Intracellular RNA sorting, transport and localization

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RNA localization and translational control are crucial for cellular fine-tuning of gene expression in space and time. A recent meeting in Tucson, Arizona pointed out mechanisms conserved across different species and cell types that contribute to the establishment of cell polarity and cell migration. Furthermore, it is becoming increasingly clear that these post-transcriptional control processes are relevant for various diseases.

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The spatial architecture of eukaryotic cells relies on an asymmetric distribution of proteins and protein complexes. This differential distribution is partly achieved through the localization of messenger RNAs. Research over the past 20 years has shown that mRNA localization is a highly interconnected multistep process, linking RNA biogenesis, nuclear export, cytoplasmic RNA transport, translation and decay[1]. During the 6th Federal Association of Experimental Biologists (FASEB) Summer Research Conference, "Intracellular RNA Sorting, Transport and Localization," held in Tucson, Arizona, USA*, it became clear that to elucidate its underlying molecular mechanism(s), RNA localization should be studied in a number of model systems, ranging from the 'simple' unicellular yeast to complex systems such as multicellular embryos or nerve cells.

The international meeting was superbly organized by Kim Mowry (Brown University) and Paul Macdonald (University of Texas at Austin) with generous financial support from FASEB, the Fragile X Research Foundation, the US National Institutes of Health and Ambion, Inc. It was held at a time when the field of RNA localization is entering a new phase. About 25 years ago, the initial observation was made that polyribosomes are present in postsynaptic compartments[2]. Later, specific dendritically targeted RNAs[3] were discovered, suggesting that they might indeed be translated locally at synapses. Since then, research has been dominated by two model systems, *Drosophila melanogaster* oocytes and *Xenopus laevis* embryos, in which a substantial number of molecules in various steps of RNA localization have now been identified and implicated in the localization process. Future research will now have to increasingly pursue more holistic (*in vivo*) approaches to assign functions to the identified key players and to look for conserved mechanisms in different systems and organisms.

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Simple model organisms for RNA localization

To understand the mechanism of mRNA localization, genetically simple model systems such as yeast are very attractive, and mRNA localization has been studied in great detail in this organism. Today, more than 20 mRNAs that localize to the bud tip of *Saccharomyces cerevisiae* have been identified. Their localization is mediated by the type V myosin motor protein Myo4p and the She proteins, the core machinery for RNA localization in yeast[4]. Jeffrey Gerst (Weizmann Institute of Science) presented new evidence that this number might still be an underestimate. He identified many additional mRNAs localized to this region of the yeast, all of which encode well-known proteins involved in the establishment of polarity or polarized secretion. As is the case with the previously identified transcripts, localization of these mRNAs depends on the She protein machinery. But surprisingly, some of the newly identified mRNAs localized independently of the She proteins during pheromone-induced polarized growth (schmooing). These new data suggest that mRNA localization in budding yeast might be more prevalent than anticipated and that mRNA localization and spatially controlled translation might have a similarly important role in the establishment of polarity as in *Drosophila* embryos.

Ralf-Peter Jansen (University of Munich) presented yet another connection between polarized secretion and mRNA localization: ribonucleoprotein particles (RNPs) containing localized mRNAs and the RNA-binding protein She2p associate with tubular endoplasmic reticulum structures that segregate into the bud. This suggests that RNA localization and at least the initial steps of protein secretion might be intimately linked.

Michael Feldbrügge (Max Planck Institute for Terrestrial Microbiology) presented an attractive new model system for the study of RNA localization. When searching for the functions of RNA-binding proteins in the plant pathogenic fungus *Ustilago maydis*, he identified a protein that might serve as a carrier of yet unknown localized mRNAs to the growing tip of this fungus. As loss of the RNA-binding ability of this protein results in shortened filaments and severely reduced pathogenicity, this might represent the first example of a role for RNA localization in plant disease.

