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Probing the Conformation of 26S rRNA in Yeast 60S Ribosomal Subunits with Kethoxal†

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ABSTRACT: The conformation and accessibility of 26S rRNA in yeast 60S ribosomal subunits were probed with kethoxal. Oligonucleotides originating from reactive sites were isolated by diagonal electrophoresis and sequenced. From over 70 oligonucleotide sequences, 26 kethoxal-reactive sites could be placed in the 26S rRNA sequence. These are in close agreement with a proposed secondary structure model for the RNA that is based on comparative sequence analysis. At least seven kethoxal-reactive sites in yeast 26S rRNA are in positions that are exactly homologous to reactive positions in *E. coli* 23S rRNA; each of these sites has previously been implicated in some aspect of ribosomal function.

In the preceding paper (Hogan et al., 1984), we described experiments designed to probe the conformation of 18S rRNA in yeast 40S ribosomal subunits. General agreement was found with a proposed secondary structure model for the 18S rRNA that was derived by comparative sequence analysis. There were, however, significant violations, some of which are in specific regions of the structure that have been previously implicated in possible biologically significant conformational rearrangements.

In this paper, we present analogous studies on the kethoxal reactivity of 26S rRNA in yeast 60S ribosomal subunits. As in the previous study, our intention is to compare the pattern of accessible, kethoxal-reactive guanines in a eucaryotic ribosome with that found for the *Escherichia coli* ribosome and at the same time test a secondary structure model for 26S rRNA derived from comparative sequence analysis. Sequences of over 70 oligonucleotides originating from kethoxal-modified sites have been determined, and 26 of these can be located in the published nucleotide sequence (Georgiev et al., 1981; Veldman et al., 1981) of yeast 26S rRNA. There is general agreement between the proposed secondary structure model and the experimental results. Furthermore, several reactive sites in 26S rRNA are in positions that are exactly analogous to reactive sites in *E. coli* 23S rRNA.

Materials and Methods

Growth and labeling of *Saccharomyces cerevisiae* (strain A364A) and preparation of ribosomal subunits were done as described in the preceding paper (Hogan et al., 1984). Kethoxal modification of 60S subunits, extraction of rRNA, and diagonal electrophoresis were carried out as described previously (Noller, 1974; Hogan et al., 1984). Repurification of oligonucleotides was done as in the preceding paper (Hogan et al., 1984).

Results

Yeast 60S ribosomal subunits were reacted with kethoxal as described in the preceding paper for 40S subunits (Hogan et al., 1984). The extracted 26S rRNA was digested with RNase T_1 and subjected to diagonal paper electrophoresis (Noller, 1974) to isolate and identify oligonucleotides originating from reactive sites. Figure 1 shows the diagonal pattern from one of the four independent experiments. The schematic diagram (Figure 1) indicates the numbering scheme used here in describing the individual oligonucleotides. After repurification, they were subjected to sequence analysis by a variety of further enzymatic digestions, followed by identification of resulting fragments (Barrell, 1971). Identification of methylated nucleotides was confirmed by diagonal electrophoresis with [^{14}C]methyl-labeled ribosomes in a fifth experiment. Sequences deduced from this information are listed in Table I.

Although over 70 oligonucleotides were sequenced, many of these cannot be located unambiguously in the 26S rRNA sequence, due to numerous occurrences of the smaller oligonucleotide sequences. As mentioned in the preceding paper (Hogan et al., 1984), some of the oligonucleotides could be placed on the basis of mobility-shift information. The number of occurrences of each oligomer in the 26S rRNA sequence and their off-diagonal mobility shifts are listed in Table I. An

