Trends in Cell Biology

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Review

The Benefits of Cotranslational Assembly: A Structural Perspective

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The faithful assembly of protein complexes in space and time is a hallmark of cellular homeostasis. Complex assembly might be seeded already during translation, if interacting subunits are recruited to the nascent chain. Here, we review recent discoveries suggesting that such cotranslational assembly is a prominent feature throughout the proteome. It might contribute to the efficiency and efficacy of assembly and occurs in coordination rather than competition with chaperones. We discuss how cotranslational assembly structurally contributes to the organizational order of assembly pathways and their surveillance. Taken together, these novel insights suggest that cotranslational assembly is intimately linked with the regulation of protein abundance, stability, and activity, offering an attractive explanation for many cellular phenomena.

Highlights

The cotranslational assembly of protein complexes in eukaryotes is a more prevalent phenomenon than previously thought.

Cotranslational assembly of protein complexes is likely to be interlinked with various biological processes including the regulation of local translation, buffering of protein complex stoichiometries, orphan subunit degradation, and protein evolution.

Structural constraints and the order of protein complex assembly pathways might necessitate cotranslational interactions.

Cotranslational assembly can increase both the efficiency and the efficacy of the process and generally occurs via N-terminally biased interaction domains in coordination with cotranslational chaperones.

Coordinating Protein Complex Assembly

Protein complexes are a key organizational unit of the proteome. The assembly of such complexes is a nontrivial task in the crowded interior of a cell, where each protein is in frequent contact with other macromolecules and therefore in competition for binding partners. Inevitably, cells had to come up with strategies to ensure faithful and efficient assembly. For many complexes, assembly based on the random collision of subunits is sufficient, as evidenced by assembly *in vitro* [1,2]. Others rely on the standard suite of cellular broad-specificity chaperones [3], have evolved dedicated chaperones [4], or even entire assembly organelles, as exemplified by ribosomal assembly in the nucleolus [5]. In all cases, premature or unintended interactions of nascent peptides are prevented, either by cotranslational binding of chaperones or by active transport to a suitable environment. There is, however, another way to achieve this; namely, through immediate cotranslational folding and concomitant association of binding partners.

As early as the 1960s, researchers showed biochemically, in prokaryotes, that nascent multimeric enzymes already possess enzymatic activity before their release from polysomes [6,7], indicating cotranslational folding and assembly. This notion later solidified with the realization that several cytoskeletal elements, including intermediate filament and sarcomere components (notably excluding actin and tubulin), are constructed cotranslationally [8]. While these examples represented homomeric or operon-encoded proteins, this concept was more recently extended to eukaryotic heteromeric complexes [9–18]. Homomers and operon-encoded heteromers of prokaryotes both emerge from a single mRNA, whereas heteromers in eukaryotes arise from several monocistronic mRNAs, thus raising intriguing new questions. Is the translation of several transcripts potentially coordinated in time and space? How does such regulation interplay with other cellular mechanisms like the degradation of orphan subunits [19] or differential assembly from different mRNA isoforms [14,20]?

To understand why cotranslational assembly has emerged as a pervasive part of the cellular assembly system, one needs to consider many aspects of protein biology: local and coordinated translation, folding, functional regulation, and cellular homeostasis. Just as every protein complex

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fulfills a specialized task in the cell, each has specific structural features that impose constraints on its assembly and evolution [21–23]. Some subunits form complexes based on promiscuous interfaces [24] and thus their subunits need to be guided to the appropriate binding partners, others are inherently aggregation prone [25], and yet others have **moonlighting** (see Glossary) functions [26,101]. A cellular assembly system needs to accommodate all of these features. How is it ensured that closely homologous proteins faithfully form functional homodimers instead of dimerizing with their close structural relatives? How is it ensured that potentially toxic proteins quickly and faithfully associate with their inhibitors? How does the cell manage to quickly produce more heteromeric ion channels on inhibition of a specific synapse? Cotranslational assembly might provide an answer to many of these questions.

The list of complexes known to assemble cotranslationally has increased rapidly in recent years and unique features and common principles have started to emerge. Here, we review recent insights into cotranslational assembly focusing on eukaryotic complexes and highlight the benefits for individual complexes and whole cells. We specifically discuss the concepts of nascent chain stabilization by interaction partners, the possibility of encoding assembly pathways by multidomain subunits, and spatially directed assembly by local translation.

Efficiency and Efficacy

Thus far, many studies of cotranslational assembly focused on its identification, and thus a major question central to any cellular phenomenon often remained unanswered: what is the benefit of cotranslational assembly of complexes?

In rapidly proliferating cells, assembling complexes correctly in a timely manner is important and thus their benefit might simply be a combination of **efficiency** and **efficacy**. However, to demonstrate this benefit experimentally would require the specific disruption of cotranslational assembly without disturbing its intimately linked translation, which has proved difficult. A successful attempt was reported for a bacterial luciferase complex [27]. When expressed from differently engineered operons encoded in *Escherichia coli*, the two subunits LuxA and LuxB preferentially assemble in ***cis*** from the same mRNA during translation. When ***trans*** assembly is enforced by artificial expression from distant sites of the genome on different mRNAs, luciferase activity is reduced by ~40%. Interestingly, full-length LuxA binds to nascent LuxB but much less so in the opposite direction, suggesting that LuxB contains a cotranslationally exposed motif that recruits earlier-translated LuxA. This positive correlation between polycistronic genes and assembly order also occurs on a proteome-wide level, with the exception of highly abundant complexes that are less prone to stochasticity [28]. The development of selective ribosome profiling (SeRP) (Box 1) allowed high-resolution positional information to be obtained about the onset of cotranslational assembly during translation, in this case on the position of the LuxB **assembly domain**.