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Dominant models: *Drosophila* and *Xenopus*

Embryos and oocytes have been the classical model systems to study mRNA localization in both *Drosophila* and *Xenopus*. Bruce Schnapp (Oregon Health & Science University) and Daniel St Johnson (Wellcome Trust, University of Cambridge, Gurdon Institute) teamed up to elucidate the function of the *Drosophila* insulin-like growth factor II mRNA-binding protein (IMP), a homolog of the vertebrate IMPs, chicken zipcode-binding protein (ZBP1) and *Xenopus Vg1* RNA–binding protein. IMP colocalizes with *oskar* mRNA at the posterior pole of the *Drosophila* oocyte. Bruce Schnapp presented evidence that point mutations in IMP-binding elements that repetitively occur in the *oskar* 3′ untranslated region (UTR) abolish the posterior localization of IMP and block translational activation of *oskar* mRNA. Surprisingly, however, in $imp^{-/-}$ mutants, *oskar* mRNA localizes and translates normally. This shows that the IMP-binding elements are required for translation and anchoring of *oskar* mRNA, but that either the IMP protein does not contribute to these processes or its role is dispensable or redundant. Crucially, these results urge caution when inferring the function of RNA-binding proteins from the effect of mutation in their binding sites.

St Johnston showed that a specialized population of follicle cells, known as border cells, do not migrate at all in $imp^{-/-}$ mutant egg chambers. This is of particular interest because vertebrate ZBP1 has an important role in the localization of β-actin mRNA to the leading edge of fibroblasts[5] and to growth cones and neurites, processes of developing neurons[6], and it thereby affects cell motility. In *Drosophila*, one of the two cytoplasmic actin isoforms, the actin 42a mRNA, localizes to the guiding protrusions of the border cells, and IMP binds specifically to this RNA. Therefore, it will be interesting to determine whether IMP is involved in the localization of this actin transcript and whether this is the reason why border cells fail to migrate in *imp* mutants. Joel Yisraeli (Hebrew University) showed that expression of a mammalian dominant-negative IMP mutant in either *Xenopus* embryos or mammalian cancer cells strongly inhibits cell movement. These findings further strengthen the idea that involvement of IMP family members in cell migration may actually be broadly conserved in both invertebrates and vertebrates. In addition, they support the proposal of a link with metastasis in cancer cells.

Multiple motors for trafficking

Microtubule-based motor proteins of the kinesin family are important in mRNA localization in oocytes of *Drosophila* and *Xenopus*. In *Xenopus* oocytes, the microtubule-dependent localization of the *Vg1* mRNA, which encodes a TGF-β–like protein, to the vegetal pole of the oocyte has been studied in great detail. Jim Deshler (Boston University) presented data suggesting that kinesin II is the motor responsible for the localization of *Vg1* mRNA[7]. Kim Mowry showed that antibodies to kinesin I co-immunoprecipitate *Vg1* RNA–binding proteins[8] and that rigor mutants of kinesin I act as dominant-negative inhibitors of vegetal RNA localization. These seemingly conflicting observations suggest that there is possibly more than one kinesin involved in RNP trafficking in *Xenopus* oocytes. In *Drosophila* oocytes, the kinesin heavy chain (KHC) of kinesin I, but not the kinesin light chain (KLC), is required for posterior localization of *oskar* mRNA[9]. It was therefore suggested that an unknown mechanism mediates the kinesin-cargo interaction in the oocyte. Isabel Palacios (University of Cambridge) reported that a newly discovered KLC-like protein, Pat1, might be the missing link in this process. Pat1 seems to interact with the KHC and, like *oskar* mRNA, accumulates at the posterior pole in a KHC-dependent manner.

How do localization elements in mRNAs bring about asymmetric distribution of these RNAs? Simon Bullock (MRC Laboratory of Molecular Biology) followed up a model originally proposed by Fusco *et al.* to explain mRNA movement in chicken fibroblasts[10]. On the basis of real-time imaging of apically localized transcripts in *Drosophila* embryos, he proposed that localization signals increase the probability for an mRNA to be picked up by a motor protein complex (in this case dynein) or, alternatively, that these signals regulate the activity of the motors by recruiting accessory factors. Daniel St Johnston showed results of an *in vivo* analysis of *oskar* mRNA localization in *Drosophila* oocytes, whereby *oskar* mRNA–containing particles are transported along microtubules in a bidirectional manner, although the net movement is toward the posterior pole. The direction, however, changes to anterior in mutants that have previously been shown to affect mRNA localization. His results, therefore, suggest that localization factors can cause an asymmetry in the movement of mRNAs, resulting in a net directional transport of localized mRNAs.

