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Review

De-centralizing the Central Dogma: mRNA translation in space and time

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SUMMARY

As our understanding of the cell interior has grown, we have come to appreciate that most cellular operations are localized, that is, they occur at discrete and identifiable locations or domains. These cellular domains contain enzymes, machines, and other components necessary to carry out and regulate these localized operations. Here, we review these features of one such operation: the localization and translation of mRNAs within subcellular compartments observed across cell types and organisms. We describe the conceptual advantages and the "ingredients" and mechanisms of local translation. We focus on the nature and features of localized mRNAs, how they travel and get localized, and how this process is regulated. We also evaluate our current understanding of protein synthesis machines (ribosomes) and their cadre of regulatory elements, that is, the translation factors.

INTRODUCTION

Proposed in the late 1950s, the Central Dogma of molecular biology first introduced a framework for how information is thought to flow within life.[1,2] It postulated some constraints on where the arrows of causality could point, namely from DNA to RNA to protein; with some proposed bidirectionality between RNA and DNA, but never from protein backward. These hand-drawn, one-dimensional arrows representing the conceptual flow of information (Figure 1) have since been expanded by a spatial dimension by asking where DNA, RNA, and proteins are within a three-dimensional cell at any given time (Figure 1). Whereas at the time of its conception, it was not certain whether RNA (messenger RNA that is) might even be a meaningful intermediate step between DNA and protein, this is now clear. This of course begs the question of why life introduced an mRNA intermediate and why it would need to be localized. Within this review, we will point out some of the key conceptual advantages of local translation as well as the essential components and some of their interesting adaptations in subcellular environments.

So, what do we mean by "local" translation and what would be the opposite? Strictly speaking, the opposite of local translation would be an entirely even distribution of mRNAs and protein products across the entire cell. As we know, however, cells possess compartments such as the nucleus, the endoplasmic reticulum (ER), mitochondria, etc. As not all proteins are present in all these compartments, it is perhaps not surprising that their corresponding mRNAs and translation follow this pattern. Furthermore, the definition of local is, of course, a function of resolution. The closer we look, the more heterogeneity we find. As an example, recent studies have uncovered the widespread co-localization of related transcripts into associated polysomes or the so-called translation factories harboring several related mRNAs.[3–8] This level of organization would have appeared entirely homogeneous only a few years ago.

The ingredients needed to enable local translation encompass all the parts needed for translation in general: a message (mRNA), a translator (the ribosome), its regulators (translation factors, RNA-binding proteins, etc.), and the individual decoders (tRNAs) (reviewed in Shirokikh and Preiss[9]). In order to impose a specific location, we also need a cellular delivery system for all of these components (the cytoskeleton, motors, and transport granules), a way to retain them once they arrive at their destination (molecular tethers) (reviewed in Mogre et al.[10]), and finally an understanding of how they interact and regulate one another. We will now introduce these components, discuss some of the systems they are most prominent in, and try to emphasize some of the big missing pieces along the way.

ADVANTAGES OF LOCAL TRANSLATION

Most locally synthesized proteins are made close to their site of action in the cell. As such, the main advantage of local synthesis is that it creates a point source of high protein concentration where it is needed, thus avoiding the expense of expressing the protein throughout the cell, when it is only needed in a small compartment. For example, during *Drosophila* development, local protein expression of several genes patterns the axial development of the embryo (see Lasko[11] for review). Indeed, in the developing embryo, ectopic or overexpression of one of these genes leads to malformation of the organism.[12] The local production of the protein also circumvents the problems associated with addressing it to the appropriate cellular destination and thus also minimizes unwanted cellular interactions that might occur *en route*. In less common scenarios, cellular signaling

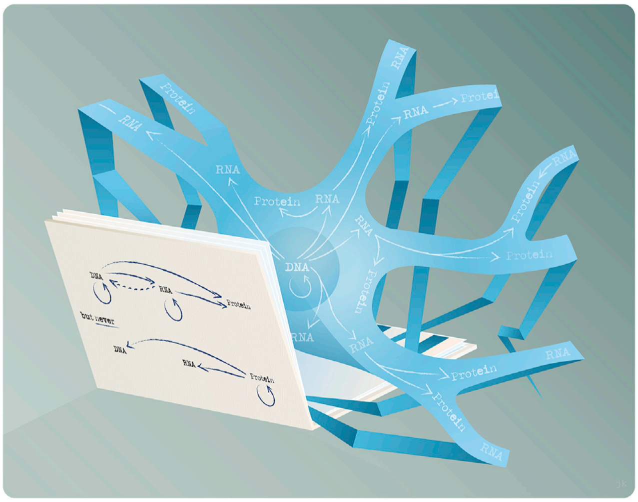

Figure 1. The Central Dogma decentralized

An originally unpublished outline sketch by Francis Crick in 1956[2] depicting a proposed flow of information in biology, represented by solid and broken arrows.

It indicates that information can flow from DNA to RNA to protein, that both DNA and RNA can likely replicate themselves, and proposes that direct DNA to protein and RNA to DNA transfer seems likely although unproven. Boldly it considered the reverse, a flow from protein back to any other form as impossible. The concept of localized translation expands this one-dimensional view by assigning spatial coordinates to each of these macromolecules within the cell, depicted via a folded paper version of a neuron. While DNA is constrained to the nucleus, both RNA and proteins can exist throughout the cell, but the unidirectional sequence of events remains unchanged as indicated by the very same arrows. Artwork by Julia Kuhl.

