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Conformational change of 23S RNA in 50S ribosome is responsible for translocation in protein synthesis

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Abstract. Since the recognition of the 'translocation' phenomenon during protein synthesis several theories have been proposed, without much success, to explain the translocation of peptidyl tRNA from the aminoacyl site to the peptidyl site. The involvement of L7/L12 proteins and therefore the L7/L12 stalk region of 50S ribosomes in the translocation process has been widely accepted. The mobility of the stalk region, as recognised by many workers, must be of physiological significance. It has recently been shown in this laboratory that 50S ribosomes derived from tight and loose couple 70S ribosomes differ markedly in quite a few physical and biological properties and it appears that these differences are due to the different conformations of 23S RNAs. It has also been possible to interconvert tight and loose couple 50S ribosomes with the help of the agents, elongation factor –G, GTP (and its analogues) which are responsible for translocation. Thus loose couple 70S ribosomes so long thought to be inactive ribosomes are actually products of translocation. Further, the conformational change of 23S RNA appears to be responsible for the interconversion of tight and loose couple 50S ribosomes and thus the process of translocation. A model has been proposed for translocation on the basis of the direct experimental evidences obtained in this laboratory.

Keywords. Ribosomes; translocation; protein synthesis; ribosomal RNA.

Introduction

During protein synthesis the peptide bond is formed by the transfer of the peptidyl moiety of the peptidyl tRNA attached to the ribosome at the *P* site to the aminoacyl moiety of tRNA attached to the *A* site (Brot, 1977). This leads to the elongation of the polypeptidyl chain by one amino acid and the former becomes associated with the tRNA at the *A* site. At the next step the peptidyl tRNA is translocated from the *A* site to the *P* site (figure 1). Apparently this translocation results in the movement of mRNA due to locking with tRNA through codon-anticodon interaction. The translocation is effected by the elongation factor-G (EF-G) and GTP during the association with 50S ribosome (Kaziro, 1978; Weissbach, 1980). Proteins L7/L12 which are quite distinct from other ribosomal proteins in many respects, have been implicated in the elongation process (Brot and Weissbach, 1981) although there is some

Abbreviation used: EF-G, Elongation factor-G.

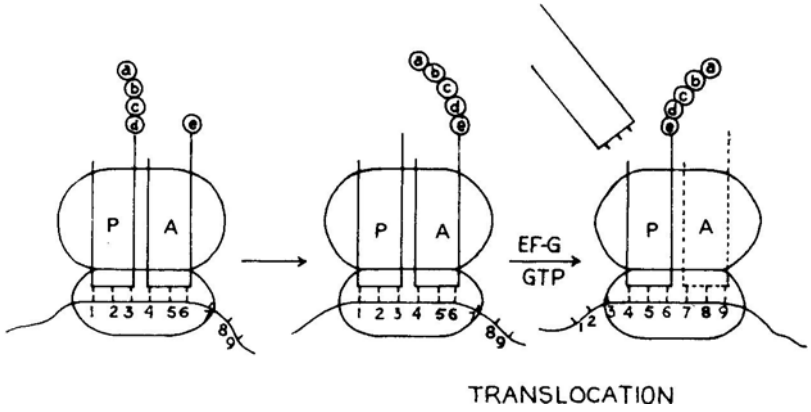


Figure 1. Translocation in protein synthesis.

controversy about this (Liljas, 1982). Similarly EF-G-dependent hydrolysis of GTP as catalysed by ribosome is thought to be connected with translocation although it is not unequivocally agreed upon (Kaziro, 1978; Nierhaus, 1982). GTPase activity is, however, found to be dependent on L7/L12 (Lockwood *et al.,* 1974).

