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The 5S RNA genes of Schizosaccharomyces pombe

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ABSTRACT

The genomic arrangement and sequences of S. pombe 5S RNA genes are reported here. The 5S gene sequences appear to be dispersed within the genome, and are found independently of other rRNA genes. The sequences of two 5S genes examined show identical coding regions of 119 base pairs but have widely varying flanking sequences. A $tRNA^{Asp}$ gene is found in the 3' flanking region of one of the 5S genes. The $tRNA^{Asp}$ gene is faithfully transcribed in an X. laevis in vitro system, while the 5S genes are not transcribed in this system.

The phylogenetic position of S. pombe is examined through comparison of 5S RNA sequences.

INTRODUCTION

In eukaryotes the rRNA genes are organized as clusters of tandem repeats that are transcribed by RNA polymerase I (1). Transcription of the repeating unit results in a single precursor, which in turn is processed to form the mature rRNA species. Usually the 5S RNA genes (5S genes) are organized as separate gene clusters but in Dictyostelium and S. cerevisiae the 5S genes are closely linked with the genes for the large ribosomal RNAs (1). Since 5S genes are transcribed by RNA polymerase III 5S RNA formation is 'independent' of transcription of the other rRNA genes. RNA polymerase III is also responsible for the formation of tRNA (Roeder, 1976).

The clustered arrangement of 5S genes may have evolutionary consequences (2,3). However, the evolutionary mechanism may not be a common one in that the 5S genes of Neurospora crassa are not tandemly repeated (4).

In an effort to learn more about the components of the translation machinery of the fission yeast Schizosaccharomyces pombe and as part of our interest in understanding the transcription of eukaryotic tRNA genes by RNA polymerase III, we initiated a study of the arrangement and nucleotide

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sequence of the 5S genes of this organism.

Our results suggest that the 5S genes of S. pombe do not display tandem repetition and are not a component of the large rRNA gene unit. Rather the 5S genes of S. pombe are apparently dispersed throughout the genome and in this respect more closely resemble the organization observed for eukaryotic tRNA genes. The nucleotide sequence of S. pombe 5S RNA differs markedly from that of S. cerevisiae. Comparative analysis of the 5S RNA sequences according to Hori (5) suggests that the evolutionary position of S. pombe may be somewhat removed from the other yeasts (6).

MATERIALS AND METHODS

General. Restriction enzymes, T4 DNA ligase, T4 RNA ligase and T4 polynucleotide kinase were obtained commercially. (γ-^{32}P)ATP was prepared by the procedure of Walseth and Johnson (7). (α-^{32}P) nucleotide triphosphates and (5'-^{32}P)pCp were commercial products. The plasmids pYM3 and pYM3.1 contain the same S. pombe HindIII insert in pBR313 and pBR322, respectively.

DNA preparation. S. pombe DNA was prepared by modifications of the procedure of Cryer et al. (8). S. pombe strain ade 6 sup 3-704 was grown in YEA broth at 30°C to late log phase and harvested. The yield was 10 g wet weight/l. The fresh cells (per 10 g) were washed with 30 ml of 50 mM EDTA, pH 7.5, treated with 20 ml of 0.3 M 2-mercaptoethanol - 50 mM EDTA for 30 min at 25°C and washed again with 20 ml of 1 M sorbitol - 0.1 M EDTA, pH 7.5. The cells were resuspended in 20 ml of sorbitol-EDTA, and 5 mg of zymolyase (dissolved in 2 ml of sorbitol-EDTA immediately before use) were added and the mixture incubated for 30 min at 37°C. The resultant spheroplasts were harvested, washed with 20 ml of sorbitol-EDTA, then resuspended in 20 ml of 0.15 M NaCl - 0.1 M EDTA - 50 mM Tris-HCl, pH 8.0 and lysed by adding 10 mg of proteinase K (in 2 ml of the same buffer) and 1 ml of 25% SDS. The mixture was incubated at 37°C for 3 hrs and then at 65°C for 30 min. The resulting lysate was deproteinized twice with an equal volume of phenol. The nucleic acids in the aqueous phase were precipitated with 2 volumes of ethanol. The pellet was resuspended in 10 ml of 0.1 x SSC and the solution was brought to a final concentration of 2 x SSC. After addition of RNase A (final concentration of 0.1 mg/ml), T1 RNase (final concentration 100 units/ml) and α-amylase (final concentration 0.1 units ml) the mixture was incubated for 1 hr at 37°C. After phenol extraction and ethanol precipitation, RNase treatment was repeated. The DNA

was further purified by banding twice in CsCl gradients using a mean CsCl density of 1.699 g/cm3. The DNA fraction was dialyzed overnight against 10 mM TrisHCl pH 7.5 - 1 mM EDTA. The yield was approximately 0.1 mg DNA/g of wet weight cells.

