The Journal of Microbiology, December 1997, p.334-340
Vol. 35, No. 4
Copyright © 1997, The Microbiological Society of Korea

Isolation and Characterization of Pre-tRNA^{Val} Splicing Mutants of *Schizosaccharomyces pombe*

Kuchan Hwang and Daemyung Kim*

Department of Genetic Engineering, Chongju University, Chongju 360-764, Korea

(Received July 30, 1997 / Accepted September 12, 1997)

A collection of 132 temperature sensitive (*ts*) mutants was generated by the chemical mutagenesis of *Schizosaccharomyces pombe* wild type strain and screened for tRNA splicing defects on Northern blots by hybridization with an oligonucleotide that recognizes the exon of the *S. pombe* tRNA^{Val} as a probe. We identified 6 mutants which accumulate precursor tRNA^{Val}. Among them, 2 mutants exhibited remarkable morphological differences compared to wild type cells. One tRNA splicing mutant showed elongated cell shape in permissive as well as non-permissive cultures. The other mutant exhibited shortened cell morphology only in nonpermissive culture. The total RNA pattern in the splicing mutants appeared to be normal. Genetic analysis of four tRNA^{Val} splicing mutants demonstrated that the mutations reside in different genes.

Key words: Pre-tRNA splicing, *Schizosaccharomyces pombe*, temperature sensitive mutants

Transcription of eukaryotic tRNA genes initiates at a purine nucleotide, usually within 10 nucleotides upstream of the tRNA coding sequence, and terminates in an oligothymidylate block which resides downstream of the coding sequence. As a result, the primary transcripts of tRNA genes are larger than mature tRNA. In addition, some tRNA genes also include intervening sequences (7). These additional sequences are removed by subsequent processing steps which include removal of 5'-and 3'-extensions, splicing of intervening sequences, modification of specific nucleotides, and addition of-CCA to the 3'-terminus (8, 9, 13, 16).

In yeast, the splicing of precursor tRNA occurs in two separate steps; endonuclease digestion which generates two half-tRNA molecules plus a free intron molecule, and an ATP-dependent ligation which joins the two half-tRNA (5, 10, 19). The size of the intron ranges from 14 to 60 nucleotides and its sequence is not conserved (18). Even though there are more than 20 different tRNA species in eukaryotic cells, it is known that only one splicing system splices all tRNA precursors that contain intron sequences (4, 20).

In *Saccharomyces cerevisiae*, there have been numerous attempts to identify genes specifically involved in tRNA splicing. The majority of these came from the *ts* mutants which are defective in tRNA splicing. To date, eight different genes are known to affect pre-tRNA splicing. These genes are *RNA1* (2, 8, 9), *LOS1* (8), *SEN1* (28), *SEN2* (6, 28), *STP1* (27), *TPD1* (25), *PTA1* (17), and *SPL1* (11). Of these, only *SEN2* is known to encode the β-subunit of tRNA endonuclease (22). Five of the genes (*RNA1, SEN1, PTA1, TDP1,* and *SPL1*) encode essential functions whereas *LOS1* and *STP1* are nonessential genes. Although most of these genes have been cloned and sequenced, the exact role of these gene products in intron excision remains to be seen.

Our laboratory recently started studying the fission yeast *Schizosaccharomyces pombe*. It is a simple unicellular eukaryote with a genome size of 14 megabases. It is an interesting organism in that certain features such as cell cycle, chromosome structure, and RNA splicing are likely to be more similar to that of mammalian cells than is *S. cerevisiae*. In addition, good genetic, cytological, biochemical, and molecular genetic techniques are available for *S. pombe*. Although *S. pombe* is such an attractive and amenable organism, there has been no attempt thus far to isolate tRNA splicing mutants in *S. pombe*. To gain a better understanding of the components required for pre-tRNA splicing in *S. pombe*, we isolated pre-tRNA^{Val} splicing mutants which accumulate precursor tRNA and studied the characteristics of some of these mutants.

Materials and Methods

Yeast strains

Table 1 lists the yeast strains used in this study.

* To whom correspondence should be addressed.

