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Cotranslational Folding of Proteins

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Abstract—We suppose that folding of proteins occurs cotranslationally by the following scheme. The polypeptide chains enter the folding sites from protein translocation complexes (ribosome, translocation machinery incorporated in membranes) directionally with the N-terminus and gradually. The chain starts to fold as soon as its N-terminal residue enters the folding site from the translocation complex. The folding process accompanies the translocation of the chain to its folding site and is completed after the C-terminal residue leaves the translocation complex. Proteins fold in sequential stages, by translocation of their polypeptide into folding compartments. At each stage a particular conformation of the N-terminal part of the chain that has emerged from the translocation complex is formed. The formation of both the particular conformations of the N-terminal chain segment at each folding stage and the final native protein conformation at the last stage occurs in a time that does not exceed the duration of the fastest elongation cycle on the ribosome.

Key words: protein folding, cotranslational folding, mechanism of folding

Two concepts (cotranslational and posttranslational) for protein folding, i.e., how the polypeptide chain folds into the native protein with a unique three-dimensional structure, are commonly considered. The cotranslational concept is that proteins fold during the synthesis of the polypeptide chain on the ribosome, while according to the posttranslational concept the protein starts to fold after the polypeptide chain has escaped from the ribosome [1].

In a previous paper [2], we attempted to show that he posttranslational concept is based on hypotheses and suggestions that, in our opinion, have no reliable theoretical or experimental basis. On the other hand, cotranslational protein folding is supported by the results of a number of experiments *in vivo* and on protein-synthesizing model systems. According to these results, polypeptides still bound to the ribosome (1) exhibit characteristic enzymatic activity of native enzymes [3-10], (2) bind antibodies specific for mature proteins [11-13], (3) form correct intra- and inter-chain disulfide cross bridges of the native proteins [14-16], (4) bind inherent cofactors or ligands necessary for the specific functioning of proteins [17-22], and (5) form protease-resistant compact structures [23-25] (for reviews [1, 26, 27]).

In this paper, we suggest what we think is acceptable reasoning why proteins must fold cotranslationally, and we propose a tentative phenomenological model for the mechanism of protein folding. For this purpose, we considered the way and the rate of translocation of polypeptides into the cytoplasm from the ribosome as well as the times supposed for protein folding *in vivo* and renaturation of denatured proteins (called *in vitro* folding).

Proteins usually fold in the place of their compartmentalization in cells. (Some proteins fold in the endoplasmic reticulum and are transported to their compartments in vesicles.) Some proteins fold near the ribosomes in the cytoplasm. The polypeptide chains of other proteins are transported to different cellular locations distant from the ribosome to fold: the periplasm, the plasma of cell organelles, and so on [28-33]. In these cases, polypeptides may cross one or more membranes on the way to the folding compartment. Some of these proteins may fold behind the membrane in its immediate vicinity. This possibly occurs with proteins of intermembrane spaces and at least with some proteins of cellular organelles. It is assumed that in the cytoplasm of bacteria, the lumen of chloroplast stroma, and the matrix of mitochondria a discrete fraction of proteins fold with the assistance of chaperone complexes [34, 35]. Thus, at least three cases of protein folding will be distinguished, i.e., protein folding at the ribosome, protein folding in the immediate vicinity of the membrane, and protein folding with the participation of chaperones.

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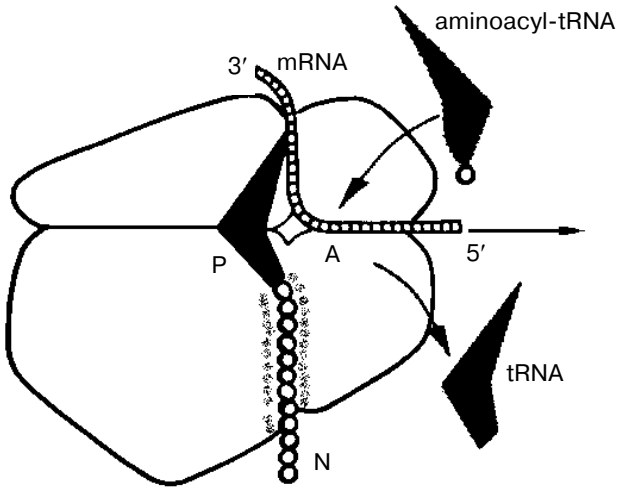
Protein folding at the ribosome and in the immediate vicinity of a membrane is considered in this paper. We shall accept that in both cases the folding process occurs without participation of any other "folding helper" proteins (such as peptidyl-prolyl-*cis-trans* isomerase, peptidyl-disulfide isomerase, and so on [29]). Thus, the emphasis is on protein folding at the ribosome, to clarify why proteins must fold cotranslationally and to advance a possible mechanism for protein folding. Then protein folding near a membrane will be discussed briefly.