can drive the local synthesis of a protein that can then travel elsewhere in the cell in a signaling capacity. For example, for some transcription factor mRNAs detected in neurons, the activity-dependent local synthesis of an immediate early gene mRNA can serve as a synapse-to-nucleus signal (e.g., *NPAS4*[13]). Another benefit of local translation concerns the associated cost of molecular trafficking of either mRNA or protein. The mRNAs and proteins that function in specific regions of the cell need to reach these subcellular compartments, which usually involves energy-dependent transport mechanisms (see below). Because an mRNA can serve as a template for upwards of 1,000 protein copies (measured in *E. coli*[14]), there is an additional multiplicative energy benefit of transporting an mRNA vs. a protein. A third advantage is temporal control and speed, that is, the ability to respond quickly to modulate the proteome. Note that local cellular signaling events that alter the translation of proteins at central locations require signaling to the site of protein synthesis and then delivery of the protein product back to the appropriate subcellular compartment. In some cells, such as neurons, the site of protein delivery can be hundreds of microns from central, somatically associated ribosomes. Indeed, several rapidly induced forms of synaptic plasticity make use of local translation.[15–17] When cellular signaling events are coupled to local translation machinery, then nascent proteins can, in principle, be delivered within minutes. Studies using fast-folding fluorescent reporters[18] or SunTag reporters enabled the rapid detection of nascent proteins.[19] In addition, localized translation can be used to favor the co-translational assembly of multi-protein complexes (see Morales-Polanco[20] for a recent review).

INGREDIENTS AND MECHANISMS FOR LOCAL TRANSLATION

The message and delivering it: mRNAs

The localization of mRNAs within the cytoplasm of cells has a long and rich history. A constellation of studies in the 1980s noted asymmetries in the localization of poly(A) mRNA and some individual mRNAs such as actin in developing sea squirts[21] and *Xenopus* eggs.[22] These early studies suggested that the localization of mRNAs within cells is important for driving the polarized development of the organism. This concept has been beautifully expanded upon in subsequent studies. For example, in the developing *Drosophila* oocyte, the localization of *bicoid/gurken* and *oskar* mRNAs to the anterior and posterior poles, respectively, is required for the ensuing polarized development of the embryo (see Martin and Ephrussi[23] for review). In an entirely different setting, the dendritic localization of the *Camk2a* mRNA[24] is required for plasticity and learning in both flies and rodents.[25,26] Outside of these specific contexts, the inhomogeneous distribution of individual mRNAs has been observed in a variety of cell types and organisms (see Martin and Ephrussi[23] and Das[27] for reviews).

From the many studies and reviews cited above, it seems reasonable to conclude that mRNA localization and local translation are likely universal features of cells. Indeed, as our resolution of the interior of cells becomes increasingly refined with both imaging and sequencing, the number of mRNAs with heterogeneous cellular localization patterns is increasing. For example, the development of high-resolution *in situ* hybridization methods led to stunning images, showing distinct mRNA patterns for 70% of transcripts imaged at a given developmental stage of *Drosophila* embryogenesis.[28] Also in neuronal dendrites, high-resolution images of >100 transcripts revealed many distinct mRNA localization patterns as a function of distance from the cell body.[29] Advances enabling sequential hybridization of multiple probes[30,31] in the same sample promise to further refine our understanding of spatial mRNA architectures. For example, applying these methods to neurons has enabled the detection of dendritic mRNAs that exhibit different proximal-distal gradient patterns as a function of distance from the cell body.[32]

Sequencing studies, often beginning with enriched subcellular fractions, have further expanded the localized mRNA repertoire. Because of their highly polarized morphology, neurons have often been exploited for these types of studies. For example, in developing and mature neurons, next generation RNA sequencing (RNA-seq) of isolated dendrites or axons has detected ~2,000–4,800 localized mRNAs in axons and dendrites. The precise number of localized mRNAs depends on the developmental stage, species, and specific compartment examined.[33–36] Studies using ribosome profiling of the same subcellular fractions have shown that the fraction of mRNAs that are translated also depends on the developmental stage and the cellular context.[33–35] For example, in developing (P5) retinal ganglion neurons, ~2,000 axonal mRNAs are translated, whereas ~1,000 mRNAs are translated in adult axons.[34] In mature

hippocampal neurons by contrast, virtually all neuropil (axon + dendrite) mRNAs that are detected are also translated to varying degrees, with over 800 mRNAs exhibiting more translation in the neuropil than in the somata.[33] Going even finer, laser-capture microdissection to isolate subcellular compartments has enabled a comparison of the mRNAs resident in individual cell bodies to those present in the dendritic arbor.[37] One can expect further refinement of subcellular mRNA populations via isolation of even smaller compartments or by the use of proximity-labeling methods, where a subcellular compartment of interest can be endowed with enzymatic activity to label neighboring molecules (e.g., Halo-Seq[38] and APEX-seq[39]). For example, APEX-seq was recently used to identify the mRNAs associated with the nucleus, nucleolus, ER membrane, mitochondrial matrix, and several other compartments. For >3,200 mRNAs, a significant enrichment in at least one compartment was observed.[39]

The nucleotide sequence of mRNAs contains the region that codes for a protein (coding sequence) flanked by regulatory sequences on either end, the 5′ and 3′ untranslated regions (UTRs). The 5′ and 3′ UTRs contain nucleotide sequences (sometimes known as "zip codes") that interact with RNA-binding proteins (RBPs) and other RNA molecules (such as microRNAs [miRNAs]) that can influence the location (see below), translation, and stability of the mRNA (see Martin and Ephrussi[23] and Mayr[40] for reviews). The number of RBPs (e.g., Castello et al.[41]) and the combinatorial nature of their regulation of a given mRNA are exciting areas that will benefit from new methods (see Hentze et al.[42] for recent review). One common feature that has emerged from many RNA-seq experiments of localized mRNAs is an expansion of the 3′ UTR, accomplished by alternative polyadenylation site selection[43–45] (Figure 2A). For example, in *Drosophila*, a set of developmental regulatory genes undergo lengthening of their 3′ UTRs during embryogenesis; these genes are selectively expressed in brain tissue.[44] In neurons, localized axonal and dendritic mRNAs contain the longest 3′ UTRs recorded,[45] which presumably expand the potential for regulation by miRNAs and other RBPs.[46] Indeed, the shortening of some 3′ UTRs is important for axonal integrity[47] and associated with plasticity.[45] In addition, while most studies have focused on motifs in the 3′ UTR, the 5′ UTR also possesses ample regulatory potential, perhaps most prominently the presence of upstream open reading frames (uORFs). uORFs can regulate the translation of the main ORF, often in response to cellular signaling events (e.g., Starck et al.[48]) (Figure 2A). Finally, regions within the coding sequence and even retained introns[49,50] have also been implicated in RNA localization.