Plasmid DNA was prepared by chloramphemicol amplification of the host E. coli HB101 and purified by CsCl-ethidium bromide density gradient ultracentrifugation according to Clewell (9).

S. pombe clone bank. S. pombe DNA was cleaved completely with HindIII and ligated to HindIII digested pBR322 DNA. After transformation of E. coli C600 SF8 (10) $Amp^{R}Tet^{S}$ clones were selected and screened (11) with in vivo labeled S. pombe (^{32}P)-5S RNA (12).

Restriction Enzyme Mapping. Restriction enzymes were obtained commercially and used according to the suppliers' recommendations. Digested DNA was analyzed by agarose gel electrophoresis (13,14) using the mapping method of Smith and Birnstiel (15). DNA was transferred from agarose gels to nitrocellulose membranes according to the method of Southern (16).

DNA Sequence Analysis. 5'-end group labeling and DNA sequence analysis were performed according to the procedures of Maxam and Gilbert (17).

End Labeling of RNA. 5S RNA was labeled at the 3'-terminus using T4 RNA ligase by the method of Bruce and Uhlenbeck (18). 5'-terminus labeling of 5S RNA was performed as follows: RNA (20-60 pmoles) in 0.005 ml 50 mM Tris-HCl, pH 8.3 was dephosphorylated at 37°C for 30 min using 0.005 units of nuclease-free calf alkaline phosphatase (19). The reaction was terminated by the addition of 0.005 ml 0.1 M potassium phosphate, pH 9.5. To this mixture were added 0.001 ml of a solution which contained 0.1 M $MgCl_2$, 20 mM spermine and 1 M KCl, 0.05-0.25 mCi (γ-^{32}P)ATP and 4 Richardson units of T4 polynucleotide kinase for a final volume of 0.01 ml. The reaction was performed at 37°C for 30 min. 5'-and 3'-terminus labeling reactions were loaded directly on to 12% acrylamide thin-gels (20) for purification of the labeled RNA.

5S RNA Sequence Analysis. Enzymatic RNA sequencing was performed using 5'- or 3'-labeled 5S RNA as described (21).

In vitro Transcription of Plasmid DNA. Xenopus germinal vesicle extracts were prepared and transcriptions performed as described previously (22).

RESULTS AND DISCUSSION

Isolation and analysis of 5S clones. A clone bank containing HindIII S. pombe DNA fragments in pBR322 was prepared as described in Materials and Methods. This bank of 4500 clones was screened with uniformly ^{32}P-labeled 5S RNA and 14 independent clones were selected for further characterization. This selection represents only a fraction of the total number of 5S genes contained in the genome. Southern analysis of genomic DNA digested with HindIII showed that at least 30 different DNA fragments hybridize to 5S RNA while only one contains sequences complementary to 5.8S RNA (Fig. 1).

The 5S clones were characterized with respect to their insert sizes and a Southern analysis was done. RNA sequence analysis indicated the presence of a PstI site within the 5S RNA gene. This Pst I site was used in the examination of the clones selected. DNA was digested with HindIII and PstI, separated by agarose gel electrophoresis, and transferred to a nitrocellulose filter. Transferred DNA was hybridized to a mixture of 5'-(^{32}P)-5S RNA and 3'-(^{32}P)-5S RNA to determine which fragments contain 5S

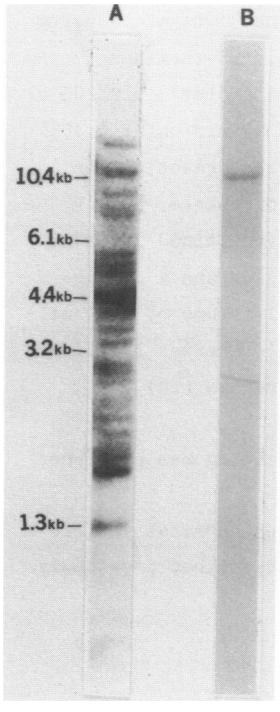

Fig. 1. Whole genome Southern hybridizations of S. pombe DNA cleaved with HindIII. Lane A - hybridized to nick translated pYM3 insert DNA containing the 5S gene; Lane B - hybridized to uniformly ^{32}P-labeled 5.8S RNA

RNA genes and to determine the number of 5S RNA genes within each HindIII insert (Fig. 2). Since two bands, representing the 5'- and 3'-halves of the gene, from the HindIII/PstI digest of each plasmid hybridized, a single 5S gene is present within each HindIII insert which hybridized to 5S RNA. The fragments resulting from HindIII and HindIII/PstI digestion of the 5S clones and the bands positive for 5S hybridization are tabulated and presented in Table 1.

Hybridization of tRNA species to the 14 5S clones proved negative except for the hybridization of a tRNA species to pYM116. Hybridization of 5.8S RNA to the 5S clones was uniformly negative. 5S and 5.8S RNA do not hybridize to the same genomic DNA fragments (see Fig. 1). Therefore the 5S RNA genes of S. pombe are not contained on a repeated DNA fragment together with the genes for the other ribosomal RNAs. A similar conclusion was reached by analysis of the S. pombe ribosomal repeat (J. Barnitz & J. Kramer, personal communication).

