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A Possible Structure of the Rabbit Reticulocyte Ribosome

AN EXERCISE IN MODEL BUILDING

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It is suggested that the location of each of the diverse ribosomal proteins in the ribosome, or in the ribosomal subparticles in their native or derived forms, is determined by the nucleotide sequence of the 16s and 23–30s RNA moieties, i.e. that the 16s and 23–30s RNA species provide a unique binding site for each species of ribosomal protein. The ways of assembling such a thread into a ribosome-like structure appear limited if the ribosome is largely stabilized by protein–protein interactions. The nucleoprotein thread was built into a structure, having the general features of a hollow cylinder, that is consistent with the known dimensions and properties of the rabbit reticulocyte ribosome. It appears possible to test the model by experiment.

The term 'ribosome' was suggested by Roberts (1958) to denote particulate ribonucleoprotein of molecular weight $2{\cdot}7 \times 10^6$–$4{\cdot}5 \times 10^6$ daltons comprising 40–65% RNA and little or no lipid. Whatever their source ribosomes appear to have the same function in protein biosynthesis, suggesting that the general features of structure are preserved irrespective of differences in size and chemical composition (for reviews see Arnstein, 1963; Petermann, 1964; Spirin, 1967).

The small RNA viruses that infect bacterial, plant and animal cells are also ribonucleoprotein particles of about the same molecular weight as ribosomes. Although the three-dimensional structure of several viruses is known the molecular architecture of the ribosome remains ill-defined. Studies of the structure of small viruses are facilitated because they are symmetrical and because the subunits of the protein coat are identical (for discussion see Caspar & Klug, 1962).

In contrast with the coat proteins of spherical RNA viruses (Crick & Watson, 1956; Caspar & Klug, 1962) ribosomal proteins are heterogeneous with respect to both primary sequence and size (10000–30000 daltons) (Möller & Chrambach, 1967; Hamilton & Ruth, 1967; Fogel & Sypherd, 1968; Moore, Traut, Noller, Pearson & Delius, 1968); thus there appear to be a number of different proteins per class of ribosome particle and a limited number of copies of a particular protein may be found in each particle. An investigation of the secondary structure of ribosomal RNA in solution led to the formulation of a hypothetical model of the rabbit reticulocyte ribosome (Cox, 1967, 1968) that was devised as a guide for further work. The aim of the present paper is to describe the model, which, although it is speculative, could lead to predictions that are capable of being tested experimentally.

The model, which conforms to the known dimensions and accounts for the known properties of the ribosome (see Table 1), was based on the following assumptions:

(1) The location of each of the diverse ribosomal proteins in the ribosome, or the ribosomal subparticles in their native or derived forms, is determined by the nucleotide sequence of the 16s and 23–30s RNA moieties, i.e. the 16s and 23–30s RNA species provide a unique binding site for each species of ribosomal protein.

(2) In the ribosome and in native subparticles protein–protein interactions are important, i.e. most of the protein subunits are in contact with at least four other protein subunits.

(3) RNA has essentially the same secondary structure within the ribosome as it has in solution after the protein is removed (for review see Cox, 1969*a*).

(4) The proteins are globular with a partial specific volume of about 0·72 (Cohn & Edsall, 1943).

(5) The protein size range is 10000–30000 daltons (cf. Hamilton & Ruth, 1967), the average being 20000 daltons. The diameter of the protein subunits (which are assumed to be globular) varies in the range 30–40 Å.

(6) Since the reticulocyte ribosome is made up of roughly equal amounts of RNA and protein, a protein subunit of 20000 daltons should associate with a sequence of about 20000 daltons (about 60

Table 1. *Properties of rabbit reticulocyte ribosomes*

Property	Value	Reference
Molecular weight	$4{\cdot}0\times10^6$–$4{\cdot}1\times10^6$	Dintzis, Borsook & Vinograd (1958)
RNA(g.)/protein(g.) ratio	1:1	Dintzis *et al.* (1958)
$S^0_{20,w}$	80 ± 2s (ribosome); 60 ± 2s (larger subparticle); 40 ± 2s (smaller subparticle)	
$[\eta]$	8ml./g.	Dintzis *et al.* (1958)
Partial specific volume	0·63ml./g.	Dintzis *et al.* (1958)
Frictional ratio (f/f_0)	1·72	Dintzis *et al.* (1958)
Dimensions from electron microscopy	250Å × 175Å (negative staining); 200Å × 155Å (positive staining)	Matthias, Williamson, Huxley & Page (1964) Matthias *et al.* (1964)
Radius of gyration	108Å	Dibble & Dintzis (1960)
Diameter in solution	340Å	Dintzis *et al.* (1958)
Axial ratio	1:1	Dintzis *et al.* (1958)
Behaviour in solution	Compact particle	Inouye, Shimagawa & Masumura (1963)
Molecular weight of RNA	3×10^4, $0{\cdot}5\times10^6$, $1{\cdot}5\times10^6$	Bachvaroff & Tongur (1966); Cox & Arnstein (1963)
Molecular weight of polypeptide chains	10000–26000 by analogy with liver ribosomal proteins	Hamilton & Ruth (1967); Low & Wool (1966)
X-ray diffraction	45–50Å reflexion	Langridge (1963)