Deliberate, directed transport of mRNA for local translation

Localized mRNAs reach their target destinations through various transport mechanisms including diffusion coupled with local anchoring (i.e., "diffusion and entrapment"),[51] restricted diffusion (e.g., microtubule lattice diffusion),[52–54] directed transport by motor proteins, and cargo "hitchhiking" on organelles (see Das[55] for review). It was initially thought that all localized mRNAs are transported in a translationally repressed state until they reach their functional sites[56,57]; however, recent findings of co-translational mRNA targeting from single-molecule and real-time translation imaging studies are beginning to challenge this long-standing notion. Co-translational targeting, which generally requires translation to produce a targeting signal in the nascent polypeptide, has emerged as a widespread mechanism to localize mRNAs to specific subcellular compartments and organelles.[5] Transport of the mRNA-ribosome-nascent chain complex is typically associated with translational arrest, as in the classic example of signal recognition particle (SRP)-mediated targeting of mRNAs encoding membrane and secretory proteins to the ER.[58,59] Apart from the ER, co-translational targeting has been reported at many other organelles (e.g., mitochondria,[60,61] peroxisomes,[62] endosomes,[5,63] the nucleus [nuclear envelope],[5,64] chloroplasts,[65] centrosomes[5,66–68]) and, in some instances, involves simultaneous mRNA transport and translation.[8,59,66,69] For further discussion of this topic, see Müntjes et al.[70] and Béthune et al.[71] for recent reviews.

In eukaryotes, upon nuclear export to the cytoplasm, the movement of most localized mRNAs shifts from passive diffusion[72,73] to active (ATP-dependent), motor-driven transport in ribonucleoprotein (RNP) granules[74,75]—membraneless condensates of mRNAs and RBPs.[76–80] Among the many types of RNP granules, both germ granules (in oocytes/embryos; for review in *Drosophila*, see Trcek and Lehmann[81]) and RNP transport granules (for review in neurons, see Fernandopulle et al.[82]) have a primary function in mRNA localization, and will hereafter be collectively referred to as "RNP granules" for simplicity. Other granules important in mRNA metabolism are stress granules (SGs) and processing bodies (P-bodies), which are generally associated with cell stress and storage of translationally repressed mRNA.[83] Inclusion of particular translation initiation factors and mRNA decay factors typify SGs and P-bodies, respectively; however, their exact composition and function remain unsettled. The dynamic association of RBPs with mRNA begins during transcription in the nucleus to regulate 5′ capping, splicing, polyadenylation, and export and continues in the cytoplasm to regulate mRNA transport, translation, and decay (primarily via 3′ UTRs, see above). The complement of bound RBPs is thus at least partially a vestige of the pre-mRNA processing that occurred in the nucleus (reviewed in Singh et al.[84]). The selection and binding affinity of associated RBPs depends on the sequence, secondary structure, and redundancy of *cis*-acting RNA elements. Given that certain RBPs can bind either directly to motor proteins or indirectly through motor protein cargo adaptors (Figure 2C), the repertoire of RBPs in a given RNP granule can influence the directionality and transport efficiency of the accompanying mRNA. Furthermore, RBPs with microtubule- or actin-binding capacity may facilitate filament track binding and thereby activation of motor proteins.[52,85]

Microtubule-based transport of mRNAs in RNP granules

The role of RBPs as mRNA-cytoskeletal machinery adapters is an elegant and evolutionarily conserved strategy for targeted and specific localization, yet it is highly contextual. The RBPs and transport machinery involved in the trafficking of a given mRNA can vary dramatically based on the cell type (e.g., central neurons vs. sensory neurons), developmental stage, repertoire of available RBPs, and target mRNA abundance. Since each mRNA can bind to multiple RBPs, and individual RBPs associate