Protein Folding at the Ribosome

Why must proteins fold cotranslationally? As is known, the ribosome complex builds up a protein polypeptide chain from the N-terminus sequentially residue by residue, the peptide bonds being formed between the residues. Next, the synthesizing polypeptide chain moves from the peptidyl-transferase center along the narrow ribosome channel to the ribosome surface and cotranslationally emerges into the cytoplasm at the exiting site on the ribosome (figure) [36, 37]. Therefore, the residues of the polypeptide chain enter the cytoplasm from the ribosome in the definite way, with the N-terminal residue and sequentially one after another. Let us suppose that the synthesized polypeptide has no signal sequence [28]. Then it is not destined for transportation and should fold in the cytosol near the ribosome.

The native conformation of a protein is formed during the numerous conformational changes of its polypeptide chain that are caused by intra- and intermolecular interactions of the amino acid residues with each other and with the molecules of the folding environment. Conformational changes of the polypeptide chain are prevented in the narrow ribosome channel by the channel walls. The potential propensities of the polypeptide to undergo conformational changes could be realized beginning from the moment when the N-terminus of the polypeptide chain emerges from the ribosome into the cytoplasm.

Next, we shall concentrate our attention on the rate of the emergence of the polypeptide from the ribosome. It is just the rate of polypeptide chain elongation (growth) on the ribosome, since polypeptides move in the narrow ribosome channel in the extended state. Average elongation rates of polypeptides during biosynthesis have been estimated for a wide variety of proteins from different cells under various conditions *in vivo* and in model systems already beginning from the 1960s (in particular, [38-47] and references cited therein). According to these results, elongation rates of polypeptides are variable and depend on the stochastic binding of the anticodons of the activated amino acid residues (ternary aminoacyl-tRNA–EF-Tu–GTP complexes) to the corresponding codons of the messenger-RNA on the ribosome [36, 37, 47]. Higher elongation rates are observed in *E. coli* and yeast and vary from 12 to 18 residues/sec per ribosome ([46] and references cited therein). The maximum elongation rate observed is 20.4 residues/sec for the *E. coli* EF-Tu polypeptide chain [47]. The absolute *in vivo* translation rates have been also determined for individual codons in *E. coli* [48, 49]. The maximum value of the absolute *in vivo* average rate is 21.6 codons/sec at 37°C, and hence the translation time per codon is 0.046 sec [49]. The latter value, which is the shortest experimentally observed elongation period of the ribosome, i.e., the time required for the elongation of a polypeptide chain by one amino acid residue during biosynthesis, is considered by us the fulcrum for our further reasoning. Taking into account this value one could suppose that the duration of a ribosome elongation cycle is on a time scale of no less 0.01 sec (see [43, 47-51] and references cited therein for discussion of this statement).



Schematic representation of polypeptide-synthesizing ribosome (following Fig. 108 from [36]).

And finally, let us consider another kind of data. It is common knowledge that the characteristic times for conformational changes of molecules by rotational degrees of freedom are 10^{-12}-10^{-7} sec [52]. The formation of α-helices and β-sheets in proteins occurs on the time scale of 10^{-7}-10^{-4} sec [53-55]. It is believed that the renaturation of denatured small proteins without disulfide bridge(s) typically requires 10^{-4}-10^{-2} sec [1, 56-60]. According to current opinions, proteins fold in the time interval from 10^{-4} to 10^{-2} sec [61-65]. Proteins fold *in vivo* quite fast too, as they exhibit functional activity just after leaving the ribosome [6-10].

