A mutation in a single gene of *Schizosaccharomyces pombe* affects the expression of several snRNAs and causes defects in RNA processing

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A bank of temperature sensitive (*ts*[−]) mutants of *Schizosaccharomyces pombe* was screened for snRNA expression mutants using an oligodeoxynucleotide that recognizes U2 RNA. One mutant with a novel phenotype was identified that has reduced steady-state levels of the spliceosomal snRNAs U1, U2, U4, U5 and U6. In addition, the mutant exhibits a temperature-dependent accumulation of aberrant U2 and U4 transcripts elongated at their 3' end. The steady-state concentration of the RNA component of RNase P is also reduced in the mutant, whereas the amount of U3 RNA, 7SL RNA, tRNA, rRNA and mRNA are the same as wild-type. Pre-mRNA, pre-tRNA and U6 RNA precursor processing are impaired in the mutant. Genetic analysis demonstrates that the snRNA defects are tightly linked to the *ts*[−] growth defect and are recessive. We have named this mutant *snm1* to indicate a defect in snRNA maintenance. The data on *snm1* suggest that a single *trans*-acting factor is essential for the maintenance of steady-state levels of several snRNAs and for proper 3' end formation of U2 and U4 RNAs.

Key words: snRNA/*Schizosaccharomyces pombe*/*snm*[−] mutant

Introduction

The nuclei of eukaryotic cells contain a ubiquitous set of abundant small RNAs known as small nuclear RNAs (snRNAs) (for reviews see Guthrie, 1988; Reddy and Busch, 1988). Although other small RNAs are present in the nucleus, the term snRNA most commonly refers to the U-class of small RNAs (U1–U12 in mammals). These RNAs are complexed with proteins in small nuclear ribonucleoprotein particles or snRNPs (reviewed in Lührmann, 1988). U1, U2, U4, U5, U6, U7 and probably U11 are located in the nucleoplasm, whereas U3 and U8 are found in the nucleolus (Reddy and Busch, 1988). U1, U2, U4, U5 and U6 have been shown to be essential for pre-mRNA splicing (reviewed in Maniatis and Reed, 1987; Steitz *et al.*, 1988). U7 is required for the formation of the 3' ends of histone mRNAs (Birnstiel and Schaufele, 1988) and U11 has been implicated in the cleavage and polyadenylation of non-histone pre-mRNAs (Christofori and Keller, 1988). Circumstantial evidence suggests that U3 participates in the processing of rRNA (Prestayko *et al.*, 1970; Zieve and

Penman, 1976; Epstein *et al.*, 1984; Lischwe *et al.*, 1985; Ochs *et al.*, 1985; Parker and Steitz, 1987; Reimer *et al.*, 1987). Another nucleoplasmic snRNA, which is a component of a RNP enzyme, is the RNA subunit of the pre-tRNA processing enzyme RNase P (Altman *et al.*, 1988; Gold *et al.*, 1988; Bartkiewicz *et al.*, 1989).

Besides their small size and nuclear location, the U-snRNAs share several other characteristics. Except for U6, the U-snRNAs all possess an unusual 2,2,7-trimethylguanosine 5' cap. U6 RNA has an unidentified cap structure (Saponara and Enger, 1969; Reddy *et al.*, 1972) and the RNase P RNAs from *Schizosaccharomyces pombe* (Krupp *et al.*, 1986) and human (Bartkiewicz *et al.*, 1989) are not capped. All snRNAs have extensive secondary structures that are phylogenetically conserved. In addition, U1, U2, U4 and U5 all contain the sequence PuA(U)_nGPu, which is the binding site for the core proteins that make up the Sm antigen recognition signal (reviewed in Mattaj, 1988; Reddy and Busch, 1988). This sequence is not present in U3, U6 and the RNase P RNA. Seven polypeptides < 30 kd in size have been found to be associated with all the U-snRNAs except U3 (reviewed in Lührmann, 1988). These polypeptides are named B', B, D', D, E, F and G. Polypeptides D, E, F and G bind to the conserved sequence described above (Mattaj, 1988). In addition, individual U-snRNAs have specific polypeptides associated with them (Lührmann, 1988). The protein constituent(s) of a eukaryotic RNase P have not yet been identified.

Most of what is known about snRNA synthesis and snRNP assembly comes from studies in vertebrate systems (for reviews see Dahlberg and Lund, 1988; Mattaj, 1988). Two conserved *cis*-acting elements required for transcription of the major U-snRNAs have been identified. A distal element, located between 200 and 250 nt upstream of the start site has an enhancer-like function and contains an octamer motif (ATGCAAAT) also found in enhancers of mRNA genes (Mattaj *et al.*, 1985; Bohmann *et al.*, 1987). The other essential sequence is a proximal element that is located ~60 nt upstream of the start site and is functionally similar to a TATA box in mRNA promoters since it fixes the position of transcription initiation (Skuzeski *et al.*, 1984; Ares *et al.*, 1985; Ciliberto *et al.*, 1985; Mattaj *et al.*, 1985; Hernandez and Lucito, 1988; Murphy *et al.*, 1987). The U3 promoter has, in addition to these two sequences, an extension of the distal element that is conserved among U3 promoters (Stroke and Weiner, 1985; Suh *et al.*, 1986). The U6 promoter includes both the proximal and distal elements and a T/A rich sequence ~25 nt upstream of the start site (Kunkel *et al.*, 1986; Krol *et al.*, 1987). U1–U5 are transcribed by RNA polymerase II (Dahlberg and Lund, 1988), whereas U6 is transcribed by RNA polymerase III in vertebrates (Kunkel *et al.*, 1986; Krol *et al.*, 1987; Reddy *et al.*, 1987). In *Saccharomyces cerevisiae* U6 RNA synthesis *in vivo* and *in vitro* has the properties expected of an RNA polymerase III-mediated process (D. Brow and

Table 1. Oligodeoxynucleotides used as probes in Northern analysis and primer extensions

Probe	Sequence (5' → 3')	Species	Nucleotides	Reference
U1	<u>TGCCAGGTAAGTAT</u>	human	1–14	Krainer and Maniatis (1985)
U2	<u>CAGATACTACACTTG</u>	human	28–42	Black <i>et al.</i> (1985)
U2-3'	<u>GTAAATATTTTCAACC</u>	<i>S.pombe</i>	+75–91	P.Brennwald and J.A.Wise (personal communication)
U3	<u>ACTATAGAAATGATCCT</u>	<i>S.pombe</i>	18–34	Porter <i>et al.</i> (1988)
U4	<u>AGTTTCAACTAGCAAT</u>	<i>S.pombe</i>	56–72	Dandekar and Tollervey (1989)
U4-3'	<u>CCACCAAATTGCTAAAG</u>	<i>S.pombe</i>	+35–51	Dandekar and Tollervey (1989)
U5	<u>CTGGTAAAAGGCAAG</u>	<i>S.pombe</i>	43–57	T.Tani and Y.Ohshima (personal communication)
U6	<u>TC(A/T)TCTCTGTATTG</u>	<i>S.cerevisiae</i>	31–44	Brow and Guthrie (1988)
U6-3'	<u>GGAAATCAAGATTGAA</u>	<i>S.pombe</i>	+27–43	Tani and Ohshima (1989)
U6I	<u>GACTCGAACCTTGGTAA</u>	<i>S.pombe</i>	62–78	Tani and Ohshima (1989)
K-RNA	<u>GCGTTCGCCCGTACGTC</u>	<i>S.pombe</i>	9–25	Krupp <i>et al.</i> (1986)
7SL	<u>TTCCAACCTCCATCGGT</u>	<i>S.pombe</i>	147–163	Brennwald <i>et al.</i> (1988a), Ribes <i>et al.</i> (1988)
Sup9 ⁺	<u>TAGATGACTAGAATACAGGATTCAAG</u>	<i>S.pombe</i>	32–57	Willis <i>et al.</i> (1984)

The sequence for each oligonucleotide is listed and 'nucleotides' refers to the sequence that is recognized in the species from which the probe is derived. The underlined segment of each sequence refers to the part that recognizes the homologous snRNA in *S.pombe*.

