The nuclear connection in RNA transport and localization

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RNA-binding proteins are involved in various aspects of RNA metabolism such as processing, translational control, stabilization, localization and transport. Many of these proteins bind several RNA targets and have multiple functions. In this review we focus on RNA-binding proteins that are implicated in RNA transport and localization and that also have a role in other aspects of RNA metabolism. These proteins might link nuclear events, such as RNA splicing, with the subsequent cytoplasmic localization of specific transcripts.

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RNA localization is recognized as a means by which to generate cellular asymmetry and polarity and to induce cell fate determination in several different types of organisms and cells [1,2]. It typically involves the interaction of *cis* elements or 'zipcodes' [3], sequences within the targeted RNA that direct its localization, and *trans*-acting factors, proteins that bind directly to the *cis* elements. Although, the specific subcellular localization of messenger RNA (mRNA) has been studied since the 1980s, most of the *trans*-acting factors that are thought to be involved in this process have been identified only in the past 5–6 years. Somehow, these factors are capable of both interacting with localized mRNAs and influencing their cytoplasmic fate.

Trans-acting factors vary in their composition, subcellular location and the structural characteristics that they recognize in RNA targets. Candidate localization factors typically contain one of three main classes of RNA-binding domains: an RNA recognition motif (RRM) or RNA-binding domain, a heterogeneous nuclear ribonuclear protein (hnRNP) K homology (KH) domain, or a double-stranded RNA-binding domain. Some proteins contain several copies of these domains in various combinations [4] (Fig. 1 and Table 1).

Trans-acting factors are often present in granules or ribonuclear protein (RNP) complexes, which are thought to contain the components necessary for RNA processing, transport, localization, anchoring and translation (Fig. 2). Further studies are needed to establish how these RNA-binding proteins interact with their RNA targets and use highly conserved RNA-binding domains to achieve specificity, as well as to identify the proteins that they interact with to form functional transport complexes.

It is becoming apparent that some part of the localization process occurs in the nucleus and that certain *trans*-acting proteins provide a nuclear component to this process that is conveyed to the cytoplasm. These proteins might interact with specific transcripts in the nucleus and could either mark them for localization and/or escort them to their ultimate destination in the cytoplasm. In this review, we discuss RNA-binding proteins that provide evidence for this dual-compartmental role.

hnRNP A/B family

hnRNP A2 transports myelin basic protein mRNA

The *trans*-acting factor hnRNP A2 is involved in the localization of myelin basic protein (MBP) mRNA to the myelin-forming processes of mammalian oligodendroctyes [5–8]. Like many of the proteins involved in RNA localization, hnRNP A2 has several other functions, including splicing [9], nuclear export [10], translational regulation [11,12] and RNA stabilization [12]. To achieve many of these functions, hnRNP A2 forms a large nuclear RNA processing complex, referred to as an 'hnRNP particle' [13], that binds and packages nascent precursor mRNAs independently of the RNA sequence [14].

As a *trans*-acting factor of MBP mRNA localization, hnRNP A2 binds to a 21-nucleotide (nt) *cis* element called the hnRNP A2 response element (A2RE), formerly known as the 'RNA trafficking sequence' (RTS), that is located in the 3′ untranslated region (UTR) of the mRNA [7,8]. In addition to its role in transporting MBP mRNA, the A2RE is involved in enhancing the 5′-cap-dependent translation of MBP mRNA [11].

The A2RE contains two partially overlapping, homologous sequences. Either sequence by itself is capable of binding hnRNP A2 and is sufficient for MBP mRNA transport, although the 5′ segment is more active. Mutations in the A2RE that disrupt hnRNP A2 binding also inhibit RNA transport [15]. In addition, treatment with hnRNP A2 inhibitory oligonucleotides decreases the transport of microinjected MBP mRNA to the peripheral myelinating processes of oligodendrocytes [15]. Although it is a predominantly nuclear protein, hnRNP A2 shuttles to the cytoplasm, by means of its M9 nucleocytoplasmic shuttling domain, where it localizes in cytoplasmic granules [16]. Microscopic analysis has shown that, in addition to MBP mRNA and hnRNP A2, these granules contain the translational components arginyl transfer-RNA synthetase, EF1-α and ribosomal RNA [17].

