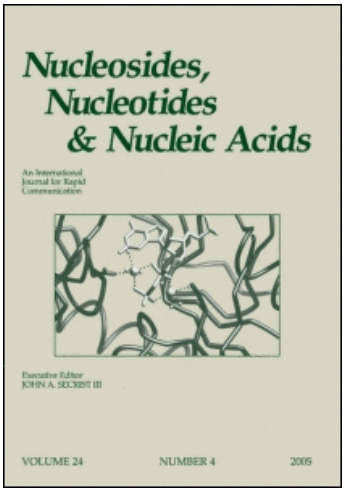

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A Novel Approach for Isolation and Mapping of Second-Site Revertants of Intron Mutations in a Ribontjcleotide Reductase Encoding Gene (*nrd*B) of Bacteriophage T4 Using the White Halo Plaque Phenotype

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A NOVEL APPROACH FOR ISOLATION AND MAPPING OF SECOND-SITE REVERTANTS OF INTRON MUTATIONS IN A RIBONUCLEOTIDE REDUCTASE ENCODING GENE (*nrd*B) OF BACTERIOPHAGE T4 USING THE WHITE HALO PLAQUE PHENOTYPE.

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ABSTRACT: The *nrd*B gene of bacteriophage T4 codes for the small subunit of ribonucleotide reductase and contains a 598 base pair self splicing intron which is closely related to other group I introns of T4 and eukaryotes. Previously, the *nrd*B intron mutations, presumably causing splicing defects of the *nrd*B transcript, were isolated and mapped in or near the *nrd*B intron. In this communication, we have isolated 181 hydroxylamine-induced revertants for the above primary *nrd*B intron mutations by strategic usage of the white halo phenotype. Also, we mapped these revertant mutations by marker rescue with subclones of the *nrd*B gene. Some of the second site mutations were mapped to regions predicted by the secondary structure model of the *nrd*B intron. To investigate the involvement of protein factors facilitating splicing of the *nrd*B transcript, we attempted to isolate extragenic revertants of *td-nrd*B double mutants by utilizing hydroxylamine mutagenesis. Mapping and sequencing of the suppressor mutations in the extragenic revertants revealed that the second-site mutations are in the *frd* gene, coding for dihydrofolate reductase. Splicing assays showed that these suppressor mutations do not reverse the splicing defects of *td-nrd*B mutants, but only affect the halo phenotype, most likely by altering the pyrimidine nucleotide metabolism.

INTRODUCTION

Group I introns are found in genes of eukaryotic organelles; nuclear, mitochondria, and chloroplast, as well as prokaryotes including bacteriophage T4 (1, 2). Group I introns, typified by the Tetrahymena thermophila nuclear 26S r-RNA gene, are removed by a series of autocatalytic processes. The splicing reaction of group I introns *in vitro* does not require the involvement of protein cofactors, thereby showing that the reactivity is intrinsic to the RNA (3, 4).

It is known that the folded structure of a group I intron is essential to its catalytic activity, as the three dimensional structure of a protein enzyme is important to its activity (5). To reveal the important structural features for catalytic activity in group I introns, several approaches were undertaken. Comparative sequence analysis among group I introns of diverse organisms revealed highly conserved primary sequence elements, conserved secondary structure, and a predicted three-dimensional structure (6, 7, 8). Site-directed mutagenesis of the Tetrahymena rRNA gene and *in vitro* splicing reactions utilizing artificial substrates revealed the splicing mechanism and specific structural elements important for self-splicing of the ribozyme of Tetrahymena (9, 10). Basic features of the predicted three dimensional structure have been proved by mutant analysis *in vitro* and by the use of specifically positioned photochemical cross linking and affinity cleavage reagents (11, 12, 13).

