Clustering of Pseudouridine Residues around the Peptidyltransferase Center of Yeast Cytoplasmic and Mitochondrial Ribosomes

Andrey Bakin,[‡] Byron G. Lane,[§] and James Ofengand*,[‡]

Roche Institute of Molecular Biology, Roche Research Center, Nutley, New Jersey 07110, and Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada M5S 1A8

Received July 1, 1994; Revised Manuscript Received August 25, 1994[⊗]

ABSTRACT: Analysis of the high molecular weight RNAs of the large ribosomal subunit of *Saccharomyces cerevisiae* cytoplasm and mitochondria by a new method [Bakin, A., & Ofengand, J. (1993) *Biochemistry 32,* 9754−9762] has for the first time located all of the pseudouridine residues present in these two RNAs. Thirty pseudouridines were found in the cytoplasmic RNA, and one was found in the mitochondrial RNA. The 30 cytoplasmic RNA pseudouridines were clustered in three regions of the RNA known to be at or near the peptidyltransferase center. The single pseudouridine in yeast mitochondrial rRNA at position 2819 was also located at the peptidyltransferase center. The localization of pseudouridines at or near the peptidyltransferase center in both cytoplasmic and mitochondrial ribosomes implies a functional role for pseudouridine in peptide bond formation. A correlation was shown to exist between the locations of the pseudouridines determined in this work and the positions of the methylated nucleotides (both 2′-OCH_3 and base-methylated) determined previously by others. In addition, this work has tentatively identified the locations of two previously unknown ribothymidine residues, at positions 955 and 2920 in the cytoplasmic rRNA.

Pseudouridine (Ψ),[1] the 5-ribosyl isomer of uridine, is a major modified nucleoside found in tRNA (Sprinzl et al., 1991), snRNA (Reddy, 1988), and rRNA (Maden, 1990), but not so far in mRNA or viral RNA. Despite being discovered over 30 years ago (Yu & Allen, 1959; Scannell et al. 1959; Cohn, 1959, 1960), no specific function for Ψ has ever been shown [reviewed in Bakin and Ofengand (1993)]. A prerequisite to understanding the function of Ψ is knowledge of the exact sequence location of the Ψ residues. Although this has up to now been an arduous task for large RNA molecules, recent developments in our laboratory have led to a rapid and facile method for sequence localization of Ψ residues. The method is based on a reverse transcription approach (Bakin & Ofengand, 1993).

Application of this method to *Escherichia coli* 23S rRNA led to the detection of five new Ψ residues, confirmation of the locations of three previously reported, and elimination of one previously assigned Ψ residue (Bakin & Ofengand, 1993, 1994). The most significant feature of this analysis was the finding that four of the five newly located Ψ residues were clustered at the peptidyltransferase ring (see Figure 4B), a secondary structural feature of 23S rRNA which is believed to be functionally involved in the process of peptide bond formation [reviewed in Bakin and Ofengand (1993), Brimacombe et al. (1993), and Noller (1993)]. Two of the previously identified Ψ residues were clustered in a different region (see Figure 3B) along with an unidentified modified U residue at position 1915 which appears to be a modified Ψ (A. Bakin, J. Wrzesinski, and J. Ofengand, unpublished results).[2] The remaining two Ψ residues, one previously identified and one newly detected (Bakin & Ofengand, 1994), define a third cluster site (see Figure 2B). These three cluster sites are geographically connected to each other as shown by both cross-linking and functional studies [reviewed in Bakin and Ofengand (1993)], and thus all three regions comprise components of the peptidyltransferase center (PTC) or its nearby vicinity.

Table 1: Number of Pseudouridylate Residues in Cytoplasmic Large Subunit Ribsomal RNAs

organism	moles of Ψ/ mole of RNA	reference
E. coli	8−9	Dubin and Günalp (1967); Gehrke and Kuo (1989)
S. carlsbergensis	~24	Brand et al. (1979)
wheat	~56	Lau et al. (1974)
X. laevis	~52	Maden (1990)
mouse	~57	Maden (1990)
human	~57	Maden (1990)

Eukaryotic ribosomal RNAs contain considerably more Ψ residues than those from *E. coli* (Table 1). Whereas Ψ comprises 1.5 percent of the U residues of *E. coli* 23S rRNA, it is 3.5 percent in yeast and *ca.* 8 percent in the large subunit

* To whom all correspondence should be addressed. Fax: 201-235-5848.

[‡] Roche Research Center.

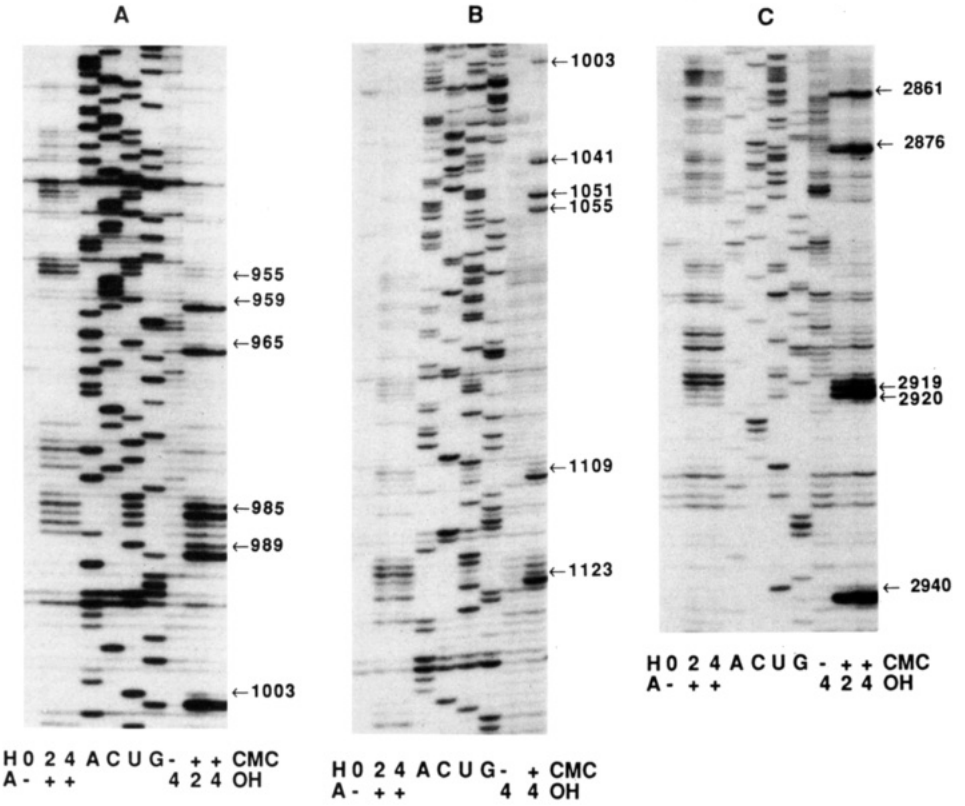
[§] University of Toronto.

