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► **To cite this version:**

Adham Safieddine, Jonathan Bizarro, Soha Salloum, Hervé Le Hir, Edouard Bertrand. Polysome sorting controls mRNA localization and protein fate. *Trends in Cell Biology*, 2025, <10.1016/j.tcb.2025.08.005>. <hal-05371249>

**HAL Id: hal-05371249**

**<https://cnrs.hal.science/hal-05371249v1>**

Submitted on 18 Nov 2025

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## Review

## Polysome sorting controls mRNA localization and protein fate

Adham Safieddine<sup>1,2,6</sup>, Jonathan Bizarro<sup>3,6</sup>, Soha Salloum<sup>4</sup>, Hervé Le Hir<sup>5,\*</sup>, and Edouard Bertrand<sup>4,\*</sup>

RNA localization and local translation are widespread phenomena that play key roles in a plethora of cellular processes ranging from embryo patterning to general cellular functions. The traditional paradigm assigns localization elements to *cis*-acting RNA sequences which assemble into complexes that regulate mRNA transport and translation, and the mRNA is generally transported while remaining translationally silent. However, recent evidence has shown that the nascent protein can also play an essential role in RNA localization and can enable polysomes to control their own transport and be delivered where and when they are needed. Two such examples are reviewed: translation factories and centrosomal mRNAs. Their comparison highlights the key role of cotranslational interactions in the spatiotemporal control of protein synthesis and protein fate.

## Translation-dependent mRNA localization is widespread

RNA localization is a post-transcriptional process in which RNA molecules accumulate in specific subcellular locations. It was traditionally described as an RNA-dependent process that relies on RNA *cis*-acting elements, termed zipcodes, which can bind to *trans*-acting factors to mediate the transport of translationally repressed mRNAs. A major long-known exception is the case of secreted proteins that rely on a signal peptide in the nascent protein to recruit the signal recognition particle (SRP) and achieve localization of their mRNAs on the endoplasmic reticulum (ER).

The SRP paradigm highlights the fact that protein synthesis, folding, and assembly into macromolecular complexes are coordinated and regulated by various factors that often act cotranslationally. The importance of cotranslational interactions is an emerging concept in the field of RNA localization and transport. In many cases it has become apparent that the polysome is transported and localized, not the silent mRNA, and the nascent protein plays a key role in the process. For instance, some polysomes are transported by their nascent chains to the site of accumulation of the mature protein, as observed in a range of species for cellular mRNAs that localize apically [1] or at centrosomes [2–7], the Golgi apparatus [6], mitochondria [8–10], the nuclear envelope [6], endosomes [6], adherens junctions [11,12], and the plasma membrane [13]. The nascent protein can also induce the accumulation of polysomes at sites unrelated to the final destination of the mature protein, and often form cytoplasmic **condensates** (see [Glossary](#)) that are visible by **single-molecule fluorescent *in situ* hybridization (smFISH)** at foci that contain many copies of the corresponding mRNA, such as of *DYNC1H1* mRNA in human cells [14–17]. These condensates, dubbed **translation factories**, are specialized sites of mRNA translation (reviewed in [18]). They appear to influence the cotranslational interactions made by the nascent protein that impact on the cotranslational assembly of protein complexes and more generally on protein fate. In this review we discuss the role of nascent protein chains in RNA transport and localization, focusing on the cases of translation factories and centrosomal mRNAs.

## Highlights

Nascent proteins can induce mRNA localization, either in translation factories or at the final compartment of protein accumulation. Translation factories form by the condensation of specific polysomes.

Chaperones are involved in organizing translation factories and compartmentalizing the translation apparatus in the cytoplasm.

Polysomes can be actively transported by molecular motors.

Nascent protein chains synergize with general RNA-binding proteins such as the exon junction complex to achieve polysome localization.

Translation-dependent mRNA localization appears to facilitate the cotranslational assembly of nascent proteins either in translation factories or in the destination compartment.

Translation factories control the fate of nascent proteins by predetermining their cotranslational interactions.

Cotranslational polysome sorting enables a single mRNA to generate multiple polysome populations that encode the same protein but which have with different functions and localizations. Polysome sorting may be a general mechanism to regulate the fate of multifunctional proteins.

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## Translation factories and cotranslational assembly of protein complexes

Cotranslational assembly (Box 1) was initially reported during the purification of several subunits of the yeast SET1C histone methyltransferase complex (Swd1, Spp1, and Shg1), which pulled down the mRNA encoding their partner subunit Set1 in a translation-dependent manner [19]. A systematic approach using multiple proteins as baits revealed that cotranslational assembly is a widespread phenomenon in yeast [20]. Sequencing of ribosome-protected mRNA fragments (Ribo-seq) revealed that, for several protein complexes, a fully translated subunit can engage its nascent partner, reciprocally or directionally (Box 1) [21]. In mammalian cells, multiple transcription complexes, such as *TFIID*, *SAGA*, and *TREX2*, assemble cotranslationally [22]. In some cases, impairment of this cotranslational pathway induces the degradation of the nascent proteins, highlighting the importance of this mechanism [23]. The dissection of the TFIID complex highlighted the predominant role of its TAF1 subunit, where its polysome acts as a central hub for the binding of partner proteins, some of which are preassembled as submodules [24]. In many cases, however, the RNA/protein pairs studied by smFISH combined with immunofluorescence showed a surprisingly low level of colocalization (2–10%), except for the *TAF1* mRNA/TAF7 protein pair (~40%) [23–25]. This low level of colocalization could be due to limited IF sensitivity, a small fraction of translated mRNAs, or the occurrence of a mixture of co- and post-translational assembly pathways. All the mRNAs cited above are randomly distributed throughout the cytoplasm and have no particular subcellular localization. They occur as single molecules that do not colocalize with each other or form condensates. By contrast, several recent reports have highlighted cases in which the cotranslational interactions occur in polysome condensates, also called translation factories [18] (Box 2). These translation factories are cytoplasmic condensates that contain multiple cotranslated mRNA molecules. The discovery of polysome condensates constitutes a conceptual advance and challenges the view that RNA condensates are repressive for translation [26,27] – as highlighted by the well-studied cases of stress granules and processing bodies (P-bodies) which contain translationally repressed mRNAs alongside

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### Box 1. Different types of cotranslational assembly mechanisms

Cotranslational assembly refers to the process of forming protein complexes while the proteins are still being synthesized by the ribosome. Cotranslational assembly pathways can be classified according to the different parameters listed below.