Despite its proven usefulness in facilitating heteromeric cotranslational assembly in prokaryotes, eukaryotes largely abandoned polycistronic transcripts during evolution. However, several studies imply that cotranslational assembly is also a prevalent mechanism in eukaryotes [12,29]. To the best of our knowledge, experimental proof that assembly is rendered more efficient by

Box 1. Sequencing Technologies Driving the Discovery and Characterization of Cotranslational Assembly

The advancement of our understanding of cotranslational assembly is intimately linked to the development of suitable technologies. Important objectives are, for example, the identification of cotranslational molecular interactions and their spatiotemporal characterization, ultimately in the cellular context.

Glossary

Assembly domain: the part of a protein that recruits interactors, here cotranslationally.
***cis*:** assembly in *cis* refers to assembly from the same (polycistronic) transcript.
Directional: directional cotranslational assembly means that a fully translated subunit is recruited to the nascent chain.
Efficacy: the efficacy of cotranslational assembly as used here refers to its reliability in producing the desired outcome (the correctly and entirely assembled complex).
Efficiency: the efficiency of cotranslational assembly as used here describes how economical it is in producing the desired outcome (time and resources).
Moonlighting: refers to proteins that have an additional function on top of their canonical role and here is used to refer to proteins that are members of multiple complexes.
Symmetrical: symmetrical cotranslational assembly means that two nascent chains interact with each other, thus linking two polysomes.
***trans*:** assembly in *trans* refers to translation from two spatially separated transcripts.

Sequencing-Based Technologies

RNA immunoprecipitation coupled to microarray (RIP-chip) or coupled to sequencing (RIP-seq)

Immunoprecipitation techniques are commonly used for the identification of RNA substrates of RBPs. The target protein is affinity enriched and the copurifying mRNAs are identified by reverse transcription and DNA microarray analysis or next-generation sequencing [92]. In contrast to crosslinking immunoprecipitation (CLIP), RIP-chip/seq captures not only primary protein–RNA interactions (Figure IA) but also indirect interactions (Figure IB) as they occur, for example, during cotranslational association with nascent chains (Figure IC). To distinguish between nascent chain interactions and other types of indirect RNA-binding events, polysomal integrity is perturbed (Figure IC′). Although the immunoprecipitation occurs via a bait protein that binds to a nascent polypeptide, it is the mRNA (as a proxy for nascent chains) that is identified. This has to be done for each interactor separately.

Selective ribosome profiling (SeRP)

SeRP offers an extension to RIP-seq and provides mRNA-positional information about cotranslational interactions [93]. Translationally arrested polysomes are subjected to affinity purification targeting the (candidate) cotranslational interactor (bait), such as another member of the desired protein complex. Ribosome protected fragments (rpfs) are prepared by nuclease digestion of both total RNA (total translatome) and the enriched RNAs and subjected to deep sequencing. The respective reads are mapped onto the corresponding ORFs and normalized to the total translatome (Figure II). Ribosomes that have not yet synthesized the relevant interaction domains that recruit the bait are not enriched and therefore do not produce rpfs for the respective part of the mRNA (Figure II, left of broken line). The resulting profiles provide positional information along the ORF about where the bait protein binds to the nascent chain [29].

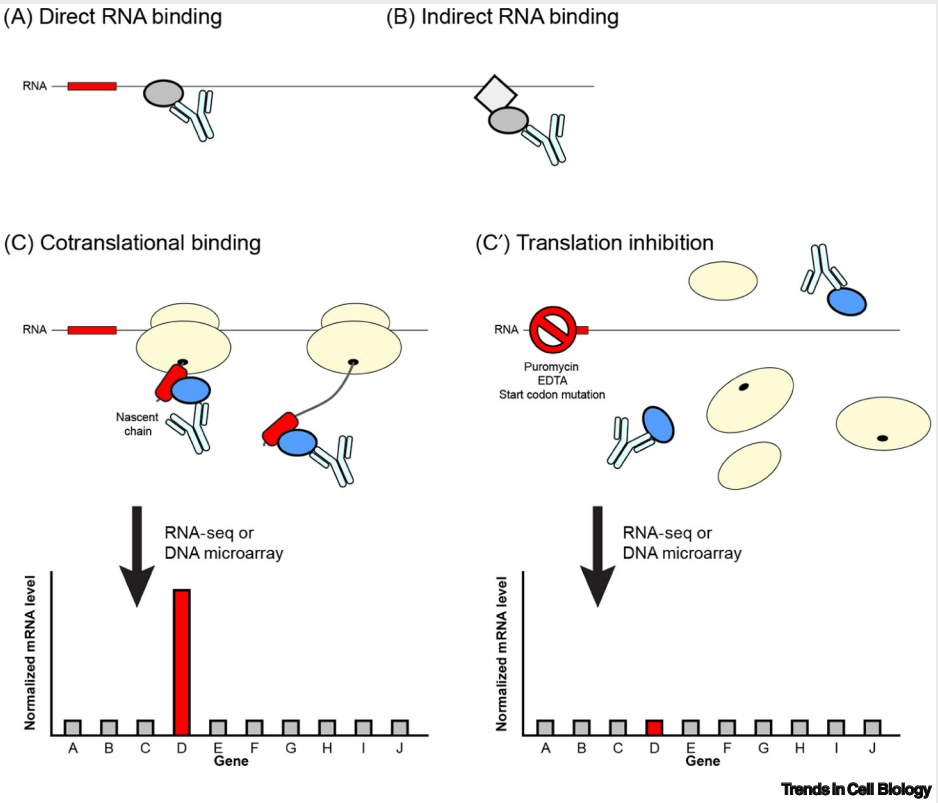


Figure I. RIP-chip or RIP-seq as Tools to Identify Protein–RNA Interactions. RIP via a candidate RBP allows (A) direct protein–RNA interactions and (B) indirect protein–protein–RNA interactions since it occurs under native conditions without crosslinking. (C) Cotranslational assembly is a special case of indirect RNA binding via the nascent chain and the ribosome. Copurified RNA is generally identified via reverse transcription followed by DNA microarray (RIP-chip) or next-generation sequencing (RNA-seq) and compared with total RNA or mock pulldowns. (C′) Disappearance of RNA enrichment after translation inhibition is commonly used as an indication of cotranslational interaction over other types of indirect binding. Adapted and extended from [12].

