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Identifying functional regions of rRNA by insertion mutagenesis and complete gene replacement in *Tetrahymena thermophila*

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The free, linear macronuclear ribosomal RNA genes (rDNA) of *Tetrahymena* are derived from a unique copy of micronuclear rDNA during development. We have injected cloned copies of the micronuclear rDNA that have been altered *in vitro* into developing macronuclei and obtained transformants that express the paromomycin-resistant phenotype specified by the injected rDNA. In most cases, these transformants contain almost exclusively the injected rDNA which has been accurately processed into macronuclear rDNA. Mutants with a 119 bp insertion at three points in the transcribed spacers and at two points in the 26S rRNA coding region were tested. Cells containing these spacer mutant rDNAs are viable, although one of them grows slowly. This slow-growing line contains the insertion between the 5.8S and 26S rRNA coding regions and accumulates more rRNA processing intermediates than control lines. One of the 26S rRNA mutants failed to generate transformants, but the other did. These transformants grew normally, and produced 26S rRNA containing the inserted sequence. A longer insertion (2.3 kb) at the same four points either abolished transformation or generated transformants that retained at least some wild-type rDNA. This study reveals that some rRNA sequences can be altered without significantly affecting cell growth.

Key words: gene replacement/insertion mutagenesis/rDNA/rRNA/*Tetrahymena thermophila*

Introduction

Though ribosomal RNAs (rRNAs) have been studied extensively in a variety of organisms and much progress has been made in defining their structural properties, only limited progress has been made in defining the functional domains of the rRNAs. Genetic studies have been hampered in most organisms by the existence of multiple copies of the genes coding for the rRNAs. This problem has been partially circumvented in *Escherichia coli* (Hui and de Boer, 1987; Jacob *et al.*, 1987), but it is still not possible to observe the *in vivo* phenotype of a population of rRNAs resulting from the transcription of a homogeneous population of ribosomal DNAs (rDNAs) that have been specifically altered *in vitro*.

Recent findings in *Tetrahymena thermophila* have made it a uniquely suitable system for functional analysis of rRNAs. *T.thermophila* has two nuclei, a diploid micronucleus (or germline nucleus) and a polyploid macronucleus (or somatic nucleus) in which the micronuclear genome has been extensively reorganized and wherein most transcription occurs during vegetative growth (reviewed by Karrer, 1986). In each diploid micronuclear genome there are two allelic copies (one on each copy of chromosome II) of the rRNA cistron encoding the 17S, 5.8S and 26S rRNAs. These copies are processed through a series of precisely regulated events during macronuclear development, producing ~9000 palindromic molecules 21 kb in size in each mature macronucleus. On each of these rDNA molecules are two copies of the rRNA cistron facing in opposite directions (reviewed by Yao, 1986).

Taking advantage of these unusual properties, methods have been devised for replacing macronuclear rDNA in *Tetrahymena*. A transformation system in which isolated macronuclear rDNA is introduced into the macronucleus by microinjection has been developed (Tondravi and Yao, 1986). The injected rDNA (derived from a C3 strain) differs from the host rDNA (derived from a B strain) in two ways: (i) the injected rDNA has a replication advantage over the host rDNA in the macronucleus, probably due to differences in their replication origins (Pan *et al.*, 1982; Larson *et al.*, 1986); and (ii) the injected rDNA confers paromomycin resistance (pm-R) as a result of a single base change near the 3′ end of the 17S rRNA coding region (Bruns *et al.*, 1985; Spangler and Blackburn, 1985). Among injected cells, up to 66% were transformed to pm-R, and nearly all of the macronuclear rDNA in the pm-R transformants was replaced by the injected type (Tondravi and Yao, 1986; Karrer and Yao, 1988). Recently pm-R transformants have also been obtained by injecting cloned, micronuclear form rDNA bearing the same genetic markers (pm-R, C3-type) into developing macronuclei. In these transformants, the injected micronuclear rDNA was correctly processed into macronuclear rDNA which completely relaced the host rDNA (Yao and Yao, 1988).

