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Molecular cloning and characterization of the *Schizosaccharomyces pombe his3* gene for use as a selectable marker

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Abstract. A DNA fragment which carries the *his3* gene of *Schizosaccharomyes pombe* has been isolated and characterized for use as a selectable marker in transformations. The *his3* gene encodes the imidazole acetol phosphate transaminase enzyme (E.C.2.6.1.9), which is responsible for converting imidazole acetol-P to histidinol-P in step 8 of histidine biosynthesis. The nucleotide sequences of a 2196 bp gene fragment and a corresponding cDNA clone were determined. Three intron sequences punctuate the 1451 bp coding region which generates a predicted polypeptide of 384 amino acids with a molecular mass of 42736 daltons. Northern analysis of *his3* mRNAs indicates that the transcript is approximately 1.6 kb in size. Steady-state levels are down-regulated by nitrogen limitation but are unaffected by histidine starvation. The deduced amino acid sequence was compared to the *Saccharomyces cerevisiae HIS5, Escherichia coli HisC,* and *Salmonella typhimurium HisC* proteins, all of which are imidazole acetol phosphate transaminases. The *S. pombe his3* protein was 49.5% identical to the *S. cerevisiae HIS5* protein and 21.5% identity was found when all four proteins were compared. The shuttle vector pBG1 was constructed by subcloning the smallest functional region of *his3* and the *S. pombe ars1* sequence into pUC18 for use in transformation of His3− *S. pombe* strains. New *S. pombe* strains in which the *his3* gene was deleted have also been constructed.

Key words: *Schizosaccharomyces pombe* – Histidine – Shuttle vector – Cloning

Introduction

There are ten steps in the biosynthesis of histidine (reviewed in Jones and Fink 1982), and nine genes required for this pathway in the yeast *Schizosaccharomyces pombe* have been identified (reviewed in Gutz et al. 1974). Seven of these have been assigned chromosomal locations (reviewed in Lennon and Lehrach 1992), and this information has been very useful in a number of genetic studies. In four cases, a correlation has been made between a gene locus and the enzyme encoded by it (Gutz et al. 1974). At the present time, no sequences of *S. pombe* histidine biosynthetic genes have been reported. In the yeast *Saccharomyces cerevisiae,* the sequences of several *HIS* genes are known (Donahue et al. 1982; Hinnebusch and Fink 1983; Nishiwaki et al. 1987; Struhl 1985), and the *HIS3* gene is used routinely as a nutritional marker (Sikorski and Hieter 1989).

In an effort to increase the number of selectable nutritional marker genes available for transformations and gene deletions in *S. pombe*, we report the isolation and sequence of the *S. pombe his3* gene, which encodes the enzyme imidazole acetol phosphate transaminase (EC.2.6.1.9) (Gutz et al. 1974). This enzyme catalyzes the eighth step in histidine biosynthesis (reviewed in Jones and Fink 1982). The amino acid sequence deduced from the nucleotide sequence is 49.5% identical to that of the *S. cerevisiae* HIS5 gene product. We have investigated the steady-state levels of *his3* mRNAs under a variety of nutritional circumstances. We have constructed a new shuttle vector containing the minimal functional region of the *his3* gene for use in *S. pombe* transformations. In addition, new yeast strains in which the *his3* gene has been deleted by a two-step gene replacement strategy have been constructed for use in *S. pombe* transformations and gene deletion experiments.

Materials and methods

Strains and media. The *Schizosaccharomyces pombe* strains used throughout this study were the multiple auxotrophs *his3-237 leu1-32 ura4-D18* h^+ and *leu1-32 ade6-M210 ura4-D18* h^-, as well as wild-type 972 h^-. Strains were grown in minimal or yeast extract medium with appropriate supplements (Moreno et al. 1991).

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For nitrogen starvation experiments, wild-type 972 h^- cells were grown in minimal medium to a density of 4×10^6 cells/ml and washed three times in minimal medium without a nitrogen source. They were then resuspended in minimal medium without nitrogen and allowed to continue growth. Aliquots were removed at 0, 4, 8, and 12 h intervals, pelleted, and frozen in a dry ice/ethanol bath. At 12 h, cells were pelleted and resuspended in fresh nitrogen-containing minimal medium and samples were taken 3 and 4 h later, pelleted, and frozen. For histidine starvation, the *his3-237* mutant was grown in minimal medium with appropriate supplements to a density of 6×10^6 cells/ml. The cells were washed three times and resuspended in minimal medium lacking histidine. Samples were taken at 0, 4, and 8 h, pelleted, and frozen. Cell cycle progression was monitored by microscopic examination of the septation index. *Escherichia coli* strains MC1061 (*araD139, Δ(ara-leu)7696, Δ(lac)174, galU, galK, hsdR2*(r_K, m_{K+}), *mcrB1, rpsL*(Str^1) and NM522 *supE, thi-1, Δ(lac-proAB)*), *Δ(hsdSM-mcrB)5*(r_{k-}, m_{k-}), [F′*proAB, lacI*^{q}*ZΔM15*] were used to retrieve plasmids from yeast cells and in other molecular cloning procedures. Bacterial cells were grown at 37° C in L-broth liquid medium or agar, with ampicillin (100 mg/ml) for plasmid selection.

Library screening and transformations. The *S. pombe* genomic library in the pUR19 vector containing the *ura4*$^+$ marker gene was a gift from Dr. A.M. Carr (Barbet et al. 1992). It was transformed into spheroplasts of the *S. pombe his3-237 leu1-32 ura4-D18 h*$^+$ strain according to standard procedures (Moreno et al. 1991). Rescuing plasmids were recovered by digesting cell walls with Zymolyase and further purified using GeneClean II (Bio 101) as described (Moreno et al. 1991). Other *S. pombe* transformations were performed by electroporation (Prentice 1991).

Competent *E. coli* cells were prepared by treating strains MC1061 and NM522 with $CaCl_2$ as described (Maniatis et al. 1982) or by electroporation as suggested by BioRad (Hercules, Calif.). To isolate *his3* cDNAs, a *S. pombe* cDNA library in the plasmid pDB20 (Fikes et al. 1990) was plated at 25 000 colonies/filter on three 110 cm Hybond-N nylon filters (Amersham). The colonies were replica-plated and screened in duplicate with a single-stranded 357 bp ^{32}P-labeled *Bam*HI-*Xho*I fragment corresponding to the 5′ end of the *his3* protein coding region.