Anchoring mRNAs

After transport, localized mRNAs are generally retained at the target site. Whether this requires specific anchoring components or whether it is the net result of constant transport of mRNAs towards the target site has been unclear for a long time. Ilan Davis (Wellcome Trust Centre, University of Edinburgh) presented a third mechanism. Using time-lapse imaging of mRNA localization in *Drosophila* embryos, he analyzed the way in which localized pair rule mRNAs are held at the apical cortex of *Drosophila* blastoderm embryos[11]. Surprisingly, the microtubule motor protein dynein seems to have a function in anchoring that is independent of its transport function. Dynein can apparently undergo a switch from a dynamic motor state to a static anchor state. Whether this attractive model of a bifunctional motor and anchor protein represents a general principle for other dynein cargo or even other motor proteins will require further investigation.

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In *Xenopus* oocytes, other anchoring mechanisms might be used. Anchoring of localized mRNAs like *Vg1* at the oocyte cortex requires cortical microfilaments and cytokeratins, but it also involves noncoding RNAs, the Xlsirts[12]. To what extent these components contribute to anchoring has been unclear. Laurence Etkin (University of Texas M.D. Anderson Cancer Center) presented a surprising link between localized RNAs and cytoskeletal elements. Destruction of either Xlsirts or *VegT* mRNA, an mRNA involved in anchoring of other localized mRNAs, results in a disruption of the cytokeratin meshwork at the vegetal cell cortex and subsequent release of localized mRNAs[13]. These results suggest a structural role for these localized RNAs in the organization of the cytoskeleton.

Keeping translation under control

RNA localization almost invariably goes together with translational control because during transport localized mRNAs must be translationally silenced. Several talks at this meeting focused on the mechanisms that control mRNA translation. Akira Nakamura (RIKEN Center for Developmental Biology) focused on translational repression of *oskar* mRNA in *Drosophila*. *oskar* repression requires two proteins: Bruno, which binds a specific sequence in the *oskar* 3′ UTR, and Cup, which binds the mRNA cap–binding factor eIF4E. The two proteins, although they bind at opposite ends of the mRNA, can interact directly, suggesting that tethering of 5′ and 3′ mRNA ends represses translation[14]. The interaction apparently depends on phosphorylation of Bruno, as hyperphosphorylated Bruno cannot bind Cup and eIF4E. This observation might lead to an understanding of how repression of *oskar* mRNA is relieved upon its arrival at its target site.

Tethering of both ends of an mRNA to repress translation is also used in controlling other mRNAs. Paul Lasko (McGill University) elucidated the way in which the *Drosophila* protein Bicoid (Bcd) controls translation of *caudal* mRNA. Bcd itself is exclusively synthesized at the anterior pole of the embryo and is required to suppress the synthesis of Caudal in the embryo's anterior half. Bcd binds the 3′ UTR of *caudal* mRNA but also a newly discovered RNA cap–binding protein, d4EHP, which is related to the cap-binding factor eIF4E[15]. Unlike eIF4E, d4EHP cannot sequester additional factors of the translation initiation complex to *caudal* mRNA, and thus it blocks the initiation of translation. In addition to that of *caudal* mRNA, d4EHP might also mediate translation inhibition of hunchback mRNA at the embryo's posterior. In this case, it interacts with a complex of the proteins Nanos, Pumilio and Brat, which assemble on the 3′ UTR of *hunchback* mRNA.

Cytoplasmic polyadenylation-induced mRNA translation is considered to be another general and conserved mechanism of translational control. Joel Richter (University of Massachusetts Medical School) gave an update on the role of the cytoplasmic polyadenylation element binding protein (CPEB) in translational regulation in both *Xenopus* oocytes and mouse neurons. He described the characterization of a CPEB1 knockout mouse[16] with specific deficits in long-term potentiation, a form of synaptic plasticity that might be relevant to learning. Furthermore, he provided evidence that other CPEB binding proteins (CPEB2–4) found in mammals might have different functions than CPEB1, as they recognize different RNA sequences. Finally, he reported that amyloid-β precursor–like protein 1 (APLP1), the function of which is not well understood, interacts with CPEB and stimulates polyadenylation and translation in oocytes. Both CPEB and APLP1 are found in a high–molecular weight complex together with maskin and CPSF100 (ref. 17) at the plasma membrane.