In the present study we have altered the cloned micronuclear rDNA of *T.thermophila* by inserting DNA segments into both coding and non-coding regions. This altered rDNA was then injected into developing macronuclei of mating cells, and, in many cases, pm-R transformants were recovered in which the injected rDNA had been processed into macronuclear rDNA and had completely replaced the host rDNA. This system allows us to observe the *in vivo* phenotype of cells with a homogeneous population of altered macronuclear rDNA and to state that some alterations clearly do not prevent rDNA function, whereas others probably do. The most striking finding is that a 26S rRNA bearing a linker insertion of 119 bases in an 'expansion' region (Gerbi, 1985; Lenaers *et al.*, 1988) near the 3′ end apparently produces functional ribosomes: cells bearing only rDNA with this altered 26S rRNA gene grow with a normal doubling time.

Results

Construction of insertion mutations

Two series of mutants were generated by inserting either a 2.3-kb *E.coli* plasmid or a 119 bp polylinker into seven

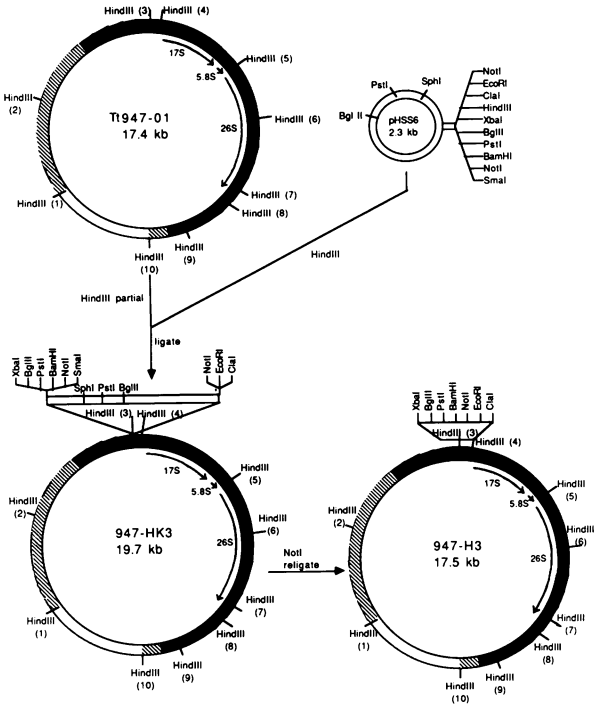

Fig. 1. Plasmid construction. Tt947-01, a plasmid consisting of pic19 plus *Tetrahymena* micronuclear form rDNA plus its flanking regions (Yao and Yao, 1988), was partially digested with *Hin*dIII (Parker *et al.*, 1977) such that most molecules were not cut or cut only once. The pHSS6 plasmid, which contains a kanamycin-resistance gene (Seifert *et al.*, 1986), was completely digested with *Hin*dIII and ligated with the partially digested Tt947-01. Transformants (selected on kanamycin and ampicillin) were obtained whose plasmid DNA had the entire pHSS6 plasmid (sometimes multiple copies; Table I) inserted at each of the 10 *Hin*dIII sites in Tt947-01 except site 9. In this figure, a plasmid with the insertion at site 3 is shown. Digestion of these plasmids with *Not*I and subsequent religation gave rise to transformants whose plasmid DNA had the 119-bp linker of pHSS6 inserted at each of the *Hin*dIII site. Black bar, *Tetrahymena* rDNA; shaded bar, *Tetrahymena* rDNA flanking regions; and open bar, bacterial plasmid sequences.

of the eight *Hin*dIII sites within *T.thermophila* rDNA. The plasmid pHSS6 (Seifert *et al.*, 1986) was linearized at its unique *Hin*dIII site and ligated with a partial *Hin*dIII digestion of the micronuclear rDNA clone Tt947-01 (Yao and Yao, 1988) in which most molecules were not cut or cut only once (Figure 1). Clones were obtained with pHSS6 (multiple copies in some cases (Table I) inserted at each of the 10 *Hin*dIII sites in Tt947-01 except site 9, and these are refered to as 947-HK1, 947-HK2, etc. (for insertion of pHSS6 at site 1, site 2, etc.). These clones were then digested with *Not*I and religated, thus removing all pHSS6 DNA except a 119-bp linker (Figure 1). These plasmids are refered to as 947-H1, 947-H2, etc. (for insertion of the linker at site 1, site 2, etc.).