Deletion mapping. Subclones of the 3.6 kb genomic DNA insert, clone 1, (see Fig.1) were constructed in pUC118 containing the *ars1* element, or in pUR19 (Barbet et al. 1992). The 3′ *Eco*RV-*Pst*I and 5′ *Sac*I-*Mlu*I fragments were blunt-ended and ligated into *Sma*I-digested and phosphatase-treated pUC118 containing the *ars1* element to create clones 1Δ3 and 1Δ1 respectively. The 3′ *Eco*RI-*Pst*I and 5′ *Sac*I-*Xho*I fragments were ligated into pUR19 digested with *Eco*RI + *Pst*I and *Sac*I + *Sal*I respectively, to create clones 1Δ5 and 1Δ2. The 3′ *Bam*HI fragment (clone 1Δ4) was prepared by removing the 5′ *Sac*I-*Bam*HI fragment from clone 1 and religating. Plasmids were tested for the presence of the *his3* gene by assessing their ability to promote colony formation of the *his3*-237 strain on plates lacking histidine.

Sequence analysis. Overlapping restriction fragments of *his3* were subcloned into pTZ18R or pTZ19R. The sequence of both strands was determined by the dideoxy chain termination method (Sanger et al. 1977) using Sequenase 2.0 (US Biochemicals) with single-stranded and double-stranded DNA templates as suggested by the manufacturer. Seven additional 17-base, single-stranded oligonucleotide sequencing primers (Operon Technologies) were needed to complete the entire sequence of both strands. The SwissProt protein database was searched using the FASTDB program (Brutlag et al. 1990) with the predicted amino acid sequence of the *his3* protein as the query sequence.

Northern and Southern blot analyses. Total RNAs from yeast were extracted from *S. pombe* wild-type 972 h^- and *his3-237* strains as described (Moreno et al. 1991). For Northern hybridizations, RNAs (5 μg total RNA/well) were size-fractionated by electrophoresis through 0.7% agarose-formaldehyde gels and transferred to a GeneScreen Plus (NEN/Dupont) membrane. Northern blots were baked at 80° C for 1 h and pre-hybridized ≥ 1 h at 65° C in 5 × SSPE (1 × SSPE is 0.15 M NaCl, 10 mM sodium phosphate, 1 mM EDTA, pH 7.4), 5 × Denhardt's, 0.5% SDS, and 100 μg hydrolyzed yeast RNA/ml. The blots then hybridized with a ^{32}P-labeled *Bam*HI-*Xho*I single-stranded fragment prepared as described (Jeffreys et al. 1985). Following hybridization, the filters were washed in 0.1 × SSPE, 0.1% SDS at 65° C for 1 h.

Plasmid DNAs were prepared by the alkaline lysis method (Maniatis et al. 1982), the cesium chloride method (Maniatis et al. 1982) or on Qiagen (Chatsworth, Calif.) Midi columns as described by the manufacturer. Probes for Southern analysis were prepared by random ^{32}P-priming of double-stranded fragments using the QuickPrime kit (Pharmacia). Hybridization conditions for Southern blots were as described for Northern blots, except that they were washed in 0.2 × SSPE, 0.2% SDS at 65° C for 1 h.

Deletion of the his3 *gene.* The *his3* gene was deleted from the *S. pombe* genome by a two-step disruption process, essentially as described (Grimm et al. 1988; Gallo et al. 1993). First, the blunt-ended *Hin*dIII *ura4*$^+$ gene fragment was subcloned into a *Hin*dIII-cut pSK(+) vector (Stratagene), which had also been blunt-ended to destroy the *Hin*dIII site in its polylinker. The entire 3.6 kb *his3* genomic clone was ligated as a *Pst*I-*Sac*I fragment into the newly constructed pSKura4 plasmid at the *Pst*I and *Sac*I sites, to create pJB1 (Fig. 2). To delete the *his3* coding region, a polymerase chain reaction (PCR)-based method was used. Two 27-base oligonucleotides were obtained, which were complementary to the *his3* flanking sequences adjacent to the protein coding region. These oligonucleotides, 5′CTAAGATCTAGTATTCAAATCAAACAT3′ and 5′TAGAGATCTTACTATACAAAAAAATGA3′, were designed such that PCR

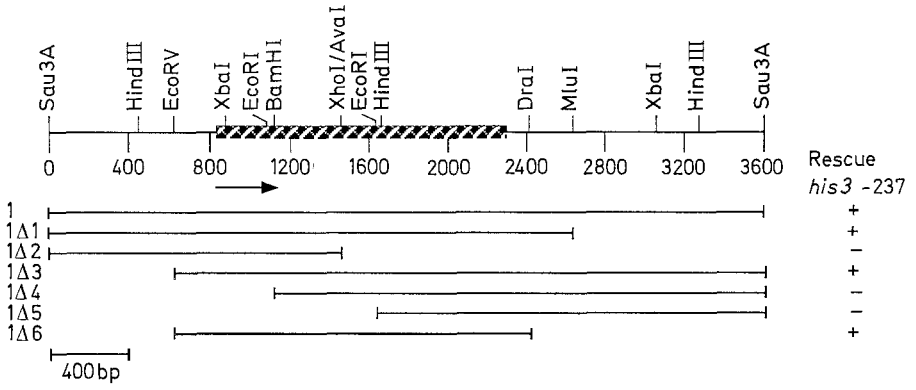

Fig. 1. Restriction map and deletion analysis of the *his3* genomic clone. Important restriction sites used are shown above the map. The 1451 bp coding region is identified as the broad *hatched rectangle*. The direction of transcription is indicated by the *arrow* below the coding region. The ability (+) or inability (−) of a deletion fragment to complement the *his3-237* mutation is indicated on the right