The prevalent model for RNA localization and translational control states that the two processes are governed by distinct yet overlapping sets of coordinated factors. Rob Singer (Albert Einstein College of Medicine) challenged this view when he reported on a previously known protein with a dual role: zipcode-binding protein 1 (ZBP1), an essential *trans*-acting factor for RNA localization in various systems. ZBP1 binds the zipcode element, a *cis*-acting localization signal in the 3′ UTR of the β-actin mRNA, and is crucial in promoting its localization to the actin-rich leading edge of the fibroblast as well as to growth cones and dendrites of neurons. ZBP1 associates with the transcript in the nucleus and is then exported into the cytoplasm. It has been postulated that transport precedes translation; however, evidence for this has been lacking. Singer, Hüttelmaier and colleagues have provided data to close the gap[18]. ZBP1 seems to act as a translational repressor, preventing premature translation in the cytoplasm while the ZBP1–mRNA complex is on its way to its final destination. Once it reaches the periphery of the cell, the nonreceptor tyrosine kinase Src phosphorylates ZBP1 and thereby modulates its RNA-binding activity, which causes dissociation of the complex. This, in turn, allows the translation of β-actin at the plasma membrane to occur.

Localization goes global and gets connected

How many transcripts are actually regulated by subcellular localization? Members of the laboratories of Henry Krause and Howard Lipshitz (both at the University of Toronto) presented their impressive efforts to tackle this question. Eric Lecuyer, from the Krause lab, presented a genome-wide screen for localized mRNAs in early *Drosophila* embryos using a high-throughput fluorescence *in situ* hybridization procedure. They analyzed transcripts that are either maternally provided and deposited into the oocyte during oogenesis or expressed in the zygote at early stages. The number of mRNAs with specific but distinct subcellular localization patterns was unexpectedly high (greater than 90%). This estimate comes as a surprise, as the majority of proteins with general cellular functions would be expected not to show a preferential localization. It will be exciting to learn the biological consequences, if any, of all these subcellular localization patterns (20–30 distinct ones observed so far) and inferred mechanisms.

One such mechanism, degradation of unlocalized mRNAs in combination with local protection, has been studied by the Lipshitz lab. A new survey presented by Howard Lipshitz indicates that around 1,000 maternally provided transcripts in the *Drosophila* oocyte are unstable and that 300 of these are locally protected from degradation at the posterior pole of the embryo. Most intriguingly, the majority of the unstable transcripts become stabilized upon mutation of the Smaug protein. Smaug has been implicated in translational repression of Nanos mRNA as well as in the specific degradation of hsp83 mRNA via recruitment of a deadenylase. The new data suggest a larger-than-anticipated role for transcript degradation in mRNA localization in general and specifically for Smaug protein.

Accumulation of mRNAs at specific locations is not restricted to sites of local protein synthesis but also seems to occur during mRNA

degradation. P-bodies are cytoplasmic foci where mRNA-decay factors are concentrated and decay occurs[19]. Roy Parker (Howard Hughes Medical Institute and University of Arizona, Tucson) showed that P-bodies might be even more versatile structures and can also serve as storage bins for nontranslated mRNAs in yeast[20]. Furthermore, microRNAs that target mammalian mRNAs for translational arrest and proteins of the RNA-mediated interference–effector complex accumulate in P-bodies as well[21]. Along these lines, Mani Ramaswami (University of Arizona, Tucson) extended to neurons this concept of P-bodies as cytoplasmic places of mRNA decay and storage. He showed that a number of proteins involved in translational repression or mRNA binding in *Drosophila* neurons accumulate in cytoplasmic granules, where their function seems consistent with their known activities in yeast P-bodies.

