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Cotranslational Assembly of Protein Complexes

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Abstract

The interaction of biological macromolecules is a fundamental attribute of cellular life. Proteins, in particular, often form stable complexes with one another. Although the importance of protein complexes is widely recognised, we still have only a very limited understanding of the mechanisms underlying their assembly within cells. In this article we review the available evidence for one such mechanism, namely the coupling of protein complex assembly to translation at the polysome. We discuss research showing that cotranslational assembly can occur in both prokaryotic and eukaryotic organisms, and can have important implications for the correct functioning of the complexes that result. Cotranslational assembly can occur for both homomeric and heteromeric protein complexes, and for both proteins that are translated directly into the cytoplasm and those that are translated into or across membranes. Finally, we discuss the properties of proteins that are most likely to be associated with cotranslational assembly.

Introduction

Proteins within the cell must carry out their important functions in an environment that is highly crowded, and are in constant physical contact with various other proteins, metabolites and macromolecules [1,2]. Apart from many transient interactions, which may or may not have important functional duties, e.g. cellular signalling [3,4], many if not most intracellular proteins function as subunits of more long-lived protein complexes [5,6]. Despite the fact that complex formation is crucial for understanding the function (and malfunction) of many proteins, the fundamental mechanisms behind the assembly of individual proteins into complexes with defined *quaternary structure* have often been neglected, and little is known about the *in vivo* assembly process. One interesting aspect of protein complex assembly is the degree to which it is coupled to the cellular translation machinery. Are the subunits fully translated before finding their interaction partners and forming their defined quaternary structure in a *post-translational* assembly pathway? Or do some protein interactions form as the individual subunits are still being translated, through *cotranslational* assembly?

The general process of the maturation of a functioning protein involves folding and translocation of the polypeptide as well as complex assembly. In recent years, there has been a growing body of evidence showing that the protein folding process can often occur whilst the polypeptide is being translated, *i.e.* cotranslationally involving the nascent polypeptide chain [7–12]. Whilst this could arise due to the basic energetics of protein folding, there are also potential functional benefits to cotranslational folding. For example, it may provide a means of tuning the potential energy landscape by lowering the energy of folding intermediates. A cotranslational folding process also contributes to the earlier formation of secondary and tertiary structures making unfavourable inter-domain aggregation events less likely.

The assembly of protein complexes can in many ways be considered analogous to protein folding, in that it typically follows a specific pathway via energetically-favourable assembly intermediates [13,14]. It is therefore natural to envisage that assembly could also sometimes occur cotranslationally, and even be functionally advantageous. Many protein complex subunits are highly dynamic or unstable in isolation [15], and so rapid assembly during translation minimises the opportunities for misfolding or aggregation. If the subunits assemble through a series of lower energy intermediates of nascent polypeptides, it would lower the overall energy of the assembly, analogous to the tuning of folding. Cotranslational protein interactions can also be viewed as a means of ensuring a precisely ordered assembly process and for avoiding unfavourable inter-subunit aggregation. Contrary to cotranslational folding, however, the kinetics of cotranslational assembly are not only a function of the rate of assembly, but also highly dependent on the concentration of available assembly partners in close proximity to the polysome.

Although cotranslational assembly has received much less attention than cotranslational folding, numerous cases have been reported over the years, beginning with observations of specific proteins associating cotranslationally with the cytoskeleton [16–19]. More recently, evidence has emerged suggesting that the phenomenon might be widespread [20]. Here we review several examples of cotranslational assembly, discussing reasons why it occurs and the functional benefits that it can provide. Although there are many transient protein-protein interactions that occur cotranslationally, *e.g.* involving chaperones [21] or targeting signal sequences for translocation [22], here we will focus on the assembly of stable protein complexes.

Cotranslational assembly of homomers

Protein complexes can be broadly split into two categories based upon their quaternary structure: *homomers*, formed from the self-assembly of a single subunit type, and *heteromers*, formed from multiple distinct subunits. There are two ways in which cotranslational homomer assembly can occur. In one, a newly translated subunit is released and then interacts with another still-translating copy of itself, most likely on the same polysome from which it was translated (Figure 1A). Alternatively, cotranslational assembly could involve interaction between two nascent chains on the same polysome (Figure 1B).