[⊗] Abstract published in *Advance ACS Abstracts,* October 15, 1994.

[1] Abbreviations: Ψ, 5-ribosyluracil (pseudouridine); PTC, peptidyltransferase center; LSU rRNA, large subunit high molecular weight ribosomal RNA; CMC, 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate; m^5U, 5-methyluridine (ribothymidine).

[2] The strong stop at this position upon reverse transcription (Bakin & Ofengand, 1993) is indicative of a residue modified at a base-pairing position. When synthetic transcripts were treated with a purified enzyme fraction from *E. coli*, a Ψ residue at U1915 was produced. These two facts, taken together, imply that the previously known modified U1915 is in reality a modified Ψ residue.

0006-2960/94/0433-13475$04.50/0



D

		CMC[b]	H[c]	985[d]	984[d] 985
	984U[a]		....	....	
A.	→U	- - -	- - -	- - -	
	U	----	....	....	....
	U		- - -	----	----
	988A		- - -	- - -	- - -

		CMC[b]	H[c]	1123[d]	1122[d] 1123	1121[d] 1123	1121[d] 1122 1123
	1120U[a]		....	....	....	....	....
B.	U	....	----	----	----	- - -	- - -
	U	- - -	----	----	- - -	....	
	→U	- - -	- - -	- - -		- - -	
	U	----	....	....	....	....	....
	1125G		----	- - -	- - -	- - -	- - -

		CMC[b]	H[c]	2919[d]	2917[d] 2919	2919Ψ[d] 2920rT
	2916U[a]		....	....	....	....
	U	....	----	----	- - -	----
C.	G	- - -	----	- - -		- - -
	→U	- - -	....			
	U	----	....	....	....	
	2921C		....	----	- - -	

FIGURE 1: Location of representative Ψ residues in yeast cytoplasmic large subunit ribosomal RNA. Panel A, reverse transcription using a primer corresponding to rRNA residues 1028−1048. Samples were treated with (+) or without (−) CMC for 20 min at 37 °C and then exposed to pH 10.4 (OH) for 2 or 4 h at 37 °C. Alternatively, samples were treated with hydrazine (H) for the indicated times in minutes followed by incubation at 60 °C for 20 min with (+) or without (−) aniline (A) as indicated. A, C, U, and G are sequencing lanes. Sequence numbers of the modified bases are indicated. Panel B, reverse transcription using primer 1182−1200. Conditions were as in panel A. Panel C, reverse transcription using primer 2978−2998. Conditions were as in panel A. Panel D, diagrammatic representation of segments of panels A−C. Relative strengths of the bands: light, •••; medium, - - -; strong, ----. [a]Sequence of segment diagrammed. Arrow indicates the deduced position of the Ψ residue. [b]Diagram of the bands experimentally found after CMC-OH treatment. [c]Diagram of the bands experimentally found after hydrazine−aniline treatment. [d]Expected hydrazine−aniline results for Ψ at the positions indicated. Each U residue was assumed to produce a light band at its position plus a medium band one residue 3′ to its position. When a medium band and a light band coincide, a strong band is assumed. Where indicated, Ψ is replaced by m^5U (rT).

high molecular weight rRNA (LSU rRNA) of higher plants and animals. The purpose of the present inquiry was to ascertain whether the increased number of Ψ residues in eukaryotes was due to an increased scatter of Ψ throughout the molecule or whether the addditional Ψ residues remain clustered at the PTC as in *E. coli*.

Table 2: Location and Structural Context of Pseudouridine Residues in LSU rRNA[a]

S. cerevisiae, cytoplasmic	*E. coli*	*S. cerevisiae*, mitochondrial
	746 (d)	
775 (e)		
959 (d)		
965 (e)		
985 (b)		
989 (b)		
1003 (a)		
1041 (e)		
1051 (a or b)		
1055 (a)		
1109 (b)		
1123 (c)	955 (a)	
2128 (b)		
2132 (a)		
2190 (b)		
	1911 (a)	
2257 (d)	1915* (d)	
2259 (d)	1917 (d)	
2263 (c)		
2265 (b)		
2313 (e)		
2339 (a)		
2348 (a)		
2350 (b)		
2415 (b)		
2730 (a)		
2822 (d)	2457 (a)	
2861 (d)		
	2504 (d)	
2876 (a)		
2919 (d)		
2940 (d)		
	2580 (a or c)	2819 (a or c)
2971 (a)	2605 (b)	

[a] The numbering system for each species is from Gutell et al. (1993). The letters in parentheses denote the structural context of the residues (see Figure 5). Numbers in the same row are equivalent or nearly equivalent residues (see Figures 2–4). Data for *S. cerevisiae* are from this work. Data for *E. coli* are from Bakin and Ofengand (1993) except for Ψ955 (Bakin & Ofengand, 1994). Ψ1051 belongs in class a on the basis of the phylogenetic model of the secondary structure of yeast LSU rRNA (Gutell et al., 1993) or in class b if an alternative base pairing between sequences 994–998 and 1049–1052 is considered. Ψ2580 of *E. coli* and Ψ2819 of yeast mitochondria (Figure 4) are assumed to have the same secondary structure in this region. This would place both in class a, adopting the structure of Figure 4B, or in class c, using the structure of Figure 4C. The asterisk denotes a modified Ψ.

MATERIALS AND METHODS

Saccharomyces cerevisiae Ribosomal RNA. A mitochondrial pellet, the gift of Steven Ellis, University of Louisville, was suspended in 300 μL of 10 mM Tris-HCl, pH 7.5, 2 mM EDTA, and 0.2% SDS; the mixture was extracted four times with an equal volume of phenol/$CHCl_3$/TE (Sambrook et al., 1989), and the RNA was precipitated from the aqueous phase with 2 vol of EtOH. After reprecipitation, the pellet was washed with 70% EtOH and the RNA was dissolved in 0.5 mL of water. From the relative strengths of the gel patterns of the reverse transcripts, the ribosomal RNA in the sample was approximately 60–70% mitochondrial and 30–40% cytoplasmic. The same preparation was used for analysis of both rRNAs by appropriate selection of primers. The sequencing lanes verified that each set of primers was specific for its own rRNA.