Nucleotide Sequence of 5S RNA genes and of 5S RNA. DNA sequence analysis was performed on the 5S gene and its flanking regions from pYM3 and on the 5S gene, the tRNA gene, and their flanking regions from pYM116. The

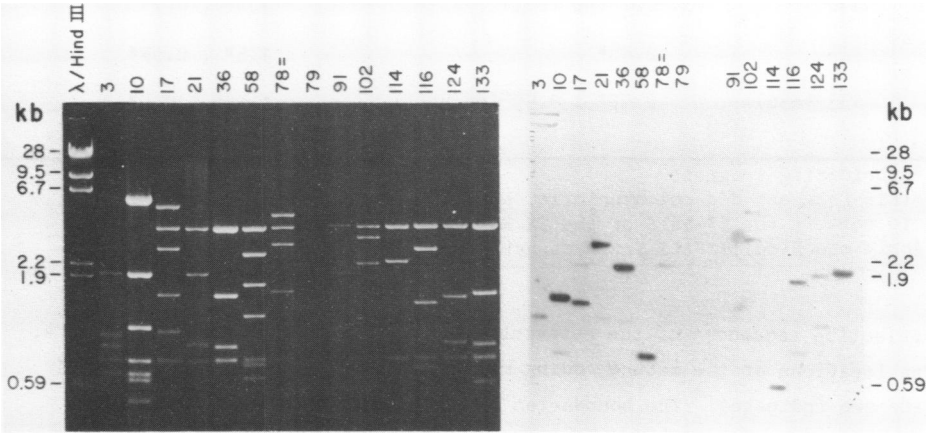

Fig. 2. Southern blot analysis of each of the S. pombe 5S RNA hybridizing plasmid DNAs. Each of the pYM clones as designated was digested with HindIII/PstI and separated on a 1% agarose gel (left half) for transfer to nitrocellulose. An autoradiograph of the hybridization pattern to a mixture of 5'-^{32}P-5S RNA (lighter bands) and 3'-^{32}P-5S RNA (darker bands) is displayed (right half). pYM78 is the same clone as pYM79. The results displayed in this figure are summarized in Table 1.

Table 1.

Characterization of S. pombe 5S clones
(Summary of hybridization results of Figure 1.)

pYM clone	HindIII NA fragments (kb)	HindIII PstI (kb)
3	2.0*	1.05+ , 0.95*
10	2.2*	1.15* , 0.6 , 0.45
17	5.6* , 5.0 (no PstI)	2.9* , 1.6* , 1.1*
21	3.0*	2.05* , 0.95*
36	2.45*	1.55* , 0.9*
58	4.55* , 2.5 (no PstI)	1.75 , 1.25 , 0.75 , 0.6# , 0.2
78 (=79)	5.8* , 2.9 (no PstI)	4.2* , 1.6*
91	2.0* , 2.0 (no PstI) , 0.85	1.1 , 0.6# , 0.3
102	5.5* , 0.56 (no PstI)	3.1* , 2.2* , 0.25
114	2.7*	2.25* , 0.45*
116	2.7 (no PstI) , 2.1*	1.4* , 0.7*
124	2.45*	1.5* , 0.95*
133 (=36)	2.45* , 0.59 (no PstI)	1.55* , 0.9*

*hybridizing fragment
+this fragment did not hybridize, but is known from DNA sequencing to contain the 5'-half of the 5S RNA gene
#only one HindIII/PstI fragment hybridized

nucleotide sequences of the relevant portions are shown in Figures 3 and 4. The locations of the mature coding region of the 5S genes and of the tRNA gene are indicated. The boundaries of the 5S gene were determined by sequence analysis of isolated 5S RNA (data not shown) using the enzymatic rapid gel sequencing methods (21). The main conclusions of this sequence analysis are: (i) the 5'-terminus is pppGU; (ii) the RNA does not contain modified nucleotides; and (iii) the 3'-terminus is $GGCU_{OH}$. The 5S RNA is 119 nucleotides long and agrees with a recently published S. pombe 5S RNA sequence (23). Comparison of the gene sequences shows that the mature coding regions in both genes are the same. Apart from the oligothymidylate

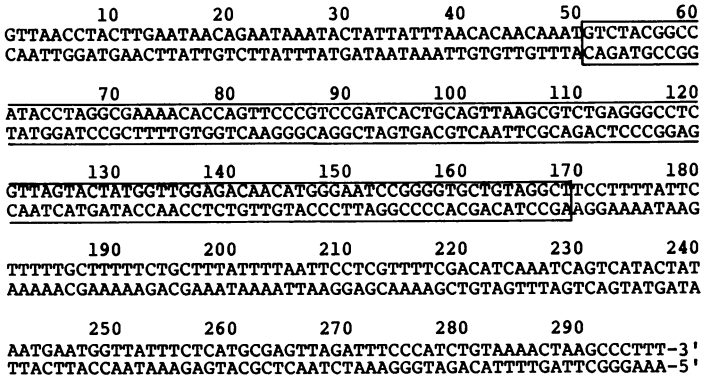

Fig. 3. DNA sequence of the 5S RNA gene regions of pYM3.

transcription terminators adjacent to the 3'-end of the coding region significant homologies between the DNA sequences of these clones have not been observed in a computer search (24).