Transformation of Tetrahymena with altered rDNA

Plasmid DNAs with insertions that fell within the transcribed region of the rDNA (*Hin*dIII sites 3–7) were injected into conjugating *Tetrahymena* cells as described (Yao and Yao, 1988). The results are summarized in Table I. Among the linker insertion mutants, all except one gave rise to pm-R transformants. This includes three insertions in the transcribed spacer regions (947-H3, 947-H4 and 947-H5)

Table I.

DNA[a]	No. pm-R/ no. injected[b]	Transformation frequency[c]
Tt947-01[d]	13/750	1.7%
947-H2	2/186	1.1%
947-H3	1/249	0.4%
947-HK3(1)	0/260	<0.4%
947-H4	1/236	0.4%
947-HK4(2)	0/196	<0.5%
947-H5	1/216	0.5%
947-HK5(2)	3/222	1.4%
947-H6	0/225	<0.4%
947-HK6(1)	0/259	<0.4%
947-H7	2/186	1.1%
947-HK7(3)	2/186	1.1%
947-H8	5/349	1.4%
947-HK8(1)	3/206	1.5%
Coinjection of negatives[e]	3/168	1.8%
Coinjection of positives[f]	8/172	4.7%

[a]These are the bacterial plasmid DNAs (described in Results) that were injected. The number in parenthesis following some of the plasmid names indicates the number of copies of pHSS6 that are inserted into the plasmid.
[b]This is the number of paromomycin resistant cells out of the total number of cells injected.
[c]This is calculated from no. pm-R/no. injected and is probably an underestimate of the true transformation frequency since not all cells injected are viable.
[d]This is an average of six experiments.
[e]This is an equimolar mixture of Tt947-01, 947-HK3, 947-HK6 and 947-H6.
[f]This is an equimolar mixture of Tt947-01, 947-H3, 947-H4 and 947-H8.

and, most significantly, one in the 26S rRNA coding region (947-H7). The one linker insertion mutant that failed to transform also contains an insertion in the 26S rRNA coding region (947-H6). These results suggest that cells containing rDNA with an insertion of 119 bp in three different locations within the transcribed spacers and one location in the coding region of the 26S rRNA are viable. This interpretation is supported by DNA studies described later. Some insertion mutants bearing the pHSS6 plasmid also generate transformants. This includes one mutant with the insertion in the transcribed spacer (947-HK5) and one with the insertion in the coding region (947-HK7). Superficially, these results suggest that cells with a large insertion at these two sites are also viable. However, the situation is more complicated as later DNA studies reveal.

Given that transformation frequencies ranged from 0.4 to 4.7% for plasmids that gave rise to pm-R transformants and that ~200 cells were injected in each of the experiments that failed to generate a transformant, negative results alone are inconclusive. However, coinjection experiments, which will be discussed later, strongly suggest that plasmids that failed to produce pm-R transformants are, in fact, incapable of transformation.

Macronuclear rDNA of the transformed cells

DNA from pm-R transformed lines grown in the presence of pm was prepared and subjected to Southern hybridization analysis to determine the structure of the macronuclear rDNA. *Bam*HI digestion of DNA of the transformants resulting from injection of 947-H3, 947-H4 or 947-H7

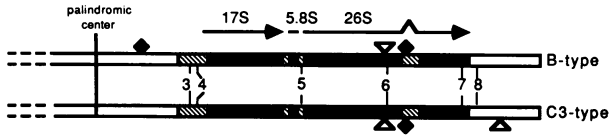

Fig. 2. Structure and restriction site polymorphisms of macronuclear rDNA. This figure shows one half of the 21 kb palindromic, macronuclear rDNA from both B type and C3 type *Tetrahymena* strains. Non-transcribed regions are shown as an open bar, transcribed spacer regions as shaded bar, and coding regions as a filled bar. The numbers (3−8) between the two rDNAs show the *Hin*dIII sites into which insertions have been made. Symbols denoting restriction sites are: filled diamond, *Sph*I and Δ or ∇, *Bam*HI.

