Xenopus laevis 28S ribosomal RNA: a secondary structure model and its evolutionary and functional implications

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ABSTRACT

Based upon the three experimentally derived models of E. coli 23S rRNA (1-3) and the partial model for yeast 26S rRNA (4), which was deduced by homology to E. coli, we derived a secondary structure model for Xenopus laevis 28S rRNA. This is the first complete model presented for eukaryotic 28S rRNA. Compensatory base changes support the general validity of our model and offer help to resolve which of the three E. coli models is correct in regions where they are different from one another. Eukaryotic rDNA is longer than prokaryotic rDNA by virtue of introns, expansion segments and transcribed spacers, all of which are discussed relative to our secondary structure model. Comments are made on the evolutionary origins of these three categories and the processing fates of their transcripts. Functionally important sites on our 28S rRNA secondary structure model are suggested by analogy for ribosomal protein binding, the GTPase center, the peptidyl transferase center, and for rRNA interaction with tRNA and 5S RNA. We discuss how RNA-RNA interactions may play a vital role in translocation.

INTRODUCTION

In order to gain an understanding of the mechanism of protein synthesis, it is essential to know the structure of the ribosomal components. The protein synthetic machinery of the bacterium Escherichia coli is by far the best characterized: the sequences of all the ribosomal RNA (rRNA) components are known, as are their sites of interaction with several of the ribosomal proteins and with other molecules needed for translation.

Models for the secondary structure of 23S rRNA of E. coli were proposed by three groups using different approaches, namely isolation of base-paired or cross-linked fragments (1), enzymatic digestion of single or double stranded regions (2), and chemical modification of single stranded regions (3). In addition, all three groups used the powerful technique of comparative sequence analysis. This method relies on the presence of evolutionarily conserved structures and compensatory mutations which give rise to primary sequence changes without destroying the secondary structure

features. While the acquisition of data on ribosome structure in eukaryotes has lagged behind that of prokaryotes, this comparative approach has recently been used to produce a secondary structure model for much of the large subunit 26S rRNA of the yeast Saccharomyces carlsbergensis by analogy to one of the E. coli models (4).

In this paper we propose a model for the 28S rRNA of Xenopus laevis based on our primary sequence alignment to E. coli, yeast and Physarum (5) and based on the three models for E. coli 23S rRNA (1-3) and the model for yeast 26S rRNA (4). In addition, we have used partial sequence data for nuclear rDNA from a number of eukaryotes (Tetrahymena: [6, 7]; Drosophila: [8-13]; mouse: [14]; Bombyx mori: F. Kafatos, personal communication; Calliphora: [15]; human: J. Drysdale, personal communication; Dictyostelium: [16] and R. Gourse, personal communication) and the recently completed sequence of rat 28S rDNA (17). Compensatory base changes between E. coli and Xenopus support our model of Xenopus 28S rRNA secondary structure and help to refine the proposed E. coli 23S rRNA secondary structures. We discuss regions deduced by analogy to E. coli to be of structural and functional significance within our model of Xenopus 28S rRNA.

The very strong conservation of rRNA secondary structure which we find reinforces the conclusion derived from primary sequence comparisons that prokaryotic and eukaryotic rRNAs are derived from the same ancestral gene. However, the transcription units of eukaryotic rDNAs are considerably larger than their prokaryotic counterparts. Although it cannot be rigorously proven whether this is due to insertions in the ancestral rDNA to give rise to the eukaryotic branch or is due to deletions in the ancestral rDNA to give rise to the shorter rRNA of the prokaryotic branch, we will treat these as insertional events to simplify discussion and to favor the commonly held bias.

We previously speculated that any of three possible evolutionary fates could befall DNA when inserted into a ribosomal RNA gene (5):

(1) Introns - the portion of RNA transcribed from DNA inserted into a stretch of evolutionarily conserved sequence cannot be tolerated in the mature rRNA as it would interrupt a functionally important site. Therefore when intron containing rDNA is expressed, the intron transcript is cut out of the precursor rRNA and a ligation step follows in the splicing reaction.

(2) Expansion Segments - the portion of RNA transcribed from DNA inserted into a stretch of evolutionarily nonconserved sequence

apparently does not destroy ribosome function as it is allowed to remain in the mature rRNA product. Such DNA insertions expand the size of eukaryotic rRNA relative to that of prokaryotes. We previously called these segments "inserts", but as this term implies evolutionary directionality and also could be confused with "introns", we rename them here "expansion segments".

(3) Transcribed Spacers - depending on the species, the RNA of certain regions within the rRNA precursor may be removed by processing cuts. Covalent ligation does not follow the spacer processing step.

METHODS

The primary sequence alignment generated by Ware *et al.* (5) which compares *Xenopus* 28S rRNA to the counterpart rRNAs from yeast, *Physarum* and *E. coli* was used as a starting point for the construction of our model of *Xenopus* 28S rRNA. Stretches of conserved sequence between *E. coli* and eukaryotes were located on the existing *E. coli* (1-3) and yeast models (4) (often in the terminal loop of hairpin structures). We then determined if the adjacent nonconserved sequences in *Xenopus* 28S rRNA could fit any of the base pairing schemes in the three *E. coli* models or in the yeast model. Where the previous 23S/26S rRNA models differed from each other, sometimes the *Xenopus* 28S rRNA sequence resolved these ambiguities by fitting only one of the proposed schemes. The interactive version (18) of the Queen and Korn (19) program was used to discover potential hairpin and long range interactions within regions of non-conserved sequence and within expansion segments. The secondary structure of the expansion segments has to be tentative, as generally there is no detectable homology between the primary sequences of *Xenopus*, *Physarum* and yeast in these areas. In some cases, the sequence of rat 28S rRNA (17), which is highly similar to that of *Xenopus*, was partly useful for deriving likely structures for the expansion segments.

RESULTS

Figures I-VII show the secondary structure of *Xenopus* 28S rRNA split into the seven domains defined by Glotz *et al*, (1) and Branlant *et al*. (2) for *E. coli* 23S rRNA. Noller *et al*. (3) have only six, as domains II and III are combined in that model. Tables I-VII compare in detail our model for *Xenopus* 28S rRNA with the three *E. coli* 23S rRNA models (1-3) and with the yeast 26S rRNA model (4). For simplicity, the three *E. coli* 23S rRNA models are hereafter referred to as the German (1), French (2) or American (3) models. The recently revised German model (20) is not used in our Tables, but is in general though not total agreement with our proposed secondary structure for *Xenopus* 28S rRNA.

It has been suspected for some time that eukaryotic 28S rRNA has a substantial amount of RNase resistant base-paired regions (21). We have

drawn 94 stem regions in the Figures of Xenopus 28S rRNA; these paired bases represent 57% of the Xenopus 28S rRNA bases. Some of the stems have bulged bases. Noller et al. have hypothesized that a bulged base (especially A) in a helical region may represent a primary recognition site for protein-RNA interactions (22). Although several bulged bases are among those conserved between E. coli and Xenopus, we do not observe a bias in base composition towards A's in bulges; however, in the proposed site of interaction of protein L24 with E. coli 23S rRNA, bulged base A_{443} of E. coli has been conserved and is equivalent to bulged base A_{346} of Xenopus 28S rRNA.

There is an only slightly higher incidence of primary sequence conservation in unpaired regions. In total, 24% of the bases in Xenopus 28S rRNA are conserved when compared to all the counterpart rRNAs of yeast, Physarum and E. coli (5), and 58% of these conserved bases are found in unpaired regions.