Analysis of the DNA sequence of the tRNA gene from pYM116 indicates that this gene codes for a $tRNA^{Asp}$ which recognizes the codon GAC. Comparison of the primary sequence of this tRNA with that of the corresponding tRNA species from S. cerevisiae shows a large sequence divergence (45%) between these tRNAs (Fig. 5). This is in line with current knowledge (6) that the tRNA sequences of these two yeasts differ drastically.

In vitro Transcription of pYM116 DNA. In the absence of an S. pombe transcription system we used pYM3.1 and pYM116 DNA as templates in a Xenopus germinal vesicle extract (22). In agreement with earlier results (25) neither 5S RNA gene supported transcription in the Xenopus extract (Fig. 6), whereas homologous (26) and Triturus (27) 5S genes are efficient templates in this extract. The tRNA gene contained in pYM116 DNA was efficiently transcribed, however. This may indicate that the Xenopus factor necessary for 5S transcription (28) does not recognize 5S genes from lower eukaryotes, while tRNA genes from lower eukaryotes are recognized by the appropriate Xenopus factors.

The autoradiogram of the gel electrophoretic separation of the transcripts shows several bands in the precursor region (Fig. 6). This indicates that transcription termination occurs as expected at various positions within the oligothymidylate stretch at the 3'-end of the gene.