The localization of MBP mRNA in oligodendrocytes has been studied extensively, and much is known

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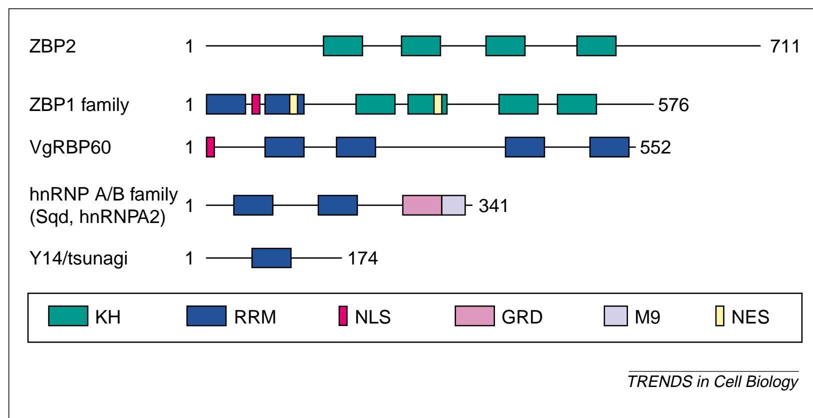

Fig. 1. Domain structures of RNA-binding proteins with a nuclear role in cytoplasmic RNA localization. The hnRNP K homology (KH) domain is coloured green, the RNA recognition motif (RRM) dark blue, the nuclear localization signal (NLS) red, the glycine-rich domain (GRD) purple, the M9 nucleocytoplasmic shuttling domain light blue, and the putative nuclear export signal yellow.

about the RNA element that mediates interaction with hnRNP A2 and subsequent transport; however, the mechanism underlying the function of hnRNP A2 remains to be elucidated.

Sqd localizes gurken *mRNA*

The *Drosophila* protein Sqd (also known as hrp40) is an RNA-binding protein of 42 kDa that is needed for the proper localization of *gurken* (*grk*) transcripts during oogenesis. Like hnRNP A2, Sqd is a member of a class of the hnRNPs that shuttle between the nucleus and the cytoplasm through an M9 shuttling motif. Homologues of Sqd include human hnRNP A1, *Saccharomyces cerevisiae* Np13 and Hrp1, and *Caenorhabditis tentans* hrp36 – proteins that are involved in RNA processing and export [18–22]. In addition, *S. cerevisiae* Hrp1 marks transcripts in the nucleus for cytoplasmic nonsense-mediated mRNA decay [20].

In *Drosophila*, the *sqd* gene is alternatively spliced [23] to produce three protein isoforms: Sqd A, Sqd B and Sqd S. These isoforms are closely related; in fact, they are identical for the first 285 amino acids. Nevertheless, they have distinct functions: Sqd A and Sqd S seem to cooperate in establishing dorsoventral patterning during oogenesis, whereas Sqd B functions in a different capacity [24].

Dorsoventral patterning in *Drosophila* requires localization of *grk* mRNA to the dorsoanterior corner of the oocyte [27,89]. The Grk protein specifies first the anterior–posterior axis and later the dorsal–ventral axis of the oocyte by signalling to adjacent cells [25,26]. The localization pattern of Grk mimics that of *grk* mRNA: it accumulates at the posterior of the oocyte during early oogenesis and later migrates to the anterodorsal region [90]. In *sqd* mutants, *grk* mRNA is mislocalized at the anterior of the oocyte, and does not accumulate anterodorsally. Sqd protein is thought to associate with *grk* mRNA in the nucleus (whether this occurs in the oocyte or nurse cell nucleus is unclear at present) and to deliver it to cytoplasmic anchors at the dorsoanterior of the oocyte. Although Sqd is known to bind directly to the 3′ UTR of *grk* mRNA, the sequences of *grk* mRNA and Sqd that are responsible for this interaction have not been identified.

An earlier study suggested that, only after exposure to Sqd or nuclear extracts, microinjected *fushi tarazu* transcripts become localized apically in *Drosophila* blastoderm embryos [28]. But subsequent analysis has shown that this is probably an artefact of the technique used to label the transcripts, because when different labelling techniques were used microinjected *fushi tarazu* and other transcripts localized rapidly without prior *in vitro* exposure to protein extracts [29,30].