Examination of Tables I-VII will reveal that, with the exception of the expansion segments, every structural element in our Xenopus 28S rRNA model except one (stem 94) has at least a partially conserved counterpart in one or more of the three E. coli models and that all but ten (stems #6, 10, 13, 31, 38, 39, 40, 51, 52, 82) have an essentially identical counterpart. Of the 74 stems which are identical between Xenopus and at least one of the E. coli stems, all are proven by compensatory base changes except for six stems (#2, 56, 61, 63, 66, 75) which have primary sequence conservation between E. coli and yeast, Physarum and Xenopus. This high degree of secondary structure conservation is reminiscent of the idea that there is a conserved core between eukaryotes and E. coli in 23S/28S rRNA structure, as deduced by their similar first phase of melting (55% G+C component [23]). A second phase of melting 28S rRNA (77% G+C for Xenopus) suggested to Cox et al. that long base-paired stretches of high G+C were variable in size and sequence between eukaryotic species, and absent in E. coli (23, 24). The nucleotides we have drawn as base-paired in the nine Xenopus 28S rRNA expansion segments (boxed-in regions in Figures 1-7) are 87% G+C, so it seems likely that the eukaryotic specific expansion segments are the components of this second melting phase.

The G+C-rich expansion segments probably represent some or all of the hairpin loop structures previously seen in spread molecules of Xenopus 28S rRNA (25). The triple loop and asymmetric double loop seen by electron microscopy of 28S rRNA vary in length and the latter also in shape between eukaryotic species (25, 26), which is a characteristic of expansion

Figures I-VII: Domains I-VII of Xenopus laevis 28S rRNA. The primary sequence is from Ware et al. (5), and the following symbols are used for ambiguities in 1% of the sequence:

P = purine R = A or U V = A or C
Q = pyrimidine S = G or C W = G or U
lower case = unclear if base is present or absent. Expansion segments are boxed-in. Other features are mentioned in the Discussion.

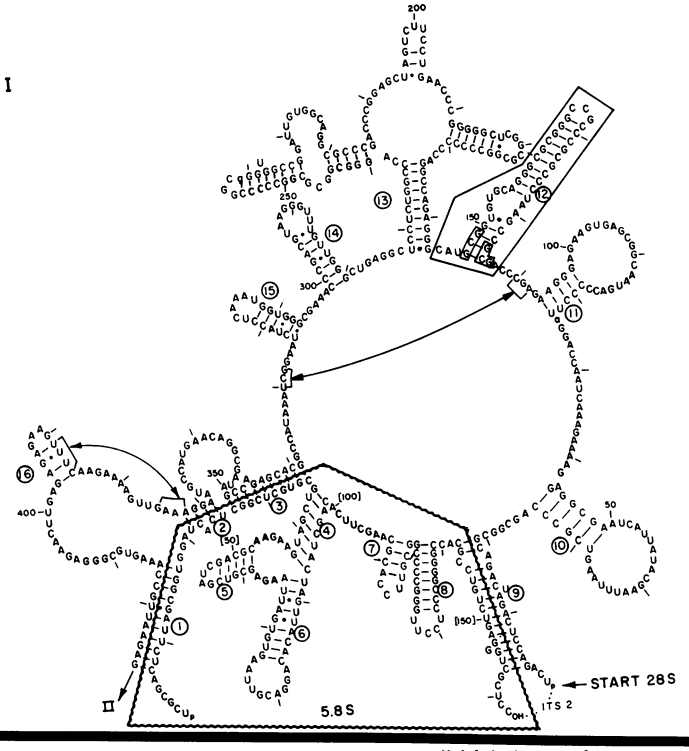


Table I: Domain I

Stem #	Xenopus bases	equivalent E. coli bases	Model in Agreement? E. coli German	E. coli American	E. coli French	yeast	notes
1	(7-17)/416-426	11-25/515-525	(+)	+	+	+	
2	(21-25)/369-372	29-34/466-470	X	X	+	X	(a)
3	(26-35)/339-349	35-44/436-446	+	+	+	(+)	(b)
4	(36-42)/(93-99)	45-51/106-111	+	X	+	+	
5	(43-61)	57-70	+	+	+	+	
6	(65-92)	74-105	(+)	(+)	(+)	(+)	
7	(108-118)	120-129	X	+	+	X	(c)
8	(119-139)	130-148	+	+	+	+	
9	(142-157)/4-19	150-176	+	+	+	+	(d)
10	26-57	183-214	(+)	(+)	(+)	+	
11	77-106	234-263	+	+	+	(+)	
12 (E.S.1)	113-156	--------	-	-	-	+	
13	157-272	270-367	(+)	(+)	(+)	(+)	(e)
14	280-301	375-396	+	+	+	+	
15	307-324	402-421	+	+	+	+	
16	386-397	484-495	X	+	+	X	

Tables I-VII Legend:

+ = identical base-paired stem
(+) = somewhat similar base-paired stem
x = dissimilar secondary structure
? = no secondary structure proposed for this region
- = equivalent bases do not exist in E. coli
E.S. = expansion segment found in eukaryotes but not in E. coli
# = primary sequence nucleotide coordinates in 23S/28S rRNA
(#) = primary sequence nucleotide coordinates in Xenopus 5.8S rRNA

Equivalent bases in E. coli 23S rRNA comparable to Xenopus 28S rRNA are taken from Ware et al. (5). Alignment of Xenopus 5.8S rRNA with the 5' end of E. coli. 23S rRNA is from Clark and Gerbi (29).

Table II: Domain II

Stem #	Xenopus bases	equivalent E. coli bases	Model in Agreement? E. coli German	American	French	yeast	notes
17	432-456	531-555	(+)	(+)	+	(+)	(f)
18	473-480/1318-1325	577-584/805-812	+	X	+	+	
19 (E.S.2)	483-976	--------	-	-	-	x	(g)
20	978-991/1167-1181	588-601/656-669	+	+	+	+	
21	994-1024	604-624	(+)	+	+	+	
22	1038-1051	638-651	+	X	+	+	
23 (E.S.3)	1052-1162	--------	-	-	-	(+)	
24	1189-1195/1307-1313	677-683/794-800	+	+	+	+	
25	1199-1288	687-775	+	+	+	+	
26	1292-1305	779-792	X	X	+	+	

Notes for Tables

Domain I

The arrow between bases 108-110 and 328-330 of Domain I is a possible long range interaction between conserved bases.

(a) The pairing shown for Xenopus differs from that drawn for yeast (4), but either is possible for both organisms. The yeast model is similar to the German model of E. coli in this area, and involves an exchange of pairing partners for stems 2 and 16, as shown by the arrow.

(b) The yeast model (4) has a bulged G rather than a bulged A, but Xenopus cannot fit that structure. The yeast sequence can however also fit the Xenopus model for this region, using a G·U base pair.

(c) Xenopus cannot fit the stem drawn here for yeast (4), but yeast fits our Xenopus model, as all bases of Xenopus stem 7 are conserved between yeast and Xenopus.

(d) The covalent linkage of 5.8S rRNA with the body of 28S rRNA is broken during processing by excision of the transcript of a transcribed spacer inserted into the terminal loop of the equivalent region in E. coli.

(e) The base of complex stem 13 is the same as the E. coli models. Our model for this region is a better fit for Xenopus and works for yeast, Physarum and E. coli.

Domain II

(f) The counterpart structure in the German model is in their domain I of E. coli 23S rRNA.