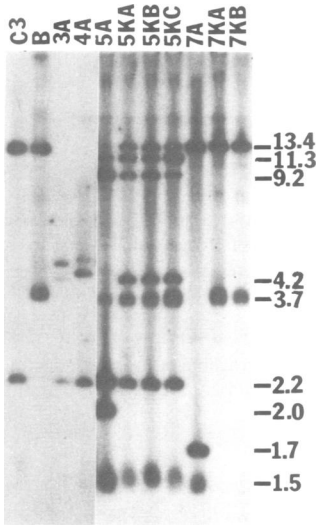

Fig. 3. *Bam*HI digestion of rDNA of pm-R transformants. *Tetrahymena* DNA was digested with *Bam*HI, electrophoresed on a 1% agarose gel, blotted and hybridized with nick-translated Tt947-01. **Lanes C3** and **B** are DNA from C3 and B strains respectively. The remaining lanes are DNA from pm-R transformants resulting from injection of: 947-H3, **lane 3A**; 947-H4, **lane 4A**; 947-H5, **lane 5A**; 947-HK5, **lanes 5KA, 5KB** and **5KC**; 947-H7, **lane 7A**; and 947-HK7, **lanes 7KA** and **7KB**. The 1.5 kb C3-specific telomeric band in lanes 3A and 4A is very faint. This may be due to poor transfer or to the lower quantity of DNA in these lanes.

(transformed lines 3A, 4A and 7A) shows that all of these transformants have virtually no host (B strain) type macronuclear rDNA (Figure 3, no 3.7 kb band). Essentially all of the rDNA has the structure expected for macronuclear rDNA derived from proper processing of the injected micronuclear rDNA. They contain the *Bam*HI site of the inserted linker (at site 3, 4 or 7) as well as the C3 strain-specific *Bam*HI site ~1.5 kb from the telomeric end of the palindromic rDNA (Figure 2 and Figure 3, lanes 3A, 4A and 7A). These DNAs also lack the B-strain-specific *Sph*I site near the center of the palindromic rDNA molecule (Figure 2 and Figure 4, lanes 4A, 7A and 7B, no 6.0 kb band). Thus, at sites 3, 4 and 7, insertion of a 119-bp linker does not appear to interfere with the processing, replication or expression of the rDNA or the processing or function of the rRNA. All transformants in this group are capable of growth while containing only (or predominantly) insert-bearing rDNA in their macronuclei.

Results obtained from the other transformants are more complicated. A transformant resulting from injection of

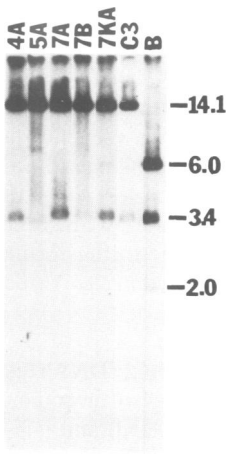

Fig. 4. *Sph*I digestion of rDNA of pm-R transformants. *Tetrahymena* DNA was digested with *Sph*I, electrophoresed on a 1% agarose gel, blotted and hybridized with nick-translated Tt947-01. **Lanes C3** and **B** are DNA from C3 and B strains respectively. The remaining lanes are DNA resulting from the injection of: 947-H4, **lane 4A**; 947-H5, **lane 5A**; 947-H7, **lanes 7A** and **7B**; and 947-HK7, **lane 7KA**.

947-H5 (transformed line 5A) (Figure 3, lane 5A) is unusual in that a minor proportion of its rDNA lacks the C3-strain-specific *Bam*HI site (3.7 kb band). This rDNA is probably generated from recombination between the host and injected rDNAs. This interpretation is supported by the observation that a small proportion of the rDNA molecules are asymmetric, having the site-5 linker insertion on only one side of the palindromic rDNA (11.3 kb band). Since most of the rDNA molecules have a linker on both sides of the palindromic rDNA (9.2 kb band), and no rDNA without a linker insertion is seen (no 13.4 kb band), it is clear that rDNA with a linker insertion in site 5 is tolerated by the cell. However, this transformant grew with a doubling time (in the presence of absence of pm) of ~4.6 h (data not shown), almost twice that of a wild-type *Tetrahymena* strain. This increase in doubling time was not due to selective cell death since 31 out of 32 individual cells examined were viable. Furthermore, after 20 generations of growth in the absence of selection, 41 out of 41 cells examined were pm-R, a result that indicates the stability of the pm-R phenotype of transformant 5A.