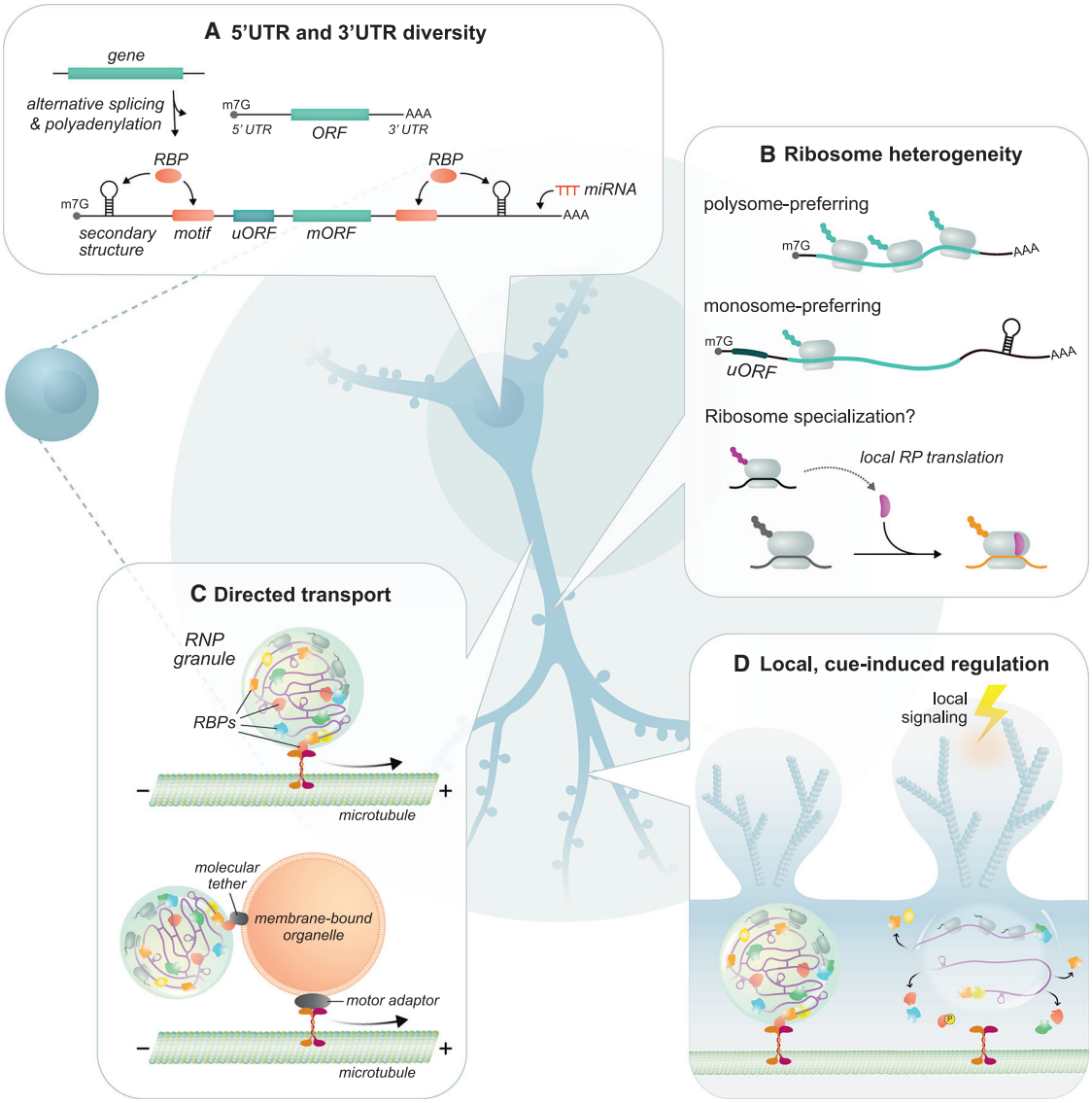

Figure 2. From yeast to neurons: Evolutionarily conserved mechanisms for regulating where, when, and how mRNAs are translated

A general depiction of select principles of mRNA localization and translation shared between a spherical cell (e.g., yeast cell; magnified in the background) and a more polarized cell (e.g., neuron; in the foreground).

(A) Alternative splicing and polyadenylation of pre-mRNAs generate mRNA isoforms that differ in their 5′ and 3′ UTRs. Dynamic interactions between isoform-specific *cis*-acting elements (e.g., sequence motifs, secondary structure) and *trans*-acting factors (e.g., RNA-binding proteins [RBPs], microRNAs [miRNAs]) lead to diverse regulatory control mechanisms affecting the localization, stability, and translation efficiency of the same mRNA.

(B) Heterogeneous ribosomes preferentially translate specific mRNAs. For example, mRNAs translated by monosomes typically contain an upstream ORF (uORF) and encode for low-abundance regulatory proteins in yeast and high-abundance synaptic proteins in neurons. On-site remodeling is hypothesized to endow ribosomes with the ability to translate specific mRNAs in response to external stimuli.

(C) Motor-driven transport of RNP granules on microtubules (and actin filaments—not depicted) occur through dynamic interactions between RBPs and the cytoskeletal machinery or by RNP hitchhiking on membrane-bound organelles such as endosomes.

(D) Local, cue-induced modification (e.g., phosphorylation) of RBPs regulate RNP anchoring at translation sites and unmasking of mRNAs, likely by altering RNP condensate properties.

Artwork by Julia Kuhl.

with overlapping subsets of mRNAs (via recognition of the same and/or distinct *cis*-acting elements with different binding affinities), this creates a competitive/cooperative interplay in RBP-mRNA binding that is shaped by the relative abundance of both RBPs and cognate mRNAs. In the case of β-actin mRNA, over 60 associated RBPs have been identified,[86] and the RBPs ZBP1 and HuD recognize distinct features in overlapping sites in the 3′ UTR in a competitive and mutually exclusive manner.[87] Much less is known about cooperative RBP binding; however, this can now be addressed by a new single-molecule approach called TRIBE-STAMP that can identify individual target mRNAs simultaneously bound by two RBPs.[88]

The need for RBP-mediated regulatory control of polarized subcellular mRNA localization is perhaps best exemplified in vertebrate neurons where axonal microtubules are unipolar (plus-end-out) and dendritic microtubules have a mixed orientation.[89] In vertebrate neurons, subcellular targeting of β-actin mRNA is critical for dendritic arborization and axon outgrowth/branching during development[90–94] as well as dendritic spine plasticity.[95] The mechanism of β-actin mRNA transport within axons vs. dendrites seems to be regulated by the developmental stage[96] and association with specific RBPs.[97,98] For example, the primary transport mode of β-actin mRNA in axon shafts may change from directed, motor-driven transport during early development[99] to slower, subdiffusive motion.[100] New developments in single-molecule localization microscopy[101] and RNA mobility assays[102,103] will allow the dissection of interaction modes between RBPs and transport machinery.