Sequencing Analysis. Preparation of primers and location of Ψ residues by the 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate [(CMC)-OH] and hydrazine–aniline reverse transcription analysis methods were carried out as described previously (Bakin & Ofengand, 1993).

RESULTS

Analysis of Ψ Locations in Yeast LSU Ribosomal RNA. For the location of Ψ residues, a new screening method was used which depends on specific chemical modification of Ψ by CMC (Bakin & Ofengand, 1993). This was accomplished by treatment of the RNA with CMC followed by alkaline hydrolysis to remove CMC from G, U, and most other U-derived bases except Ψ (Ho & Gilham, 1971). Subsequent reverse transcription results in a stop one base 3′ to the Ψ residue due to attachment of the CMC group to the N-3 of Ψ, which blocks base-pairing.

The entire LSU rRNA of *S. cerevisae* cytoplasm was screened except for the 40 3′-terminal nucleotides, which could not be analyzed by reverse transcription methods. Representative gels (Figure 1) were chosen to illustrate the more difficult assignments. Interpretation of the gels depends on our previous finding (Bakin & Ofengand, 1993) that CMC modification of Ψ results in a strong stop one base 3′ to the site of modification and a weaker "stutter" stop at the modification site itself, as is seen for example at residue 989. Sometimes the stutter stop is very weak, however, as at residues 959, 965, and 1003, while in other cases it is quite strong, as at residue 985. The reason for this is unknown. Examples of both types of behavior have been observed previously (Bakin & Ofengand, 1993). This effect can lead to misassignment of Ψ residues when Ψ occurs in a run of U residues as is the case with Ψ985 (Figure 1A). For example, the CMC pattern alone cannot distinguish between Ψ985 and Ψ984Ψ985. This was accomplished by means of the complementary hydrazine reaction. Under our conditions this reaction destroys U residues but not ribothymidine (m^5U) or Ψ, resulting in a strong band one base 3′ to the U and a weaker stutter band at the U itself (Bakin & Ofengand, 1993). m^5U and Ψ residues, being resistant, do not yield bands, leaving blanks at the appropriate gel positions. The diagrams in Figure 1D(A) show the experimental CMC and hydrazine results as well as the expected hydrazine results for either Ψ985 or Ψ984Ψ985. Clearly only one Ψ is present, at position 985. Examination of the hydrazine band pattern for U953U954U955 showed the expected bands at 954 and 955 for U953 and U954, but there was no band at 956 corresponding to U955, indicating the presence of either m^5U or Ψ. However, since there was also no band at 956 in the CMC lane, the residue cannot be Ψ. On the basis of these criteria, we propose that U955 is a m^5U residue.

Analysis of position 1123 (Figure 1B) proceeded similarly. From the CMC pattern, Ψ formation at any of the 5 U residues could have occurred, although to very different extents. However, the presence of bands in the hydrazine lanes at 1121 and 1125 ruled out Ψ at 1120 or 1124. The remaining possibilities are diagrammed in Figure 1D(B). Clearly, the pattern most consistent with the experimental results is that for Ψ1123 alone. Note that while Ψ1109 also occurs in a row of U residues, its reaction pattern with CMC was unambiguous. Likewise, there was no difficulty in making the assignments of Ψ1003, Ψ1041, Ψ1051, and Ψ1055.