would extend these primers outward from the coding region, resulting in the amplification of pJB1 with specific excision of the *his3* coding region. The oligonucleotides were also engineered to create unique *Bgl*II restriction sites at each end of the PCR fragment (Fig. 6). The PCR reactions were performed using 10 ng of plasmid DNA template and *Pfu* DNA polymerase (Stratagene), with denaturation at 95° C for 2 min, annealing at 50° C for 2 min, and extension at 72° C for 10 min. The 6.7 kb PCR product was gel-purified, cut with *Bgl*II, and self-ligated to create a new plasmid lacking the coding region. This was linearized at the unique *Mlu*I restriction site present in the 3′ flanking region of the *his3* gene and transformed into the *ade6-M210 ura4-D18 leu1-32 h*$^-$ strain by electroporation (Fig. 6). Transformants prototrophic for uracil were selected and stable *ura4*$^+$ integrants were generated by replicating the colonies four times to yeast extract agar containing uracil, before switching back to minimal agar supplemented only with adenine and leucine. These procedures allow recovery of a strain in which the *ura4*$^+$ gene has inserted into the genome between the wild-type *his3* gene and the *his3* deletion construct (Fig. 6). Stable *ura4*$^+$ integrants were grown for approximately five generation times to allow recombination to occur between the two versions of the *his3* locus. Such recombination can result in the excision of the *ura4*$^+$ gene and one copy of the *his3* locus (Fig. 6). Cells is which this event had occurred were selected by plating on minimal agar plates containing 5-fluroorotic acid (5-FOA) at a final concentration of 1 mg/ml and uracil at 50 μg/ml (Grimm et al. 1988).

Results

Cloning of the his3 *gene*

A *S. pombe* genomic DNA library in the pUR19 vector (Barbet et al. 1992) was transformed into spheroplasts of the *S. pombe* histidine auxotrophic strain *his3-237 leu1-32 ura4-D18 h*$^+$. Approximately 15 000 transformants were replica-plated onto agar lacking histidine at 32° C to select for *his*$^+$ colonies. Plasmids recovered from four *his*$^+$ colonies were able to confer the *his*$^+$ phenotype on the *his3-237* strain upon re-transformation. These four plasmids contained three different but related inserts with the smallest being approximately 3.6 kb long (Fig. 1).

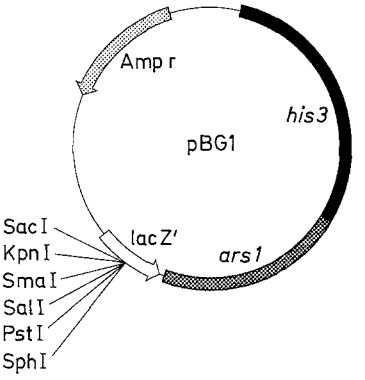

Fig. 2. The pBG1 shuttle vector utilizing the *S. pombe his3* gene. The *ars1* element and the *his3* gene were inserted into the blunt-ended *Nde*I site of the pUC18 vector as described in Materials and methods. Unique cloning sites in the plasmid are listed

To define the position of the *his3* gene within this 3.6 kb fragment, a series of deletions was constructed and tested for the ability to rescue the *his3-237* mutant (Fig. 1). From this analysis, it appeared that the essential region of *his3* was contained within approximately 1750 nucleotides between the *Eco*RV and *Dra*I sites (Fig. 1). All constructs that did not contain this region were unable to complement the *his3-237* mutant.

The *his3* gene maps genetically to chromosome II and is genetically linked to the *cdc2* gene (Lennon and Lehrach 1992). Hybridization of the *his3* gene clone to a library of *S. pombe* cosmids (Hoheisel et al. 1993; Maier et al. 1993) also places the *his3* gene in close physical proximity to the *cdc2* gene (data not shown.)

A shuttle vector utilizing the *his3* gene, pBG1, was created in the pUC18 vector (Pharmacia). The 1782 bp *Eco*RV-*Dra*I *his3* fragment (clone 1Δ6) and the 1.2 kb *Eco*RI *ars1* element were blunt-ended and ligated into the pTZ19R (Pharmacia) *Sma*I and *Acc*65I (*Kpn*I) polylinker sites, respectively. They were removed as a single fragment from pTZ19R by digesting with *Pst*I and *Sac*I. This fragment was then blunt-ended and ligated into the blunt-ended *Nde*I site of pUC18 to create pBG1 (Fig. 2).