An early example of cotranslational assembly of a homomer came from investigations into the well-characterised bacterial homotetramer β-galactosidase [23,24]. The enzymatic activity of β-galactosidase is only evident after the conformational changes that are required for tetramer formation have taken place. In these experiments it was shown that the enzymatically active form of the complex could be observed at the same time as nascent polypeptide chains. It became clear that not only the folding, but also the assembly into the functioning enzyme occurred in a cotranslational manner. The authors suggested that this was due to the proximity of the nascent polypeptides, as monomers from adjacent ribosomes dimerized before forming the final tetrameric structure.

The reovirus attachment protein σ1 forms a homotrimer and can be divided into two segments: an N-terminal tail that is anchored in the virion and a globular C-terminal domain that is responsible for virion attachment (Figure 2). Curiously it was found that the trimerisation of the two regions takes place using two different mechanisms [25]. Assembly of the N-terminal region, which is translated first, was found to occur cotranslationally, at neighbouring ribosomes that had passed the midpoint of the mRNA strand. In contrast, trimerisation of the globular C-terminal region, which is translated last, was found to be highly chaperone- and ATP-dependent and occurs post-translationally.

The tumour suppressor p53 forms a homotetramer with dihedral symmetry. Whilst both alleles of p53 are often mutated or non-functional in cancer cells, mutations in a single allele often display a dominant-negative effect. Depending on the location of the mutation, numerous factors contribute to this effect, but a key aspect relates to the mechanism of p53 tetramer assembly and the extent to which it is coupled to translation. McLure et al. demonstrated that this process occurs by an initial cotranslational dimerization of p53, with tetramers forming separately, and post-translationally [26]. The suggested driving force for this assembly mechanism was the stabilisation of the dimer through hydrophobic interactions between the N-termini. A direct effect of this cotranslational assembly is that the possible stoichiometries of the fully assembled complex are constrained: p53 dimers will always be homomers of either the wild-type or mutant version of the protein. Consequently, 1/4 of the resulting homotetramers will be fully wild-type, as opposed to only 1/16 in a situation where cotranslational dimerization does not occur. This suggests that the cotranslational dimerization step has a strong influence on the magnitude of the dominant-negative effect observed.

NF-κB1 (nuclear factor of kappa light polypeptide gene enhancer in B-cells 1) is a member of the NF-κB family of transcription factors and is involved in regulation of several cellular processes, particularly the inflammatory response [27]. The complex exists predominantly as a heterodimer of p50 and p105 subunits, with the p50 subunit being a truncated form of p105. Full-length p105 is comprised of an N-terminal Rel homology domain (RHD), and a larger C-terminal ankyrin-repeat domain that functions as an I-κB-like inhibitor of mature NF-κB1.

Between these two domains lies a nuclear-localisation signal and a glycine-rich region that acts as the site of endoproteolytic cleavage by the 26S proteasome [28]. Active NF-κB1 requires cleavage and degradation of the C-terminal domain of p105 to form mature p50 [29]. A key question arising from this observation is how the proteasome degrades p105 whilst sparing p50. Building on early observations that free p50 rapidly associates with other Rel family proteins *in vitro*, Lin et al. demonstrated *in vivo* that p50-p105 heterodimers assemble on the same polysome via cotranslational homodimerization of p50 RHDs [30,31] (Figure 3). This is coupled with cotranslational processing by the proteasome – crucially, it is the process of dimerization that appears to act as a physical barrier to degradation of p50. In support of this, it was shown that deletion of the second subdomain of RHD (essential for dimerization) led to a significant reduction in the amount of p50 observed upon expression of the mutant *NF-κB1* gene. This suggests that in the absence of dimerization, p105 is completely degraded. If so, this provides a clear example of how cotranslational assembly can be functionally important; in this case, cotranslational assembly is essential for the production of mature NF-κB1, with the active p50 subunit being placed under immediate control of the inhibitory p105 subunit by the process. Subsequent post-translational activation then depends on phosphorylation and ubiquitin-mediated cleavage of the remaining p105 ankyrin-repeat domain.