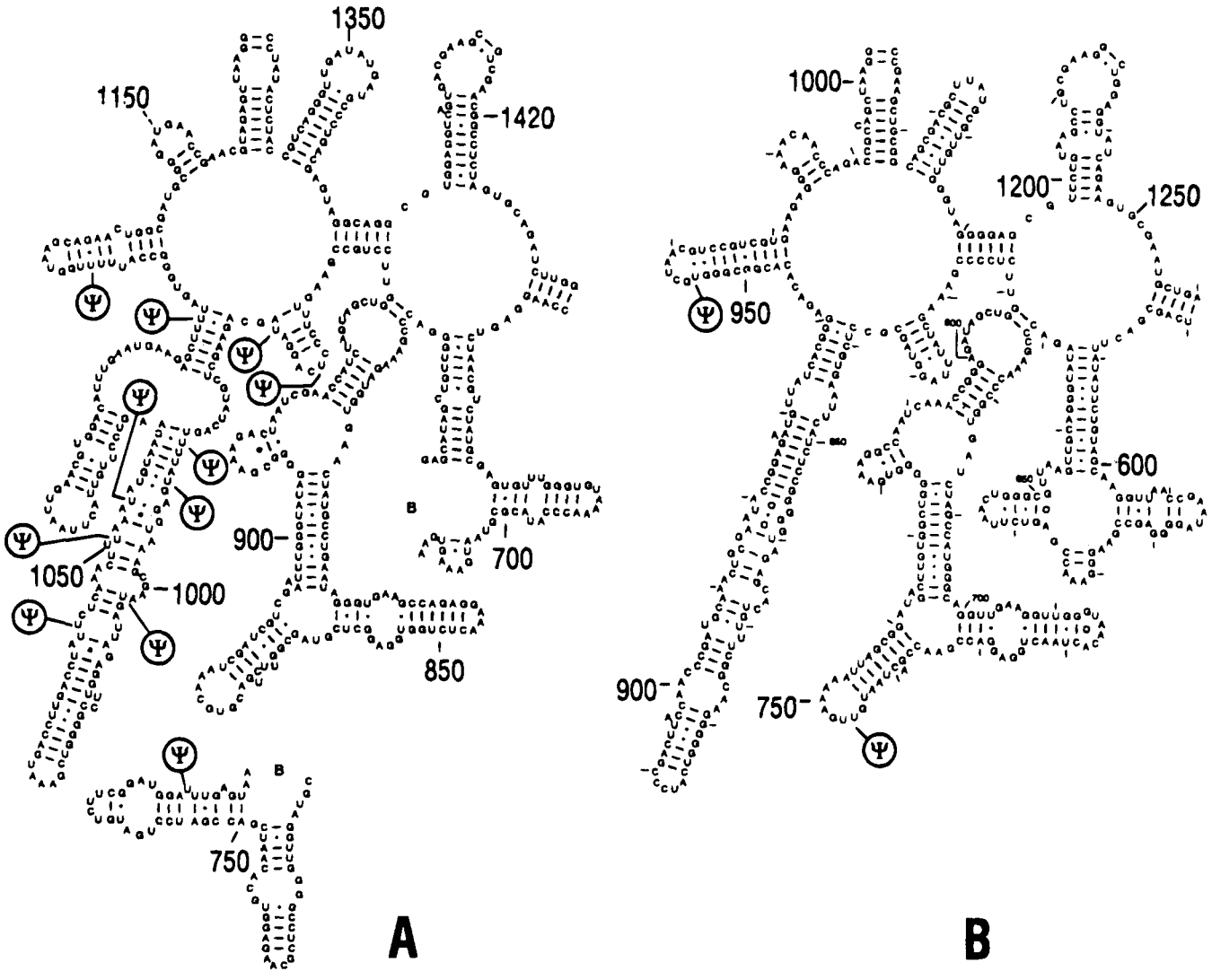


FIGURE 2: Comparative location of Ψ residues in the 5′ region of yeast cytoplasmic (A) and *E. coli* (B) LSU rRNA. Data for *E. coli* are from Bakin and Ofengand (1993) except for Ψ955, which is newly determined (Bakin & Ofengand, 1994). Secondary structures are from Gutell et al. (1993). Segment A shown here may also be found on the left side of Figure 6, which provides an overall orientation.

Analysis of positions 2919 and 2920 illustrates an additional complexity. The experimental results are shown in Figure 1C and diagrammed in Figure 1D(C). Ψ at 2916 or 2917 was ruled out by the presence of strong bands in the hydrazine lane at 2917 and 2918 despite the relatively strong stop at 2918 in the CMC lane. The only other possibility is Ψ2919, which apparently results in a double stop at 2919 and 2920. There is no Ψ at 2920 because of the lack of a strong stop at 2921 in the CMC lane. Nevertheless, there is no strong stop in the hydrazine lane at 2921 as expected if 2920 were U. This leads us to propose that, like U955, U2920 may be modified to m^5U, despite the fact that the experimental band pattern intensities do not exactly match those expected for Ψ2919 and m^5U2920. Identification of Ψ2861, Ψ2876, Ψ2940, and all of the remaining Ψ sites was unambiguous and therefore is not shown. We emphasize that only the most difficult assignments are shown in Figure 1.

Clustering of Ψ Residues at the PTC of Yeast Cytoplasmic LSU rRNA. A total of 30 Ψ residues were found in yeast LSU rRNA (Table 2), in reasonably good agreement with the earlier estimate listed in Table 1. The most surprising feature, however, is that, despite the more than 3-fold increase in number compared to *E. coli*, the Ψ residues cluster in the same three regions of the secondary structure. Eleven Ψ residues cluster in the 5′ region of yeast LSU rRNA, whereas two were found in *E. coli* (Figure 2). *E. coli* Ψ955 has a counterpart in yeast Ψ1123, but *E. coli* Ψ746 has no obvious counterpart in yeast. In the 950–1140 sequence of yeast LSU rRNA, which contains 10 of the 11 Ψ residues, 17% of the U residues were modified to Ψ. In the central region (Figure 3), Ψ2257 and Ψ2259 in yeast are present at the same positions as *E. coli* Ψ*1915 and Ψ1917, but instead of *E. coli* Ψ1911, yeast has Ψ2263 and Ψ2265. Yeast has seven additional Ψ residues nearby which are absent in *E. coli*. In the 3′ region (Figure 4), which includes the peptidyltransferase ring, the four Ψ residues in *E. coli* have approximate counterparts in yeast (Table 2), but yeast has four additional residues. Interestingly, Ψ2919 of yeast is adjacent to the U residue which was thought to be pseudouridylated in *E. coli* until our previous work (Bakin & Ofengand, 1993).

Number and Location of Pseudouridines in the LSU rRNA of Yeast Mitochondrial Ribosomes. The Ψ content of yeast mitochondrial ribosomes has been investigated previously, with the conclusion that there was not more than one Ψ in either the small or the large subunit RNA (Klootwijk et al., 1975). Sequence analysis of the entire yeast mitochondrial