Table 1. Yeast strains used in this study

Yeast strain	Genotype	Source
972	*h* : wild type *S. pombe* strain	H. B. Kim
975	h^{+}: wild type *S. pombe* strain	H. B. Kim
ED665	h^{-}, *ade6-M210*$^{-}$, *leul-32*$^{-}$, *ura4-D18*$^{-}$	H. B. Kim
ED666	h^{-}, *ade6-M210*$^{-}$, *leul-32*$^{-}$, *ura4-D18*$^{-}$	H. B. Kim
ED667	h^{+}, *ade6-M216*$^{-}$, *leul-32*$^{-}$, *ura4-D18*$^{-}$	H. B. Kim
SKC144	h^{+}, *ts14*$^{-}$	This study
SKC148	h^{-}, *ts14*$^{-}$	This study
SKC213	h^{+}, *ts21*$^{-}$	This study
SKC216	h^{-}, *ts21*$^{-}$	This study
SKC562	h^{-}, *ts56*$^{-}$	This study
SKC563	h^{+}, *ts56*$^{-}$	This study
SKC884	h^{+}, *ts88*$^{-}$	This study
SKC886	h^{-}, *ts88*$^{-}$	This study

Media and growth conditions

Rich (YE) media was prepared according to Alfa *et al.* (1). Wild type strains were grown at 23°C. Temperature sensitive strains were grown at 23°C for permissive temperature or at 37°C for non-permissive temperature. Sporulation agar (SPA: 1% glucose, 0.1% KH_2PO_4, 1.5% agar, and vitamin mixture: personal communication with Tomohiro Matsumoto) was used in sporulation experiments.

EMS mutagenesis

Wild type strain was mutagenized with the alkylating agent ethylmethansulfonate (EMS) by the modification of the method described in Sherman *et al.* (24). *S. pombe* was cultured to a cell density of 10^7-10^8 cells/ml, the pellet resuspended in 50 mM sodium phosphate buffer (pH 8.0), and treated with 3.5% EMS for 60 min at 30°C. Mutagenized cells were washed 3 times with sterile water and resuspended in 1 ml of sterile water. The cells were plated on YE plates at a concentration of 200~300 cells per plate and incubated at 23°C for 5 days. These colonies were replica-plated on two sets of YE plates. One set was incubated at 23°C while the other set was cultured at 37°C. Colonies that grew poorly or did not grow at the non-permissive temperature were retested for their growth defect and the strains which still exhibited strong growth defect were included in the temperature sensitive mutants bank.

Temperature shift and RNA preparation

The *ts*$^{-}$ mutant cells were grown to mid-log phase at 23°C and then shifted to 37°C for 5 h. Cells were harvested and washed once with distilled water. Extraction of total RNA from the pellet was performed by the modification of the method described in Nischt *et al.* (15). Cells were resuspended in 100 µl of 10 mM Tris-HCl pH 7.5, 10 mM EDTA, 100 mM LiCl, 1% lithium lauryl sulfate and an equal volume of sterile glass beads was added. Cells were broken by 8 times of 30 seconds vortexing and the volume was brought to 500 µl by the addition of 10 mM Tris-HCl pH 7.5, 1 mM EDTA, 100 mM LiCl. An equal volume of phenol/chloroform (1:1) was added and the mixture was vortexed for 10 seconds. The aqueous phase was extracted with chloroform and RNA was precipitated by the addition of LiCl to a final concentration of 0.5 M and an equal volume of ethanol. Using this procedure, 100~200 µg of RNA was usually obtained.

Northern analysis

About 20 µg of total RNA from each mutant was used for Northern analysis. The RNA was added to a formamide dye mix (95% formamide, 20 mM Tris-HCl pH 8.0, 5 mM EDTA, 0.1% bromophenol blue and 0.1% xylene cyanol), denatured at 95°C for 5 min and then fractionated on 10% polyacrylamide (acrylamide:bisacrylamide=29:1) gel containing 8 M urea. After electrophoresis, the gel was stained with 0.5 mg/ml ethidium bromide for 5 min and a picture of the gel was taken under UV. The RNA was blotted onto a Genescreen Plus membrane (Du Pont) in 10 mM Tris-acetate (pH 7.8), 5 mM sodium acetate, 0.5 mM EDTA for 16 hrs at 20 V and crosslinked on the membrane by baking at 65°C for 2 hrs. Prehybridization, hybridization, and washing of the membranes were done as described in Potashkin *et al.* (21). The blots were prehybridized at 42°C in a solution containing 6 X SSPE (23), 0.1% SDS, 20 X Denhardt's (23), and 5 mg/ml herring sperm DNA for 2 hrs. After complete removal of prehybridization solution, hybridization of the membranes were performed at 42°C for 16 hrs in 6 X SSPE, 0.1% SDS, and 1 X 10^6 cpm/ml of probe. The membrane was washed 3 times in 6 X SSPE, 0.1% SDS at 25°C for 15 min. The blots were exposed to X-ray film at -70°C with intensifying screen for 2~3 days.