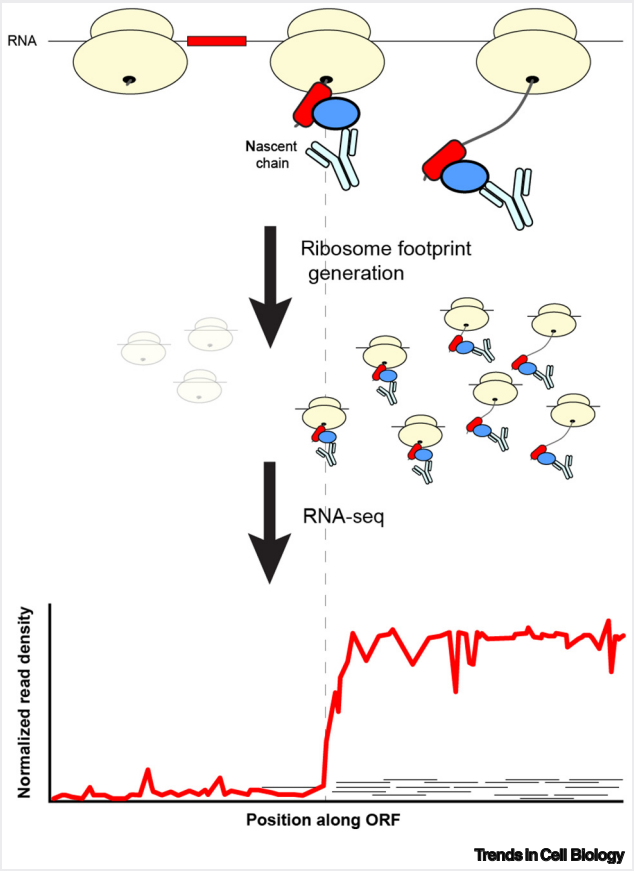


Figure II. SeRP Allows Identification and Positional Characterization of Cotranslational Interactions. Extending RNA immunoprecipitation, selective ribosome profiling (SeRP) allows high-resolution mapping of cotranslational interactions. Protein–ribosome nascent chain complexes (RNCs) are copurified efficiently only if a sufficient portion of the transcript was translated to allow the emergence of the interaction domains from the ribosome. Ribosomes prior to this position on the mRNA will not be captured. Immunopurified RNCs are further digested with nuclease to produce ribosome protected fragments (rpfs), reverse transcribed, and subjected to deep sequencing. Mapping of identified rpfs onto the respective open reading frames (ORFs), normalized to the total translatome, allows precise determination of the degree of interactions along the ORF.

cotranslational association is still missing for eukaryotic heteromers. However, several groups have shown that this assembly mode is advantageous in preventing aggregation or degradation [11,15,16,29]; in short, the efficacy of assembly. By deleting one subunit at a time and assaying the effects on abundance and solubility of the remaining members, a recent study has confirmed that the nascent subunit is generally more aggregation prone than its interactors [29]. Nine of 12 preselected stable *Saccharomyces cerevisiae* complexes underwent cotranslational assembly and all that were tested showed an increased misfolding propensity of the nascent chain subunit. The remaining three complexes have a well-characterized set of assembly chaperones potentially fulfilling the same role. However, even for those that do assemble cotranslationally, their interactions seem to be coordinated with ribosome-associated Hsp70-family chaperones [29]. This crosstalk between the nascent quality control system and the to-be-recruited partners that subsequently stabilize the nascent chain highlights the ribosome exit tunnel as a central hub for cellular surveillance [30,31].

In addition to chaperones, N-terminal biasing of interaction domains [32] and translational pausing at specific sites along the open reading frame (ORF) [9,33] have previously been suggested to aid folding and assembly [32,34–37], potentially by providing additional time. Various studies indicated the importance of these features [9,16–18,32,33,38–40] for cotranslational assembly, but methodological limitations, in particular regarding precise positional information pinpointing

interaction onset, had prevented more generic insights. This technical challenge has been addressed with the introduction of SeRP (Box 1). Shiber and colleagues confirmed an enrichment of interaction domains towards the N terminus in their dataset. They observed that the emergence of the full interaction domain from the ribosome exit tunnel almost perfectly coincided with cotranslational binding and subsequently remained stable [29]. Although the N-terminal biasing of assembly domains could explain how cells efficiently organize the assembly of heteromers, it does not seem to apply to homomers [16]. In case of heteromers, cotranslational interactions can conceptually occur either while both partners are still being synthesized and tethered to the ribosome (here termed **symmetrical**) or with one mature subunit binding a nascent subunit (here termed **directional**). Interestingly, more complexes seem to assemble in a directional fashion, with some notable exceptions [16,17,29]. While it is certainly too early to draw conclusions about cellular preferences, symmetrical assembly might require significantly more regulatory effort to ensure the coordinated translation of more than one polypeptide simultaneously in time and space (see Outstanding Questions). In the future, systematic mechanistic dissection of the kinetics of both translation and chaperone involvement, as well as the placement of interaction and stalling domains within ORFs, will be needed to better understand the underlying causality.