The ZBP1 family: transport of β-actin, *Vg1* and *VegT* mRNAs

The zipcode-binding protein-1 (ZBP1) family of RNA-binding proteins is a well-conserved protein family [31–40]. ZBP1 family members have been associated with various aspects of RNA metabolism including RNA localization, mRNA stability and translational control. Specifically, chick ZBP1 and *Xenopus* Vg1 RNA-binding protein (Vg1RBP; also called Vera) have been implicated in the localization of β-actin mRNA [31,41] and *Vg1* and *VegT* mRNAs [33,42], respectively.

Table 1. Properties of *trans*-acting factors associated with localized mRNAs in the nucleus and the cytoplasm[a]

Protein	Organism	Cell type	mRNA	Homologues	Associated functions	Refs
hnRNP A/B family						
HnRNPA2	Human	Oligodendrocyte	MBP		Splicing	[8–12,17]
Sqd (hrp40)	*Drosophila*	Oocyte	*gurken*	Human hnRNPA1, *S. cerevisiae* Np13, Hrp1, *C. tentans* hrp36	mRNA processing and transport, NMD	[18,20–24]
ZBP1 family						[31–42,50,53]
ZBP1	Chicken	Fibroblast, neuron	β-Actin	CRD-BP, IMP1, IMP2, IMP3, KOC, HCC, dIMP	mRNA stability, translational regulation, overexpressed in cancer	[31,41,45]
Vg1RBP	*Xenopus*	Oocyte	*Vg1, Veg T*	See above	See above	[32–34,42,50,53]
ZBP2	Chicken	Fibroblast, neuron	β-Actin	KSRP, MARTA	Splicing	[56,57]
She2p	*S. cerevisiae*	Budding yeast	*ASH1*	NK	NK	[80]
Loc1p	*S. cerevisiae*	Budding yeast	*ASH1*	NK	NK	[78]
Tsunagi	*Drosophila*	Oocyte	*Oskar*	Human Y14	EJC, NMD	[59,67–71]

[a]Abbreviations: EJC, exon–exon junction complex; NK, not known; NMD, nonsense-mediated mRNA decay.

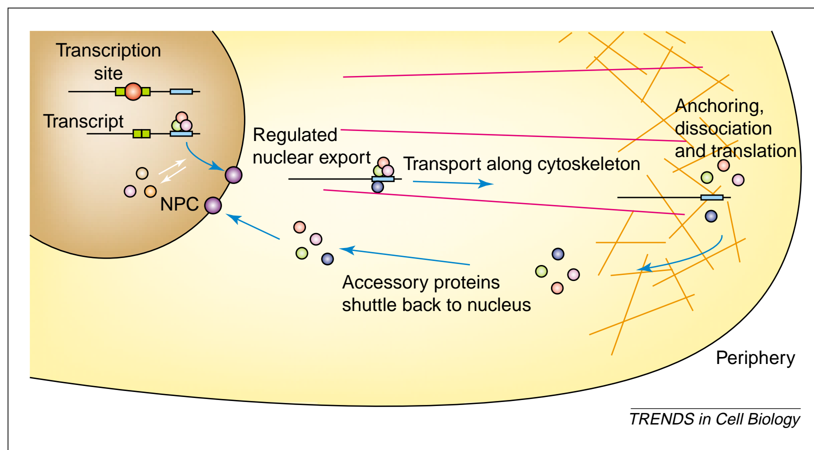

Fig. 2. Model of localization factors shuttling between the nucleus and the cytoplasm. Factors associate with the RNA at the transcription site to form a localization complex or 'locasome' – a particle that is designated to localize associated RNAs, by riding with the RNA through the nuclear pore complex (NPC) where additional factors join and assemble motors. The locasome then travels to the cell periphery directed by the polarity of the cytoskeletal filaments and anchors on the isotropic microfilament network, where it releases the shuttling factors and becomes competent for translation. The shuttling factors then return to the nucleus. The cell periphery might be the growth cone of a neuron, the lamellipod of a fibroblast, the vegetal pole of a *Xenopus* oocyte or the posterior pole of a *Drosophila* embryo.