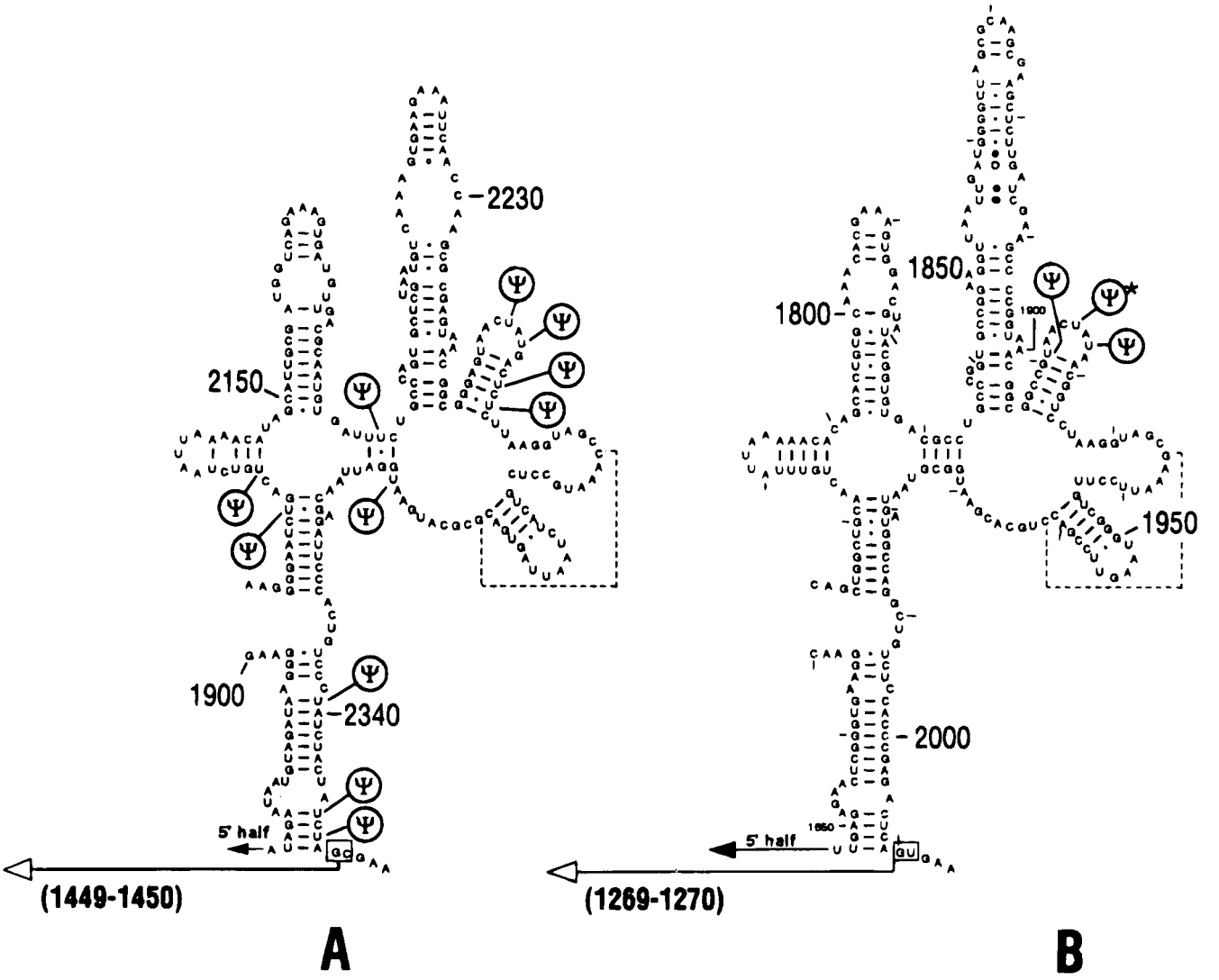


FIGURE 3: Comparative location of Ψ residues in the central region of yeast cytoplasmic (A) and *E. coli* (B) LSU rRNA. Data for *E. coli* are from Bakin and Ofengand (1993). Secondary structures are from Gutell et al. (1993). Segment A is also shown in the central part of Figure 6, which provides an overall orientation.

LSU rRNA has now revealed a single site for Ψ, at position 2819 (Figure 4C). This site is equivalent to *E. coli* Ψ2580 (Figure 4B), placing the single Ψ in mitochondrial LSU ribosomes squarely in the peptidyltransferase ring. This location of the single Ψ residue of yeast mitochondria LSU ribosomes underscores the potential functional role for Ψ at the PTC. This point is further emphasized by the fact that both human and mouse mitochondrial ribosomes have only one Ψ in the three cluster regions described in this work, and the Ψ residue is at precisely the same site as in yeast mitochondrial ribosomes and *E. coli* ribosomes (Bakin & Ofengand, 1994).

It should be noted that, as in the case of the yeast cytoplasmic and mitochondrial ribosomal RNAs described above, the entire *E. coli* 23S rRNA molecule has now been screened except for the 3′ 21 residues. This work resulted in the detection of only one additional Ψ residue, at position 955 (Bakin & Ofengand, 1994). The result clearly illustrates the fact that, in both this organism and yeast, Ψ residues are distributed in a highly nonrandom manner.

DISCUSSION

Number and Location of Pseudouridine Residues. There has been relatively little work on the Ψ content of yeast ribosomal RNAs beyond the early studies of Brand et al. (1979) who reported 23−24 Ψ residues per molecule of 26S rRNA of *Saccharomyces carlsbergensis.* We find exactly 30 Ψ residues by direct sequencing of the entire molecule from *S. cerevisiae.* Only four Ψ residues had been positioned in the RNA sequence prior to our work (Veldman et al., 1981). Our results confirm and more precisely locate these four residues. Moreover, the CUmAΨ fragment reported by Veldman et al. (1981) can now be identified. The only such sequence in the entire 26S RNA molecule is located at Ψ2348. Thus our results confirm the five Ψ locations reported previously and extend these observations by an additional 25 residues. We can also account for all of the Ψ-containing oligonucleotides reported by Brand et al. (1979).

Despite the dispersion of these three cluster sites in two-dimensional space, there is a considerable body of evidence, at least in *E. coli,* which links all three regions by both intra-rRNA cross-linking and functional studies [reviewed in Bakin