Nascent Chain Stabilization and Orphan Protein Degradation

That certain subunits of a multiprotein complex are inherently unstable in isolation is well known to many biochemists and particularly structural biologists. Many complexes can therefore be efficiently produced recombinantly only by coexpression of their subunits [41,42]. Similarly, in cell biology, there are often complex-specific limits to the overexpression of a certain subunit that cells will tolerate before the excess is degraded [43]. Last, the deletion or reduction of a

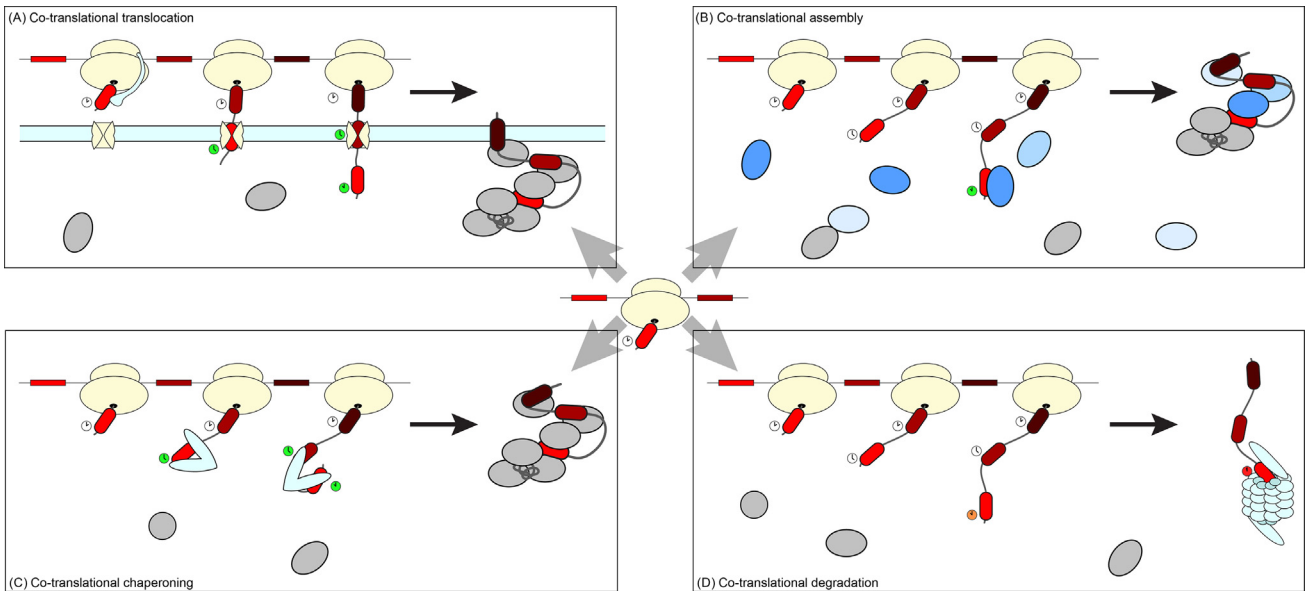
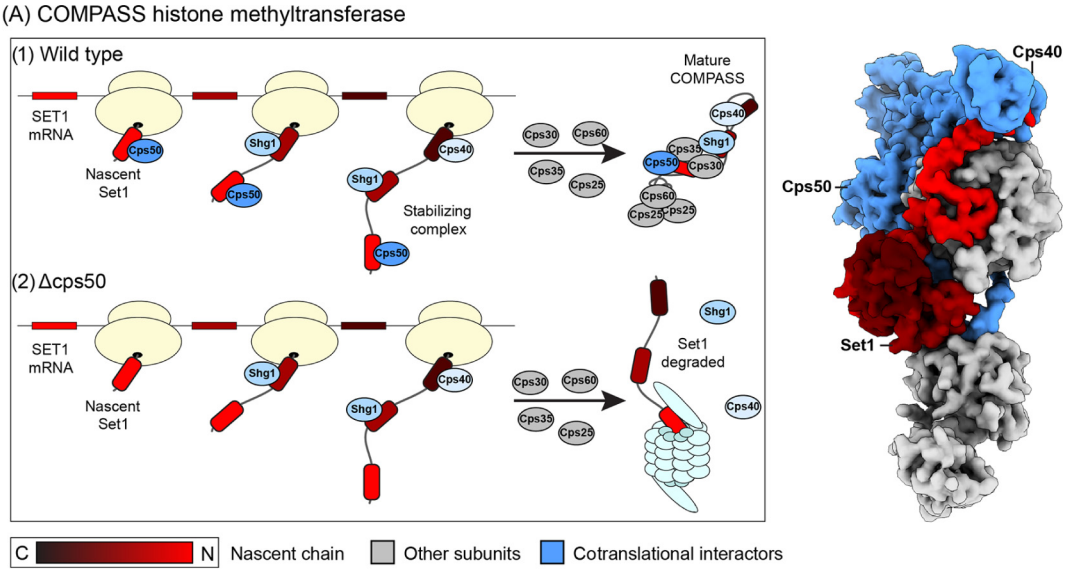
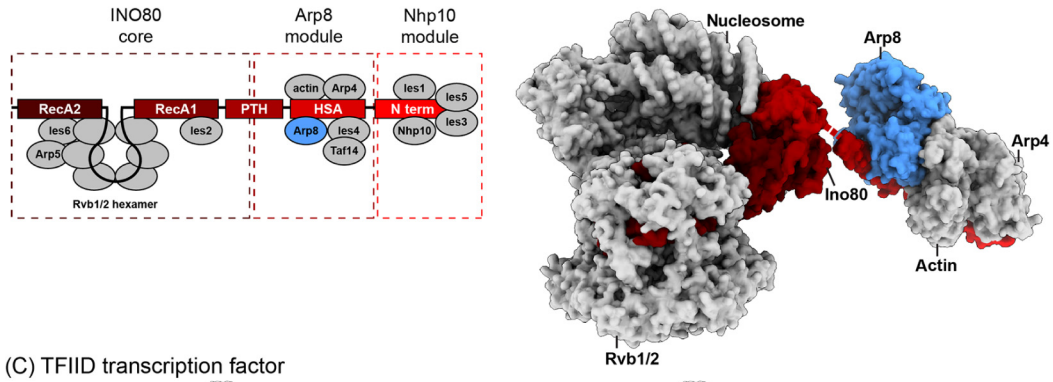
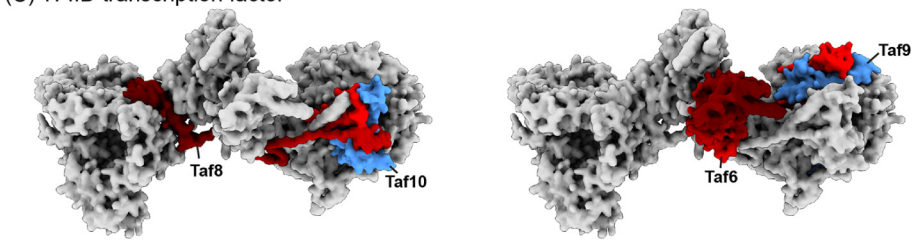
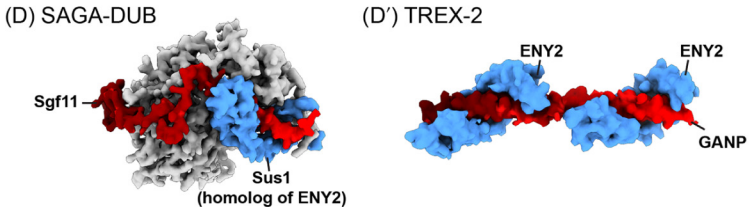