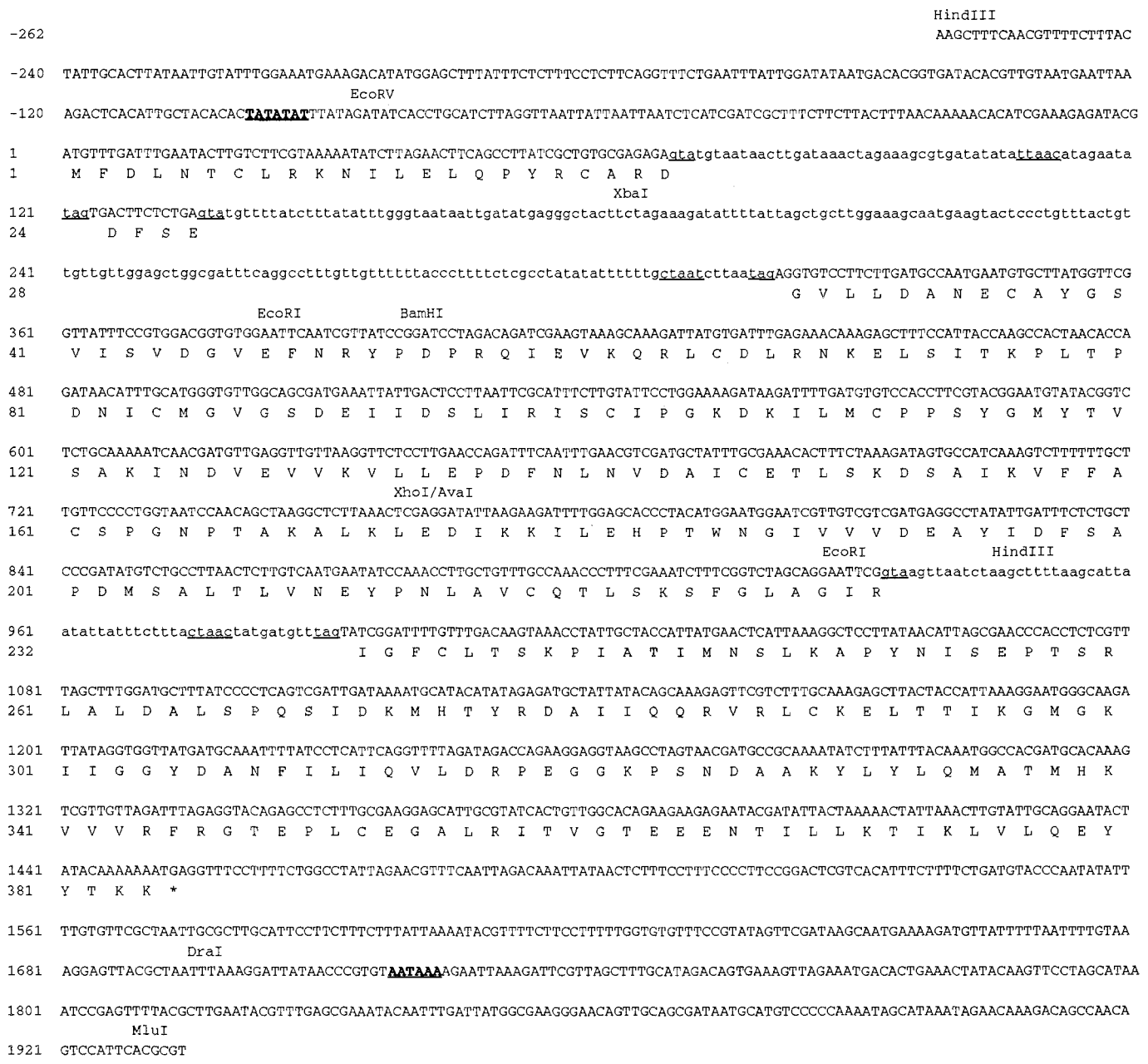

Fig. 3. The nucleotide sequence of the *S. pombe his3* gene. The 1451 bp *his3* coding region is initiated with the ATG start codon at position 1 and continues to the TGA stop codon indicated by an *asterisk*. Three intron sequences are present *(lowercase lettering)* with their splice donor, splice acceptor, and branch point sequences *underlined*. Important restriction sites are shown above the sequence. A possible TATA box in the 5′ untranslated region and polyadenylation consensus sequence in the 3′ untranslated region are in *boldface type* and *underlined*. The derived amino acid sequence of the openreading frame is in standard single letter notation

The nucleotide sequence of his3

The nucleotide sequence of the *Hin*dIII-*Mlu*I *his3* fragment that extends in both directions from the minimal functional fragment was determined on both strands. A 1451 bp coding region was identified in which three putative introns were present (Fig. 3). To confirm the intron/exon boundaries, eight overlapping cDNA clones corresponding to the *his3* gene were obtained, and three were sequenced. The cDNA clones were found to lack putative intron sequences corresponding to positions 69-123, 136-320, and 933-991 bp from the ATG initiation codon in the genomic clone (Fig. 3). Each of these intron sequences contained consensus splice donor, splice acceptor, and branch point sequences known to be present in *S. pombe* introns (Russell 1989). In the 262 bp, 5′ noncoding sequence, a possible Hogness box or TATA consensus sequence (Locker and Buzard 1990) is present at position −97 to −100 upstream of the predicted initiation codon. The 483 bp 3′ untranslated region contains