Structure of S. pombe 5S RNA. The 5S RNA sequences of three yeasts, S. pombe, S. cerevisiae and T. utilis, and that of D.

```
      10        20        30        40        50        60        70        80        90       100       110       120       130
CCAATTTGGCTAGCTTTTTTAAAATATTTCCTAAATTAAATTCGTACAGACAAGATTTCAATAATTGTACATCTCGTGCAAAGTATAAGAATATGGATATTAAAAATTTTACAGTTCATTGACAAAAGAG
GGTTAAACCGATCGAAAAAATTTTATAAAGGATTTAATTTAAGCATGTCTGTTCTAAAGTTATTAACATGTAGAGCACGTTTCATATTCTTATACCTATAATTTTTAAAATGTCAAGTAACTGTTTTCTC

      140       150       160       170       180       190       200  5S ⟶     220       230       240       250       260
TTTAGTATATAACATTAGAGTATCTCTTTTATATTGGAATAATATATATAGTGCAATTATAAGAACAAGTCGTCTACGGCCATACCTAGGCGAAAACACCAGTTCCCGTCCGATCACTGCAGTTAAGCGT
AAATCATATATTGTAATCTCATAGAGAAAATATAACCTTATTATATATATCACGTTAATATTCTTGTTCAGCAGATGCCGGTATGGATCCGCTTTTGTGGTCAAGGGCAGGCTAGTGACGTCAATTCGCA

      270       280       290       300       310       320       330       340       350       360       370       380       390
CTGAGGGCCTCGTTAGTACTATGGTTGGAGACAACATGGGAATCCGGGGTGCTGTAGGCTTCTTTTTTTTAAATTCCAAAACAATATTATTAATTCTCTACTTTTTAATGCACTCATTGGGTTTGTGCTA
GACTCCCGGAGCAATCATGATACCAACCTCTGTTGTACCCTTAGGCCCCACGACATCCGAAGAAAAAAAATTTAAGGTTTTGTTATAATAATTAAGAGATGAAAAATTACGTGAGTAACCCAAACACGAT

      400       410       420       430       440       450       460       470       480       490       500       510       520
TTTAGCTCAACAACATTTGTCATTCTTATTTTAATTTCGTACTCAATCGCATTAGCCCGAATTCTATTAACATTTTTACACTAAAAGCTATTTTACGGTATATTTAGTAAAGGTAAAGAAACACAGCTAT
AAATCGAGTTGTTGTAAACAGTAAGAATAAAATTAAAGCATGAGTTAGCGTAATCGGGCTTAAGATAATTGTAAAAATGTGATTTTCGATAAAATGCCATATAAATCATTTCCATTTCTTTGTGTCGATA

      530       540       550       560       570       580       590       600       610       620       630       640       650
GAAAGTGCTGCCAAAAATTTCAATTGACGAAATCAGCCATCAATGGCTGTAACTATTGAATTCCATTGTCTTTCAACTTTTGAATTATCGCAGCCTACAACAAAAAATGAAATTAATCACAATCTCTTCA
CTTTCACGACGGTTTTTAAAGTTAACTGCTTTAGTCGGTAGTTACCGACATTGATAACTTAAGGTAACAGAAAGTTGAAAACTTAATAGCGTCGGATGTTGTTTTTTACTTTAATTAGTGTTAGAGAAGT

      660       670       680       690       700       710       720       730       740       750       760       770       780
ATCTTCATCGTTACAGATAAATATAATACATGCGAGACGTGTAGGGAAGAAGACCAAAGAAAGTAATTTTAGAGATAAATTCTTACCTTCTTTCTTCACTGTATCCGAAGCCTTCAAAAACTGACTAAAC
TAGAAGTAGCAATGTCTATTTATATTATGTACGCTCTGCACATCCCTTCTTCTGGTTTCTTTCATTAAAATCTCTATTTAAGAATGGAAGAAAGAAGTGACATAGGCTTCGGAAGTTTTTGACTGATTTG

      790       800       810       820       830       840       850  ASP ⟶    870       880       890       900       910
TATTTAAGTTGCTCATGCAACACAAAGTACACGACAATTATATTTTGCAAATTAGTAATCCTCACAATAATCTCCTTTAGTATAGGGGTAGTACACAAGCCTGTCACGCTTGCAGCCCGGGTTCGAATCC
ATAAATTCAACGAGTACGTTGTGTTTCATGTGCTGTTAATATAAAACGTTTAATCATTAGGAGTGTTATTAGAGGAAATCATATCCCCATCATGTGTTCGGACAGTGCGAACGTCGGGCCCAAGCTTAGG

      920       930       940       950       960       970       980       990      1000      1010      1020      1030      1040
CGGAGGGAGAGTTTTTAACTAAAATATTTTTTAAATTACCATATTTAACAAAAGAGGATAATAATGAGGATATGCTAGTAAAATTTTACTATCGAACCTTAATGAGTCGAAGAAGCAAATACAATAAACC
GCCTCCCTCTCAAAAATTGATTTTATAAAAAATTTAATGGTATAAATTGTTTTCTCCTATTATTACTCCTATACGATCATTTTAAAATGATAGCTTGGAATTACTCAGCTTCTTCGTTTATGTTATTTGG

     1050      1060      1070      1080      1090      1100      1110      1120      1130      1140      1150      1160      1170
TTCTTGGAAGGTTGTATACATTGCTGCAAAATATCTCATATATTGAATAATGAATATGTTTCATTAATAACAATAAGTTATGTGTTCATTAAGACTCCGAGCTTAAAAGCTCTCGATTATGATGGCATGC
AAGAACCTTCCAACATATGTAACGACGTTTTATAGAGTATATAACTTATTACTTATACAAAGTAATTATTGTTATTCAATACACAAGTAATTCTGAGGCTCGAATTTTCGAGAGCTAATACTACCGTACG