Preparation of probe

The oligonucleotide used in this study was VAL-E: 5'-TCGAACTTGGGACCGTC-3', complementary to 3'-half of *S. pombe* valine tRNA precursor (12) synthesized from Korea Biotech. Inc. The oligonucleotide was labeled at its 5' end using [γ-^{32}P] ATP (Amersham) and T4 polynucleotide kinase (NEB). Unincorporated [γ-^{32}P] ATP was removed by centrifugation through a Sephadex-50 column (23).

Genetic cross and spore manipulation

Mating between opposite mating types was caerried out by mixing pairs of mutants on SPA and allowing mating by incubation at 23°C for 2 days.

Mating was confirmed by the observation of asci formation under microscope. The asci were resuspended in 1 ml of distilled water, added 10 μl of glusulase, and incubated overnight at 23°C. The suspension was centrifuged in a microcentrifuge for 1 min and resuspended in 100 μl of distilled water. The number of spores was counted in a hematocytometer and the spores were plated on YE at a concentration of about 300 spores per plate.

Results and Discussion

Isolation of temperature sensitive mutants

We isolated *ts*⁻ mutants by EMS mutagenesis and dual temperature replica plating technique. For optimization of mutagenesis, the concentration of EMS was varied from 0% to 3.5% and number of the colonies survived at 23°C was counted. Table 2 shows the persentage of killing and yield of *ts*⁻ mutants from EMS mutangensis. The persentage of killing increased as expected from 0% to 87%. An EMS concentration that produced 80% killing was 3-3.5% which is consistent to that of same mutagenesis of *S. pombe* (David Frendewey: personal communication). In this EMS concentration, the *ts*⁻ mutants represented about 0.2% of the survivors. We performed 9 mutageneses using 3.5% EMS and collected 132 *ts*⁻ mutants. This frequency of *ts*⁻ mutants was 6 fold less than what has been reported for *S. cerevisiae* (26). However their *ts*⁻ mutants were about 1-2% of the survivors which is about 5~10 fold more than that of ours. The 6 fold difference in the frequency of *ts*⁻ mutant generation may due to differences in the organisms used in mutagenesis.

Screening for pre-tRNA splicing mutants

In this study, 132 *ts*⁻ mutants were screened for pre-tRNA splicing defects by Northern analysis. The procedure consisted of 4 steps: (1) the extraction of total RNA from cells cultured at 23°C to mid-log phase and then shifted to 37°C for 5 hrs; (2) Fractionation of total RNA by electrophoresis in acrylamide-urea gel and blotting of RNA onto a nylon membrane (3) probing the membrane with tRNA specific oligonucleotide labeled with ^{32}P at the 5'-end (Fig. 1A).

We searched GenBank sequence database for tRNA sequences of the *S. pombe*. Thus far, 13 nuclear tRNA sequences have been reported and 4 tRNA genes are known to contain an intron sequence. However, their introns are usually short; some of them are not long enough to design an intron specific probe. Among four tRNA specific probes we tested (SER-I: $tRNA^{Ser}$ intron specific probe, SER-E: $tRNA^{Ser}$ exon specific probe, VAL-E: $tRNA^{Val}$ exon specific probe, and LYS-E: $tRNA^{Lys}$ exon specific probe), the VAL-E probe generated the strongest signal and lowest background level (data not shown). Therefore, we chose the VAL-E probe for analysis of pre-tRNA accumulation. This oligonucleotide is complementary to the 3' half of $tRNA^{Val}$ hence, hybridization of the blot with this probe allows the detection of pre-tRNA, mature tRNA, and 2/3 molecule which contains the 3' half of tRNA.

A representative Northern blot from a primary screen of 13 *ts*⁻ mutants is shown in Fig. 1B. Two

Table 2. Yield of *ts*⁻ mutants from EMS mutagenesis[a]

EMS conc. (%)	% killing	% of *ts*⁻ mutants
0.0	0	0.00
0.5	15	0.00
1.0	29	0.04
1.5	39	0.05
2.0	53	0.07
2.5	66	0.12
3.0	76	0.18
3.5	87	0.20

[a] The result is a mean of the data obtained from three independent experiments in which the dose of mutagen was varied. % killing=100 X [1-(number of survivor/number of cells in 0% EMS)]. % *ts*⁻=100 X (number of *ts*⁻ colonies/number of survivors).