C.Guthrie, personal communication). In addition to the conserved sequences in the 5' flanking region there is a loosely conserved *cis*-acting sequence found in the 3' flanking region (3' box) of U1–U5 (Dahlberg and Lund, 1988), which is required for proper 3' end formation of U1 (Hernandez, 1985; Hernandez and Weiner, 1986; Ciliberto *et al.*, 1986; Neuman de Vegvar *et al.*, 1986), U2 (You *et al.*, 1985) and U3 (Stroke and Weiner, 1985; Ach and Weiner, 1987). Proper initiation of an snRNA promoter is essential for proper termination of transcription (Hernandez and Weiner, 1986; Hernandez and Lucito, 1988; Neuman de Vegvar *et al.*, 1986). Termination of U6 occurs in a stretch of Ts, as is true of other RNA polymerase III genes. The human RNase P RNA (H1 RNA) is transcribed by RNA polymerase III (M.Bear, T.Nilsen, C.Costigan and S. Altman, personal communication).

Recently, several groups have begun to study snRNA synthesis in fission yeast. Homologs of the U1–U6 RNAs have been identified and their genes have been sequenced (Tollervey, 1987; Tollervey and Mattaj, 1987; Brennwald *et al.*, 1988b; Porter *et al.*, 1988; Dandekar and Tollervey, 1989; Tani and Ohshima, 1989), revealing a striking similarity to their vertebrate counterparts in both size and predicted secondary structure. The fission yeast snRNAs, like their vertebrate counterparts, have a 2,2,7-trimethylguanosine cap at their 5' ends and are packaged into Sm-precipitable snRNPs (Tollervey and Mattaj, 1987; Brennwald *et al.*, 1988b). The gene for the *S.pombe* RNase P RNA, termed K RNA, has also been sequenced (Krupp *et al.*, 1986). Very little is presently known about the promoter regions and termination signals of fission yeast snRNAs. In this report we characterize a unique recessive mutant of *S.pombe*, which maintains reduced steady-state levels of U1, U2, U4, U5, U6 and K RNAs. In addition, aberrant longer transcripts of U2 and U4 accumulate in the mutant at the non-permissive temperature.

Results

Screening the *ts*[−] mutants for snRNA maintenance defects

A bank of 70 *ts*[−] mutants (Potashkin *et al.*, 1989) was screened for mutants that are defective in the maintenance

Fig. 1. Northern blot analysis of the *ts*[−] mutants. Total RNA (20 µg/sample) from cultures that had been shifted from growth at 23 to 37°C for 2 h was fractionated on a formaldehyde–agarose gel and blotted onto nylon membranes. The blots were probed with an oligonucleotide that recognizes U2 RNA. The blot was exposed to X-ray film with an intensifying screen for 1 day.

of snRNAs. Each of the *ts*[−] mutants was grown to mid-log phase at 23°C and then shifted to 37°C for 2 h. Total RNA was isolated from each mutant, fractionated on formaldehyde–agarose gels and transferred to nylon membranes. The blots were probed with an oligonucleotide, L15 (Table 1; Black and Steitz, 1986), that is complementary to human U2 RNA and had previously been shown to recognize an RNA of the expected size in *S.pombe* (Ares, 1986). The U2 RNA of *S.pombe* is identical to the mammalian homologs in this region (Brennwald *et al.*, 1988b). We chose U2 for screening of snRNA expression mutants because it is one of the most abundant snRNAs in *S.pombe* (Brennwald *et al.*, 1988b).

Most of the *ts*[−] mutants produce a strong mature U2 RNA signal. However, one mutant, *ts45*, had a reduced amount of U2 RNA and a longer U2-specific transcript that was not present in any of the other mutants (Figure 1). RNAs prepared from a wild-type strain and *ts45* grown at 37°C were compared directly by Northern analysis to confirm this initial observation (data not shown). In addition to the longer U2-specific transcript, a shorter transcript of 110 nt was reproducibly observed in *ts45* at 37°C when a polyacrylamide denaturing gel was used to fractionate the RNA (data not shown).

Initial characterization of *ts45*

A direct comparison of the growth of *ts45* with a wild-type strain in complete medium at both the permissive and the non-permissive temperatures showed that *ts45* grew more slowly than wild-type at the permissive temperature and was

Fig. 2. Northern blot analysis of the snRNAs from the progeny of each of four spores from two tetrads. *ts45* was mated to a wild-type strain and allowed to undergo meiosis. Tetrads were dissected and total RNA was prepared from the progeny of each of the four spores (1, 2, 3 and 4 in A and B) after a shift to 37°C for 5 h. RNA was fractionated on a denaturing polyacrylamide gel, blotted and probed with a series of oligonucleotide probes (see Table I). The blots were exposed to X-ray film with an intensifying screen for 1–5 days depending upon the probe used. The polyacrylamide gel in (C) was stained with ethidium bromide.

incapable of increasing its growth rate at 37°C. *ts45* was crossed to a wild-type strain and tetrad analysis showed that the *ts⁻* growth defect segregated 2:2. To be sure that the strain contained a single defect, *ts45* was back-crossed three times to a wild-type strain. In addition, the RNA from the progeny of four spores of several tetrads was examined by Northern analysis. The RNA blots were probed with L15 to quantitate the steady-state level of U2 RNA. Two representative tetrads are shown in Figure 2. A 2- to 3-fold reduction in the steady-state level of U2 was observed in two of the four spores and this correlated with the *ts⁻* growth defect. A longer exposure of the RNA blot showed that the larger transcript observed on the RNA blot shown in Figure 1 is present only in the progeny of mutant spores (data not shown).

A radioactively labeled antisense RNA derived from the U2 gene was used in a ribonuclease protection assay to determine more accurately the length of the longer transcript. The RNase A and RNase T1 resistant fragments indicate several larger transcripts ranging in size from ~20 to 200 nt longer than the normal U2 RNA (Figure 3, lane 5). The larger U2 transcripts are detected only in *ts45* and not in the wild-type grown under the same conditions. A much longer exposure of the gel shown in Figure 3 revealed a small amount of the aberrant U2 products at 23°C in the mutant, while no larger RNAs were seen in the wild-type. The longer U2 transcripts are, therefore, specific to the mutant and their production is greatly enhanced after a period of growth at 37°C.

