markers.

A 2.2 kb *BamH*I/*Bgl*II fragment, 3'end-labelled at its *BamH*I site (located within codon 342), was used to determine the 3'ends of the different actin mRNAs. As expected, three major protected fragments of 160 bp, 520 bp and 725 bp were observed (Figures 6A and B). As only the smallest of the three protected fragments was fully sequenced, the exact 3'end of the shortest mRNA was again determined with a 420 bp *BamH*I/*Hae*III fragment which was end-labelled at the *BamH*I site. As can be seen in Figure 6A, the 3'end maps to the A or T residue 59 or 60 nucleotides downstream from the translation termination codon. A putative polyadenylation signal sequence, AATAA, ends 15 or 16 nucleotides in front of the 3'end (see Figure 2).

The three mRNAs therefore have the same 5'end but they differ significantly in the length of their 3'untranslated regions which are, without poly(A) tail, 60, 420 and 625 nucleotides long.

**The amino acid sequence of the *S. pombe* actin**

As deduced from the DNA sequence, the *S. pombe* actin is 374 residues long and resembles, in this respect, the *S. cerevisiae* actin (2-4) and the vertebrate cytoplasmic actins which are shorter by one residue than the skeletal muscle actins (11). Interestingly, the degree of homology between the *S. pombe* actin and the mammalian cytoplasmic γ-actin (33 amino acid

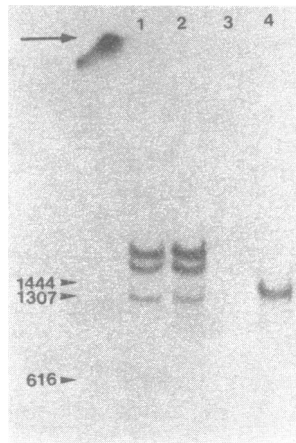


**Figure 4.** RNA blot analysis of *S. pombe* actin gene transcripts generated in *S. pombe* and *S. cerevisiae*. Polyadenylated RNA from *S. pombe* strain L972, either logarithmically growing (lane 1) or stationary (lane 2), and from the *S. cerevisiae* strain YNN27 untransformed (lane 3) or transformed with the recombinant plasmid YRp70/PA which harbours the *S. pombe* actin gene with flanking sequences (lane 4), was denatured by glyoxylation, separated on a 1.5 % agarose gel, transferred to a nitrocellulose filter and hybridized to an actin-coding fragment shown in Figure 1. Note that the homologous *S. cerevisiae* actin gene transcript of 1440 nucleotides (7) does not crosshybridize with the *S. pombe* hybridization probe under the conditions used (5 x SSC, 50 % formamide, 42°C; lane 3). Numbers to the left indicate the position and lengths (in nucleotides) of *TaqI* pBR322 DNA fragments used as size markers. The long arrow indicates the start of the gel.

differences) is higher than that between the two ascomycete actins (39 differences; underlined in Figure 2). In particular, within the N-terminal region, which is highly variable in different actin isoforms of one species and in actins from different eukaryotes (12), *S. pombe* actin exhibits perfect homology for the first 39 residues with the mammalian γ-actin whose N-terminal residues Glu-Glu-Glu- together with Ile-5 have been regarded as diagnostic for this actin isotype (12).

The comparison between the *S. pombe* and the *S. cerevisiae* actin (Figure 2) shows a certain clustering of amino acid exchanges. Whereas the region between residues six and 100 is highly conserved (with only three conservative changes), there is a conspicious accumulation of exchanges between residues 160 to 180 and 260 to 280 (with six out of 17 and six out of 15 amino acids differing in the two regions, respectively). Another noticeable feature of the *S. pombe* actin is the tyrosine residue in position 370. In all other actins sequenced so far, position 370 is invariably occupied by a histidine residue.

**Transcription of the *S. pombe* actin gene in *S. cerevisiae***

As a first step in studying the functional expression of the *S. pombe* actin gene in the evolutionary distant budding yeast *S. cerevisiae*, we transformed the haploid *S. cerevisiae* strain YNN27 with the recombinant plasmid YRp70/PA containing the *S. pombe* actin gene with 1.6