Actin-based transport of mRNAs in RNP granules

It is generally understood that long-range and short-range directed transport involves the microtubule and actin cytoskeleton, respectively. In cases where the traversed distance is relatively short (≤15 μm), mRNA transport seems to depend solely on actin filaments. Consider the three fungal species: the budding yeast *Saccharomyces cerevisiae*, and the two pathogens *Candida albicans* and *Ustilago maydis* that undergo yeast-to-hyphal transitions during pathogenesis. During *S. cerevisiae* budding, actin-dependent transport is required for asymmetric localization of ∼30 mRNAs (e.g., *ASH1* mRNA). Despite the rapid switch to hyphal growth during infection, the transport of 40 mRNAs to hyphal tips in *C. albicans* is not dependent on microtubules but rather the same actomyosin-based transport system found in *S. cerevisiae*;[104] however, the maximal distance between the hyphal tip and proximal nucleus is only 15 μm. This is in stark contrast to hyphal tip growth in *U. maydis*, which can be ∼50 μm from the closest nucleus.[104] Here, mRNA transport occurs on microtubules by hitchhiking on endosomes[105]; more on this discussed below.

In many cases, both cytoskeletal networks are required for spatially precise cargo delivery. If cytoskeletal filaments are interconnected cellular roadways, one can think of microtubules as the highways and actin as the side streets – both are needed to arrive at a faraway destination. This can be demonstrated in filamentous fungi[106] and the classic examples in *Drosophila*[107] and *Xenopus* oocytes[108] and in mammalian neurons[109] where actin-dependent mRNA anchoring occurs after microtubule-based transport. Classic exceptions are the apical anchoring of mRNA in *Drosophila* embryos, where microtubules, but not actin, are required,[110] and β-actin mRNA localization in fibroblasts, where both cytoskeletal networks are involved in transport and anchoring.[85,111] In neurons and filamentous fungi, actin and microtubule networks interact and are tightly coupled at cell tips to coordinate polarized growth.[112,113] Cytoskeletal track switching occurs during the trafficking, polarized sorting, and remodeling of membrane organelles[114–117]; however, less is known about the mechanisms underlying cytoskeletal crosstalk during the sorting, subcellular targeting, and anchoring of RNPs.

For navigating complex cellular terrains such as mixed-polarity microtubules in proximal dendrites,[89] bidirectional transport of membrane organelles is often described as a "tug-of-war" between plus-end directed kinesin and minus-end directed dynein motors.[118] To what extent membraneless RNPs are associated with multiple motor types is less clear. One study demonstrated that the cargo adaptor huntingtin (HTT) mediates dendritic transport of β-actin mRNA as an RNP complex containing RBPs, kinesin-1, and dynein.[119] Here, phosphorylation of Htt acts as a molecular switch between anterograde (kinesin-1-mediated) and retrograde (dynein-mediated) vesicular transport,[120] but whether this also affects RNP transport remains to be determined. More recently, it was shown that TDP-43 cooperates with two other RBPs, FMRP and Staufen1, to regulate the anterograde (KIF5-mediated) and retrograde (dynein-mediated) transport, respectively, of Rac1 RNPs in dendrites.[121] Since it is generally thought that dynein mediates selective transport into dendrites,[122–124] continued study of dynein-mediated neuronal RNP transport will help to understand not only bidirectional movement but also axon-dendrite sorting.

RNP tethering on organelles

In addition to directed transport facilitated by RBP-cytoskeletal machinery interactions, microtubule- and actin-based transport can occur through RNP tethering or hitchhiking on various membrane-enclosed organelles such as the ER,[125,126] mitochondria,[61,127] and endosomes (see Müntjes et al.,[70] Béthune et al.,[71] and Vargas et al.[128] for recent reviews). Cargo hitchhiking is an economical mode of transport as it reduces energy expenditure and occupancy of transport machinery. Notably, organelles of the endocytic pathway are common vehicles for RNP hitchhiking in filamentous fungi[129] and neurons[63,69,130,131] (Figure 2C). Why endosomes are commonly used as vehicles may be due to their high motility, activity-dependent trafficking,[132,133] capacity for long-distance, bidirectional movement on both microtubule and actin networks, and their role as platforms for integration of signaling pathways. Furthermore, endosomes are central components of the tip-growth machinery, coordinating endocytosis and exocytosis to drive rapid and polarized growth of cell tips. They also serve as platforms for local translation. In *U. maydis*, septin *cdc3* mRNA translation and filament assembly take place on Rab5a-positive early endosomes (EEs) for subsequent delivery to growth poles.[129] Similarly, in axons of *Xenopus* retinal ganglion cells (RGCs), mRNAs encoding mitochondrial proteins are translated on Rab7a late endosomes (LEs) to sustain mitochondrial function[69]; however, how RNPs are tethered to LEs remains unknown. Mechanistic insight comes from other vertebrate models of neuronal RNP transport, where the lipid-binding protein annexin A11[130] and a novel Rab effector complex[131] serve as molecular tethers onto lysosomes and EEs, respectively.

Regulating mRNA localization and translation through phase separation

Cytosolic RNP granules are heterogeneous in size, function, and biophysical properties. The regulation of mRNA condensation through liquid-liquid phase separation (LLPS) has emerged as an important phenomenon underlying RNP granule assembly and function (see Tauber et al.[134] for review). RBPs with prion-like domains and other intrinsically disordered regions (e.g., FUS,[135,136] hnRNPA1,[137] TIA-1,[138] TDP-43,[139] and FMRP[139]) function as scaffold proteins that drive LLPS. Mounting evidence

suggests that dynamic compartmentalization through phase separation may be advantageous for long-distance mRNA transport as well as serve as a regulatory mechanism for activity-dependent translation. During *Drosophila* embryonic development, the RBPs with prion-like domains Bruno and Hrp48 phase separate with *oskar* mRNA into liquid-like condensates that rapidly mature into a solid state, and preventing this liquid-solid phase transition impairs *oskar* mRNA localization.[77] Similarly, post-translational modifications (e.g., phosphorylation) of scaffolds can trigger RNP granule disassembly and this is thought to "unmask" mRNAs for activity-dependent translation[139,140] (Figure 2D).