nucleotides) of RNA. A sequence of about 60 nucleotides will have a well-defined secondary structure (Cox, 1966). This is believed to be a linear array of hairpin loops. On the basis of the estimated sizes of the hairpin loops of reticulocyte ribosomal RNA (25 ± 5 residues on average for RNA from the smaller subparticle and 30 ± 6 to 35 ± 7 residues for RNA from the larger subparticle; Cox, Gould & Kanagalingam, 1968), interaction between one protein subunit and a length of RNA comprising two hairpin loops is most likely. Other possibilities cannot be entirely eliminated. When scale models of reticulocyte ribosomal RNA (see Plate 1*a*) were built according to the features reported by Cox *et al.* (1968) it was found that a sphere representing a globular protein of diameter 30–40Å fitted between two hairpin loops (see Plate 1*b*). On this basis the binding site is a unique three-dimensional structure that is made up of the single-stranded region joining one hairpin loop to another, the unpaired regions within the hairpin loops and the double-helical segments of the two loops. A feature of a ribonucleoprotein thread formed in this way is that each unit of one protein subunit and two hairpin loops is linked through a single-stranded region that would confer flexibility upon the thread and also render it very sensitive to hydrolysis by ribonuclease, since the hydrolysis of any diesterified phosphate linkage within this region would immediately affect the molecular weight.

Assembly of the ribonucleoprotein thread into a ribosome-like structure

The feasibility of packing a ribonucleoprotein thread into an ordered structure with the known dimensions and properties of rabbit reticulocyte ribosomes (Table 1) was then considered. The assumption that protein–protein interactions approach maximum excludes conformations produced by randomly coiling the ribonucleoprotein thread. The thread could be coiled into a spiral forming an irregular hollow cylinder (Cox, 1967). Alternatively the thread may fold backwards and forwards upon itself to form a sheet (see Figs. 1*a* and 1*b*) about 80Å thick, which could then be folded into a horseshoe shape of the correct dimensions having a cleft of not less than about 20–30Å (Plate 2). The length of the ribonucleoprotein thread will determine the size of the sheet and hence the characteristics of the horseshoe. The smaller subparticle,

EXPLANATION OF PLATE 1

Hypothetical model of reticulocyte ribosomal RNA and ribonucleoprotein. (*a*) Part of the RNA chain of the smaller subparticle. The ribose phosphate backbone is represented by wire and the base residues are represented by the short tags. Each hairpin loop was assumed to comprise seven base pairs, 3Å apart, and nine unpaired residues. The flexible region joining one hairpin loop to another was taken to be five to ten residues long. It is likely that the unpaired residues within the loop may tend to stack one upon another, so that the hairpin loop may have the general features of the anticodon loop of transfer RNA proposed by Fuller & Hodgson (1967). (*b*) Part of the ribonucleoprotein chain: one protein per two hairpin loops. The protein subunits (40Å diameter) are represented by the white balls. The single-stranded regions might be in intimate contact with protein. The flexible region joining one unit of a protein plus hairpin loops to another permits protein–protein interactions between adjacent protein subunits. The proteins are heterogeneous (see, e.g., Hamilton & Ruth, 1967; Möller & Chrambach, 1967; Fogel & Sypherd, 1968; Moore *et al.* 1967; Chersi, Dzionara, Donner & Wittman, 1968; Nomura & Traub, 1968).

which is too small to form a horseshoe, could form a sheet of five-by-four repeating units that fits like a cap on to the larger subparticle. The complete structure of this 'horseshoe and cap' model has the dimensions 160–200 Å × 160–200 Å × 240 Å (Plate 3) and appears to us the most likely. Neither the orientation of the double-helical segments at the surface nor the site of interaction of the subparticle is known. The model illustrated in Plate 3 was constructed from RNA chains in which the hairpin loops are of uniform size, with the result that the RNA moiety is distributed equally between the inside and outside surfaces. If the hairpin loops are not uniform in size the RNA will be unequally distributed between the inside and outside surfaces. In constructing the illustrated model hairpin loops were placed alternately on either surface with most of the proteins in contact with six neighbours. A plan of the proposed model is shown in Plate 4. There might be other ways of folding the polynucleotide chain, but the condition that most of the proteins should be in contact with at least four other subunits generates structures for the larger subparticle that have a groove or a cavity and structures for the smaller subparticle that are sheet-like.