Figure 1. The Multiple Fates of Nascent Polypeptides. On emergence from the ribosome exit tunnel, nascent proteins can engage in a variety of processes depending on cellular context and function. These include but are not limited to the following. (A) Engagement of signal recognition particles (SRPs) and delivery to the rough endoplasmic reticulum for cotranslational translocation. (B) Cotranslational folding and association with other complex members. (C) Cotranslational chaperoning to aid proper folding and subsequent handoff to interaction partners. Successful engagement by the nascent chain in any of these processes generally leads to stabilization as indicated by a clock turned green. If these processes are not satisfied and completed within a certain timeframe, the cellular quality control system takes over (clock turned orange and red). (D) Cotranslational degradation by the cellular quality control system.









Trends in Cell Biology

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protein complex member often results in a reduction of other members alongside [44]. Each of these widely observed phenomena could be explained by the clearance of unassembled subunits, termed orphan protein degradation [19]. Although it was long suspected, it is only recently that more and more studies have found cotranslational assembly to be involved in mediating this quality control pathway [11,16,29].

Analogous to other cotranslational events such as translocation or chaperoning, assembly is likely to protect correctly associated subunits from degradation (Figure 1). Depending on the fate of the nascent chain, this is mediated by quality control mechanisms that sense the completion of each complex's cellular maturation program. Failure to do so, due to lack of interaction partners, improper folding, or mislocalization, generally results in clearance (Figure 1D) [19]. These events are not mutually exclusive but interconnected, as exemplified by the cotranslational assembly of transmembrane proteins [39] or the coordination with Hsp70 chaperones [29]. In this section, we discuss recent literature that highlights the interplay between cotranslational assembly and orphan subunit degradation.

The catalytic subunit Set1 of *Saccharomyces cerevisiae* has a central role during the cotranslational assembly of the histone methyltransferase complex COMPASS [11]. Identified during a test for COMPASS RNA binding by RNA immunoprecipitation coupled to microarray (RIP-chip) (Box 1), Set1's own RNA appeared as the top hit. A subcomplex of three core proteins (Swd1/Cps50, Shg1, and Spp1/Cps40) stabilized the nascent Set1 protein. Deletion of either Swd1/Cps50 or its partner Swd3/Cps30 led to drastically reduced Set1 levels (Figure 2A, left). As the remaining stabilizing interactors still bound to Set1's nascent chain and its translation was undisturbed, the authors concluded that excess Set1 that fails to find its partners in time is degraded. Complementary strong RNA overexpression led to only a very modest increase in Set1 concentration, presumably again due to the lack of stabilizing partners. The authors suggested that this removal of unbound nascent protein might occur via the proteasome, and later work identified N-terminal degradation signals [45]. Interestingly, the human Set1 homolog MLL is a substrate of the E2/E3 ubiquitin ligase Ube2O, marking it for degradation by the proteasome [46]. How exactly Ube2O recognizes orphan proteins to trigger their degradation [47,48] remains to be further characterized but could entail the recognition of exposed degradation signals that would otherwise be shielded in the fully assembled complex [47]. It thereby seems to compete for the same binding sites as nuclear transport receptors and assembly chaperones and prefer basic and hydrophobic stretches as shown for the ribosome or α-globin [47,48].