*Bam*HI digestion of pm-R transformant DNA from any one of the three transformants resulting from injection of 947-HK5 (Figure 3, lanes 5KA, 5KB and 5KC) shows that these transformants contain significant quantities of host rDNA (3.7 kb band) which cannot be eliminated by 40 generations of growth in pm (data not shown) as well as the injected rDNA. The injected rDNA can be lost when selection is released. It is therefore likely that host rDNA is essential for the survival of these pm-R transformants. In addition, there are asymmetric molecules (11.3 kb band) that bear the linker on only one side of the rDNA palindrome. These must have resulted from recombination, which has also been observed recently in other studies (Lovlie *et al.*, 1988; Yu *et al.*, 1988), or from deletion. Despite the unstable phenotype, these three transformants grow at a normal rate (2.5 h doubling time) in the presence of selection.

Macronuclear rDNA from both pm-R transformants produced by injection of 947-HK7 (transformed lines 7KA, 7KB) contains only B-type, host telomeric ends with no

pHSS6 insertion at site 7 (Figure 3, lanes 7KA and 7KB; 3.7 kb band). However, both rDNAs lack the B-strain-specific *Sph*I site (Figure 4, lane 7KA; no 6.0 kb band). Thus it is likely that recombination between the host and injected rDNA has taken place between the pm-R site, which is 4.2 kb from the center of the palindromic rDNA (Kiss and Pearlman, 1981; Engberg *et al.*, 1984; Spangler and Blackburn, 1985), and site 7. Since no rDNA containing an insert was found in these transformants, it is likely that rDNA with pHSS6 inserted in site 7 is deleterious to the cell.

Coinjection experiments

To determine whether altered rDNAs that failed to transform are truly incapable of doing so, an equimolar mixture of three of these (947-HK3, 947-HK6 and 947-H6) plus unaltered Tt947-01 DNA was injected into conjugating *Tetrahymena* cells. DNA from the resulting pm-R transformants (Table I) was analyzed by Southern hybridization. Only rDNAs derived from 947-HK6 and 947-HK3 should be cut by *Sma*I (Figure 1). A *Sma*I digestion of DNA from the pm-R transformants (Figure 5, panel A) shows that rDNA derived from 947-HK6 or 947-HK3 is not present in significant quantities. With long exposures, a faint band is visible in two of the transformant DNAs at ~4.0 kb (Figure 5, panel A, lanes M−A and M−C), which may represent the rDNA derived from 947-HK3. A *Bam*HI digestion shows only bands expected for rDNA derived from Tt947-01 or host rDNA (Figure 5, panel B). For comparison, a coinjection experiment using an equimolar mixture of four DNAs, unaltered Tt947-01, 947-H3, 947-H4 and 947-H8, that had given rise to pm-R transformants was performed. DNAs from the resulting pm-R transformants (Table I) were analyzed by Southern blotting. Mature rDNAs derived from all four species of injected DNA are found in significant quantity in at least one of the eight pm-R transformants (Figure 5, panel C). In addition, more than one species of injected DNA had been processed into macronuclear rDNA in many of the transformants. These results confirm the negative transformation results obtained earlier and suggest that the insertions that failed to produce transformants interfere with the normal processing, replication or function of these rDNAs.

Analysis of rRNA from pm-R transformants

In *Tetrahymena* the mature 17S, 5.8S and 26S rRNAs are initially transcribed as a 35S precursor (Kumar, 1970; Prescott *et al.*, 1971; Sutton *et al.*, 1979). After the removal of the intervening sequence (Din *et al.*, 1979; Cech and Rio, 1979), the 35S precursor is cleaved, producing the 17S rRNA and the 26S rRNA precursor. The 26S rRNA precursor is subsequently cleaved to produce three molecules: two of roughly equal size (~17S), which comprise the mature 26S rRNA, and the 5.8S rRNA, which may reach maturity via a 7S intermediate (Eckert *et al.*, 1978; Kister *et al.*, 1983). To analyze the transcription and processing of rRNAs from the mutated rDNAs, RNA was prepared from the pm-R transformants and subjected to Northern hybridization studies.