Cotranslational assembly of heteromers

The assembly of heteromers is inherently more complex than for homomers, due to the fact that it involves interactions between distinct proteins that are usually encoded by different genes. Those interacting proteins must somehow find each other within the cell. Cotranslational interaction, in which a fully translated protein finds its way to the nascent chain of another protein (Figure 1C), provides a way to minimise the stochasticity of assembly by increasing the chance of subunit encounter.

One example of a heteromeric interaction with both co- and post-translational assembly mechanisms is the covalent disulphide bond formation between heavy and light chains in the immunoglobulin molecule. Despite earlier evidence of post-translational formation of the disulphide linkers, it was shown that over-production of light chains in the endoplasmic reticulum (ER) in certain cell types lead to light-heavy chain heterodimerization on the nascent heavy chain, purely due to light chain abundance in the proximity of the translating heavy chain transcript [32]. Thus protein expression levels and abundance are likely to be important regulators of whether or not heteromeric assembly occurs cotranslationally.

The yeast histone methyltransferase, COMPASS, is comprised of eight different subunits. In work originally designed to investigate the role of mRNA in COMPASS function, a four-member subcomplex of COMPASS (Swd1p, Spp1p, Shg1p, Set1p) was found to interact with *SET1* mRNA [33]. Crucially, formation of the mRNA-associated subcomplex was found to be dependent on active translation, indicating that the subunits are binding to the nascent Set1p protein as it is translated. Furthermore, whilst structural data is not available for the full complex, it appears that the binding sites of Shg1p, Swd1p and Spp1p are localised to the N-terminal or central region of Set1p; this is consistent with cotranslational assembly as these regions are translated earlier [33,34].

More recently, the first systematic analysis of cotranslational assembly has been performed. Duncan and Mata used ribonucleoprotein immunoprecipitation-microarray (RIP-Chip) experiments to identify mRNA sequences associated with 31 proteins from *Schizosaccharomyces pombe* [20]. Here they found that 12 of these (38%) co-purified with

the mRNAs of known interaction partners. Importantly, as for the COMPASS example, copurification was found to be dependent on active translation, indicating that interactions are probably occurring between proteins and nascent peptides, rather than protein and mRNA. These interactions were also found to be highly specific – Cdc2p for example was found to copurify with just two mRNAs, despite having a large number of known and hypothesised protein interaction partners. Interestingly, the fact that these mRNA-protein interactions are so specific has since been used by the same group to predict novel protein-protein interactions [35].

Cotranslational assembly of secreted and membrane complexes

The above examples involve cotranslational assembly within the cytoplasm, but many proteins are directly translocated into or across membranes during translation. In eukaryotes, membrane and secreted proteins are translated at the rough ER. In investigations into the assembly of the extracellular human tenascin protein, responsible for cell adhesion, it was shown that the hexameric complex is formed without any assembly intermediates being observed [36]. As soon as the tenascin protein is experimentally detectable it appears to be cotranslationally assembled into its active hexamer structure. In this case the authors suggested that the arrangement of the membrane-bound polysome at the ER, where the ribosomes have been seen to form various circular loops and spirals, directly resulted in the homomer acquiring its circular hexamer shape.

A further example of ER membrane influence on cotranslational assembly is seen in voltage-gated potassium cation channels. These channels are tetrameric with interfaces located at the N-terminal region of the subunits (referred to as the tetramerisarion (T1)-domains). In experiments using *Xenopus laevis* oocytes, it was shown that T1-T1 association occurred between ribosome-attached subunits, and the ER membrane was postulated to regulate the local concentration of the interacting domains [37]. In the related human ether-à-go-go related gene (*hERG1*), responsible for the potassium channel hERG, the two subunits hERG1a and hERG1b are isoforms of *hERG1*, arising from two mRNA splice-variants [38]. The isoforms are identical apart from the important N-termini, and it was observed that the two N-termini localise and bind to each other cotranslationally. This mechanism is crucial to avoid unfavourable aggregation events involving the hERG1b subunits and is mediated by the ER, which ensures colocalisation of the transcripts.