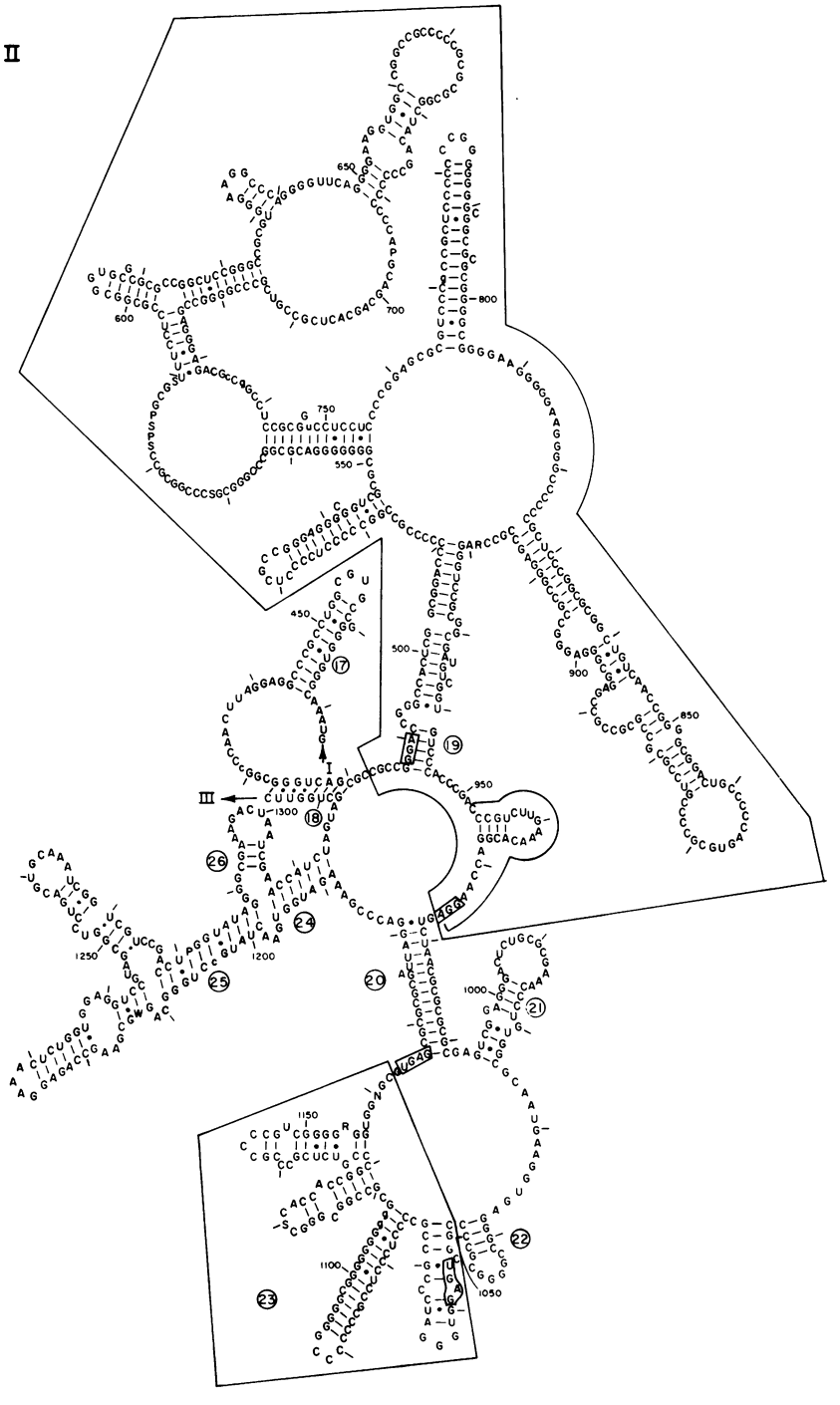
(g) Xenopus bases 952-976, shown by a bracket, is the only stretch >5 bases conserved between all known eukaryotic 26S/28S expansion segment sequences.

Domain III

(h) Xenopus does not fit the German or French models for the counterpart region of E. coli, but Xenopus does fit the American model quite closely except for a difference in the apex of Xenopus stem 31. The proposed base of the yeast stem (4) is the same as drawn here for Xenopus; the apex of the yeast stem can be redrawn to fit our Xenopus model, thereby increasing the number of yeast base pairs.

(i) The large internal loop between Xenopus stems 37 and 38 fits the open structure of the American E. coli model based on their bisulfite data. The base pairing suggested instead for this large internal loop in the yeast and other E. coli models does not work in an identical fashion for Xenopus. There are fewer base pairs in Xenopus stem 38 than in the E. coli models.

(j) This region is slightly larger in Xenopus than E. coli, but was not sufficiently larger to qualify as an expansion segment for Xenopus (5). However, its increased length in Physarum and rat would qualify it as an expansion segment in those species. There also appear to be extra bases here in yeast mitochondria (35).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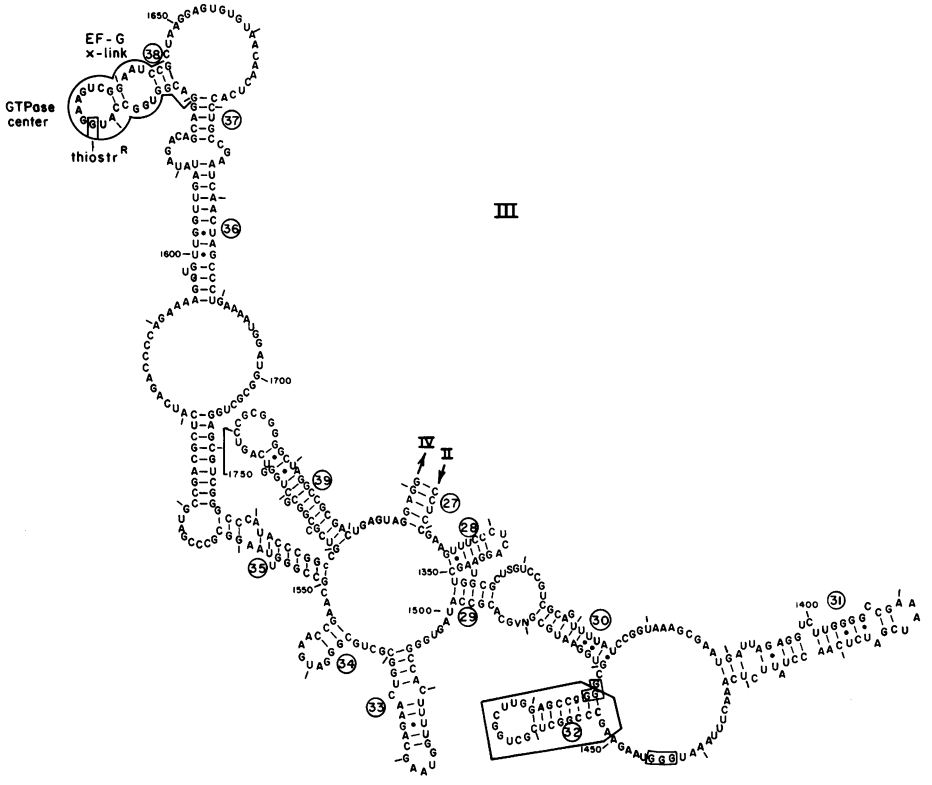