All the T4 introns (*td*, *nrd*B, *nrd*D) are capable of self-splicing, and contain the conserved structural features folded into a common secondary structure of group I introns (5, 14, 15). The *td* intron is the best characterized and has contributed a great deal of information to the understanding of important structural features of the group I introns (16, 17). To examine the predicted secondary and tertiary structures of group I introns, random mutagenesis utilizing novel techniques with T4 is very useful. The advantage of the approach with T4 is based on a phenotypic screening methods (18), and easy mapping of mutations due to the promiscuous recombination system of T4 (19).

The *nrd*B gene codes for the small subunit of ribonucleotide reductase and contains a 598 base pair (bp) intron. Recently, 159 hydroxylamine induced *nrd*B mutants were isolated (20). Thirty of them contain mutations which map in or near the intron. Most probably, those thirty mutants are *nrd*B$^-$ due to splicing defects caused by mutations within the intron. The isolation of second-site revertants, which restore splicing activity of the *nrd*B intron mutants by introducing complementary mutations at second-sites, will lead to the understanding of secondary and tertiary interactions in the splicing process *in vivo*. We report the isolation and mapping of 181 second-site revertants of *nrd*B intron mutants. The screening, isolation, and mapping of these second site revertants has been conducted by the strategic usage of the white halo phenotype.

Although most pre-mRNAs containing group I introns are self-splicing *in vitro*, there is compelling evidence that splicing *in vivo* is promoted by protein factors (21, 22, 23). Therefore, we sought extragenic revertants which reverse splicing defects of both *td* and *nrd*B transcripts in a *td-nrd*B double mutant. Such revertants might affect a protein factor promoting splicing of both *td* and *nrd*B pre-mRNA.

MATERIALS AND METHODS

Strains and plasmids: T4D$_Q$, an osmotic shock resistant mutant of T4D was used as the standard (wild type) T4 strain. The *td*8S series of T4 mutants were from previous collection (D. Hall). The *frd*1SL series of mutants were from Sunil Lal's collection which was isolated after hydroxylamine mutagenesis of *frd*1 mutants (20). The *nrd*B series of plasmid subclones were from Dr. David Shub (SUNY Albany) (Fig. 1).

Hydroxylamine mutagenesis of *frd*$^-$*nrd*B$^-$double mutants and *td*$^-$*nrd*B$^-$ double mutants: Hydroxylamine mutagenesis of extracellular phage particles was performed as previously described (24).

Screening of revertants utilizing the white halo plaque phenotype: After the hydroxylamine mutagenesis of *frd*$^-$*nrd*$^-$double mutants and *td*$^-$*nrd*$^-$ double mutants, false revertants phenotypically normal for ribonucleotide reductase production (*nrd*$^+$) and defective in thymidylate synthase production (*td*$^-$) or dihydrofolate reductase production (*frd*$^-$), were screened by testing phage for reappearance of the white halo plaque phenotype (Fig. 2) (18). The white halo phenotype was obtained by plating *E. coli*. OK305 cells and mutant phage on GPTG plates as described previously (18, 20, 25). The *E. coli* OK305 strain requires uracil , and has a defect in cytidine deaminase. When a lawn of OK305 is grown on Petri dishes with synthetic medium (GPTG) containing cytidine as the sole pyrimidine source, there is a uracil deficiency which can be remedied, if some of the cells are infected with a T4 mutant defective either in dTMP synthase (*td*) or in dihydrofolate reductase (*frd*). Either one of these mutations in an infecting phage causes dUMP accumulation which can lead to the release of uracil from infected cells for utilization by neighboring uninfected cells. This causes mutant plaques to be surrounded by a white halo due to increased bacterial growth around the lysed cells. This halo phenotype is prevented by *nrd*B mutations in *frd*$^-$*nrd*B$^-$ and *td*$^-$*nrd*B$^-$ double mutants, which block dUMP synthesis. After hydroxylamine mutagenesis of those double mutants, the halo phenotype can only reappear by introduction of new mutations that cause an *nrd*B$^+$ phenotype (Fig. 2). Revertants exhibiting the halo phenotype were isolated and mapped.

