STRUCTURAL TRANSFORMATIONS OF RIBOSOMES (DISSOCIATION, UNFOLDING AND DISASSEMBLY)

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Received 2 January 1974

1. Introduction

Besides morphological descriptions the following is known about ribosomes: (1) each complete ribosome is always constructed from two loosely associated unequal subparticles (subunits); (2) each subparticle consists of one molecule of high-polymer RNA (plus low molecular-weight RNA for the large subparticle) and numerous molecules of ribosomal protein; (3) all, or almost all the ribosomal proteins in the ribosomal subparticle are different; (4) the molecule of high-polymer ribosomal RNA ensures the assembly of these different proteins into a ribonucleoprotein particle, and, in one way or another, unites them on the basis of its covalent structure; (5) as a whole the particle (subparticle) is characterized by a unique, asymmetric and at the same time rather compact packing of its components.

The discovery of structural transformations of the ribosomal particles in vitro such as dissociation, unfolding and disassembly, has played an important role in the formation of contemporary knowledge and concepts on ribosomes.

2. Dissociation

2.1. *Discovery*

During earlier attempts to isolate cellular ribonucleoprotein particles (ribosomes) from various organisms it soon became clear that to maintain the stability of the particles the presence of essential concentrations (10^{-3} M–10^{-2} M) of Mg^{2+} or Ca^{2+} cations in the medium was necessary [1,2]. In 1957 Chao first reported that isolated yeast 80 S ribosomes cleave into two smaller unequal components, the 60 S and the 40 S, upon lowering the concentration of these cations in the medium [2]. On again increasing the cation concentration, the 60 S and 40 S particles re-associated into 80 S particles. Soon after analogous data were reported for ribosomes from pea seedlings [3,4], *Azotobacter* [5], rat liver [6] and *E. coli* [7,8]. Tissières et al. gave the most detailed and unambiguous description of the process of reversible dissociation for *E. coli* 70 S ribosomes on lowering of the Mg^{2+} concentration [9]. The dissociation of ribosomes into two unequal subunits or subparticles proved to be a universal phenomenon intrinsic to ribosomes of all organisms:

70 S → 50 S + 30 S, or

80 S → 60 S + 40 S.

2.2. *Construction of the ribosome from two subparticles*

The discovery of dissociation showed that each complete ribosomal particle is constructed from two unequal subparticles. The sub-division of the ribosome into two unequal parts was corroborated by electron microscopic observations [10–12]. Thus, the construction of the ribosome from two unequal subparticles proved to be a universal *structural principle* of ribonucleoproteins of this type.

In addition to this important structural conclusion, it was the discovery of ribosome dissociation that opened the possibility of isolating individual ribosomal subparticles [9]. The consequences were invaluable. Investigations on the structure of separate

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subparticles, isolation and study of individual 16 S and 23 S ribosomal RNA's, and isolation and study of ribosomal proteins of the separate subparticles were carried out on this basis. Besides this, the study of partial functional activities of isolated ribosomal subparticles (template RNA binding, aminoacyl-tRNA binding, peptidyltransferase function, GTPase function, etc.) became possible.

2.3. *Conditions for maintaining the associated state*

From reports on dissociation of ribosomes it became obvious that Mg^{2+} or Ca^{2+} is necessary to maintain the associated state of ribosomes. This correlates with the fact that Mg^{2+} or Ca^{2+} in a definite concentration is necessary to maintain the functional activity of the ribosome in cell-free polypeptide-synthesizing systems as well as for the self-assembly of ribosomal particles in vitro.

The absolute concentration of Mg^{2+} (or Ca^{2+}) ions required for maintaining the association of ribosomal subparticles strongly depends both on the functional state of the ribosome and on the physico-chemical factors of the medium (for a more detailed consideration of functional and external factors see Review [13]).

The subparticles in the translating ribosome are joined rather firmly and this state is apparently irreversible. In any case, to dissociate the translating bacterial ribosomes, the Mg^{2+} concentration in the medium must be decreased to a much greater extent than for ribosomes that have terminated translation [13–21]. On the contrary, 'uncharged' (deprived of peptidyl-tRNA and aminoacyl-tRNA) ribosomal subparticles are associated very loosely [20], and a dynamic equilibrium can exist between the free subparticles and their couples (70 S $\rightleftharpoons$ 50 S + 30 S) [22; see also 23–25]. Ribosomes containing one of the substrates of the protein-synthesizing system, e.g., peptidyl-tRNA or the template-bound aminoacyl-tRNA, are intermediate in their association stability [17,20; see also 18,21,26]. Besides this, there are indications of the existence of a number of special protein factors ('dissociation factors' [27–33] and 'association factors' [34–37]) which can weaken or strengthen the coupling between subparticles. The more stable the coupling between subparticles the lower is the Mg^{2+} concentration required to induce dissociation.