Using a synthetic oligonucleotide complementary to the inserted linker (given the known orientation of pHSS6 in the altered Tt947-01 plasmids which, for unknown reasons, was identical at every insertion site) as a hybridization probe, we found that the linker sequence is present in the 35S rRNA

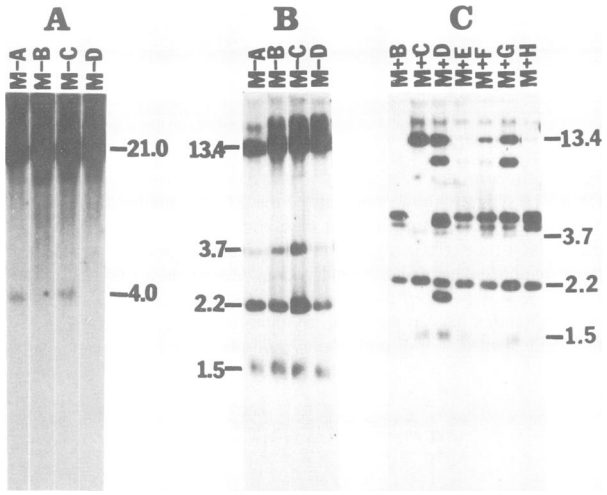

Fig. 5. Analysis of rDNA from pm-R transformants resulting from coinjections. **Panel A.** DNAs from four transformants (M-A to M-D) resulting from injection of an equimolar mixture of Tt947-01, 947-HK3, 947-HK6 and 947-H6 are digested with *Sma*I, electrophoresed on a 1% agarose gel, blotted and hybridized with nick-translated Tt947-01. **Panel B.** This is the same as panel A except that the DNAs were digested with *Bam*HI. **Panel C.** DNAs from seven transformants (M+B to M+H) resulting from injection of an equimolar mixture of Tt947-01, 947-H3, 947-H4 and 947-H8 were digested with *Bam*HI, electrophoresed on a 1% agarose gel, blotted, and hybridized with nick-translated Tt947-01.

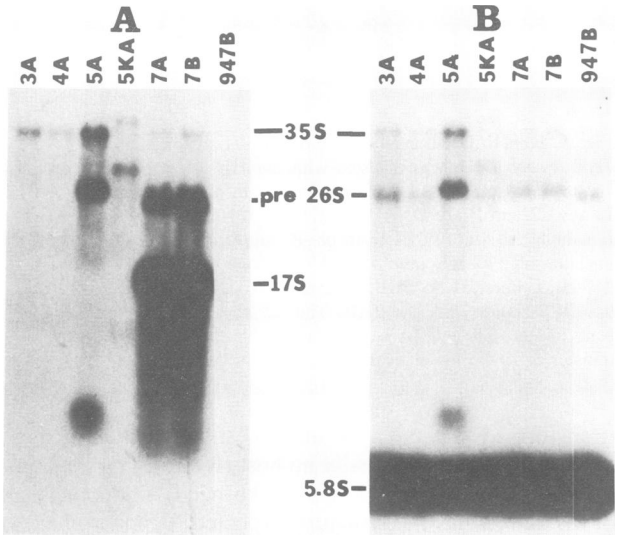

Fig. 6. Northern analysis of rRNA from pm-R transformants. RNA from pm-R transformants was prepared, electrophoresed, blotted and hybridized wtih an oligonucleotide complementary to the pHSS6 linker (**panel A**) or with an oligonucleotide complementary to a portion of 5.8S rRNA (**panel B**). In each lane is RNA from pm-R transformants resulting from the injection of: 947-H3, **3A**; 947-H4, **4A**; 947-H5, **5A**; 947-HK5, **5KA**; 947-H7, **7A** and **7B**; and unaltered Tt947-01, **947B**.

precursor of transformed lines 3A, 4A, 5A, 5KA, 7A and 7B as well as in mature 26S rRNA (which migrates as two 17S molecules) of 7A and 7B (Figure 6, panel A). In addition, 5KA contains RNA species that are visibly larger than the pre-26S and the 35S precursors of the other transformants, strongly suggesting that the 2.3 kb pHSS6 insertion is transcribed. A small molecule (~300 bases) is

also detected in 5A RNA. This is presumably a processing intermediate. Longer exposures reveal what may be an analogous band in the 5KA lane. These data show that the inserted DNA is transcribed as a part of the rRNAs in these transformed lines.