#### Types of cotranslational interactions

There are several different types of cotranslational interactions. (i) Interactions between a mature protein and the nascent polypeptide of a different protein that is still being synthesized by a ribosome, forming the cotranslational/post-translational (co-post) pathway. For example, in yeast the fully translated NAA10 subunit of the N-acetyltransferase A complex binds cotranslationally to its nascent partner subunit NAA15 [21]. (ii) Interactions between two nascent proteins that are still being translated by the ribosome, forming the cotranslational/coassembly (co-co) pathway. This can occur in *trans*, involving two different mRNA species, or in *cis*, involving two ribosomes translating the same mRNA. For example, two nascent lamin A proteins assemble into a homodimer during their translation [49]. (iii) Interactions between a nascent protein that is still being translated and a dedicated assembly chaperone, forming a recently described pathway dubbed cha-cha from 'chaperone channeling'. In this case the assembly chaperone accompanies the fully translated subunit across the cell until it binds to its partners. This is illustrated through the R2TP chaperone that binds to nascent POLR2A and POLR2B polypeptides and facilitates their assembly with other subunits of the RNA polymerase II complex [31].

#### Directionality of cotranslational interactions

Cotranslational interactions can be (i) reciprocal, when the nascent peptide of subunit A can bind to its mature protein partner B, and vice versa (mature subunit A binds to nascent subunit B); and (ii) unidirectional when the interaction occurs between a specific nascent subunit A and a mature subunit B, but not the other way around. An example of reciprocal interactions is the heterotrimeric multi-aminoacyl-tRNA synthetase complex where each of its three subunits (GLURS, METRS, and ARC1P) can bind cotranslationally to one or both remaining subunits mainly via their glutathione-S-transferase (GST)-like folds at the N terminus [21]. Unidirectional interactions apply to the hetero-dodecameric fatty acid synthase (FAS,  $\alpha_6\beta_6$ ) complex where only the fully mature  $\beta$  subunit (the last 389 aa) binds to the nascent  $\alpha$  subunit (the first 94 aa) to stabilize it [21]. By contrast, the  $\alpha$  subunit does not associate with the nascent  $\beta$  subunit.

### Box 2. Classification of translation factories

Translation factories are polysome condensates that can be classified based on several criteria.

#### Composition

Homotypic translation factories contain several copies of a single mRNA species (e.g.,  $\beta$ -catenin translation factories [43]). Heterotypic factories contain different mRNA species (e.g., mRNAs encoding various dynein heavy chains [29]).

#### Mechanism of formation

Translation factories can be translation-dependent, in which case the presence of the nascent polypeptide is required for factory formation, or translation-independent, when the nascent polypeptide is not required (e.g., TIS11B condensates) [18]. This review focuses on translation-dependent translation factories.

#### Functions

Translation factories can be classified according to their function. Currently known functions include the assembly of protein complexes, post-translational modifications, and protein degradation.

proteins involved in RNA decapping, decay, miRNA silencing, and translation inhibition. In contrast to these compartments, translation factories generate a local environment that is permissive for translation and which influences protein biogenesis.

### Functions of translation factories

In metazoans, translation factories have so far been described for the mRNAs encoding peripherin, DYNC1H1, ASPM, BUB1,  $\beta$ -catenin, **POLR2A**, **PRPF8**, **dynein axonemal** heavy chain, and the proteasome subunits RPT1/PSMC2 and RPT2/PSMC1 [6,15,16,28–31]. In many of these cases, the factories control protein fate by favoring and determining cotranslational interactions. As such, they have been implicated in the assembly of protein complexes and in marking newly made proteins with degradative post-translational marks. For instance, RPT2/PSMC1 and RPT1/PSMC2 are both subunits of the proteasome, and their cotranslation in the same heterotypic factories during stress promotes their cotranslational assembly [28].

#### R2TP translation factories

The translation factories of *POLR2A*, *PRPF8*, and axonemal dynein mRNAs all contain high levels of the **R2TP chaperone**, which is specialized in the assembly of protein and ribonucleoprotein (RNP) complexes and links the HSP90 chaperone with its cofactors and clients (Figure 1A,B). Indeed, R2TP assembles nuclear RNA polymerases, short nuclear/nucleolar RNPs (sn/snoRNPs), PIKKs, and axonemal dyneins (reviewed in [32,33]). It binds cotranslationally to many of its clients, including pairs of subunits that associate with each other, such as the POLR2A/POLR2B subunits of RNA polymerase II, EFTUD2 and PRPF8 of the U5 snRNP, and EP400 and BDR8 of the chromatin remodeling complex TIP60 [31]. The POLR2A and PRPF8 translation factories sometimes mix and colocalize (Figure 1A). By contrast, their partner subunit mRNAs occur as dispersed single molecules that display little colocalization with these factories, although they themselves also bind cotranslationally to the R2TP chaperone [31]. Therefore, there are no nascent chain–nascent chain interactions and the co-co model (Box 1) does not apply. Instead, the R2TP chaperone binds cotranslationally but independently to the different subunit mRNAs that they chaperone, and accompany the fully translated subunits across the cytoplasm until they associate. The R2TP thus alternates between co- and post-translational steps, and this cycle was shown to be regulated by its ATPase activity [31]. This may allow the R2TP chaperone to buffer the transient excess of a subunit, for example if one subunit is produced in excess because of the stochasticity of transcription or other aspects of gene expression. Indeed, studies in yeast have shown that the expression of subunits from the same complexes is generally uncorrelated as a result of **gene expression bursting** and expression noise [34]. The translation factories may

### Glossary

**Axonemal dynein:** a family of multi-subunit molecular motors that localize to motile cilia and flagella and enable their movements. Their heavy chains contain the motor domain and work as either homo- or heterodimers. They are cell type-specific.