Several mechanisms have been proposed for the translocation process. One of the earlier models, the locking and unlocking ribosome of Spirin (1969) assumes that 70S ribosomes can exist in two different states, locked (with the 30S and 50S subunits closely associated) and unlocked (with the 30S and 50S subunits drawn somewhat apart). The two subunits are envisaged as being hinged to each other and the locking and unlocking of the subunits of the ribosomes are assumed to provide the driving mechanism for translocation. According to the inchworm theory of Hardesty *et al.* (1969), a 'kink' is created in the mRNA during the enzymatic binding of aminoacyl tRNA to the *A* site of ribosome. EF-G and GTP are assumed to straighten out the 'kink' and in doing so peptidyl tRNA is translocated from the *A* site to the *P* site of ribosome. Leder's (1973) model is based on the substrate-product relationship for an enzyme. The weakness of this hypothesis has been discussed in detail by Nierhaus (1982). The latter has proposed a new hypothesis arguing that there is a third site, 'exit site' (*E* site) for the binding of aminoacyl tRNA. None of these models, however, provide satisfactory explanation of the translocation process itself and each one lacks strong experimental support. In this connection the nonenzymatic translocation *i.e.*, translocation in the absence of EF-G, reported by Pestka (1969) and Gavrilova and Spirin (1971) is quite intriguing. However, the efficiency of such a system is only 1% of the enzymatic one. It may not be out of place to mention here that Woese (1970) suggested a model as an alternate to the translocation from *A* to *P* site. It involves the conformational change of tRNA. Similar conformational change of tRNA has been implicated by Lake (1980) to explain the switch over of tRNA from *R* (recognition) site to aminoacyl site, however, without resulting in translocation. Although the mechanism of translocation still remains obscure experimental evidences will be summarised here to indicate that the conformational change of 23S RNA is responsible for translocation.

Involvement of L7/L12 stalk region in translocation

As already mentioned in the 'introduction', L7/L12 proteins are involved in translocation (Brot and Weissbach, 1981). No GTPase activity is observed in the absence of these proteins. There was some controversy about the location of L7/L12 (Liljas, 1982) but Strycharz *et al.* (1978) unequivocally demonstrated that these are located at a region known as 'stalk' region, protruding out of the main body. All the 4 copies of L7/L12 are thought to be located in this region and bound to 23S RNA through the protein L10 (Schrier *et al.,* 1973; Dijk *et al.,* 1979; Pettersen 1979; Tokimatsu *et al.,* 1981). They are bound to L10 through N-terminals and their C-terminals protrude out. However, it is quite interesting to note that proton magnetic resonance studies indicated an exceptional mobility of L7/L12 in the ribosome (Gudkov *et al.,* 1972) and supported the proposal of L7/L12 being a dimeric motile protein (Kischa *et al.,* 1971). ESR studies also indicated that L7/L12 might be a flexible domain of the 70S ribosome (Tritton, 1978). Further, Moller *et al.* (1983) have proposed from experimental data that each 50S ribosomal particle possesses two binding sites, each site being involved in the binding of one dimer of L7/L12. Binding of L7/L12 dimer at one site gives rise to the formation of the L7/L12 stalk whereas the binding at the other site has no effect on the number of visible stalks. Crosslinking experiments of Traut *et al.* (1983) also indicate that the L7/L12 stalk may not be a static structure. Conformational changes in L7/L12 upon binding of tRNA to the ribosomes have been suggested by Lee *et al.* (1981). These observations have naturally led to the speculation that the mobility of L7/L12 stalk may be connected with the tanslocation process.

Extensive work has been carried out in this laboratory on the structure of ribosomal subunits with the help of the single strand-specific enzyme RNase I (Burma, 1979a,b, 1982, 1984a,b; Burma *et al.,* 1980). The 30S ribosome is highly resistant to the action of RNase I in presence of Mg^{2+} at a concentration of 1 mM or so whereas 50S subunit is quite susceptible under such condition. In the presence of high Mg^{2+} concentrations (20 mM or so) 50S ribosomes also become resistant but only after L7/L12 and L10 proteins (along with L4) are removed (Raziuddin *et al.,* 1979) and 23S RNA is split in the stalk region (A. K. Srivastava, unpublished results). Subsequently it was shown that 50S ribosomes become resistant to RNase I when L7/L12 proteins are removed by treatment with 1M NH_4Cl and ethanol (Byasmuni and Burma, 1982) indicating thereby that L7/L12 proteins unfold the structure of 23S RNA in the stalk region. Recently two populations of 50S ribosomes have been isolated (to be discussed later), one population being highly sensitive to RNase I whereas the other one, highly resistant to the enzyme (Burma *et al.,* 1984b). These data clearly indicate that some part(s) of 23S RNA (most probably in the stalk region) is capable of assuming two conformations, one folded and the other one, having open structure, highly susceptible to the action of RNase I. This will be discussed in detail later.

