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Cotranslational Assembly of Oligomeric Proteins

Jaime Santos,[1] Sander J. Tans,[2,3] Nenad Ban,[4] Günter Kramer,[1] and Bernd Bukau[1]

[1]Center for Molecular Biology of the University of Heidelberg (ZMBH), DKFZ-ZMBH Alliance, Heidelberg, Germany; email: bukau@zmbh.uni-heidelberg.de, g.kramer@zmbh.uni-heidelberg.de

[2]AMOLF, Amsterdam, The Netherlands

[3]Bionanoscience Department, Kavli Institute of Nanoscience Delft, Delft University of Technology, Delft, The Netherlands

[4]Department of Biology, Institute of Molecular Biology and Biophysics, ETH Zurich, Zurich, Switzerland

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Abstract

The assembly of newly synthesized proteins into functionally active oligomers has long been regarded as a posttranslational process driven by random collision of subunits. However, growing evidence indicates that, for many proteins, assembly occurs cotranslationally, tightly coupling synthesis, folding, and subunit assembly. This fundamentally different mechanism enables the spatial and temporal coordination of assembly, promotes the hierarchical formation of multisubunit assemblies, enhances the stability of involved subunits, enlarges the space of feasible protein structures including complexes with intertwined subunits, and has profound effects on protein evolution and function. In this review, we describe the molecular mechanisms, cellular requirements, and functional implications of cotranslational assembly and discuss its relevance to human disease, its evolutionary significance, and its transformative potential in synthetic biology and recombinant protein production.

Contents

1. INTRODUCTION

Protein complexes are central to nearly all biological processes, executing cellular functions by establishing complex molecular networks (1). Accurate and timely complex assembly is therefore essential for maintaining proteome integrity and cellular viability (2, 3), and errors in this process lead to protein misfolding and aggregation (4–7) linked to a range of diseases (8, 9).

Protein complex assembly has traditionally been viewed as a posttranslational, diffusion-driven process in which fully synthesized subunits randomly encounter each other in the crowded cellular milieu (10, 11). The stochastic nature of this process is inherently prone to off-target interactions, because the same physicochemical features that enable productive protein–protein interactions also trigger nonspecific interactions and aggregation (12–15). To bias this competition toward functional interactions, cells employ a multilayered quality control machinery, including (*a*) dedicated chaperones and assembly factors (16–18) that guide complex subunits toward productive assembly; (*b*) quality control proteins, which detect and resolve nonnative assemblies (19, 20); (*c*) proteolytic machineries, which remove destabilized or misfolded subunits

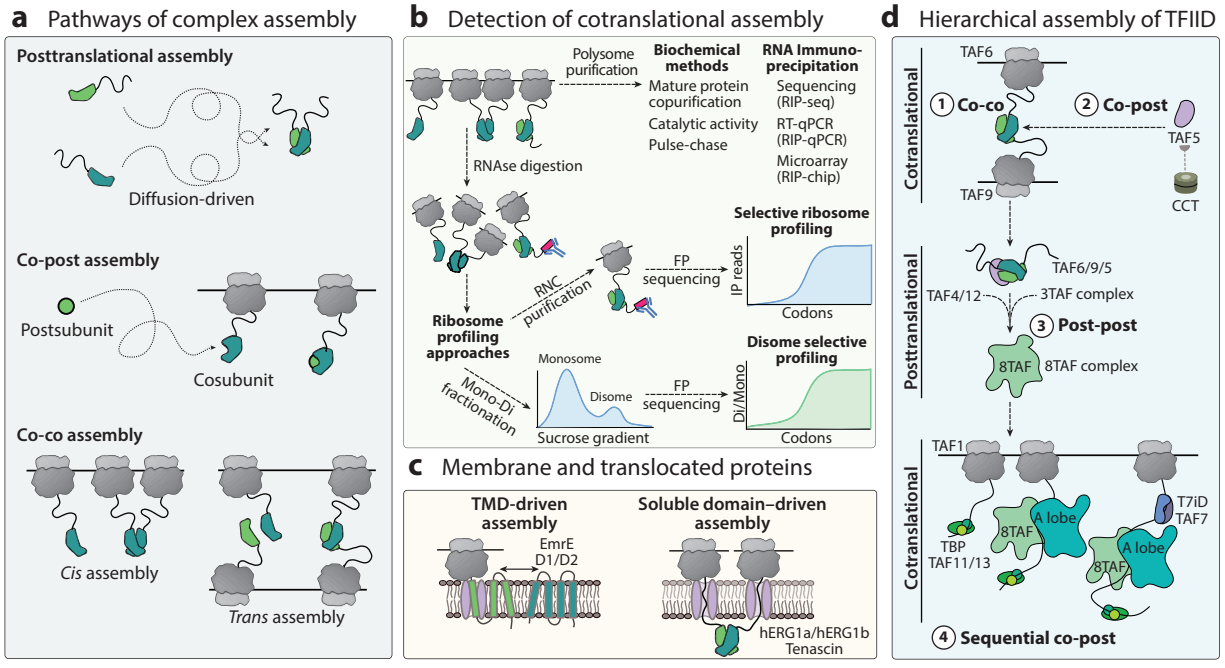


Figure 1

Overview of cotranslational assembly. (*a*) Pathways of protein complex formation. (*b*) Methods for detection of cotranslational assembly. (*c*) Schematic representation of two modes of cotranslational assembly at membranes. (*d*) Schematic representation of hierarchical assembly of TFIID based on Reference 48 using different assembly mechanisms. Abbreviations: FP, ribosome footprint; IP, immunoprecipitation; RIP-chip, RNA immunoprecipitation chip; RIP-Seq, RNA immunoprecipitation followed by sequencing; RIP-qPCR, RNA immunoprecipitation followed by quantitative polymerase chain reaction; RNC, ribosome–nascent chain; RT-qPCR, real-time quantitative polymerase chain reaction; TFIID, transcription factor II D; TMD, transmembrane domain.

(21, 22); and (*d*) surveillance of subunit stoichiometry and orphaned subunits, i.e., proteins that failed to find their partners (3, 23–25). While this robust machinery protects the proteome, it also consumes substantial cellular energy and resources. Streamlining complex formation, in both efficiency and specificity, has therefore likely been a significant force in evolution.

Recently, cotranslational assembly has emerged as a widespread and universally conserved mechanism for efficient protein complex formation (5, 26–29). In cotranslational assembly, complexes begin to form while at least one of the interacting subunits is still being synthesized by the ribosome. Two primary modes of cotranslational assembly have been described (**Figure 1*a***): the co-post assembly route, where a fully synthesized subunit (postsubunit) interacts with a nascent polypeptide still emerging from the ribosome (cosubunit or nascent subunit) (5, 30), and the co-co assembly route, where two or more nascent chains interact (26, 31–34). The interactions between nascent-chain pairs generate disomes (or higher-order ribosome assemblies) composed of connected ribosomes, a fundamental feature allowing the proteome-wide analysis of co-co assembly (for a detailed description, see Section 3).

Cotranslational assembly offers diverse functional advantages, even as the extent of these benefits remains incompletely understood. By coupling protein synthesis, folding, and complex formation, these early interactions can shape cotranslational folding pathways (35), improve the efficiency and specificity of complex assembly (30), minimize promiscuous interactions (29), and limit the accumulation of orphaned subunits (36), thereby alleviating reliance on general

chaperones and degradation systems. Additionally, cosubunits do not need to fold independently and be stable and soluble in the cytoplasm while searching for their partners. Cotranslationally assembling proteins can therefore populate a distinct section of the folding landscape inaccessible during posttranslational assembly (28, 37, 38), driving the evolution of new protein folds.

At the same time, cotranslational assembly introduces new challenges. It requires precise spatial organization of the translation apparatus, including intracellular colocalization of the involved mRNAs, coordination of translation kinetics, regulation of subunit stoichiometries, and targeted action of quality control pathways. Our understanding of these principles remains in its infancy, yet recent work has begun to delineate their emerging foundations. In this review, we present these recent findings and outline the key challenges lying ahead.

2. METHODS FOR ANALYSIS OF COTRANSLATIONAL ASSEMBLY

Early insights into cotranslational assembly emerged from classical biochemical methods, such as pulse-chase labeling, copurification of polysomes with mature proteins, and detection of complex-specific activities in polysome fractions (39–43) (**Figure 1*b***). Recent advances have been driven by sequencing-based approaches that enable in vivo profiling of cotranslational protein complex formation (26, 33, 34, 44, 45) (**Figure 1*b***). Unlike traditional biochemical methods that target a specific complex, these techniques capture proteome-wide interactors and, in the case of ribosome profiling–based techniques, can even provide codon-level resolution. These methods rely on sequencing mRNAs or ribosome-protected fragments, which, when combined with a selective step, can reveal interactions with nascent chains. Key techniques include RNA immunoprecipitation followed by sequencing (RIP-seq), selective ribosome profiling (SeRP), and disome-selective profiling (DiSP).