Table III: Domain III

Stem #	Xenopus bases	equivalent E. coli bases	Model in Agreement? E. coli German	E. coli American	E. coli French	yeast	notes
27	1326-1330/1777-1781	813-817/1190-1194	+	+	+	+	
28	1334-1350	821-835	+	+	+	(+)	
29	1351-1355/1495-1499	836-840/937-941	+	+	+	+	
30	1365-1375/1480-1489	848-856/923-932	(+)	+	+	(+)	
31	1389-1432	868-909	X	(+)	X	X	(h)
32 (E.S.4)	1451-1477	--------	-	-	-	+	
33	1506-1530	948-971	+	+	+	+	
34	1535-1546	976-987	+	+	+	+	
35	1550-1579/1707-1728	991-1019/1142-1163	+	+	+	+	
36	1595-1608/1677-1689	1030-1043/1112-1124	X	+	(+)	+	
37	1616-1620/1669-1673	1051-1056/1104-1108	+	+	+	+	
38	1622-1646	1058-1081	(+)	(+)	(+)	+	(i)
39	1730-1770	1165-1183	(+)	(+)	(+)	(+)	(j)

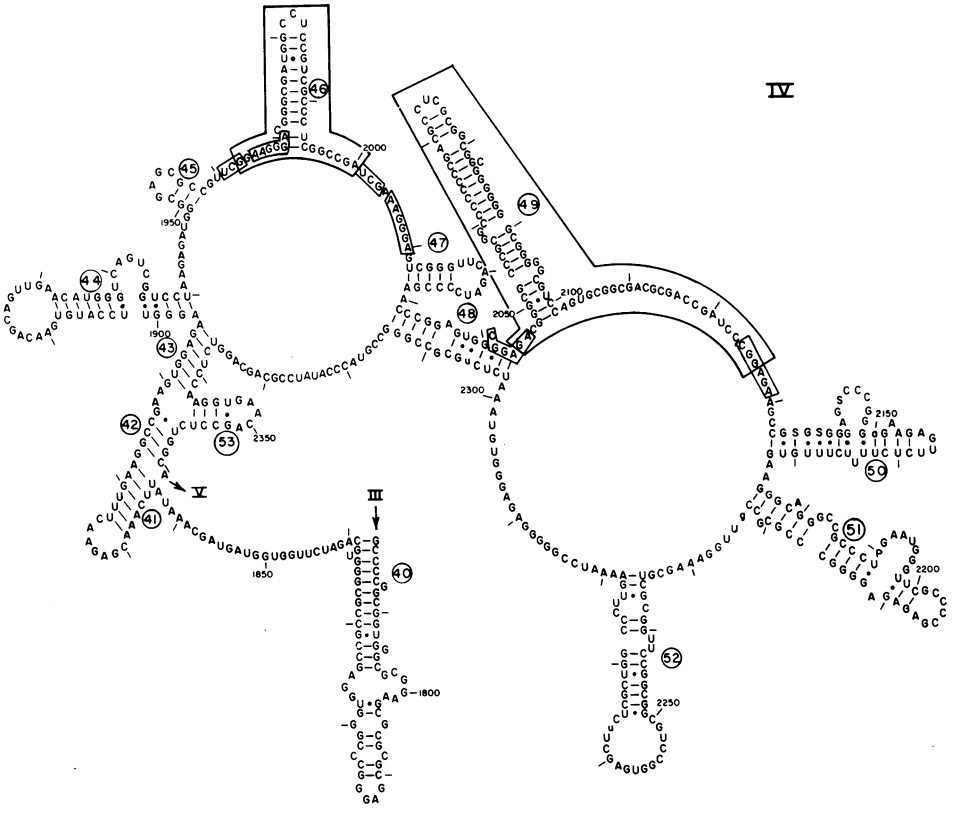

Table IV: Domain IV

Stem #	Xenopus bases	equivalent E. coli bases	Model in Agreement? E. coli German	E. coli American	E. coli French	yeast	notes
40	1782-1839	1195-1251	(+)	(+)	(+)	(+)	(k)
41	1864-1882	1276-1294	+	+	+	+	
42	1884-1887/2357-2360	1296-1299/1641-1644	X	+	+	X	(1)
43	1891-1896/2337-2342	1303-1307/1621-1626	+	+	+	X	(m)
44	1897-1940	1308-1351	(+)	(+)	+	(+)	(1,n)
45	1949-1958	1360-1370	(+ alt)	X	+	X	(1,o)
46 (E.S.5)	1963-2000	--------	-	-	-	(+)	(1)
47	2012-2029	1385-1402	+	+	+	X	(1)
48	2031-2044/2302-2314	1404-1417/1587-1598	+	+	+	X	(1)
49 (E.S.6)	2045-2126	--------	-	-	-	?	
50	2133-2172	1424-1463	+	X	X	?	
51	2175-2223	1466-1514	(+)	(+)	(+)	?	
52	2235-2278	1526-1569	(+)	X	X	?	
53	2343-2355	1627-1639	+	+	+	+	

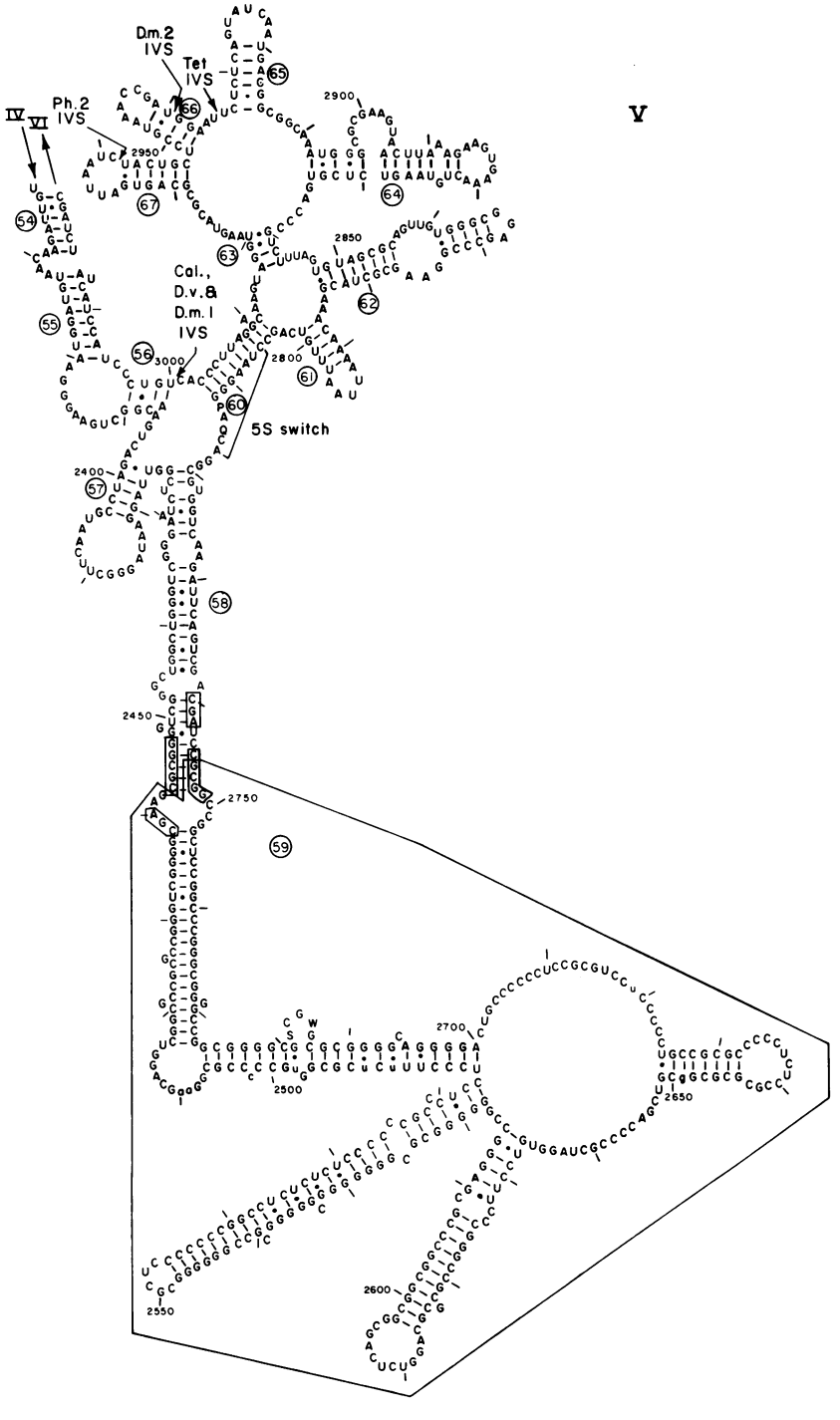
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Table V: Domain V