     1180      1190      1200      1210      1220      1230      1240      1250      1260      1270      1280      1290      1300
TATGTCTTTACCACCATCTAAACTGGCTTAACTGAAACATAAGAATGTTATATCGATTGAAGTTGAGTTTTAGAAAATCAAGGTATCTTTTTTTCATCATCAGCTGACCTTTCAACCCTTCAATTTATAT
ATACAGAAATGGTGGTAGATTTGACCGAATTGACTTTGTATTCTTACAATATAGCTAACTTCAACTCAAAATCTTTTAGTTCCATAGAAAAAAAGTAGTAGTCGACTGGAAAGTTGGGAAGTTAAATATA

     1310      1320      1330      1340      1350      1360      1370      1380      1390      1400      1410      1420      1430
TTATTTGAAGACTATGCAAATGCTTTGTATAATAGCATCCATCCTTAGTTGGTGAGCCATTGGATATGATTTTATATATAAGCCAAAGTTACATTAATAACCTGTTAGTTGCTATAACAGTCAAAAAGTC
AATAAACTTCTGATACGTTTACGAAACATATTATCGTAGGTAGGAATCAACCACTCGGTAACCTATACTAAAATATATATTCGGTTTCAATGTAATTATTGGACAATCAACGATATTGTCAGTTTTTCAG

     1440      1450      1460      1470
ATTGGCTTGGAAATCACGATGAACTATCTAACGAAACACCCAAGTTC-3'
TAACCGAACCTTTAGTGCTACTTGATAGATTGCTTTGTGGGTTCAAG-5'
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Fig. 4. DNA sequence of the 5S RNA and tRNA gene region of pYM116. The direction of transcription for the 5S rRNA gene and the $tRNA^{Asp}$ gene is indicated by an arrow.

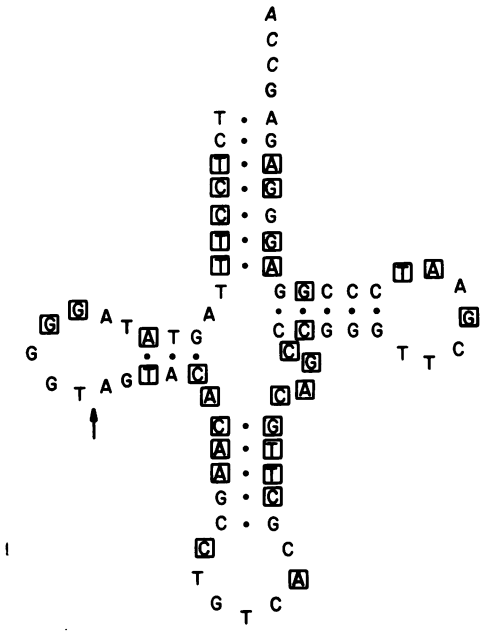

Fig. 5. The cloverleaf structure of S. pombe tDNA^{Asp}. The boxed nucleotides differ from those in S. cerevisiae tRNA^{Asp} (38). The arrow indicates the position of an additional nucleotide in this S. cerevisiae tRNA.

melanogaster are compared in Fig. 7. S. pombe 5S RNA differs in 38 nucleotides from the 5S RNA sequence of S. cerevisiae, in 37 nucleotides from T. utilis 5S RNA, and in 36 from D. melanogaster 5S RNA. Nevertheless, there are regions in these four 5S RNAs which show considerable conservation of primary structure.

Figure 8 shows the S. pombe 5S RNA sequence arranged in the secondary structure model proposed by Stahl et al. (29). This model is based on an earlier structure proposed by Fox and Woese (30). Results from Raman (31) and IR-Spectroscopy (32) suggested higher degrees of base pairing in pro- and eukaryotic 5S RNAs than expected from Watson-Crick base pairs alone. In this new 5S RNA model non-Watson-Crick base pairs are included. The existence of such non-Watson-Crick pairing in 16S rRNA has been discussed (33). As shown in Fig. 8, the S. pombe 5S RNA secondary structure has 41 base pairs of which 14 are A:U, 20 are G:C, 5 are G:U and 2 are G:A pairs.

Figure 8 also indicates nucleotide positions which are conserved

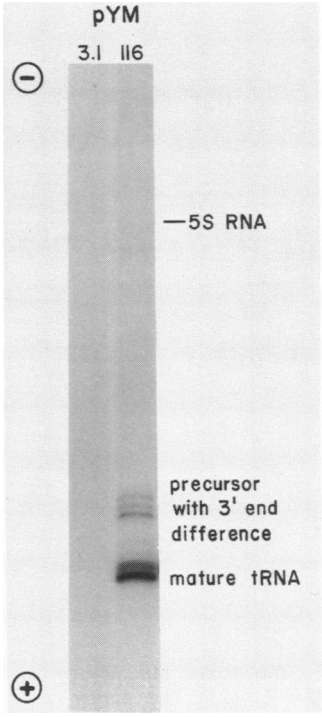

Fig. 6. Autoradiogram of an polyacrylamide gel electrophoresis of RNA transcription product from pYM3.1 and pYM116. The positions of precursor and mature tRNA and of a 5S RNA marker are indicated.

in the primary structure of all four 5S RNAs compared. Nucleotides that are conserved in three out of the four 5S RNA species are underlined. With the exception of nucleotide 88, the most variable positions occur in base paired

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                1         10        20        30        40        50        60
S. POM.   PPPGUCUACGGCCAUACCUAGGCGAAAACACCAGUUCCCGUCCGAUCA CUGCAGUUAAGCGU
S. CER.   PPP-GU-G--------U---CCA----G----GU--------------A---N--------UG
T. UTI.   PPP-GU-G--------U----CA----G----GU-CU-----------A---U--------UG
D. MEL.   PPP-C-A---A-------AC-CU---U---U-G----U---------- ---A-A------AG

                70        80        90        100       110       120
S. POM.   CUGAGGGCCUCGUU AGUACUAUGGUUGGAGACAACAUGGGAAUCCGGGGUGCUGUAGG CUOH
S. CER.   G-A--A----GACCG----G-G-A--G--U---C-U-C-C---A-UCA-------C-AU --
T. UTI.   --A--A----GA-CG----G-G-A--G--U---C-U-C-C---A-UCA-------C-AU --
D. MEL.   -GUC----GCG--- ------UA-A-G--G---CG-U------CA-C-C---U---U--C--
```