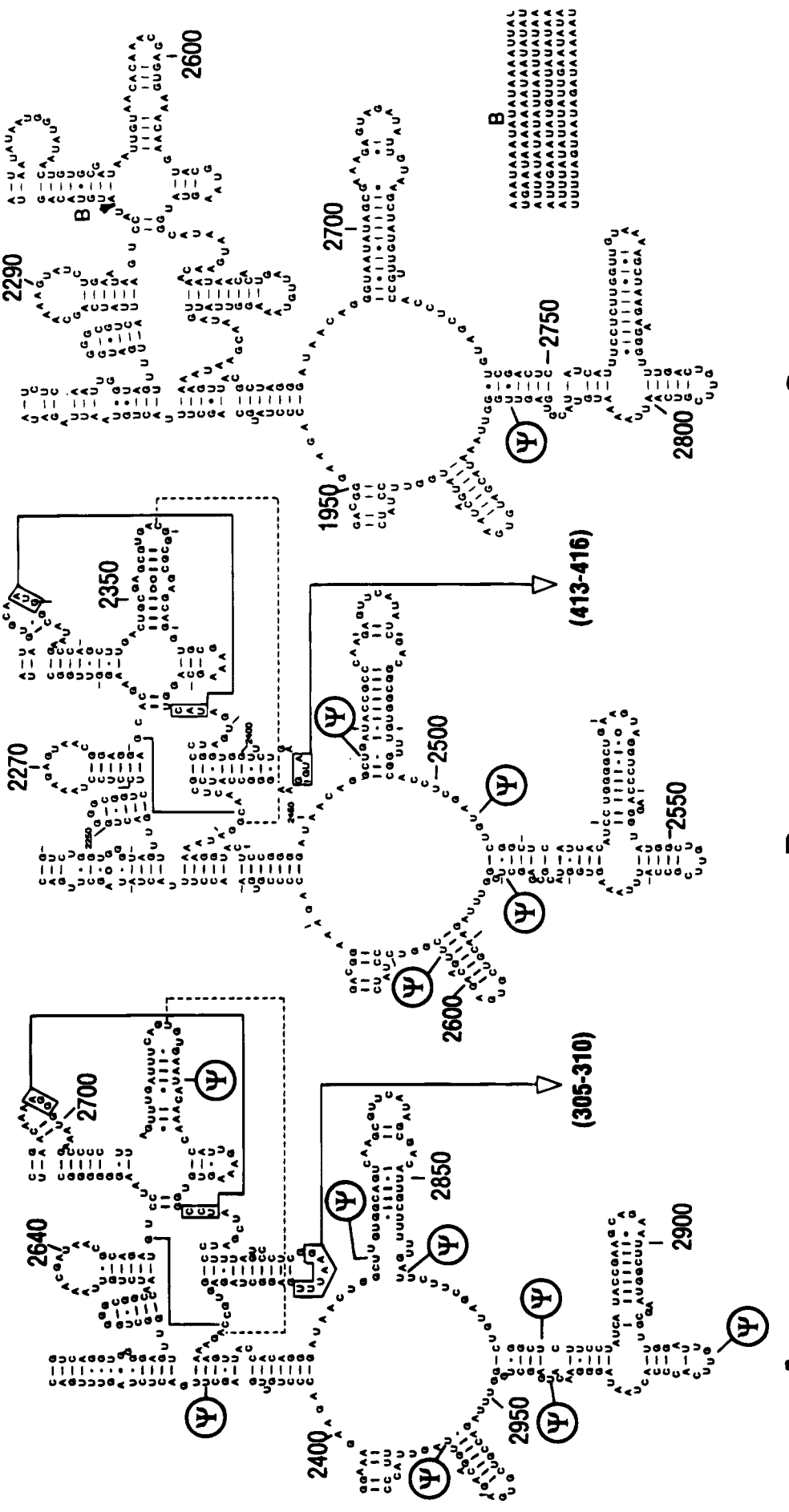


FIGURE 4: Comparative location of Ψ residues in the 3′ region of yeast cytoplasmic, (A) *E. coli*, (B), and yeast mitochondrial (C) LSU rRNA. Data for *E. coli* are from Bakin and Ofengand (1993). Secondary structures are from Gutell et al. (1993). Segment A is also shown in the right-hand part of Figure 6, which provides an overall orientation.

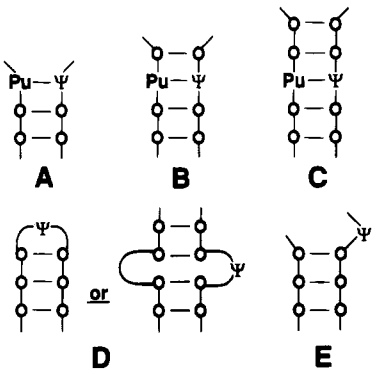


FIGURE 5: The five classes of structural features in which Ψ residues can be found: A, Pu-Ψ base pair closing a loop or bulge; B, Pu-Ψ base pair in a helix 1 base pair removed from a loop or bulge; C, Pu-Ψ base pair in a helix surrounded by at least 2 base pairs on either side; D, Ψ in loops or single-stranded regions but not adjacent to a base pair; E, Ψ in loops or single-stranded regions adjacent to a base pair.

and Ofengand (1993)]. In adddition, the 820−970 region of *E. coli* LSU rRNA, which is the counterpart of the 950−1140 segment of yeast cytoplasmic LSU rRNA, has been juxtaposed to the PTC by the cross-linking of 979−984 to G2029, three nucleotides away from A2032, which was in turn cross-linked to A2054C2055 (Döring et al., 1991). A2054 is base-paired to U2615, which is adjacent to the peptidyltransferase ring and is near Ψ2605. Also, a cDNA oligomer complementary to nucleotides 803−811 of *E. coli* LSU rRNA inhibited the interaction of the CCA end of tRNA with the ribosome, an action which should occur at the PTC (Hill et al., 1990). Thus it appears not only that Ψ residues are clustered in a nonrandom way but that most or all are in or near the PTC. The location of the single Ψ residue in the LSU rRNA of yeast mitochondria two residues from the peptidyltransferase ring supports this view.

Structural Features of Ψ Residues in rRNA. The availability of 40 Ψ sites in a known secondary structural context has allowed us to make a preliminary analysis of the structural features in which Ψ residues occur (Figure 5 and Table 2). Ψ residues were found in both helical and single-stranded regions. In the helical regions, we could distinguish three classes of purine−Ψ base pairs. The most prevalent ones were those that closed a loop or bulge (Figure 5A, 11 members) and those that were 1 base pair removed from the loop or bulge (Figure 5B, 10 members). Less frequent (Figure 5C, 4 members) were purine−Ψ base pairs which were contained in a helix consisting of at least 2 base pairs on either side. In this regard it is worth noting that even in the four examples of class C only yeast Ψ1123 (Table 2) is truly surrounded on both sides by two conventional base pairs. In the other three examples, one of the surrounding base pairs is a G-U mismatch, which is thought to be slightly less stable than a standard A-U or G-C pair (Chastain & Tinoco, 1991). While it may be that Ψ predominates near the ends of helical regions because it is easier for the enzyme to react with a quasi-single-stranded element, it is also possible that a Ψ-Pu base pair at or near a loop may have been selected for because its presence stabilizes loop closure. This might occur by virtue of the additional H-bond of Ψ in the major groove of its helix which might aid in triplex or other helix−helix interactions, as well as in recognition by protein.

The single-stranded sites could be divided into two subclasses, those which occurred in large loops or bulges separated by at least one residue from the closing base pair (Figure 5D) and those which, while single-stranded, were adjacent to the closing base pair (Figure 5E). Of the 15 members of the single-stranded class, 73% fall into class D, while only 27% are in class E. Separation of Ψ from the helical region as in class D increases the role of primary structure in specifying Ψ formation. In the case of class E, the adjacent single-stranded nucleotide is believed to stack on the closing helix (Chastain & Tinoco, 1991), which would confer partial helical properties on the Ψ residues of this class. Despite the expectation that Ψ would predominate in the single-stranded areas since the flexibility inherent in single-stranded regions should allow a more ready access by the synthase as well as a more facile placement of the extra N-1 proton either for functional or structural purposes, Ψ was found to a slightly greater extent (62%) in helical regions.

Biosynthesis of the Ψ Residues. Are there 30 enzymes, one for each of the Ψ residues in the LSU of yeast cytoplasmic ribosomes? No information is as yet available for yeast, but on the basis of our results with *E. coli* extracts (J. Wrzesinski, B. G. Lane, and J. Ofengand, unpublished results), it is likely that multiple enzymes exist, although some probably modify more than one position. In this context, it is worth emphasizing that Ψ formation is an extraordinarily economical way to introduce a modification into RNA, as the energy requirement for the conversion reaction is nil. By contrast, methylation, Nature's other popular modification mechanism, is quite costly since for each methylation not only is one *S*-adenosylmethionine (SAM) consumed, but a methyl group is sequestered from eventual oxidation to CO_2. The net energy cost of this has been estimated to be equivalent to that of the hydrolysis of 12−13 molecules of ATP to ADP plus P_i (Stock & Simms, 1988; Atkinson, 1977).