Stem #	Xenopus bases	equivalent E. coli bases	Model in Agreement? E. coli German	American	French	yeast	notes
54	2363-2368/3015-3020	1646-1651/2006-2011	X	+	X	(+)	
55	2373-2380/3006-3014	1656-1663/1997-2005	+	+	+	+	
56	2390-2393/3000-3003	1673-1676/1991-1994	+	X	+	+	
57	2399-2423	1682-1706	+	+	+	+	
58	2425-2457/2755-2780	1707-1756	(+)	+	+	?	(p)
59 (E.S.7)	2458-2754	--------	-	-	-	?	
60	2788-2796/2988-2997	1764-1772/1979-1988	+	+	(+)	(+)	
61	2799-2813	1775-1790	+	+	+	+	
62	2816-2852	1793-1828	+	+	(+)	(+)	
63	2857-2860/2980-2983	1833-1836/1971-1974	X	+	+	X	(q)
64	2866-2908	1842-1899	+	(+)	(+)	(+)	(r)
65	2915-2933	1906-1924	X	+	+	+	
66	2937-2952	1928-1943	X	+	+	+	
67	2953-2971	1944-1962	(+)	+	+	+	

Notes for Tables, continued

Domain IV

(k) All three E. coli models are identical here, and Xenopus fits them except that a small internal loop near the base of the E. coli stem is missing in Xenopus, and the apex of Xenopus stem 40 has some mismatched bases making it less stable than in E. coli. The proposed yeast model (4) is identical to that drawn here for Xenopus, except the yeast model invokes additional base pairs to diminish the size of the internal loop within the stem. These additional base pairs cannot be drawn for Xenopus.

(l) The yeast model differs from ours drawn here for Xenopus. The yeast sequence can be fitted to our model very well, and is then more similar to the E. coli models.

(m) There is a duplication in yeast nucleotides 1482-1488 and 1538-1544, and the counterpart of the former rather than the latter are used to form Xenopus stem 43. The counterpart of the latter (yeast 1538-1544) are part of Xenopus stem 45.

(n) Our structure is partially comparable to the yeast model, but they incorrectly designated this stem as an expansion segment in yeast (4), despite its partial sequence conservation with E. coli (5).

(o) Our Xenopus model is partially consistent with the German alternative structure (+ alt) for this region of E. coli.

Domain V

(p) Xenopus has a few extra bases at the base of stem 58 relative to E. coli, and the internal and apical loops of the E. coli counterpart are a part of the Xenopus stem. With some minor adjustments, the yeast sequence can be fitted to our Xenopus model of stem 58.

(q) The Xenopus sequence of stem 63 is totally conserved with that of yeast, so either the yeast model (4) or our Xenopus model can work for both species.

The revised German model (20) suggests that E. coli bases 1834-1836 pair with 1964-1966 to stabilize this stem, but this does not work well for Xenopus.

(r) Due to conserved sequence, either the yeast model (4) or our Xenopus model can fit both species.

Domain VI

(s) The American model has a smaller internal loop just next to the apical loop than the other E. coli models, and compensatory base changes in Xenopus support the American model.

(t) The looped out region in Xenopus between stems 77 and 78 and the comparable region in yeast cannot be drawn as the base-paired side stem shown in the E. coli models.

(u) The 3-4 base pairs at the base of this stem in the E. coli cannot be drawn for Xenopus nor yeast. An alternate form for these eukaryotes, but not drawn here as it does not also work for E. coli, is to extend the base pairing at the apex of the stem, thereby reducing the size of the apical loop.

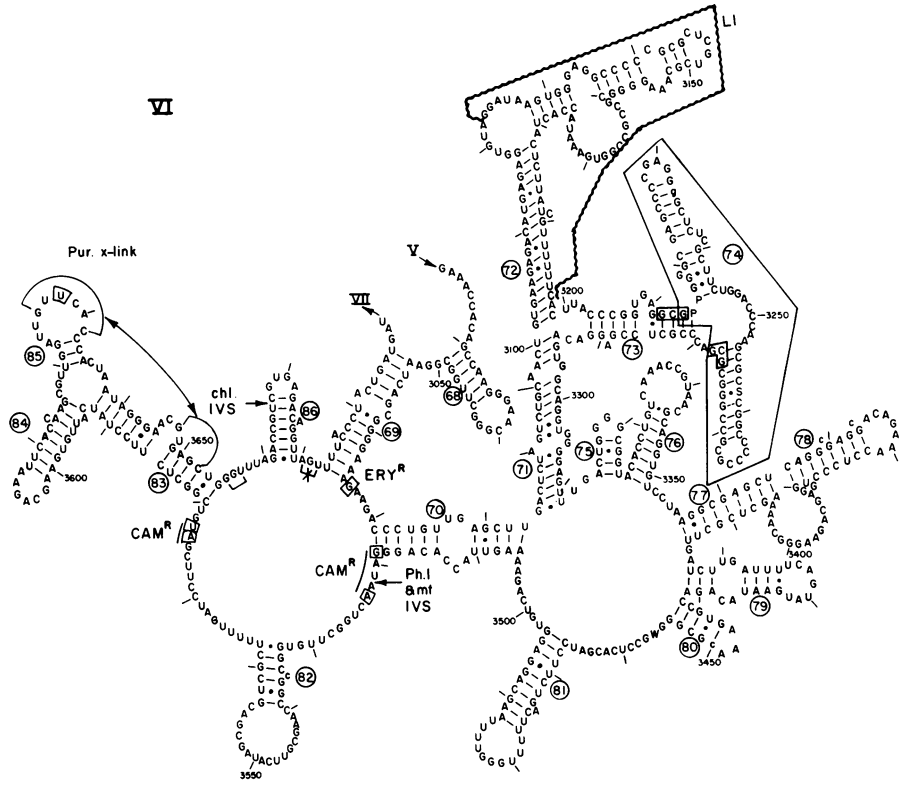


Table VI: Domain VI

Stem #	Xenopus bases	equivalent E. coli bases	Model in Agreement? E. coli German	E. coli American	E. coli French	yeast	notes
68	3030-3049	2022-2041	X	+	+	+	
69	3053-3065/3683-3696	2045-2057/2610-2623	(+)	+	(+)	(+)	
70	3071-3083/3506-3519	2063-2075/2434-2447	+	(+)	+	+	
71	3084-3100/3294-3310	2076-2092/2227-2243	(+)	+	(+)	(+)	
72	3101-3199	2093-2197	(+)	(+)	(+)	(+)	(s)
73	3203-3213/3281-3291	2201-2224	+	(+)	(+)	+	
74 (E.S.8)	3214-3278	--------	-	-	-	X	
75	3314-3324	2247-2257	+	+	+	+	
76	3325-3351	2258-2283	+	+	+	+	
77	3356-3364/3406-3414	2288-2296/2336-2344	+	+	+	+	
78	3365-3390	2297-2320	(+)	+	+	(+)	(t)
79	3417-3441	2347-2370	+	+	+	+	
80	3442-3454	2371-2382	+	+	+	+	
81	3467-3497	2394-2425	+	+	+	+	
82	3533-3562	2461-2489	(+)	(+)	(+)	+	(u)
83	3580-3592/3638-3654	2507-2519/2565-2581	X	+	+	+	
84	3593-3616	2520-2543	(+)	+	+	+	
85	3620-3634	2547-2561	X	+	+	+	
86	3661-3679	2588-2606	(+)	+	+	+	