Finally, there is evidence that the plant D1 transmembrane protein assembles cotranslationally into the photosystem II complex [39,40]. This is an interesting example as the D1 protein frequently experiences photodamage and experiences a high rate of turnover. Thus the ability of D1 to be translated directly into the chloroplast membrane and cotranslationally assemble allows photosystem II to be quickly repaired. It is also notable that translational pausing is known to occur at specific sites during the translation of D1 [41], potentially allowing time for assembly to occur [42], analogous to how translational pausing can facilitate protein folding [12].

Perspectives

Here we have highlighted a number of examples of homomeric and heteromeric protein complexes that assemble cotranslationally. However, we still have very little idea about the frequency of the phenomenon. One systematic analysis suggested that it might be quite widespread, yet this work considered only a very small fraction of known proteins in fission yeast [20]. In addition, questions remain about the specific mechanisms by which

cotranslational interactions occurs. For example, it is unclear whether binding events are limited to those occurring between one nascent and one fully folded chain, or whether dimerization ever occurs whilst both chains are being translated. Thus, there is considerable future potential for both large- and small-scale screens looking for evidence of cotranslational assembly.

Why do some protein complexes assemble cotranslationally while others do not? While possible functional benefits have been discussed here, it is important to remember that cotranslational assembly has not necessarily been selected for evolutionarily in all cases. Cotranslational assembly could occur simply because a free subunit encounters a nascent chain and their interaction is energetically favourable. In fact, for some proteins, there may be evolutionary pressure to avoid cotranslational assembly. Although we only have experimental evidence of cotranslational assembly for a fairly small number of complexes, we can make some predictions about which complexes might be most likely to assemble cotranslationally:

- All things being equal, homomers should be more likely to cotranslationally assemble than heteromers, since interacting subunits can be translated from the same polysome and local subunit concentration will be high.
- Many prokaryotic complexes are encoded in operons, so that interacting proteins are often translated off the same polycistronic mRNA. This ensures that interacting subunits are in close physical proximity upon translation and facilitates a higher rate of complex assembly [43]. Thus we can predict that operon-encoded heteromers should be more likely to undergo cotranslational assembly.
- For both homomers and heteromers, the likelihood of cotranslational assembly should be greater for highly abundant proteins, as this will increase the chance that an interaction partner encounters and binds a nascent chain still in the process of being translated.
- Localisation towards N-terminal regions is likely to be a general feature of interfaces that form cotranslationally, since this will allow more time for cotranslational assembly to occur. Therefore, complexes with N-terminal interfaces should be more likely to have formed cotranslationally.
- Subunits that are highly flexible or disordered in isolation [15] could benefit from cotranslational assembly, as this would avoid them spending unnecessary time free and susceptible to proteases in the cell.
- The first step of a protein complex assembly pathway is the most probable to occur cotranslationally. Thus we may be able to use experimental characterisation or structure-based prediction of assembly order [13,14] to identify subunits and interfaces that are most likely to form cotranslationally.

Finally, there are major questions remaining about how cotranslational assembly is regulated and how proteins are localised to polysome, especially for heteromers with subunits translated from different mRNA molecules. This is especially important for eukaryotic complexes, which have a much greater propensity to form heteromers [44,45], compared to bacterial proteins, which are more likely to self-assemble into homomers [46] or be encoded in operons. Much more work is needed to fully understand how the assembly of heteromeric complexes occurs within eukaryotic cells, both co- and post-translationally, and how it is regulated.

Acknowledgements

References

1. Ellis RJ. (2001) Macromolecular crowding: An important but neglected aspect of the intracellular environment. Curr. Opin. Struct. Biol. **11**, 114–119.

2. McGuffee SR, Elcock AH. (2010) Diffusion, crowding & protein stability in a dynamic molecular model of the bacterial cytoplasm. PLoS Comput. Biol. **6**, e1000694.

3. Nooren IMA, Thornton JM. (2003) Structural characterisation and functional significance of transient protein-protein interactions. J. Mol. Biol. **325**, 991–1018.

4. Landry CR, Levy ED, Abd Rabbo D, Tarassov K, Michnick SW. (2013) XExtracting insight from noisy cellular networks. Cell. **155**, 983–989.

5. Perica T, Marsh JA, Sousa FL, Natan E, Colwell LJ, Ahnert SE, et al. (2012) The emergence of protein complexes: quaternary structure, dynamics and allostery. Biochem. Soc. Trans. **40**, 475–491.