Many external physico-chemical factors of the medium also affect the stability of coupling between subparticles and thus determine the minimal Mg^{2+} concentration inducing dissociation. Among them, the increase of the ionic strength at the expense of monovalent cations (K^+, NH_4^+, etc.) is well known to promote dissociation [2,4,6,9,24,25,38–43]. Na^+ and Li^+, even in relatively low concentrations, have an especially strong dissociating effect [6,41–46]. While both Mg^{2+} and Ca^{2+}, as well as Mn^{2+} and Co^{2+}, are effective in maintaining association [2,4,6,24,42,47–49], the competing cations of other bi-valent metals such as Sr^{2+}, Ba^{2+}, Cd^{2+}, Hg^{2+}, Ni^{2+} and Zn^{2+} are ineffective or possess strong dissociating effect [2,6, 42,48,49]. Organic bi- and polyvalent cations such as spermidine and putrescine [24,50–56], as well as aminoglucoside antibiotics (streptomycin, neomycin, kanamycin [55–57]) prevent dissociation and can be regarded as synergists of Mg^{2+}.

For the *E. coli* 70 S ribosome it was shown that the factors promoting dissociation, i.e., antagonists of Mg^{2+}, in addition to increased ionic strength, are also elevated temperature [24,41,43,58], high pH [41, 43,49] and urea [59]. Factors preventing the dissociation of the 70 S ribosome, i.e., synergists of Mg^{2+}, are methanol [59,60], ethanol, dimethyl sulphoxide and other water-soluble hydrophobic solvents, acting approximately proportionally to their hydrophobicity and concentration [59], as well as low ionic strength, low temperature and low pH [41,43]. These results were confirmed in studies of 80 S ribosomes [61; see also 2,4,6,62,63].

Among the purely physical factors, the increase of hydrostatic pressure (e.g., in the tube during centrifugation) was also specially noted as a factor promoting the dissociation of ribosomes [64–66].

It is likely that the dissociation of the ribosome into subparticles is in some way associated with certain conformational changes within the subparticles themselves, recorded by the change in hydrogen exchange [67] and circular dichroism [25,68,69]. However, at present it is difficult to assert conclusively when an influence on the structure of the subparticle has a primary effect and dissociation occurs as a result [69], and when the agent directly disrupts the junction between the subparticles and the conformational change (if any) is secondary.

The same remark can also be made concerning the

present data on direct modifications of the structure of ribosomal subparticles. Thus, it is not clear what is the mechanism of the dissociating effect of agents blocking on the SH-groups of ribosomal proteins [70–72], H_2O_2 [73], γ-radiation [74], etc.

2.4. *Functional designation of the two-subparticle construction*

The discovery of reversible dissociation of ribosomes suggested that ribosomal subparticles have in principle the possibility of drawing apart from each other and that this may be needed for something during the functioning of the ribosome. Indeed, in experiments in vivo and in cell-free systems it was shown that the ribosomes can exchange their subparticles, i.e., periodically dissociate and re-associate [75–80]. It was ascertained that the ribosome, necessarily associated during elongation, passed into the dissociated or equilibrium (70 S ⇌ 50 S + 30 S) state after termination of translation, and that initiation of translation requires the presence of free (not associated into the complete ribosome) 30 S subparticles; re-association occurs in the process of initiation of translation [17,31,81–86].

The impression is created, however, that the lability of subparticle association is also required in the course of elongation, despite the continuous maintenance of the associated state. Thus, excessive stabilization of association, no matter how it be induced, seems to inhibit elongation [13,87]. Some years ago a hypothesis was proposed on the cyclic locking–unlocking of the subparticles as the driving mechanism of translocation in the process of elongation [88–90]. The hypothesis presumes that during elongation the subparticles remain permanently joined only by some restricted site, or hinge, while their mutual, relative to each other, mobility must be maintained within definite limits to ensure their dynamic functioning.