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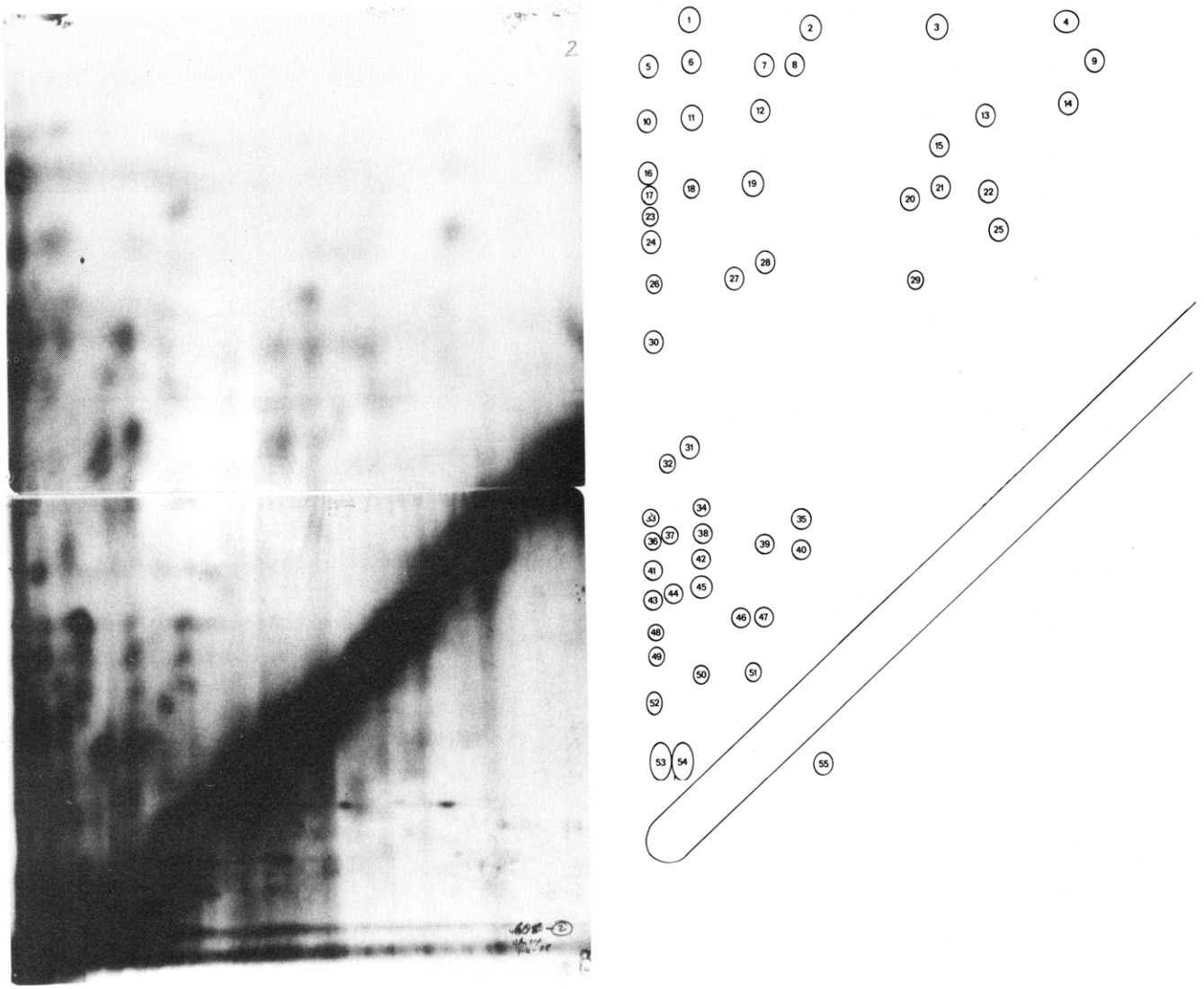


FIGURE 1: Diagonal paper electrophoresis (Noller, 1974) of oligonucleotides from kethoxal-modified yeast 60S ribosomal subunits. First dimension is from left to right and second dimension from bottom to top. The numbers of the various spots are indicated in the schematic diagram shown at the right.

example of this method of assignment is the placement of oligonucleotides 11c and 14, both of which have the sequence $UACG_{OH}$. Absence of the 3′-phosphate indicates that the site of kethoxal modification is a G residue immediately 5′ to the sequence (Noller, 1974). The mobility-shift number, based on the mobility of the spot in the second electrophoretic dimension relative to its mobility in the first dimension, is a measure of the base composition of the "partner" oligonucleotide, the RNase T_1 oligomer immediately 5′ to the oligomer in question (or 3′, in the case of 3′-phosphorylated oligonucleotides). Each G or U of the partner approximately doubles the second-dimension mobility of the other oligonucleotide, and the effect of three A's is equivalent to that of one U or G (Barrell, 1971). Oligonucleotide 11c has a mobility shift of 4, whereas oligonucleotide 14 has a shift of 1. A search of the 26S rRNA sequence shows that the sequence GUACG occurs only at positions 398, 2109, and 3019. The 5′ partners at each position are AAAAAG, G, and AACUUAG, respectively. These sequences would give rise to mobility shifts of $2^2/_3$, 1, and 4, respectively. The only possible way to account for the shift of oligonucleotide 14 is to place it at position 2109, and placement of 11c at position 3019 fits its mobility shift of 4. It might conceivably have been assigned alternatively to position 398, except that for the following: (a) This would also require a second kethoxal hit at position 392, in which case some single hit product would be expected, and this is not observed. (b) The resulting mobility shift from a second hit would be close to 5, and this is not observed. (c) Modification of position 398 would give AAAAAGp as an off-diagonal spot, which would migrate to a position near spot 31; no such oligomer was detected.

