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REVIEW ARTICLE

Molecular motors: directing traffic during RNA localization

James A. Gagnon* and Kimberly L. Mowry

Department of Molecular Biology, Cell Biology & Biochemistry, Brown University, Providence, RI, USA

Abstract

RNA localization, the enrichment of RNA in a specific subcellular region, is a mechanism for the establishment and maintenance of cellular polarity in a variety of systems. Ultimately, this results in a universal method for spatially restricting gene expression. Although the consequences of RNA localization are well-appreciated, many of the mechanisms that are responsible for carrying out polarized transport remain elusive. Several recent studies have illuminated the roles that molecular motor proteins play in the process of RNA localization. These studies have revealed complex mechanisms in which the coordinated action of one or more motor proteins can act at different points in the localization process to direct RNAs to their final destination. In this review, we discuss recent findings from several different systems in an effort to clarify pathways and mechanisms that control the directed movement of RNA.

Keywords: RNA localization, transport, molecular motor, cell polarity, posttranscriptional control

Introduction

A rapidly growing number of localized mRNAs have been identified in both somatic cells and oocytes (reviewed in Holt and Bullock, 2009; Martin and Ephrussi, 2009). Since the types of proteins these mRNAs encode are highly diverse, the reasons for localization can be broadly characterized. In somatic cells, localized mRNAs act to provide regional functional specialization. One well-studied example is in fibroblasts, where sorting of mRNAs encoding actin isoforms in fibroblasts influences cell motility and morphology (Figure 1A; reviewed in Condeelis and Singer, 2005). In neuronal cells, mRNA localization and local protein synthesis impacts cell polarity and synaptic plasticity (reviewed in Bramham and Wells, 2007). The localization of maternal mRNAs in many developing organisms provides the basis for both initial polarity during oogenesis and subsequent patterning during embryogenesis. Prominent examples of this phenomenon are found in *Drosophila melanogaster* (Figure 1B), where localized mRNAs underlie patterning along both the anterior–posterior and dorsal–ventral axes (reviewed in Minakhina and Steward, 2005), and in *Xenopus laevis* (Figure 1C), where localized maternal mRNAs generate developmental polarity along the animal–vegetal axis (reviewed in King et al., 2005). It is increasingly clear that RNA localization is an important posttranscriptional regulatory mechanism. Indeed, a high-throughput screen in *Drosophila* embryos revealed that up to 70% of endogenous transcripts exhibit subcellular localization (Lecuyer et al., 2007), underscoring a broad role for RNA localization in the regulation of gene expression.

Analysis of localized transcripts in both invertebrate and vertebrate organisms has shed light into conserved mechanisms of RNA localization (reviewed in Holt and Bullock, 2009; Martin and Ephrussi, 2009). A theme has emerged in which multiple components cooperate to recognize and bind the RNA, then subsequently assemble with additional factors into a localization-competent RNP (ribonucleoprotein) complex. Some of these proteins are conserved across species, whereas others are evolutionarily diverse but are related in function. Initially, RNA-binding proteins recognize *cis*-acting motifs or "localization signals" in the RNA (reviewed in Hamilton and Davis, 2007). These targeting sequences

Address for Correspondence: Kimberly L. Mowry, Department of Molecular Biology, Cell Biology & Biochemistry, Box G-L268, Brown University, Providence, RI 02912. E-mail: Kimberly_Mowry@brown.edu
*Present address: Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA.