RIP-seq detects cotranslational interactions by immunoprecipitating a target protein and sequencing the associated transcripts (46) (**Figure 1*b***). This reveals whether a protein complex subunit is physically connected with actively translated mRNAs encoding a partner subunit. Additional treatments to dissociate translating ribosomes are typically used to confirm that these interactions are mediated by nascent chains. Variants of RIP, coupled with microarray analysis (47) or quantitative polymerase chain reaction (qPCR) (28, 48), have also been used to study cotranslational interactions.

SeRP builds on a similar principle but provides sequences of ribosome-protected mRNA fragments (ribosome footprints) rather than full-length transcripts (44, 49, 50) (**Figure 1*b***). These footprints are ~30 nt long and isolated together with ribosomes from nuclease-treated cell lysates. The footprint sequence reports the ribosome position along the translated mRNA, and thus the nascent protein length. By sequencing footprints that copurify with a bound protein of interest, for example, a subunit of a protein complex, SeRP can reveal the nascent protein identity and when during translation the interaction takes place (5, 45). DiSP is another adaptation of ribosome profiling that sequences footprints from isolated disome pairs of ribosomes connected via their nascent chains (26) (**Figure 1*b***). By sequencing solely the short 30-nt footprints, these disomes can be distinguished from collided ribosomes, which protect 60-nt footprints from nuclease digestion. This approach has enabled the detection of co-co translational assembly and provided proteome-wide information on cotranslational assembly of homo-oligomers (26, 28).

The ability of RIP-seq and SeRP to detect co-post or co-co interactions depends on the location and accessibility of the affinity tag or targeted epitope. If the purification-targeted region is exposed early (N-terminal or internal motifs), both co-co and co-post interactions may be captured, though they cannot be distinguished. Targeting a C-terminal epitope that becomes accessible only after translation allows for the specific detection of co-post interactions.

Noteworthily, while RIP-seq and SeRP can suggest co-co assembly, these methods cannot differentiate between co-co interactions and bidirectional co-post assembly, in contrast to DiSP, which provides more direct evidence. In this review, candidates for co-co assembly are discussed as reported by the original studies. In addition, we note that these methodologies rely on pulldowns or other selection procedures and are therefore biased toward detecting more stable interactions. This suggests that the true prevalence of cotranslational assembly is likely underestimated.

The information gathered by the aforementioned biochemical and sequencing methodologies can be further exploited and complemented through a variety of orthogonal approaches, each providing unique insights into cotranslational assembly. High-resolution fluorescence microscopy has been used to study the colocalization of mRNAs and complex subunits within cells (48, 51, 52), while mass spectrometry–based approaches have provided detailed information on conformational changes during nascent β-galactosidase folding and assembly (35). Single-molecule optical tweezers were able to recapitulate the simultaneous folding and assembly of nuclear lamins (37) and BTB domains (38), and single-molecule fluorescence techniques offer fundamental insights into the dynamics of cotranslational interactions (53). Structural approaches, including nuclear magnetic resonance, cryo–electron microscopy (cryo-EM), and cryo–electron tomography (cryo-ET), have also been applied to study cotranslational folding, interactions, and polysome organization (53–57). Integrating these complementary approaches will be pivotal for uncovering the mechanistic basis of cotranslational assembly.

3. PATHWAYS OF COTRANSLATIONAL PROTEIN COMPLEX ASSEMBLY

The two main routes for cotranslational complex assembly, co-post and co-co assembly (**Figure 1*a***), can be further classified based on the genetic context of the subunit-encoding transcripts: *cis*-assembly of subunits translated from the same polysome (i.e., mRNA molecule) or *trans*-assembly of subunits translated from different polysomes. Hence, cotranslational assembly can proceed through diverse pathways:

1. Co-post assembly is a widespread strategy, from bacteria to humans, for facilitating heteromer formation. In bacteria, this route is exemplified by LuxA–LuxB heterodimers, translated from the *luxA–luxB* bicistronic mRNA (30). Disruption of the operon reduces assembly efficiency, suggesting that *cis* assembly of heteromers is facilitated by the operon organization, although a systematic analysis of this principle is still lacking. In yeast, co-post assembly appears to be highly prevalent: One study found that approximately 40% of the examined complexes (30 in total) assembled cotranslationally (47), while an independent work reported cotranslational assembly in 9 out of 12 complexes (5). In human cells, several studies suggest a similar prevalence (28, 48, 58, 59). For the formation of heteromeric complexes encoded by distinct mRNAs, as is the case in eukaryotic cells, the *trans* assembly mode is mandatory. While direct experimental evidence is still sparse, co-post assembly in the *cis* mode may also be possible for homomeric proteins in bacteria and eukaryotes [e.g., ACC1 (28, 39)].
2. Co-co assembly in *cis* is considered to provide a general route for homomer formation (26, 28) and may also mediate heteromer formation from polycistronic mRNAs in bacteria, although this remains largely unexplored. Co-co assembly in *trans*, in principle, can enable both homomer and heteromer formation from subunits encoded in different transcripts, strictly relying on mRNA colocalization. Systematic proteome-wide analyses of *trans* co-co assembly are limited by the difficulty of identifying the connected ribosome–nascent chain (RNC) pairs. However, several examples have been described (48, 58, 60), and

computational work estimated it may account for ~20% of heteromeric complexes in both bacteria and eukaryotes (28).

This diversity of assembly pathways provides a versatile toolkit that may be tailored to each complex's structural, functional, and regulatory needs. For example, *cis* co-co assembly produces mRNA-specific homomers by involving exclusively nascent chains from the same mRNA (26). This precision is not achievable through co-post or posttranslational assembly and provides several biological advantages, including the possibility of using oligomers encoded by duplicated genes to functionally diversify without interference by cross-assembly (see Section 9). Also, these mechanisms are not mutually exclusive. A single complex may assemble via both co-co and co-post pathways, offering robustness to the process, e.g., by allowing fully synthesized unassembled subunits to reengage their nascent partners. This versatility likely echoes the evolutionary tuning of assembly routes, enabling complexes to optimize assembly pathways according to biophysical, structural, and expression properties. It also underscores the distinct cellular requirements and regulatory mechanisms imposed by each pathway. Thus, while co-co assembly demands tight temporal and spatial coordination of the translating ribosomes, co-post assembly relaxes these constraints but requires the posttranslational partner to remain stable and available to engage its nascent-chain partner.

4. COTRANSLATIONAL ASSEMBLY OF MEMBRANE AND TRANSLOCATED PROTEINS

While cotranslational assembly is increasingly documented for cytoplasmic and nuclear proteins, its role in the biogenesis of membrane proteins and proteins translocated across organellar membranes remains less clear. Nonetheless, accumulating evidence indicates that membrane and translocated proteins can also assemble cotranslationally during their synthesis, insertion into, and translocation across membranes.

Proteome-wide profiling of ribosome pairs connected by nascent chains during translation [disome selective profiling (DiSP)] provided the first information showing that co-co translational assembly of membrane-associated and organelle-targeted proteins is prevalent in human cells (26). However, as stated by the authors, this work did not formally exclude the possibility that certain large complexes of ribosomes with the translocation machinery may migrate to the disome fraction, resulting in false positives. Thus, while these experiments are suggestive, the true extent of cotranslational assembly in membrane contexts remains open, highlighting the need for refined approaches to explore this process. Up to this point, more detailed information exists only for a few examples.

Regarding the assembly of integral membrane proteins, in *Escherichia coli*, the transmembrane domains of the small inner-membrane protein EmrE dimerize through co-post assembly during membrane insertion (**Figure 1*c***), demonstrating that membrane integration and oligomerization can be coupled (61). Similarly, in chloroplasts, the D1 transmembrane protein cotranslationally associates with D2 during its incorporation into photosystem II, forming an early assembly intermediate (62, 63). Mitochondria offer another compelling example of cotranslational assembly of a complex composed of nuclear- and mitochondrial-encoded subunits. During the biogenesis of cytochrome c oxidase (Complex IV), the mitochondrially encoded COX1 subunit is cotranslationally engaged in the inner mitochondrial membrane by the assembly factors C12ORF62 and MITRAC12 (36), which reside in the inner membrane. Thereafter, COX1 translation is paused until the nuclear-encoded COX4 is imported and incorporated. Overall, this assembly follows a co-post mechanism that links translation termination to subunit availability and association within

the bilayer of the inner mitochondrial membrane, enforcing proper stoichiometry and preventing accumulation of orphaned COX1.

The cotranslational assembly of membrane proteins is not limited to transmembrane domain interactions. N-terminal domains transported into the lumen of the endoplasmic reticulum (ER) or cytoplasmic domains can also mediate early assembly (**Figure 1*c***). This has been proposed for human voltage-gated potassium-channel tetramers (64) and hERG1a–hERG1b heterodimers (65), where soluble domains drive oligomerization during insertion into the ER membrane en route to the plasma membrane.