All of the ZBP1 family members that have been examined so far are cytoplasmic and present in cytoskeleton-associated RNA-containing granules. Despite their predominantly cytoplasmic localization, the presence of nuclear localization and export signals within the protein, and their similarity to hnRNP proteins, suggests that they might spend some of their time in the nucleus. In support of this, treating chick embryo fibroblasts with Leptomycin B, an inhibitor of Crm-1-dependent nuclear transport, results in a nuclear accumulation of ZBP1 (Y. Oleynikov and R.H. Singer, unpublished). In addition, in serum-stimulated fibroblasts, ZBP1 tagged with green fluorescent protein is recovered at transcription sites of β-actin mRNA after photobleaching (Y. Oleynikov and R.H. Singer, unpublished).

Surprisingly, members of this protein family recognize different RNA target sequences despite their high homology. ZBP1 binds to a 54-nt *cis* element in the 3′UTR of β-actin mRNA. This mRNA localizes in the lamellae of chick embryo fibroblasts [43] and the growth cone of chick embryonic neurons [44]. Its *cis* element contains short tandem repeats that are necessary for direct interaction between the protein and the RNA. ZBP1, the first member of this family to be identified, was isolated by its affinity for the β-actin mRNA *cis* element [31]. The functional relationship between ZBP1 and β-actin mRNA localization was confirmed by showing that antisense oligonucleotides of the *cis* element, which delocalize β-actin mRNA, disrupt the interaction between ZBP1 and β-actin mRNA ([41,45]; and Y. Oleynikov and R.H. Singer, unpublished).

Vg1RBP, *Vg1* mRNA and *VegT* mRNA seem to be distributed homogeneously during early *Xenopus* oogenesis. In later stages both mRNAs form a wedge that extends from the nucleus to a strong band at the vegetal cortex [46]. Each mRNA is directed by localizing elements (*Vg1* LE and *VegT* LE) that are present in the 3′ UTR.

The *Vg1* LE and the *VegT* LE are much larger and more complex than is the β-actin mRNA *cis* element. The *Vg1* LE comprises 340 nt [47] and contains a series of repeats, E1–E4, that are involved in both localization and the interaction between the RNA and Vg1RBP [32–34,42,48]. The *VegT* LE comprises 300 nt [49] and, although it does not possess an overall homology to the *Vg1* LE, it does contain some sequence elements, such as E2 sequences, that are present in the *Vg1* LE [42,49]. It is unclear, however, which sequences of the *Vg1* LE and *Veg T* LE mediate binding with Vg1RBP and are sufficient for localization of the RNAs. The published data vary depending on which method is used to examine localization and how the mutant constructs are designed [32–34,42,47,48,50–52].

Mutagenesis studies have shown that the binding of ZBP1 and Vg1RBP to their target RNAs correlates with the localization of both the RNA and the protein. Both proteins are expressed developmentally and colocalize with their RNA targets through a mechanism mediated by the cytoskeleton. Localization of both ZBP1 and Vg1RBP involves the actin and microtubule cytoskeletal systems, but the primary cytoskeletal system used by each protein differs. The microtubule system predominates in the transport of β-actin mRNA by ZBP1 in neurons and in that of *Vg1* or *VegT* mRNAs by Vg1RBP [34,41,44,53]. But actin seems to be more important for the transport of β-actin mRNA and ZBP1 in embryonic fibroblasts ([54,55]; and Y. Oleynikov and R.H. Singer, unpublished).

It has not been established whether these *trans*-acting proteins provide a direct link between the RNA and the cytoskeleton or whether there is another, unidentified intermediate involved. In studies of ZBP1, the third and/or fourth KH domains of the protein are responsible for the specific interaction between the protein and the *cis* element. Interaction with an intermediate protein most probably occurs at the amino terminus of the protein (K.L. Farina and R.H. Singer, unpublished).

ZBP2 involvement in β-actin mRNA localization

The most recently identified RNA-binding protein involved in RNA localization comes from studies of β-actin mRNA in fibroblasts and neurons. Gu *et al.* [56] used affinity purification in chick brain extracts to identify a protein of 92 kDa that, like ZBP1, specifically binds to the 54-nt zipcode in the 3′ UTR of β-actin mRNA. This protein, named ZBP2, is homologous to human KH-domain-containing splicing regulatory protein (KSRP), a nuclear mRNA splicing factor [57]. In addition to four KH domains, ZBP2 contains a glutamine-rich carboxy terminus and an amino-terminal domain rich in proline and glycine.