Now let us compare the above time scales. It becomes obvious that the polypeptide residues enter the folding site, i.e., the cytoplasm, from the ribosome far more slowly as compared to both the characteristic times for conformational changes of the molecules by the rotational

degrees of freedom and the formation of α-helices and β-sheets in proteins. Moreover, the native conformation *per se* of a protein is formed more quickly as compared to the least time required for polypeptide chain elongation by one amino acid residue during biosynthesis. Therefore, while the ribosome joins a residue to a polypeptide, either α-helices, β-sheets, compact states during nucleated collapse, and specific conformation could be formed in any N-terminal part of the polypeptide that has emerged from the ribosome. This simple and obvious conclusion, in our opinion, is a true and reliable justification for the conclusion that proteins fold during biosynthesis of their polypeptide chains, i.e., cotranslationally.

A model for the mechanism of cotranslational protein folding (temporal formation of native protein conformation). As noted above, the residues of a polypeptide chain enter the cytoplasm from the ribosome sequentially and gradually from the N-terminal residue; the times suggested for protein folding are less than the fastest elongation period of the ribosome. Based only on these data and taking into account that no association of polypeptide with the ribosome is observed either during the biosynthesis or after escaping the ribosome, the following tentative phenomenological model can be proposed for the mechanism of cotranslational protein folding.

– Folding of protein starts as soon as the N-terminal residue of its polypeptide chain enters the cytosol from the ribosome (beginning from this moment the N-terminus of the polypeptide is able to undergo conformational changes under the action of molecular forces causing the folding). Then the folding process accompanies the translocation of the chain and is completed after the C-terminal residue leaves the ribosome.

– Protein folding occurs in sequential stages. The stages and their sequences are similar to those of the translocation of the polypeptide chain residues from the ribosome. The number of stages is roughly the same as the number of translation events during biosynthesis of the polypeptide chain.

– At each folding stage, a specific conformation of the N-terminal part of the chain that has emerged from the ribosome is formed. (In a simple approximation, at the first stage a specific conformation of the N-terminal residue is formed, at the second stage a specific conformation of the N-terminal part consisting of two residues is formed, and so on.) This conformation serves as a starting structure for the formation of the N-terminal part that has elongated by one amino acid residue at the next stage of folding. (The conformation of the elongated segment is determined by the interactions of residues of the structure that was formed at the previous stage with the residue that has just entered into the folding site and with the molecules of the folding environment. It is formed during the generation of new stabilizing bonds and the breaking of old ones (hydrogen bonds, hydrophobic interactions, and so on).)

– The secondary structure elements of the polypeptide, i.e., α-helices, β-sheets, and turns, formed at each stage of folding can be either disrupted or changed, or remain unchanged at the next stage.

– Formation of a specific conformation of the N-terminal part of the chain at every folding stage, as well as of the native protein final conformation at the last stage, occurs during a time not exceeding the shortest elongation period of the ribosome. The total time for the native protein spatial structure formation is comparable with the duration of biosynthesis of its polypeptide chain.

As far as we know, three hypotheses have been formulated for the possible mechanism of cotranslational protein folding. According to one of these hypotheses, the route of folding of some proteins is determined by *in vivo* translation rates of different sections of polypeptides during biosynthesis [66]. The other hypothesis suggests that the spatial folding of a synthesized polypeptide is controlled by both the pauses and rates of translation of different sections of the mRNA that are determined by both the degenerate nature of the genetic code and clusters of rare codons [67]. A somewhat different hypothesis was suggested according to which, as the polypeptide emerges from the ribosome into the surrounding environment in α-helical conformation, breaking of the conformation occurs at some distance from the ribosome with simultaneous changes into the elements of protein tertiary structure that are subsequently joined, avoiding the molten globule state. In this process, the initial sites of α-helix breaking and termination of its unfolding are determined by the initiating and terminating amino acid "codons" on the α-helix [68]. Avoiding a detailed analysis of the above hypotheses, we would like to note briefly that they seem to have at least one serious disadvantage. They do not consider the total codon sequence of the protein gene as well as the chain segments between the "initiating" and "terminating" signaling codons of the secondary structure elements of the protein. In other words, both the secondary and tertiary structures do not depend on amino acid sequences of proteins.