Thus, a number of useful experimental criteria for placement of oligonucleotides are inherent in the diagonal pattern. In all, 26 kethoxal-reactive sites can be placed in the 26S rRNA, and these are summarized in Table II.

Discussion

As discussed in the previous paper, the results of kethoxal probing of 26S rRNA are of interest not only as an experimental test of secondary structure models but also as a measure of the similarity between the exposed regions of ribosomal rRNA in eucaryotic and eubacterial ribosomes. Our previous studies on the kethoxal reactivity pattern of *E. coli* 50S ribosomal subunits (Herr & Noller, 1978) provide the basis for this comparison.

A secondary structure model for yeast 26S rRNA is shown

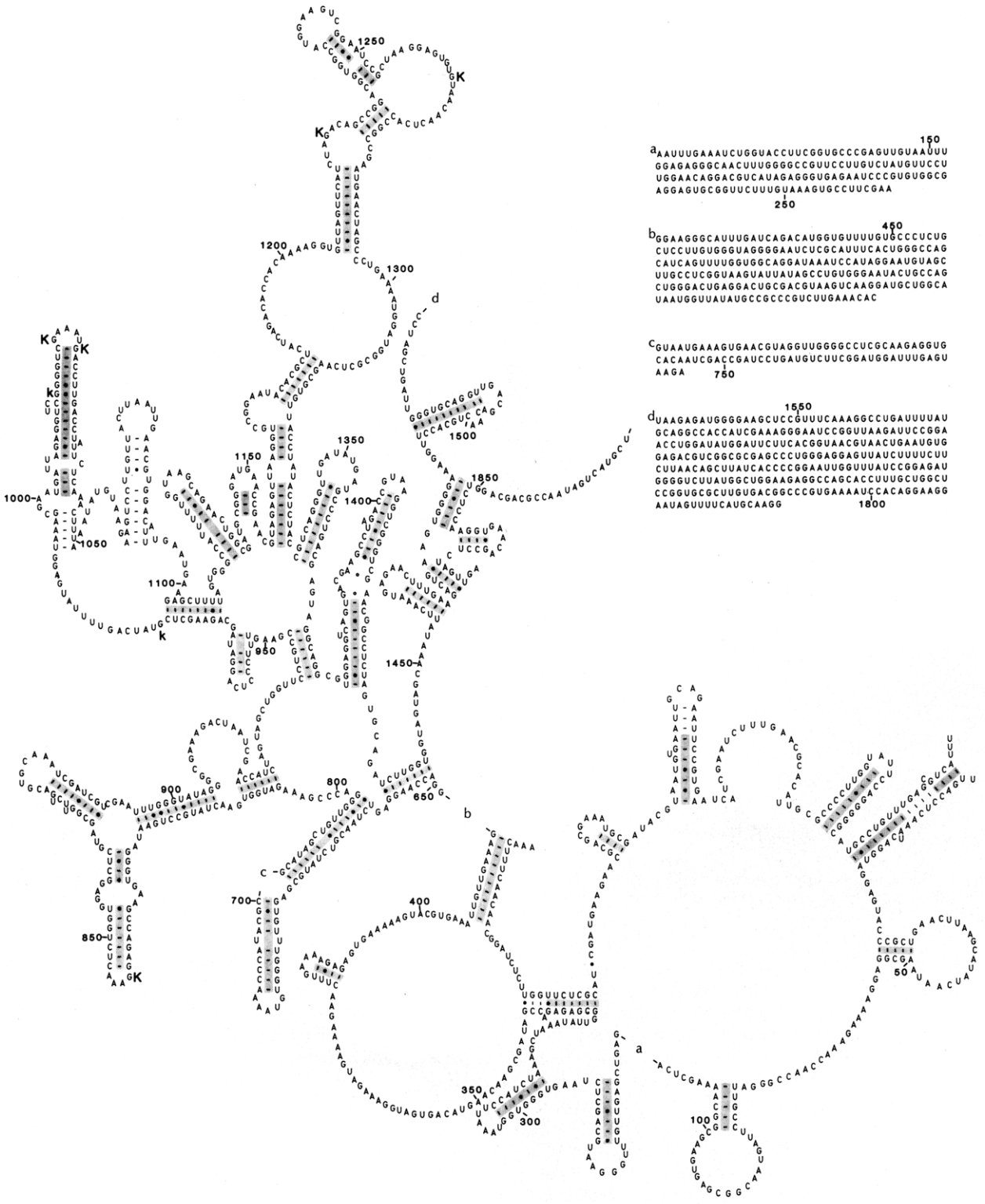
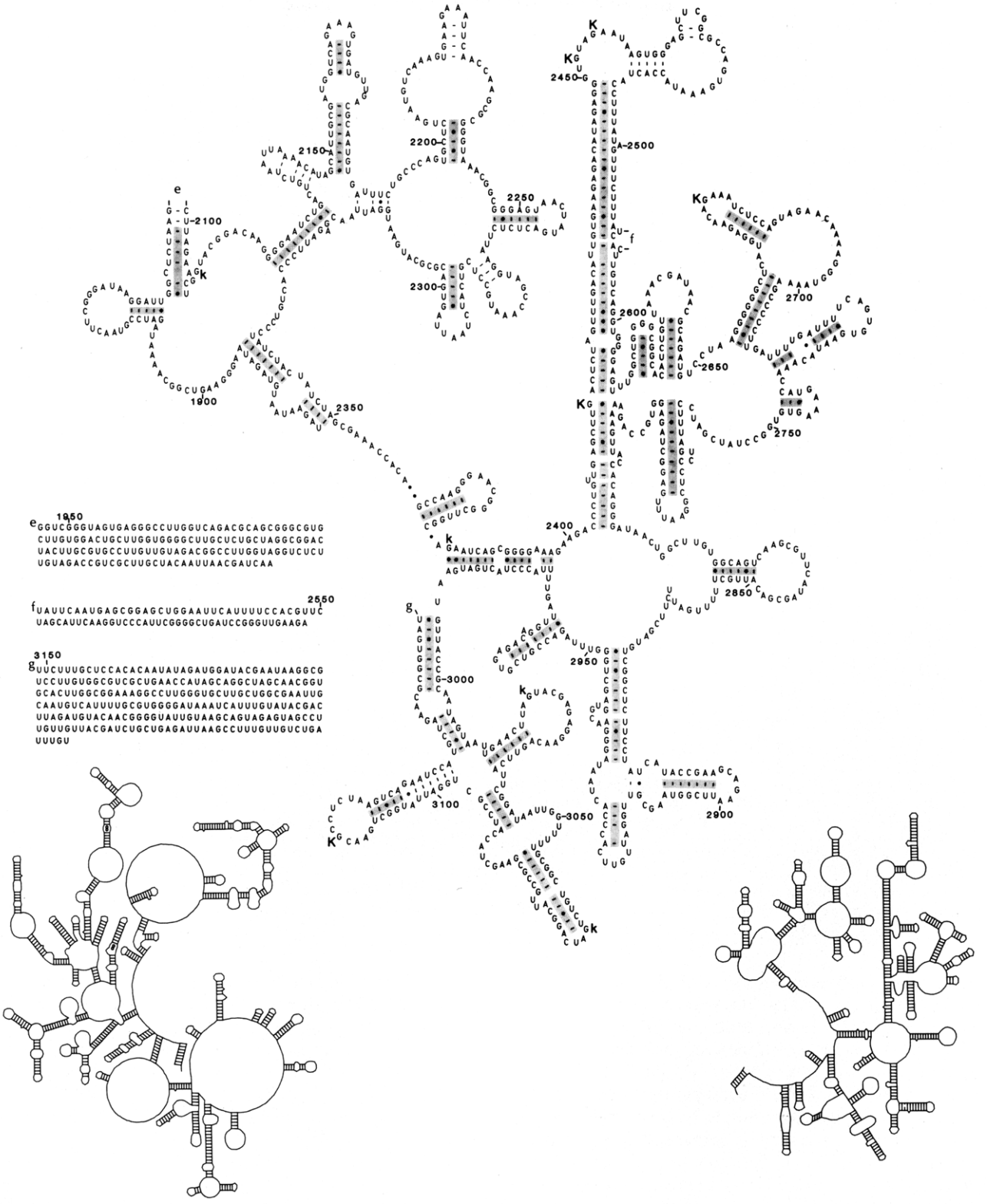