Marker rescue mapping: To map secondary mutations, cells containing a cloned *nrd*B subfragment were infected with phage revertants and it was determined whether recombinant *nrd*$^-$ progeny (non-halo) were present in the marker rescue lysate (19). A schematic diagram of the *nrd*B subclones is shown (Fig. 1).

Dot-blot splicing assays: For RNA preparation, conditions for cell growth, T4 infection, RNA extraction, and hybridization were done as previously described (26). The oligonucleotide probes were designed in such a way that their binding to the specific

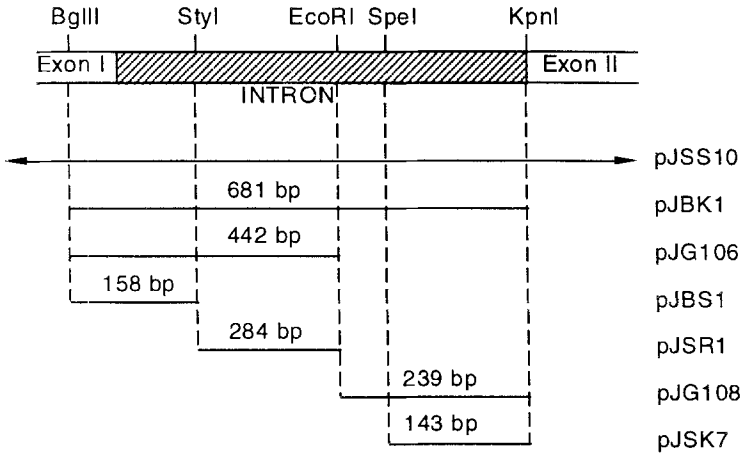

FIGURE 1. A Schematic Diagram of the *nrd*B clones
pJSS10, whole *nrd*B gene clone; pJBK1, *nrd*B intron subclone; pJG108, pJBS1, pJSR1, and pJSK7 devide the sequence covered by pJBK1 into finer regions. The mutants which mapped to pJG108 region but show negative marker rescue with pJSK7 were recorded as -JSK7. *Bgl*II, *Sty*I, *Eco*RI, *Spe*I and *Kpn*I are restriction sites used to generate the subclone fragments

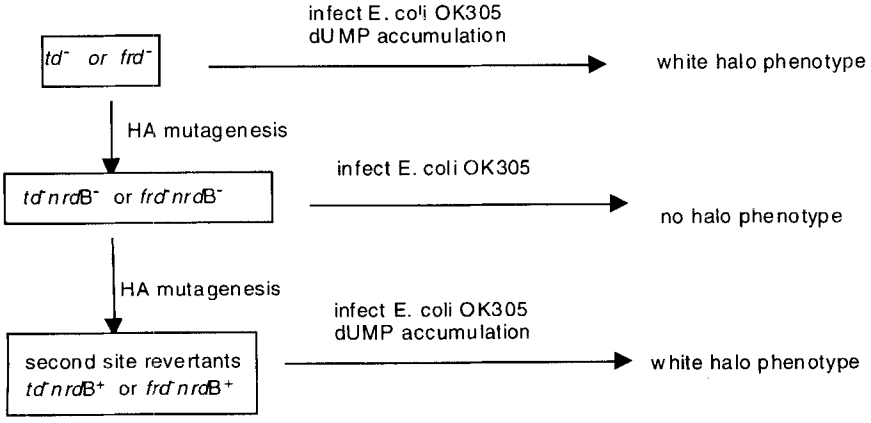

FIGURE 2. The white halo plaque phenotype of *nrd*B mutants. The halo is due to more cell growth around the plaques.

regions on the RNA was dependent on the stages of spliced or unspliced *nrd*B RNA as illustrated (Table 1).