Proteins entirely translocated across membranes can also benefit from cotranslational assembly, even though this process has more constraints. These proteins are generally translocated individually across the bilayer via translocons, implying that assembly can occur only once nascent chains emerge at the distal site of the membrane, e.g., in the ER lumen or the periplasmic space of gram-negative bacteria. For instance, in specific myeloma cell lines, immunoglobulin biosynthesis involves co-post dimerization of heavy chains (e.g., MOPC-21, IgG1) or heavy–light chain pairings (e.g., MPC-11, IgG2b) during ER import, resulting in distinct pathways of immunoglobulin oligomerization (66). Tenascin hexamers have also been proposed to assemble cotranslationally in human cells during translocation via N-terminal coiled-coil interactions (41).

5. LARGE COMPLEXES AND MACROMOLECULAR ASSEMBLIES

Beyond binary interactions, cotranslational assembly plays a central role in the formation of higher-order complexes in a modular and hierarchical manner. By integrating co-co, co-post, and posttranslational steps, cells can segregate and organize intricate assembly pathways by aligning synthesis, folding, and interaction events with the structural logic of the final complex.

The processes of assembling the transcriptional regulators TFIID, SAGA, and ATAC exemplify this layered strategy (32, 48, 52, 58) (**Figure 1*d***). For TFIID, nascent TAF6 first engages nascent TAF9 in co-co mode while establishing co-post interactions with TAF5. This initial complex then assembles posttranslationally with TAF4 and TAF12 and the 3TAF complex, which itself is built through cotranslational interactions among TAF2, TAF8, and TAF10, to form the 8TAF complex. Finally, the nascent TAF1 polypeptide acts as a scaffold that is cotranslationally engaged by the 8TAF complex, the partial A lobe, TAF7, TBP, and TAF11–TAF13. These multilayered cotranslational and posttranslational assembly events converge to build the full TFIID complex on a nascent scaffold, using the vectorial nature of TAF1 translation to enforce temporal order and assembly hierarchy in this last assembly step (**Figure 1*d***). A similar scaffold strategy is employed for the assembly of the SET1C complex, with Set1 acting as a nascent-chain scaffold (67).

Nuclear pore complexes (NPCs) also illustrate the hierarchical nature and spatiotemporal precision of cotranslational assembly (29, 68). In the cytoplasm, Nsp1 binds nascent Nup57 in co-post mode, Nup49 joins later posttranslationally, and the resulting complex cotranslationally engages nascent Nic96 (29). Other subunits are cotranslationally recruited to the NPC at later stages, providing a temporal order and spatial organization of the assembly pathway (68). Disruption of these steps impairs complex formation and compromises cellular fitness (29). Eukaryotic initiation factor 3 and the human multi-tRNA synthetase complex are other examples of multisubunit complexes that use cotranslational pathways to sequentially recruit and assemble subunits in a defined order (59, 69).

Cotranslational mechanisms also initiate the assembly of large macromolecular structures of the cell. For example, intermediate filament proteins such as vimentin form homodimers through co-co assembly (26) that then associate into higher-order polymers posttranslationally (70). Similarly, nuclear lamins dimerize cotranslationally (26) before being imported to the nucleus to

assemble into the lamina. Through this early dimerization mechanism, nascent chains shield one another from misfolding (37) and form the first building blocks able to remain stable in the cellular environment.

Generally, hierarchical cotranslational assembly limits promiscuous interactions (29) and enforces ordered complex formation. Cotranslational mechanisms can then determine how and when multiprotein complexes are built. Yet, our current understanding of the full extent of these regulatory mechanisms, and their prevalence at a proteome-wide level, remains limited due to technical constraints. For instance, proteome-wide insights into co-post mechanisms are still lacking; co-co assembly pairs cannot be identified at a proteome-wide level; and higher-order co-co assemblies, beyond disomes, remain unexplored.

6. STRUCTURAL AND MOLECULAR DETERMINANTS OF COTRANSLATIONAL ASSEMBLY

Whether a protein complex assembles posttranslationally or cotranslationally, via either co-co or co-post pathways, appears to be governed by a multilayered regulatory network. Localized translation, polysome architecture, and translation kinetics may contribute to controlling when and where subunits meet. However, beyond these cellular mechanisms, the protein sequence and structure can contribute to the assembly pathway as well. Features such as domain architecture, protein length, the position of the oligomerization domains, the geometry of the final complex, subunit folding pathways, and the physicochemical properties of the interface can all contribute to determining the route of assembly (**Figure 2*a***). This section reviews emerging insights into the structural and molecular principles of co-co and co-post assembly.

6.1. Structural and Molecular Principles of Co-Co Assembly

The identification of co-co assembling proteins in human and bacterial cells by Bertolini et al. (26) using DiSP provided a proteome-wide dataset for uncovering principles of cotranslational assembly (**Figure 2*a***).

One of the clearest properties of the oligomerization domains involved in co-co assembly is that they are predominantly N terminal. This positioning enables early interaction between nascent chains emerging from neighboring ribosomes and provides a time window for productive assembly before translation is completed. In contrast, proteins that assemble posttranslationally tend to have their oligomerization domains toward the C terminus (6), which underscores the temporal emergence of interaction domains as a key determinant of the assembly pathway. Mispositioned oligomerization domains can lead to misassembly and aggregation (6).

Nature frequently reuses a specific set of homodimerization domains as co-co assembly modules. These include coiled-coil, BAR (Bin-Amphiphysin-Rvs), BTB (broad-complex, tramtrack, and bric-a-brac), RHD (Rel homology domain), and SCAN (SRE–ZBP, CTfin51, AW-1, and Number 18 cDNA) domains (26) (**Figure 2*b***). Among these, BTB, RHD, and SCAN are compact globular domains that typically assemble shortly after the entire domain has emerged from the ribosome exit tunnel. This tight coupling between domain exposure and assembly minimizes the time during which an exposed oligomerization domain remains unpaired, reducing the risk of misfolding and aggregation. In contrast, coiled-coil and BAR domains rely on the pairing of complementary α-helices for oligomerization and can initiate oligomerization even before the full domain is exposed. Coiled coils, in particular, begin dimerizing once sufficiently long segments of both helices have emerged. These domains exemplify how the structural logic of the interaction interface modulates the timing of cotranslational assembly.

Expanding this initial study, Mallik et al. (28) used computational tools to further elucidate the structural determinants of cotranslational complex assembly. By mapping the experimental

