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# Regulation, evolution and consequences of cotranslational protein complex assembly

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Most proteins assemble into complexes, which are involved in almost all cellular processes. Thus it is crucial for cell viability that mechanisms for correct assembly exist. The timing of assembly plays a key role in determining the fate of the protein: if the protein is allowed to diffuse into the crowded cellular milieu, it runs the risk of forming non-specific interactions, potentially leading to aggregation or other deleterious outcomes. It is therefore expected that strong regulatory mechanisms should exist to ensure efficient assembly. In this review we discuss the cotranslational assembly of protein complexes and discuss how it occurs, ways in which it is regulated, potential disadvantages of cotranslational interactions between proteins and the implications for the inheritance of dominant-negative genetic disorders.

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## Introduction

Many proteins can assemble into protein complexes [1,2•]. Although there is tremendous diversity in the types of quaternary structures that can be formed [3,4••], at the simplest level, protein complexes belong to two categories: homomers, formed from multiple copies of the same protein subunit, and heteromers, which have at least two distinct subunits with different amino-acid sequences. While homomers and heteromers are both prevalent across evolution, most prokaryotic complexes are homomers, while most eukaryotic complexes are heteromers [5–7].

Protein complexes are crucial for a large number of biological functions, and different types of protein quaternary structures have been shown to facilitate different biological functions and allosteric regulation [8•,9–12]. A large number of other benefits have been proposed [4••,13]. For example, considering the possibility of acquiring mutations during transcription and translation, it is more efficient to synthesize a larger structure in modules of subunits. Importantly, it also allows fine spatial and temporal regulation, and reduces folding complexity in forming unique shapes such rings or filaments. It has also been shown that multiple identical domains of the same polypeptide chain are prone to aggregation [14] due to formation of domain-swapped structures during cotranslational folding [15•]. Therefore, translating these domains as separate polypeptides that later assemble into a large complex can be less risky. Finally, it is important to emphasize that, while clearly there are many advantages to protein complexes, protein oligomerization is not always functionally beneficial and the result of evolutionary selection, but may be explained by simple nonadaptive processes [6,16].

In recent years, we have learned a considerable amount about the processes by which proteins assemble into complexes. We know that proteins generally assemble via ordered pathways that tend to be evolutionarily conserved [17,18]. Moreover, these assembly pathways appear to be biologically important both in prokaryotes [19] and eukaryotes [20]. However, there are still unanswered questions about how the cell regulates protein complex assembly, and where assembly actually occurs within the cell. A logical place to begin addressing this is in the initial stages of protein synthesis and folding.

## Cotranslational folding and assembly

The phenomenon of cotranslational folding has received considerable attention in recent years. Although the exact frequency at which cotranslational folding occurs in either prokaryotes or eukaryotes is unknown, there is a large body of computational [21–23] and experimental work [24,25••,26,27••] supporting and defining its likelihood. Significantly, these works emphasize the balance between the rate of translation, for example, as a function of charged-tRNA availability [28] or mRNA secondary structure [29–31], and the rate of protein folding. For reviews on the topic we recommend [32–34].

There are several reasons why proteins might acquire secondary structure during translation, sometimes even while still inside the ribosome exit tunnel [24,35,36]. For example, folding cotranslationally can modify the potential energy landscape to avoid nonproductive intermediates that would prevent the protein from reaching its native state [28]. However, cotranslational folding also reduces the propensity of deleterious non-specific interactions with the crowded cellular milieu or with other polypeptides on the same polyribosome. In other words, the protein primarily folds to protect itself from nonspecific interactions, but in doing so also allows assembly with native partners.

Given the prevalence of cotranslational folding, it is natural to imagine that assembly could also occur cotranslationally, especially given that folding and assembly are so intimately related [37]. This could potentially be beneficial for many of the same reasons as cotranslational folding; in particular, it could protect the protein from non-specific interactions, which is crucial due to the presence of the exposed interfaces making the unassembled subunits very sensitive to aggregation. This is particularly true for soluble homomers, which typically form larger hydrophobic interfaces than heteromers, and are thus more prone to misinteraction [38]. Although cotranslational assembly has received far less attention than cotranslational folding, it has been known of for a long time, with the first example we are aware of being homotetrameric β-galactosidase published in 1964 [39]. More recently, evidence is emerging that the phenomenon may be widespread [34,40••].

## How does cotranslational assembly occur within the cell?

During cotranslational assembly, at least one of the protein subunits begins to assemble while it is still in the process of being translated, that is, the interaction involves a nascent chain. This can occur via either *cis* or *trans* mechanisms. The *cis* mechanism (Figure 1a) involves the assembly of polypeptides from the same mRNA; this can refer either to the case where an interaction occurs while both chains are still in the process of being translated, or when a nascent chain binds to a fully translated protein released by the same mRNA. In contrast, the *trans* mechanism (Figure 1b) involves the assembly of a polypeptide from one mRNA with the product of another, and can apply to either heteromeric or homomeric assembly.

The rate at which cotranslational assembly will occur is a function of the affinity of the subunits for one another, and their effective concentration. However, concentration in this case is not purely determined by the number of proteins in solution, but also by the density of nascent polypeptides on the polyribosome. An important parameter influencing this is the length of time a nascent polypeptide spends attached to the mRNA, which in turn depends on numerous factors, including mRNA secondary structure [30], the availability of charged-tRNAs, the overall length of the mRNA, and elements such as anti-Shine-Dalgarno sequences in mRNA [41]. Thus, concentration is a function of multiple variables, but for simplicity can be summarized as the total number of nascent polypeptides within the polyribosome's sphere of influence at a particular point in time.

At this point, we would like to propose an additional role to the secondary structure of mRNA. As mentioned above, the secondary structure of mRNA affects translation rate, thus regulating nascent chain folding into its correct fold. However, it is likely that many mRNAs form more complex structures than that of the two-dimensional structure, and thus the polyribosome and consequently the ribosome tunnels will be orientated in a particular way. These trajectories will influence both the probability of clashing between nascent chains, which will affect the stability of monomers, and the probability of cotranslational complex assembly. It is therefore important to understand the native three-dimensional of the polyribosome, continuing recent efforts [42••,43•].

The cell broadly regulates both *cis* and *trans* mechanisms. For *cis*, the number of ribosomes, which is a function of 'initiation rate' (how many), 'elongation' (how long), and 'termination', will determine its frequency of occurrence. For the *trans* mechanism, concentration can be increased by active transport of the same-gene mRNAs transcripts to a specific location in the cell, a mechanism which has been observed in both eukaryotes [44] and prokaryotes [45,46]. It is worth mentioning that this factor is rarely discussed in the literature, and should be taken into account while discussing mRNA localization of protein complexes.

## Cotranslational assembly of operon-encoded complexes

At this juncture, it is important to highlight the stark differences between eukaryotic and prokaryotic assembly of protein complexes, specifically for heteromers. In eukaryotes, cotranslational assembly of heteromers must occur in *trans*, either through co-localization of mRNAs encoding interacting proteins, or through localization of fully folded proteins to active polysomes (Figure 1b). In contrast, prokaryotes often encode protein complex subunits in operons, whereby distinct protein subunits can be translated from the same polycistronic mRNA molecule [47,48]. Thus, for operon-encoded complexes, cotranslational assembly of heteromers can occur in *cis* in much the same way as it does for homomers (Figure 2).

To this end, there are multiple strands of evidence pointing to the important role operons play in facilitating