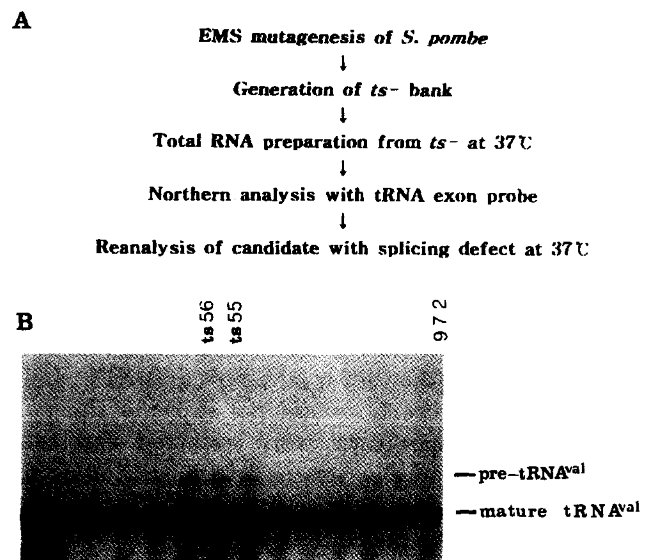

Fig. 1. (A) Scheme used for the isolation of tRNA splicing mutants; (B) A representative Northern blot from a primary screen of 13 temperature sensitive mutants. The cells were grown at 23°C to mid-log phase and then shifted to 37°C for 5 hrs. Total RNA (20 μg/sample) was fractionated on a 10% acrylamide, 8 M urea gel and blotted onto positively charged nylon membrane. To test the accumulation of precursor tRNA, the blots were then probed with ^{32}P-labeled Val-E which recognizes 3'-exon of *S. pombe* valine tRNA.

distinctive tRNA species were detected by the tRNA^{Val} exon specific probe. The size of long RNA (about 80 nucleotides) matched that of the precursor tRNA^{Val} (83 nucleotides) and short RNA (about 70 nucleotides) was close in length to that of mature tRNA^{Val} (73 nucleotides) based on the known sequence of tRNA^{Val} (12). We did not examined the RNA which migrated almost to the end of the gel.

As a negative control, pre-tRNAs present in the unmutagenized wild type 972 were examined. A detectable steady-state level of unspliced tRNAs was found to already exist in vegetatively growing wild type cells. Of the 132 *ts*⁻ mutants screened, 10 mutants were initial candidates for defects in tRNA splicing and were chosen for rescreening. All candidate st- rains from the primary screen were colony-purified and retested for the tRNA splicing defect. In this retest, 6 mutants (*ts14, ts21, ts55, ts56, ts88,* and *ts93*) were observed to significantly accumulate unspliced tRNA^{Val} precursor under the non-permissive condition, judging by the increased intensity of the hybridized bands relative to that of the wild type strain (Fig. 2). These 6 mutants were selected as tRNA^{Val} splicing mutants and used for the experiments below.

The frequency of tRNA splicing mutants in this experiment (6 mutants outof 132 *ts*⁻ mutants) is slightly higher than that in the *S. cerevisiae* experiment (about a dozen tRNA splicing mutants out of about 500 *ts*⁻ mutants) (6). Although its mechanism is different from tRNA splicing, its pre-mRNA splicing of *S. cerevisiae* involves at least 26 gene products directly or indirectly (26), suggesting that more tRNA splicing mutants can be found.

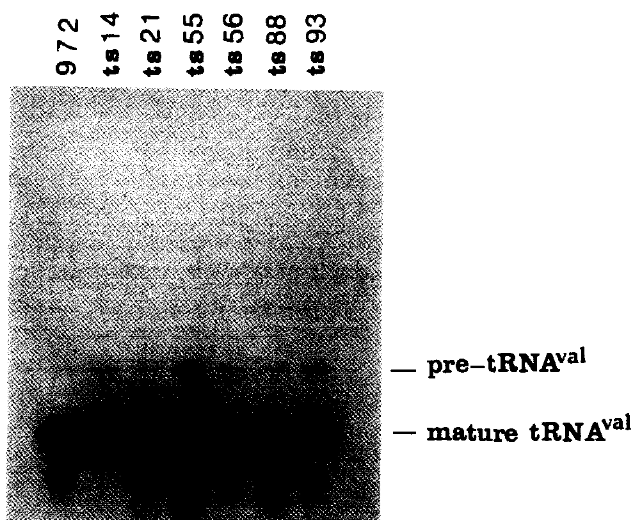

Fig. 2. Northern analysis of tRNA splicing mutants. The splicing mutants were grown at 23°C to mid-log phase and then shifted to 37°C for 5 hrs. Total RNA (20 μg/sample) was fractionated, blotted, and hybridized as in Fig. 1 legend.