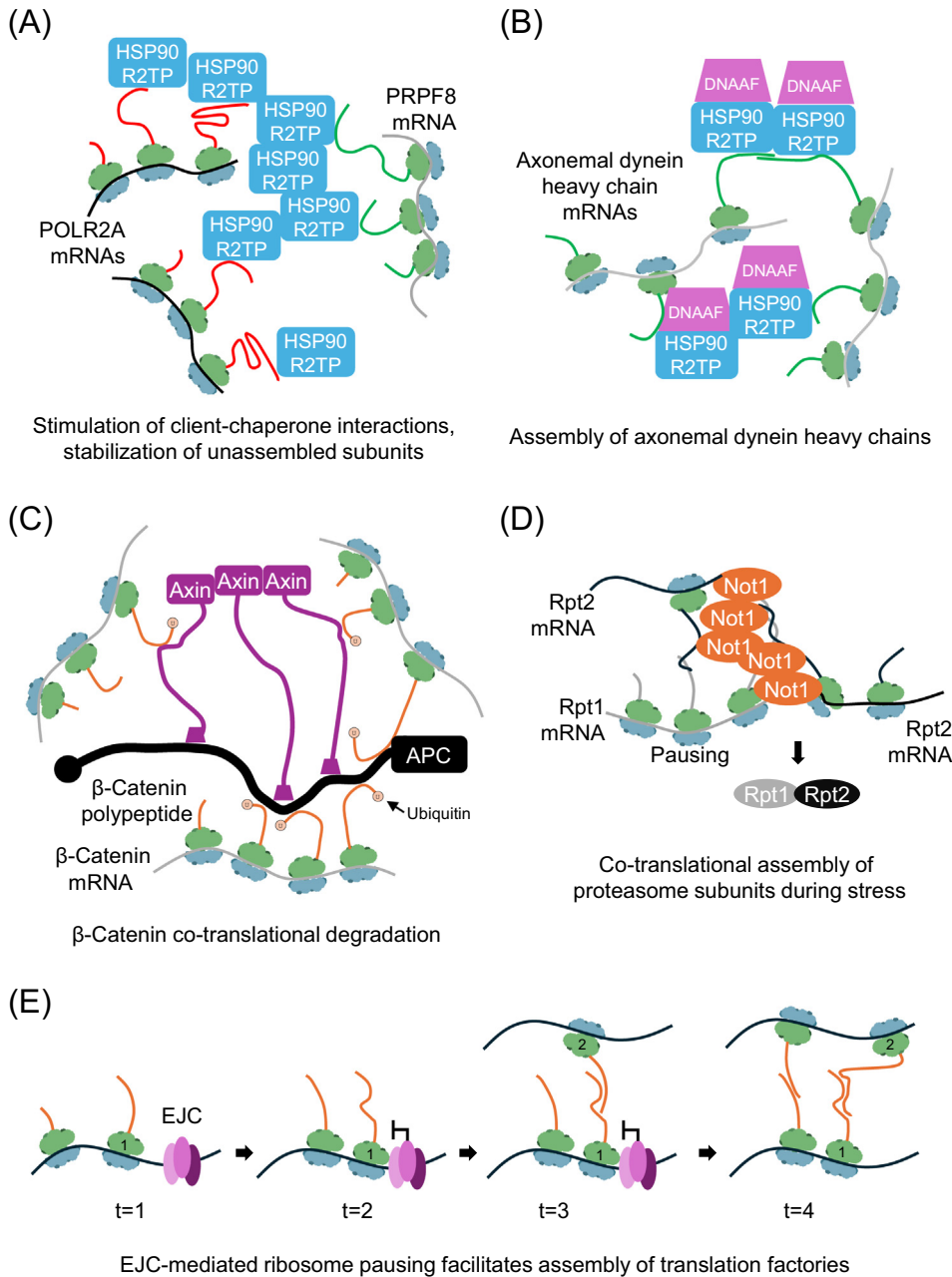
**Condensates:** membraneless organelles that form via multivalent interactions that lack defined stereospecificity and can scale with addition of components. They often contain proteins and nucleic acids and have varying levels of compositional complexity. They are present in diverse cells, tissues, and subcellular compartments. Typical cytoplasmic examples include processing bodies (P-bodies), stress granules, and germ granules.

**Exon junction complex (EJC):** a complex that is deposited onto mRNA by the spliceosome upstream of spliced junctions and is removed in part by ribosomes in the cytoplasm. It is composed of a core of three proteins (EIF4A3, MAGOH, and RBM8A) and participates in all stages of mRNA life including transcription, splicing, methylation, export, translation, and RNA decay. The role of the EJC in mRNA localization was first observed in *Drosophila* in the case of *oskar* mRNA, and more recently in humans ([72] and references therein).

**Gene expression bursting:** stochastic fluctuations of transcription activity observed at steady-state in all species because of the intrinsic dynamic properties of promoters.

**Germ granules:** specific ribonucleoprotein (RNP) condensates that play a key role in the development and functions of germ cells and also contribute to the patterning of embryos. Germ granules are involved in the storage and translational activation of specific mRNAs, and play distinct roles during different stages of germline development from late oocytes to maturing gametes. They are composed of homotypic clusters of RNAs and have two subcompartments, an internal compartment where storage and translational repression occurs, and a peripheral compartment that is involved in translation.

**NOT1 assemblyosomes:** condensates formed by the NOT1 protein that associate with specific polysomes to



promote the cotranslational assembly of protein complexes.

**POLR2A:** RNA polymerase II subunit A, the large subunit of RNA polymerase II.

**Polysome sorting:** a mechanism to sort polysomes to different intracellular locations depending on their cotranslational interactions.

**PRPF8:** pre-mRNA processing factor 8, a large nuclear protein and splicing factor that is part of the U5 small nuclear ribonucleoprotein (snRNP) complex and is a major component of the catalytic core of the spliceosome.

**R2TP chaperone:** a highly conserved chaperone dedicated to the assembly of protein and RNP complexes that is composed of the RUVBL1 and RUVBL2 AAA+ ATPases and the RPAP3 and PIH1D1 adapters. It associates with a prefoldin-like complex to form the PAQosome (particle for arrangement of quaternary structure). In the cytoplasm, the R2TP complex cooperates with HSP90 to fold proteins and assemble multiple macromolecular complexes, including C/D and H/ACA small nucleolar RNPs (snoRNPs), the telomerase RNP, U4 and U5 spliceosomal snRNPs and various cellular complexes such as nuclear RNA polymerases and axonemal dyneins.

**Ribosome pausing:** a process in which ribosomes pause or stall after translating part of the nascent polypeptide. This provides a window to fold nascent proteins and further regulate polysome composition: for example by recruiting binding partners to the nascent polypeptide that are necessary for protein complex assembly, or adaptors or motors that are required for the subcellular trafficking of polysomes.

**Single-molecule fluorescent *in situ* hybridization (smFISH):** a method that uses arrays of fluorescent oligonucleotides corresponding to a given mRNA to enable robust single-molecule sensitivity.

**Translation factories:** polysome condensates in which the translation of specific mRNAs occurs. Translation factories contain several mRNA molecules (from two to tens) and can be homotypic if formed by a single mRNA species or heterotypic if they contain multiple mRNA species. Translation-dependent translation factories generally require multivalent interactions that are dependent on the nascent polypeptide and are sensitive to translation inhibitors

**Figure 1. Examples of translation factories and possible assembly mechanisms.** (A) R2TP translation factories. The HSP90/R2TP chaperone binds cotranslationally to POLR2A and PRPF8 and condenses their polysomes. HSP90 is dimeric, R2TP has a hexameric ring of RUVBL1/RUVBL2, and polysomes are intrinsically multivalent with regard to the nascent protein chains. The multivalency of the system may drive condensation. (B) R2TP translation factories for dynein axonemal heavy chains. Legend as in (A), except that several types of dynein heavy-chain mRNAs colocalize in the translation factories and heteromerize during their translation. (C) β-Catenin translation factories. APC and AXIN1 bind to β-catenin cotranslationally and, because of the multivalent nature of the interaction system, condense β-catenin polysomes. (D) The NOT1 assembly. The NOT1 complex condenses upon oxidative stress and brings together the *Rpt1/Rpt2* mRNAs coding for yeast proteasomal subunits (*PSMC2/PSMC1* in humans). This enables their cotranslational interaction which

(Figure legend continued at the bottom of the next page.)

serve to favor the cotranslational interactions of some clients with R2TP, which may protect the subunits from degradation [35] and promote efficient translation [36,37]. Another benefit of chaperone-assisted assembly is that it may stabilize partially assembled complexes which may otherwise dissociate before additional subunits join the complex, especially if their concentration is low [38].