FIGURE 2: Secondary structure model of yeast 26S rRNA. The secondary structure was derived by comparative sequence analysis (see text). The primary sequence is from the DNA sequence of the 26S rRNA gene (Georgiev et al., 1981; Veldman et al., 1981). The letters a–f designate "extra" sequences that are found only in eucaryotic large subunit rRNAs; their positions in the structure are indicated by the corresponding

in Figure 2. This model was derived by comparative sequence analysis (Woese et al., 1983) and is based on 16 complete 23S-like sequences. A detailed account of the structure will be presented elsewhere. Veldman et al. (1981) have proposed a model for the secondary structure of yeast 26S rRNA that contains many features in common with the model presented here. Some 16 helices present in our model are missing from that of Veldman et al. (1981), and many of the helices shown in the latter model do not meet our criteria on the basis of comparative sequence analysis (two or more pairs of compensating base changes to establish the existence of a helix; Noller et al., 1981). There is general agreement, however, concerning base pairing in the portion of the molecule shown in Figure 1. Like the eubacterial 23S rRNA structure (Noller et al., 1981), it is organized by long-range interactions into six domains, as is evident from comparison with the schematic



letters. Positions of kethoxal modification (Table II) are shown by K (strongly reactive) or k (weakly reactive). Helices considered proven by comparative sequence analysis are shaded.

drawings of the *E. coli* 23S rRNA structure, provided in Figure 2. The increased size of the yeast 26S rRNA compared with *E. coli* 23S rRNA (3390 vs. 2904 nucleotides) is accounted for mainly by six major insertions, relative to the eubacterial structure. These are at ca. positions 110 (46 nucleotides), 455 (189 nucleotides), 720 (61 nucleotides), 1060 (32 nucleotides), 1960 (110 nucleotides), and 2525 (51 nucleotides). The 5.8S rRNA is included in the structure in a position analogous to the 5′ 160 nucleotides of eubacterial 23S rRNA, as previously discussed (Noller et al., 1981). Nearly all of the individual helices proposed for eubacterial 23S rRNA (Noller et al., 1981) have counterparts in the 26S rRNA structure, although details of the individual structures are often different. Two of the helices, at positions 2220 and 2470, are truncated with respect to the eubacterial structure. As in the case of small-subunit rRNAs, highly conserved sequences appear in the same secondary structure context in the 23S and 26S rRNAs. The significance of this observation is borne out

Table I: Sequences of Oligonucleotides from Kethoxal-Reactive Sites in Yeast 26S rRNA[a]