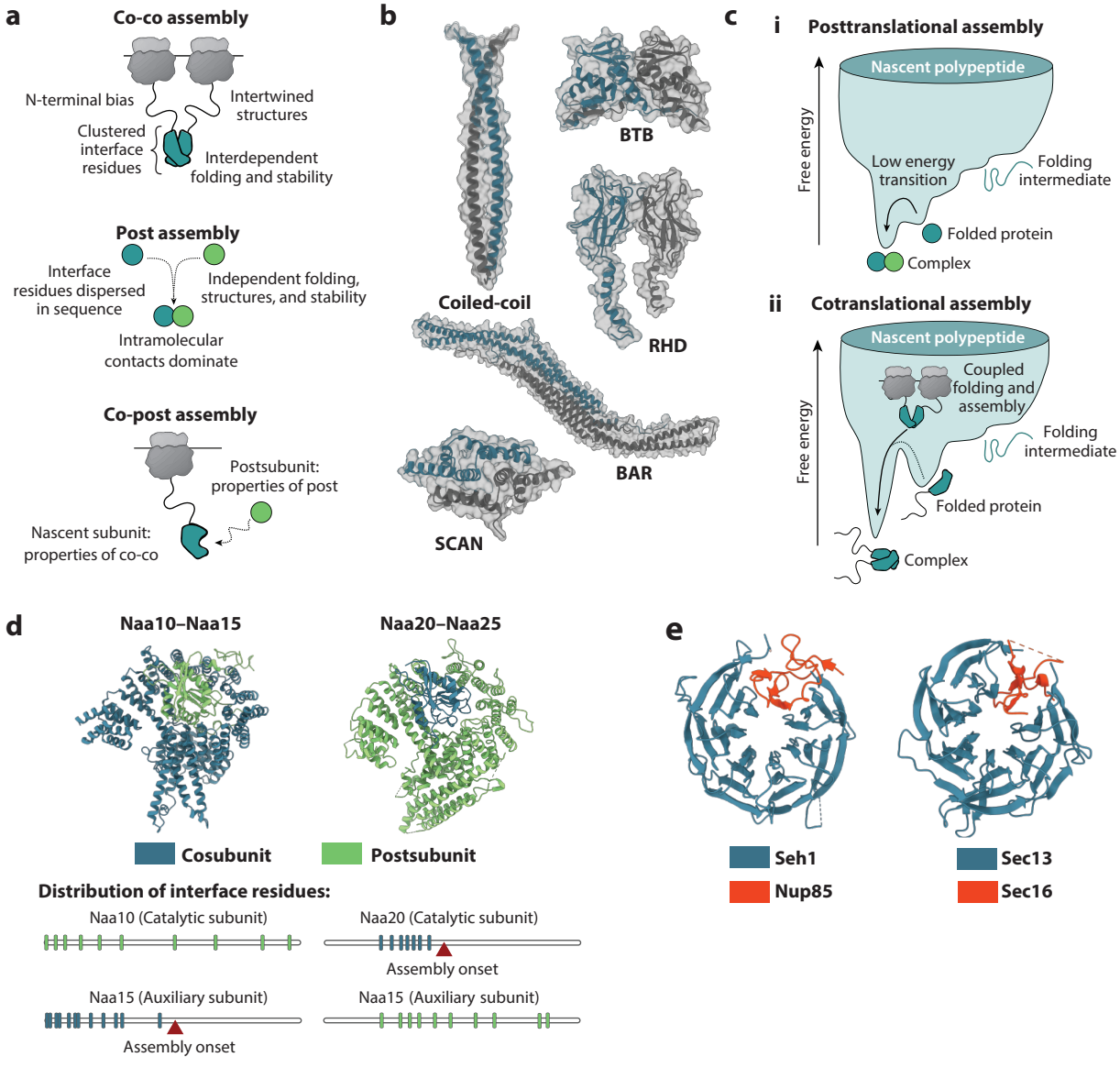


Figure 2

Structural basis of cotranslational assembly. (*a*) Summary of the structural features of complex assembly pathways. (*b*) Structures of the principal domains involved in co-co assembly: coiled-coil (PDB ID: 1X8Y), BTB (PDB ID: 9ETW), RHD (PDB ID: 1K3Z), BAR (PDB ID: 3Q0K), and SCAN (PDB ID: 3LHR). (*c*) Folding landscape of (*i*) post- and (*ii*) cotranslational assembly. In co-co assembly, the intertwined topology of the complex structure implies a high energy barrier between the folded monomers and the complex. By coupling folding and assembly cotranslationally this barrier is naturally overcome during synthesis. (*d*) Structures of NatA (PDB ID: 6HD5) and NatB (PDB ID: 6VP9). The nascent subunit is colored in blue and the postsubunit in green. A schematic representation of the distribution of residues contributing to the interface energy is shown, based on Reference 73. (*e*) Structures of the Seh1–Nup85 and Sec13–Sec16 exemplifying domain invasions in co-post assembly. Abbreviations: BAR, Bin–Amphiphysin–Rvs domain; BTB, broad-complex, tramtrack, and bric-a-brac domain; RHD, Rel homology domain; SCAN, SRE–ZBP, CTfin51, AW-1, and number 18 cDNA domain.

co-co data (26) onto AlphaFold-derived structures, they identified structural signatures characteristic of co-co assembly in humans. Co-co assembling proteins tend to show a higher degree of structural interdependence, adopting more intertwined topologies with a higher ratio of inter- to intramolecular contacts. Co-co assembling proteins also have greater clustering of interface residues along the sequence, consistent with cotranslational assembly initiating before the entire polypeptide has emerged from the ribosome. This aligns with previous observations of cotranslational assemblies being enriched in large and intertwined interaction surfaces (34, 71) and of cooperative stabilization of co-co assembling partners (72). Overall, these analyses confirm that co-co subunits are structurally dependent on their translation partner for proper folding and stabilization. This principle has been experimentally validated through single-molecule optical tweezer experiments on human lamin A in a minimal in vitro system, where cotranslational dimerization was found to be essential for preventing misfolding (37). These findings are intuitive, as the long lamin A monomers are likely to be structurally unstable and offer diverse misfolding options, and suggest that co-co assembly expands the range of possible protein structures.

An important consequence of the intertwined nature of co-co assembled complexes is that subunit folding and oligomerization are coupled, creating a folding energy landscape distinct from those of postassembling complexes (28) (**Figure 2*c***). As a consequence, monomeric and assembled states are separated by a high energy barrier (28), which is naturally overcome during simultaneous cotranslational folding and assembly (**Figure 2*c***). This endows the resulting assemblies with high kinetic stability, making them more resistant to subunit exchange or disassembly. As shown recently for BTB dimers using single-molecule methods, co-co assembly can establish a temporal order of key folding and assembly events (38). Due to the close spatial proximity of neighboring ribosomes, early interactions can form between incomplete BTB domains that prevent the formation of closed monomeric states, thus opening up novel folding–assembly pathways. Hence, the lengthy domain-swapping process that would likely be required to turn closed BTB monomers into a dimer can be circumvented.

Altogether, co-co assembling complexes can occupy distinct energy landscapes in which subunit folding and assembly are deeply intertwined. This not only expands the conformational diversity of protein complexes but also imparts kinetic stability that restricts subunit exchange, highlighting the assembly pathway itself as a mechanism to control complex dynamics.

6.2. Structural and Molecular Principles of Co-Post Assembly

While the proteome-wide identification of co-co assembling complexes using DiSP has laid the foundation for defining the principles of co-co assembly, a comparable systematic analysis of co-post assembly is still lacking, representing a major gap in the field. Nonetheless, evidence from individual complexes suggests that co-post assembly exhibits a hybrid behavior. This hybrid nature is evident in the NatA and NatB heterodimers (73). Although NatA and NatB share highly similar structures (**Figure 2*d***), they follow inverted co-post assembly paths: In NatA, the catalytic subunit is the postsubunit, whereas in NatB, it serves as the cosubunit. This reversal correlates with an inverted distribution of interaction determinants (**Figure 2*d***). In the cosubunit, interface residues are clustered and located upstream of the onset of assembly (73), consistent with co-co assembly patterns, while in the postsubunit, they are more dispersed (**Figure 2*d***). Assembly is triggered when the ribosome exposes a hotspot region within the cosubunit that contributes a high binding energy to complex formation, as shown by molecular dynamics simulations (73). This emphasizes how cotranslational assembly impacts the structural organization of protein interfaces.

Domain position also emerges as a key determinant of co-post assembly. In TAF10–TAF8 (48, 58), the cosubunit has an N-terminal oligomerization domain, enabling early engagement, while the postsubunit's interaction domain is C terminal. Swapping the oligomerization domains

between co- and postsubunit did not alter the order of assembly. This suggests that it is the relative position of a domain within the nascent chain, rather than its specific sequence identity, that dictates the directionality of co-post assembly (58). TAF6–TAF9 uses oligomerization domains homologous to those of TAF10–TAF8, but both are N terminal; in this case the assembly mode is co-co. As seen in co-co complexes, the onset of co-post assembly closely follows the emergence of interaction domains from the ribosome tunnel (5, 29).

Mirroring structural features of co-co assembly, some co-post complexes also show intertwined architectures, as exemplified by domain invasion motifs in nucleoporin assembly. Seh1 and Sec13 contain incomplete β-propellers that are structurally completed by their cotranslational partners Nup85 and Nup16, respectively (**Figure 2*e***). Likewise, coiled-coil interactions mediate the assembly of nascent Nup57 with Nup1 (29). As in co-co complexes, nascent cosubunits in co-post pairs often rely on their partners for folding and stability, while postsubunits tend to remain stable in the absence of their partner (5, 28, 48, 58, 67).

Overall, co-post assembly bridges co-co and posttranslational modes: The nascent subunit gains the folding and stabilization benefits of cotranslational assembly, while the postsubunit retains flexibility and independence until engagement. However, proteome-wide characterization of co-post assembly remains a critical milestone to fully unravel the principles and evolutionary constraints guiding this hybrid mechanism.

7. EMERGING HALLMARKS OF COTRANSLATIONAL ASSEMBLY

The complexity of cotranslational assembly imposes high cellular demands to ensure that interactions occur efficiently, accurately, and on time, and consequently, cells invest substantial resources into this process. In this section, we highlight emerging hallmarks of cotranslational assembly, including the coordination of translation kinetics; polysome organization; localized translation; and molecular mechanisms that guide, assist, and monitor assembly (**Figure 3**).