Defect in snRNA maintenance

To determine if the defect in *ts45* was specific for U2 RNA, we screened the RNA blots derived from the tetrads with

Fig. 3. Ribonuclease protection assay of U2 RNA in *ts45*. (A) An antisense RNA derived from the *S.pombe* U2 gene was prepared with T3 RNA polymerase from a *Bam*HI linearized template. The filled arrow denotes the position and transcriptional orientation of the U2 RNA coding sequences in *S.pombe*. (B) The antisense RNA was used in a ribonuclease protection assay with total RNA prepared from *ts45* and a wild-type strain (972) grown at either 23°C or after a shift to 37°C for 5 h. The gel was exposed to X-ray film with an intensifying screen for 1 day.

a set of probes (Table I) that recognize the other major snRNAs (U1, U4, U5 and U6) known to be essential for pre-mRNA splicing. The U4 oligonucleotide was derived from *S.pombe* sequences (Dandekar and Tollervey, 1989). The U1 oligonucleotide is complementary to the human snRNA but recognizes an RNA of the expected size in *S.pombe* (Potashkin *et al.*, 1989). The U5 probe was derived from the *S.cerevisiae* U5 (snR7) sequence (Patterson and Guthrie, 1987). The *S.pombe* U5 sequence is identical to the budding yeast sequence in this region (T.Tani and Y.Ohshima, personal communication). The U6 probe was an antisense RNA derived from the human U6 gene (gift of N.Hernandez, Cold Spring Harbor). The results are shown in Figure 2(A). In addition to U2, there is a 2- to 5-fold reduction of U1, U4 and U5 RNAs in the progeny from the temperature-sensitive spores compared with the wild-type spores. There are two U5 transcripts in the wild-type. The major U5 species (~115 nt) co-migrates with the human U5 RNA on polyacrylamide gels (data not shown), whereas the larger U5 transcript is ~140 nt. Similar observations have been made by T.Tani and Y.Ohshima (personal communication). Both U5 RNAs are detected on a blot of RNAs immunoselected by an anti-trimethylguanosine antibody (J.Potashkin, A.Krainer and D.Frendewey, unpub-

Fig. 4. Mapping of the 5' end of the U2, U4, U5 and U6 transcripts. Total RNA was prepared from a wild-type strain and *ts45* after growth at either 23°C or after a shift to 37°C for 5 h. This RNA was used as template for primer extension analysis with oligonucleotide primers that recognize sequences within the RNA coding regions of each gene.

Fig. 5. Primer extension analysis of the U2, U4 and U6 long transcripts. Total RNA (20 µg/reaction) was prepared from a wild-type strain (972) and *ts45* after growth at either 23°C or after a shift to 37°C for 5 h. RNA was used as template for primer extension analysis using oligonucleotide primers which recognize sequences in the 3' flanking regions of U2, U4 and U6 genes (U2-3', U4-3' and U6-3' respectively) or within the U6 intron (U6I). The gel was exposed to X-ray film with an intensifying screen for 1 day.

lished results). Two U5 RNAs that differ by 35 nt at the 3' end have also been identified in *S.cerevisiae* (Patterson and Guthrie, 1987). Both of the *S.cerevisiae* U5 RNAs are transcribed from the same gene (Patterson and Guthrie, 1987) and are found in the spliceosome (Pikielny *et al.*, 1986). In Figure 2(A), the larger U5 is reduced or absent in the mutant and the smaller U5 is reduced ~2-fold compared with the wild-type (see also Figure 4). We also observe a reduction in the U6 RNA signal in *ts45*. This reduction is not obvious in the overexposure shown in Figure 2(A). We have shown this exposure to illustrate the appearance of a larger U6 transcript (~50 nt larger) in the progeny of the mutant spores. A shorter exposure (data not shown) and primer extension analysis (Figure 4) indicate a 2-fold reduction of U6 RNA in *ts45* compared to wild-type. The mutant also produces an abnormally large U4 RNA (~70 nt larger) that is not seen in the wild-type (Figure 2A).

The results described above concern the spliceosomal snRNAs U1, U2, U4, U5 and U6. To determine if the defect in *ts45* was specific for this group of transcripts, we examined two other snRNAs: K RNA and U3 RNA. The steady-state amount of K RNA was drastically reduced (~10-fold) in *ts45* compared to wild-type (Figure 2A). The mutation in *ts45* does not affect all snRNAs, however. The amount of U3 RNA, a nucleolar snRNA, is the same in the mutant and the wild-type (Figure 2B).

We also analyzed the steady-state content of several cytoplasmic RNAs. 7SL RNA, the RNA component of signal recognition particle, is the same in *ts45* as in wild-type (Figure 2B). In addition we detected no differences in the amounts of the abundant, stable cytoplasmic rRNAs and tRNAs (Figure 2C). Since equal mass amounts of total RNA were loaded in each lane for the Northern analyses, we expected equal amounts of rRNA (>80% of total RNA mass; Watson *et al.*, 1987) to be present in each sample. The 5.8 S rRNA signal shown in Figure 2(C) and the ethidium bromide staining pattern for the larger rRNAs seen on agarose gels (data not shown) confirm this expectation. The reductions in the mass proportions of the snRNAs that we observe in *ts45* are relative to rRNA or tRNA content, which remains constant in all of the analyses shown in Figure 2. We also observed that the amounts of mRNA, either from an intronless gene (alcohol dehydrogenase) or from an intron-containing gene (β -tubulin) were identical in *ts45* and wild-type (data not shown). To summarize, the quantities of U1, U2, U4, U5, U6 and K RNA, as proportions of total RNA mass, are reduced 2- to 10-fold in the *ts45* mutant at 37°C compared to the wild-type grown under identical conditions. In addition, larger transcripts are produced for U2, U4 and U6 RNAs in the mutant at the non-permissive temperature. U3 RNA as well as the major cytoplasmic RNAs are not affected.

Characterization of the longer transcripts

To determine if any of the larger RNAs (U2, U4 and U6) were the result of upstream transcription initiation, we mapped their 5' ends by primer extension. The same oligonucleotides that were used as probes on RNA blots were also used as primers. A single 5' end was detected for each RNA in the wild-type and the mutant at both temperatures (Figure 4). These results supported the reductions that we observed by Northern analysis (Figure 2), especially for U4, U5 and U6 RNAs. (Compare the 37°C signals for the wild-type and *ts45*.) Longer exposures of the gels did not reveal any additional 5' ends that could explain the larger transcripts. For U2, U4 and U6 in particular, mutant-specific extension products were not observed. These data suggested that the larger RNAs are the results of 3' extensions or unspliced introns (in the case of U6). The primer extension experiment also indicated that the larger, less abundant U5 RNA is extended at its 3' end. A probe derived from the 3' flanking region (sequences provided by T.Tani and Y. Ohshima) hybridizes specifically to the longer U5 transcript (J.Potashkin, A.Krainer and D.Frendewey, unpublished results). Therefore, *S.pombe* may be similar to *S.cerevisiae* (Patterson and Guthrie, 1987).