In *S. cerevisiae*, the tRNA splicing mutants exhibited three distinctive pre-tRNA species representing precursors differing in the extent of processing at their 3' and 5' ends (6). It is not clear why such multiple pre-tRNA bands were not observed in our experiments. It may be due to differences in the organisms used for the experiments; in *S. pombe*, the 5' and 3' end processing may be fast reactions whereas in *S. cerevisiae*, the reactions may be slow processes. However, the extent of pre-tRNA accumulation is well correlated to that of a tRNA splicing mutant of *S. cerevisiae, Sen1* (see reference 28, figure 6), in which the mature tRNA formed a strong and broad band while the pre-tRNA showed a weak and sharp band and the intensity was slightly but significantly increased compared to wild type.

It is interesting to ask whether our tRNA splicing mutants are specific to valine tRNA or not. Unfortunately, Northern analyses using other tRNA probes, as mentioned above, failed to generate signals strong enough to examine other tRNA splicing defects. Thus the specificity of the tRNA splicing mutants was not determined. The tRNA splicing mutants of *S. cerevisiae*, isolated by a procedure basically same as above, are not specific to a single tRNA (6, 17), suggesting that the tRNA splicing mutants obtained in this research may not be specific to valine tRNA. Further experiments using suppresser tRNA can determine this fact.

Morphology of tRNA splicing mutants

The morphology of the splicing mutants was examined under microscope. Exponentially growing cultures of the wild type strain and tRNA^{Val} splicing mutants at 23°C were shifted to 37°C for 5 hrs and the morphology of the cells was observed under microscope (Fig. 3). The morphologies of most tRNA splicing mutants were not different from that of wild type cells. However, *ts93* showed elongated cells in 37°C culture as well as in 23°C culture. Compared to the wild type cells, these mutant cells were elongated 1.5-3 fold in their length. The mutants *ts55* is interesting in that the cells in 37°C culture exhibited shortened cell morphology. It is known that cell cycle mutants can produce elongated cells (3). Such elongated cells are produced when their DNA synthesis or nuclear division is impaired (14). It is interesting that some of our tRNA^{Val} splicing mutants exhibited the characteristic morphology of cell cycle mutants. Further experiments such as DAPI (4', 6-diamidino-2-phenylindole) staining can determine whether *ts55* or *ts93* are real cell cycle mutants.

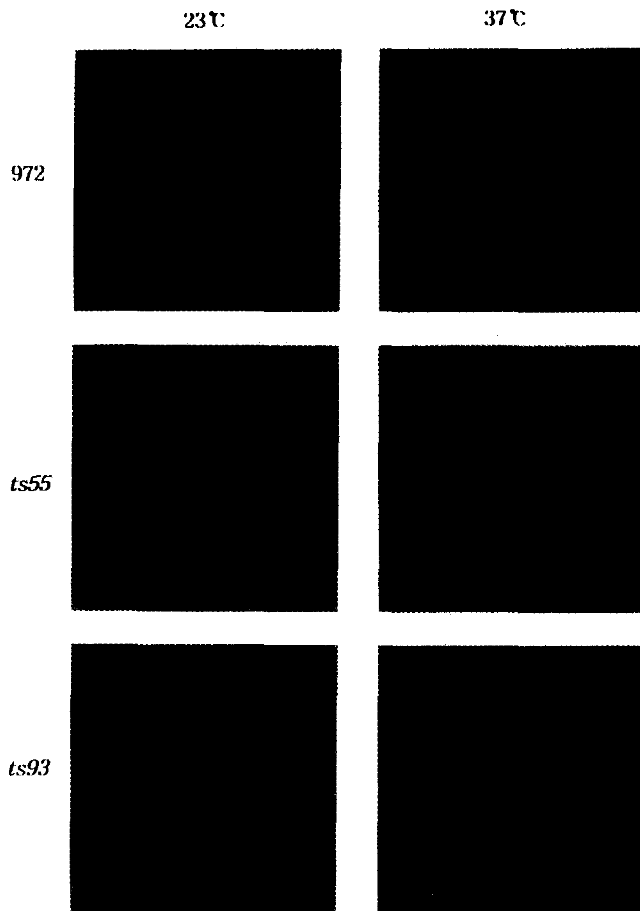

Fig. 3. Cellular morphology of *ts55* and *ts93*. Cells were grown at 23°C to mid-log phase and shifted to 37°C for 5 h. The pictures were taken under Olympus CH-2 microscope at a final magnification of x 400.