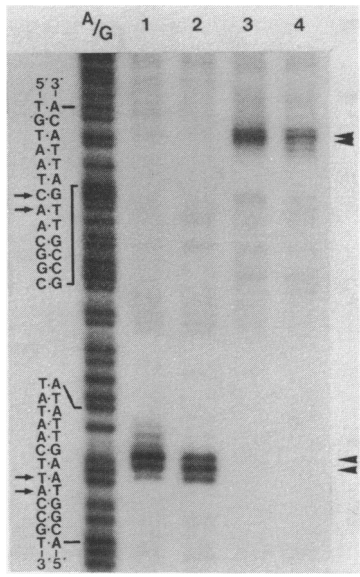


**Figure 5.** S1 nuclease protection analysis to determine the transcription start sites of the *S. pombe* actin gene in *S. pombe* (lanes 1 and 2) and in *S. cerevisiae* (lanes 3 and 4). Total cellular RNA was hybridized to the 5'end-labelled *NcoI/HhaI* fragment (see Figure 1) and hybrids formed at 37°C for 3 hours were digested with either 20 units/ml (lanes 1 and 3) or 100 units/ml of S1 nuclease (lanes 2 and 4). Protected fragments were separated on a 6 % polyacrylamide-urea sequencing gel in parallel with the fragments generated by chemical cleavage at A and G residues of the same DNA fragment used for the nuclease protection experiment. Arrows at the sequences shown indicate the position of the 5'start sites (corrected by 1.5 nucleotides) as mapped with the single strand-specific nuclease.

kb and 2.1 kb flanking the 5'and 3'end of the protein-coding region. An RNA blot analysis revealed that only one *S. pombe*-specific transcript with a length of about 1330 nucleotides was formed in *S. cerevisiae* (Figure 4). As shown by S1 nuclease protection experiments (Figures 5 and 7), the transcription initiation site as well as the 3'end of the transcript differed from those of the actin mRNAs synthesized in *S. pombe*. The *S. cerevisiae* transcript of the *S. pombe* actin gene initiated 39 nucleotides further upstream (at the C or A residue at position -96/-97; Figure 5) and terminated about 28 nucleotides further downstream (at position +85/+88; Figure 7) from the respective sites used in *S. pombe*. It is also noteworthy that there was no indication for the usage of the two other termination regions observed in *S. pombe*. This result supports the conclusion drawn from experiments by Russel (13) that the transcription initiation mechanism in *S. pombe* and *S. cerevisiae* differs and in addition it shows that the mechanism of transcription termination (or 3'processing) as well has diverged between these two distantly related yeast species.

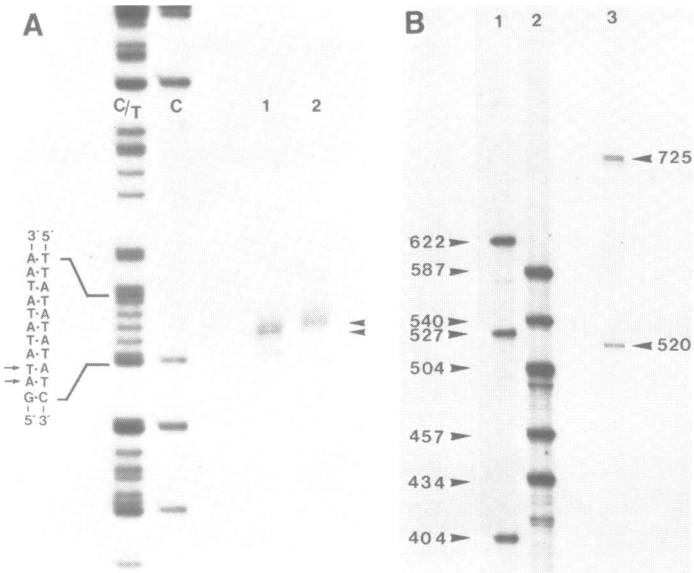


**Figure 6.** S1 nuclease protection analysis of 3'ends of *S. pombe* actin gene transcripts. The experiments were performed with total cellular RNA of *S. pombe* and either the 3'end-labelled *BamHI/HaeIII* fragment (A) or the 3'end-labelled *BamHI/BglII* fragment (B) shown in Figure 1. S1 nuclease was used at concentrations of 10 and 20 units/ml (lanes 1 and 2; A) and 20 units/ml (lane 3; B). As length markers for the identification of the 3'end of the shortest of the three mRNAs served the fragments of the sequencing ladder of the 3'end-labelled *BamHI/HaeIII* fragment (C/T- and C-specific chemical cleavage; A) or the 5'end-labelled fragments generated by *HpaI* (lane 1; B) or *HaeIII* (lane 2; B) digestion of pBR322. In (A), the position of the protected fragments with regard to the sequence, corrected by 1.5 nucleotides (7), is indicated by arrows on the left. In (B), arrows to the right indicate the position and the approximate length of the protected fragments corresponding to the 3'ends of the two larger mRNAs.