Tight and loose couple ribosomes and translocation

It is well known that there are two types of 70S ribosomal populations, the tight couples which remain associated at low Mg^{2+} concentrations (4 mM or so) and loose couples

which require higher Mg^{2+} concentrations (10 mM or so) for association. It has been suggested that the ribosomes which dissociate readily (loose couples) may be damaged ones whereas tight couples are active particles (Noll *et al.,* 1973a,b; Hapke and Noll, 1976). There were a number of reports suggesting that the ribosomes undergo conformational change during translocation (Schrier and Noll, 1971; Chuang and Simpson, 1971; Waterson *et al.,* 1972). The suggestion was made on the basis of the observed differences in the sedimentation values of pre- and post-translocation ribosomal complexes during high speed centrifugation. The interpretation of the results was, however, questioned since this could be an artifact of pressure-induced dissociation (Infante and Baherlein, 1971; Infante and Krauss, 1971). However, Van Diggelen *et al.* (1971) showed that the ability to form the two association products is the intrinsic property of 50S subunits. These workers studied the association properties of 'native' subunits (which are present in the free state in the extract) and 'derived' subunits which are obtained by the dissociation of 70S ribosomes. It is most likely that the derived subunits are 50S ribosomes present in tight couples and native subunits are components of loose couples. No difference in the constituents of the two populations of 50S ribosomes was, however, detected although these workers (Van Diggelen and Bosch, 1973; Van Diggelen *et al.,* 1973) strongly felt that the two types of ribosomes differ in a ribosomal protein.

It has been shown in this laboratory that there is specific interaction between 16S and 23S RNAs which form the bimolecular complex under two well-defined conditions (Nag and Burma, 1982; Burma *et al.,* 1983; Nag *et al.,* 1984). In order to demonstrate that such association reflects the mechanism of association of 30S and 50S subunits, the method of kethoxal treatment, as extensively used by Noller and his coworkers (Herr and Noller, 1978, 1979; Herr et al., 1979) for the modification of ribosomes, was followed (Nag and Burma, 1984). As shown by Noller and his coworkers, 30S ribosomes on treatment with kethoxal completely lose their capacity to associate with 50S ribosomes. Surprisingly enough, 16S RNA isolated from kethoxal-treated 30S ribosomes or directly treated with kethoxal also loses its capacity to associate with 23S RNA. However, on treatment of 50S ribosomes with kethoxal only half of the population lost their capacity to associate with 30S ribosomes. This was at variance with the observation of Herr and Noller (1979) who observed complete loss. Similarly, only half of the population of 23S RNA either derived from kethoxal-treated 50S subunits or directly treated with kethoxal lost their capacity to associate with 16S RNA. These results indicated that there are two distinct populations of 50S ribosomes which perhaps originate from the two populations of 23S RNA. But the most important observation is that the two RNAs have quite different conformations and thus behave differently to kethoxal treatment.

As mentioned above, the results obtained with the 50S ribosomes in this laboratory were at variance with those of Herr and Noller (1979). The difference may be due to the fact that they had used 50S ribosomes derived from tight couples whereas the mixture of tight and loose couples was used in our experiments. Actually it has now been shown in this laboratory (Burma *et al.,* 1984b) that the two populations of 50S ribosomes are actually derived from tight and loose couples. 50S ribosomes isolated from tight couples completely lose their association capacity on treatment with kethoxal whereas those from loose couples do not lose the capacity at all on such treatment. The same is