Fig. 7. Comparison of the sequence of four 5s RNAs. The 5S RNA sequence from S. pombe is shown. Positions being different between S. cerevisiae, T. utilis, or D. melanogaster towards S. pombe are written out. The numbering is based on the sequence of S. cerevisiae and T. utilis 5S RNAs (121 nucleotides), while D. melanogaster has 120 and S. pombe 119 nucleotides. Gaps were introduced in the alignment in order to match up homologous sequences.

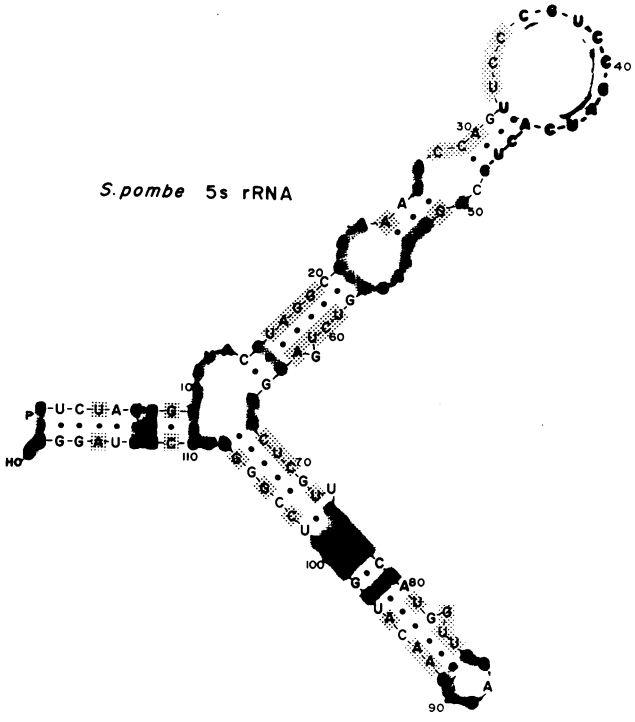

Fig. 8. Sequence of S. pombe 5S RNA arranged in the model by Stahl et al. (29). Shaded areas represent nucleotides that are conserved within the four compared species. Dotted areas represent homology in three out of four species.

and stacked regions, while the nucleotides surrounding the base of the fork in the model and the nucleotides in loops around nucleotide positions 40 and 90 seem to be highly conserved (34).

Evolution of S. pombe 5S RNA. In order to help determine the position of S. pombe in the phylogenetic tree of yeasts, we calculated the Knuc values (5) for the four different 5S RNAs presented in Fig. 7. These data, completed by a comparison of the percent sequence homology within the four 5S RNAs, are shown in Figure 9. Utilizing Hori's phylogenetic tree of 5S RNAs (5) we have placed S. pombe closest and S. cerevisiae farthest of the three yeasts from D. melanogaster.

Based on the sequence comparison of S. pombe 5S RNA, we suggest that S. pombe may have split somewhat later from the evolutionary line leading to D. melanogaster than did the yeasts T. utilis and S.

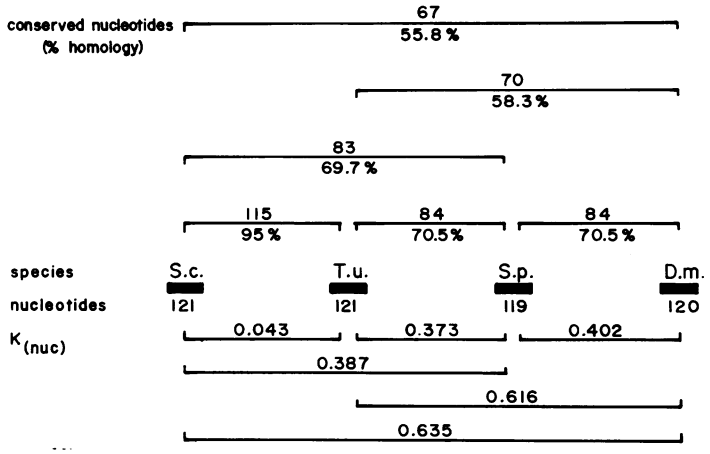

Fig. 9. 5S RNA sequences of S. cerevisiae (S.c.), T. utilis (T.u.), S. pombe (S.p.), and D. melanogaster (D.m.) are compared. The number of conserved nucleotides and the degree of homology between species is shown. K_{nuc} values were calculated by the method of Hori (5).

cerevisiae. A similar analysis of the phylogeny of $tRNA^{Phe}$ species (35) supports this conclusion. The sequence of cytochrome C (36) and the organization of the pathways of tryptophan biosynthesis (37) suggests that S. pombe is closer to Neurospora than to S. cerevisiae. Sequence data on other genes are needed to establish more securely the evolutionary relationships among lower eukaryotes.