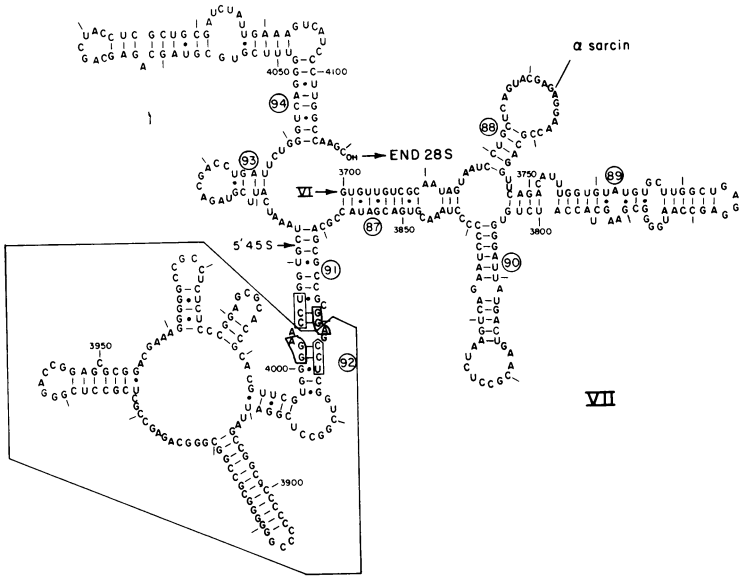


Table VII: Domain VII

Stem #	Xenopus bases	equivalent E. coli bases	Model in Agreement? E. coli German	American	French	yeast	notes
87	3700-3714/3842-3857	2628-2642/2772-2787	X	+	+	+	
88	3719-3745	2647-2673	(+)	+	+	+	(v)
89	3748-3803	2676-2731	(+)	(+)	+	+	
90	3805-3839	2735-2769	(+)	(+)	+	+	
91	3861-3870/4005-4013	2791-2809	+	+	+	+	
92 (E.S.9)	3871-4004	--------	-	-	-	x	
93	4019-4036	2815-2832	+	+	+	+	(w)
94	4041-4106	2837-2902	X	X	X	X	(x)

Notes for Tables, continued

Domain VII

(v) The slightly smaller apical loop shown in the German model of E. coli due to two added base pairs, is also possible for Xenopus and yeast but is not drawn as such here. This is the only difference between the three E. coli models.

(w) Stem 93 in Xenopus is somewhat shorter than the corresponding stem in yeast or E. coli; a stronger stem can be formed for Xenopus between 4015-4019 and 4033-4037 by slippage relative to the other models.

(x) The base of stem 94 in Xenopus is the same as in the yeast model, and the 3' end of this stem's base corresponds to the 3' end of 23S rRNA which can bind to the 5' end of 23S rRNA in E. coli but not in eukaryotes. The rest of stem 94 differs between our Xenopus model and that drawn for yeast (D-21), but both models work for both species.

segments. Based on position within Xenopus 28S rRNA (after correcting the earlier Xenopus ultrastructure map with regard to polarity [27, 28]) and based on hybridization results (Rein C. Brand, personal communication), the triple loop seen by electron microscopy and previously estimated to be 220-300 base pairs (25, 26) may be within expansion segment #2 (= complex

stem 12 of domain II). Similarly, the asymmetric double loop seen in Xenopus 28S rRNA may be within expansion segment #7 (= complex stem 59 of domain V). Since the length, and structure of expansion segments may vary between species, proof of their structure by compensatory base changes is often not possible and other models of their conformation may still be worked out.

DISCUSSION

DNA Insertions

We propose that there are three categories of DNA insertions into rDNA: introns, expansion segments, and transcribed spacers. The equivalent positions of DNA insertions of these three classes in a variety of organisms are indicated on our secondary structure model, and are discussed below.

(1) Introns

Introns have been found in the rDNAs in several organelles and in eukaryotic nuclei (though not in Xenopus nor other vertebrates). They appear to be of two types. The first type is represented by the introns sequenced in the rDNA of dipteran flies: Drosophila virilis (8), Drosophila melanogaster introns 1 and 2 (9-12), and Calliphora erythrocephala (15). This class of introns resembles transposable elements in structure and appear to lead to the inactivation of the intron containing rDNA (reviewed in 30). The second type of intron is found in the rDNA of organelles (chloroplasts: [31], and mitochondria: [32-35]) and in the nuclei of lower eukaryotes (Physarum introns 1 and 2: [36], and Tetrahymena: [6, 7]). In this class, the intron containing rDNA is transcribed, and in one case it has been found that the intron transcript can self-excise (37).

Despite their vastly different characteristics, both types of intron are found only in a limited area of the rDNA, corresponding to six sites in domains V and VI (Figs. V and VI). All introns are found in highly conserved regions of rDNA sequence (38, 39), but there are many other conserved regions in rDNA in which no introns have been found. What feature attracts introns to insert at only these six sites? There appears to be no primary sequence specificity for the site of intron insertion, except that a T residue occurs adjacent to the 5' (40) or 3' side of the intron. Intron transcripts interrupt both single-stranded and base-paired regions of the rRNA secondary structure. Therefore, rDNA sequence and the secondary structure of the rRNA transcript do not appear to direct the position of intron insertion. The only striking correlation we find is that all introns

are found in or very close to regions of rRNA which may be implicated in tRNA interactions (see below), but how this fact may be related to a mechanism for intron insertion is unclear.

(2) Expansion Segments

We previously defined expansion segments as eukaryotic specific inserted sequences $\geq$ 20 bases relative to E. coli (5); expansion segments can be found in both 18S and 28S rRNA. Using this definition, Xenopus 28S rRNA has nine expansion segments: they are found in all seven domains and are indicated as boxed-in regions in Figures I-VII. Our previous primary sequence alignment (5) has shown that these nine expansion segments occur in identical positions in all eukaryotic nuclear 26S-28S rDNAs sequenced to date (yeast: [4, 41]; Physarum: [36]; rat: [17]), but are absent from prokaryotic 23S rDNA. A few additional expansion segments beyond the nine we defined in Xenopus occur in Physarum and also in one case in yeast (5). Only expansion segments 1 and 4 are about the same length and secondary structure in Xenopus, yeast and Physarum 26S-28S rRNA. The other seven expansion segments differ in length between these three species, and it is difficult to draw a consensus secondary structure which fits all three species. The expansion segments often share properties reminiscent of mobile elements; in all nine of them there appears to be a small base duplication near the boundaries of the expansion segment, as shown by boxed-in groups of bases in the Figures. Although such short oligonucleotides are expected to occur fairly often by random chance, their position near the expansion segment boundaries is intriguing. Also, other sequences near the borders of the expansion segments are inverted repeats. Despite the general primary sequence divergence of any given expansion segment between vertebrates and lower eukaryotes, it is likely that a particular expansion segment evolved in all those species from the same original insertion as the insertion site into rDNA is conserved between species.