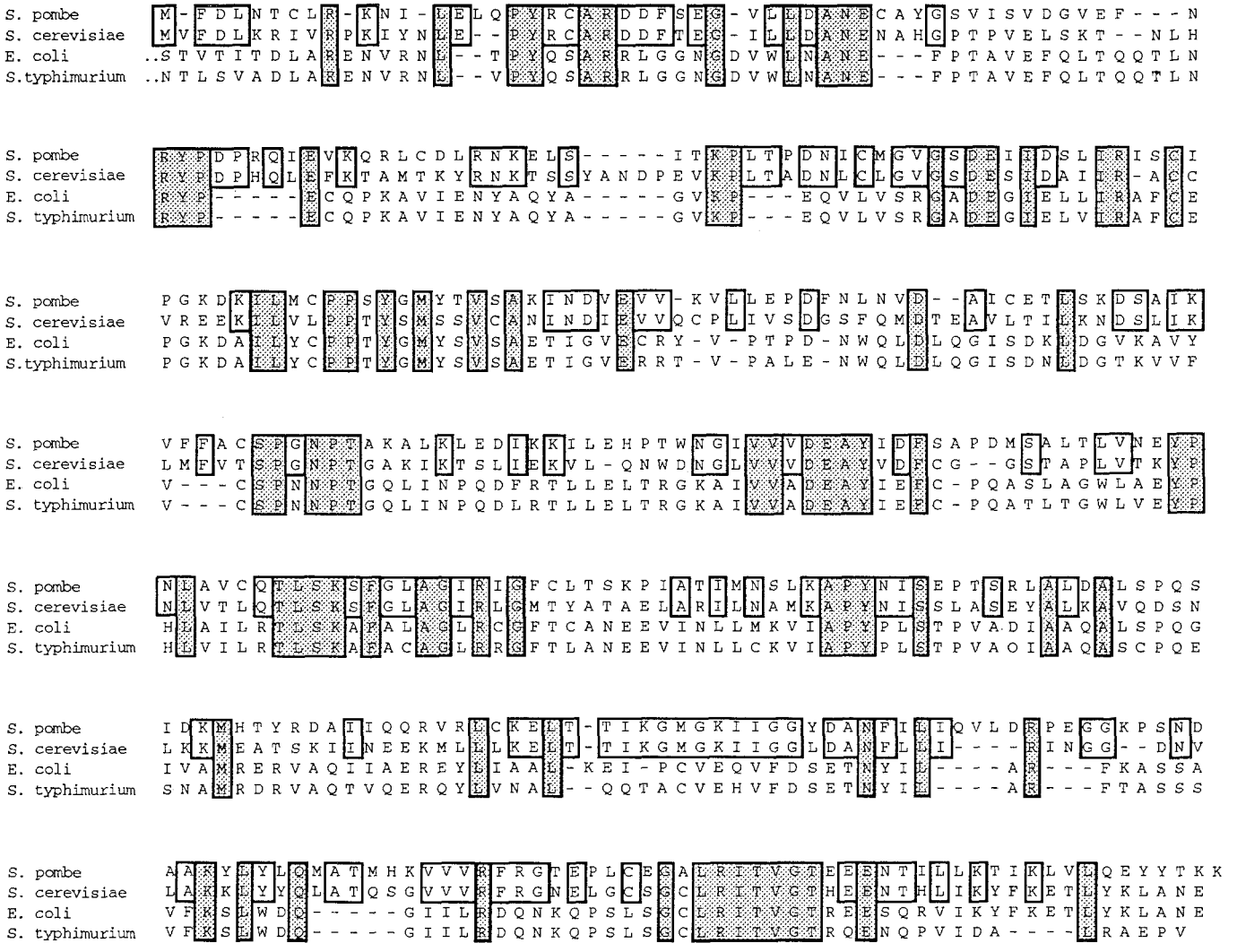

Fig. 4. Alignment of imidazole acetol phosphate transaminase sequences. The amino acid sequence of the *S. pombe his3* protein is aligned with the *S. cerevisiae* HIS5 (Nishiwaki et al. 1987), *E. coli* HisC (Carlomagno et al. 1988) and the *S. typhimurium* HisC (Carlomagno et al. 1988) proteins. Amino acid residues identical in the two yeast proteins are in *open boxes,* whereas residues identical in all four proteins are highlighted by *shaded boxes*. Gaps have been introduced to maximize the alignment

a eukaryotic polyadenylation consensus signal (5′AATAAA3′) (Proudfood and Brownlee 1976) at positions 1717 to 1722 relative to the ATG initiation codon.

The 1451 bp coding region of *his3* predicted a protein composed of 384 amino acids with a predicted molecular mass of 42.7 kDa and a pI of 5.69 (Fig. 3). The SwissProt protein database was searched with the deduced amino acid sequence of *his3*, and homologs were found in many species. The five proteins containing the greatest homology are imidazole acetol phosphate transaminase enzymes (E.C.2.6.1.9) which convert imidazole acetol-P to histidinol-P at step eight of histidine biosynthesis (Jones and Fink 1982). In Fig. 4, the amino acid sequence of the *S. pombe his3* protein is compared with that of the *S. cerevisiae* HIS5 (Nishiwaki et al. 1987), *E. coli* HisC (Carlomagno et al. 1988), and the *Salmonella typhimurium* HisC (Carlomagno et al. 1988) proteins. The two yeast protein sequences were found to be 49.5% identical, with at least 21.5% identity among all four proteins.

Transcriptional analysis of his3

Northern blot analysis of total RNA from wild-type cells showed that the *his3* transcript is approximately 1.6 kb in size, which is in good agreement with the size of the coding region (Fig. 5a). To test whether the gene is transcriptionally controlled by general nutrient availability, steady-state *his3* mRNA levels were examined in wild-type 972 cells during a period of nitrogen starvation (Fig. 5a). Levels of *his3* transcript were reduced as the cells stopped dividing and entered stationary phase (8–12 h of nitrogen starvation). To ensure that equal amounts of mRNA were present on the blot, it was reprobed with a loading control, the *cdc3* gene. The steady-state levels of *cdc3* do not change upon nitrogen starvation (Burke and Gould, unpublished data) and *cdc3* mRNA levels were constant on this blot (data not shown). Induction of re-entry into the cell cycle by feeding the nitrogen-starved cells rich medium led to a return of *his3* mRNA to starting levels (Fig. 5a).

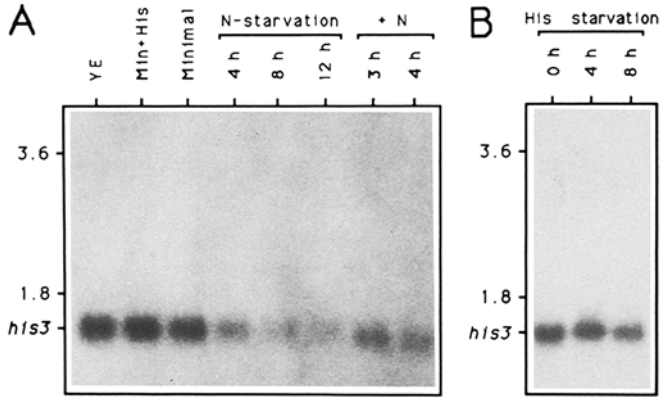

Fig. 5A, B. Northern blot analysis of *S. pombe his3* mRNA. Northern blots were probed with an internal *Bam*HI-*Xho*I single-stranded fragment as described in Materials and methods. The sizes and positions of the ribosomal RNAs are indicated. The *his3* mRNAs are approximately 1.6 kb in size. **A** RNAs extracted from wild-type 972 h^- cells. mRNAs from cells growing in: lane 1, yeast extract with supplements; lane 2, minimal medium with histidine (7.5 mg/ml); lane 3, minimal medium without supplements; lanes 4, 5, and 6, minimal medium lacking nitrogen for 4, 8, and 12 h respectively. The mRNAs in lanes 7 and 8 were extracted from the nitrogen-starved cells 3 and 4 h after nitrogen had been re-introduced into the medium. **B** mRNAs from the *his3-237* strain grown in minimal medium for 0, 4, and 8 h in the absence of histidine