Axonemal dyneins are key motors that enable the motility of cilia and flagella. They are multisubunit machines assembled by R2TP and R2TP-like chaperones, and their heavy-chain mRNAs form translation factories in zebrafish and *Drosophila* cells, and also possibly in the human airway epithelium [29,30,39] (Figure 1B). These factories contain R2TP but exclude the other dynein subunit mRNAs, and thus also preclude a co-co assembly pathway. However, they contain several types of dynein axonemal heavy chains which heteromerize to produce functional motors. These factories thus serve not only to favor chaperone client interactions but also heavy-chain homo- and heteromerization. They are also likely important to deliver dyneins directly where they are needed because they localize at the distal part of the axoneme in *Drosophila* spermatocytes, the place where the motors are added to the growing flagella [30].

#### $\beta$ -Catenin translation factories

$\beta$ -Catenin is a bifunctional protein that functions at adherens junctions to link E-cadherin to the actin cytoskeleton, and also as an inducible transcription factor of the WNT pathway. In the presence of WNT,  $\beta$ -catenin is stable and enters the nucleus to activate the transcription of target genes, while in its absence  $\beta$ -catenin is degraded by AXIN1 and adenomatous polyposis coli protein (APC), which together drive its phosphorylation and ubiquitination [40,41]. The binding of  $\beta$ -catenin to APC, AXIN1, and E-cadherin is mutually exclusive, and membrane  $\beta$ -catenin is thus resistant to APC- and AXIN-mediated degradation. Membrane  $\beta$ -catenin also works as a mechanosensor, and tissue strain triggers its release from adherens junctions to activate target genes [42]. It was recently shown that the stability of the  $\beta$ -catenin protein is regulated entirely at the cotranslational level, and  $\beta$ -catenin mRNAs generate two pools of polysomes [6,43]. The first pool binds cotranslationally to E-cadherin and localizes at the membrane to produce the membrane pool of  $\beta$ -catenin protein. The second pool binds cotranslationally to APC and AXIN1 and accumulates in translation factories (Figure 1C). It produces  $\beta$ -catenin proteins that are cotranslationally targeted for degradation. Upon WNT signaling, APC and AXIN1 become inactivated and the  $\beta$ -catenin translation factories are dissolved, enabling  $\beta$ -catenin mRNA to produce a stable protein that will function as a transcription factor [6,43]. By concentrating nascent proteins and degradation factors,  $\beta$ -catenin translation factories likely prevent leakage of the system by favoring cotranslational interactions that lead to protein degradation. Indeed, APC is a multivalent protein, and one APC protein is able to bind to as many as ten molecules of  $\beta$ -catenin [40,41]. This ensures that, once it has been loaded onto a  $\beta$ -catenin polysome, APC can efficiently bind to newly initiated  $\beta$ -catenin protein chains emerging from the same ribosome, similarly to a person walking a treadmill. Importantly, the regulation is entirely driven by the nascent  $\beta$ -catenin protein and the cotranslational interactions that it makes [6,43]. This contrasts a newly discovered mechanism observed for mRNAs that localize to cytoplasmic protrusions in a 3' untranslated region (3'-UTR)-dependent manner, where the localization of the mRNA controls the partners of the encoded protein [44]. Overall, the cotranslational sorting of  $\beta$ -catenin

that dissociate the ribosome from the mRNA (e.g., puromycin).

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relieves ribosome pausing and enables production of the Rpt1/Rpt2 heterodimer. (E) A hypothetical model for the role of the exon junction complex (EJC) in the formation of translation factories. During the first round of translation, the EJC stalls incoming ribosomes, and thus enables the nascent polysome to interact with another polysome via their nascent protein chains. Once formed, the polysome-polysome interactions within translation factories are stable because new proteins are continuously produced. Abbreviation: DNAAF, dynein axonemal assembly factor.

polysomes is an efficient system that enables a single mRNA to generate two polysome populations that code for the same protein but with different functions and localizations. **Polysome sorting** may be a general mechanism to regulate the fate of multifunctional proteins.

### Formation of translation factories

The formation of translation factories can in principle involve different types of interactions, including protein–protein [6,43], protein–RNA [28], and RNA–RNA [7] (Box 2). For translation-dependent translation factories, a key event appears to be the synthesis of the nascent peptide because translation inhibitors such as puromycin, which dissociate the nascent protein chains from the mRNAs, generally rapidly dissolve the factories [6]. Polysomes contain multiple nascent protein chains, making them inherently multivalent. A multivalent cotranslational partner could thus crosslink these polysomes and drive their condensation. Indeed, this is believed to be a general mechanism underlying their formation.

$\beta$ -Catenin is one of the best-understood examples of translation factories [6,43].  $\beta$ -Catenin polysomes, APC, and AXIN1 form a highly multivalent system (Figure 1C). APC can dimerize and bind about ten  $\beta$ -catenin molecules. It also binds to AXIN1, which itself also binds to  $\beta$ -catenin and oligomerizes [40,41]. By binding to nascent  $\beta$ -catenin proteins, APC and AXIN1 can thus crosslink  $\beta$ -catenin polysomes and promote their condensation. In the initial phase of condensation, polysomes bearing multiple chains of nascent  $\beta$ -catenin may act as seeds to drive the initial clustering of APC and AXIN1. This may induce their condensation and the subsequent recruitment of additional  $\beta$ -catenin polysomes. Once in the condensate, a polysome is unlikely to dissociate because of the high local concentration of APC and AXIN1 as well as the continuous production of nascent proteins.

Remarkably, the HSP90 and R2TP chaperones have been reported to drive the formation of translation factories, leading to the remarkable conclusion that these chaperones are not only involved in protein folding and assembly but also in organizing and compartmentalizing the translation apparatus in the cytoplasm [29,30,35]. The R2TP complex is composed of an RPAP3/PIH1D1 heterodimer associated with the RUVBL1/RUVBL2 AAA+ ATPases, which form a hetero-hexameric ring that is intrinsically multivalent [32,33]. This property could possibly enable R2TP to crosslink its client polysomes and to assemble translation factories (Figure 1A,B). Of the many clients of R2TP, it is unclear why only *POLR2A*, *PRPF8*, and dynein axonemal heavy-chain (*DNAH*) mRNAs form translation factories. These mRNAs may associate more strongly with R2TP, or their nascent proteins may bind multiple times to the chaperone, thereby increasing the valency of the system. It is also possible that ribosomes could pause during the translation of these mRNAs (**ribosome pausing**), giving more time for polysome–polysome interactions (Figure 1E) [29].