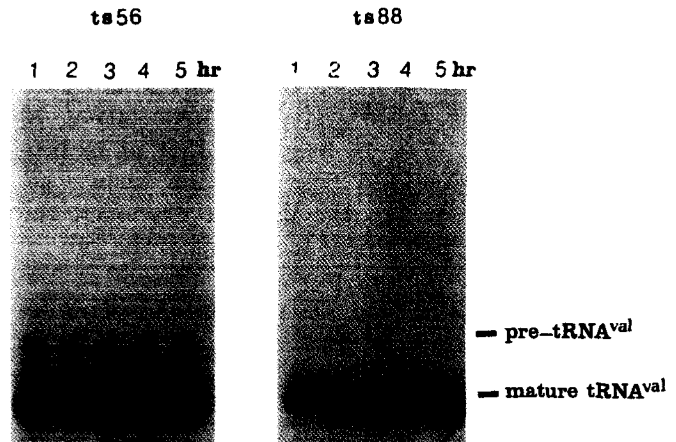

Fig. 4. Northern blot analysis of accumulation of pre-tRNA in *ts56* and *ts88*. Cells were grown at 23°C and shifted to 37°C for 1-5 hrs. Total RNA (20 μg/sample) was analyzed by Northern blotting as in Fig. 1.

Pre-tRNA accumulation in *ts56* and *ts88*

To further characterize the temperature sensitivity of *ts56* and *ts88* with regard to their splicing defect, RNA prepared from each mutant grown at 23°C and shifted to 37°C for 1-5 h was analyzed on Northern blot (Fig. 4). The kinetics of pre-$tRNA^{Val}$ accumulation differed slightly in each of the mutants. The amount of precursor declined gradually in *ts56*, whereas pre-tRNA levels appeared to peak at 2hrs in *ts88*. These results are consistent with the observation that in *ts56*, pre-tRNA accumulated at both permissive and nonpermissive temperatures, but in *ts88*, accumulation of precursor was found only at nonpermissive temperature (data not shown).

Analysis of other RNAs

The results presented above concerned pre-$tRNA^{Val}$ splicing in the *ts*⁻ mutants. It is also interesting to determine whether other RNAs are defective in these splicing mutants. We compared the total RNA profile for the wild type and the splicing mutants. Total RNA was prepared from each splicing mutant and from wild type cells that had been grown to mid-log phase at 23°C and shifted to 37°C for 5 hrs. Equal amounts of each RNA were fractionated on a 10% acrylamide gel containing 8 M urea. The gel was stained with ethidium bromide and visualized by fluorescence under UV illumination. Although there were small variations of RNA loaded, the total RNA patterns for wild type and mutants were essentially indistinguishable; the amount and position of 5.8S, 5S, and tRNA are the same in all samples (Fig. 5). Therefore, the pre-$tRNA^{Val}$ splicing defects exhibited in our tRNA splicing mutants are not due to changes in the amounts of these RNAs.

Genetic analyses in *ts14*, *ts21*, *ts56*, and *ts88*

The ts^- mutants in this report were obtained by random mutagenesis using 3.5% EMS. This EMS concentration is known to generate mostly single mutation in *S. pombe* (Judy Potashkin: Personal Communication). Multiple mutation can be removed by backcrossing ts^- mutant strains to a wild type strain 2~3 times (David Beach: Personal communication). To remove any possible multiple mutations, the $tRNA^{Val}$ splicing mutants were backcrossed three times to wild type strain and ts^- phenotype of resulting progeny cells were followed by replica plating. Northern analysis of cells from the third generation of each splicing mutant showed that they were still harboring the tRNA splicing defect as shown in Fig. 2 (data not shown).

To determine whether the tRNA splicing mutants carry mutations in the same or separate genes, each splicing mutant (the third generation) was crossed with one other and the temperature