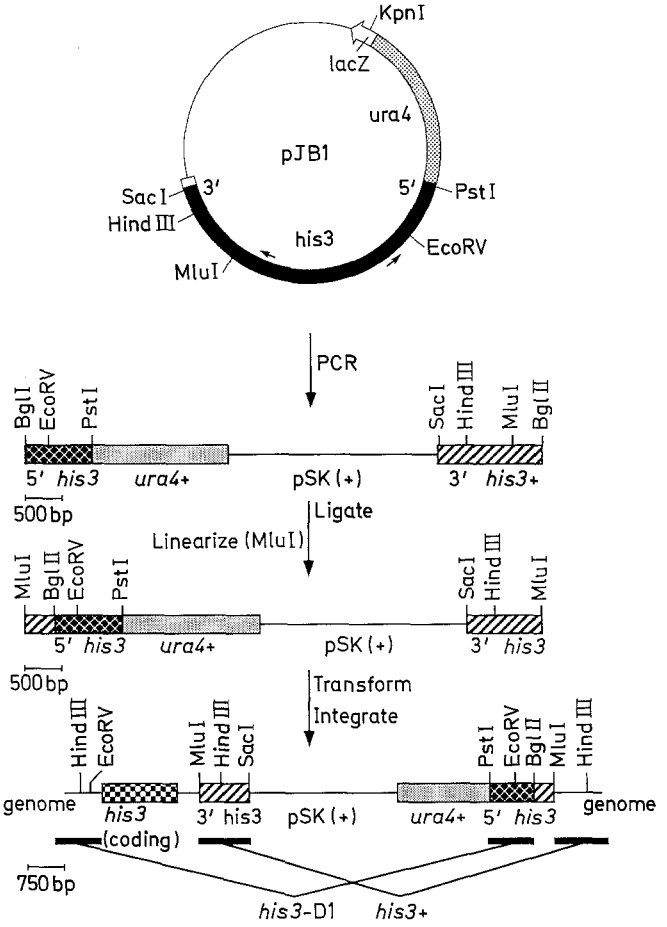

Fig. 6. Schematic diagram of the *his3* gene disruption. The pJB1 construct was produced by inserting the 3.6 kb *his3 (black)* and the *ura4*$^+$ *(dotted)* genes into the pSK(+) vector as described in Materials and methods. The arrows in the *his3* gene segment indicate the approximate positions and orientations of the oligonucleotides used for PCR amplification. The oligonucleotides, extending outward from the protein coding region, were designed to add *Bgl*II restriction sites to the ends of the PCR fragment (see Materials and methods). The PCR fragment was ligated to itself at the *Bgl*II site to form a circular plasmid, and then linearized at the unique *Mlu*I site. The linearized *Mlu*I fragment was transformed into the *ade6-M210 ura4-D18 leu1-32 h*$^-$ strain and integrated into the genome. An integrant produced by recombination within the 3′ noncoding region is shown. Integrants were grown under non-selective conditions to allow recombination to occur between the homologous regions of the *his3* genes. Recombinants that have lost *ura4*$^+$ were selected on 5-FOA plates as detailed in Materials and methods. Depending on the type of crossover, such recombinants were either *his3*$^+$ or deleted for the *his3* gene *(his3-D1, his3-D2, his3-D3)*

To test whether the gene is transcriptionally controlled by histidine availability, as is its cognate in *S. cerevisiae* (Nishiwaki et al. 1987), the steady-state level of *his3* mRNA was examined in wild-type cells growing in the absence of exogenously added histidine, and in the *his3-237* strain during a period of histidine deprivation. Levels of *his3* transcript were the same in wild-type cells grown in the presence or absence of histidine (Fig. 5a). Levels of *his3* mRNA also did not change during the course of histidine starvation (Fig. 5b), although the cells stopped growing as judged by their reduced size and reduced septation index (data not shown).

Disruption analysis of the his3 *gene*

The *his3* gene was deleted in a two-step gene disruption process (Grimm et al. 1988; Gallo et al. 1993). The *his3* and *ura4*$^+$ genes were subcloned into a bacterial expression vector (pJB1), and the coding region was then precisely removed by PCR methodology. The resulting construct (Fig. 6) was linearized and integrated into the *S. pombe* genome by homologous recombination to create strains containing both the wild-type *his3* gene and the deleted version of the *his3* gene with the *ura4*$^+$ gene in between (Fig. 6). Growth of these strains in media containing uracil allowed recombination to occur between the homologous regions of the two *his3* genes with the elimination of the intervening *ura4*$^+$ gene. Such strains were selected by their ability to grow on medium containing 5-FOA (Fig. 6). A significant portion of the 5-FOA-resistant cells had lost the wild-type version of the *his3* gene as evidenced by their inability to grow on minimal agar plates lacking histidine (Fig. 7a). Three of these, *his3-D1, his3-D2* and *his3-D3,* were characterized further. Southern blotting confirmed that the wild-type version of the *his3* gene had been deleted in these strains (Fig. 7b). Plasmids carrying the *his3* gene (pBG1) and clone 1) restored the ability of the new *his3* deletion strains to grow in the absence of histidine. An example is shown in Fig. 7c.