The mRNA encoding cytoplasmic dynein heavy chain (DYNC1H1) also forms translation factories [16], although this motor is not known to be assembled by the R2TP chaperone. Interestingly, the DYNC1H1 factories require not only the nascent protein chains but also the presence of an **exon junction complex (EJC)** [45]. EJCs are mainly disassembled during translation [46] and therefore mark transcripts that have not yet been entirely translated. Therefore, it is possible that, during the initial round of translation, the EJC could slow down or arrest incoming ribosomes and promote polysome–polysome interactions (Figure 1E). Another interesting aspect of DYNC1H1 is that its polysomes display rapid rectilinear motion indicative of motor-dependent movements [16]. Such a mechanism could facilitate the assembly of translation factories. Alternatively, it could transport entire factories and deliver them to where they are most needed. If axonemal dynein factories display similar properties, this could be particularly relevant because these factories

are sometimes localized to specific sites to promote the delivery of axonemal dyneins where they are needed [30].

A ribosome pausing model has been clearly demonstrated for the **NOT1 assemblyosomes** (Figure 1D) [28]. Evidence from yeast and mammalian cells suggests that, after translating an N-terminal region of the RPT1/PSMC2 and RPT2/PSMC1 subunits of the proteasome, ribosomes stall and the messenger RNP (mRNP) complexes form heavy particles that also contain NOT1 (the scaffold of the CCR4–NOT deadenylase complex). Fusion of these heavy particles then facilitates cotranslational interactions between the nascent RPT1/PSMC2 and RPT2/PSMC polypeptides which alleviate the ribosome pause and allow the assembly of the two subunits [28]. Moreover, upon oxidative stress, NOT1 forms macroscopically visible condensates which are necessary to bring together the two polysomes encoding RPT1/PSMC2 and RPT2/PSMC1. This is thought to enhance cotranslation interactions and proteasome production in response to proteotoxic stresses [28].

Finally, a novel aspect of translation factories was recently highlighted for *Drosophila* **germ granules**. These structures are essential for germ cell development and functions. They are made of homotypic RNA clusters and are involved in both translational repression and activation at specific times during development [47,48]. It was found that germ granules have two subcompartments – an internal compartment where RNA storage occurs and a peripheral compartment where translated mRNAs are located. Moreover, when repression is relieved, mRNAs move from the interior to the surface of the condensates [47,48]. The internal organization of translation factories may thus be functionally relevant.

### Are translation factories widespread?

Only a few translation factories have been described so far and it is unclear how many are present in a cell. This question is particularly pressing for heterotypic translation factories because these are difficult to find by systematic microscopy screens in view of the enormous number of possible combinations between mRNAs. The sequencing of disome footprints held by a protein linker identified thousands of cotranslational assembly events, suggesting that the co-co pathway, and thus translation factories, may be widespread [49]. Computational analysis also suggests that heterotypic translation factories could perhaps be frequent in yeast [50]. However, two-color smFISH detecting mRNAs coding for pairs of subunits assembling together found only a very small fraction of mRNA/mRNA colocalization (a few percent) [25,50]. The extent of cotranslated mRNAs and the frequency of heterotypic translation factories is thus an open question. Systematic identification of homotypic and heterotypic translation factories will be necessary to clarify the compartmentalization and organization of the translation apparatus in the cytoplasm, an outstanding question.

### A conserved family of mRNAs localizes to centrosomes in a translation-dependent manner

Centrosomes are evolutionarily conserved organelles that serve as microtubule (MT) organizing centers [51]. RNA localization on centrosomes and MT-related structures was first described *in vivo* for cyclin B1 (*CCNB1/Cycb*) mRNA during the early division of *Drosophila* [52] and *Xenopus* embryos [53], and for a series of mRNAs during asymmetric divisions of mollusc embryos [54]. Since then, systematic imaging [4,55] and biochemical approaches [56,57] have identified several mRNAs that accumulate on centrosomes in a variety of cell types and organisms [58]. Some mRNAs such as *cycB* localize in a translation-independent way and rely on the binding of the CPEBP protein to its mRNA 3'-UTR [53,59]. The sections below focus on centrosomal mRNAs whose localization is translation-dependent. Remarkably, many of these mRNAs are

localized in both vertebrates and invertebrates, suggesting functional significance: pericentrin (*PCNT/plp*), centrocortin (*cen*), abnormal spindle-like microcephaly associated (*ASPM/asp*), coiled-coil domain containing 88C (*CCDC88C/girdin*), nuclear mitotic apparatus protein 1 (*NUMA1/Mud*), ninein (*NIN/Bsg25D*), and BICD cargo adaptor 2 (*BICD2/Bicd*). These mRNAs encode centrosomal proteins and their localization is regulated during the cell cycle [2–4,6,7,60] (Figure 2). Their localization is sensitive to polysome-disrupting drugs such as puromycin, and reporter assays showed that their coding sequence (CDS) is sufficient for mRNA localization, provided that it is translated [2,4,7,60]. Thus, information encoded in the nascent polypeptide is necessary and largely sufficient to cotranslationally target these mRNAs towards centrosomes, akin to the well-known case of secreted proteins that are targeted to the ER [61].