Significance of the Ψ Residues. What might the role of Ψ be? By virtue of its available N-1 position, which can be readily acylated to yield a relatively unstable adduct (Spector & Keller, 1958), Ψ might be an intermediate acceptor of the carboxyl end of the growing peptide chain before it reacts with the amino group of the incoming amino acid (Lane et al., 1992). In this context, it makes perfect sense that Ψ residues are clustered in the vicinity of the PTC. The recent report that mutations in *E. coli* 23S rRNA at G2505 and G2583, in intimate proximity to Ψ2504 and Ψ2580, are lethal *in vivo* (Saarma & Remme, 1992) lends some support to this idea, as does our preliminary observation that *E. coli* mutant Ψ2580C also appears to be lethal *in vivo* (A. Bakin and J. Ofengand, unpublished results). The variation in number and exact position of the Ψ residues between yeast and *E. coli* might be explained, as we argued previously (Bakin & Ofengand, 1993), if Nature had devised the Ψ network within the PTC to ensure the availability of at least one acylation site at all times. While this explanation could conceivably hold for the nine Ψ residues in *E. coli*, it is unlikely to be valid for all 30 Ψ residues in yeast cytoplasmic LSU rRNA. However, it may be that *some* of the Ψ residues play this role, while others may have a different function. An example of such a different function could be use of the H-bonding potential of the N-1 proton to provide a type of cross-strut stabilization of an essential

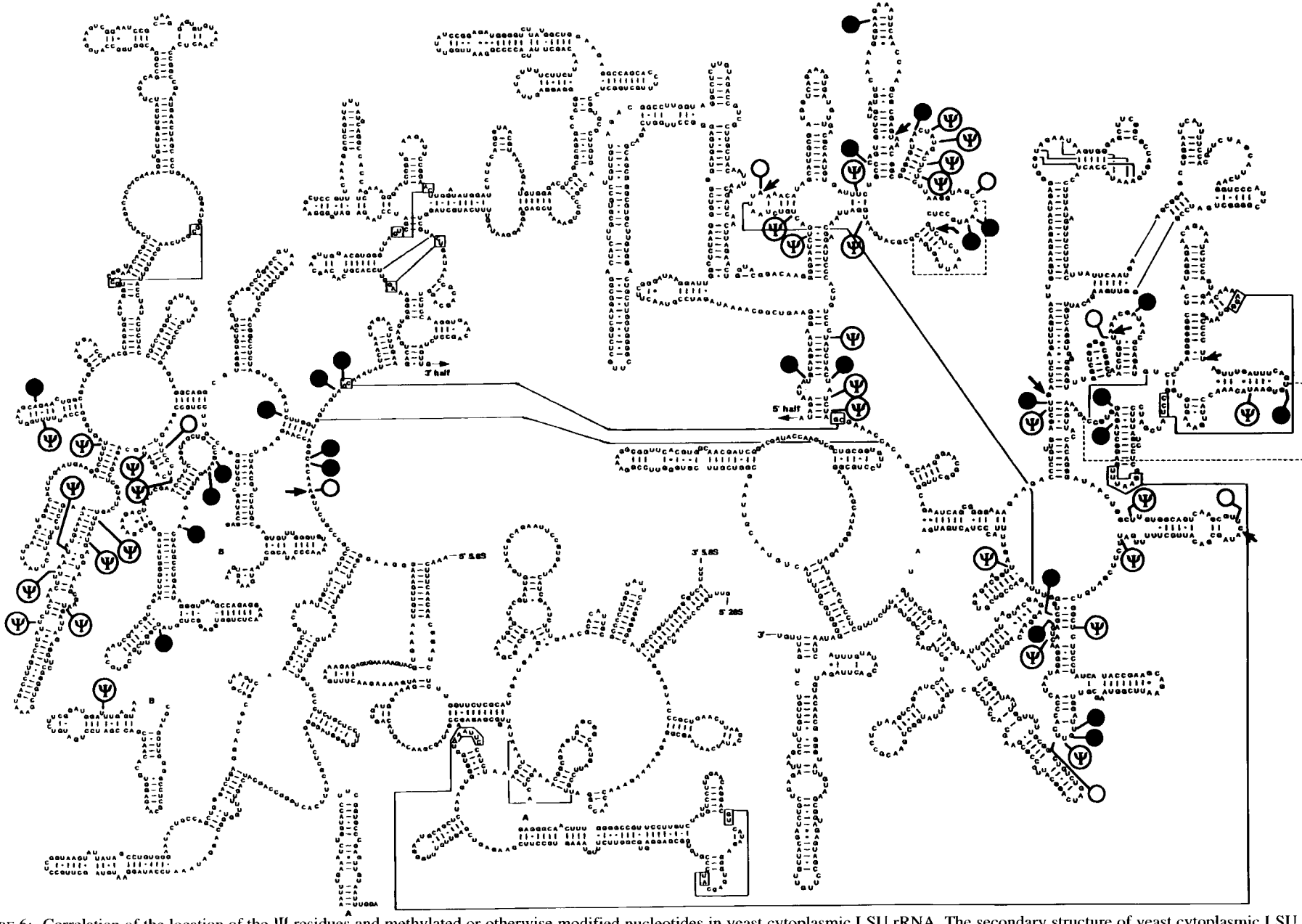


FIGURE 6: Correlation of the location of the Ψ residues and methylated or otherwise modified nucleotides in yeast cytoplasmic LSU rRNA. The secondary structure of yeast cytoplasmic LSU rRNA is from Gutell et al. (1993). The location of the methylated residues is from Veldman et al. (1981). The Ψ residues (this work) are as indicated. Solid circles, 2′-OCH_3 residues. Open circles, base-methylated residues. Arrow, reverse transcriptase stops (this work). Base methylations at 955 and 2920 and Um at 2346 should be considered preliminary (see text).

tertiary RNA structure at the PTC. This hypothesis could also easily accommodate variable numbers of Ψ at slightly different positions in the secondary structure, since each rRNA secondary structure could fold up in a slightly different way to achieve the same three-dimensional result.