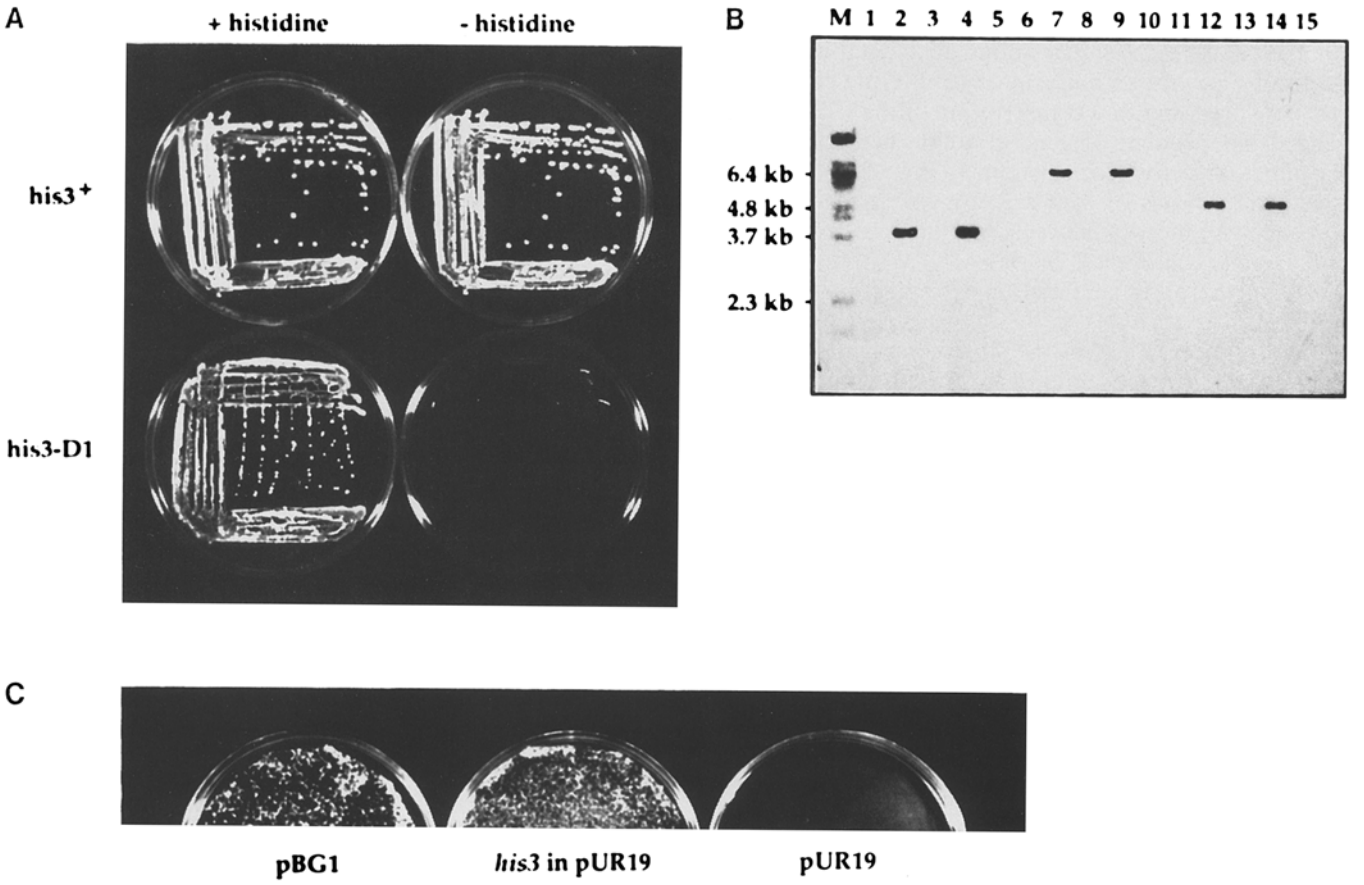

Fig. 7A–C. Analysis of *his3* deletions. **A** The *ade6-M210 ura4-D18 leu1-32* and *ade6-M210 ura4-D18 leu1-32 his3-D1* strains were replica-plated from yeast extract plates onto minimal agar in the presence or absence of histidine (7.5 mg/ml), and allowed to grow at 32° C for 48 h. **B** Southern blot analysis of the *his3* deletion strains. Lambda DNA digested with *Bst*EII served as molecular size marker (M). Genomic DNAs (1 μg/lane) in lanes, 1, 6, and 11 were from *his3-D1*, lanes 2, 7, and 12 from *his3-D2*, and in lanes 3, 8, and 13 from *his3-D3*. Wildtype 972 h^- genomic DNAs (1 μg/lane) were loaded in lanes 2, 4, 7, 9, 12, and 14. The genomic DNAs were digested with *Eco*RV (lanes 1–5), *Bgl*II (lanes 6–10), or *Pst*I (lanes 11–15), resolved by gel electrophoresis and transferred to GeneScreen Plus (NEN/Dupont) membrane. The blot was probed with the *Bam*HI-*Xho*I fragment from the *his3* coding region. The bands were visualized by autoradiography after a 36 h exposure at −70° C. **C** Complementation of the *his3-D1* strain. The *ade6-M210 ura4-D18 leu1-32 his3-D1* strain was transformed with pUR19, the 3.6 kb *his3* genomic clone in pUR19, or pBG1. The transformants were plated onto minimal agar plates containing uracil, leucine and adenine (7.5 mg/ml) but lacking histidine and allowed to grow at 32° C for 3 days

Discussion

We have cloned and characterized the *his3* gene from *S. pombe* in order to create an additional nutrient marker for use in transformations and gene disruptions. The *his3* gene was proposed to correspond to the enzyme, imidazol acetol phosphate transaminase (Gutz et al. 1974). Our DNA sequence analysis of the *his3* gene confirms this.

Three introns with consensus splice donor, splice acceptor, and branch point sequences (Russell 1989) punctuate the *his3* protein coding region. A possible TATA consensus sequence (Locker and Buzard 1990) was found at positions −94 to −100 upstream of the ATG initiation codon. A potential polyadenylation signal (5′AATAAA3′) (Proudfoot and Brownlee 1976) was found in the 3′ untranslated region of the gene, 263 bp downstream of the stop codon.

When the predicted 43 kDa *his3* protein was compared to other proteins in the SwissProt protein database, homologs were found both in other lower eukaryotes and in prokaryotes. Proteins which exhibit the greatest similarity to the *his3* protein are other imidazole acetol phosphate transaminases (E.C.2.6.1.9.). When compared with the *S. cerevisiae* HIS5 protein (Nishiwaki et al. 1988), the *S. pombe his3* protein shares 49.5% identitical amino acids. The prokaryotic enzymes encoded by *E. coli hisC* (Carlomagno et al. 1988) and *S. typhimurium hisC* (Carlomagno et al. 1988) are significantly less conserved but still exhibit 21.5% identity. Presumably, the longer stretches of conservation correspond to residues involved in catalysis (see Fig. 4).

In *S. cerevisiae*, many genes encoding enzymes of the histidine biosynthetic pathway are under general amino acid control, and transcription of these genes is coordinately stimulated by amino acid deprivation (Jones and Fink 1982). In addition, these genes are transcriptionally stimulated by drugs that block the final steps in histidine

biosynthesis, mimicking histidine starvation (Jones and Fink 1982). Since *S. pombe* amino acid metabolism is not subject to general amino acid control (Gutz et al. 1974), it is not surprising that transcription of the *S. pombe his3* gene is independent of general amino acid control and specific starvation for histidine (Fig. 5b). However, steady-state levels of *his3* mRNA were observed to be somewhat reduced when cells are starved for nitrogen and exit the cell cycle (Fig. 5a).