Depending on N-terminal amino acid residues (N-end rule), acetylation status, translation efficiency, or disorder, a large proportion of nascent chains may never be completed in the first place [49–51], as a considerable portion of the proteome is subject to cotranslational degradation. The N-end rule-mediated degradation of orphan proteins has been shown for several

Figure 2. Structural Commonalities of Cotranslationally Assembling Complexes. Structural models of several complexes utilizing cotranslational assembly. Molecular structures are displayed as surface representations and color coded based on the role of the subunit during assembly. The nascent chain is further color coded from the C terminus (dark red) to the N terminus (bright red). (A) *Saccharomyces cerevisiae* COMPASS histone methyltransferase [Protein Data Bank (PDB): 6BX3 [65]]. The catalytic Set1 extends from the center of the complex to the periphery, forming interactions with the previously identified cotranslational interactors Cps40/Cps50 [11]. The box shows a proposed model of Set1 assembly (adapted from [11]). Cotranslational formation of an initial stabilizing complex is followed by maturation via the recruitment of additional partners. Deletion of one of these stabilizers leads to the degradation of Set1. (B) Model of two partial chimeric Ino80 chromatin remodeler structures bound to a nucleosome (PDB: 6fml [68], 5nbn [67]) and schematic representation (both adapted from [67]). The central Ino80 ATPase subunit extends throughout the complex, recruiting three distinct modules along its path. Depicted in the structure is the deep C-terminal insertion into Rvb1/2 and the cotranslational interface with Arp8. (C) Model of the human TFIID BC core (PDB: 6mzc [70]). Highlighted are two distinct pairs of identified cotranslational interactors. Left: The nascent Taf8 again stretches throughout the entire complex, interacting with Taf10 at its N-terminal domain. Right: Taf6 and Taf9 are assembled symmetrically via the same domain. (D,D') Model of *S. cerevisiae* SAGA-DUB (D) and human TREX-2 (D') with the shared subunit ENY2/Sus1. The two homologs adopt highly similar conformations, embracing the different central nascent subunits. Cotranslational assembly has been identified for both complexes in human cells [16], but yeast SAGA-DUB is shown as the human structure is not available.

complexes and could be rescued by coexpression of its partners [52]. In the case of fungal $\alpha_6\beta_6$ fatty acid synthase (FAS), orphan Fas α is ubiquitinated by the N-end rule ubiquitin ligase Ubr1 and degraded by the proteasome in collaboration with Hsp70-family chaperones. Consequently, Fas α is prone to aggregation during stress and to degradation in the absence of Fas β [29]. Mature Fas β binds to nascent Fas α, which also appears to be coordinated with Hsp70 chaperone binding [29]. Given the highly intertwined interaction domains, it is unsurprising that such an interface has to form concomitantly with folding [29,53].

The question of how well expression of protein complex members is coordinated remains subject to active research [54–56], but it is likely that inherent stochasticity at each level from transcriptional to post-translational regulation ultimately prevents perfect coordination. It is interesting to note that the stoichiometry of protein complexes is post-translationally buffered [57] and less clearly reflected in ribosomal profiling and gene expression data than protein abundances [57,58]. Fully assembled complexes, however, seem to need a sufficient concentration of structurally relevant subunits to be stable [57]. The concept of 'driver subunits' [59], whose expression levels ultimately determine the abundance of the entire complex and thus are more tightly regulated than other subunits that are produced in excess, might be in good concordance. Further, many complexes have evolved shared or moonlighting subunits [26], expanding the complexity of coordinated expression. The generation of orphan proteins thus seems inevitable. It has been estimated that >10% of the proteome undergoes nonexponential decay predominantly within the first hours after synthesis and a large fraction of the affected proteins are known protein complex members [60]. Since cotranslational assembly and orphan subunit degradation appear to be intimately interlinked processes, it is interesting to ask how exactly the cell detects and removes orphan proteins (reviewed in [19]) (see Outstanding Questions). While cotranslational chaperones are in control over the fate of many proteins, designated interaction partners in a protein complex seem to fulfill a similar role for a surprising number of them. One might thus envision a continuum with certain proteins in high need of chaperone attention on the one end and proteins that are content with their natural partners on the other.

Extended Nascent Subunits as Common Structural Features of Cotranslationally Assembling Complexes

The order in which protein complexes assemble appears to be important and subject to evolutionary pressure. It is often preserved across species [61], despite the impact of drastic evolutionary events such as gene fusions. To abide by this evolutionary constraint and retain a degree of flexibility in subunit composition and relative positions, it is appealing to envision a coordinating subunit that encodes assembly order within the polypeptide chain. Like pearls on a string, this protein uses a sequence of small domains or short linear motifs (SLIMs) to form interactions and 'capture' its partners one by one as its domains emerge from the ribosome. Intrinsic disorder would greatly facilitate this, by acting as 'molecular glue' to bring together various subunits while retaining flexibility [62,63]. As mentioned earlier however, disorder would potentially also attach a sort of molecular timer to these interactions as disordered proteins are more prone to degradation if sensitive sites are not sequestered [64]. By kinetic competition, each interaction would have to be shielded or satisfied within a certain timeframe to escape quality control (Figure 1B,D), which might focus the stoichiometric control of an entire complex onto a single rate-limiting 'driver' subunit [59].

Many of the recently identified nascent chains that form cotranslational interactions with other complex members appear to be built according to this blueprint. In two recent high-resolution structures of *S. cerevisiae* COMPASS [65,66], the central Set1 polypeptide winds throughout the entire complex, forming contacts with all but one peripheral subunit (Figure 2A). On its path,

it interacts with Cps30, Cps40, and Cps50, and its catalytic SET domain is positioned in the central junction of the complex. These are three of the four subunits mentioned earlier that were found to be crucial in stabilization [11]. Interestingly, the most important early stabilizer, Cps50 [11], itself winds throughout the entire complex as well, providing a scaffold alongside [65,66]. Unsurprisingly, the entire N terminus of Set1 harboring the identified degradation signals [45] has been actively removed during the structure determination projects, pointing to additional contacts that yet remain to be unraveled.