Primer Extension Analysis of Transcripts with dGTP or dTTP omission: For 5' splicing assay, end-labeled oligonucleotide 5 (Table 1) that hybridizes to the 5' end of the intron was used to prime cDNA synthesis from RNA (30*ug*/reaction) extracted from cells infected at 30°C with wild-type T4 or with T4 *nrd*B mutants (27). To stop extension at the first G, dGTP was omitted and a high concentration of ddGTP (ddGTP:dNTP=3.3:1) was added to the reaction tube. The ddGTP termination of primer extension from primer 5 resulted in a 35nt fragment, B (26nt primer + 9nt extension) from unspliced message and a 33nt fragment, A (26nt primer + 7nt extension) from spliced message. The reactions were run on 5% polyacrylamide gel for good resolution of short oligonucleotides.

RESULTS

a) Mutant isolation and mapping: Thirty double mutants containing *nrd*B$^-$ and *frd*$^-$ or *td*$^-$ mutations were previously isolated by Lal and Hall (20). These *nrd*B$^-$ mutations were mapped within or near the intron (Table 2). The thirty hydroxylamine induced *nrd*B$^-$ mutants were treated again with hydroxylamine under conditions which induce only unidirectional transition mutations (GC to AT). This second treatment should produce only false revertants. Phage pseudorevertants (*nrd*$^+$) were isolated by this halo phenotype (Table 2). All hydroxylamine induced revertants were independent, because the phage did not replicate during mutagenesis. In the case of six mutants (for example, *td*8S22 and *frd*1SL335), no revertants were found even with several mutagenesis and screening attempts.

The second site mutation in selected pseudorevertant phage were mapped by marker rescue in subclones containing defined portions of the *nrd*B gene (Fig. 1). JG106 and JG108 divide the JBK1 region into two. JG106 is divided into JBS1 and JSR1. JG108 has a region which is covered by JSK7. This mapping was based on the fact that the plasmid subclones that contain the wildtype DNA sequence corresponding to the one altered in the phage mutant will recombine to give a wildtype gene in the phage, which is called positive marker rescue (29). For some pseudorevertants, such as frd1-SL2-SH2-2, the suppressor and the original mutation mapped into the same subclone locus and gave very weak positive marker rescue, indicating the secondary mutation is very close to the primary mutation. Interestingly, revertants of *frd*1-SL1 and *frd*1-SL183 did not show positive marker rescue with JBK1, JG108, and JG106, covering the 3' end of exon I and the whole intron, but did show rescue only with the whole *nrd*B clone, JSS10. Therefore,

TABLE 1. Description of Oligonucleotides

Probe	nt[a]	specificity	Sequences(5'-3')[b]	Complement of[b,c]
1	22	Exon II	CACGTGATGAACAGCTTCACCT	1865-1886
2	25	Splice Junction	TATCTTTTGCGT.GTACCTTTAACTT	1189-1200.1799-1811
3	23	Exon I-Intron	TATCTTTTGCGT.AAAATGCGCCT	1189-1211
4	23	Intron-Exon II	TCGAACATACG.GTACCTTTAACT	1788-1810
5	26	Intron (5' end)	GCCTTTAAACGGTAACGTTTATCGAA	1208-1233

[a]nt = number of nucleotides in oligomer.

[b]Dots represent sequence junctions.

[c]The numbering of the nucleotide complementary to the oligomer is given (numbering as Sjöberg B. -M. *et al.* 1986 (28))

TABLE 2. Isolation of revertants of *nrd*B intron mutants and marker rescue mapping

primary mutant	mapping position	frequency of revertants	revertant	mapping of revertant
frd1SL1	JSK7	1/20,000	SH1-1	JSS10
frd1SL2	JSK7	11/5,000	SH2-2,4,6	JSK7
frd1SL183	JSK7	9/700	SH183-1,2,3	JSS10
frd1SL293	JSK7	2/9,000	SH293-1,2	JG106
frd1SL335	JSK7	0/5,000	none	--
td8S22	-JSK7[a]	0/10,000	none	--
td8S57	-JSK7[a]	10/8,000	SH57-1,2	frd