The 3' flanking sequences of the U2, U4 and U6 genes have been determined (Dandekar and Tollervey, 1989; Tani and Ohshima, 1989; P.Brennwald and J.A.Wise, personal communications), so we were able to design oligonucleotide

Fig. 6. Ribonuclease protection assay of $\alpha 1$ -tubulin transcript in *ts45*. (A) An antisense RNA complementary to portions of the intron and exon 2 of the *S. pombe* $\alpha 1$ -tubulin gene was prepared with T7 RNA polymerase from an *AccI* linearized template. (B) The antisense RNA was used in a ribonuclease protection assay with total RNA prepared from *ts45*, *prp2* (a pre-mRNA splicing mutant) and a wild-type strain (972) grown at either 23°C or after a shift to 37°C for 5 h. E2 is the exon 2 protected fragment, P is the pre-mRNA protected fragment and Probe marks the position of undigested antisense RNA. The gel was exposed to X-ray film with an intensifying screen for 2 days.

primers that are complementary to sequences downstream of each of these coding regions (U2-3', U4-3' and U6-3' in Table I) based on the sizes that we estimated for the longer transcripts from our Northern assays. Extended cDNAs of the expected sizes are produced from the U2-3' and U4-3' primers only in the RNA samples prepared from the mutant grown at 37°C (Figure 5), indicating that the larger U2 and U4 RNAs seen in *ts45* are extended at their 3' ends at least as far as the site of primer recognition. No extension was observed from the wild-type RNA, which suggests that the longer transcripts observed in *ts45* might be aberrant products, rather than normal precursors that are processed to form the mature RNAs. However, since our experiments address only steady-state RNA levels, the larger RNAs could have very short half-lives in the wild-type and therefore may not have been detected by primer extension (Figure 5), Northern blotting (Figures 1 and 2A) or RNase protection (Figure 3). Northern assays confirmed the results of the primer extension experiments (data not shown).

The results with the U6-3' primer extension are different from those of U2 and U4. There are no extension products observed in either the wild-type or *ts45* in any of the samples (Figure 5), indicating that the longer transcript does not possess 3' flanking sequences complementary to the probe. As mentioned above, the larger U6 transcript observed on Northern blots for U6 is the size expected for the unspliced U6 precursor. We therefore performed another primer extension experiment with an oligonucleotide that recognizes the U6 intron (U6I in Table I). The results of this analysis demonstrate that the mutant accumulates larger amounts of unspliced U6 RNA precursor after growth at 37°C than the wild-type grown under identical conditions (Figure 5). Approximately equal amounts of unspliced U6 pre-RNA are present in the wild-type and *ts45* grown at 23°C. We have previously found that the steady-state level of U6 pre-RNA in the wild-type is dependent on the growth temperature

Fig. 7. Northern blot analysis of tRNA processing in the progeny of each of four spores from two tetrads. (A) Diagrammatic representation of the processing pathways of the $tRNA^{Ser}$ and $tRNA^{Met}$ dimeric precursor. (B) *ts45* was mated to a wild-type strain and allowed to undergo meiosis. Tetrads were dissected and total RNA was prepared from the progeny of each of the four spores (1, 2, 3 and 4) after shift to 37°C for 5 h. RNA was blotted and probed with an oligonucleotide probe Sup9+ (see Table I) which recognizes the $tRNA^{Ser}$ precursor. The blot was exposed to X-ray film with an intensifying screen for 1 day.

(Potashkin and Frendewey, 1989). In the wild-type a small amount of U6 precursor is detectable at 23°C, but after a 5 h incubation at 37°C (the time used for the experiments shown in Figure 5) no unspliced U6 precursor is seen. The defect in U6 splicing seen in *ts45* (Figure 5) is qualitatively the same as that observed in three pre-mRNA splicing mutants (Potashkin and Frendewey, 1989).

RNA processing in *ts45*

Because the steady-state levels of the major spliceosomal snRNAs are reduced in *ts45*, we expected to see an effect on pre-mRNA splicing in the mutant. However, when we initially screened our bank of *ts*⁻ mutants for defects in pre-mRNA splicing, *ts45* was not identified as a pre-mRNA splicing mutant (Potashkin *et al.*, 1989). This screen relied on the detection of unspliced precursors by a Northern blot assay. Here we used a more sensitive ribonuclease protection assay to re-examine pre-mRNA splicing in *ts45*. The probe was an antisense RNA complementary to the fission yeast $\alpha 1$ -tubulin transcript. The spliced mRNA protected a 393 nt fragment and unspliced RNA protected a 448 nt fragment (Figure 6A). The results show that there is a slight accumulation of pre-mRNA in *ts45* at 37 but not at 23°C (Figure 6B), whereas no precursor is observed in the wild-type. By comparison, the pre-mRNA splicing mutant *prp2* shows a more severe defect. Therefore, pre-mRNA splicing is impaired in *ts45*, but not to the same extent as the *prp*⁻ mutants that were initially identified as pre-mRNA

splicing mutants by the Northern assay (Potashkin *et al.*, 1989).

Since the steady-state level of K RNA is also reduced in *ts45* (Figure 2), we assayed for defects in pre-tRNA processing. In fission yeast, tRNA^{Ser} and tRNA^{Met} are processed from a single primary transcript (Willis *et al.*, 1984). RNase P cleaves this dimeric precursor in two places to produce two monomeric pre-tRNAs with proper 5' ends (Figure 7A). The monomeric precursors are then trimmed at their 3' flanks, CCA is added and the intron of pre-tRNA^{Ser} is removed by splicing (Willis *et al.*, 1984, 1986). The RNA blot of the tetrad analysis shown in Figure 2 was reprobed with an oligonucleotide that recognizes the intron of the *sup9*⁺ tRNA^{Ser} precursor (Table I). The *sup9*⁺ probe detects both monomeric and dimeric tRNA^{Ser} precursors, but not the mature tRNA^{Ser}. The results of this probing show that there is a significant accumulation of both types of tRNA^{Ser} precursors in the mutant compared to wild-type (Figure 7B), indicating a severe impairment of pre-tRNA processing in *ts45*.

Analysis of the temperature sensitivity of the snRNA phenotype

To analyze the temperature sensitivity of *ts45*, we prepared total RNA from a wild-type strain and *ts45* after growth at the permissive and non-permissive temperature. Cells were grown at 23°C to mid-log phase and then part of each culture was shifted to 37°C for various time periods up to 5 h and the RNA was analyzed by Northern assay. When the RNA blots were probed with a U4-specific oligonucleotide, the amount of mature U4 RNA in the wild-type appears to decrease slightly after the initial shift to the elevated temperature but normal, abundant steady-state levels return within 2 h (Figure 8A). The U4 pattern in *ts45* is different. At the permissive temperature the amount of U4 RNA is slightly lower than wild-type and remains at this low basal level throughout the time course. The longer aberrant U4 transcript is detectable by 2 h and increases at 5 h. Therefore, the lower amount of the mature U4 transcript in the mutant is not a temperature-sensitive phenotype, whereas the accumulation of the longer transcript is temperature dependent. This observation was confirmed by an experiment in which total cellular RNA from cells grown at 23, 30 or 37°C was analyzed on RNA blots probed with the U4-specific oligonucleotide. The longer transcript was present only in the 37°C sample (data not shown). A modification of the time course was performed in which the *ts45* culture, after growth at 37°C for 5 h, was shifted back to 23°C for 2 and 16 h. After 2 h the longer transcript is still present in the whole cell RNA, but after 16 h the longer transcript is no longer detected (data not shown).