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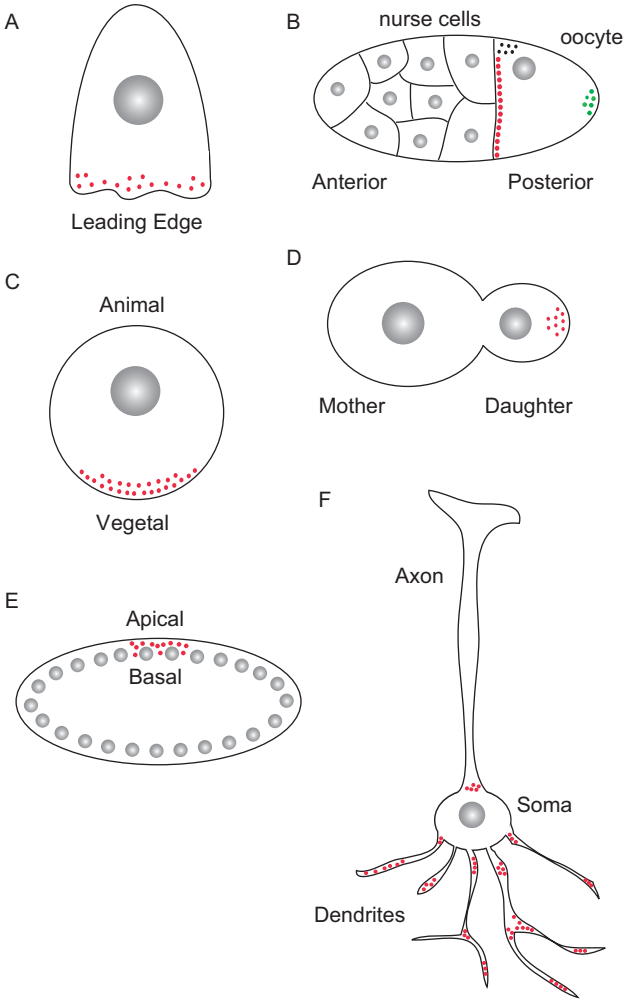

Figure 1. Examples of RNA localization. (A) β-Actin mRNA (red stipples) is localized to the leading edge of chick embryo fibroblasts by a myosin-V motor. (B) Localization of RNAs in the *Drosophila* oocyte. *Oskar* mRNA (green stipples) is transported to the posterior pole of oocyte by kinesin-1. *Bicoid* mRNA (red stipples) is transported to the anterior of the oocyte by dynein. Dynein also localizes *gurken* mRNA (black stipples) to the dorsoanterior corner of the oocyte. (C) Vg1 mRNA (red stipples) is transported to the vegetal pole of the *Xenopus* oocyte by kinesin-1 and kinesin-2. (D) Localization of *ASH1* mRNA (red stipples) to the daughter cell in budding yeast is mediated by a myosin motor. (E) Localization of the pair-rule mRNAs (red stipples) to the apical side of nuclei in the *Drosophila* syncytial blastoderm embryo mediated by dynein. (F) mRNAs localized to growth cones and dendrites of neurons mediated by kinesin. For all panels, nuclei are shown in gray.

are usually (but not always) found in the 3′ untranslated region (UTR) of localized transcripts and interact with the RNA-binding proteins to form a core RNP in the nucleus (reviewed in Lewis and Mowry, 2007). These early (nuclear) interactions are critical for recognition of RNA as destined for localization as well as for translational repression of the mRNA (reviewed in Besse and Ephrussi, 2008). After export, additional factors are recruited to direct cytoplasmic fate (reviewed in Lewis and Mowry, 2007). These can include molecular motors, which actively transport these translationally silenced mRNPs to a subcellular compartment using cytoskeletal networks (reviewed in Tekotte and Davis, 2002; Bullock et al., 2006; Hirokawa, 2006). The targeted mRNPs are often anchored at their final destination, and ultimately, local translation initiates to generate polarized gene expression. Although some mechanisms for mRNA localization rely on diffusion and entrapment or local protection from degradation (reviewed in Bashirullah et al., 1998; Lipshitz and Smibert, 2000; St. Johnston, 2005; Martin and Ephrussi, 2009), the most abundant examples involve motor-driven RNA transport (reviewed in Tekotte and Davis, 2002; St. Johnston, 2005; Bullock et al., 2006; Hirokawa, 2006).