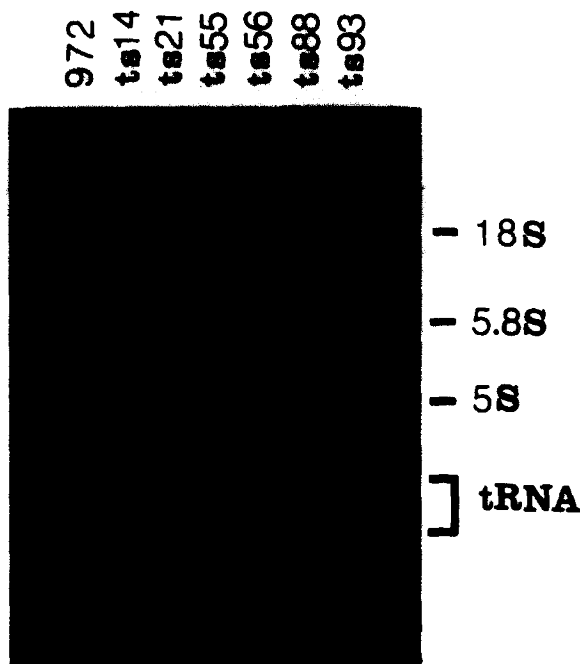

Fig. 5. Total RNA profile in tRNA splicing mutants. Total RNA (20 μg/lane) from wild type (972) or mutants cells grown to mid-log at 23°C and then shifted to 37°C for 5 hrs was fractionated on a polyacrylamide/urea gel. The gel was stained with ethidium bromide and exposed to UV irradiation to visualize the RNAs.

sensitive phenotypes of the progeny cells were observed by replica plating (Table 3). In the crosses between two identical ts^- mutants as a control, no ts^+ progeny were observed implying that the two mutations are identical. In four splicing mutants we tested (*ts14, ts21, ts56*, and *ts88*), the portions of the ts^+ progeny ranged from 39% to 72%, suggesting that the mutations of all four tRNA^{Val} splicing mutants reside in different genes.

We did not determine whether the mutations of our tRNA splicing mutants are recessive or dominant. These mutations are probably recessive because most ts^- mutations generated by chemical mutagenesis are recessive (David Frendewey: Personal Communication). Furthermore, the wild type gene corresponding to one of our tRNA splicing mutants (*ts88*) was cloned by complementation and the plasmid harboring the 7.2 kb insert was recovered (data not shown), implying that the mutation of *ts88* is recessive and resides within a single gene.

It is our hope that our mutants *ts14, ts21, ts56*, and *ts88* can be used for further studies to elucidate the mechanism of pre-tRNA splicing. The cloning and sequencing of the wild type genes that are defective in these strains may shed light in understanding the nature of a factor required for pre-tRNA splicing in *S. pombe*.

Table 3. Genetic analysis in some tRNA splicing mutants

Yeast strains	Description	# of total colony	# of ts^+ colony	% of ts^+ colony
SKC144 X SKC148	*ts14* self	350	0	0%
SKC144 X SKC216	*ts14* X *ts21*	362	222	61%
SKC144 X SKC562	*ts14* X *ts56*	259	186	72%
SKC144 X SKC886	*ts14* X *ts88*	288	154	53%
SKC213 X SKC216	*ts21* self	238	0	0%
SKC213 X SKC562	*ts21* X *ts56*	237	109	46%
SKC213 X SKC886	*ts21* X *ts88*	300	118	39%
SKC563 X SKC562	*ts56* self	447	0	0%
SKC563 X SKC886	*ts56* X *ts88*	258	104	40%
SKC884 X SKC886	*ts88* self	281	0	0%

Acknowledgments

We thank Dr. Hyong Bai Kim for his kind donation of *S. pombe* strains. We are very grateful to Dr. Kwan Hee You for offering space and facilities for experiments dealing with radioactive material. This work was supported by Non Directed Research Fund, Korea Research Foundation, 1992.

References

1. **Alfa, C., P. Fantes, J. Hyams, M. McLeod, and E. Warbrick.** 1993. Experiments with fission yeast: A laboratory course manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, New York.
2. **Atkinson, N.S., R.W. Dunst, and A.K. Hopper.** 1985. Characterization of an essential *S. cerevisiae* gene related to RNA processing: Cloning of *RNA1* and generation of a new allele with a novel phenotype. *Mol. Cell. Biol.* **5**, 907-915.
3. **Bonatti, S., M. Simili, and A. Abbondandolo.** 1972. Isolation of temperature sensitive mutants of *Schizosaccharomyces pombe*. *J. Bacteriol.* **109**, 481-491.
4. **Gandini-Attardi, D., I. Margarit, and G.P. Tocchini-Valentini.** 1985. Structural alterations in mutant precursors of the yeast leucyl tRNA gene which behave as defective substrate for a highly purified endoribonuclease. *EMBO J.* **4**, 3289-3297.
5. **Greer, C.L., C.L. Peebles, P. Gegenheimer, and J. Abelson.** 1983. Mechanism of action of a yeast RNA ligase in tRNA splicing. *Cell* **32**, 537-546.
6. **Ho, C.K., R. Rauhut, U. Vijayraghavan, and J. Abelson.** 1990. Accumulation of pre-transfer RNA splicing 2/3 intermediates in a *S. cerevisiae* mutant. *EMBO J.* **9**, 1245-1252.
7. **Hopper, A.K. and N.C. Martin.** 1992. Processing of yeast cytoplasmic and mitochondrial precursor tRNAs, p. 99-141. *In* E.W. Jones, J.R. Pringle, and J.R. Broach (eds.), The molecular biology of the yeast *Saccharomyces*: Gene expression. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, New York.
8. **Hopper, A.K., F. Banks, and V. Evangelidis.** 1978.