The U4 probe was stripped from the RNA blot and the blot was reprobed with an oligonucleotide that recognizes K RNA. As with U4, the steady-state level of K RNA in the wild-type tends to decrease after shifting to 37°C, but a normal signal is observed within 2 h (Figure 8A). The amount of K RNA in *ts45* is reduced at least 10-fold at both 23 and 37°C compared to wild-type. The same pattern in both the wild-type and *ts45* was observed for the other snRNAs with the exception of U3. As seen in Figure 8(A), the U3 signal is identical in *ts45* and the wild-type. In addition, ethidium bromide staining of the gel prior to

Fig. 8. The effect of temperature shift on snRNA concentrations. Cells were grown to mid-log phase and then a part of each culture was maintained at 23°C and the rest grown at 37°C. Aliquots of cells were removed at various times after growth at 37°C. Total RNA (20 µg/sample) was fractionated on a denaturing polyacrylamide gel and blotted onto nylon membranes. (A) The blots were probed with oligonucleotides which recognize U4, K and U3 RNAs. The blot was exposed to X-ray film with an intensifying screen for 1 day in the case of U3 and 4 days for U4 and K RNA. (B) The polyacrylamide gel was stained with ethidium bromide.

transfer revealed no differences between *ts45* and wild-type with respect to the abundant cytoplasmic RNAs (Figure 8B). The 5.8S rRNA is constant throughout the time course of the experiment as expected for an equal mass loading. Agarose gel analysis of the 28S and 18S rRNA confirms that equal amounts of rRNA were loaded in each lane (data not shown). Relative to the larger rRNA, the 5S RNA and tRNA decrease upon temperature shift but return to normal after 2 h. This pattern was identical in both the wild-type and the mutant. Therefore, the defect in RNA synthesis or maintenance that we observe in *ts45* appears to be specific for a particular group of snRNAs. We presently have no explanation for the loss of 5S RNA and tRNA upon temperature shift, but it may be related to the transient decrease in the rate of transcription and processing of rRNA observed in *S.cerevisiae* upon shift from 23 to 36°C (Warner and Udem, 1972).

In the above series of experiments, equal mass amounts of RNA were loaded on each lane of the gel. Therefore, the results of the RNA analysis are standardized against rRNA content. We have repeated the time course experiments by analyzing total RNA on a per cell basis (see Materials and methods). The results were identical to those seen in Figure 8 (data not shown). Therefore, *ts45* exhibits a decreased steady-state level of U1–U6 and K RNAs compared to the wild-type by both methods of analysis.

Genetic analysis

The mutation in *ts45* was tested for dominance by mating it to a wild-type strain of the opposite mating type carrying a complementing *ade6⁻* allele. Diploids were selected on minimal medium at 23°C and then streaked on complete medium and grown at 37°C. The diploids were not temperature sensitive for growth. In addition, whole-cell RNA was prepared from the diploids and from each of the haploid parental strains after growth at both the permissive and the non-permissive temperatures. The RNA prepared from the diploids was examined by Northern analysis using the U4-specific oligonucleotide. There was no reduction in the steady-state level of U4 in the diploid compared to the wild-type parental strain, and there was no accumulation of the aberrant elongated transcript (data not shown). Both the ability of the diploids to grow at 37°C and the absence of an snRNA phenotype at the elevated temperature indicates that the mutation is recessive.

The molecular phenotype of *ts45* is very different from that observed in the three previously identified pre-mRNA splicing mutants of fission yeast. In these mutants the steady-state levels of U1, U2, U4 and U5 are the same as wild-type (Potashkin *et al.*, 1989). The amount of U6 is slightly reduced in the pre-mRNA splicing mutants, as with *ts45*, because of inefficient splicing of the U6 precursor (Potashkin and Frendewey, 1989). In addition, the pre-mRNA splicing defect is more severe in the *prp⁻* mutants previously identified (Potashkin *et al.*, 1989) compared to *ts45* (Figure 6). To test whether *ts45* could complement the growth defects in the *prp⁻* mutants, *ts45* was crossed with *prp1*, *prp2* and *prp3*. An *h⁺ ade6-M210* strain of *ts45* was mated with *prp1*, *prp2* and *prp3*, which are *h⁻* and carry the complementing *ade6-M216* allele. The diploids were selected on minimal plates and then streaked onto complete medium and incubated at 37°C to check for a *ts⁻* growth defect. All the diploids were capable of growing at the non-permissive temperature, placing *ts45* in a different complementation group from *prp1*, *prp2* and *prp3*. Because we do not yet know whether the *ts45* mutation affects the synthesis, processing or turnover of snRNAs we have given this mutant the more general name *snm1* to indicate a defect in snRNA maintenance.

Discussion

We have identified an *S.pombe* mutant (*ts45*) that is temperature sensitive for growth and expresses a novel molecular phenotype: it maintains reduced steady-state amounts of the spliceosomal snRNAs U1, U2, U4, U5 and U6 compared to the wild-type. Interestingly, the level of K RNA in *ts45* is also considerably below normal. Thus, K RNA is related to the major nucleoplasmic snRNAs with regard to some aspect of its synthesis or maintenance. The concentrations of the nucleolar snRNA U3 as well as the major cytoplasmic RNAs (tRNA and the rRNAs), mRNA and 7SL RNA appear to be normal in *ts45*. We have assayed the quantities of all the major classes of cellular RNA encoded by the nuclear genome and, specifically, all of the *S.pombe* snRNAs for which we were able to obtain probes. Although this survey is obviously not complete, we infer from our data that the *ts45* mutation affects a limited number

of snRNAs that are presumed to reside in the nucleoplasm as opposed to the nucleolus.

Probably as a consequence of the reduced snRNA concentrations, *ts45* exhibits defects in pre-mRNA and U6 pre-RNA splicing. The larger U6 RNA seen in the progeny of the *ts⁻* spores at 37°C is the unspliced precursor. The mutant accumulates unspliced U6 pre-RNA when grown at 23 and 37°C. By comparison, in the wild-type small amounts of U6 pre-RNA are detected at 23°C and immediately after shifting from 23 to 37°C (Potashkin and Frendewey, 1989), but no precursor is observed in cells grown at 30°C (Tani and Ohshima, 1989) or after 5 h at 37°C. Thus, the accumulation of U6 pre-RNA in *ts45* is qualitatively similar to that seen in three pre-mRNA splicing mutants (Potashkin and Frendewey, 1989). Consistent with the U6 RNA splicing defect, we also observe a slight accumulation of unspliced pre-mRNA in *ts45* relative to the wild-type, indicating a mild impairment in pre-mRNA splicing but only at 37°C. The fact that the apparent efficiency of pre-mRNA splicing (inferred from the ratio of precursor to product) is only marginally diminished in *ts45* despite its low snRNA content implies that the spliceosomal snRNAs are normally in excess and that a reduction in snRNA levels greater than the 2- to 5-fold exhibited by the mutant would be required to elicit a more severe splicing defect. The reason that pre-mRNA accumulates exclusively at 37°C, even though the snRNA content of *ts45* is the same at the permissive and non-permissive temperatures, is not clear. One possible explanation is that the pre-mRNA splicing efficiency (number of pre-mRNA molecules spliced per unit time) in *ts45* is identical at 23 and 37°C, but the rate of pre-mRNA transcription increases in response to the shift to 37°C. This would increase the pre-mRNA pool, but not change the steady-state amount of mRNA. In any case, the snRNA concentration in *ts45* is insufficient to allow complete processing of the pre-mRNA and U6 pre-RNA that is made at 37°C. As expected from the ~10-fold reduction in K RNA levels, tRNA processing in *ts45* is impaired. The type of pre-tRNAs that accumulate are indicative of a reduced efficiency of RNase P cleavage (Willis *et al.*, 1984). These results strongly suggest that one of the affected snRNAs, most likely K RNA (Krupp *et al.*, 1986), is required for efficient pre-tRNA processing.