Nuclear chromatin remodeling complexes also appear often to assemble cotranslationally, possibly in encoded order. The central Ino80 subunit of the eponymous *S. cerevisiae* remodeler is a prime example, where its N-terminal domain recruits a subcomplex of four proteins [67], its central domain binds to a set of actin-related proteins (Arps) that are shared among many remodelers [67], and its C terminus is inserted into the RuvA/B helicase [68] (Figure 2B). In doing so, it adapts an extended conformation that could recruit one module after the other on emergence from the ribosome. The same general architecture also applies to other family members, such as SWR1, SWI/SNF, and RSC [67,69]. Several *Schizosaccharomyces pombe* Arp-family members have indeed been found to bind cotranslationally to their respective nascent central subunits including Ino80 and the SWI/SNF central subunit Snf22 [12]. Another recently discovered example is the nuclear transcription factor TFIID. It is assembled in the cytoplasm, where its subunit Taf10 binds to the nascent Taf8 subunit and the Taf10 protein colocalizes with *TAF8* mRNA in cytoplasmic foci. This interaction was lost on mutation of the N-terminal interaction domain of Taf8 [16]. In the complex, Taf8 is extended, forming interactions with the majority of subunits and binding Taf10 at its N-terminus (Figure 2C, left) [70]. In total, TFIID houses three identified pairs of cotranslational assembly, all relying on the same histone-fold domains as also shown by Taf6–Taf9 (Figure 2C, right) [16]. As a final example, ENY2 (Sus1 in yeast) is a shared component of the TREX-2 and SAGA complexes. In human cells, it stabilizes different nascent chains in the two complexes (Figure 2D,D′) [16], exemplifying how moonlighting is accomodated by cotranslational assembly. With the rapidly growing structural repertoire of large complexes, cotranslational assembly might be predicted from structural databases in the future or even exploited to engineer artificial and novel complex topologies.

Protein Complexes Can Associate Cotranslationally with Their Regulators

To prevent toxicity, the activity of certain proteins has to be effectively inhibited in time or space without leakage. This can be achieved by cotranslationally attaching specific regulators to their targets. The caspase-activated DNase CAD is one of these particularly sensitive cases. In proliferating cells, CAD is kept inactive by its inhibitor ICAD, and acute depletion of ICAD triggers nuclear fragmentation and cell death [71]. Fittingly, CAD can fold only in the presence of ICAD [72]. Dissection of the molecular timing in *in vitro* translation systems has proved that Hsp70–Hsp40 cotranslational chaperones are needed to keep CAD in a ‘quasi-native state’ after which the handoff to ICAD for maturation occurs [72].

While not as deleterious for the cell as CAD, cyclin-dependent kinases (CDKs) take center stage in terms of cell cycle timing and progression and are therefore regulated at several levels. This includes cyclin-dependent activation, phosphorylation, localization, and inhibition [73]. In *S. pombe* the CDK protein Cdc2 is the only member and is sufficient to drive the cell cycle with a single cyclin partner [74]. In their systematic investigation into *S. pombe* cotranslationally interacting proteins, Duncan and Mata probed the interactors of this essential kinase. The authors emphasize that, despite dozens of known interactors and substrates of Cdc2, it specifically enriched two mRNAs encoding the cyclin *cdc13* (a positive regulator) and the CDK inhibitor *rum1* (a negative regulator) [12], although other interactors are several-fold more abundant in

the cell. Why only one of its cyclins – Cdc13 – and not others requires this type of rapid assembly remains enigmatic.

Localized Construction of Protein Complexes

Despite the lack of operons in eukaryotic cells, there arguably is a yet-bigger need for spatial coordination because they are considerably larger. Eukaryotic mRNAs are packed into messenger ribonucleoprotein (mRNP) particles, clusters of protein and mRNA that regulate the fate of a given transcript. In a theoretical framework termed the 'post-transcriptional operon' hypothesis, it is these contained RNA-binding proteins (RBPs) that substitute for physical operons by coregulating functionally related mRNAs [75]. By tethering them together in an area, the problem of slow, diffusion-based encounters of subunits is reduced dramatically and may be more achievable within the timeframe of translation.

The regulation of RNA localization has received tremendous attention since the realization that a large percentage of transcripts display specific subcellular localization [76]. While RNA localization, local translation, and cotranslational assembly are distinct mechanisms, and can occur independently of each other, their benefits for the efficiency and regulation of protein complex activity are often complementary. The regulation of local translation jointly with cotranslational assembly would offer an attractive concept to control the composition of protein complexes, in particular those containing moonlighting or closely related subunits that potentially assemble in a promiscuous manner. However, generating direct experimental evidence for the association of the two processes remains challenging and is often restricted to specialized tissues or biological conditions. Examples include the formation of the higher-order structure of sarcomeres into the typical band pattern in muscle cells, which requires the coordinated expression and assembly of the myosin heavy chain, titin, and other proteins. Nascent chains of several of these proteins were found stably attached to the cytoskeleton while still bound to ribosomes (reviewed in [8]). The localization of the corresponding transcripts using fluorescence *in situ* hybridization (FISH) (Box 2) revealed the same sarcomeric periodicity [77] and ribosomes appeared to be arranged in regular arrays in the muscle filament lattice [78]. In a more recent study, two mRNAs encoding regulatory subunits of the human proteasome were found to colocalize to cytosolic foci coined 'assemblysomes' under stress conditions [17]. In the same study, their *S. cerevisiae* homologs were shown to assemble symmetrically during translation, indicating evolutionary conservation.

Box 2. RNA, Protein, and Translation Visualization Techniques

While SeRP provides positional information about interactions along the mRNA sequence, the cell lysis during the procedure eliminates any higher-order spatial information about their position within the cell. As protein–nascent chain interactions have to occur in proximity to the translating polysomes, visualizing them in the cellular context is important.