7.1. Translation Kinetics and Pausing

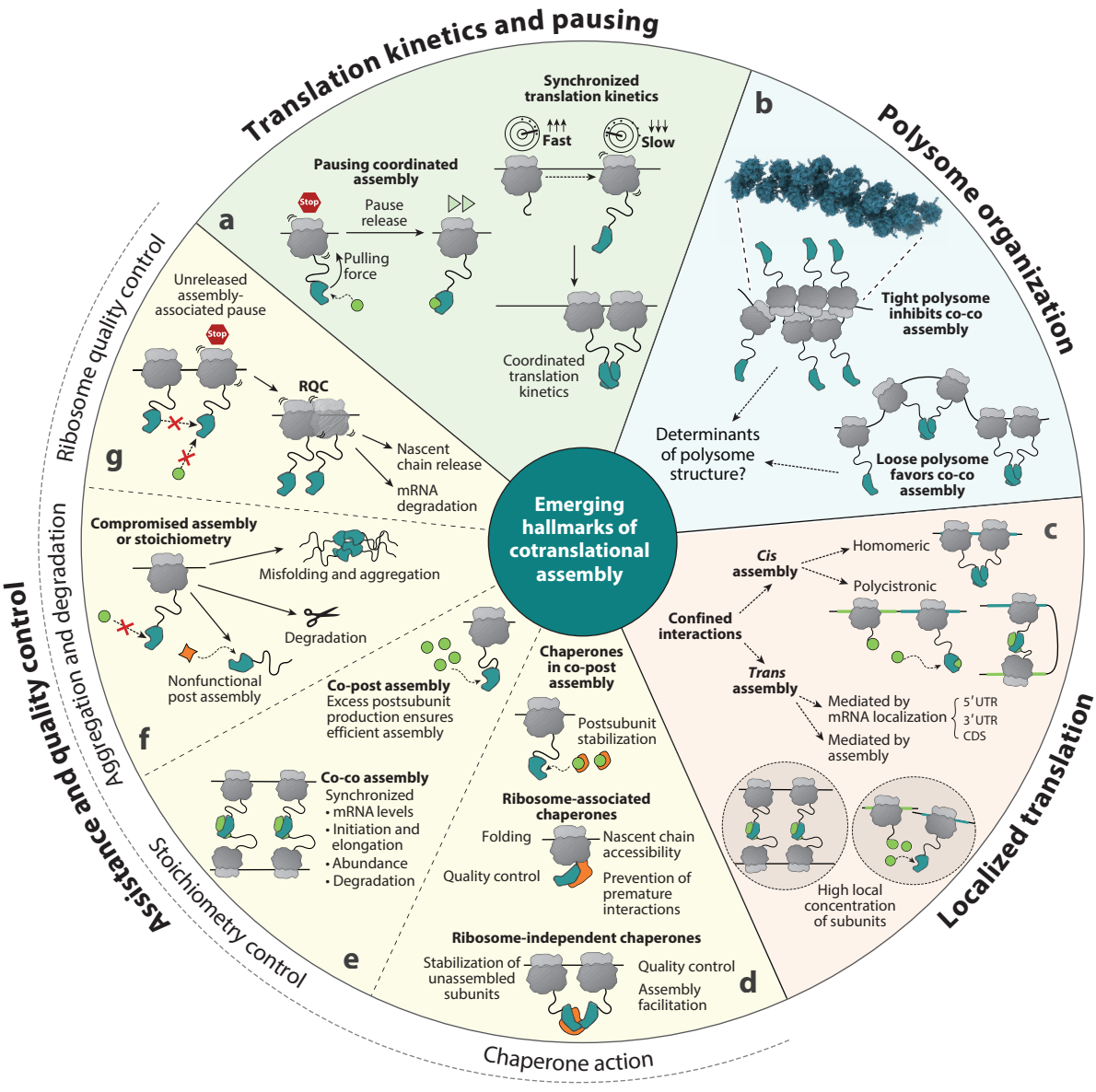
Translation kinetics are inherently nonuniform and can be modulated by a range of different mechanisms, such as through codon usage, mRNA structure formation, and nascent-chain interactions with the ribosomal peptide tunnel. These local variations in translation speed play a central role in nascent-chain biology, influencing protein folding, membrane targeting, and translocation and even regulating signaling pathways (74–79).

The most extreme form of translation speed modulation is pausing, which can act as a translation checkpoint and is resolved by mechanical forces generated by the nascent chain upon folding or membrane translocation (79–81). This principle may extend to cotranslational assembly (**Figure 3*a***), where subunit binding may relieve ribosomal pausing, as suggested for EmrE dimerization (61). The co-co assembly of the yeast proteasome subunits RPT1 and RPT2 is also coordinated through a pausing site that increases assembly efficiency and is overcome upon assembly (60). Similar pausing sites have been observed during cotranslational assembly of D1 into photosystem II (82) as well as COX1 assembly into cytochrome c oxidase (36), which stalls until its assembly partner, COX4, becomes available (both reviewed in Section 5). In theory, pausing sites could also contribute to co-co assembly by synchronizing the translation of a ribosome pair. However, to our knowledge, there is currently no direct experimental evidence supporting this mechanism.

Beyond discrete pauses, modulation of translation initiation and global elongation rates can also fine-tune the timing of cotranslational folding and complex assembly (**Figure 3*a***). Computational analysis of proteome-wide data suggests that co-co assembling subunits have more correlated translation kinetics than postassembling pairs (28), which is not surprising since proper

timing and stoichiometry are critical for efficient co-co assembly. Additionally, in the co-post assembly of TAF1 (48) (see Section 6), its three TAF6-binding motifs are translated rapidly (these motifs correspond to a region of low ribosome occupancy, indicative of rapid translation), and this is followed by translation slowdown. This speed change may provide a time window for partner engagement before translation proceeds.

Together, these findings suggest that translation kinetics act as a regulatory layer for cotranslational assembly, akin to their role in protein folding and membrane targeting, e.g., by the signal recognition particle (83). Yet, the mechanistic implications of initiation and elongation dynamics for assembly are only beginning to be explored.



(*Caption for* ***Figure 3*** *appears on following page*)

Figure 3 (*Figure appears on preceding page*)

Emerging hallmarks of cotranslational assembly. (*a*) Translation kinetics and ribosome pausing are proposed to coordinate cotranslational assembly by temporally controlling subunit interactions at specific points during translation. (*b*) Polysome organization may inherently inhibit or promote cotranslational assembly. How this is regulated and the determinants of polysome structure are still open questions. (*c*) Localized translation confines cotranslational interactions to a specific cellular location, increasing assembly efficiency or even making it possible. Localized translation is either genetically encoded in operons or supported by mRNA colocalization. mRNA localization can be determined by different features encoded in the 5′ UTR, the CDS, or the 3′ UTR. Assembly itself may act as an independent determinant sustaining colocalization. (*d*) Chaperone action in co-post and co-co assembly. Chaperones may have multifunctional actions at different points of the assembly process and depending on the specific complex. (*e*) The stoichiometry between different subunits must be tightly coordinated to ensure efficient assembly while preventing aberrant interactions and the generation of orphaned subunits. (*f*) Upon failure to assemble, nascent subunits may misfold, aggregate, be degraded, or engage in nonnative or promiscuous interactions. (*g*) RQC emerges as a potential mechanism to ensure subunit stoichiometry and removal of unassembled subunits even before the end of translation. Abbreviations: CDS, coding sequence; RQC, ribosome quality control; UTR, untranslated region.

7.2. Polysome Organization in the Cytoplasm

Polysomes were traditionally considered disordered clusters of ribosomes with little spatial coordination distributed along an mRNA. However, cryo-ET analysis revealed that some polysomes can adopt highly ordered, densely packed architectures (57, 84). For instance, left-handed helical arrangements with regularly spaced and uniformly oriented ribosomes have been observed in both bacteria and eukaryotes (57, 84).

Although the impact of polysome architecture on cotranslational assembly remains poorly understood, helical configurations pose a significant barrier to interactions between nascent chains of adjacent ribosomes. In these configurations, ribosomal exit tunnels face away from one another, spatially segregating emerging polypeptides (**Figure 3*b***). While this is considered an evolutionary strategy to minimize aberrant interactions (84), it also hinders co-co assembly in *cis*. Instead, *cis* co-co assembly demands loosely packed, flexible polysome arrangements that provide enough spatial freedom for nascent chains to bridge the gap between ribosomes, especially if one assumes nascent-chain interactions to occur between adjacent RNCs on a polysome (**Figure 3*b***). Such loosely packed architectures have indeed been observed in cryo-EM tomography analysis (56, 85), though their prevalence and significance remain unclear, partly due to the fact that previous computational analyses were focused on ordered structures. Since cryo-ET allows polysomes to be visualized in situ, this method is useful to demonstrate how ribosome arrangements could sterically permit or hinder nascent-chain interactions.

Polysome architecture is therefore unlikely to be static; indeed, it may be actively remodeled to facilitate cotranslational assembly. One proposed mechanism involves mRNA secondary structures altering ribosome positioning and exit tunnel orientation into more favorable configurations (86). Alternatively, local modulation of translation kinetics could distort ribosome spacing and temporarily create assembly-permissive geometries. The same principles could be exploited to prevent premature interactions as well as ribosome collisions.

Given the widespread nature of co-co assembly, such local rearrangements in polysome structure may represent a critical yet underexplored regulatory layer in cotranslational complex formation.

7.3. Polysome Organization at Membranes

Unlike cytoplasmic polysomes, membrane-bound polysomes are confined to a two-dimensional (2D) space, aligned along the membrane surface (87, 88). Such polysomes are less densely packed and exhibit a defined orientation, with their polypeptide exit tunnels facing the membrane (87, 88). This semiordered 2D organization could be compatible with co-co assembly, as nascent

chains translated from the same mRNA laterally translocate into the membrane and emerge at the luminal side in close proximity.

A key limitation for cotranslational subunit association, however, is nascent-chain length, since the nascent polypeptide must be long enough to span the distance between adjacent translocons. This has been proposed for the assembly of tenascin hexamers (41), where the cotranslational formation of a six-arm structure is structurally feasible due to the large size of the protein (2,201 residues) and the spatial layout of the translocons. While experimental confirmation is lacking, similar mechanisms may operate in other large membrane-associated or secreted proteins.

7.4. Localized Translation in Cotranslational Complex Assembly

Efficient assembly of protein complexes requires precise regulation of both the spatial proximity and local concentration of subunits. Localized translation has emerged as a key strategy to control these parameters, playing a central role in both co- and posttranslational assembly (51, 89–92). For co-co assembly in *cis* and *trans*, physical proximity between translating ribosomes is essential. Co-post assembly does not strictly require ribosome proximity but still benefits from localized translation, since this increases the likelihood of productive encounters between nascent and postsubunits (30). In this section, we explore how cells spatially coordinate translation from different mRNAs to drive efficient cotranslational complex formation.