[a]-JSK7=JG108 except JSK7

the second site mutations, likely located within the intron, are probably very near to the 3' end of the intron, and could not be rescued by intron subclones. The revertants of *frd*1-SL293 were mapped to the JG106 region, whereas the primary mutation was located in the JG108 region. The frequency of second site revertants and their mapping is summarized in Table2.

b) Attempts to find extragenic suppressor mutations: In order to look for other genes promoting splicing of T4 introns, *td*$^-$*nrd*B$^-$ double mutants which have mutations in both introns were subjected to hydroxylamine mutagenesis. False revertants, which suppress presumed splicing defects in both introns by assisting folding of mutant RNA, could be recognized as *td*$^+$*nrd*B$^+$ phenotypes. Such revertants would most likely be extragenic suppressors. After hydroxylamine mutagenesis of *td*$^-$*nrd*B$^-$ mutants (*td*8S22, *td*8S57), revertants were screened for halos in the presence of daraprim, which inhibits the function of dihydrofolate reductase and made *td*$^+$*nrd*B$^+$ have a halo plaque phenotype. Several attempts of hydroxylamine mutagenesis of *td*8S22 involving scanning of 10,000

plaques did not yield any revertants with the halos. Five revertants of *td*8S57 were isolated and plated for halos without daraprim to distinguish $td^{+}nrdB^{+}$ and $td^{-}nrdB^{+}$. All the revertants showed halos in the presence or absence of daraprim, which indicated that all of them are $td^{-}nrdB^{+}$. To locate the secondary mutations, marker rescue mapping utilizing *nrd*B subclones was done for each revertant of *td*8S57. The secondary mutations were not rescued by *nrd*B subclones, while the primary mutations were mapped to the subregion of JG108 as expected. The possibility that the secondary mutations are in the *frd* gene was examined by marker rescue with *frd* clone pGS38. All *td*8S57 revertants were rescued by the *frd* subclone, indicating that the secondary mutations are in the *frd* gene. The dideoxy sequencing of these revertants reveals the presence of the primary mutation, but not the secondary mutations in the *nrd*B intron. These revertants were subjected to splicing assays involving dot-blot hybridization, primer extension, and primer extension with dNTP omission. In the dot-blot assay, synthetic oligonucleotide probes, designed in such a way that their binding to the complementary regions on the RNA is dependent on the presence of spliced or unspliced *nrd*B RNA, were used as probes (Table 1). The control probe 1, binding to both premessage and ligated message, gave a signal of the same intensity with wildtype, *td*8S57, and *td*8S57-SH57-1 RNAs, which indicated the same amount of RNA. The probe 3, specific to the 5' pre-mRNA splice sites gave more intense signals with both *td*8S57 and *td*8S57-SH57-1 than wildtype. The probe 2, directed against the ligated splice junction, gave less intense signals with *td*8S57 and *td*8S57-SH57-1 than wildtype RNAs. These dot blot splicing assays show that suppressor mutations do not alter the splicing defects of the *td*8S57 primary mutation (Fig. 3).

Also, the primer extension of *td*8S57-SH57-1, using the primer close to the 5' end of the intron also shows defects in 5' cleavage similar to *td*8S57 (data not shown). The dNTP omission splicing assay, which produced the characteristic band indicating the splicing status of the transcript, was done to compare splicing activity of *td*8S57 and *td*8S57-SH57-1. For 5' cutting, ddGTP termination of extension with primer N121 specific to the 5' end of intron is expected to produce 35 nt and 33 nt bands from unspliced and spliced messages, respectively. The primer extension of *td*8S57-SH57-1 RNA resulted in similar intensities of 35 nt and 33 nt bands just as *td*8S57 RNA, indicating that this revertant does not alter the splicing defects of *td*8S57 (Fig. 4).