Yeast mutant which accumulates precursor tRNAs. *Cell* **14**, 211-219.
9. **Knapp, G., J.J. Beckmann, P.F. Johnson, S.A. Furhman, and J. Abelson.** 1978. Transcription and processing of intervening sequences in yeast tRNA gene. *Cell* **14**, 221-136.
10. **Knapp, G., R.C. Ogden, C.L. Peebles, and J. Abelson. 1979.** Splicing of yeast tRNA precursors: Structure of the reaction intermediates. *Cell* **18**, 37-45.
11. **Kolman, C.J.** 1990. Isolation and characterization of a yeast mutant involved in transfer RNA splicing. Ph.D. thesis. Yale University, New Haven, CT.
12. **Kuhn, R.M., L. Clarke, and J. Carbon.** 1991. Clustered tRNA genes in *Schizosaccharomyces pombe* centromeric DNA sequence repeats. *Proc. Natl. Acad. Sci. USA* **88**, 1306-1310.
13. **Melton, D.A., E.M. DeRobertis, and R. Cortese.** 1980. Order and intracellular location of the events involved in maturation of a spliced tRNA. *Nature* **284**, 143-148.
14. **Mitchison, J.M. and J. Creanor.** 1971. Further measurements of DNA synthesis and enzyme potential during cell cycle of fission yeast *Schizosaccharomyces pombe*. *Exp. Cell. Res.* **69**, 244-247.
15. **Nischt, R., E. Thuroff, and N.F. Kaufer.** 1986. Molecular cloning of a ribosomal protein gene from the fission yeast *Schizosaccharomyces pombe*. *Curr. Genet.* **10**, 365-370.
16. **Nishikura, K., J. Kurjan, B.D. Hall, and E.M. DeRobertis.** 1983. Genetic analysis of the processing of a spliced tRNA. *EMBO J.* **1**, 263-268.
17. **O'Connor, J.P. and C.L. Peebles.** 1992. PAT1, an essential gene of *Saccharomyces cerevisiae* affecting pre-tRNA processing. *Mol. Cell. Biol.* **12**, 3843-3856.
18. **Ogden, R., M.-C. Lee, and G. Knapp.** 1984. Transfer RNA splicing in *Saccharomyces cerevisiae*: Defining the substrates. *Nucl. Acids Res.* **12**, 9367-9382.
19. **Peebles, C.L., R.C. Ogden, G. Knapp, and J. Abelson. 1979.** Splicing of yeast tRNA precursors: A two-stage reaction. *Cell* **18**, 27-35.
20. **Peebles, C.L., P. Gegenheimer, and J. Abelson.** 1983. Precise excision of intervening sequences from precursor tRNA by a membrane-associated yeast endonuclease. *Cell* **32**, 525-536.
21. **Potashkin, J., R. Li, and D. Frendewey.** 1988. Pre-mRNA splicing mutants of *Schizosaccharomyces pombe*. *EMBO J.* **8**, 551-559.
22. **Rauhut, R., P.R. Green, and J. Abelson.** 1990. Yeast transfer RNA-splicing endonuclease is a heterotrimeric enzyme. *J. Biol. Chem.* **265**, 18180-18184.
23. **Sambrook, J., E.F. Fritsh, and T. Maniatis.** 1989. Molecular cloning: A laboratory manual (2nd. ed.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, New York.
24. **Sherman, F., G.R. Fink, and J.B. Hicks.** 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, New York.
25. **van Zyl, W.H., N. Wills, and J.R. Broach.** 1989. A general screen for mutants of *Saccharomyces cerevisiae* deficient in tRNA biosynthesis. *Genetics* **123**, 55-68.
26. **Vijayraghavan, U., M. Company, and J. Abelson.** 1989. Isolation and characterization of pre-mRNA splicing mutants of *Saccharomyces cerevisiae*. *Genes Dev.* **3**, 1206-1216.
27. **Wang, S.S. and A.K. Hopper.** 1988. Isolation of a yeast gene involved in species-specific pre-tRNA processing. *Mol. Cell. Biol.* **8**, 5140-5149.
28. **Winey, M. and M.R. Culbertson.** 1988. Mutations affecting tRNA splicing endonuclease activity of *Saccharomyces cerevisiae*. *Genetics* 118, 609-617.