Like KSRP, ZBP2 has a predominantly nuclear localization; however, it is occasionally seen at the leading edge of fibroblasts and in neuronal growth cones [56]. The two proteins differ in a segment of

47 amino acids located just before the first KH domain that is present in ZBP2 but not in human KSRP. There seems to be no other homologue to KSRP in chicken.

ZBP2 is enriched in brain extracts much more than in fibroblasts, which perhaps indicates a stronger role for ZBP2 than ZBP1 in brain development. Although both ZBP1 and ZBP2 bind to the same region of β-actin mRNA, they segregate into separate complexes in ultraviolet crosslinking studies. This suggests that they do not bind to the *cis* element simultaneously. Gu *et al.* [56] propose that the β-actin transcript is somehow marked for localization in the nucleus by ZBP2 and then passed to ZBP1 for transport. Overexpression of the 'central domain', which contains the 47-residue segment and the four KH domains, results in partial delocalization of β-actin mRNA in chick embryo fibroblasts. These results clearly show that ZBP2 has a role in the localization of β-actin mRNA. The identification of this nuclear splicing factor that is also involved in RNA localization comes at an exciting time in the field when the idea that these two processes are related has come to the forefront [58,59].

VgRBP60 and hnRNP I involvement in Vg1 and VegT mRNA localization

An 85-nt subelement of the *Vg1* LE, which is distinct from the sequence recognized by Vg1RBP, is required for localizing *Vg1* mRNA to the vegetal pole [48], and a hexanucleotide sequence within this 85-nt subelement – the *Vg1* motif 1 (VM1) – is essential for this localization. Using the 85-nt element, Cote *et al.* [52], identified VgRBP60, a protein of 60 kDa present in oocyte extracts, that forms crosslinks specifically with the VM1 but not with a mutant VM1 motif [52]. The same mutations in the VM1 that abolish its interaction with VgRBP60 also disrupt localization of the *Vg1* RNA in *Xenopus* oocytes.

But a recent report by Kwon *et al.* [42] indicates that the VM1 is not required for localization of *Vg1* mRNA, making the role of VgRBP60 unclear [42]. It should be noted that studies verifying whether binding between the mutated *Vg1* LE (used by Kwon *et al.* [42]) and VgRBP60 was disrupted were not carried out. Bubunenko *et al.* [49] have shown that, in addition to binding the *Vg1* LE, VgRBP60 also binds to regions containing VM1-like sites in the *VegT* LE [49]. Mutations in the *VegT* LE that diminish VgRBP60 binding, but not Vg1RBP binding, also result in impaired localization, supporting a role for VM1-like sequences in localization of the RNA.

VgRBP60 is a homologue of human hnRNP I and the polypyrimidine tract-binding protein (PTB) – proteins that arise from alternative splicing of the same transcript. VgRBP60 is more closely related to hnRNP I, sharing 87% identify with this human protein. VgRBP60 contains four RRM domains. Studies of hnRNP I and PTB indicate that the third and fourth RRM domains are responsible for RNA binding and that the amino-terminal portion of the protein mediates protein–protein interactions [60,61]. A putative bipartite nuclear localization signal identified in hnRNP I and PTB has been also found in VgRBP60 [60,62,63]. *Vg1* mRNA colocalizes with VgRBP60 at the vegetal cortical cytoplasm in *Xenopus* oocytes [52]. Because mammalian counterparts of VgRBP60 are associated with mRNA splicing and transcriptional regulation [64], it is possible that *Xenopus* VgRBP60 has functions other than its implicated role in RNA localization.

Y14 and Tsunagi involvement in cytoplasmic localization of RNA

The predominantly nuclear RNA-binding protein Y14 is associated with nonsense-mediated mRNA decay [59,65,66] and RNA localization [67,68]. Homologues of Y14 have been found in *Drosophila, Xenopus*, mouse, *C. elegans* and *S. pombe*. Originally Y14 was identified in a yeast two-hybrid screen of a human fetal brain library using Magoh as bait [69]. The *Drosophila* homologue of Magoh, Mago nashi, is required for germ cell assembly during *Drosophila* oogenesis [67,70]. Bound to Magoh, Y14 associates with spliced mRNAs in the nucleus as part of the nuclear exon–exon junction complex and maintains this association during translocation to the cytoplasm [59,70]. The interaction between Y14 and Magoh is conserved between the *Drosophila* homologue of Y14, Tsunagi, and *Drosophila* Mago nashi [67]. It has been suggested that, during nonsense-mediated mRNA decay, Y14 might participate in a complex that influences the ultimate fate of the transcript by 'marking' it in the nucleus [13,58].