6. Marsh JA, Teichmann SA. (2014) Structure , Dynamics , Assembly , and Evolution of Protein Complexes. Annu. Rev. Biochem. **84**, 551–575.

7. Komar AA. (2009) A pause for thought along the co-translational folding pathway. Trends Biochem. Sci. **34**, 16–24.

8. O'Brien EP, Vendruscolo M, Dobson CM. (2012) Prediction of variable translation rate effects on cotranslational protein folding. Nat. Commun. **3**, 868.

9. Gloge F, Becker AH, Kramer G, Bukau B. (2014) Co-translational mechanisms of protein maturation. Curr. Opin. Struct. Biol. **24**, 24–33.

10. Ciryam P, Morimoto RI, Vendruscolo M, Dobson CM, O'Brien EP. (2013) In vivo translation rates can substantially delay the cotranslational folding of the Escherichia coli cytosolic proteome. Proc. Natl. Acad. Sci. U. S. A. **110**, E132–40.

11. Pechmann S, Frydman J. (2013) Evolutionary conservation of codon optimality reveals hidden signatures of cotranslational folding. Nat. Struct. Mol. Biol. **20**, 237–43.

12. Zhang G, Hubalewska M, Ignatova Z. (2009) Transient ribosomal attenuation coordinates protein synthesis and co-translational folding. Nat. Struct. Mol. Biol. **16**, 274–280.

13. Levy ED, Boeri Erba E, Robinson C V, Teichmann S a. (2008) Assembly reflects evolution of protein complexes. Nature. **453**, 1262–5.

14. Marsh JA, Hernández H, Hall Z, Ahnert SE, Perica T, Robinson C V, et al. (2013) Protein complexes are under evolutionary selection to assemble via ordered pathways. Cell. **153**, 461–470.

15. Marsh JA, Teichmann SA, Forman-Kay JD. (2012) Probing the diverse landscape of protein flexibility and binding. Curr. Opin. Struct. Biol. **22**, 643–650.

16. Isaacs WB, Kim IS, Struve a., Fulton a. B. (1989) Biosynthesis of titin in cultured skeletal muscle cells. J. Cell Biol. **109**, 2189–2195.

17. Isaacs WB, Fulton a B. (1987) Cotranslational assembly of myosin heavy chain in developing cultured skeletal muscle. Proc. Natl. Acad. Sci. U. S. A. **84**, 6174–6178.

18. L'Ecuyer TJ, Noller JA, Fulton AB. (1998) Assembly of tropomyosin isoforms into the cytoskeleton of avian muscle cells. Pediatr. Res. **43**, 813–822.

19. Isaacs WB, Cook RK, Van Atta JC, Redmond CM, Fulton AB. (1989) Assembly of vimentin in cultured cells varies with cell type. J. Biol. Chem. . **264** , 17953–17960.

20. Duncan CD, Mata J. (2011) Widespread cotranslational formation of protein complexes. PLoS Genet. **7**, e1002398.

21. Zhang Y, Ma C, Yuan Y, Zhu J, Li N, Chen C, et al. (2014) Structural basis for interaction of a cotranslational chaperone with the eukaryotic ribosome. Nat. Struct. Mol. Biol. **21**, 1042–1046.

22. Park E, Rapoport TA. (2012) Mechanisms of Sec61/SecY-Mediated Protein Translocation Across Membranes. Annu. Rev. Biophys. **41**, 21–40.

23. Zipser D, Perrin D. (1963) Complementation on Ribosomes. Cold Spring Harb. Symp. Quant. Biol. **28**, 533–537.

24. Kiho Y, Rich A. (1964) Induced Enzyme Formed on Bacterial Polyribosomes. Proc. Natl. Acad. Sci. U. S. A. **51**, 111–118.

25. Leone G, Coffey MC, Gilmore R, Duncan R, Maybaum L, Lee PWK. (1996) C-terminal trimerization, but not N-terminal trimerization, of the reovirus cell attachment protein is a posttranslational and Hsp70/ATP-dependent process. J. Biol. Chem. **271**, 8466–8471.