oligonucleotide no.	sequence	no. of occurrences	mobility shift	position	oligonucleotide no.	sequence	no. of occurrences	mobility shift	position
1	UG_{OH}		4		29	$AAUCAG_{OH}$	1	1+	2383
2	UG_{OH}		3		30	$AAUAAG_{OH}$	2	≥4	2452, 2455
3	UG_{OH}		2		31	AACAGp	4	3	2674
4	UG_{OH}		1		32	UUG_{OH}	16	4	
5	Gp		≥4		33	UGp		≥4	
6	Gp		3		34a	$CUUG_{OH}$	3		
7	Gp		3		34b	UGp		3	
8	Gp		3		35a	UGp		1+	
9	Gp		1		35b	$CUUG_{OH}$	10	1	
10a	AUG_{OH}	19	≥4		36	UGp		≥4	
10b	CGp		≥4		37a	UGp		3	
11a	CGp		4		37b	$CCUUCG_{OH}$	1	3[b]	254, 256
11b	AUG_{OH}	19	4		38	CUGp	17	3	
11c	$UACG_{OH}$	3	4	3019	39	UCGp		1	1022
12a	CGp		3		40	UGp		1+	
12b	AUG_{OH}	19	3		41a	UAGp	20	≥4	
13	CGp		1+		41b	(C,U)Gp		≥4	
14	$UACG_{OH}$	3	1	2109	42	CUGp	17	3	
15	$UAAG_{OH}$	8	1+		43	AUGp	19	≥4	
16	AGp		≥4		44	$ACUC_mUAG_{OH}$	1	3	2416
17a	$AAUG_{OH}$	2	≥4		45	$UAUCAG_{OH}$	1	2+	976
17b	$ACAG_{OH}$	2	≥4	1220	46	$CCUCUAAG_{OH}$	1	1+	3113
18a	$AUCAG_{OH}$	2	3+	3066	47	$AAUCUCG_{OH}$	1	1+	474
18b	$AAUG_{OH}$	9	3+		48	CAAUGp	2	≥4	3273
18c	CAGp	17	3+		49	$AAAUCUG_{OH}$	1	≥4	118
18d	AGp	9	3+		50a	$AAAUCUCCAG_{OH}$	1	2	2674
19a	$AAUG_{OH}$	9	3		50b	$UAACAACUCACCG_{OH}$	1	2	1266
19b	AGp		3		50c	AAAUGp	1	2	1027
20	CAGp	17	1+		51	$AAACUCUG_{OH}$	1	1	843
21	ACGp	13	1+		52	AAUNGp		≥4	
22	$AAUG_{OH}$	9	1		53a	AUAUGp	2	≥4	1621, 1622
23	$UAAAG_{OH}$	2	≥4	249	53b	$UUCUAG_{OH}$	1	≥4	2546
24	AAGp	22	≥4		54a	AAUCUCGp	1	~2	481
25	ACG_{OH}	13	1		54b	CUU_mGp	1	~2	2416
26	$AAUAAG_{OH}$	2	≥4	2452, 2455	54c	UUGp	16	~2	
27	AACGp	8	3	3113	55	UUCCGp	1	<0	1016
28	$AAAUG_{OH}$	1	1+	1022					

[a] Sequences were determined as described in the preceding paper (Hogan et al., 1984). Oligonucleotide numbers refer to those given in Figure 1. Where a spot gave rise to more than one oligonucleotide, they are distinguished by letters (e.g., 10a, 10b, etc.). The number of occurrences of each oligonucleotide sequence was determined by a computer search of the complete sequence (B. Weiser, unpublished results). Mobility shifts and position are determined as described in the text. [b] The predicted mobility shift for oligonucleotide 37b is 4, whereas the observed shift is 3. The basis of this disagreement is not presently understood.

by consideration of the kethoxal-modification results.

Of the 26 kethoxal-reactive sites that can be placed in the 26S rRNA sequence, 16 are located in the portion of the molecule included in the secondary structure model presented in Figure 1, providing a spot check of the latter structure as it exists in our 60S ribosomal subunit preparations. There is good general agreement between the kethoxal probe results and the proposed structure; sites of kethoxal attack are found, for the most part, in unpaired regions of the 26S rRNA (Figure 2). Some discrepancies are found, however, and these require further discussion. A strong kethoxal hit at position 1027 and a weak one at position 1016 contradict the proposed helix 1015–1021/1027–1033 (Figure 2). Comparative sequence analysis of the available eucaryotic 23S-like rRNAs argues strongly for the existence of the proposed helix, however (Table III). One kethoxal site is involved in a G-U pair adjacent to another G-U pair, a structure of very low predicted stability (Tinoco et al., 1973); the other is at a terminal C-G pair and could "breathe" under the conditions of chemical modification, although the high reactivity at this position (G_{1027}) does not support this possibility. Yet another option is that multiple conformational possibilities may exist for this region of 26S rRNA, a possibility considered for certain regions of the 16S-like RNAs in the preceding paper (Hogan et al., 1984). The other two discrepant sites are positions 2383 and 2416, both of which are shown in A-G pairs. Normal Watson–Crick pairs are found at analogous positions in many 23S-like rRNAs, supporting the suggested A-G pairs in the yeast structure. Perhaps these pairings are weak and become disrupted during the modification experiment; alternatively, they may in fact be unpaired in 26S rRNA.