Transport of translation machinery

The composition of RNP granules is best characterized in neurons, where a large diversity exists in the identity of the mRNA(s) and RBPs present. Evidence from many studies points to directed transport in RNP granules as a primary means of localizing translational machinery to the cell periphery. Several studies demonstrate enrichment of initiation factors as well as ribosomal RNA (rRNA), ribosomal proteins (RPs), individual subunits, and entire ribosomes in neuronal RNPs,[56,74,141–147] although there are reports where ribosomal machinery components are few (e.g., a few RPs)[148] or excluded entirely.[76,149,150] The presence of ribosomes may be characteristic for only subsets of RNP granules (e.g., TDP-43- and FMRP-positive RNPs),[151,152] and may even vary based on the RBP isoform present (e.g., ribosome-free vs. ribosome-containing Stau2-positive RNP granules).[153,154] The presence of ribosomes does not preclude translational repression of mRNAs during transport. Mounting structural evidence suggests that ribosomes in RNP granules are stalled on mRNAs in a pre-translocation state[141,146]. Since translation initiation is a rate-limiting step, this is an attractive strategy for rapid mRNA/ribosome "unmasking" for cue-induced translation at discrete sites[140,155] (Figure 2D).

Apart from transport in RNP granules, alternative mechanisms to localize components of the translation machinery may include: (1) non-granule-associated directed transport; (2) via exosome delivery from neighboring cells[156]; (3) association with mobile ER-derived "ribosome-associated vesicles" (RAVs),[157] and (4) the trafficking of translation machinery-encoding mRNAs instead of the proteins themselves[152,158] (see below). For example, in adult *C. elegans* mechanosensory neurons, polysomes and monosomes outside of RNA granules localize to presynaptic terminals within an hour after axon injury for local protein synthesis-mediated regrowth.[159] While this accumulation of ribosomes was shown to be dependent on microtubules and regulated by a kinesin-1 cargo adaptor, it remains unclear whether the ribosomes interface directly with the microtubule transport machinery or if other proteins mediate binding.

Core translation machinery: Ribosomes

How are ribosomes localized in cells? In some cases, ribosome localization arises simply from the passive exclusion from membrane-bound or membraneless compartments[160] or the tethering of the ribosome by virtue of interactions of its nascent peptide chain.[161,162] In bacteria, for example, both ribosomes and a fraction of the cellular tRNA pool (that are perhaps ribosome-associated) are somewhat excluded from the nucleoid.[163–167] This localization appears to be dependent on active transcription though,[168] indicating that already in prokaryotes, ribosome localization involves active processes.

But are there systems where ribosomes are actively transported and retained to remote outposts to provide a local reservoir for translation? Yes, and cells with obvious polarity have been the front-runners in localized ribosome detection. For example, one hot-spot for the detection of localized ribosomes is the leading edge of migrating cells, including neuronal growth cones (more on this below). Co-localization of core ribosome components with components of the cell adhesion machinery have been observed repeatedly[169–171] and can be dependent on the type of substrate,[169,171] an intact actin cytoskeleton, and enacted force.[169] Attachment to the correct substrate has widespread and drastic effects on the cell's translatome.[172,173] The recruitment of the core translation machinery to these transient cell adhesion sites might be mediated through the multifaceted ribosomal protein RACK1,[170,174–176] likely via its integrin-binding site.[169,177] Interestingly, RACK1 is a peripheral component of the ribosome, exhibiting one of the highest exchange rates.[178] RACK1 dynamics may therefore contribute to ribosome heterogeneity and the tuning of translation.[179–181] For example, it could be that some ribosomes are recruited to nascent focal adhesion sites via RACK1, which could lead to the specific translation of a subset of adhesion-relevant mRNAs.

The polarized compartments of neurons, including axons and dendrites, also represent an abundant source of localized ribosomes. One of the earliest known neuronal studies detected ribosomes in electron micrographs of dendritic "synaptic knobs" in primate spinal cord neurons.[182] Twenty years later, Steward and colleagues used electron microscopy (EM) to detect polyribosomes within dendritic spines in the hippocampus.[183] Subsequently, evidence was accumulating for the presence of ribosomes in presynaptic axon terminals,[184,185] confirmed and expanded by more recent studies.[34,186] Serial EM and 3D reconstruction have provided a more quantitative view of ribosome abundance in hippocampal dendrites and detected polyribosomes in about 10% of the postsynaptic spines under control conditions; this fraction rose to 40% after plasticity, suggesting a movement of local ribosomes into synapses.[187] Importantly, these EM studies possess strict criteria for the classification of objects as polyribosomes, including at least three ribosomes in a particular arrangement. Smaller ribosome formats such as monosomes, which carry out a substantial fraction of translation near synapses[188] will thus be missed. A recent study using super-resolution microscopy which can detect both monosomes and polysomes has estimated a much higher ribosome population in dendrites.[189]