26. Nicholls CD, McLure KG, Shields MA, Lee PWK. (2002) Biogenesis of p53 Involves Cotranslational Dimerization of Monomers and Posttranslational Dimerization of Dimers: IMPLICATIONS ON THE DOMINANT NEGATIVE EFFECT. J. Biol. Chem. **277**, 12937–12945.

27. Jurk D, Wilson C, Passos JF, Oakley F, Correia-Melo C, Greaves L, et al. (2014) Chronic inflammation induces telomere dysfunction and accelerates ageing in mice. Nat. Commun. **2**, 4172.

28. Lin L, Ghosh S. (1996) A glycine-rich region in NF-kappaB p105 functions as a processing signal for the generation of the p50 subunit. Mol. Cell. Biol. **16**, 2248–2254.

29. Fan CM, Maniatis T. (1991) Generation of p50 subunit of NF-kappa B by processing of p105 through an ATP-dependent pathway. Nature. **354**, 395–398.

30. Chen FE, Huang DB, Chen YQ, Ghosh G. (1998) Crystal structure of p50/p65 heterodimer of transcription factor NF-kappaB bound to DNA. Nature. **391**, 410–413.

31. Lin L, DeMartino GN, Greene WC. (2000) Cotranslational dimerization of the Rel homology domain of NF-kappaB1 generates p50-p105 heterodimers and is required for effective p50 production. EMBO J. **19**, 4712–4722.

32. Bergman LW, Kuehl WM. (1979) Formation of intermolecular disulfide bonds on nascent immunoglobulin polypeptides. J. Biol. Chem. **254**, 5690–5694.

33. Halbach A, Zhang H, Wengi A, Jablonska Z, Gruber IML, Halbeisen RE, et al. (2009) Cotranslational assembly of the yeast SET1C histone methyltransferase complex. EMBO J. **28**, 2959–2970.

34. Dehé P-M, Géli V. (2006) The multiple faces of Set1. Biochem. Cell Biol. **84**, 536–548.

35. Duncan CD, Mata J. (2014) Cotranslational protein-RNA associations predict protein-protein interactions. BMC Genomics. **15**, 298.

36. Redick SD, Schwarzbauer JE. (1995) Rapid intracellular assembly of tenascin hexabrachions suggests a novel cotranslational process. J. Cell Sci. **108 (Pt 4**, 1761–1769.

37. Lu J, Robinson JM, Edwards D, Deutsch C. (2001) T1-T1 interactions occur in ER membranes while nascent KV peptides are still attached to ribosomes. Biochemistry. **40**, 10934–10946.

38. Phartiyal P, Jones EMC, Robertson G a. (2007) Heteromeric assembly of human ether-à-go-go-related gene (hERG) 1a/1b channels occurs cotranslationally via N-terminal interactions. J. Biol. Chem. **282**, 9874–9882.

39. Zhang L, Paakkarinen V, Van Wijk KJ, Aro EM. (1999) Co-translational assembly of the D1 protein into photosystem II. J. Biol. Chem. **274**, 16062–16067.

40. Zhang L, Aro EM. (2002) Synthesis, membrane insertion and assembly of the chloroplast-encoded D1 protein into photosystem II. FEBS Lett. **512**, 13–18.

41. Kim J, Klein PG, Mullet JE. (1991) Ribosomes pause at specific sites during synthesis of membrane-bound chloroplast reaction center protein D1. J. Biol. Chem. **266**, 14931–14938.

42. Képès F. (1996) The "+70 pause": hypothesis of a translational control of membrane protein assembly. J. Mol. Biol. **262**, 77–86.

43. Sneppen K, Pedersen S, Krishna S, Dodd I, Semsey S. (2010) Economy of operon formation: Cotranscription minimizes shortfall in protein complexes. MBio. **1**, 3–5.

44. Lynch M. (2012) The evolution of multimeric protein assemblages. Mol. Biol. Evol. **29**, 1353–1366.

45. Marsh JA, Teichmann SA. (2014) Protein Flexibility Facilitates Quaternary Structure Assembly and Evolution. PLoS Biol. **12**, e1001870.