EXPLANATION OF PLATES 2–4

Model of a hypothetical structure of the rabbit reticulocyte ribosome. Ribosomal RNA (see Plate 1) was built of wire and tags according to the data for RNA summarized by Cox *et al.* (1968). Each sphere represents a unique protein (two-thirds have a diameter corresponding to 40 Å and one-third a diameter corresponding to 30 Å) and the hairpin loops are not identical, so that the surface of the model is heterogeneous. Plate 2 shows the larger subparticle, which was built from a thread of 45 protein subunits and 90 hairpin loops. The cleft or groove has a minimum width of 20–30 Å, but could be wider. A cleft about 40 Å wide could accommodate transfer RNA. Plate 3 shows the whole ribosome. The smaller subparticle, which was constructed in the same way as the larger subparticle, is fitted like a cap on to the larger subparticle. It is envisaged that messenger RNA is bound on the inside surface of the smaller subparticle, possibly parallel to the axis of the cylinder, with the growing polypeptide chain being extruded through the groove. The possibility exists that the binding sites of both amino acid transfer RNA and polypeptide transfer RNA lie within the groove. We suggest that aminoacyl-transfer RNA enters at one end where it is bound first to the aminoacyl-transfer-RNA-binding site and then, on the formation of the peptide bond, is bound to the peptidyl-transfer-RNA-binding site and is subsequently expelled through the other end of the cleft. This hypothetical model serves to show that the form of a hollow cylinder is feasible. The essential features of the model would be preserved if other ways of folding the nucleoprotein thread were used, provided that most of the proteins were in contact with at least four other subunits (see, e.g., Fig. 1). The essential features would also be retained by a less regular structure, e.g. one in which occasional protein subunits were missing, if there were an excess of protein, or if the number of repeating units varied from layer to layer. It is possible, on the basis of the estimates of the number of proteins and of the size of the hairpin loops, that the larger subparticle comprises about 60 proteins and about 120 hairpin loops. A sheet of six rows of ten subunits folded into a horseshoe shape would have dimensions (200 Å × 200 Å × 180 Å) that are acceptable for the larger subparticle. This hypothetical model and the one proposed by Spirin (1963) appear to have common features. Plate 4 shows a plan of the proposed model. The protein subunits of the smaller subparticle are denoted by stippling and bars, the protein subunits of the larger subparticle are shown by stippling alone, and the hairpin loops of RNA, which are placed alternately inside and outside the cylinder, are represented by the smaller open circles.

Comparison of the properties of the proposed model with those observed for ribosomes

Function in protein biosynthesis. The protection of 30–35 amino acids of the growing polypeptide chain from proteolytic enzymes (Malkin & Rich, 1967) and the inaccessibility of a segment of messenger RNA, about 30 nucleotides long, to nucleases (Takanami & Zubay, 1964; Takanami, Yan & Jukes, 1965) argue for the presence of a protected environment inside the ribosome. The inner groove of the model fulfils this requirement provided that the messenger is bound to the inside surface of the smaller subunit and the nascent chain is extruded through the central cavity.

This is in accord with the view that the subparticles themselves play an important role in protein biosynthesis. The first step appears to be the association of messenger RNA with the 30s ribosomal subunit and the initiating transfer RNA, which is followed by the reaction of this complex with the 50s subunit (Ghosh & Khorana, 1967) to form the messenger RNA–ribosome complex necessary for peptide-bond formation. There is evidence that when the end of the message is reached the ribosome dissociates into subparticles, when they join a common pool and are then recycled (Kaempfer, Meselson & Raskas, 1968; Colombo, Vesco & Baglioni, 1968).

The proposal that messenger RNA is bound on the inside of the smaller subparticle provides an explanation for the observation that polyribosomes dissociate directly into subparticles without first forming ribosomes as intermediates (e.g. Bonanou, Cox, Higginson & Kanagalingam, 1968).