7.4.1. Genetically encoded localized translation: *cis* assembly. The simplest strategy to ensure nascent-subunit proximity is to encode them on the same mRNA. This *cis* coexpression inherently couples the synthesis of interacting partners in space, bypassing the need for additional localization machinery (**Figure 3*c***). As a result, *cis* assembly is expected to be particularly efficient, whether through co-co or co-post mechanisms.

As demonstrated for lamin homodimers (26), co-co assembly in *cis* of nascent chains translated from one polysome is favored over heterodimer assembly in *trans* of nascent splicing isoforms translated from distinct mRNAs (**Figure 3*c***). In bacteria, operon organization provides a natural framework for *cis* heteromer formation, with multiple subunits translated from the same polycistronic mRNA (**Figure 3*c***). A paradigmatic example is the luxA–luxB operon, whose products assemble via a co-post mechanism. When luxA and luxB are expressed from separate mRNAs instead of a bicistronic mRNA, luciferase activity drops by ∼40% (30). Noteworthily, gene order within operons often mirrors the assembly pathway of the complex (93), which could potentially allow upstream open reading frame (ORF)-translated subunits to engage nascent chains encoded in downstream ORFs, as in the luxA–luxB example. While such an effect has not yet been observed for co-co assembly, a similar principle could be expected (**Figure 3*c***). In this way, genome architecture itself may serve as a blueprint for cotranslational assembly.

7.4.2. *Trans* assembly through mRNA proximity. Advanced fluorescence microscopy revealed that mRNAs encoding functionally related proteins often colocalize in the cytoplasm to enhance productive interactions (51, 60, 90, 92, 94). These mRNA clusters, frequently termed translation factories, concentrate ribosomes translating related subunits (reviewed in 92).

Recent studies highlight different examples of co-post assembly facilitated by mRNA colocalization (**Figure 3*c***). Shade et al. (51) identified significant colocalization of mRNAs encoding three co-post-assembling complexes in yeast: fatty acid synthase (FAS1–FAS2), phosphofructokinase (PFK1–PFK2), and the aminoacyl-tRNA synthetase complex (GUS1–ARC1). Disruption of FAS1–FAS2 colocalization leads to growth impairment, consistent with assembly defects. NUP1 and NUP2 mRNAs cotranslationally associate with the NPC, allowing their translation and assembly to be coordinated with their final destination (68). Another example is β-catenin, which has been observed by fluorescence microscopy to cotranslationally assemble into the destruction

complex at specific cytoplasmic foci (46) but can alternatively co-post assemble with E-cadherin at the plasma membrane (46). This example underscores how localized translation not only facilitates nascent-chain assembly but also can sort subunits into distinct assembly pathways.

While localized translation can improve co-post assembly, *trans* co-co assembly strictly depends on it (**Figure 3*c***), as partnering nascent chains must be spatially close to interact. Though still poorly understood, early evidence indicates colocalization of mRNAs for TAF6–TAF9, YEATS2–ZZZ3, and Rpt1–Rpt2 pairs (52, 58, 60). More recently, Mallik and colleagues (28) demonstrated strong colocalization of RPB1 and RPB2 mRNAs, which were initially predicted computationally and later experimentally validated as co-co assembling partners.

In general, mRNA localization in the cytosol is directed by a composite code of interacting signals (91, 95), including *cis*-encoded elements in the 3′ untranslated region (UTR), the 5′ UTR, or the coding sequence that act as molecular zip codes (96, 97) (**Figure 3*c***). These are recognized by RNA-binding proteins (98, 99) and complemented by RNA–RNA interactions (100, 101), which allow anchoring to subcellular structures (102), as well as nascent polypeptide-encoded signals that engage targeting machinery during translation (79, 103, 104). Two recent articles provide a detailed overview (105, 106). Although the specific roles of these localization mechanisms in cotranslational assembly are still unclear, early evidence suggests a complex interplay of distinct elements, including the 3′ UTR, 5′ UTR, and coding sequence (51) (**Figure 3*c***). Notably, cotranslational assembly may itself promote mRNA localization (see the next section).

7.4.3. Nascent-chain interactions as drivers of mRNA colocalization. A compelling idea is that *trans* co-co interactions between nascent chains might not only depend on mRNA colocalization but also reinforce it. Indeed, co-co interactions suffice to physically tether polysomes, promoting sustained colocalization over multiple rounds of translation. Supporting this idea, treatment with the nascent chain–releasing antibiotic puromycin more strongly disrupts mRNA colocalization for co-co than for co-post pairs (28, 51). Co-co interactions might act as molecular zippers, maintaining the proximity of their source mRNAs. If such interactions are multivalent, involving more than two polysomes, they could give rise to self-organizing structures, or co-co assembly factories, in which numerous polysomes are held together by the very proteins they produce.

According to this model, a single colocalization event may be sufficient to initiate co-co assembly, which could in turn sustain continued mRNA colocalization. Interestingly, both bacterial and eukaryotic cells have mechanisms to colocalize mRNAs early in gene expression. In bacteria, cotranscriptional translation ensures identical transcripts are translated in close proximity. In eukaryotes, functionally related mRNAs can be cotranscriptionally bundled into assemblies called transperons, which can exit the nucleus through the same pore and remain colocalized in the cytoplasm (66, 107, 108). This early spatial coupling could allow downstream polysome tethering via co-co assembly, reinforcing translational organization without requiring a complex mRNA localization machinery.

7.5. Assistance and Quality Control of Cotranslational Assembly

While cotranslational assembly increases assembly efficiency, reducing misfolding risks, it demands precise coordination between protein synthesis, folding, and oligomerization, all while avoiding nonnative interactions. This complexity hints at the existence of dedicated quality-control mechanisms that limit errors during the entire assembly process. In this section, we review key molecular strategies that guide, regulate, and provide quality control during cotranslational assembly (**Figure 3*d–g***).

7.5.1. Chaperone action in cotranslational assembly. Cells rely on a specialized network of molecular chaperones that act cotranslationally to support the biogenesis of nascent polypeptides. The strong correlation between domain exposure and the onset of complex assembly (5, 26) (see Section 7) suggests that chaperone activity preceding assembly must occur in close proximity to the ribosomal tunnel exit. This spatial constraint positions ribosome-associated chaperones (27, 31)—such as bacterial trigger factor (TF), the yeast Hsp70 homolog Ssb, and the ubiquitous eukaryotic chaperones ribosome-associated complex and nascent-polypeptide-associated complex (NAC)—as key coordinators of early complex formation. Indeed, both TF and Ssb have been implicated in the regulation of co-post assembly pathways. TF prevents premature interactions between the LuxA and LuxB subunits in *E. coli*, regulating the timing of subunit engagement and promoting directional assembly by favoring the interaction of fully synthesized LuxA with nascent LuxB (30). It was recently shown that TF can stabilize and promote the partial folding of nascent domains, while DnaK can limit premature interactions between domains, which likely plays a role in cotranslational assembly as well (109–111). Ssb assists in the folding of domains involved in cotranslational assembly (5), although its precise contribution to assembly remains unclear, as Ssb-deficient cells exhibit widespread aggregation. These findings support a model in which ribosome-associated chaperones act as first-line responders, shielding hydrophobic surfaces, stabilizing and promoting the folding of nascent domains, and preventing premature or nonproductive interactions (**Figure 3*d***). Their universal presence and positioning at the ribosomal tunnel exit make them ideally suited to coordinate both monomer folding and complex assembly.

Among the ribosome-associated factors, NAC stands out with its multifunctionality as a chaperone and organizing hub for multiple factors (112–114). It modulates the access and activity of the signal recognition particle, methionine aminopeptidases, N-terminal acetyltransferases (53, 55, 115), N-myristoyltransferase 1 (116), and the eEF1A-specific chaperone Chp1 (117, 118). Moreover, it was shown recently that NAC directly interacts with nascent chains across the proteome and guides structure formation (114). These regulatory functions raise the intriguing possibility that NAC also gates assembly interfaces or coordinates the sequential engagement of chaperones and assembly factors, thus defining when and how subunits interact (**Figure 3*d***).

Beyond ribosome-bound factors, nonribosome-associated chaperones likely contribute to cotranslational assembly as well. Such chaperones may assist during various stages of assembly: stabilizing nascent subunits before engagement, facilitating productive interactions during assembly, or acting downstream to reverse off-pathway associations and aggregation of partially synthesized protein partners (**Figure 3*d***). In particular, complex assembly frequently relies on substrate-specific assembly factors, such as those involved in tubulin and proteasome biogenesis (16, 119), suggesting that analogous factors also operate during cotranslational assembly.

Chaperones play a critical role in co-post assembly pathways by maintaining posttranslational subunits in a soluble and assembly-competent state until their cotranslational partners become available (**Figure 3*d***). This role mirrors classical posttranslational assembly mechanisms and is exemplified by the chaperonin TRiC/CCT, which maintains TAF5s in a soluble, assembly-competent state while they await association with the nascent TAF6 complex (18, 32, 48).