46. Marsh JA, Rees HA, Ahnert SE, Teichmann SA. (2015) Structural and evolutionary versatility in protein complexes with uneven stoichiometry. Nat. Commun. **6**, 6394.
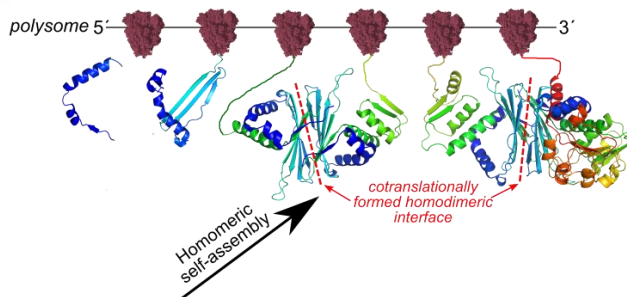
Figures

A) Cotranslational homomer assembly
between one nascent chain and one full subunit

polysome 5´ ——— 3´

cotranslationally
formed homodimeric
interface

Homomeric self-assembly
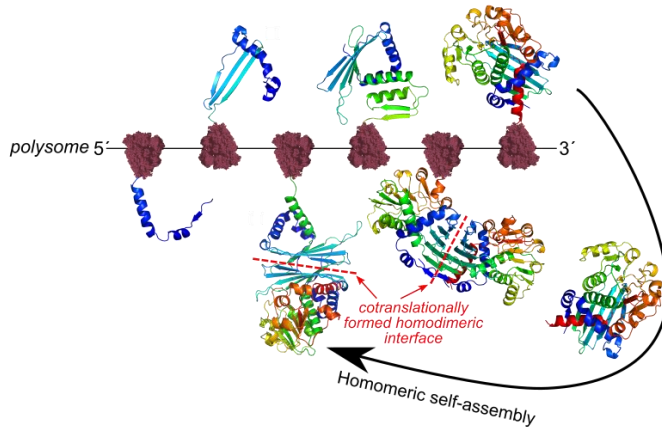
B) Cotranslational homomer assembly
between two nascent chains