Using a plasmid containing an rDNA fragment (extending from site 7 to site 8 in Tt947-01) as a hybridization probe, we found that all of the pm-R transformants contain mature 26S rRNAs that appear normal in size (data not shown). The transformant line 5KA was found to contain larger, as well as normal-sized, 35S and 26S precursors; this is expected since the 2.3 kb insertion is present in ~50% of its rDNA.

To determine whether the processing of the rRNAs is defective in the slow-growing transformant 5A, the same RNA blot was probed with a synthetic oligonucleotide complementary to a portion of the 5.8S rRNA. Densitometric analysis of the result (in Figure 6, panel B) shows that there is three times as much 26S precursor and 47 times as much 35S precursor in 5A as there is in a control line transformed with unaltered Tt947-01. This result suggests a processing defect in converting the 35S precursor into the 26S and 17S precursors. In addition, an RNA ~300 bases long (the same size as the band that hybridized with the linker oligonucleotide) is seen in 5A. This molecule is probably a processing intermediate that includes the 5.8S sequence as well as the inserted sequence between the 5.8S and 26S rRNAs. No similar molecule is seen in the control line transformed with unaltered 947-01.

Discussion

We describe here a new approach to the study of the functional regions of rRNA. The method involves microinjection of cloned, micronuclear form, pm-R rDNA bearing a specific alteration into developing macronuclei, where it is processed into palindromic, macronuclear rDNA which can completely replace the host rDNA. Thus it has been possible to observe the phenotype of cells containing a homogeneous population of specifically altered rDNAs.

The altered versions of Tt947-01 that have been injected can be divided into four classes on the basis of their ability to transform *Tetrahymena* and replace the host rDNA: (1) those that fail to yield transformants; (2) those that produce transformants in which the alteration has been eliminated by recombination, i.e. 7KA and 7KB; (3) those that produce transformants in which the injected rDNA cannot completely replace the host rDNA even after extensive selection, i.e. 5KA, 5KB and 5KC; and (4) those that produce pm-R transformants in which the host rDNA is completely replaced by the injected rDNA. Clearly, mutant clones belonging to the last class (class 4) produce fully functional rDNA and rRNA. Class 3 mutant rDNAs are probably deleterious to the cell due to some functional defect; but since their presence is tolerated by the cell, this defect must be recessive in nature. On the other hand, altered rDNAs that fail to produce transformants (class 1 mutants) must have a defect in rDNA or rRNA maintenance or function that is dominant in nature since their presence in the cell is not tolerated even when the alteration is in the 26S rRNA coding region (as in the cases of 947-H6 and 947-HK6) rather than in the 17S rRNA coding region wherein the pm-R mutation lies. The class 2 mutant rDNAs probably also carry dominant (but not necessarily lethal) defects in rDNA or rRNA function, but the site of alteration is sufficiently distant from the pm-R site to allow recombination to take place at a high frequency. Consistent with this explanation, the sites of alteration that failed to yield pm-R recombinants (3, 4 and 6) are about half as far from the pm-R site as the site of alteration that did yield pm-R recombinants (site 7).

Two of the sites altered in this study (sites 6 and 7) fell within 'expansion regions' (Gerbi, 1985) or 'divergent domains' (Lenaers *et al.*, 1988) in the coding region of the 26S rRNA. The substantial size variation seen in 23S-like eukaryotic rRNAs is due to the occurrence of these non-conserved sequences of varying lengths at well conserved sites within the rRNA. Injection of altered rDNA with the 2.3 kb insertion at either of these sites did not give rise to transformants that carried the insertion, suggesting that this insertion in the rDNA or rRNA is deleterious to the cells. Although no transformants with the 119 bp linker insertion at site 6 were obtained, there were two transformants with the linker insertion at site 7. Moreover, these transformants contained only (or predominantly) rDNA with the linker insertion at site 7, and their mature 26S rRNA also bore the linker insertion. This striking finding provides the first evidence that an expansion region can be flexible in its sequence requirements.