true for naked 23S RNAs or 23S RNAs isolated from treated tight and loose couple 50S ribosomes. Further, the Mg^{2+} dependent associations (with 16S RNA) of the two 23S RNAs are quite different. At Mg^{2+} concentrations below 20 mM, the association capacity of tight couple 23S RNA is more than that of loose couple 23S RNA. Further, at 2·5 mM Mg^{2+} 50S ribosomes from loose couples are highly resistant to the action of RNase I whereas tight couple ones are quite susceptible. This was expected from the behaviour of kethoxal-treated 50S ribosomes. It has already been mentioned that L7/L 12 stalk region of 50S ribosomes is susceptible to the action of RNase I. It is quite likely that the difference between the tight and loose couple 50S ribosomes lies in the 23S RNAs and that too in the stalk region. The 23S RNA isolated from loose couple 50S ribosomes binds more ethidium bromide than loose couple ones, as determined by fluorescence measurements (Burma *et al.,* 1984b). Further, loose couple 23S RNA is found to have slightly more hyperchromicity (on heat treatment) than tight couple 23S RNA. Thus it appears that the loose couple 50S ribosomes have more folded structure (may be in the L7/L12 stalk region) than tight couple 50S ribosomes. Not only that tight couple 50S ribosomes are more biologically active than loose couple ones, 23S RNA isolated from tight couple 50S ribosomes are also more biologically active than the loose couple 23S RNA (Burma *et al.,* 1984b) as measured by the ribosome-like activity of 16S.23S RNA complex (Burma *et al.,* 1984a). The properties of tight and loose couple 50S ribosomes as well as 23S RNAs have been summarised in table 1. It appears most likely that the major difference between tight couple and loose couple lies in the conformation of 23S RNA.

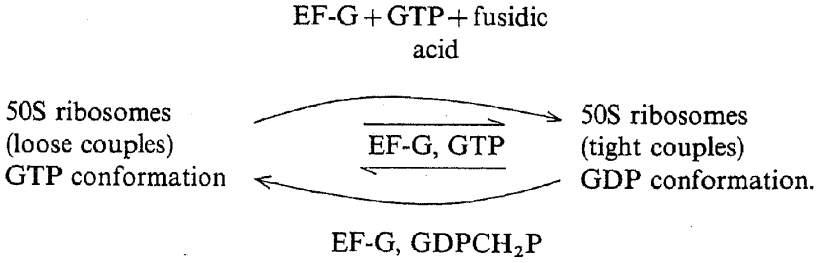
Table 1. Comparison of the properties of 50S ribosomes and 23S RNAs derived from tight couple and loose couple 70S ribosomes.

Properties	Tight couple	Loose couple
50S ribosomes		
Capacity to associate with 30S ribosomes at 4 mM Mg^{2+}	100%	Nil
Effect of kethoxal treatment on the association capacity at 10 mM Mg^{2+}	Complete loss	No loss
Sensitivity to RNAse I at 2·5 mM Mg^{2+}	Susceptible	Resistant
Biological activity	More	Less
23S RNAs		
Capactity to associate with 16S RNA at 4 mM Mg^{2+}	40%	5%
Effect of kethoxal treatment on the association capacity at 20 mM Mg^{2+}	Complete loss loss	No loss
Biological activity	More	Less
Binding of ethidium bromide	Less	More
Hyperchromicity on thermal denaturation	Less	More

Interconversion of tight and loose couples

The strongest evidence in favour of the involvement of tight and loose couples in translocation came from the following unpublished results from this laboratory. On

treatment of loose couple 50S ribosomes with EF-G, GTP and fusidic acid they were converted to tight couples as tested by (i) association capacity with 30S ribosomes, (ii) effect of kethoxal treatment of this association capacity, (iii) sensitivity to RNase I, and (iv) biological activity. 23S RNA isolated from the converted 50S ribosomes also behaved like 23S RNA derived from tight couple 50S ribosomes indicating thereby that the change has taken place at the RNA level. It is well known that fusidic acid, the inhibitor of protein synthesis forms a complex with EF-G and GTP and allows the association of EF-G-GTP with 50S ribosomes. It also allows the hydrolysis of GTP but prevents the dissociation of EF-G-GDP from the ribosomes (Kuriki *et al.,* 1970; Bodley *et al.,* 1970). It should also be pointed out here that EF-G is known to bind directly to 23S RNA (Girschovich *et al.,* 1982; Skold, 1983). Therefore it is quite likely that 50S ribosome (rather 23S RNA) is locked in one conformation (tight couple one) due to treatment with EF-G, GTP and fusidic acid. The conversion of tight couples to loose couples could be effected by EF-G and non-hydrolysable GTP analogue $GDPCH_2P$ but the conversion is not so efficient. However, the treatment at 70S tight couple level instead of 50S tight couple, leads to somewhat better conversion. It is well known that the binding of $GDPCH_2P$ to 50S ribosome is rather weak although it has been claimed that translocation takes place in the absence of hydrolysis of GTP (Karizo, 1978). Thus it is expected that $GDPCH_2P$ will lock 50S ribosomes (rather 23S RNA) in the other conformation (loose couple conformation). It is also highly interesting to note that on treatment of either tight or loose couple 50S ribosomes with EF-G and GTP a mixture of the two is produced. These results clearly show that the tight and loose couple 50S ribosomes are interconvertible forms and the interconversion takes place with the help of the agents responsible for translocation. The reactions are shown below.