Several recent studies of Tsunagi and Mago nashi indicate that the complex they form is involved in cytoplasmic mRNA localization [59,67,68]. During *Drosophila* oogenesis, the localization of *oskar* mRNA to the posterior pole is required for cell-fate determination. Tsunagi and Mago nashi colocalize to the posterior pole during this process [67,70]. The localization of *oskar* is dependent, in part, on Mago nashi [71,72] and the RNA-binding proteins Staufen [73] and Tsunagi [67,68]. Specifically, Mago nashi anchors the Staufen–*oskar* mRNA complex at the posterior pole. In the absence of Mago nashi function, *oskar* mRNA remains at the anterior pole of the oocyte. Tsunagi is required during the translocation of *oskar* mRNA from the anterior to the posterior pole [67,68].

In *tsunagi* mutant flies, both *oskar* mRNA and Tsunagi translocate from the nurse cell nuclei to the anterior pole of the oocyte but are unable to localize to the posterior pole [67,68]. In mutant flies that mislocalize Staufen–*oskar* mRNA, Tsunagi colocalizes with the mRNA complex [68]. Y14 is a clear example of an RNA-binding protein that is implicated in RNA localization that functions in both the nucleus and the cytoplasm.

She2p and Loc1p localize *ASH1* mRNA in budding yeast

In the budding yeast, *S. cerevisiae*, Ash1 protein localizes to the bud nucleus during late anaphase and

is a transcriptional repressor that is needed for mating-type switching [74,75]. The asymmetrical localization of this protein results from the localization of *ASH1* mRNA to the bud tip [76,77]. *ASH1* mRNA localization requires the presence of four *cis* elements located throughout its coding region and 3′ UTR [78]. Recently, two *trans*-acting factors, She2p and Loc1p, responsible for the localization of *ASH1* mRNA have been identified. Unlike the other *trans*-factors discussed here, She2p or Loc1p have no known homologues nor do they contain any known homology domains. Despite this lack of conserved RNA-binding domains, She2p and Loc1p can bind directly to *ASH1* mRNA ([79]; and S. Huettelmaier and R.H. Singer, unpublished) .

She2p was identified in genetic screens for defects in mating-type switching [80] and for RNA localization to the tip of the bud [81–83]. The second factor, Loc1p was identified in a three-hybrid screen for proteins that interact with *ASH1* mRNA [79]. Because of the strict nuclear localization of Loc1p, it is thought that Loc1p functions by 'marking' the *ASH1* mRNA in the nucleus for subsequent localization in the cytoplasm. It is possible that Loc1p passes the RNA to another protein, such as She2p, that can shuttle between the nucleus and cytoplasm. Nuclear 'marking' of transcripts and shuttling might be functions that converged into single hnRNPs in higher eukaryotes. Although we lack a clear picture of the mechanism underlying *ASH1* mRNA localization, it is evident that this is a process that begins in the nucleus with the interaction between *ASH1* mRNA and its *trans*-acting factors.

Concluding remarks

Research in the field of RNA localization has moved beyond the study of localized transcripts themselves to the *trans*-acting protein factors that bind to the transcripts and direct them to subcellular locations. Here we have focused on several proteins that bind directly to *cis* elements in localized RNAs and have both a nuclear and cytoplasmic presence. Other, well-characterized, strictly cytoplasmic RNA-binding proteins that are involved in localization but not other functions – such as *Drosophila* Staufen and *S. cerevisiae* She3p – have been reviewed elsewhere [84–88].

The study of the multifunctional RNA-binding proteins involved in localization offers insight into directed RNA transport. One such revelation has been that many of the *trans*-acting factors are hnRNP-like or actual homologues of hnRNPs. It is becoming increasingly apparent that many of these proteins have a nuclear function that is linked to their role in localizing RNAs. This is clearly demonstrated by the role of Sqd in localizing *grk* mRNA [23,24] and the participation of Tsunagi in o*skar* mRNA localization [67,68].