3. Unfolding

3.1. *Discovery*

In 1963 it was discovered that the further, more exhaustive, removal of Mg^{2+} from *E. coli* ribosomal subparticles results in their abrupt transformation into a less compact state, especially at low ionic strengths [91,92]. This loosening and unfolding of ribosomal particles was displayed by a decrease of the sedimentation coefficient and a simultaneous increase of specific viscosity of the particles, without their fragmentation or splitting off of noticeable amounts of ribosomal protein. Despite the retention of the complete protein content, the compactness of the ribosomal particles in the fully unfolded state decreased to the level of compactness of free RNA, according to hydrodynamic data.

To achieve unfolding two main ways were devised for removing Mg^{2+} from the ribosomal particles: competitive displacement of Mg^{2+} by a high monovalent cation (e.g., NH_4^+) concentration with a following decrease of ionic strength [91–94], or direct removal of Mg^{2+} by EDTA at low ionic strength [95–97].

The process of unfolding was studied in especial detail with *E. coli* ribosomal particles [93,96]. Transitions between discrete conformational states can be schematically represented in terms of sedimentation coefficients as follows:

50 S → 35 S → 22 S gradually down to ≈ 5 S;

30 S → 26 S → 15 S gradually down to ≈ 5 S.

Later on, different aspects of the unfolding of *E. coli* ribosomal particles were investigated in a number of laboratories [97–106]. It was confirmed that there is indeed no change in either the protein content of the particles nor in the set of ribosomal proteins as a result of the unfolding. However, the 5 S RNA in unfolded ribosomes proved to be bound very loosely and readily exchanges with exogenous 5 S RNA if the latter is added to the medium [102]; some authors have observed the loss of the 5 S RNA during unfolding [99,107–109]. In contrast to the original compact ribosomal particles, the unfolded particles were found to be very sensitive to nucleases [97,98,110].

The unfolding of ribosomal particles can also be induced by heating [111–113]. It is very interesting that the exhaustive replacement of Mg^{2+} by putrescine, spermidine or alien metal cations such as Sr^{2+} or Ba^{2+} also leads to a loosening of the ribosomal particles [114–116].

Subparticles of 80 S ribosomes were also found to unfold in a magnesium-free medium, especially in the presence of Mg^{2+}-chelating anions such as EDTA, pyrophosphate and others, and the process also proceeds through several stages. Some loosening of

the subparticles seems to accompany the dissociation of the 80 S ribosomes if it is done by a drastic enough removal of Mg^{2+} [4,6,117–120]. The loosening of the isolated yeast 60 S subparticle into the 50 S component was specially studied [121,122]. Further unfolding stages of animal ribosomal subparticles were also investigated [123,124].

The question of reversibility of the unfolding is complicated. The first stage of the unfolding seems to be reversible, i.e., the particles can spontaneously recover their initial compactness and biological activity upon the restoration of the Mg^{2+} content [21,96, 97,100]. Probably this does not occur if the 50 S (or 60 S) subparticle loses its 5 S RNA [99,107,108]. The final stage of unfolding may not reverse to biologically active particles upon the restoration of the Mg^{2+} content [96–98,125,126]; however, the recovery of the initial structure and biological activity can be attained in this case if the particles are subjected to heat activation [125,126].

3.2. *Construction of the subparticles from the ribonucleoprotein strand*

The discovery of unfolding of ribosomal subparticles primarily showed that each ribosomal particle can be considered as a compactly folded ribonucleoprotein strand. This established another fundamental principle of structural organization of the ribosome.

Indeed, the final state of unfolding of the ribosomal particle represents an RNA strand, with or without secondary structure (depending on the ionic strength and temperature), but with a complete set of ribosomal proteins attached to it. The unfolded ribonucleoprotein practically does not differ in its hydrodynamic characteristics from the free ribosomal RNA under analogous conditions of ionic strength and temperature, displaying a much lesser compactness in comparison with the original ribosomal particle [91–97,101]. In an electron microscope the unfolded ribosomes can be observed as strands differing little from RNA strands [91,92]. The transition itself from the compact particle into the unfolded 22 S or 15 S ribonucleoprotein is not accompanied by a noticeable disruption or change of the RNA secondary structure [97,100,127], and only a further decrease of the ionic strength or rise in temperature leads to the melting of the RNA secondary structure within the ribonucleoprotein [127,128], in parallel with the gradual decrease in sedimentation coefficient (22 S or 15 S down to $\approx$ 5 S) and increase of specific viscosity [93,101].