While it is expected that cotranslationally acting chaperones serve as central orchestrators of complex biogenesis, experimental evidence detailing their specific roles in co-post and co-co assembly remains limited. This likely reflects a highly context-dependent landscape in which chaperone requirements and activities are finely tuned by the needs of specific complexes and nascent chains.

7.5.2. Stoichiometry control in cotranslational assembly. Accurate assembly of protein complexes requires strict stoichiometric balance of their subunits (24, 120). While posttranslational assembly depends primarily on protein abundance (24), cotranslational pathways also demand temporal coordination of subunit synthesis, especially for *trans*-encoded partners, where asynchronous expression can hinder productive interactions. To control stoichiometry, cells can regulate mRNA transcription and degradation, translation initiation and elongation rates, and postsynthesis subunit stability to ensure synchronized subunit availability (25, 36, 79, 121–123).

In predicted co-co assembly pairs (28), transcriptional, translational, and proteome parameters (mRNA abundance, initiation, and elongation and protein levels) are tightly matched (28), enabling synchronous synthesis and subunit interactions at the ribosome (**Figure 3*e***). Co-post pairs display intermediate coordination, while posttranslational pairs show reduced coupling (28). Interestingly, in the co-post assembly of TAF10 with TAF8, the postsubunit is expressed at higher levels than the nascent one (58) (**Figure 3*e***). This imbalance may facilitate efficient capture and stabilization of the emerging nascent chain by ensuring a minimal pool of unassembled postsubunits, thereby increasing the effective k_{on} of assembly. In these systems, the nascent chains are typically the vulnerable components, and their stability may rely on rapid engagement with the postsynthesized partners. Thus, co-co assembly imposes stringent stoichiometric constraints, enforced through combined transcriptional and translational regulation, while co-post pathways likely benefit from an excess of stable postsubunits to enhance assembly efficiency.

When upstream stoichiometric control fails, degradation of unassembled subunits becomes essential to restore balance and prevent toxicity. While often harmful, aggregation may also serve as a last-resort mechanism to sequester excess subunits (123). During cotranslational assembly, loss of a partner subunit leads not only to reduced subunit levels but also to mRNA degradation of its nascent counterpart, pointing to a feedback loop that limits unassembled subunit production (58, 124).

7.5.3. Aggregation and degradation of unassembled subunits. Disruptions in stoichiometry or impaired complex assembly can result in the accumulation of unassembled subunits. This is common to every assembly pathway but particularly important in cotranslational assembly, since nascent subunits often depend on cotranslational interactions for stability (see Section 7). Without their partners, they are prone to misfolding and aggregation (**Figure 3*f***), as shown in single-molecule studies of lamin A (37) and observed more broadly in vivo. In a study of 46 yeast strains (28), perturbation of partner stoichiometry, via depletion or overexpression, led to increased formation of cellular puncta, indicative of aggregation. These effects are more pronounced for co-co heteromers, where both partners are vulnerable, whereas in co-post assembly, only the nascent subunit is destabilized when its partner is missing, while the postsubunit remains soluble (5, 48).

To prevent such outcomes, unassembled subunits are often targeted for degradation (**Figure 3*f***), a mechanism broadly active across protein complexes (125, 126). Proteome-wide analyses reveal that subunits in cotranslationally assembled complexes tend to be degraded more following partner depletion (28, 72). Co-co partners exhibit coordinated degradation kinetics under normal conditions (28), and degradation increases when assembly is rendered deficient, e.g., by mutations of the subunit interface (19, 20). In co-post systems, the nascent-chain partner is more sensitive to degradation. For example, depletion of TAF10 (postsubunit) led to significant reductions in TAF8 (cosubunit) mRNA and protein levels, but TAF10 expression was unaffected by TAF8 deletion (58). Interestingly, in both cases, other subunits of the TFIID complex remained stable, suggesting that this destabilization is specific to cotranslational pairs. Another example of a nascent subunit that is degraded in the absence of its partner is Set1 (67).

If the subunits are stable enough, orphaned cosubunits may still oligomerize via posttranslational assembly. Such a situation increases the risk of forming nonfunctional complexes (**Figure 3*f***), as observed for Nup57, which forms a promiscuous heterotrimer with Nup49 if cotranslational Nsp1 binding is disrupted (29). This is particularly relevant in *cis* co-co assembly, because disrupting cotranslational assembly removes the mechanism that normally enforces paralog- or isoform-specific pairing (26). Without this safeguard, incorrect paralog combinations can form, which undermines the evolutionary advantage of maintaining strict interaction specificity (see Sections 8.3–8.5).

7.5.4. Ribosome quality control in cotranslational assembly. The degradation of mRNAs when cotranslational assembly is compromised (58, 124) suggests a quality-control mechanism responsive to complex formation and stoichiometry (58, 124). While this may be the result of complex-specific responses, here, we speculate that a broader, conserved mechanism exists.

Translational pausing sites that coordinate assembly (see Section 8.1) could, upon assembly failure, lead to prolonged stalling and subsequent ribosome collisions (127, 128). These collisions would recruit ribosome quality control (RQC) factors that terminate translation and degrade incomplete nascent chains (129, 130). Since the RQC response also leads to mRNA degradation (129, 130), it can simultaneously act as a regulator of mRNA stoichiometry (**Figure 3*g***). Beyond resolving failed events, if prolonged pausing reflects substoichiometric partner levels, RQC-triggered mRNA degradation could help restore balance. This hypothesis would provide a mechanism to couple cotranslational assembly, translation kinetics, and stoichiometry control, with RQC playing a broader role in synchronizing complex assembly with gene expression.

8. EVOLUTIONARY ADVANTAGES OF COTRANSLATIONAL ASSEMBLY

Cotranslational assembly is a conserved and widespread strategy for protein biogenesis across all domains of life. Experimental and computational studies estimate that at least 10–20% of protein complexes assemble cotranslationally in bacteria, yeast, and humans (28). This mode of assembly is linked to specific oligomerization modules, structural features, and interaction-domain positioning within coding sequences (see Section 7), linking the evolutionary trajectories of these domains with cotranslational assembly. Cotranslational assembly is not only a mechanism to enhance efficiency and fidelity in complex formation but also a selection force with profound evolutionary implications. In this section, we review some evolutionary advantages of cotranslational assembly.

8.1. Cotranslational Buffering of Protein Solubility as a Driving Force for the Emergence of New Folds and Assemblies

By tightly coupling folding, stability, and assembly (28, 35, 37), cotranslational assembly pathways contribute to the emergence of novel folds and protein architectures (see Section 7). Nascent chains are protected early during translation through an interaction with a cotranslational partner, allowing simultaneous folding and assembly while stabilizing aggregation-prone or transient intermediates that would misfold in a posttranslational context (5, 26, 28, 37). This protective effect allows nascent chains to bypass the constraints of independent stability and solubility, enabling them to explore new structural solutions that are accessible only to cotranslationally assembled complexes. In this way, cotranslational assembly acts as a permissive force, expanding the accessible sequence and structure space during protein evolution. It not only enhances efficiency and fidelity in complex formation but also facilitates structural innovation. Protein dimers that are formed via swapping of secondary structure elements such as β-strands provide an example (38).

8.2. Cotranslational Assembly Orchestrates Hierarchical Assembly and Competing Pathways

A universal feature of protein complex biogenesis is the hierarchical assembly of subunits in a defined order (131, 132). Several examples discussed in this review (e.g., TFIID and NPC assembly; see Section 6) demonstrate how cotranslational assembly acts as a key mechanism to enforce this principle (29, 32, 48, 52, 58, 68), while preventing the formation of off-pathway intermediates (29).

In addition, increasing evidence suggests that cotranslational interactions can act as a triaging system to guide moonlighting proteins toward distinct complexes. For instance, during the assembly of the NPC, Seh1, Sec13, and Nsp1 selectively engage cotranslationally with Nup85, Sec31, and Nup57 but not with alternative partners such as the Sea complex, Sec16, Nup145C, Mtc5, or Nup82 (29). Similarly, β-catenin engages cotranslationally with either the destruction complex or E-cadherin at the plasma membrane, depending on the cellular context (46). How cells balance such competitive assembly pathways remains unclear. One possibility is that nascent proteins are produced in sufficient quantities to saturate a dominant partner (likely the earliest cotranslational interaction), allowing a controlled leakage to secondary complexes. Alternatively, an inefficient assembly pathway with a certain failure rate may be evolutionarily advantageous by nurturing a moonlighting complex with unassembled subunits. This hypothesis would imply that cells may deliberately fine-tune, not maximize, assembly efficiency to serve multiple functions simultaneously. In addition, it is possible that assembly pathway triaging is governed by yet-unknown regulatory signaling processes.