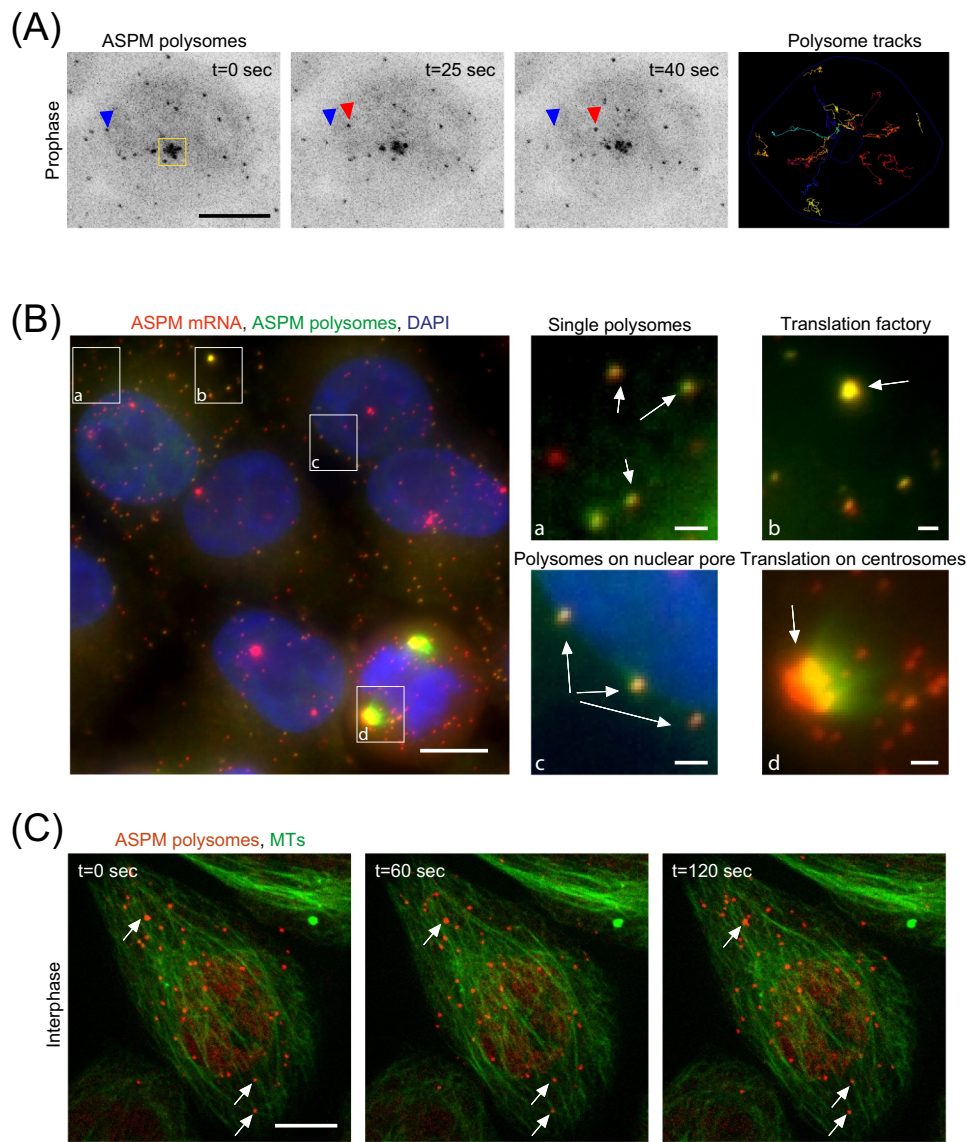
### Centrosomal mRNA localization involves active polysome transport

Centrosomal accumulation of the translation-dependent mRNAs is sensitive to MT and dynein disruption, indicating that MT-based motorized transport is required [2–4,60]. The ability to image single polysomes in living cells using nascent peptide chain labeling methods (reviewed in [62,63]) has allowed a better understanding of polysome transport towards centrosomes. One example is *ASPM* mRNA, which encodes a microtubule-binding protein that accumulates on spindle poles during mitosis alongside its mRNA. Direct visualization of *ASPM* polysomes revealed rapid trafficking towards centrosomes at the onset of mitosis, precisely when this mRNA starts to be localized (Figure 2A) [4]. This appears to take place on MTs using two distinct pathways: (i) active sliding of the polysome, with speeds and patterns that are typical of motor-based transport, and (ii) pulling MTs with *ASPM* polysomes attached to them as MTs are remodeled during mitotic entry. After arriving at their destination, translating mRNAs show more restricted dynamics and are anchored to centrosomes, the pericentriolar material (PCM), or spindle MTs. Similar observations have also been made for *NUMA1* mRNA, suggesting that the active transport of polysomes is a general mechanism [4].

*ASPM* polysomes are not only localized at centrosomes during mitosis but also display a remarkable diversity of intracellular localizations in interphase: polysomes can be stably anchored to nuclear pores, to MTs, or form translation factories (Figure 2B,C) [6]. The factors determining these distinct localizations of *ASPM* polysomes are currently unclear. However, they appear to be regulated according to the cell cycle because *ASPM* polysomes and protein accumulate on centrosomes exclusively during mitosis [3,4,6]. One possibility is that polysome localization is related to the multifunctionality of the protein. In interphase, *ASPM* has a predominantly nucleoplasmic pool that contributes to DNA metabolism [64,65], but it also has a cytoplasmic MT-binding pool [66]. New puromycin rescue experiments found that the centrosomal localization of *ASPM* mRNA in metaphase was not fully restored even after 4 h of puromycin washout, suggesting a requirement for the nascent peptide before the trafficking itself occurs [67]. A model thus emerges in which *ASPM* polysomes are bound to MTs in interphase and only become competent for mitotic centrosomal transport during a specific time-window (likely mid to late S or G2 phase). This likely requires cell cycle-specific regulation of polysomal components which could involve nascent peptide post-translational modifications, programmed ribosomal pausing, and motor or nascent chain binding-partner availability. The result is that MT-bound polysomes in interphase are primed for centrosomal transport upon mitotic entry. This would be consistent with the rapid wave of *ASPM* polysome transport towards the centrosomes that occurs in prophase [4] (Figures 2A and 3).

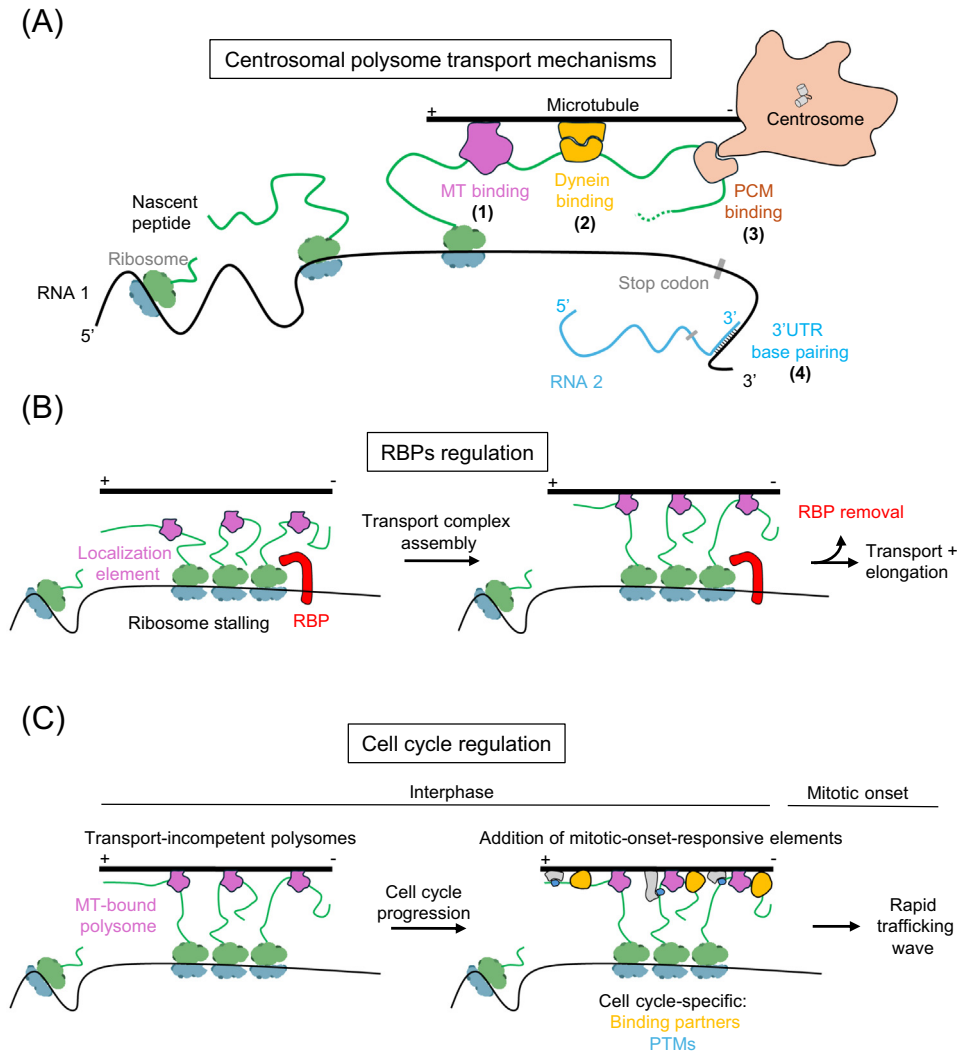
### Polysome localization elements in nascent centrosomal proteins