The study of unfolding has revealed at least three important characteristics of compact packing of the ribonucleoprotein strand within the original ribosome. Firstly, the conditions requisite for the unfolding indicate that Mg^{2+} (and perhaps Ca^{2+}) are necessary to maintain the appropriate compact state. Secondly, the study of unfolding and re-folding as well as the comparison of the original particles with the unfolded ones and with free RNA definitely indicate that the compact packing is a function of the ribonucleoprotein and not RNA. Only the presence of ribosomal protein ensures the compact packing of ribosomal RNA as a whole, forming its tertiary structure and the quaternary structure of the particle. Thirdly, the disruption of intraribosomal compact packing during unfolding proceeds in a cooperative manner, through discrete stages. This poses a very important question on the principles of the packing. Apparently such cooperative conformational transitions can result either from breaking down of some regularity (order) in packing, or from disruption of a unique junction (cross-link, tie) fixing a structure as a whole. This question is one of the most important in studies of the quaternary structure of ribosomal particles.

3.3. *The framework role of ribosomal RNA*

Another important result of the discovery of the unfolding is that numerous molecules of ribosomal protein could be retained on RNA independently of the quaternary structure of the ribosomal particle as a whole. From this it followed that the high molecular-weight ribosomal RNA can be considered as a single covalently continuous framework for the molecules or groups of the molecules of ribosomal protein. It is likely that its biological role lies just in this.

On the basis of the unfolding studies the following schematic model of organization of the ribosomal particle was suggested [92]: 1) the covalent chain of the high molecular-weight ribosomal RNA forms a secondary structure analogous in general to that in solution, with numerous short double-helical regions successively connected through intervening single-stranded regions into an RNA strand; 2) different protein molecules or groups of molecules interact

with different RNA regions predominantly along the single-stranded regions of the chain so that the secondary structure of RNA is not essentially disturbed and, as a result, the ribonucleoprotein strand is formed; 3) the ribonucleoprotein strand in its turn is folded in a definite compact manner, with the participation of the proteins, forming the ribosomal subparticle.

The assumption that protein interacts mainly with the single-stranded regions of ribosomal RNA immediately followed from the behaviour of the unfolded ribonucleoprotein: the melting of the RNA secondary structure recorded by the decrease of the sedimentation coefficients to 3–5 S or by direct optical methods did not lead to the detachment of the protein; and, vice versa, the preservation of the full protein content in the unfolded ribonucleoprotein exerted no influence on the melting of the helices [93,100,101, 127,128]. Evidence in favour of this assumption was also given by studies of base composition of the RNA fragments protected from nucleases by ribosomal proteins in unfolded ribonucleoproteins [110]. The binding of proteins by RNA single-stranded regions is more directly confirmed by comparison of CD spectra of RNA in the free state and within the ribosome as well as in the process of unfolding [129]. Even more straightforward data were obtained in studying interactions of 16 S RNA fragments with protein S4 [130]. However, the possibilities of some changes of double-helical regions of ribosomal RNA during formation of the native compact structure at the expense of protein interactions is not excluded [126].

Naturally, the idea of the ribonucleoprotein strand being formed as a result of attachment of different ribosomal protein molecules along the RNA strand raised the question of specific protein–RNA recognitions. It is just due to this recognition that the necessary specificity and uniqueness of the spatial arrangement of various protein molecules or groups of molecules must be achieved on the RNA framework, which in its turn must unambiguously determine the acquisition of the unique quaternary structure of the particle. In the last few years it has been shown that individual ribosomal proteins of the *E. coli* 30 S subparticle, such as S4, S7, S8, S15 and S20 are actually attached directly to the 16 S ribosomal RNA, each being attached very specifically only to a strictly definite region (or regions) of this RNA [131–135]. The attachment of other 30 S subparticle proteins depends on the proteins indicated above. In the case of the *E. coli* 50 S subparticle proteins, the specific and independent settling on different regions of the 23 S RNA was shown for individual proteins L2, L6, L16, L17, L19, L20, L23 and L24 [135,136]. The majority of ribosomal proteins however, cannot be bound to the RNA as independent individual molecules, and their retention in connection with the RNA or generally within the ribonucleoprotein depends both on the proteins mentioned above and on each other.