The diversity in sequence and often in length for each expansion segment suggests the possibility that they may not play a function in the ribosome. Indeed, in some cases described below, the transcripts of expansion segments may be removed during processing. The major exception to the nonconservation of expansion segment sequence, which we previously pointed out (5), is 25 completely conserved bases at the 3' end of expansion segment 2, shown by a bracket in Figure II (Xenopus nucleotides 952-976). These 25 bases may carry out a function common to eukaryotic ribosomes, but

altered or absent in prokaryotes. Furthermore, 2-3 conserved methylated nucleotides occur within this 25 base stretch in both yeast (4) and higher eukaryotes including Xenopus (42, 43). The only other stretch of bases conserved in expansion segments of Xenopus, yeast and Physarum 26S-28S rRNA is found in expansion segment 9 (Xenopus nucleotides 3958-3962).

(3) Transcribed Spacers

5.8S RNA found in all eukaryotes, is homologous to the 5' end of E. coli 23S rRNA (29, 44, 45). The coding region for 5.8S RNA is separated from the main body of the 26S-28S RNA gene by the internal transcribed spacer 2 (ITS 2). The ITS 2 is missing in prokaryotes, and may have an evolutionary origin similar to introns or to expansion segments. Like introns, the portion of RNA transcribed from the ITS 2 is removed by processing cuts, but unlike introns this is not followed by covalent ligation. Such ligation may not be necessary since both ends of 5.8S RNA are held by hydrogen bonds to 28S rRNA (46, 47), as can be seen in Figure I.

In some species, the expansion segments are also treated as transcribed spacers, such that the RNA transcripts of these regions are removed by processing cuts. In insects and many lower eukaryotes, 28S rRNA is subdivided into α and β halves; the transcribed spacer ("gap") separating the α and β halves of fungus fly 28S rRNA has been sequenced (Rainer Renkawitz, personal communication) and includes the fifth expansion segment corresponding to Xenopus stem #46 (Fig. IV). Similarly, the ninth expansion segment corresponding to Xenopus stem #92 (Fig. VII) is cleaved out during processing of higher plant chloroplast rRNA. The RNA 3' to this "spacer" is chloroplast 4.5S RNA (Fig. VII), and corresponds to the 3' terminus of E. coli 23S rRNA (29, 48-50).

Functional Sites

We have indicated on our secondary structure drawings some positions we deduce as candidates of functional importance for Xenopus 28S rRNA in the translation process. These functions are discussed below:

(1) Protein Binding Sites

In E. coli 17 of the 32 proteins of the 50S ribosomal subunit can bind to 23S rRNA, but only half of these 17 have been implicated as strong binding proteins needed for the first step of 50S reconstitution (51), and these are: L1, (L2), L3, L4, L9, L11, L20, L23, and L24 (52). Of these, L24 binds to the 5' end of 23S rRNA and is believed to be the initiator protein for normal 50S subunit biogenesis (53). The regions on 23S rRNA to which several of these proteins can independently bind have been studied by RNase

protection experiments and more recently their coordinate positions have been correlated with the E. coli 23S rDNA sequence (2):

ribosomal protein	E. coli 23S rRNA coordinate #	reference
L24	10-530	54-56
L4	615 (cross-link)	57
L20	(perhaps 800-850)	58
L11	1052-1112	59
L23	1342-1416 and 1588-1619	2, 58
L1	2084-2224	16, 60, 61

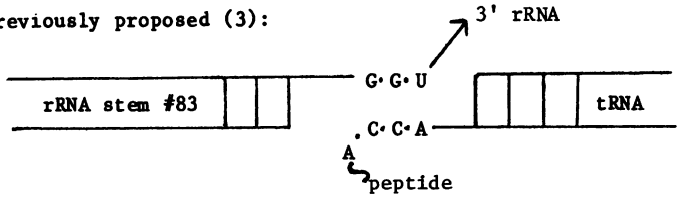
Although counterpart ribosomal proteins which are important for 60S subunit biogenesis must exist in eukaryotes, little is known about their rRNA binding sites. However, in the case of L1, the E. coli ribosomal protein can bind to Dictyostelium 26S rRNA (16); the corresponding position for heterologous L1 protein protection from RNase is shown in Fig. VI at nucleotides 3123-3197 on Xenopus 28S rRNA. It is likely that the region recognized by L1 may extend further down the left side of stem #72 to about nucleotide 3100 (Fig. VI), although this left side of the stem is not protected from RNase when L1 binds to eukaryotic rRNA. We previously drew a more open loop within the secondary structure of the L1 region (16), but compensatory base changes in other species, including archaebacteria, support the additional base pairing which we now show for Xenopus in Fig. VI (D.L. Thurlow, P.B. Cahill, M.L. Zeller, and R.A. Zimmermann, personal communication). Xenopus nucleotides 3118-3136 and 3161-3179 are highly conserved in sequence between eukaryotes and E. coli, suggesting their importance for L1 recognition. Within the conserved sequence, E. coli nucleotides 2112 and 2116 (Xenopus equivalents 3120 and 3124) are protected from kethoxal in polyribosomes (62).

(2) Peptidyl transferase center and tRNA sites

Peptidyl transferase effects the transfer of the nascent peptide chain from the acceptor stem of tRNA in the P site to the amino-acylated tRNA of the A site. Erythromycin inhibits peptide elongation, and a mutation to erythromycin resistance in yeast mitochondria has been mapped to the equivalent of E. coli rRNA base 2058 (63) (Xenopus equivalent base: 3066; ERY^R in Fig. VI). This base is in close proximity in our secondary structure model of Xenopus 28S rRNA to the equivalent sites of mitochondrial resistance mutations which remove the inhibitory effects of chloramphenicol on peptidyl transferase (32, 63-64) (E. coli equivalent 23S rRNA bases: 2447, 2451, 2503, 2504; Xenopus equivalent 28S rRNA bases: CAM^R 3519, 3523, 3576, 3577; Fig. VI). This open, single stranded region of conserved sequence in our model likely forms part of the peptidyl transferase center

in eukaryotes as well as in prokaryotes and organelles.

Coaxial stacking might help align and stabilize the position of tRNA on the ribosome for nascent peptide transfer (3). The location of a G G U trinucleotide adjacent to the base of a stem in E. coli 23S rRNA, and which was proposed for pairing leading to coaxial stacking with the 3' end of tRNA (3) is not conserved in Xenopus; this interaction which cannot work in Xenopus is depicted in Figure VI by a bracket which has been crossed out. However, there is a G G U trinucleotide nearby, indicated by the bracket in Fig. VI, which is adjacent to stem #83 of Xenopus 28S rRNA, and is conserved in sequence between all eukaryotes and E. coli. Pairing with this G G U would allow coaxial stacking with tRNA in the same manner as previously proposed (3):



Our candidate site for potential coaxial stacking with tRNA is still within the peptidyl transferase center proposed above.