It is also possible to convert loose couple 23S RNA to tight couple one by heat treatment.

Involvement of 23S RNA in translocation

Originally ribosomes were thought to be inert platform for the synthesis of the proteins but as the involvement of ribosomal proteins in the various steps of protein synthesis became gradually known it was realised that ribosomes play an active role in protein synthesis. So long ribosomal RNAs appeared to be the requisite structures for holding the ribosomal proteins in position and thus allowing the latter to function. But this

concept also needs revision as ribosomal RNAs are also found to participate directly in several reactions catalysed by ribosomes. Some of these are as follows: (i) recognition of mRNA by the 3′-end of 16S RNA (Shine and Dalgarno, 1974; Steitz and Jakes, 1975; Dunn *et al.,* 1978), (ii) binding of tRNA (Noller and Chaires, 1972; Schwarz and Ofengand, 1978; Ofengand. *et al.,* 1982; Brow and Noller, 1983), (iii) antibiotic sensitivity and resistance (Heiser *et al.,* 1972; Lai *et al.,* 1973; Yamada *et al.,* 1978), (iv) binding of 5S RNA by 23S RNA through proteins (Gray *et al.,* 1972; Spierer *et al.,* 1979; Tewari and Burma, 1983), (v) binding of initiation factors, at least IF3 (Gualerzi and Pon, 1973; Nag *et al.,* 1983), (vi) binding of EF-G (Girschovich *et al.,* 1982; Skold 1983), (vii) subunit association (Nierhaus, 1982; Herr and Noller, 1978, 1979; Herr et al., 1979; Shanter and Shane, 1977; Steitz, 1979; Van duin *et al.,* 1976; Nag and Burma, 1984), and (viii) binding of poly U and phenylalanyl tRNA, GTP hydrolysis, peptidyl transferase activity and polyphenylalanine synthesis as demonstrated in this laboratory (Burma *et al.,* 1984a).

Although it was suspected for quite a long time that RNA-RNA interaction plays a major role in the association of 30S and 50S subunits (references cited above) no direct evidence was, however, available. It was demonstrated for the first time in this laboratory that 16S and 23S RNAs form a specific bimolecular complex under two Well-defined conditions: (i) reconstitution condition under which 30S and 50S ribosomes can be reassembled from their constituent RNAs and proteins (Traub and Nomura, 1968; Nierhaus and Dohme, 1974) and (ii) alcohol condition under which 16S and 23S RNAs assume structural features of 30S and 50S ribosomes respectively (Vasiliev *et al.,* 1978; Vasiliev and Zalite, 1980). Subsequently it was shown that 5S RNA can also be incorporated into such complex but only with the help of three ribosomal proteins, L5, L18, L15/L25 (Tewari and Burma, 1983). L5, L18 and L25 are 5S RNA binding proteins (Gray *et al.,* 1972; Home and Erdman, 1972; Yu and Wittman, 1973) and L15 has been involved in the assembly of 5S RNA into 50S ribosomes (Nierhaus, 1982; Rohl and Nierhaus, 1982). These studies indicated the involvement of ribosomal RNAs in the association of ribosomal subunits as well as binding of 5S RNA through proteins. The former was confirmed by the kethoxal treatment of 30S and 50S ribosomes as well as 16S and 23S RNAs, the results of which have been described elsewhere (Nag and Burma, 1984). As discussed already, the kethoxal treatment led to the observation that 50S ribosomes occur in two distinct forms and that also due to two different conformations of 23S RNA. These studies further indicated the involvement of 23S RNA in the translocation reaction (Burma *et al.,* 1984b). That rRNAs have distinct roles to play in the various steps of proteins synthesis has been shown by demonstrating the ribosome-like activity of 16S.23S RNA complex (Burma *et al.,* 1984a). The various steps of proteins synthesis as well polyphenylalanine synthesis could be demonstrated with the addition of the factors involved in protein synthesis and a limited number of ribosomal proteins.