DISCUSSION

The ability of group I introns to catalyze their own splicing is mainly due to the precise folded structure. The folding of the intron RNA results in placing substrate sites and catalytic sites which are widely separated in the primary sequence, in close proximity, and

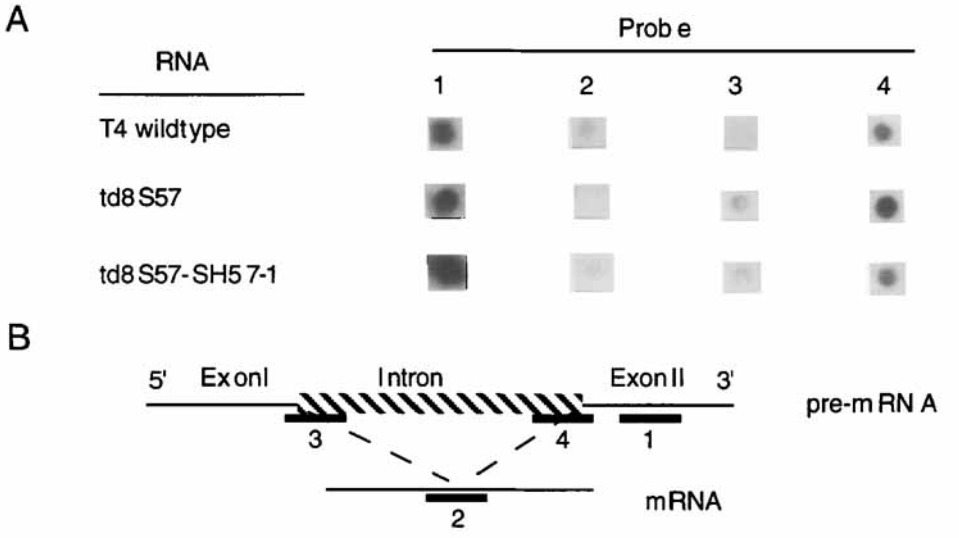

FIGURE 3. A. dot blot hybridization splicing assay. The numbering in (A) corresponds to the hybridization probe used (B and Table 1). B. Schematic description of oligonucleotides used in dot blot assay.

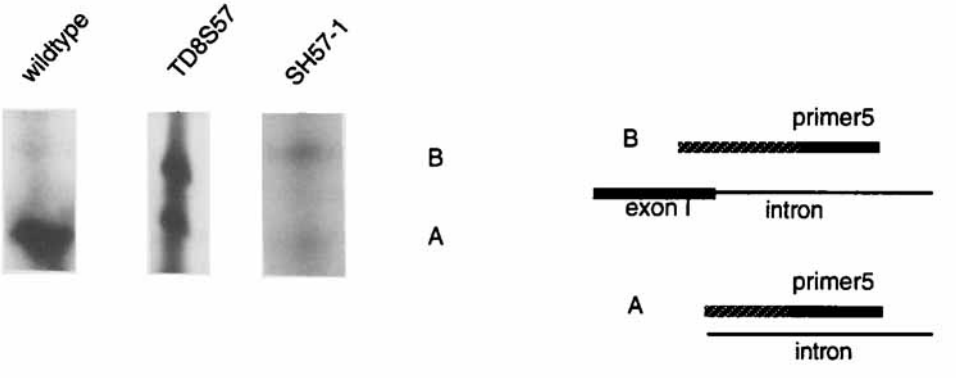

FIGURE 4. Primer Extension Analysis of Transcripts with dGTP Omission
The ddGTP termination of primer extension from primer 5 resulted in a 35nt fragment, B,(26nt primer (solid box)+9nt extension(dashed box)) from unspliced message and a 33nt fragment, A, (26nt primer(solid box) +7nt extension (dashed box)) from message cleaved at the 5' splice site.