Hydrodynamic properties. The proposed model has dimensions (180 Å × 180 Å × 240 Å) that agree with the observed values of 185 Å × 200 Å obtained be electron microscopy (Dibble & Dintzis, 1960). The axial ratio of the model is small (1·3:1) and would not be distinguishable from the ratio 1:1

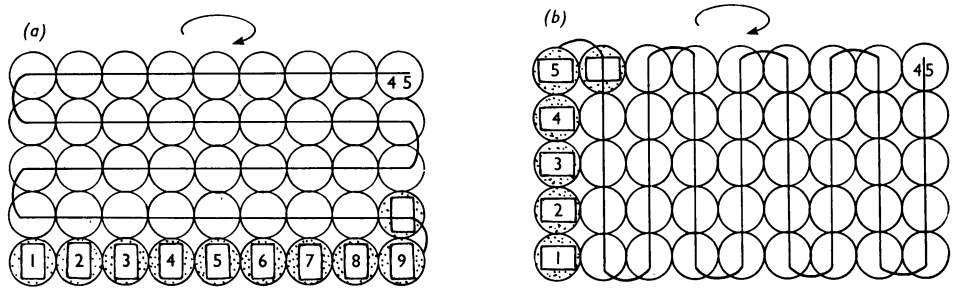

Fig. 1. Diagram of sheets formed by folding the thread constructed on the basis of the assumption that one protein subunit interacts with two hairpin loops. The thread is shown in its most compact form, comprising 45 repeating units and measuring 80 Å × 200 Å × 360 Å. The arrow indicates the axis around which the sheet is folded to give a horseshoe. The orientation of the double-helical segments with respect to the cylindrical axis of such a particle is not known. In (*a*) there are five rows of nine subunits and the RNA double-helical regions are shown parallel to the cylindrical axis. In (*b*) there are nine rows of five subunits and the double-helical segments are shown perpendicular to the cylindrical axis. The protein subunits are represented by circles and the double-helical segments of RNA by the rectangles (for clarity only a few are shown). The direction of the ribosomal RNA chain is indicated by the continuous line. About half the protein subunits shown in the figure are essential for the maintenance of the sheet-like conformation. For example, most of the odd-numbered protein subunits would still make four contacts with other proteins (see assumption 2) even if all the even-numbered proteins were removed. Similarly, if the 45 protein subunits are arranged so that most make six contacts, 17 may be removed without violating assumption 2. Thus the essential features of our model may be preserved even though the ratio of RNA to protein may vary widely.

by hydrodynamic methods. The frictional ratio, f/f_0, found for ribosomes is unusually high (about 1·7) and is attributable to hydration rather than to asymmetry (Dintzis *et al.* 1958). The proposed model has two features that may increase the frictional ratio: first, the surface area of the model is about 1·7–1·9 times that of the equivalent compact sphere, so that a substantial increase in hydration would be expected; secondly, the cleft in the centre might also increase f/f_0. Petermann & Pavlovec (1969) found that the larger subparticle appears to be more highly hydrated than the smaller subparticle of rat liver ribosomes. From this it was inferred that there might be a hole or a groove in the larger subparticle. Although the proposed model is a compact particle (in agreement with the high value of $S_{20,w}$ and low value of $[\eta]$ observed), it is not a uniform hard sphere and so agrees with the low-angle X-ray-diffraction data (Dibble, 1964).

Structural features. (*a*) X-ray-diffraction studies. A 45–50 Å reflexion was reported for rabbit reticulocyte ribosomes (Langridge, 1963). This was interpreted as evidence for arrays of four or five double-helical segments of RNA separated by 45–50 Å. The proposed model has features consistent with this interpretation.

(*b*) Electron microscopy. The electron-microscopic studies of ribosomes in general (*Escherichia coli* ribosomes are the commonly studied species) do not conflict with the model. Horseshoe-shaped larger subparticles have been reported (Bruskov & Kiselev, 1968). An eye-like region in the centre of the ribosome was reported by Hart (1965) and by Nanninga (1967). The presence of sharp edges was deduced (Lubin, 1967; Nanninga, 1968). The model does not conflict with the observations of Sabatini, Tashiro & Palade (1966) on the attachment of mammalian ribosomes to membranes, or with the presence of a channel within the ribosome (Redman & Sabatini, 1966; Florendo, 1968).