Dissection of the nascent peptide-encoded localization signal has enabled a better understanding of the underlying mechanisms. The CDS of *plp* mRNA encodes discrete peptidic *cis*-elements



**Figure 2. ASPM polysome localization and dynamics.** (A) Time-lapse microscopy images highlighting the dynamics of ASPM polysomes in a living prophase HeLa cell. Individual polysomes are labeled with the SunTag system and appear as foci. The accumulation of polysomes and mature protein on the centrosome is highlighted by a yellow square. Blue and red arrowheads indicate the starting and current position of a polysome, respectively. On the right is an overlay showing ASPM polysome tracks over ~2 min. Scale bar, 10  $\mu$ m. (B) Localization patterns of ASPM mRNA and polysome during the cell cycle in HeLa cells. ASPM mRNAs (in red) and polysomes (as well as the mature protein, in green) are revealed using single-molecule FISH and the SunTag system, respectively. Insets highlight individual polysomes, polysomes forming translation foci, polysomes on the nuclear envelope, and polysomes on centrosomes. (C) ASPM polysomes (in red, indicated with white arrows) are stably anchored to MTs (in green) in a living interphase HeLa cell. Modified from Safieddine *et al.* [4]. Scale bars are 10  $\mu$ m in images and 1  $\mu$ m in insets. Abbreviations: ASPM, assembly factor for spindle microtubules; DAPI, 4',6-diamidino-2-phenylindole nuclear stain; FISH, fluorescent *in situ* hybridization; MTs, microtubules.

that are necessary and sufficient to drive centrosomal accumulation of its mRNA in *Drosophila* embryos [60]. It was found that a specific *plp cis*-element directly interacts with the motif CM2 of centrosomin (Cnn) protein, a major component of the PCM that is crucial for centrosome



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**Figure 3. Summary of known and speculative polysome localization mechanisms.** (A) Various molecular interactions contribute to centrosomal mRNA trafficking, including (1) direct interaction of the nascent peptide (e.g., ASPM) with MTs, (2) interaction of the nascent peptide with dynein or dynein adaptors (e.g., Cen), (3) interaction of the nascent peptide with PCM components (e.g., Plp), or (4) 3'-UTR base pairing between two mRNA molecules. (B,C) Speculative mechanisms for how polysome trafficking can be regulated by (B) RBP-induced ribosome stalling (e.g., EJC, U2AF) or (C) cell cycle-dependent changes. Abbreviations: ASPM, assembly factor for spindle microtubules; MT, microtubule; PCM, pericentriolar material; RBP, RNA-binding protein; 3'-UTR, 3'-untranslated region.

assembly [68,69]. A substitution in the Cnn sequence that impairs both PCM assembly and its interaction with Plp protein causes *plp* mRNA to delocalize away from the fragmented PCM [60]. This revealed a role for the PCM and its scaffold *cnn* in centrosomal mRNA localization and anchoring. Another study analyzed the CDS of *cen* mRNA in *Drosophila* embryos [2]. Splitting the *cen* CDS into N-terminal and C-terminal halves abolished centrosomal RNA localization of either fragment, although the N-terminal protein fragment accumulates at centrosomes. Thus, multiple information encoded in each half of the CDS must cooperate for proper cotranslational transport (Figure 3A). Among these, the N-terminus of the Cen protein appears to play a special role because it contains a coiled-coiled domain that is similar to the CC1 box

that mediates interaction with dynein light intermediate chains. Moreover, mutating this sequence abolished mRNA localization, suggesting that the nascent Cen protein chain directly interacts with dynein to transport its polysome. Along these lines, it was shown that *DLGAP5* mRNA localizes to centrosomes in human cells in a translation-dependent way [70]. This mRNA contains a duplicated MT-binding domain at its N terminus which is essential for centrosomal mRNA localization. These domains are also sufficient, provided that the downstream coding sequence of it is long enough. This indicates that the ribosome elongation time is an essential feature of the transport mechanism. It is worth noting that many of the centrosomal mRNAs code for proteins that directly bind to motors and MTs, and possibly also themselves provide a direct link to active transport systems. Finally, it is worth mentioning that a case of intermolecular interactions between RNAs was described: this allows one RNA (*Ik2*) to piggyback on *cen* mRNA via 3'-UTR intermolecular interactions [7] (Figure 3A).

### Interplay between ribosome elongation, RNA-binding proteins (RBPs), and polysome localization

mRNAs encoding secreted proteins represent the paradigm for translation-dependent RNA localization. In this case, the nascent protein bears a signal peptide at its N-terminus. It is recognized by the SRP, which induces ribosome pausing until it reaches the SRP receptor on the ER, where elongation resumes [61]. It is currently unknown whether a similar mechanism applies to other mRNAs that are transported in a translation-dependent manner. However it was established that unfolded protein domains favor ribosome pausing [36,37]. Thus, for centrosomal proteins with an obligatory cotranslational assembly pathway, a non-localized mRNA would most likely generate a nascent protein with unfolded domains that may trigger ribosome pausing. This would provide time for the polysome to reach its target compartment, where the unfolded domain would bind to its partner and enable the ribosomes to resume elongation. Measurement of ribosome pausing *in vivo* indicated that pausing can last as long as 5 min [71], which would be sufficient for transport by diffusion, or by motors as in the case of centrosomal mRNAs.