The involvement of 23S RNA in translocation was, however, indicated from an earlier observation in this laboratory (Raziuddin *et al.,* 1979). The L7/L12 stalk region of 50S ribosome was found to be susceptible to the action of single-strand specific enzyme RNase I. This was strengthened by the subsequent observation in this laboratory that 50S ribosomes become resistant to the action of RNase I on removal of L7/L12 (Byasmuni and Burma, 1982), contrary to the expectation. On addition of

L7/L12 back to the core particle it again becomes susceptible to RNase I. Although it is normally believed that L7/L12 do not directly interact with 23S RNA but are attached to it through L10 (Schrier *et al.,* 1973; Tokimatsu *et al.,* 1981) it is quite logical to conclude from the above mentioned observations that L7/L12 have the capacity to organise structures of rRNA in the stalk region either by direct or indirect interaction with 23S RNA. Actually Marquardt *et al.* (1979) have shown weak interaction of L7/L12 with 23S RNA. The observations made in this laboratory are of great significance as L7/L12 are known to be directly involved in the translocation. Further, elongation factor EF-G seems to bind directly to 23S RNA at a site close to L10 (Girschovich *et al.,* 1982; Skold, 1983). Bochkareva and Girschovich (1984) have recently shown that the binding of EF-G interferes with the action of RNase I on 23S RNA near the EF-G binding region. These observations clearly indicate that 23S RNA (specially the domain present in stalk region) may be directly involved in translocation. This is further substantiated by the very recent observation in this laboratory (which has been mentioned already) that the tight couple 50S ribosomes are highly susceptible to the action of RNase I whereas loose couple 50S ribosomes are quite resistant in the presence of 2·5 mM Mg^{2+} (Burma *et al.,* 1984b). Based on the earlier observation in this laboratory (Byasmuni and Burma, 1982) and the recent observation that the tight couple and loose couple 50S ribosomes are the products of translocation process itself, as evident from their interconversion by the agents responsible for translocation, it can justifiably be proposed that 23S RNA is directly involved in the translocation process. Most probably it undergoes conformational change during this process. Further, the change in conformation of 23S RNA is most likely in the stalk region and manifested in the alteration of the association capacity of 50S ribosomes with 30S ribosomes. This association takes place most probably through the interaction between 16S and 23S RNAs.

Proposed model and justifications

Although no direct evidence can be provided at present for the mechanism of translocation, yet the observations made in this laboratory and other laboratories, as summarised above, have tempted us to provide the following tentative model for translocation (figure 2). EF-G associated with GTP (or, in other words, EF-G; GTP complex) binds directly to 23S RNA of 50S ribosome at the site close to the binding site of L10 through which proteins L7/L12 are bound to 50S ribosome. It should be pointed out here that electron microscopy of 50S particles to which EF-G was crosslinked indicated a position close to the stalk portuberance (Girschovich *et al.,* 1981). This binding induces conformational change in 23S RNA in the stalk region (the conformation induced by such binding may be designated as GTP conformation). The conformational change is most probably induced by L7/L12 which appear to be motile proteins. The motility is being assumed to be due to the interaction of EF-G-GTP with not only 23S RNA but also L10 to which L7/L12 proteins are bound. Subsequent to this event, GTP is hydrolysed to GDP and P_i through some unknown mechanism and 23S RNA goes back to original conformation (which may be called as GDP conformation). Since EF-G-GDP can not remain bound to 23S RNA it comes off and