Correlation with the Location of the Methylated Residues. In addition to the clustering of Ψ residues, the methylated nucleosides of yeast (Veldman et al., 1981) are also found concentrated within the same three domains (Figure 6). A similar phenomenon occurs in *E. coli* (Bakin & Ofengand, 1993; Brimacombe et al. 1993; Smith et al., 1992). Although this clustering is more obvious for the central and 3′ domains than for the 5′ domain, the methylated residues in the 5′ domain might be just as correlated with Ψ as in the other regions if the actual three-dimensional folding of the RNA were known. Figure 6 shows the 30 methylated residues located by Veldman et al. (1981), the two m^5U residues at 955 and 2920 tentatively identified in this work, and Um2346, located by comparing the CUmAΨ sequence described by Veldman et al. with our Ψ positions. Of the 33 residues, 26 are 2′-OCH_3 and seven are base-methylated. Another 11 2′-OCH_3 residues and one m^5C are in oligonucleotides which could not be placed in the 26S rRNA sequence (Veldman et al., 1981). There are eight sites where reverse transcriptase paused or stopped which presumptively identify base-modified residues. Four are at known sites of modification. Note that the pause occurs (and is shown) one base 3′ to the actual site of modification, except in the case of the m^1A residues. For unknown reasons, in these two cases, m^1A644 and m^1A2141, the stop occurred at the modified base position itself. The same behavior was previously observed for m^1G (Bakin & Ofengand, 1993) and may therefore be connected somehow to methylation at the N-1 position of purines. The remaining four pause sites have not been characterized. Overall, the data indicate the presence of 12 base-modified residues and 37 2′-OCH_3 groups. Thus, there are 1.5 times more methylated nucleosides than Ψ residues in yeast LSU rRNA. A slightly lower ratio (1.3) is found in other eukaryotic species (Maden, 1990), whereas the ratio of 1.6 in *E. coli*, which has 9 Ψ residues to 15 methylated nucleosides (Bakin & Ofengand, 1993), is slightly higher.

The methyl groups contribute a hydrophobic patch except for residues like m^7G where a charged base is generated, whereas Ψ formation results in the generation of a new hydrophilic H-bond donor. Both classes of modified nucleosides are located on the interface surface of the large ribosomal subunit (Brimacombe et al., 1993; Mitchell et al., 1991, 1992). This is the surface which contacts both the small subunit and ligands such as tRNA and mRNA. It is possible, therefore, that both classes of modifications participate together in the formation of an interfacial surface studded with hydrophobic patches and with hydrophilic groups for the express purpose of fixation of tRNAs and mRNA on the ribosome in the desired orientation. This hypothesis leads to a new view of the mechanism of interaction of tRNA and mRNA with the ribosome. Whereas the prevailing view up to now has been that base pairing between rRNA and tRNA or mRNA is involved, the interaction may instead utilize the special functional groups of the modified nucleosides, possibly ignoring classical base pairing entirely. Whatever the outcome, it is clear that Nature has found both classes of modification highly desirable. It now remains for the experimenter to divine their precise function.

ACKNOWLEDGMENT

We thank Steven Ellis, University of Louisville, Louisville, KY, for his very generous gift of a preparation of *S. cerevisiae* mitochondria.

REFERENCES

Atkinson, D. (1977) *Cellular Energy Metabolism and Its Regulation,* p 75, Academic Press, New York.
Bakin, A., & Ofengand, J. (1993) *Biochemistry 32,* 9754−9762.
Bakin, A., & Ofengand, J. (1994) *FASEB J. 8,* A1270.
Brand, R. C., Klootwijk, J., Sibum, C. P., & Planta, R. J. (1979) *Nucleic Acids Res. 7,* 121−134.
Brimacombe, R., Mitchell, P., Osswald, M., Stade, K., & Bochkariov, D. (1993) *FASEB J. 7,* 161−167.
Chastain, M., & Tinoco, I., Jr. (1991) *Prog. Nucleic Acid Res. Mol. Biol. 41,* 131−177.
Cohn, W. E. (1959) *Biochim. Biophys. Acta 32,* 569−571.
Cohn, W. E. (1960) *J. Biol. Chem. 235,* 1488−1498.
Döring, T., Greuer, B., & Brimacombe, R. (1991) *Nucleic Acids Res. 19,* 3517−3524.
Dubin, D., & Günalp, A. (1967) *Biochim. Biophys. Acta 134,* 106−123.
Gehrke, C. W., & Kuo, K. C. (1989) *J. Chromatogr. 471,* 3−36.
Gutell, R. R., Gray, M. W., & Schnare, N. N. (1993) *Nucleic Acids Res. 21,* 3055−3074.
Hill, W. E., Tassanakajon, A., & Tapprich, W. E. (1990) *Biochim. Biophys. Acta 1050,* 45−50.
Klootwijk, J., Klein, I., & Grivell, L. A. (1975) *J. Mol. Biol. 97,* 337−350.
Lane, B. G., Ofengand, J., & Gray, M. W. (1992) *FEBS Lett. 302,* 1−4.
Lau, R. Y., Kennedy, R. Y., & Lane, B. G. (1974) *Can. J. Biochem. 52,* 1110−1123.
Maden, B. E. H. (1990) *Prog. Nucleic Acid Res. Mol. Biol. 39,* 241−303.
Mitchell, P., Osswald, M., Schueler, D., & Brimacombe, R. (1990) *Nucleic Acids Res. 18,* 4325−4333.
Mitchell, P., Osswald, M., & Brimacombe, R. (1992) *Biochemistry 31,* 3004−3011.
Noller, N. F. (1993) *FASEB J. 7,* 87−89.
Reddy, R. (1989) *Methods Enzymol. 180,* 521−532.
Saarma, U., & Remme, J. (1992) *Nucleic Acids Res. 20,* 3147−3152.
Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989) in *Molecular Cloning: A Laboratory Manual,* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Scannell, J. P., Crestfield, J. P., & Allen, F. W. (1959) *Biochim. Biophys. Acta 32,* 406−412.
Smith, J. E., Cooperman, B. S., & Mitchell, P. (1992) *Biochemistry 31,* 10825−10834.
Spector, L. B., & Keller, E. B. (1958) *J. Biol. Chem. 232,* 185−192.
Sprinzl, M., Dank, N., Nock, S., & Schön, A. (1991) *Nucleic Acids Res. 19,* 2127−2171.
Stock, J. B., & Simms, S. (1988) in *Advances in Posttranslational Modifications of Proteins and Aging, Advances in Experimental Medicine and Biology,* (Zappia, V., Galletti, P., Porta, R., & Wold, F., Eds.) Vol. 231, pp 201−212, Plenum Press, New York.
Veldman, G. M., Klootwijk, J., deRegt, V. C. H. F., Planta, R. J., Branlant, C., Krol, A., & Ebel, J.-P. (1981) *Nucleic Acids Res. 9,* 6935−6952.
Yu, C.-T., & Allen, F. W. (1959) *Biochim. Biophys. Acta 32,* 393−406.