activating phosphodiester bonds at splice sites (5). The catalytically important secondary and tertiary structures of group I introns have been studied with several approaches as described in the Introduction (6, 7, 8). All group I introns contain several conserved sequence elements, P, Q, R, and S, which exhibit pairwise complementarity. This sequence complementarity causes all group I introns to fold into strikingly similar structures involving specific pairing between the conserved sequences; P4, P6, and P7. This central core was proposed to bring the ends of the intron into close proximity, but deletion studies showed that some conserved paired regions can be deleted without affecting catalytic activity (29). Also, some unpaired sequences between paired regions were more highly conserved in primary sequences. It was thought that the paired regions do have a structural role to form secondary structures and the junction sequences do have a functional role to catalyze self-splicing reactions. One way to understand the functional relevance of each sequence in paired and unpaired regions is to isolate splicing defective *nrd*B intron mutants and false revertants of them, which have a compensatory mutation and restore the splicing activity. Characterization of compensatory mutations involving sequencing and splicing assays will lead to more comprehensive understanding of secondary and tertiary interactions for *nrd*B intron catalytic activity. Even though most primary mutants gave false revertants after hydroxylamine mutagenesis, some mutants (for example, *td*8S22 and *frd*1SL335) did not give revertants. This could be because some bases are involved in interactions other than regular Watson-Crick base pairs to form catalytically active structure; and therefore a primary change can not be compensated by a second mutation in one of interacting bases.

Although several pre-mRNAs containing group I introns are self-splicing *in vitro* , there is compelling evidence that splicing *in vivo* requires proteins. In the case of yeast mitochondrial RNA splicing, these proteins have been shown to include maturases encoded by ORF of its own group I introns (31). Several trans-acting mutations have been identified, which block splicing of group I introns. Even though T4 introns are self-splicing *in vitro*, the rate of splicing is significantly lower than that *in vivo*. In the case of *td* RNA, a nonphysiologically high temperature (60°C) is required to drive the self-splicing reaction efficiently *in vitro*, compared to *in vitro* splicing at 37°C or in an *E. coli* protein-synthesizing S-30 fraction, which suggests the participation of host or phage proteins (32). Also, the Neurospora CYT-18 protein, a tyrosyl-tRNA synthetase, which functions in splicing group I introns in mitochondria, was reported to promote *in vitro* splicing of mutants of the distantly related bacteriophage T4 *td* intron (33). The model to explain the enhancement of group I intron splicing by various protein factors is that the protein binds to the intron and helps the RNA to achieve its self-splicing conformation. Therefore, one of the goals of this study was to isolate extragenic revertants, which promote splicing of

splicing defective *nrd*B mutants by extragenic mutation. The mapping and splicing assays of *td*8S57 revertants showed that the secondary mutation is in the *frd* gene, but does not restore splicing of *td*8S57. The explanation for the characteristics of *td*8S57 revertants would be that a second mutation in the *frd* gene, causes accumulation of dUMP, producing weak halos by compensating for the effects of leaky *nrd*B mutations. Thus the revertants of *td*8S57 would be extragenic revertants, which do not affect splicing of *nrd*B RNA, but affect the halo phenotype of *td*8S57. The absence of *td*8S22 revertants showed that this td8S22 mutation is nonleaky, which can not be compensated with *frd* mutation. Overall, this result suggests that protein factors involved in *nrd*B RNA splicing, may not be encoded by the T4 genome, but possibly by the host chromosome.