A single recessive mutation in *ts45* is responsible for the *ts⁻* growth phenotype, the abnormal snRNA pattern and the defects in RNA processing. *ts45* complements the *ts⁻* growth defect of each of three pre-mRNA splicing mutants (*prp1*, *prp2* and *prp3*) that we previously isolated (Potashkin *et al.*, 1989), placing it in a separate complementation group. We have assigned *ts45* the formal name *snm1* (snRNA maintenance). This designation reflects the primary aspect of the molecular phenotype: the maintenance of a reduced steady-state snRNA content.

The molecular phenotype of *snm1* indicates either a reduction in the rate of synthesis and/or an acceleration in the rate of turnover of a group of snRNAs. The primary defect caused by the *snm1* mutation could manifest itself in three general areas of snRNA metabolism: transcription, processing and transport, or snRNA maintenance. Given the connection between snRNA transcription and 3' end formation in vertebrates (Hernandez and Weiner, 1986; Hernandez and Lucito, 1988; Neuman de Vegvar *et al.*,

1986) and the reduction in snRNA content associated with 3'-extended transcripts in *snm1*, it is tempting to speculate that the *snm1* lesion is in a gene required for snRNA transcription. For example, a mutation in a component of the snRNA transcription complex could cause an attenuated rate of transcription initiation, resulting in a lower steady-state snRNA content. If the same component also participated in termination of transcription, then the mutation could lead to the production of read-through transcripts extended at their 3' ends. To explain the *snm1* phenotype by this sort of mutation requires that the initiation defect be the same at all temperatures, whereas the errors in termination would be temperature sensitive, i.e. more frequent at 37°C. For this hypothesis to accommodate all aspects of snRNA synthesis in *snm1*, it must be reconciled with several observations: (i) U3 RNA content is normal in *snm1*; (ii) both presumptive RNA polymerase (Pol) II and III products are affected; (iii) 3'-extended transcripts are detected only for U2 and U4 RNAs; (iv) a 3'-truncated U2 RNA is produced; and (v) K RNA is affected.

U3 RNA, like U1, U2, U4 and U5 RNAs, is transcribed by Pol II in vertebrates (Dahlberg and Lund, 1988). However, the U3 promoter is slightly different from the other snRNA Pol II promoters (Stroker and Weiner, 1985; Suh *et al.*, 1986). This difference, together with the possibility that U3 RNA is transcribed in the nucleolus, suggests that U3 RNA transcription might not require the identical set of factors as those used for the synthesis of the other Pol II snRNAs. For example, U3 RNA transcription might utilize an additional factor that compensates for the *snm1* mutation.

U6 RNA, which is a Pol III transcript in vertebrates (Kunkel *et al.*, 1986; Reddy *et al.*, 1987) and most likely in budding yeast (D.Brow and C.Guthrie, personal communication) and in fission yeast (Tani and Ohshima, 1989), is reduced to approximately the same extent in *snm1* as the U1, U2, U4 and U5 RNAs. This similarity of effect could be explained by the fact that U6 promoters share elements with those of Pol II snRNAs (Krol *et al.*, 1987). Alternatively, the lower U6 RNA concentration in *snm1* could be accounted for by the splicing defect alone. However, the steady-state quantity of U6 RNA in *snm1* is reduced to approximately the same extent as that seen in *prp1*, *prp2* and *prp3*, which exhibit much more severe splicing defects (data not shown). If the reduction in U6 RNA in *snm1* were a consequence of a partial or complete block in splicing, then the mature U6 RNA signal should decrease as the precursor signal increases, as is the case in *prp1*, *prp2* and *prp3*.

If one result of the *snm1* mutation is a ts^- defect in transcription termination, then why do we observe 3'-extended products only for U2 and U4 RNAs? The longer U2 and U4 transcripts may be able to assume secondary structures that impart unusual stability or make these RNAs poor substrates for the snRNA 3' processing nuclease. Run-through transcription could be occurring on the U1 and U5 genes, but these transcripts might be unstable or are rapidly processed to mature forms. U6 RNA transcription is expected to terminate in a run of uridines (Tani and Ohshima, 1989), as with all Pol III transcripts. The 3'-truncated U2 RNA could be a degradation product of the longer aberrant transcript (Konings and Mattaj, 1987; Shuster and Guthrie, 1988). The length (110) of the smaller U2 RNA implies a 3' end near the 3' border of the

Sm-binding region. Therefore, as proposed by Konings and Mattaj (1987), 3' to 5' exonucleolytic degradation of the longer RNA might be halted by the snRNA core proteins bound at the Sm site, producing a relatively stable shorter U2 RNA.

Our current ignorance of RNase P RNA transcription in *S.pombe* precludes a complete discussion of the severe reduction in K RNA levels that we observe in *snm1*. However, the human RNase P RNA (H1 RNA) is transcribed by Pol III *in vitro* and its gene has a promoter structure similar to the U6 gene (M.Bear, T.Nilsen, C. Costigan and S.Altman, personal communication). If K RNA is also transcribed by Pol III, then the *snm1* results suggest that the K gene should have a U6-like promoter. The essential elements of the K RNA promoter have yet to be determined.

We have focused this discussion on the possibility that the primary effect of the *snm1* lesion manifests itself at the level of transcription. Alternatively, a primary defect in snRNA 3' processing could produce 3'-extended transcripts at 37°C, but a processing error does not readily explain the maintenance of constant low levels of the affected snRNAs in the absence of precursor accumulation. If the *snm1* mutation is in a gene for a factor that mediates snRNA maintenance via snRNP integrity, such as an snRNP protein, snRNAs could be exposed to degradation because of aberrant snRNP assembly or stability. This type of model predicts that at least one protein is shared by the spliceosomal snRNPs and RNase P. However, anti-RNase P sera do not immunoprecipitate the spliceosomal snRNPs and anti-Sm sera, which recognize a subset of the proteins common to the spliceosomal snRNPs, do not immunoprecipitate RNase P (Gold *et al.*, 1988; Bartkiewicz *et al.*, 1989). This mechanism also does not directly account for the extended snRNAs, unless the mutated protein is also required for processing. Despite these arguments against the alternative explanations, our data do not eliminate either of these possibilities.