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REFERENCES

1. Long, E. D. and Dawid, I. B. (1980) Ann. Rev. Biochem. 49, 727-764.
2. Petes, T. D. (1980) Cell 19, 765-774.
3. Szostak, J. W. and Wu, R. (1980) Nature 284, 426-431.
4. Selker, E. U., Yanofsky, C., Driftmier, K., Metzenberg, R. L., Alzner-DeWeerd, B., and RajBhandary, U. L. (1981) Cell 24, 819-828.
5. Hori, H. and Osawa, S. (1979) Proc. Nat. Acad. Sci. 76, 381-385.
6. Mao, J., Schaack, J., Sharp, S., Yamada, H., Kohli, J. and Söll D. (1981) in Molecular Genetics in Yeast (von Wettstein, D., Friis, J.,

Kielland-Brandt, M., and Stenderup, A., eds.) Munksgaard, Copenhagen, pp. 276-290.
7. Walseth, T. F. and Johnson, R. A. (1979) Biochim. et Biophys. Acta 526, 11-31.
8. Cryer, D. R., Eccleshall, R. and Marmur, J. (1975) Meth. Cell Biol. 12, 39-44.
9. Clewell, D. B. (1972) J. Bact. 110, 667-676.
10. Wensink, P. C., Finnegan, D. J., Donelson, J. E., and Hogness, D. S. (1974) Cell 3, 315-325.
11. Grunstein, M. and Hogness, D. S. (1975) Proc. Nat. Acad. Sci. 72, 3961-3965.
12. Rubin, G. (1975) Meth. Cell Biol. 12, 45-64.
13. Sharp, P. A., Sugden, B. and Sambrook, J. (1973) Biochem. 12, 3055-3063.
14. Maniatis, T., Jeffrey, A., and van de Sande, H. (1975) Biochem. 14, 3787-3794.
15. Smith, H. O. and Birnstiel, M. L. (1976) Nucl. Acids Res. 3, 2387-2398.
16. Southern, E. M. (1975) J. Mol. Biol. 98, 503-517.
17. Maxam, A. M. and Gilbert, W. (1980) in Methods in Enzymology 65 (Grossman, L. and Moldave, K., eds.) Academic Press, New York. pp. 499-560.
18. Bruce, A. G. and Uhlenbeck, O. C. (1979) Nucl. Acids Res. 5, 3665-3677.
19. Efstratiadis, A., Vournakis, J. N., Donis-Keller, H., Chaconas, G., Dougall, D. K., and Kafatos, F. C. (1977) Nucl. Acids Res. 4, 4165-4174.
20. Sanger, F. and Coulson, A. R. (1978) FEBS Lett. 87, 107-110.
21. Silverman, S., Heckman, J. S., Cowling, G. J., Delaney, A. D., Dunn, R. J., Gillam, I. C., Tener, G. N., Söll, D., and RajBhandary, U. L. (1979) Nucl. Acids Res. 6, 435-442.
22. Schmidt, O., Mao, J., Silverman, S., Hovemann, B., and Söll, D. (1978) Proc. Nat. Acad. Sci. 75, 4819-4823.
23. Komiya, H., Myazaki, M. and Takemura, S. (1981) J. Biochem. 89, 1663-1666.
24. Sege, R., Söll. D., Ruddle, F. H., and Queen, C. (1981) Nucl. Acids Res. 9, 437-444.
25. Schmidt, O., Hovemann, B., Silverman, S., Yamada, H., Mao, J., and Söll, D. (1979) in Enzyme Regulation and Mechanism of Action (Mildner, P. and Ries, B., eds.) Pergamon Press, Oxford. pp. 179-188.
26. Birkenmeier, E. H., Brown, D. D., and Jordan, E. (1978) Cell 15, 1077-1086.
27. Kay, B. K., Schmidt, O., and Gall, J. G. (1981) J. Cell Biol. 90, 323-331.
28. Engelke, D. R., Ng, S. Y., Shastry, B. S., and Roeder, R. G. (1980) Cell 19, 717-728.
29. Stahl, D. A., Luehrsen, K. R., Woese, C. R., and Pace, N. R. (1981) Nucl. Acids Res. 9, 0000-0000.
30. Fox, G. E. and Woese, C. R. (1975) Nature 256, 505-507.
31. Luoma, G. A. and Marshall, A. G. (1978) J. Mol. Biol. 125, 95-105.
32. Stulz, J., Ackermann, T., Appel, B., and Erdmann, V. A. (1981) Nucl. Acids Res. 9, 3851-3861.
33. Woese, C. R., Magnum, L. J., Gupta, R., Siegel, R. B., Stahl, D. A., Kop, J., Crawford, N., Brosius, J., Gutell, R., Hogan, J. J., and Noller, H. F. (1980) Nucl. Acids Res. 8, 2275-2293.
34. Erdmann, V. A. (1980) Nucl. Acids Res. 8, r31-r47.
35. Cedergren, R. J., LaRue, B., Sankoff, D., and Grosjean, H. (1981) Crit. Rev. Biochem. 11, 35-104.

36. Simon-Becam, A. M., Claisse, M. and Lederer, F. (1978) Eur. J. Biochem. 86, 407-416.
37. Schweingruber, M. E. and Dietrich, R. (1973) Experientia 29, 1152-1154.
38. Gangloff, J., Keith, G., Ebel, J. P., Dirheimer, G. (1972) Biochim. Biophys. Acta 259, 210-222.