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REFERENCE

1. Cech, T. R. (1988) Gene 73, 259-271.
2. Saldanha, R., Mohr, G., Belfort, M., and Lambowitz, A. M. (1993) FASEB J. 7, 15-24.
3. Cech T. R., and Bass, B. L. (1986) Annu. Rev. Biochem. 55, 599-629.
4. Kruger, K., Grabowski, P. J., Zaug, A. J. Sands, J., Gottschling, D. E., and Cech, T. R. (1982) Cell 31, 147-157.
5. Waring, R. B., and Davies, R. W. (1984) Gene 28, 277-291.
6. Davies, R. W., Waring, R. D., Ray, J. A., Brown, T. A., and Scazzacchio, C. (1982) Nature 300, 719-724.
7. Burke, J. M. (1988) Gene 73, 273-294.
8. Michel, F., and Westhof, E. (1990) J. Mol. Biol. 216, 585-610.
9. Cech, T. R. (1990) Annu. Rev. Biochem. 59, 543-568
10 Pyle, A. M., Murphy, F. L., and Cech T. R. (1992) Nature 358, 123-131.
11. Burke, J. M., Esherick, J. S., Burfeind, W. R., and King J. L. (1990) Nature 344, 80-82.
12. Wang, J. -F., and Cech, T. R. (1992) Science 256, 526-529
13. Wang, J. -F., Downs, W. D., and Cech, T. R. (1993) Science 260, 504-508
14. Belfort, M. (1990) Annu. Rev. Biochem. 24, 363-385.
15. Shub, D. A., Gott, J. M., Xu, M. Q., Lang, B. F., Michel, F., Tomaschewski, J., Pedersen-Lane, J., and Belfort, M. (1988) Proc. Natl. Acad. Sci. USA 85, 1151-1155
16. Hall, D. H., Povinelli, C. M., Ehrenman, K., Pedersen-Lane, J., Chu, F., and Belfort, M. (1987) Cell 48, 63-71.
17. Heuer, T. S. Chandry, P. S., Belfort, M., Celander, D. W., and Cech T. R. (1991) Proc. Natl. Acad. Sci. USA 88, 1105-1109.
18. Hall, D. H., Tessman, I., and Karlstrom, O. (1967) Virology 31, 442-448.
19. Broker, T. R., and Doermann, A. H. (1975) Ann. Rev. Genet. 9, 213-244.
20. Lal., S. K., and Hall, D. H. (1993) Biochem. Biophys. Res. Commun. 196, 943-949

21. Lazowska, J., Claisse, M., Gargouri, A., Kotylak, A., Spyridakas, A., and Slonimski, P. P., (1989) J. Mol. Biol. 205, 275-290.
22. Mohr, G., Zhang, A. X., Gianelos, J. A., Belfort, M., and Lambowitz, A. M. (1992) Cell 69, 483-494.
23. Coetzee, T., Herschlag, D., and Belfort, M. (1994) Genes & Dev. 8, 1575-1588.
24. Hall, D. H., and Tessman, I. (1966) Virology 29, 339-345.
25. Karlstrom. O. (1968) J. Bacteriology 95, 1069-1077.
26. Belfort, M., Pedersen-Lane, J., West, D., Ehrenman, K., Maley, G., Chu, F., and Maley, F. (1985) Cell 41, 375-382.
27. Wallace, R. B., Johnson, M. J., Hirose, T., Miyake, T., Kawashima, E. H., and Itakura, K. (1981) Nucl. Acids Res. 9, 879-894.
28. Sjöberg, B. -M. , Hahne, S., Mathews, C. Z., Mathews, C., Rand, K. N., and Gait, M. J. (1986) EMBO J 5, 2031-2036.
29. Mattson, T., Van Houwe, G., Bolle, A., Selzer, G., and Epstein, R. (1977) Mol. Gen. Genet. 154, 319-326.
30. Barfod, E. T., and Cech, T. R. (1988) Genes & Dev. 2, 652-663.
31. Garriga, G., and Lambowitz, A. M. (1986) Cell 46, 669-680.
32 Belfort, M., Pedersen-Lane, J., Ehrenman, K., Chu, F. K., Maley, G. F., Maley, F., McPheeters, D. S., and Gold, L. (1986) Gene 41, 93-102.
33. Mohr, G., Zhang, A., Gianelos, J. A., Belfort, M., and Lambowitz, A. M. (1992) Cell 69, 483-494.

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