The current gold standard for RNA visualization in fixed tissues is single-molecule FISH (smFISH) and variations thereof. By hybridization of short (20–50 nt) direct fluorescently labeled DNA oligonucleotides to target RNAs, transcripts are visualized with single-molecule sensitivity [94,95]. Proteins can be imaged in the same samples using immunofluorescence microcopy but generally lack the same single-molecule sensitivity. Direct one-to-one comparison of individual RNA and protein molecules is therefore very limited for traditional immunofluorescence and fluorescent protein tagging. This might, however, become more feasible thanks to the development of bright molecular probes (e.g., peptide arrays coupled to fluorescent binders as described below) and advanced imaging techniques in the future. For live imaging of RNAs, genetically encoded aptamers such as the MS2 system are widely used [96,97].

To visualize translating mRNAs and nascent polypeptides simultaneously, orthogonal techniques were developed that utilize genetically encoded N-terminal arrays of short epitopes, which are recognized by fluorescently labeled binders [88,98–100]. Although this relatively new class of techniques has, to the best of our knowledge, not yet been used to probe cotranslational assembly, the simultaneous visualization of RNA and nascent protein holds great potential and allows the spatial characterization of translation parameters such as elongation speed, pausing, or binding events.

Arguably the most familiar cellular location for local translation, however, is the rough endoplasmic reticulum (ER). Either actively tethered by RBPs or passively attached via nascent chain–translocon interactions (Figure 1A), numerous mRNAs are actively translated in polysomes on its surface, giving it the characteristic rough appearance [79]. However, even within the broad surface of the ER, there is suborganellar RNA localization that is relevant for cotranslational events. In example, both homomeric [39] and heteromeric [80] voltage-gated K^+ ion channels (Kv) assemble cotranslationally via their N termini on insertion into the ER [39]. One family member, the cardiac Kv channel hERG [14], is a pseudoheteromer that is assembled from two isoforms of the same transcript differing in those N termini. While the cotranslational interactions of the proteins are mediated by the respective domains, the association of the two transcripts is translation independent and possibly mediated by RBPs [14]. Furthermore, the translation of these voltage-gated ion channels regulated on a higher order spatial scale. The mRNA encoding the largely brain-specific Kv1.1 channel is locally translated in dendrites on mTOR or synaptic inhibition [81]. In dendrites, its transcripts form large, immobile clusters termed 'translational hotspots', from which new protein emerges. It is likely that this local translation still occurs on distant outposts of ER and mRNAs are possibly even transported alongside ER vesicles, as has been shown for several examples (reviewed in [82]).

Besides secretory or membrane proteins, the ER is a cellular hub for the translation of various cytosolic and nuclear proteins [83]. In this, mRNAs are not distributed evenly but follow a higher-order, mesoscale organization [83]. Consistent with this is the recent discovery of an ER subdomain called the TIGER domain [84], which opens an intriguing new level of cellular complexity. Mediated by the broad-specificity RBP TIS11B, this biomolecular condensate selectively enriches or depletes transcripts and thereby influences their fate by biasing protein complex compositions. This results in scenarios where identical polypeptides such as CD47, expressed from isoforms with varying 3′ UTRs (untranslated regions), end up at either the plasma membrane or the ER [20]. On a molecular level, this is achieved by interactions of RNA-bound effector proteins with the nascent chain inside but not outside the granule.

In light of the prominence of shared subunits and evolutionarily related proteins among complexes assembling cotranslationally, it is tempting to speculate that biasing of certain complex compositions by differential recruitment of mRNA isoforms is a more commonly used mechanism. This would allow tuning of the interactome despite identical or highly similar polypeptides. Among those identified so far are three kinesin and kinesin-like proteins [12], all five *S. pombe* myosins [85] as well as the myosin-like Nup211 [12], five TATA-binding protein (TBP)-associated factors and TBP [16], three chromatin remodeler ATPase subunits [12], eight Arps [12], and several voltage-gated ion channels [14,39]. While there is no direct evidence of localization-based interactome biasing for these related proteins so far, several members of these protein families were recently found to form so-called 'assembly particles' or translation hotspots [81,85–91]. At some point during assembly, their nascent proteins seem to exist in large cytosolic foci surrounded or filled by their own mRNAs. Often colocalized with chaperones, these granules are speculated to provide the proper milieu for translation, folding, and assembly prior to the release of their matured contents for their final function [85].

Concluding Remarks

In summary, it appears likely that the cotranslational assembly of protein complexes in eukaryotes is more prevalent than previously thought, but challenging to characterize because only a very minor fraction of the molecular species in a cell are engaged in assembly. It however offers an attractive explanation for various commonly observed phenomena, such as that faithful protein complex assembly requires the coexpression of multiple subunits, the fact that the order of

Outstanding Questions

What are the molecular determinants for and what fraction of the proteome is subject to cotranslational assembly? Proteome-wide RIP-Seq/SeRP studies and the development of bioinformatics predicting cotranslational assembly will represent important milestones to answer these questions in the future.

Orphan subunit degradation and cotranslational complex assembly seem to compete for nascent chain association. Whether the fate of the nascent polypeptide is chosen solely based on kinetic restraints or regulated in a more multifaceted manner remains unclear.

Does the combination of local translation and cotranslational association prevent promiscuous assembly and allow biasing of protein complex composition?

How do the protein subunits involved in cotranslational complex assembly find each other to begin with? For **symmetrical** assembly, what are the relative contributions of RBP-mediated versus nascent chain-mediated recruitment? For **directional** assembly, do the mature partners find their corresponding nascent chains via simple diffusion or is an active transport mechanism involved?

assembly pathways imposes a strong evolutionary restraint, the post-translational buffering of complex stoichiometries, the necessity of orphan subunit degradation, and the existence of driver subunits whose regulation seems to ultimately control protein complex abundance. Further characterization of these processes might be fundamental for our understanding of protein homeostasis in eukaryotic cells that is of high relevance for aging and human disease (see Outstanding Questions).

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