Protein Folding near a Membrane

It is well known that the translocation of a polypeptide chain across the plasmatic membrane in both prokaryotes and eukaryotes and across the organelle membranes of eukaryotes occur via a protein-conducting channel that is formed by a protein-translocating complex incorporated into the membrane [28-33, 69-72]. The polypeptide crosses the channel in an extended state from its N-terminus. Supposing that a second signal sequence is lacking in the polypeptide, then it is not destined for further transportation and should fold in the immediate vicinity of the other site of the membrane.

To our knowledge, there are no data about the rate of polypeptide chain translocation across the membrane

channel. Nonetheless, at least in the case when the ribosome is anchored to the endoplasmic reticulum membrane and pushes the synthesized polypeptide across it into the lumen, the polypeptide chain residues are translocated across the membrane with the periodicity of the elongation cycle of the ribosome. Based on this observation, one can suggest that in almost all cases *in vivo*, upon translocation the polypeptide chain across the membrane channel, the residues cross the membrane at periodicity comparable to the elongation period of the ribosome. This suggestion seems to us quite reasonable, so it could be justified by the similarity of some aspects of polypeptide elongation on the ribosome and polypeptide translocation across the membrane channel. First, simple Brownian motion (diffusion) is believed to be responsible for both the first [36, 37] and the second [70, 71] of these processes. Besides, both processes are conjugated with the complicated recognition event (protein–protein, protein–nucleotide, codon–anticodon, in the first process [36, 37], and chaperone–polypeptide, protein–protein, protein–nucleotide, in the second process [28-33, 69-73]). And finally, both processes are periodic chemomechanical processes that are driven by the chaotic binding either of GTP (during elongation) or ATP (upon translocation) by their followed hydrolysis and removal that leads to the linear periodic spatial transfer (translocation) of the polypeptide.

Thus, upon protein folding near the membrane the residues of its polypeptide chain enter the folding site sequentially with the N-terminal residue and gradually, with periodicity that is comparable with the elongation period of the ribosome, i.e., similar to the protein folding at the ribosome. Therefore, one can suggest that protein folding in the immediate vicinity of the membrane occurs according to the scheme described above for protein folding at the ribosome.

To formulate the model for the protein folding mechanism, we proceeded from the fact that the polypeptide chains enter the folding sites from the narrow channels in the protein complexes (ribosome, translocation apparatus in the membrane). We believe the areas of the surfaces of these protein complexes near which polypeptide chains fold into native proteins (the vicinity of the exiting site) consist of polar amino acid residues and are strongly hydrated. The hydrate shell is supposed to shield the folding polypeptide to associate with the translocation complex. The prerequisite for our suggestion is that protein conducting channels via which polypeptides are translocated across the membrane are composed of polar amino acid residues and comprise aqueous pores in the membrane [29, 69-73]. It is quite possible that a similar situation holds also in the ribosome channel [74, 75]. For this reason, we suppose that translocation complexes themselves (i.e., the ribosome and the translocation apparatus), from which polypeptide chains enter into their folding places, do not play an active role in the folding of proteins.

Thus, the residues of polypeptide chains enter different aqueous cell compartments to fold from the translocating protein complexes in a similar way directionally with the N-terminal residue, sequentially one after another, and temporally with periodicity that is comparable with the elongation period of the ribosome.

We believe that proteins fold cotranslationally by the tentative scheme described in this article. According to this scheme, protein folding is a dynamic process of self-organization of the spatial structure of a temporally elongating polypeptide chain that occurs in sequential stages.

Polypeptides enter cell compartments some distance from the ribosome posttranslationally, i.e., when they have escaped from the ribosome (exceptions are polypeptides synthesized on ribosomes anchored to the endoplasmic reticulum and are imported into the reticulum) [29-33, 69-73]. Therefore, the folding of many proteins is not coupled with the biosynthesis of the polypeptide chain. Because of this, in our opinion, cotranslational protein folding means that folding of a polypeptide chain into its native protein occurs in parallel with the translocation of the chain into the folding place.

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