8.3. *Cis* Co-Co Assembly Promotes mRNA-Specific Homomers

Cells express many protein complexes with distinct functions but similar protein interfaces and assembly pathways. How cells prevent aberrant interactions between identical or highly compatible oligomerization domains remains incompletely understood, particularly when such interactions would yield complexes of similar thermodynamic stability. A paradigmatic example involves lamin A and lamin C, isoforms encoded by alternatively spliced transcripts of the same gene. Despite sharing an identical N-terminal dimerization domain, they form exclusive homodimers (133). Bertolini et al. (26) demonstrated that *cis* co-co assembly can enforce such specificity by ensuring selective homomerization of subunits translated from the same mRNA. This principle is referred to as mRNA-specific homomerization.

While the full extent of this mechanism remains to be uncovered, other co-co assembling domains likely benefit similarly. For instance, the human proteome encodes over 200 distinct BTB-domain proteins, most of which form homodimers. Aberrant heterodimerization of BTB-domain proteins is detrimental, and cells have evolved a dedicated quality-control system to detect and disassemble these aberrant complexes, even when heterodimers are as stable as the native homodimers (19, 20). Since BTB domains co-co assemble, *cis* assembly likely serves as a key strategy to preventively enforce homodimerization. This reduces the burden on the BTB quality-control machinery, which would then primarily act on dissociated or unassembled subunits.

This mRNA-specific homomerization has broad implications for protein evolution and the buffering of dominant-negative effects and deleterious mutations, which we explore further in the next sections.

8.4. Paralogs and Divergence Following Gene Duplication

Gene duplication events provide the basis for evolutionary innovation. However, newly duplicated genes—paralogs—face the challenge of avoiding unwanted interactions with their ancestral or sibling proteins. In the early stages after duplication, heteromerization between paralogs can

compromise the functionality of the original homomeric complex. This imposes evolutionary constraints, as both paralogs must balance acquiring new functions while avoiding interference with the ancestral role (134–136).

Among the various cellular strategies that accelerate paralog divergence, *cis* co-co assembly was identified as the most prominent one (134). By restricting interactions to products of the same transcript (see the previous section), *cis* co-co assembly enables each paralog to follow an independent evolutionary trajectory, effectively insulating them from each other's interaction networks. This is not surprising, since co-co assembly can be inherited from the ancestral protein, while other diverging mechanisms appear with a delay after duplication (134).

8.5. Cotranslational Assembly Buffers Dominant-Negative Mutations

Dominant-negative mutations arise when a mutant allele produces a defective subunit that interferes with the function of the wild-type protein (137). This is particularly disruptive in the context of homomeric multisubunit complexes, where incorporation of even a small fraction of mutant subunits can poison the entire complex (138–140). As a result, dominant-negative mutations often exert effects that are disproportionate to the dosage of the mutant allele.

Similarly to paralog proteins, mRNA-specific *cis* cotranslational assembly results in allele-specific oligomerization, acting as a barrier preventing mutant alleles from incorporating into complexes formed by wild-type subunits (26, 86, 141). It has been observed that homomeric proteins associated with autosomal dominant inheritance are significantly depleted in co-co assembly, and genes known to act via dominant-negative mechanisms are among the least likely to assemble cotranslationally (141). Thus, co-co assembly contributes to mutational robustness by restricting the pathological consequences of monoallelic loss-of-function mutations (141).

A second layer of protection is provided by the intrinsically ingrained biophysical properties of cotranslationally assembled complexes (see Section 7). The higher stability and lower dissociation rates of cotranslationally assembly complexes prevent posttranslational subunit exchange, further extending this initial buffering through the life of the protein.

9. IMPLICATIONS FOR PROTEIN DESIGN AND SYNTHETIC BIOLOGY

Protein complex assembly is evolutionarily optimized for the native cellular context (142), but this coordination is rarely recapitulated in heterologous expression. This has profound implications for biotechnology and synthetic biology, where proteins are expressed in nonnative settings. Accordingly, misfolding and aggregation pose major challenges for biotechnology and synthetic biology, reducing yield and functionality and jeopardizing entire production pipelines (143, 144).

For cotranslationally assembling proteins, this challenge is amplified, as heterologous expression often disrupts the multilayered regulatory network controlling cotranslational assembly, including translation kinetics, mRNA localization, and chaperone assistance. Unsurprisingly, recombinant expression of subunits from cotranslationally assembling complexes often leads to unstable or insoluble subunits, unless coexpressed (58, 145, 146), while inefficient cotranslational complex formation reduces protein yield and activity (35). These issues extend to protein design driven by artificial intelligence (AI) and synthetic biology efforts, where reprogramming of cellular circuits may neglect the temporal and spatial coordination required for proper complex formation. How, then, can the principles of cotranslational assembly be harnessed to optimize complex formation in these settings?

The cotranslational assembly of immunoglobulins illustrates this challenge and opportunity. Antibody therapies had a market value of 186 billion USD in 2021 (147), but their production

often suffers from poor antibody folding and inefficient assembly. For this reason, antibody redesign is an active area of research. Determining whether a given antibody uses co-post assembly (see Section 5) will help in such endeavors, i.e., by prioritizing solubilizing mutations on the postsubunit (the one that waits for its partner) or redesigning assembly pathways.

Enhancing localized translation represents another promising approach to promote cotranslational assembly. This can be achieved by tethering mRNAs to specific cellular compartments using scaffold RNAs, localization signals, or engineered phase-separated condensates (104, 148–150). In bacteria, colocalization of the LuxA and LuxB subunits of luciferase on the same operon has been shown to significantly improve heteromer assembly (30). This principle can be extended to eukaryotic systems via polycistronic expression strategies, such as those employing 2A-skipping peptides (151) or viral cleavage sequences (152, 153). Alternatively, coexpression of molecular chaperones that specifically stabilize the postassembly subunit in co-post interactions can aid in efficient folding and complex formation. For proteins that assemble via *cis* co-co assembly, optimizing the ribosome load per mRNA, translation efficiency, or codon-driven pausing events are promising approaches to directly impact assembly success.

In addition, it is intriguing to propose that proteins not naturally evolved for cotranslational assembly may be engineered to do so. The recent rise of AI-based protein-structure predictions together with the observation that cotranslational assembly can be predicted from structure suggest that this may be achievable. Alternatively, synthetic fusion of known N-terminal co-co assembly domains, such as coiled coils, BAR domains, or BTB domains, could be used to drive early dimerization, seeding the subsequent assembly of downstream domains. These modules could then be cleaved after assembly using engineered protease cleavage sites to yield native-like proteins.

In sum, embracing the principles of cotranslational assembly offers a powerful strategy to enhance the fidelity, efficiency, and robustness of protein complex biogenesis in biotechnological and synthetic contexts.

10. CONCLUSIONS

The study of cotranslational assembly has reshaped our understanding of protein biogenesis, revealing that translation, folding, and complex formation are not sequential events but simultaneous and coordinated processes. This spatial and temporal coupling provides a powerful regulatory layer that ensures assembly fidelity, defines interaction hierarchies, and shields nascent complexes from inappropriate interactions with paralogs, misfolded subunits, or competing factors.

What had long been considered the exclusive realm of posttranslational control is now understood to begin cotranslationally, pointing to previously understudied regulatory mechanisms, including domain positioning, translation kinetics, ribosome-associated factors, and polysome structure. Just as posttranslational assembly was revealed to be remarkably intricate over the past century, we can now anticipate that the cotranslational landscape will prove equally complex and equally fundamental to understanding how the proteome is built, maintained, and regulated.

Looking forward, the field is poised to tackle key open questions: How broadly does cotranslational assembly operate across different organisms and cell types? Can we predict and redesign cotranslational pathways? How does cotranslational assembly integrate with quality control systems, and how do cells deal with inefficient cotranslational assembly? What is the role of cotranslational assembly in protein evolution? Ultimately, dissecting how cotranslational assembly is regulated and embedded in the broader proteostasis network represents a key frontier in understanding cellular homeostasis and disease.

SUMMARY POINTS

1. Cotranslational assembly is a prevalent process that couples polypeptide synthesis, folding, and subunit association to create protein complexes with distinct structural properties that determine their biological activities.
2. Many multisubunit complexes use cotranslational assembly to impose hierarchical assembly, prevent promiscuous interactions, and control stoichiometry.
3. The cellular factors that coordinate cotranslational assembly are only beginning to be defined, with emerging evidence suggesting roles for translation kinetics, operon organization, polysome structure, localized translation, and chaperone action.
4. Cotranslational assembly provides evolutionary advantages. It facilitates the emergence of novel folds and enforces mRNA-specific homomerization, which shapes paralog evolution and buffers dominant-negative mutations.

FUTURE ISSUES

1. What is the prevalence of cotranslational assembly across the proteome? How frequently do complexes assemble through co-post versus co-co mechanisms, in *cis* versus *trans*, or at membranes?
2. What thermodynamic and kinetic principles govern nascent-chain folding and subunit engagement during cotranslational assembly?
3. How do distinct cellular processes (e.g., translation kinetics, polysomes structure, localization) enable and support efficient cotranslational assembly?
4. How does the quality-control network including molecular chaperones and E3 ubiquitin ligases surveil cotranslational assembly? Do dedicated surveillance systems act on nascent complexes?
5. How does misregulation of cotranslational assembly contribute to disease?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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