Using the minimal functional fragment of *his3*, the pBG1 shuttle vector (Fig. 2) was constructed for use in transformation of *S. pombe* strains carrying either the *his3-237* mutation or the *his3* deletion. The α-complementation system of pUC18 has been retained in pBG1 for ease of identification of cloned inserts. The length of the *his3* fragment used in pBG1 is 1782 bp, a manageable size for marker genes in yeast shuttle vectors (Russell 1989; Grimm et al. 1988; Sikorski and Hieter 1989). The minimalization of the flanking sequences does not appear to have compromised the ability of the gene to function, at least on a plasmid, since cells with the *his3-237* mutation grow as well when they carry pBG1 as they do when they harbor the original full-length genomic clone (data not shown).

Since its development, the *ura4*$^+$ deletion strain, *ura4-D18* (Grimm et al. 1988), has been very useful in a variety of molecular genetic experiments in *S. pombe*. We have generated similar strains deleted for the *his3* gene (*his3*-D1, -D2, and -D3) to use in gene deletion, integrative transformations, and genetic crosses. At the present time, the *S. cerevisiae LEU2* (Beach and Nurse 1981) and *S. pombe ura4*$^+$ (Grimm et al. 1988) genes are the most widely used marker genes in *S. pombe*. Given that the *S. cerevisiae LEU2* gene may not efficiently complement the *S. pombe leu1-32* mutation in a single copy, it is not often used as a gene replacement marker (Hayles and Nurse 1992). Since it would be predicted to complement His3$^-$ strains in a single copy, the *his3*$^+$ gene has the potential to be used as a gene replacement marker. The use of *his3* for creating gene disruptions would also allow one to introduce plasmids carrying the *ura4*$^+$ gene and greatly facilitate experiments involving plasmid shuffling.

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References

Barbet N, Muriel WJ, Carr AM (1992) Versatile shuttle vectors and genomic libraries for use with *Schizosaccharomyces pombe*. Gene 114:59–66

Beach D, Nurse P (1981) High frequency transformation of the fission yeast *Schizosaccharomyces pombe*. Nature 290:140–142

Brutlag DL, Dautricourt JP, Maulik S, Relph J (1990) Improved sensitivity of biological sequence database searches. Comp App Bio Sci 6:237–245

Carlomagno MS, Chiariotti L, Alifano P, Nappo AG, Bruni CB (1988) Structure and function of the *Salmonella typhimurium* and *Escherichia coli* K-12 histidine operons. J Mol Biol 203:585–606

Donahue TF, Farabaugh PJ, Fink GR (1982) The nucleotide sequence of the *HIS4* region of yeast. Gene 18:47–59

Fikes JD, Becker DM, Winston F, Guarente L (1990) Striking conservation of TFIID in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*. Nature 346:291–294

Gallo GJ, Prentice H, Kingston RE (1993) Heat shock factor is required for growth at normal temperatures in the fission yeast *Schizosaccharomyces pombe*. Mol Cell Biol 13:749–761

Grimm C, Kohli J, Murray J, Maundrell K (1988) Genetic engineering of *Schizosaccharomyces pombe:* a system for gene disruption and replacement using the *ura4* gene as a selectable marker. Mol Gen Genet 215:81–86

Gutz H, Heslot H, Leupold U, Loprieno N (1974) *Schizosaccharomyces pombe*. In: King RC (ed) Handbook of genetics, vol 1. Plenum Press, New York, pp 395–446

Hayles J, Nurse P (1992) Genetics of the fission yeast *Schizosaccharomyces pombe*. Annu Rev Genet 26:373–402

Hinnebusch AG, Fink GR (1983) Repeated DNA sequences upstream from *HIS1* and several other coregulated genes in *Saccharomyces cerevisiae*. J Biol Chem 258:5238–5247

Jeffreys AJ, Wilson V, Thein SL (1985) Hypervariable minisatellite regions in human DNA. Nature 314:67–73

Jones EW, Fink GR (1982) Regulation of amino acid and nucleotide biosynthesis in yeast. In: Strathern JN, Jones EW, Broach JR (eds) The molecular biology of the yeast *Saccharomyces:* metabolism and gene expression. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp 181–299

Hoheisel JD, Maier E, Mott R, McCarthy L, Grigoriev AV, Schalkwyk LC, Nizetic D, Francis F, Lehrach H (1993) High-resolution cosmid and P1 maps spanning the 14-Mbp genome of the fission yeast *Schizosaccharomyces pombe*. Cell 73:109–120

Lennon GG, Lehrach H (1992) Gene database for the fission yeast *Schizosaccharomyces pombe*. Curr Genet 21:1–11

Locker J, Buzard G (1990) A dictionary of transcription control sequences. J. DNA Seq Map 1:3–11

Maier E, Hoheisel JD, Lehrach H (1993) Ordered clone libraries as high resolution mapping tools. In: Albergina L, Frontali L, Sensi P (eds) Proc 6th Eur Cong Biotechnol, in press

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

Moreno S, Klar A, Nurse P (1991) Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. Methods Enzymol 194:795–823

Nishiwaki K, Hayashi N, Irie S, Chung D-H, Harashima S, Oshima Y (1987) Structure of the yeast *HIS5* gene responsive to general control of amino acid biosynthesis. Mol Gen Genet 208:159–167

Prentice HL (1991) High efficiency transformation of *Schizosaccharomyces pombe* by electroporation. Nucleic Acids Res 20:621

Proudfoot NJ, Brownlee GG (1976) 3′ Non-coding region sequences in eukaryotic messenger RNA. Nature 263:211–214

Russell P (1989) Gene cloning and expression in fission yeast. In: Nasim A, Young P, Johnson BF (eds) Molecular biology of the fission yeast. Academic Press, San Diego, pp 244–271

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467

Sikorski RS, Hieter P (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics 122:19–27

Struhl K (1985) Nucleotide sequence and transcriptional mapping of the yeast *pet56-his3-ded1* gene region. Nucleic Acids Res 13:8587–8601