## DISCUSSION

Actin is an ubiquitous eukaryotic protein with an extraordinarily high conserved primary structure. Whereas higher eukaryotic species express several actin isoforms (11,12,14), lower eukaryotes like yeasts (2-4, and this report), *Neurospora crassa* (B. Buchholz, P. Mertins and D. Gallwitz, unpublished results) and *Acanthamoeba castellanii* (15,16) express only one major form of actin. However, the only organisms identified so far to contain a single actin gene copy per haploid genome are the yeasts *S. cerevisiae* (1-4) and *S. pombe* (this report) and the filamentous fungus *Neurospora crassa* (B. Buchholz, P. Mertins and D. Gallwitz, unpublished results).

The structure of actin genes has been studied in a large number of phylogenetically very distant species. It has been found that most of these genes are split with the introns interrupting the coding region at many different positions. Like the actin genes of the slime mold

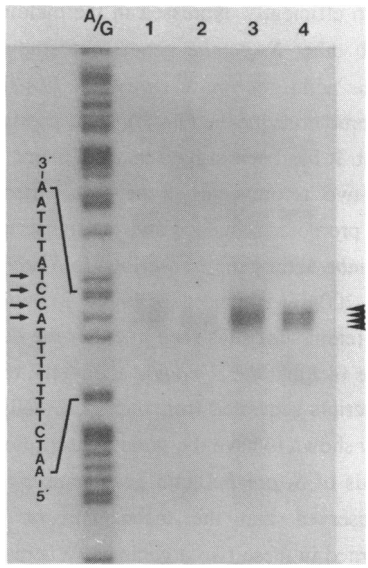


**Figure 7.** 3'end mapping of the *S. pombe* actin gene transcript generated in *S. cerevisiae*. Total RNA from *S. cerevisiae* strain YNN27 transformed with the recombinant plasmid YRp70/PA was hybridized to the 3'end-labelled *BamHI/Hae*III fragment as described in Figure 6. Fragments protected after digestion with 50 units/ml (lanes 1 and 3) or 100 units/ml of S1 nuclease (lanes 2 and 4) at 50°C (lanes 1 and 2) or 37°C (lanes 3 and 4) were separated electrophoretically on a 6 % sequencing gel alongside the fragments generated by chemical cleavage at A/G residues of the same 5'end-labelled fragment used for nuclease protection. Arrows to the left point to the nucleotides in the sequence to which the protected fragments map.

*Dictyostelium discoideum* (17) the single *S. pombe* actin gene is not split. The protein it codes for resembles the mammalian cytoplasmic γ-actin in having its characteristic N-terminus with three glutamic acid residues in the first three positions and an isoleucine residue in position five. In fact, the overall homology between the *S. pombe* actin and the mammalian γ-actin is higher than that between the actins of the two ascomycetes. This tends to support the now widespread notion that the fission yeast is more similar to vertebrate cells than the budding yeast (18).

A somewhat surprising observation was that three mRNAs with significant length differences are transcribed from the single *S. pombe* actin gene. These mRNAs are formed in comparable amounts and regardless of whether the cells are actively dividing or are resting. The three mRNAs differ in the length of their 3'untranslated regions and this is reminiscent of the situation in higher eukaryotes where similar observations have been made in a number of instances (19-24).

In accord with previous experimental findings (13,25,26) showing that several *S. pombe* genes are being transcribed in *S. cerevisiae*, although with different start sites, we found that the

*S. pombe* actin gene was also efficiently expressed in the budding yeast. However, in marked contrast to the findings with other *S. pombe* genes, our study shows that the transcription initiation site of the *S. pombe* actin gene in *S. cerevisiae* lies further upstream and therefore closer to the likely TATA promoter sequence (TATATAA, position -146 to -140; see Figure 2) than in the homologous yeast. It has been argued that the major difference of the transcription initiation mechanism in the two ascomycetes is the requirement in *S. cerevisiae* of a larger distance between the TATA promoter sequence and the transcription start site (13). This does either not hold for the *S. pombe* actin gene transcribed in *S. cerevisiae* or else another TATA sequence (the region around -200, for instance; see Figure 2) could fulfill the promoter function in the budding yeast. The different start sites seen in either the homologous or the heterologous yeast are not explained by the fact that the *S. pombe* actin gene on a plasmid was transcribed in the budding yeast since transcripts generated from chromosomally located and plasmid-borne *S. pombe* genes were previously shown to have the same transcription start points (13).

In studying the 3'ends of *S. pombe* actin gene transcripts in the homologous and the heterologous yeast, we observed that the mechanism of transcription termination (or 3'processing) have also diverged in these two organisms. Whereas three 3'ends several hundred nucleotides apart are generated in *S. pombe*, only one was found in the heterologous yeast and this was located about 28 nucleotides downstream from the 3'end of the homologous transcript terminating closest to the translation termination codon. This observation adds to the many mechanistic differences noted that distinguish gene expression in these evolutionary rather distant ascomycetes (18).

## ACKNOWLEDGEMENTS

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