In most non-polarized cells, however, it has proved more difficult to identify an explicitly localized population of ribosomes. This difficulty arises because ribosomes are highly abundant and have to translate all of the mRNAs of a cell. It is clearly easier to detect localized mRNAs. One approach, then, is to focus on cells with biased translatomes, where only a small portion of mRNAs account for the majority of nascent proteins, and where many of those transcripts are localized. Muscle cells represent

one such system, where e.g., in cardiac muscle, the most abundant 11 transcripts account for almost 13% of the entire transcriptome.[190] Here, and in skeletal muscle, many major mRNAs are localized to the periodic contractile unit, the so-called sarcomeres.[53,190–193] This localization appears to depend on active transport along the microtubule cytoskeleton and coincides with markers of protein synthesis.[190,194,195] This and other biochemical evidence of rapid incorporation of nascent sarcomeric proteins led to the suggestion of co-translational insertion of nascent muscle fiber components into the sarcomere.[196–198] Correspondingly, ribosomes have also been visualized at these sites both via immunofluorescence or *in situ* hybridization[53,190,194] and by EM,[199] especially in developing muscle or upon forms of injury or myofiber induction. Importantly, this localization does not appear to follow a simple steric exclusion effect of tightly packed muscle fibers but rather depends on an intact microtubule cytoskeleton and the presence of the microtubule motor kinesin-1.[194] A common theme that emerges between these discussed examples of specific ribosome localization at the leading edge, neuronal processes, and the contractile apparatus of muscles, is the cytoskeleton. As discussed below, there is ample visual and biochemical evidence for a strong interplay between the translational apparatus and either F-actin or microtubules,[198,200–203] and these ties go beyond a simple means of transporting and tethering them to the right place at the right time.

More recently, it has become clear that RPs can also selectively be translated at protrusion sites[198] and the basal/apical regions of gut epithelial cells.[204] Similar to muscle, all of these cells are (temporarily) polarized enough to detect an enrichment of total translation. Interestingly, in intestinal epithelium of fasted mice, RP-mRNAs (alongside many other transcripts) are localized opposite of its protein products and consequently exhibit low translation.[204] This picture changes upon re-feeding, where RP-mRNAs are actively relocated to the apical side where the translation machinery is anchored and consequently exhibit an increased translational efficiency. Also, for migrating cells, RP-mRNAs are translated at the leading edge, thus in both cases potentially creating a positive feedback loop of locally synthesized RPs. And although at least in migrating cells, the majority of nascent RPs are shuttled back into the nucleus for increased *de novo* ribosome assembly,[204] it is possible that a fraction of locally synthesized RPs are used to repair or specialize ribosomes.

Ribosome heterogeneity/dynamics

Our understanding of translation regulation has recently expanded to include the possibility of selective translation by "specialized or dynamic ribosomes" (e.g., Shi and Barna[205]). The classic view of the ribosome as a generic, static assembly of four rRNAs and ~80 RPs has been challenged by demonstrations of intra-/intercellular compositional heterogeneity and context-dependent, on-site remodeling in a variety of organisms. Ribosome heterogeneity can manifest through myriad ways including the incorporation/exclusion of RP paralogs,[206,207] differences in core RP stoichiometry,[208] RP post-translational modifications,[209,210] rRNA chemical modifications,[211] rRNA isoforms,[212] and association with distinct ribosome-associated proteins.[213,214] Because we now know that monosomes are key contributors to the local translatome,[188,215] heterogeneity can be further extended to include differential translation by ribosomal subpopulations (monosomes vs. polysomes) (Figure 2B). For example, monosome-enriched mRNAs often encode low-abundance regulatory proteins in yeast[215] and high-abundance synaptic proteins in neurons.[188] In both yeast and neurons, monosome-enriched mRNAs often contain an upstream open reading frame (uORF), the presence of which is generally associated with reduced translation of the downstream ORF.[216] Translation of the uORF or mainORF is regulated during global protein synthesis inhibition following phosphorylation of eukaryotic initiation factors (eIFs) and/or eukaryotic elongation factors (eEFs). Apart from canonical stress signaling pathways (see Costa-Mattioli and Walter[217] for recent review), translation factor phosphorylation is enhanced in neurons during many forms of protein-synthesis-dependent synaptic plasticity through activation of distinct calcium signaling cascades. Even in the absence of evoked/action-potential-driven neural activity, dendritic spines are maintained in a translationally repressed state through miniature-synaptic-event-induced phosphorylation of eEF2.[218,219] While this suggests that global translation inhibition during neural activity enables uORF-controlled translation of proteins important for plasticity, direct evidence for this is lacking.

Almost 50 years ago, radioactive labeling and two-dimensional gel electrophoresis experiments in HeLa cells demonstrated that a subset of RPs can exchange between ribosome-bound and -free states in the cytoplasm.[220] Since then, metabolic labeling and quantitative mass-spectrometry-based proteomics studies have shown context-specific RP exchange in response to changes in nutrient availability in budding yeast[221] and rodent liver tissue[222] as well as for purposes of ribosomal repair in response to chemical damage in *E. coli*[223] or oxidative stress in rodent neurons.[80,178] Furthermore, dynamic RP exchange on mature, translationally competent ribosomes in developing *Xenopus* RGC axons[92] and rodent neurites[178] is fueled by a local supply of newly synthesized RPs. It is thought that on-site remodeling of the ribosome could lead to changes in mRNA selectivity and translational efficiency (Figure 2B), although direct functional evidence of specialized ribosomes remains elusive. For example, yeast ribosomes containing specific RP paralogs necessary for respiratory growth confer more efficient translation of mitochondrial proteins[224]; yet, it is unclear as to whether these paralog-specific ribosomes form independently of the nucleolus through on-site incorporation of locally synthesized RP paralogs.