The discovery of the unfolding and the concepts formulated on the framework role of ribosomal RNA in arranging ribosomal proteins directly suggested the possibility of self-assembly of ribosomal particles [92]. The first experiments on the physical self-assembly of compact 50 S and 30 S particles in vitro from 25 S and 19 S ribonucleoprotein particles of the precursor type (accumulating in *E. coli* cells in the presence of chloramphenicol and containing only about half of the ribosomal protein) and free protein were performed as early as in 1963 [92,137,138]. Later, three laboratories independently performed the self-assembly of biologically active 50 S and 30 S subparticles from analogous ribonucleoproteins representing artificially stripped subparticles and split ribosomal protein, or from some lesser stripped ribonucleoproteins and split protein [139–142]. The finale of the story was the self-assembly of biologically active ribosomal particles, first the 30 S [143] and then the 50 S [144,145], from ribosomal RNA and a complete set of ribosomal proteins. The concept of the framework role of ribosomal RNA in the formation of the ribosomal ribonucleoproteins was entirely confirmed.

4. Disassembly

4.1. *Discovery*

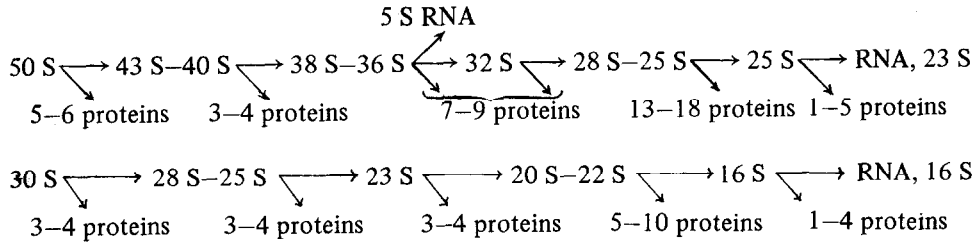
In 1964 Meselson et al. [146] reported that ribosomal particles can give rise to denser 42 S and 23 S derivative particles during centrifugation in CsCl gradients; it was presumed that they lose part of their ribosomal protein, and the loss of about 20% of ribosomal protein could explain the observed increase

in the buoyant density of the particles. In the next year it was shown by direct chemical analysis of the particles that incubation at high CsCl concentrations actually results in the splitting off of protein from ribosomal particles and that this splitting passes through several successive discrete stages, down to particles containing only about half of the original ribosomal protein (step-wise disassembly) [147]. Stepwise disassembly was later studied in more detail, and it was shown that it can be reversed, i.e., reassembly of ribosomal particles was done by removing CsCl and introducing Mg^{2+} [139]:

$$\begin{bmatrix}50\ S\\30\ S\end{bmatrix} \rightleftharpoons \begin{bmatrix}43\ S \rightleftharpoons 36\ S\\28\ S \rightleftharpoons 23\ S\end{bmatrix} + \text{protein}' \rightleftharpoons \begin{bmatrix}28\ S\\22\ S\end{bmatrix} + \text{protein}''$$

RNA: protein = 1.7, RNA: protein = 2–2.5, RNA: protein = 4–5.

These and subsequent investigations of successive stepwise disassembly of *E. coli* ribosomal particles under the action of high concentrations of CsCl, LiCl and other monovalent salts and then of salt and EDTA or salt and urea [148–155] allow one to propose the following general scheme:



Stepwise stripping of proteins with high salt concentrations was also achieved with some animal ribosomes [156–160].

4.2. *Diversity of ribosomal proteins*

The discovery of the stepwise disassembly of ribosomal particles provided evidence of the physical heterogeneity of ribosomal proteins within the particles. This was in conformity with the chemical variety of ribosomal proteins first demonstrated by Waller [161,162] and thus substantiated the formulation of the diversity of the component proteins as one more very important principle of the structural organization of the ribosome.

The study and identification of individual proteins split off in the course of disassembly of *E. coli* ribosomal particles [163,153,154] has shown that the most easily split off proteins of the 30 S subparticle are S1, S2, S3, S5, S9, S10 and S14; the group of proteins S11, S12, S18 and S21 are harder to split off; the S4, S6 and S16 proteins are retained more firmly in the ribonucleoprotein; the most firmly retained during complete disassembly are proteins S7, S8, S15, S17 and S19. In the 50 S subparticle among the easiest proteins to split off are L16, L26 and L33, then L7, L8, L10, L28 and after them L6, L11, L12, L15, L25, L27, L31; proteins L3, L4, L13, L17, L19, L21, L22, L23, L24 and L29 are firmly retained within the ribonucleoprotein [153,154].