Interaction with small molecules. It is evident from the model that most of the 16s and 30s RNA species lie on the surface and should be freely accessible to those molecules that are small enough to penetrate the cleft. Thus the proposed model provides an explanation for the accessibility of the nucleic acid moiety to cationic dyes (Furano, Bradley & Childers, 1966; McPhie & Gratzer, 1966; Miall & Walker, 1967) and to cations (Edelman, Ts'o & Vinograd, 1960; Bohn, Farnsworth & Dibble, 1967; Sheard, Miall, Peacocke, Walker & Richards, 1967; Choi & Carr, 1967). Once the proteins were 'fixed', presumably by cross-linking the protein subunits one with another, formaldehyde was found to react as readily with at least 80% of the base residues of the RNA moiety of ribosomes as with the base residues of free RNA. As a consequence of the reaction with formaldehyde the conformation of the RNA moiety was converted from a partly double-helical into a single-stranded form. This conformational change is accommodated without altering either the sedimentation coefficient or the appearance of the ribosome as seen through the electron microscope (Cox, 1969*a*). These results

support the notion that the RNA moiety is on the surface of the ribosome.

Changes in the conformation of the subparticles. The reversible conformational changes of the ribosomal subparticles (Gavrilova, Ivanov & Spirin, 1966; Gesteland, 1966) brought about by changing the ionic environment imply that their shape can vary between wide limits, and could be explained on the basis of the 'horseshoe and cap' model. For the larger ribosomal subparticle one can visualize a transformation from a compact faster-sedimenting horseshoe form to a slower-sedimenting open sheet; further changes in sedimentation velocity might be regarded as a co-operative unwinding of the layers of the sheet. The smaller ribosomal subparticle might also be expected to undergo changes in shape on changing the ionic strength, as is, in fact, reported.

Effect of ribonuclease (*see Cox*, 1969b). The molecular weight of EDTA-subparticles decreased rapidly after exposure to small amounts of ribonuclease. This is in accord with the structure of the hypothetical ribonucleoprotein thread in which every other single-stranded region joining one hairpin loop to another is unprotected by protein. The findings that 20–30% of the RNA moiety may be digested away, that the 30s RNA component from the larger subparticle is broken at about 40 sites separated by about 100 nucleotides and that the 16s RNA component from the smaller subparticle has about 20 sites that are sensitive to ribonuclease are all in accord with the model, which predicts that the sites of the 16s and 30s RNA components that are sensitive to the enzyme are the hairpin loops (about 20 for the smaller and about 45 for the larger subparticle) on the outside surface of the hollow cylinder. The large proportion of chain scissions that are 'hidden' by double-helical secondary structure also suggests that the sensitive sites are the hairpin loops. The observation that a substantial proportion of the major RNA components are protected from enzymic hydrolysis agrees with the proposal that RNA is partly located on the inner surface of a groove or hole within the ribosome. Once the ribosome is formed the particle survives the hydrolysis of the principal RNA components, as would be expected if protein–protein interactions are important to stability. Thus the effects of digestion with ribonuclease on reticulocyte ribosomes are in accord with the proposed model.

Role of 5s *RNA*

The second structural RNA component, 5s RNA, of the larger subparticle (Rosset, Monier & Julien, 1964; Bachvaroff & Tongur, 1966; Forget & Weissman, 1967; Brownlee, Sanger & Barrell, 1968) becomes attached to the larger subparticle at a late stage in the maturation of the ribosome and then remains in permanent association (Kaempfer & Meselson, 1968). The 5s RNA remains attached to the larger subparticle even after 20% of the protein is removed, but is liberated when more protein is lost or on titration with EDTA (Morell & Marmur, 1968; see also Comb & Sarkar, 1967). These observations suggest that 5s RNA might be required to maintain the proposed horseshoe conformation.

Comparison with other models

Apart from models based on electron-microscopic studies (Hart, 1965; Nanninga, 1968; Bruskov & Kiselev, 1968), other models have been proposed. For example, the studies of Cotter, McPhie & Gratzer (1967) (cf. Cox, 1967) with yeast ribosomes led to the principal tentative conclusions: (*a*) that the conformation of the RNA in the ribosome is similar to that in the free state, and contains about 60% of paired bases in short double-helical segments; (*b*) that proteins are not associated with the double-helical parts of the RNA, and may be packed into the non-helical loops, or associated with each other in some of quaternary structure, or both; (*c*) that the surface of the ribosome consists chiefly of RNA and not protein; (*d*) that this surface RNA is, by inference, largely double-helical, and the helices probably project outwards from the surface. In contrast, Worcel, Goldman & Sachs (1968) suggested that ribosomal RNA forms an inner core of the ribosomes of *Mycobacterium tuberculosis* with the protein subunits arranged as a coat around the central RNA backbone. We believe that our more detailed model agrees more closely with the known properties of ribosomes. Our aim in undertaking model-building studies was to provide a guide for further work, and the proposed model appears to fit the known data for ribosomes and their constituents sufficiently well to serve this purpose. We emphasize that our model is speculative and that we cannot exclude other ways of producing feasible structures.

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