Other more complicated explanations of the *snm1* phenotype such as a combination of transcription, processing and stability or a mutation in the heat shock response can be envisaged, but we feel the simplest explanation is a primary defect in transcription. This hypothesis makes three predictions: (i) the *snm1* defect should be mediated through the promoters of the affected RNAs; (ii) the promoters of the affected RNAs should have at least one element (e.g. the octamer of the vertebrate snRNA promoters) in common or share at least one transcription factor; and (iii) the transcription rate for all of the affected RNAs should be reduced relative to the wild-type and this difference should be maintained at both 23 and 37°C. Experiments to test these predictions as well as to characterize the *snm1* gene are in progress.

Materials and methods

Yeast strains and genetic assay

All *S.pombe* strains used in this study were derived from strains 972 (h^-) or 975 (h^+) introduced by U.Leupold. The following strains were used: $h^- ade6-M216$ and $h^+ ade6-M210$. The standard genetical procedures for *S.pombe* were used in this study (Gutz *et al.*, 1974). The bank of temperature-sensitive mutants was prepared by mutagenizing the wild-type strain 972 with ethylmethanesulfonate (EMS, Aldrich) by the method described for

S. cerevisiae (Sherman *et al.*, 1986). Strains which did not grow or grew slowly at 37°C were selected as previously described (Potashkin *et al.*, 1989).

Media and growth conditions

Yeast were grown in YE medium (0.5% yeast extract, 3% glucose), PM (*S. pombe* minimal) medium, which is a modification of EMM2 medium (Mitchison, 1970; Nurse, 1975) or PMA medium which in addition contains 75 mg/l of adenine.

Temperature-sensitive mutants were grown at 23°C for the permissive temperature and 37°C for the non-permissive temperature. These temperatures were chosen to create a wide difference in the optimal growth temperature of the mutants.

Preparation of RNA

To prepare total RNA from each of the mutants, cells were grown to mid-log phase at 23°C. An aliquot of 5 ml was shifted to 37°C for the time indicated in the figure legends. Cells were harvested, washed once with distilled water and then cell pellets were frozen at -20°C. RNA was extracted from the cell pellet by disruption with glass beads followed by phenol/chloroform/isoamylalcohol extractions (Nischt *et al.*, 1986). Between 100 and 200 µg of RNA could readily be obtained by this method. Larger preparations of RNA were done as previously described (Potashkin *et al.*, 1989).

Northern analysis

Whole-cell RNA (20 µg) was fractionated on formaldehyde-agarose gels (Maniatis *et al.*, 1982) or 1.5 mm thick 10% polyacrylamide/urea gels (Maxam and Gilbert, 1980). RNA was transferred from formaldehyde-agarose gels to Gene Screen Plus (New England Nuclear) by capillary blot as described by the manufacturer. Transfer of RNA from polyacrylamide gels to Gene Screen Plus was by the electroblot procedure as described by the manufacturer using the Hoeffer Scientific Industries transfer apparatus at 30 V overnight. Pre-hybridization, hybridization and removal of probes from RNA blots was as previously described (Potashkin *et al.*, 1989).

To analyze the effect of temperature shift on steady-state levels of snRNA, the wild-type and *ts45* were grown to mid-log phase and then a part of each culture was maintained at 23°C and the rest was grown at 37°C. Aliquots of cells were removed at various times after growth at 37°C. In one series of experiments, equal mass quantities of RNA were loaded onto each of the wells. In another series of experiments, equal numbers of cells were harvested and 10⁴ d.p.m. of [³H]RNA prepared from yeast was added to each of the cell pellets prior to cell breakage and preparation of RNA. Equal amounts of [³H]RNA were then loaded onto each of the wells of the gel.

Preparation of probes

All oligonucleotides were synthesized on the Applied Biosystems 380A or 380B DNA synthesizers. Oligonucleotides were then gel purified and 5' end labeled with [^γ-³²P]ATP (ICN) as previously described (Potashkin *et al.*, 1989).

Plasmids used to synthesize antisense RNAs for the ribonuclease protection assays were constructed as follows. A *Bam*HI-*Xba*I fragment carrying the fission yeast U2 gene (Brennwald *et al.*, 1988b) was subcloned into the vector Bluescribe (Stratagene) in an orientation such that an antisense RNA could be prepared using T3 RNA polymerase (Figure 4). The template was cut with *Bam*HI so that a full-length antisense RNA could be prepared. The construction of pBSα1.1, used in the ribonuclease protection assay, was as described (Potashkin *et al.*, 1989).

Molecular analysis

RNAse protection assays were done using ³²P-labeled T3 or T7 RNA polymerase transcripts under conditions previously described (Zinn *et al.*, 1983). The RNA samples were fractionated on 8% polyacrylamide/urea sequencing gels (Maxam and Gilbert, 1980). Gels were exposed to Kodak X-ray film for 1 and 3 days.

Primer extension assays were done as described (Potashkin and Frendewey, 1989).