The more recent discovery [56] that a nuclear splicing factor is involved in the localization of β-actin mRNA also links events in the nucleus with the subsequent cytoplasmic localization of specific transcripts. In addition, several proteins required for mRNA localization are homologues of either nuclear or shuttling hnRNPs, many of which are involved in splicing. The idea that RNA splicing in the nucleus begins a process that affects RNA localization is a new and intriguing idea [13,58]. So far, Y14 and ZBP2 provide the strongest evidence of a link between these two processes. But the evidence is still circumstantial: ZBP2 has not been shown to be involved in the splicing of β-actin mRNA and Y14 has not been shown to bind directly to a localized RNA.

Although it is not clear how nuclear events influence the downstream localization of certain transcripts, it is likely that they begin with the assembly of a protein complex during transcription (Fig. 2). Whether this happens in concert with splicing or is a subsequent event remains to be seen. Perhaps there is cooperation between the two processes. Proteins in the localization complex presumably function together to direct transcripts along the transport pathway. The complex is most probably dynamic, with proteins entering and exiting *en route*. If so, this will make analyses of the localization machinery difficult to interpret. The best understanding will emerge by studying different organisms, and the continued application of new technology and innovative high-resolution imaging should allow investigators to overcome the technical hurdles posed by each system.

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SMN-mediated assembly of RNPs: a complex story

Gunter Meister, Christian Eggert and Utz Fischer

Although many RNA–protein complexes or ribonucleoproteins (RNPs) assemble spontaneously *in vitro*, little is known about how they form in the environment of a living cell. Insight into RNP assembly has come unexpectedly from functional analyses of the survival motor neuron (SMN) protein, a gene product that is affected in the neuromuscular disease spinal muscular atrophy. These studies show that the assembly of spliceosomal U-rich small nuclear RNPs *in vivo* depends on the activity of two large protein complexes, one of which contains the SMN protein. These complexes might also facilitate the assembly of other cellular RNPs.

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The list of cellular processes that depend on the action of RNPs continues to grow. Some of the most prominent examples are involved in translation (ribosomes), protein translocation into the endoplasmic reticulum (signal recognition particle [SRP]), telomere synthesis (telomerase) and RNA processing (U-rich small nuclear RNPs [U snRNPs] and U small nucleolar RNPs [snoRNPs]).

For function in their respective cellular pathways, RNPs are assembled from RNA and protein in a process that can be controlled and/or facilitated at several stages. The synthesis and processing of RNP components have to be coordinated tightly so that all components are available in sufficient amounts when assembly is initiated. Because RNA is normally transcribed in the nucleus and proteins are generated in the cytoplasm, transport processes must ensure that all components of the RNP reach the subcellular site where assembly takes place. Finally, the assembly process itself (i.e. the binding of proteins to the RNA) can be subject to regulation and/or assistance.

This review focuses on progress that has been made in understanding the last step in the biogenesis of RNPs – the assembly of RNA and proteins to form functional particles. Early studies indicated that many RNPs (including the ribosome, which is one of the most complex) could assemble spontaneously *in vitro* when purified RNP components were incubated under appropriate conditions [1]. But the lack of appropriate test systems prevented those studies from determining whether 'self-assembly' is also the way in which RNPs are generated *in vivo*. Insight into the mechanism of RNP assembly has come recently from molecular analyses of a common genetic disorder called spinal muscular atrophy (SMA). These studies have shown that, unlike the situation *in vitro*, the assembly of the spliceosomal class of RNPs *in vivo* is an active process that is mediated by several factors, including the product of the *SMN* gene that is affected in SMA.

Mutations in the *SMN1* gene cause spinal muscular atrophy

Spinal muscular atrophy is an autosomal recessive disorder that affects roughly 1 in 6000 live births and is one of the most frequent genetic causes of early death in infants. Affected individuals suffer from progressive paralysis of trunk and limbs caused by degenerating motor neurons in the spinal cord [2,3]. The disease clearly correlates with mutations in the *survival motor neuron* (*SMN*) gene, which is duplicated in a 500-kilobase region at locus 5q13 [4].

Most individuals with SMA have homozygous mutations or deletions in one gene copy (*SMN1*),

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