Perhaps even more intriguing is the similarity of the reactivity patterns of *E. coli* 23S rRNA and yeast 26S rRNA in their respective ribosomal subunits. At least seven of the reactive sites in 26S rRNA have reactive counterparts at homologous positions in 23S rRNA (Table II). This correspondence is all the more remarkable if one considers that sequence differences between yeast and *E. coli* often preclude the possibility of kethoxal reaction at homologous positions in the two molecules. Several of the sites in common between *E. coli* and yeast have already been identified with specific aspects of ribosomal function. Position 277 in *E. coli* 23S rRNA (position 118 in yeast 26S rRNA; cf. Figure 2) is kethoxal reactive in 70S ribosomes but protected in polysomes (Brow & Noller, 1983). Position 1093 (1266 in yeast) is in a conserved structural region very near A_{1067}, the site of me-

Table II: Positions of Kethoxal-Reactive Guanines in Yeast 26S rRNA[a]

position	oligo-nucleotide	reactivity	corresponding reactive site in *E. coli* 23S rRNA	position	oligo-nucleotide	reactivity	corresponding reactive site in *E. coli* 23S rRNA
118	49	++	277	1621	53a	++	
249	23	+		1622	53a	++	
254	37b	++		2109	14	+	
256	37b	++		2383	29	+	
474	47	++		2416	44, 54b	++	
481	54a	++		2452	26, 30	++	2112
843	51	++		2455	26, 30	++	2115
976	45	+		2546	53b	++	
1016	55	+		2674	31, 50a	++	2308
1022	28	+		3019	11c	+	2655
1027	50c	++		3066	18a	+	
1220	17b	++		3113	46, 27	++	2751
1266	50b	++	1093	3273	48	++	

[a] Positions are from Table I. Reactivities of individual guanines are classified arbitrarily as strong (++) or weak (+). *E. coli* positions were previously determined (Herr & Noller, 1978).

Table III: Comparative Sequence Analysis of the 1000–1050 Region of 26S rRNA[a]

yeast[b]	AAUGAUUAGAGGUUCCGGGGUCGAAAUGACCUUGACCUAUUCUCAAACUUU
Physarum[c]	AAUGAUUAGGAGCACCGGGCGGUUUGACCGUUCGGCUCAUUCUCAAACUUU
rat[d]	AAUGAUUAGAGGUCUUGGGGCCGAAACGAUCUCAACCUAUUCUCAAACUUU

[a] Numbering corresponds to the yeast 26S rRNA sequence. Overlining shows proposed base-paired sequences. [b] Sequence is from Georgiev et al. (1981) and Veldman et al. (1981). [c] Sequence is from Otsuka et al. (1983). [d] Sequence is from Chan & Wool (1983).

thylation conferring resistance to the antibiotic thiostrepton (Thompson et al., 1982) and the site of cross-linking of elongation factor EF-G to 23S rRNA by diepoxybutane (Sköld, 1983). Positions 2112 and 2115 (2452 and 2455 in yeast) are protected from kethoxal attack in *E. coli* ribosomes when they are engaged in protein synthesis (i.e., in polysomes) but not in vacant 70S ribosomes (Brow & Noller, 1983). Position 2308 (position 2674 in yeast) is strongly protected from kethoxal in 70S ribosomes, placing this part of the molecule at the subunit interface (Herr & Noller, 1979). Position 2655 (3019 in yeast) is in a highly conserved loop, whose scission at position 2661 by the toxin α-sarcin inactivates the ribosome, preventing interaction with elongation factors (Fernandez-Puentes & Vazquez, 1977; Endo & Wool, 1982). Finally, position 2751 (3113 in yeast) is partially protected from kethoxal in 70S ribosomes (Herr & Noller, 1979) but strongly protected in polysomes (Brow & Noller, 1983), implicating this region in ribosome function as well. Thus, all seven of the kethoxal-reactive sites common to *E. coli* and yeast large-subunit rRNAs are in some way implicated in ribosome function.

Although we have probed only 26 of the over 3000 nucleotides of 26S rRNA, and a similar proportion of 23S rRNA, these results begin to suggest that the secondary structure homology between eucaryotic and eubacterial rRNA, especially around the more highly conserved sequence regions, underlies homologies between their three-dimensional structures. Very few guanines (probably less than 50 out of 700–800) are exposed in either the *E. coli* or yeast large ribosomal subunit. As for the small-subunit rRNAs, it is probably not coincidental that many of these exposed sites occur in highly conserved sequences and are contained by nearly identical secondary structure elements. This should not come as a complete surprise, since it is known, for example, that the transfer RNAs of these two organisms are recognized by each other's ribosomes. It is not implausible that the tRNA binding sites in these ribosomes could be virtually identical in structure, in spite of the vast number of detailed differences between their molecular components.

Registry No. Kethoxal, 27762-78-3; guanine, 73-40-5.

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