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References

- Ach, R.A. and Weiner, A.M. (1987) *Mol. Cell. Biol.*, **7**, 2070-2079.
- Altman, S., Gold, H.A. and Bartkiewicz, M. (1988) In Birnstiel, M.L. (ed.), *Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles*. Springer-Verlag, Berlin, pp. 183-195.
- Ares, J.M. (1986) *Cell*, **47**, 49-59.
- Ares, J.M., Mangin, M. and Weiner, A.M. (1985) *Mol. Cell. Biol.*, **5**, 1560-1570.
- Bartkiewicz, M., Gold, H. and Altman, S. (1989) *Genes Dev.*, **3**, 488-499.
- Birnstiel, M.L. and Schaufele, F.J. (1988) In Birnstiel, M.L. (ed.), *Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles*. Springer-Verlag, Berlin, pp. 155-182.
- Black, D.L. and Steitz, J.A. (1986) *Cell*, **46**, 697-704.
- Bohmann, D., Keller, W., Dale, T., Scholer, H.R., Tebb, G. and Mattaj, J.W. (1987) *Nature*, **325**, 268-272.
- Brennwald, P., Liao, X., Holm, K., Porter, G. and Wise, J.A. (1988a) *Mol. Cell. Biol.*, **8**, 1580-1590.
- Brennwald, P., Porter, G. and Wise, J.A. (1988b) *Mol. Cell. Biol.*, **8**, 5575-5580.
- Brow, D.A. and Guthrie, C. (1988) *Nature*, **334**, 213-218.
- Christofori, G. and Keller, W. (1988) *Cell*, **54**, 875-889.
- Ciliberto, G., Buckland, R., Cortese, R. and Philipson, L. (1985) *EMBO J.*, **4**, 1537-1543.
- Ciliberto, G., Dathan, N., Frank, R., Philipson, L. and Mattaj, J.W. (1986) *EMBO J.*, **5**, 2931-2937.
- Dahlberg, J.E. and Lund, E. (1988) In Birnstiel, M.L. (ed.), *Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles*. Springer-Verlag, Berlin, pp. 38-70.
- Dandekar, T. and Tollervey, D. (1989) *J. Mol. Biol.*, **208**, 371-379.
- Epstein, P., Reddy, R. and Busch, H. (1984) *Biochemistry*, **23**, 5421-5425.
- Gold, H.A., Craft, J., Hardin, J.A., Bartkiewicz, M. and Altman, S. (1988) *Proc. Natl Acad. Sci. USA*, **85**, 5483-5487.
- Guthrie, C. (1988) In Birnstiel, M.L. (ed.), *Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles*. Springer-Verlag, Berlin, pp. 196-211.
- Gutz, H., Heslot, H., Leupold, U. and Loprieno, N. (1974) In King, R.C. (ed.), *Handbook of Genetics*. Plenum Press, New York, pp. 395-446.
- Hernandez, N. (1985) *EMBO J.*, **4**, 1827-1837.
- Hernandez, N. and Weiner, A.M. (1986) *Cell*, **47**, 249-258.
- Hernandez, N. and Lucito, R. (1988) *EMBO J.*, **7**, 3125-3134.
- Konings, D.A.M. and Mattaj, J.W. (1987) *Exp. Cell Res.*, **172**, 329-339.
- Krol, A., Carbon, P., Ebel, J. and Appel, B. (1987) *Nucleic Acids Res.*, **15**, 2463-2478.
- Krupp, G., Cherayil, B., Frendewey, D., Nishikawa, S. and Söll, D. (1986) *EMBO J.*, **5**, 1697-1703.
- Kunkel, G.R., Maser, R.L., Calvet, J.P. and Pederson, T. (1986) *Proc. Natl Acad. Sci. USA*, **83**, 8575-8579.
- Lischwe, M.A., Ochs, R.L., Reddy, R., Cook, R.G., Yeoman, L.C., Tan, E.M., Reichlin, M. and Busch, H. (1985) *J. Biol. Chem.*, **260**, 14304-14310.
- Lührmann, R. (1988) In Birnstiel, M.L. (ed.), *Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles*. Springer-Verlag, Berlin, pp. 71-99.
- Maniatis, T. and Reed, R. (1987) *Nature*, **325**, 673-678.
- Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Mattaj, J.W. (1988) In Birnstiel, M.L. (ed.), *Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles*. Springer-Verlag, Berlin, pp. 100-114.
- Mattaj, J.W., Linehard, S., Jiricny, J. and DeRobertis, E.M. (1985) *Nature*, **316**, 163-167.
- Maxam, A.M. and Gilbert, W. (1980) *Methods Enzymol.*, **65**, 499-560.
- Mitchison, J.M. (1970) *Methods Cell Physiol.*, **4**, 131-165.
- Murphy, J.T., Skuzeski, J.M., Lund, E., Steinberg, T.H., Burgess, R.R. and Dahlberg, J.E. (1987) *J. Biol. Chem.*, **262**, 1795-1803.

- Neuman de Vegvar,H.E., Lund,E. and Dahlberg,J.E. (1986) *Cell*, **47**, 259–266.
- Nischt,R., Thuroff,E. and Käufer,N.F. (1986) *Curr. Genet.*, **10**, 365–370.
- Nurse,P. (1975) *Nature*, **256**, 547–551.
- Ochs,R.L., Lischwe,M.A., Spohn,W.H. and Busch,H. (1985) *Biol. Cell*, **54**, 123–134.
- Parker,K.A. and Steitz,J.A. (1987) *Mol. Cell. Biol.*, **7**, 2899–2913.
- Patterson,B. and Guthrie,C. (1987) *Cell*, **49**, 613–624.
- Pikielny,C.W., Rymond,B.C. and Rosbash,M. (1986) *Nature*, **324**, 341–345.
- Porter,G.L., Brennwald,P.J., Holm,K.A. and Wise,J.A. (1988) *Nucleic Acids Res.*, **16**, 10131–10152.
- Potashkin,J. and Frendewey,D. (1989) *Nucleic Acids Res.*, **17**, 7821–7831.
- Potashkin,J., Li,R. and Frendewey,D. (1989) *EMBO J.*, **8**, 551–559.
- Prestayko,A.W., Tonato,M. and Busch,H. (1970) *J. Mol. Biol.*, **47**, 505–515.
- Reddy,R. and Busch,H. (1988) In Birnstiel,M.L. (ed.), *Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles*. Springer-Verlag, Berlin, pp. 1–37.
- Reddy,R., Ro-Choi,T.S., Henning,D., Shibata,H., Choi,Y.C. and Busch,H. (1972) *J. Biol. Chem.*, **247**, 7245–7250.
- Reddy,R., Henning,D., Das,G., Harless,M. and Wright,D. (1987) *J. Biol. Chem.*, **262**, 75–81.
- Reimer,G., Pollard,K.M., Penning,C.A., Ochs,R.L., Lischwe,M.A., Busch,H. and Tan,E.M. (1987) *Arthritis Rheum.*, **30**, 793–800.
- Ribes,V., Dehoux,P. and Tollervey,D. (1988) *EMBO J.*, **7**, 231–237.
- Saponara,A.G. and Enger,M.D. (1969) *Nature*, **223**, 1365–1366.
- Sherman,F., Fink,G. and Hicks,J. (1986) *Laboratory Course Manual for Methods in Yeast Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Shuster,E.O. and Guthrie,C. (1988) *Cell*, **55**, 41–48.
- Skuzeski,J.M., Lund,E., Murphy,J.T., Steinberg,T.H., Burgess,R.R. and Dahlberg,J.E. (1984) *J. Biol. Chem.*, **259**, 8345–8352.
- Steitz,J.A., Black,D.L., Gerke,V., Parker,K.A., Kramer,A., Frendewey,D. and Keller,W. (1988) In Birnstiel,M.L. (ed.), *Structure and Function of Major and Minor Small Nuclear Ribonucleoprotein Particles*. Springer-Verlag, Berlin, pp. 115–154.
- Stroke,I.L. and Weiner,A.M. (1985) *J. Mol. Biol.*, **184**, 183–193.
- Suh,D., Busch,H. and Reddy,R. (1986) *Biochem. Biophys. Res. Commun.*, **137**, 1133–1140.
- Tani,T. and Ohshima,Y. (1989) *Nature*, **337**, 87–90.
- Tollervey,D., (1987) *J. Mol. Biol.*, **196**, 355–361.
- Tollervey,D. and Mattaj,I.W. (1987) *EMBO J.*, **6**, 469–476.
- Warner,J.R. and Udem,S.A. (1972) *J. Mol. Biol.*, **65**, 243–257.
- Watson,J.D., Hopkins,N.H., Roberts,J.W., Steitz,J.A. and Weiner,A.M. (1987) *Molecular Biology of the Gene*. Benjamin/Cummings, California.
- Willis,I., Frendewey,D., Nichols,M., Hottinger-Werlen,A., Schaack,J. and Söll,D. (1986) *J. Biol. Chem.*, **261**, 5878–5885.
- Willis,I., Hottinger,H., Pearson,D., Chisholm,V., Leupold,U. and Söll,D. (1984) *EMBO J.*, **3**, 1573–1580.
- You,C., Jr and Weiner,A.M. (1985) *Cell*, **42**, 193–202.
- Zeive,G. and Penman,S. (1976) *Cell*, **8**, 19–31.
- Zinn,K., DiMaio,D. and Maniatis,T. (1983) *Cell*, **34**, 865–879.

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