There is little direct data on tRNA binding sites of the large ribosomal subunit. Presuming that initiator $tRNA^{met}$ directly enters the ribosome at the P site (rather than binding to a distinct I site; see ref. 65 for discussion on this issue), complementarity between $tRNA^{met}_{init}$ and 28S rRNA might at least define candidates for the P site for future experimental testing. Three potential interactions are:

Xenopus $tRNA^{met}_{init}$	Xenopus 28S rRNA	Comments
1-8/70-75 (acceptor stem)	2928-2943 (domain V)	Must open stems 65 and 66 in Xenopus 28S rRNA and acceptor stem of $tRNA^{met}_{init}$ for this interaction (38).
13-20 (D-loop)	2993-3001 (domain V)	First suggested for E. coli (66), and also works in chloroplasts (67).
10-18 (D-loop)	3524-3532 (domain VI)	Must open stem in E. coli 23S rRNA for this same interaction.

Some meager evidence exists for the A site of the large ribosomal subunit. A derivative of puromycin, which mimics amino-acylated tRNA, has been cross-linked to E. coli 23S rRNA (68, 69), and although there is some ambiguity in the data it has been argued that the cross-link is to

nucleotide #2555 (2). Nucleotide #3628 of Xenopus 28S rRNA, which corresponds to this deduced cross-link site for puromycin in E. coli, is indicated by a box in Fig. VI. A conserved methylated U is found in this region in mitochondrial rRNA (70) and corresponds to Xenopus base 3625 (E. coli 2552). Xenopus nucleotides 3625-3629 are conserved in primary sequence between eukaryotes and E. coli, supporting the idea that this loop which is suggested as the A site of peptidyl transferase in E. coli is also likely to play the same role in Xenopus.

Since α-sarcin inhibits EF-T_1 dependent binding of amino-acylated tRNA (71-73), the α-sarcin cleavage site in domain VII has been proposed as part of the A site. α-sarcin cleaves E. coli, yeast and rat rRNA (74-76) and the corresponding cleavage position is shown after nucleotide #3733 of Xenopus 28S rRNA in Fig. VII.

(3) GTPase center

GTP is hydrolyzed both during the initiation of translation and during the translocation of tRNA from the A site to the P site in prokaryotes. E. coli ribosomal protein L11 is needed for protein L10 binding and subsequent association with L7/L12 which forms the GTPase stalk of the 50S subunit (77). The antibiotic thiostrepton binds to E. coli 23S rRNA + L11, blocking EF-G association to the ribosome and therefore stopping EF-G dependent GTPase activity and inhibiting translocation (78). Protein L11 protects bases 1052-1112 of E. coli 23S rRNA, suggesting that the stem and loop represented by these nucleotides may form part of the GTP hydrolysis center. Moreover, methylation of A_{1067} by a methylase from Streptomyces azureus (a producer of thiostrepton) blocks thiostrepton binding and its inhibitory effects (78). Eukaryotes such as Xenopus have a G residue at the position of A_{1067} of bacteria (thiostr^R and boxed-in G in Fig. III) which may help explain the decreased sensitivity of eukaryotes to thiostrepton. Finally, nucleotides 1055-1081 of E. coli 23S rRNA, which include this proposed GTPase center, can be cross-linked to EF-G (79). The conserved loop+stem #38 which corresponds to the EF-G cross-linked region and which we propose as the eukaryotic GTPase center is shown by a bracket in Fig. III for Xenopus 28S rRNA. It is interesting that G_{1071} of the GTPase center of E. coli (Xenopus equivalent base 1636) is protected from kethoxal by mRNA/tRNA association in polyribosomes (62).

(4) 5S RNA interaction with 28S rRNA

The complex of E. coli ribosomal proteins L5, L18, and L25 plus 5S RNA has been found associated with nucleotides # 2282-2389 of E. coli 23S RNA

(80) (Xenopus equivalent bases 3350-3461 of domain VI). Mammalian mitochondrial ribosomes seem to lack 5S RNA, and they also lack this binding region of the large ribosomal RNA (1). Reconstituted E. coli 47S particles (which lack 5S RNA, L5, L16, L18, and L25) are inactive for the peptidyl transferase reaction; they can be reactivated by addition of 5S RNA (81). Notice that the proposed peptidyl transferase center is nearby to the rRNA region associated with 5S RNA, L5, L18, and L25 in domain VI. Tantalizing though the data are, the evidence is not yet sufficient to state whether 5S RNA interacts directly with this region in domain VI of rRNA, or instead if 5S RNA association to domain VI is simply mediated via protein interactions.

The evidence for 5S RNA association with another region of 23S rRNA is stronger. In this case, analysis of hydrogen bonded fragments by the German group showed that in E. coli 5S RNA is found hydrogen-bonded to residues #1759-1768 of 23S rRNA (1). The same interaction can also be drawn for maize chloroplasts (67) and for domain V of Xenopus (nucleotides 2782-2794; Fig. V) and is supported by compensatory base changes in these organisms. Notice that the rRNA stem implicated in this reaction must open up so that one side of the stem can hydrogen bond to 5S RNA.

(5) RNA-RNA Switches

It has been hypothesized that a series of switches in RNA-RNA pairing may be fundamental to the translocation mechanism (reviewed in [82]). Three alternative pairing interactions in E. coli 23S rRNA have been deduced by the German group as possible candidates for a role in switching (1).

The first candidate switch for E. coli 23S rRNA can also be drawn in Xenopus. Xenopus 28S rRNA stem 60 (Fig. V) could open up so that one side of the broken stem might pair with 5S RNA and the other side of the broken stem might pair with the D-loop of $tRNA^{met}_{init}$. A dynamic model incorporating both the $tRNA^{met}_{init}$ and 5S rRNA interaction at this particular site in E. coli 23S rRNA has been proposed by Dr. Christian Zwieb (personal communication). He has shown by simple model building that these three molecules can be arranged in a three dimensional structure in support of his proposal.

A second candidate switch of the German group involves E. coli 23S RNA bases 184-242 (Fig. IIa of reg. [1]), but it has no parallel in eukaryotes. However, their third possible RNA-RNA alternative interaction does work for both prokaryotes and eukaryotes. In this case in E. coli 23S rRNA there could be pairing either between bases 2553-2557 and 2575-2579 or else between 2506-2512 and 2576-2582 (1). The same possibility for alternative

partners exists in Xenopus 28S rRNA as shown by the double-headed arrow in Fig. VI. As discussed above, it is likely that E. coli 23S rRNA base #2555 (contained within this putative switch) has been cross-linked to a puromycin derivative, suggesting that this may be part of the A site. Changing from the structure shown in Fig. VI to the switched conformation could conceivably accompany or drive the A to P site transition of the tRNA. A full appreciation of how putative RNA switches may work awaits derivation of the three-dimensional structure of rRNA and other future experiments.

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Noted added in proof: After this paper was accepted, two reports on the secondary structure of 28S rRNA of rat and mouse, respectively, came to our attention (A.A. Hadjiolov, O.I. Georgiev, V.V. Nosikov, and L.P. Yavachev, Nucleic Acids Res. 12: 3677-3693 [1984]; B. Michot, N. Hassouna, and J.-P. Bachellerie, Nucleic Acids Res. in press [1984]). These two studies generally agree with ours on the structure of the conserved core of 28S rRNA, but their models of certain expansion segments differ. Hadjiolov et al. propose for expansion segments 2 and 7 a triple loop and asymmetric double loop, but they are not held together at their base by long range interactions. On the other hand the different triple loop and asymmetric double loop of Michot et al. are each held together by long range interactions, as expected from S1 nuclease data ([26] and R. Brand and S. Gerbi, unpublished results). However, these long range interactions involve extensive pairing of expansion segment with 28S conserved core sequence, contrary to a mechanism of simple insertion of expansion segment into a pre-existing conserved core structure. Clearly more experimental work needs to be done in order to have conclusive structures for the expansion segments.