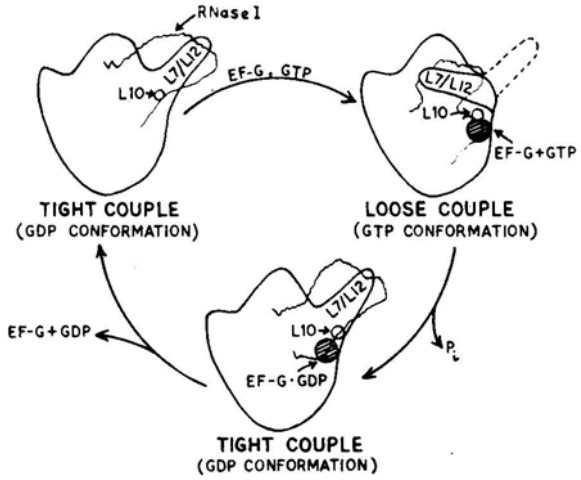


Figure 2. Model for translocation.

EF-G dissociates from GDP, reassociates with another molecule of GTP and the whole process is repeated again. The only advantage of the proposed model is that it is based on some direct experimental data and vulnerable to experimental assault.

The model is capable of explaining some of the observations made in this laboratory (table 1). Since 23S RNA in tight couple ribosomes is in unfolded form in the stalk region, it is sensitive to RNase I. Similarly, the binding of more ethidium bromide to loose couple 23S RNA and also more hyperchromicity on melting of this RNA, in comparison to tight couple 23S RNA, also fit in with the assumption of more folding of RNA in the former case.

Tight couple 50S ribosomes or 23S RNA lose their capacity to associate with 30S ribosomes and 16S RNA respectively on treatment with kethoxal due to availability of guanine residues for modification whereas in loose couple 50S ribosomes or 23S RNA such guanine residues are not available due to the folding of RNA. Further, due to this folded structure the association capacity of loose couple 50S ribosomes (or 23S RNA) is lower than that of tight couple 50S ribosomes (or 23S RNA). This is due to ready availability of the sites of association in the latter case in comparison to the former. The interconversion of loose couple and tight couple 50S ribosomes can also be explained on the basis of the proposed model. When loose couple 50S ribosomes are treated with EF-G, GTP and fusidic acid, the conformation of 23S RNA of 50S ribosomes is converted to tight couple conformation (GDP conformation) but since EF-G can not be released due to binding with the fusidic acid loose couple 23S RNA is locked in tight couple or GDP conformation. In the case of conversion of tight couple 50S ribosomes by EF-G and $GDPCH_2P$, the reverse situation happens. 23S RNA is locked in GTP conformation as $GDPCH_2P$ is not hydrolysable. Similarly, on treatment of either type of 50S ribosomes with EF-G and GTP a mixture of the two are obtained due to the cyclic operation and the fact that there is equal chance of both the conformations to be produced. On heat treatment 23S RNA of loose couple 50S ribosomes are converted to

23S RNA of tight couple 50S ribosomes, most probably due to unfolding of the RNA chain in the stalk region.

The higher biological activity of tight couple 50S ribosomes (or 23S RNA) can also be explained on the basis of the proposed model. Loose couple (or GTP conformation) is a transient conformation and cannot normally occur free from EF-G and GTP but may be obtained as an artifact of the isolation procedure. EF-G and GTP are removed during isolation and as a result 23S RNA is locked in GTP conformation. EF-G and GTP normally bind to tight couple 50S ribosomes having GDP conformation which is a natural process. It is rather difficult for EF-G (and GTP) to bind to 23S RNA in the loose couple conformation due to already folded conformation of 23S RNA. Under physiological condition no such binding is expected to take place. Tight couples are thus ready for translocation whereas loose couples have to be forced to bind with EF-G and GTP for conversion to tight couple and initiation of translocation. This model also emphasises that the hydrolysis of GTP is not necessary for translocation (Kaziro, 1978). The conformational change of 23S RNA required for translocation is effected by the binding of EF-G and GTP to 50S ribosome. The hydrolysis of GTP is subsequently necessary to bring it back to the earlier conformation. Work is in progress in this laboratory to test the model still further and it is being hoped that the details of the mechanism of conformational change of 23S RNA during translocation will be learnt soon.

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