Nervous systems to study RNA localization

John Carson (University of Connecticut Health Center) provided the first mechanistic insight on how myelin basic protein (MBP) RNA is transported inside a living mammalian cell, into the processes of cultured oligodendrocytes[22]. He then identified a short RNA-transport sequence in the 3′ UTR of this mRNA that confers cues for both transport and translation, which has now been renamed the heterogenous nuclear RNP (hnRNP) A2 recognition element, as this RNA element binds hnRNP A2. An attractive model was postulated as to how and when RNA granule assembly occurs in oligodendrocytes, as follows. hnRNP A2 is imported into the nucleus via transportin 1, where it binds MBP mRNA. As the nuclear concentration of hnRNP A2 is very high, tetramers of hnRNP A2 are formed. These prevent RNA granule formation in the nucleus by a mechanism not yet fully understood. In the cytoplasm, however, the tumor-overexpressed gene product (TOG) can bind hnRNP A2, thereby dissociating the A2 tetramers on the RNA. This, in turn, allows granule formation. In other systems, TOG orthologs interact with kinesins possibly regulating transport activity. The identification of an interaction between TOG and hnRNP A2 by Elisa Barbarese (University of Connecticut Health Center) prompted her to investigate the role of TOG[23] in hnRNP A2 granule formation. Surprisingly, small interfering RNAs directed against TOG suppressed expression of MBP mRNA but had no effect on granule formation or on localization of MBP RNA. TOG may therefore act as an activator of translation. Finally, Ross Smith (University of Queensland) presented a detailed molecular characterization of the four major isoforms of hnRNP A2 (A2, A2b, B1, B1b) in neurons and oligodendrocytes. Interestingly, the alternatively spliced isoforms have different subcellular locations. It seems that it is the minor cytoplasmic A2b and B1b isoforms that participate in mRNA trafficking.

Yet another group of important *trans*-acting factors in RNA localization are Staufen proteins, the third conserved family of RNA-binding proteins along with hnRNPs and ZBPs/IMPs. In mammals, two Staufen (*Stau*) genes exist that both have been implicated in dendritic RNA localization. No clear function, however, has been assigned to this protein family in mammals. Michael Kiebler (Medical University of Vienna) reported on an approach using RNA-mediated interference in cultured mature hippocampal neurons to assay for a possible function of the brain-specific Staufen2. Inactivation of Staufen2 in these cells substantially reduces the number of dendritic spines, the postsynaptic endings of synapses. Instead, unusually long filopodia appear. In these neurons, the underlying actin cytoskeleton is altered and one of the transcripts known to be dendritically targeted, β-actin, is downregulated as compared to control cells. These data suggest that mammalian Staufen2 might be involved in the formation of synapses in the hippocampus, their maintenance or both.

Finally, a *Drosophila* model for spinal muscular atrophy (SMA) was announced at this meeting. Mutations in human *SMN1* account for more than 95% of the cases of SMA. SMN protein is required for the biogenesis of small nuclear RNAs (snRNAs); however, the link to the etiology of SMA *in vivo* is unclear. To address this question, Greg Matera (Case Western Reserve University) has generated flies that contain mutations in key components of the snRNA biogenesis pathway, including SMN and PHAX, an snRNA export factor. In *Drosophila*, hypomorphic mutations of *smn* and null alleles of *phax* result in interesting phenotypes resembling SMA. Both *smn* and *phax* and seem to be essential for viability and fertility; however, a fraction of the expected homozygous mutants escape embryonic and larval lethality and survive to adulthood. Only a fraction of the expected homozygous mutants survive to adulthood. These 'escapers' are sterile and unable to jump or fly, with defects in flight muscle myofibril organization. As U1 and U2 snRNA levels are downregulated in *phax* mutants, Matera has put forward the hypothesis that reductions in overall levels of splicing factors may result in defects in alternative splice site choice.

Putting things into perspective

The current studies clearly show an urgent need for interdisciplinary approaches to understand RNA localization. On the one hand, we still need to unravel the basic molecular mechanisms and their underlying key players for each step in RNA localization. The tradeoff for doing so is often a highly reductionist approach using simplified *in vitro* systems. On the other hand, there is a pressing need to design more holistic *in vivo* approaches that address true functions for the identified key players and compare these in different systems and organisms. It is one remarkable achievement of the organizers of the Tucson meeting to have brought different experts studying diverse model organisms together to explore as many distinct experimental avenues as possible to approach the ultimate goal: the advancement of our understanding of how RNA localization occurs and how we may possibly cure individual molecular defects in the future. ■

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