In addition to such a 'built-in' mechanism, ribosome pausing may be enhanced by the binding of *trans*-acting factors to the mRNA that act as ribosome roadblocks (Figure 3B). For instance, the localization of *cen* mRNA at centrosomes in *Drosophila* requires the RBP Egl [2]. Similarly, the localization of *NIN* mRNA to centrosomes at the base of primary cilia in quiescent human cells was shown to require not only the nascent protein but also the EJC [72]. Likewise, *Zo-1/ZO1*, *Afdn/AFDM*, and *Scrib/SCRIB* mRNAs localize to adherens junctions in *Drosophila* and human epithelial cells, concomitantly with their encoded proteins, and this localization also depends on EJC proteins [12]. Newer data using a specific inhibitor that prevents EJC assembly in human cells showed that the EJC is required for the localization of many translation-dependent mRNAs [73]. The inhibitor not only alters the centrosomal localization of *NIN* mRNA as expected but also the localization of *NUMA1*, *ASPM*, *PCNT*, and *AKAP9* mRNAs, as well as the formation of the DYNC1H1 translation factories. Such a widespread role of the EJC would be consistent with a general mechanism in which the EJC transiently blocks ribosome elongation to enable polysome transport. There is currently no direct evidence for this mechanism and other models are possible. For instance, the EJC plays an important role in mRNA packaging [74–76] which may be an important feature that slows down the ribosome or which may be necessary to generate a transport-competent and partially translated mRNP particle. More direct evidence for a ribosome pausing mechanism comes from a recent study on the role of U2AF in mRNA localization at mitochondria [77]. U2AF is a heterodimeric splicing factor that shuttles between the nucleus and the cytoplasm [78], and it was also shown to bind to cytoplasmic mRNAs and regulate their translation [79]. In particular, U2AF binds to many nucleus-encoded mitochondrial mRNAs immediately downstream of the mitochondrial signal peptide. In addition, U2AF binding correlates

with increased disome (collision complex between two ribosomes) signals upstream from its binding site, indicating that it stalls incoming ribosomes [77]. One such target, *HSPD1* mRNA, accumulates at mitochondria in hematopoietic primary cells and this localization is disrupted by mutants of U2AF that bind less well to the mRNA [77].

All these data are very consistent with a model in which polysome localization is favored by ribosome pausing, itself induced by the binding of RBPs, possibly in combination with unfolded domains of the nascent protein (Figures 1E and 3B). However, this model remains speculative and raises several questions.

First, cDNAs lacking introns can produce mRNAs that localize in a translation-dependent way. It is thus unclear how the EJC can be necessary for mRNA localization. One possibility would be that the polysome contains a multitude of binding sites for RBPs which play partly redundant roles, and while a cDNA may be sufficient for mRNA localization, it may be less efficient than in the native context. In agreement, centrosomal localization of *Cen* mRNA in *Drosophila* requires multiple signals that are dispersed along the mRNA, and deletion of some generated a partial phenotype [2]. Likewise, U2AF mutation decreases but does not abolish mitochondrial mRNA localization [77].

Second, ribosome pausing is known to trigger collisions that lead to mRNA decay; likewise, ribosome stalling on transcripts that have EJCs can also induce mRNA decay [80]. If ribosome pausing is important for mRNA localization, how then do these mRNAs escape degradation? One possibility could be that the ribosome pauses near the N terminus of the protein such that collisions cannot form, as in the case of SRP- or U2AF-dependent mitochondrial mRNAs [77]. Another possibility is that the conformation of the stalled ribosome and/or the transient nature of the pause precludes degradation by quality control mechanisms, possibly aided by *cis*-acting factors that bind to the mRNA [71,81]. Indeed, some mRNAs are stable despite having long-lived EJCs [46], and it was recently observed that colliding ribosomes can cooperate to overcome pausing signals instead of inducing RNA degradation [71].

Finally, it is likely that RBP binding, ribosome stalling, transport, and elongation resumption upon arrival at the destination are coordinated events that ensure proper delivery of proteins to where needed. How these events are integrated into an efficient process is currently not understood. In the future it will be essential to carefully analyze ribosome pausing and its regulation as the mRNA becomes localized.

### Concluding remarks

The reasons that mRNAs are locally translated can be numerous. However, for mRNAs that are transported by partially translated nascent protein, a plausible hypothesis is that these proteins need to be cotranslationally assembled in the target compartment, as is the case for secreted proteins that are translated on the ER as well as for some nucleus-encoded mitochondrial proteins ([10]; reviewed in [61,82]). It is possible that the complexity of the structure of centrosomes and their highly dynamic compositions during the cell cycle imposes constraints on their assembly that can be best fulfilled by a cotranslational mechanism. The highly conserved nature of the centrosomal mRNAs between human and *Drosophila* is consistent with this hypothesis, and researchers now have tools that enable them to delocalize mRNAs at will to test these hypotheses [83,84]. Similarly, the translation of some mRNAs in dedicated factories is often related to issues of cotranslational assembly, for instance to favor some partners at the expense of others (as in the case of  $\beta$ -catenin) or to promote their association with partners or assembly chaperones (as in the case of dynein axonemal heavy chains and R2TP translation factories). A unifying theme of

### Outstanding questions

Is translation-dependent RNA localization required for protein function? Does it facilitate cotranslational assembly of nascent proteins?

Does translation-dependent RNA localization require direct/indirect interactions with motors?

Does translation-dependent RNA localization require ribosome pausing? Do general RBPs such as the EJC play a common role in pausing? How does this relate to nascent protein folding, and how is pausing regulated spatially?

If ribosome pausing occurs, how could ribosomal collisions and quality control pathways be prevented from degrading the RNA?

How many translation factories are present in a typical cell? Are some cell types particularly prone to the formation of translation factories (e.g., multi-ciliated cells and neurons)?

Are polysomes that code for different proteins frequently colocalized and cotranslated? Is this always related to cotranslational assembly of multi-protein complexes, or does it also occur for proteins that do not belong to the same complex? What is the level of spurious polysome association for unrelated proteins?

Do chaperones play a general role in organizing and compartmentalizing the translation apparatus in the cytoplasm?

cotranslational mRNA localization may thus be cotranslational assembly of protein complexes. Translation factories may enable preassembly of protein complexes, and mRNA delivery to various cellular locations may allow cotranslational assembly with the target compartment. Future research should rigorously address the functions of cotranslational mRNA transport and the role of ribosome pausing in the process (see [Outstanding questions](#)). In particular, the roles of the R2TP chaperone and the EJC need to be clarified because they appear to play important roles in the same set of cotranslational transport systems, including the formation of dynein translation factories and the transport of mRNAs to centrosomes [31,73].

### Acknowledgments

The work was funded by grants from La Ligue Nationale Contre le Cancer (Équipe Labélisée) and MSDAvenir.

### Declaration of interests

The authors declare no competing interests.

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