Three of the sites altered (3, 4 and 5) are within the transcribed spacer regions, sites 3 and 4 being upstream of the 17S rRNA coding region and site 5 being between the 5.8S and 26S coding regions. Transformants containing linker inserts at these sites were obtained, suggesting that rRNA processing is not susceptible to such insertions. This is the first demonstration of successful *in vivo* rRNA processing with altered spacer regions. However, transformants with the 2.3 kb insertion at sites 3 or 4 were not obtained, suggesting that the larger insertion at these sites is deleterious to the cells. Transformants with the 2.3 kb insertion at site 5 were obtained, but the injected rDNA failed to replace the host rDNA even after extensive selection, suggesting that the injected rDNA was not fully functional or was deleterious to the cells.

The one stable transformant with a linker insertion at site 5 (called 5A) grew with a doubling time almost twice that of a wild-type strain. RNA analysis suggests that the 35S rRNA precursor is ~50 times more abundant in this transformant than it is in control cells, and the 26S precursor is ~3 times more abundant. This result implies that 5A is defective in processing the 35S precursor. In addition, another RNA molecule of ~300 bases that contains 5.8S RNA sequences as well as sequences downstream of 5.8S RNA is observed only in this line. Kister *et al.* (1983) have observed a similarly sized molecule in normal, macronuclear RNA that hybridizes to a probe consisting of the 5.8S RNA coding region and some of the spacer sequences surrounding it. The molecule we have observed could be a product of the normal processing pathway whose quantity is increased in 5A or an aberrant processing product induced by the presence of the alteration. Since nothing is known about the specific enzymes that process rRNA in eukaryotes or the sites they recognize and act upon, a processing mutant of this kind may be valuable.

The system described here clearly offers a unique opportunity for studying rRNA *in vivo*. It should now be possible to carry out a detailed genetic analysis, including

site-directed mutagenesis, to study specific functional regions of rRNAs in *Tetrahymena*.

Materials and methods

Cells and culture

T.thermophila strains CU427 and CU428 were obtained from P.Bruns. *Tetrahymena* cells were maintained and grown in axenic media as described earlier (Gorovsky *et al.*, 1975).

Analysis of Tetrahymena DNA

DNA was prepared from whole cells as described by Austerberry and Yao (1987). DNA was electrophoresed in agarose gels as described previously (Yao *et al.*, 1981). Southern blotting was performed essentially as described (Southern, 1975). Plasmid probes were labeled with ^{32}P by nick translation (Rigby *et al.*, 1977). Oligonucleotide probes were synthesized using an Applied Biosystems oligonucleotide synthesizer and labeled by addition of labeled phosphate to the 5′ ends (Maniatis *et al.*, 1982).

RNA preparation and analysis

Log phase cultures (100 ml at $1-2 \times 10^5$) of *Tetrahymena* were pelleted, washed with 10 mM Tris, pH 7.5, 1 mM EDTA, and resuspended in 8 ml of 10 mM Tris, pH 7.5, 10 mM EDTA at 0°C, the temperature at which all subsequent steps took place. Cells were lysed by the addition of 8 ml of 7 M urea, 2% SDS, 20 mM NaAc, 10 mM EDTA, pH 7.0 and treatment with a dounce homogenizer. Nucleic acids were extracted by first adding 1 vol of water-saturated phenol and shaking for 10 min and then adding 1 vol of chloroform. The resulting aqueous phase was extracted twice more with 1 vol of chloroform and precipitated by adding 1/10 vol of 1 M NaAc, pH 6.0 plus 2 vols of 95% ethanol. The resulting pellet yielded 1–2 mg of RNA. Gels for Northern blots were run in glyoxal, blotted onto nitrocellulose filters and hybridized as described by Thomas (1980).

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