Ideally, we would like to know the position, composition, translational status, and mRNA engagement of individual ribosomes. With the recent advent of structural cell biology, specifically *in situ* cryoelectron tomography and subtomogram averaging, the first three of these now seem feasible. In fact, the ribosome appears to represent the ideal target as it (1) is sufficiently large, (2) displays good contrast due to heavy electron scattering, and most of all (3) is highly abundant. This combination of attributes has made the ribosome the current poster child of in-cell structure determination and resulted in record resolutions of up to 3.5 Å.[225–227] This has allowed us to unravel many of its distinct states and compositional differences within the cell.[226,227] While

this has approach has been applied to single-celled organisms with limited polarization so far, it is likely that we will soon see a number of studies examining the spatial arrangement and translational states of ribosomes in many of the local translation systems discussed here. Over the years, numerous biochemical and molecular biology studies have uncovered important aspects concerning ribosome occupancy,[228] preferential translation on monosomes vs. polysomes,[188] or ribosomal stalling,[229] and it will be a tremendous step forward to finally visualize these with extraordinary spatial precision directly within cells.

Translation factors and other regulatory factors

Besides the presence of ribosomes, other auxiliary factors are required for successful (local) translation. These translation factors (TFs) are broadly divided into the three stages of translation they participate in: initiation factors, elongation factors, and termination factors. Besides being essential for the process to function, they also heavily modulate all aspects of translation such as fidelity, initiation/elongation speed, resolution of roadblocks, and many others.[230] Similarly to ribosomes, TFs can be viewed as part of the underlying translational infrastructure of the cell and hence need to be available wherever even a single mRNA is to be translated. Indeed, the cellular levels of these factors seem to reflect this: elongation factors are among the most abundant proteins within the cell, reaching up to 2–5× the levels of ribosomes and 40–100× that of all mRNAs in *S. cerevisiae*.[231] Perhaps unsurprisingly then, at least in yeast, most TFs appear to be homogeneously distributed throughout the cytoplasm.[232] There are, however, some notable exceptions such as eIF2 and eIF2B, which are found in specific granules from yeast[232] to mammals,[233] or eEF1A at the cytoskeleton (more below).[234] As with other components of the translational machinery, this changes under various forms of cellular stress, where many translation factors are relocated to a variety of storage and/or degradation granules.[235]

A recurring theme in TF localization, and the entire translation machinery for that matter, is an association with the cytoskeleton.[236] Aside from its canonical function of delivering aminoacyl-tRNAs to the elongating ribosome, the elongation factor eEF1A has a well-established moonlighting function as the regulator of the actin and microtubule cytoskeleton.[234,237–239] This role is mutually exclusive with its canonical role in elongation.[240] The regulatory relationship is reciprocal, as when the actin cytoskeleton is disrupted (e.g., via eEF1A mutation), translation is inhibited at least partially via phosphorylation of the initiation factor eIF2α[241,242]—a common hub for translational modulation.[243] Numerous other TFs have been found in association with the cytoskeleton over the years,[236,244] including several eEF1B subunits[245,246] and different components of the eIF3 complex, which alternatively interact with F-actin,[247] microtubules,[248] and keratin. TFs can also be found at cytoskeleton-rich structures such as centrosomes,[5] focal adhesions,[249] or protrusions more broadly.[171,250] eEF1A, alone or when attached to F-actin at the leading edge, can also bind and tether β-actin mRNA, and can be released by addition of competing peptides both *in vitro* and *in vivo*.[251]

In the context of neurons, activities such as memory consolidation via synaptic plasticity critically depend on both structural changes via a re-modeled cytoskeleton[252] and local translation of effector proteins.[16] The two are undoubtedly connected, as induction of long-term potentiation (LTP) leads to an F-actin-dependent influx of RNPs[109,253] and TFs such as the cap-binding protein eIF4E into spines.[254,255] Similar to previously discussed examples of RPs, the mRNA of eIF4E is also located in dendrites[33] and locally recruited to synapses,[256] thus likely providing a fresh supply of new translation factors via local translation itself. At the synapse, eIF4E can boost translation in response to synaptic activity via relieved repression by FMRP-CYFIP1,[257] and pharmacological inhibition of eIF4F complex assembly impairs memory formation in certain learning paradigms[9]—a deficit shared with eIF2α impairment.[258] During translation initiation, eIF4E as part of the eIF4F pre-initiation complex is responsible for 5′-cap binding and thus recruitment of mRNA.[9] On the other end of the reaction, the multimeric eIF3 complex as part of the 43S pre-initiation complex brings along the 40S small ribosomal subunit and initiator Met-tRNA.[9] Not surprisingly then, also eIF3 subunits have been credited with several crucial functions in neurons, including dendritic pruning of developing *Drosophila* sensory neurons[259] and modulation of neuronal excitability in *C. elegans* motor neurons.[260] On the whole, most if not all translation factors have been identified in neurites and synapses to various degrees,[261,262] and mis-regulation of many of them is evident in diverse forms of neurodegenerative diseases.[263]

CONCLUDING REMARKS

The original depiction of the Central Dogma (Figure 1) concerned the unidirectional flow of genetic information from DNA to RNA to protein. In this review, we have examined how the latter part of this flowchart is distributed within cells to enable rapid translational responses to signals and economize on trafficking demands. We have noted that as technology has increased our resolution of the cell interior, we have come to appreciate that the localization of mRNAs and protein synthesis machines in subcellular compartments is ubiquitous across cells and organisms. We have highlighted the features of the elements needed for local translation, how they move to different locations within cells and how translation is carried out and regulated by its components. Although as originally formulated, the Central Dogma did not make any statements on the spatial relationships of its components, we believe that our ever-increasing knowledge about functionally specialized micro- and nano-domains within cells has shown how mRNA localization and translation is both needed and exploited. We imagine that the coming years will bring an even more refined view of what we can call ‘‘local’’ and provide much needed information on the temporal dynamics/stasis of these local translation domains.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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