polysome 5´ ——— 3´

cotranslationally
formed homodimeric
interface

Homomeric
self-assembly

C) Cotranslational heteromer assembly

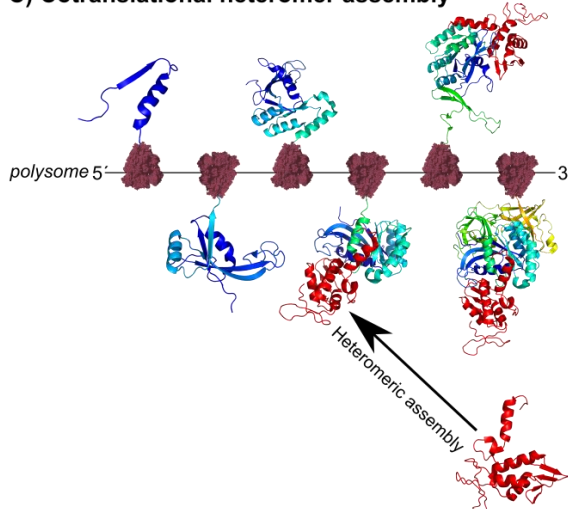*polysome* 5´ ——— 3´

Heteromeric assembly

Figure 1. Cotranslational assembly of protein complexes. In all panels, moving from left (5´) to right (3´) on the polysome (*i.e.* the mRNA bound to multiple ribosomes), we can see increasingly long nascent chains being translated. Homomer assembly can occur in two ways. In (A), a full-length subunit is released and binds to a nascent chain, forming a cotranslationally assembled homodimer. In (B), two nascent chains from the same polysome interact with each other. For heteromer assembly (C), a different subunit (red) encoded by a different gene binds to a nascent chain, forming a cotranslationally assembled heterodimer. These are hypothetical examples of cotranslational assembly based upon PDB ID: 2I99 (homodimer) and PDB ID: 2DCU (heterodimer).
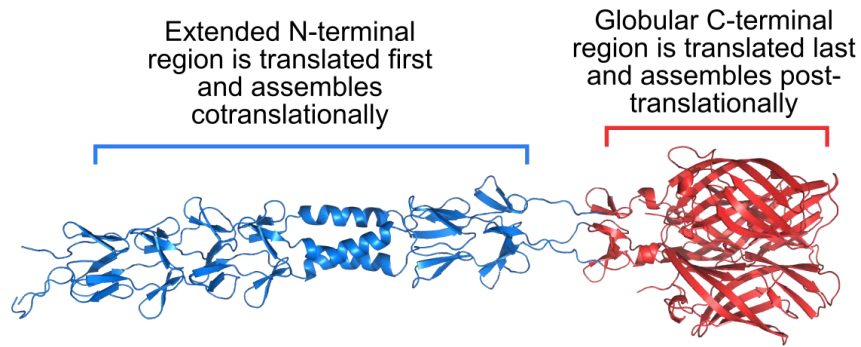
Figure 2. Structure of the reovirus attachment protein σ1. In this homotrimeric complex (PDB ID: 3S6X), the extended N-terminal region (blue) is known to assemble cotranslationally, while the globular C-terminal region (red) assembles only post-translationally. This highlights the idea that cotranslationally forming interfaces should generally localised towards the N-termini of proteins, as they will spend more time as part of a nascent chain and have more time to cotranslationally interact.
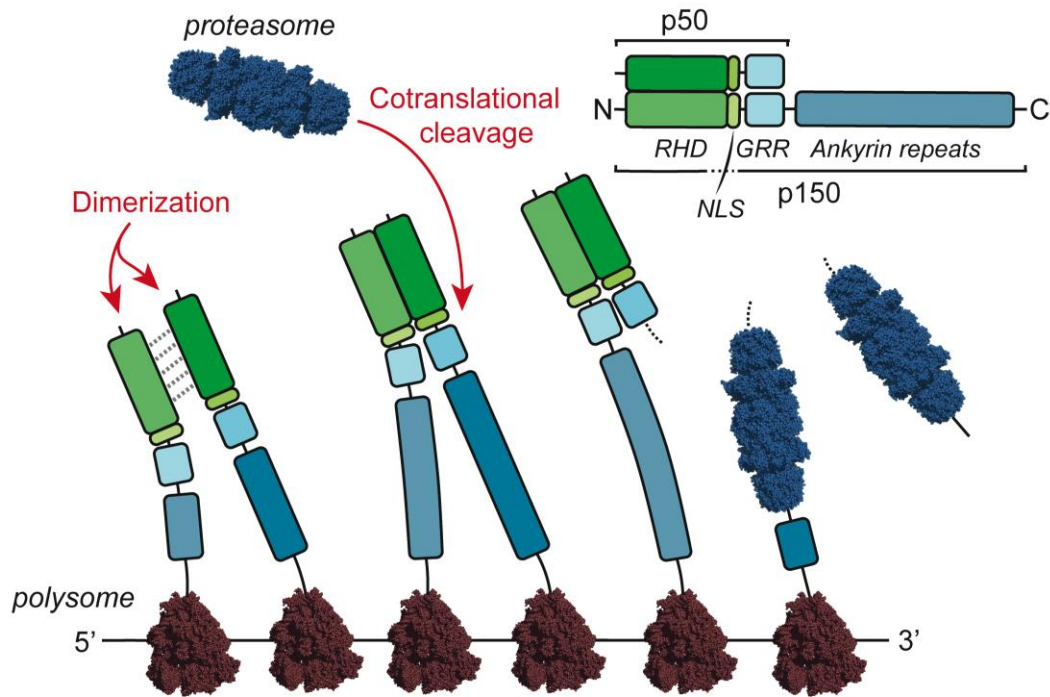
Figure 3. Cotranslational assembly of p50-p105 heterodimer. The p50 protein is ~400 residues in length and is comprised of a Rel homology domain (RHD), nuclear localisation signal (NLS) and C-terminal glycine-rich region (GRR), which is targeted by the proteasome. The p105 protein differs only in that it contains an additional ankyrin-repeat domain. During translation, the RHDs of two nascent polypeptides dimerize, though it is unclear as to whether this occurs whilst both chains are being actively translated (as shown here) or between freshly synthesized p105 and the actively translating chain, as in Figure 1A. As very rapid dimerization of the RHDs is essential to prevent complete degradation of p50/p105 by the proteasome (which also occurs cotranslationally), it seems plausible that the former scenario is correct.