It should be noted that in the process of disassembly the large ribosomal subparticle loses not only the proteins, but its 5 S RNA as well. The splitting of 5 S RNA proceeds simultaneously with the splitting off of a definite group of proteins; it has been established that on disassembly of *E. coli* ribosomes, the 43 S–40 S particles (and perhaps the 38 S–36 S particles as well) still contain 5 S RNA, while the 28 S–25 S particles are already completely devoid of it [99,148, 164].

An important and characteristic feature of the disassembly process is that the splitting off of at least a part of the proteins at high monovalent salt concentrations proceeds by small *cooperative groups* and not as completely independent protein molecules; correspondingly, the transformation of ribosomal particles into stripped ribonucleoprotein particles passes through several discrete stages [139,147,150]. It seems that sometimes, if not always, such a group can consist of spatially neighbouring proteins forming together some functional center of the ribosomal particle. In any case, it was recently shown that proteins S11, S18, S21 and probably S12 are neighbours [165] and this correlates with their more or less cooperative detachment from the 30 S subparticle in the course of disassembly (see above).

4.3. *Self-assembly of ribosomal particles*

The disassembly of ribosomal particles proved to be reversible and this made it possible to demonstrate the reconstitution of biologically active ribosomes in vitro [139–142]. The reconstitution of biologically active 30 S subparticles [143] and then the 50 S subparticles [144,145] from the free RNA and a complete set of corresponding proteins was a fine end of this discovery and proved the possibility of full *self*-assembly of the ribosome.

The experiments on the reconstitution of ribosomes immediately opened possibilities for studying the functional role of individual ribosomal proteins and the participation of the proteins in the formation of various functional centers of the ribosome [166]. The usual way for doing this was the reconstitution of the particle without a definite protein followed by the testing of the functional activities of the reconstituted ribosome. Though this method did not yield as much as was expected due to the strong functional interdependence of different proteins on each other and their interconnections with the general structure of the ribosome, several interesting conclusions were nonetheless made. First of all it was shown, for example, that the proteins of the 30 S subparticle, such as S4, S7, S8, S9, S15, S16, S17, S19, play a structural role, being obligatory for the formation of a compact particle or for the course of further self-assembly [167]. Other proteins such as S1, S2, S3, S10, S11, S12, S13, S14, S18, S21, do not play such a decisive structural role [167] but are vitally important in forming the mRNA-binding and aminoacyl-tRNA-binding sites of the 30 S subparticle [166,168–171].

Even the first experiments on the stepwise disassembly and reassembly of ribosomal particles permitted one to assume that the discrete ribonucleoprotein intermediates obtained may more or less correspond to natural precurosors which are formed in the course of biogenesis (assembly) of ribosomes in vivo [139,147]. Indeed, the formation of ribosomes in vivo passes through several successive discrete stages, through several protein-deficient ribonucleoprotein precursor particles ('neosomes') [172,173]. The ribonucleoprotein precursors have sedimentation coefficients [173–178] analogous to those of intermediate particles during artificial disassembly and reassembly:

23 S RNA → 25 S(28 S) → 32 S → 38 S → 43 S → 50 S;

16 S RNA → 18 S–22 S → 26 S–28 S → 30 S.

The set of ribosomal proteins in natural ribosome precursors and in artificially stripped or reconstituted intermediates are more or less similar [153,179–183]. Consequently, the sequence of ribosome assembly from RNA and protein in experiments on in vitro reconstitution, and the interdependent, partly cooperative character of this assembly [131] seems to serve as a satisfactory approximation to the sequence and character of the in vivo ribosome assembly (biogenesis).

5. Conclusion

At present, among different lines of investigation of ribosomes, structure, neighbouring groups, topography of individual ribosomal proteins and their involvement in the functional sites are being studied most intensively. This is undoubtedly a very promising way to attempt to understand the molecular basis of the structure and functioning of ribosomes. This approach, however, will never give a complete and *integral* picture unless thorough studies are made of the rules of the ribonucleoprotein formation (RNA–protein and protein–protein recognitions and interdependences), of the manner of ribonucleoprotein strand packing as a whole, of mobility of the entire ribosomal subparticles relative to each other and other possible conformational changes of the ribosome in the course of functioning. A more profound comprehension of the structural transformations of ribosomes is required for the development of the integral approaches, and it can be predicted that they will evoke further interest in the next few years.

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