Motor-driven RNA transport

Molecular motors use the energy produced by ATP hydrolysis to generate conformation changes that result in directional movement on the cytoskeletal tracks. RNA cargoes are moved by motors from all three motor families: myosins, kinesins, and dynein (Figure 2A–2D). In general, kinesin motors transport cellular cargos, including RNAs, vesicles, and organelles, to the plus ends of microtubules (reviewed in Hirokawa et al., 2009), whereas cytoplasmic dynein transports cargos to the minus ends of microtubules (reviewed in Vallee et al., 2004). A type V myosin motor is necessary for localization of *ASH1* mRNA (Figure 1D) in budding yeast (Münchow et al., 1999; Takizawa and Vale, 2000), and localization of the zygotic pair-rule transcripts (Figure 1E) in the *Drosophila* embryo is dependent upon cytoplasmic dynein (Wilkie and Davis, 2001). Both dynein and kinesin motors have been implicated in RNA localization in *Drosophila* oocytes (Brendza et al., 2000; Schnorrer et al., 2000; Cha et al., 2002; Duncan and Warrior, 2002; Januschke et al., 2002). In vertebrates, kinesin-1 plays a role in transport of several RNAs in cell types within the nervous system (Carson et al., 1997; Aronov et al., 2002; Kanai et al., 2004), and both kinesin-1 and kinesin-2 are involved in RNA transport in *Xenopus* oocytes (Betley et al., 2004; Yoon and Mowry, 2004; Messitt et al., 2008). Roles for molecular motors in RNA transport can provide for directional targeting of mRNAs to specific regions of the cell cytoplasm. However, the mechanisms by which molecular motors attach to and asymmetrically transport RNA cargos have, until recently, been unclear.

RNA transport by kinesin motors

The kinesin superfamily spans diverse classes of molecular motors, which transport cargo such as organelles, proteins, and mRNAs on the intracellular microtubule network of many eukaryotic cells (reviewed in Vale, 2003; Hirokawa et al., 2009). Most well-studied are the N-kinesins, which are plus end-directed motors. Significant variation can be seen in component organization within the N-kinesin family, but common to all are a kinesin motor domain and a structurally important coiled coil domain (reviewed in Hirokawa et al., 2009).

This family is most clearly exemplified by kinesin-1 (Vale et al., 1985). Kinesin-1 (Figure 2B), also known as conventional kinesin, is a tetrameric protein complex consisting of two identical kinesin heavy chains (KHC) and two kinesin light chains (KLC). The KHC motor domain is responsible for binding microtubules and hydrolyzing ATP (Yang et al., 1989), whereas the KLC homodimer appears to work with KHC to bind cargo (Hirokawa et al., 1989). Kinesin-1 was first implicated in RNA transport through studies of myelin basic protein mRNA localization in oligodendrocytes (Carson et al., 1997), and roles in RNA transport have since been uncovered in systems as diverse as oocytes and neurons.

Due to their highly polarized nature, neurons provide numerous examples of localized mRNAs. RNAs are transported over long distances in neurons (Figure 1F), both to the synapses of dendrites and within the axon (reviewed in Hirokawa, 2006; Sossin and DesGroseillers, 2006; Vuppalanchi et al., 2009). System-wide approaches have characterized hundreds of transcripts that are localized in mammalian neurons (Eberwine et al., 2002; Poon et al., 2006; Suzuki et al., 2007), where local translation of RNA is thought to facilitate a rapid response to signals at the synapse, and may mediate synaptic plasticity (reviewed in Holt and Bullock, 2009; Wang et al., 2010). Since cytoplasmic extensions in neurons contain highly polarized microtubule networks, with the minus ends of microtubules at the cell body and plus ends at the cell periphery, kinesin-1 was a clear candidate for transport of mRNAs and other cargos in an anterograde fashion to the synapse. Evidence supporting a role for kinesin-1 in transport of RNAs came from biochemical purification and mass spectrometric analyses of kinesin-containing complexes from mouse brain tissue, which revealed numerous RNA-binding proteins, as well as localized RNAs (Kanai et al., 2004). Transport of these large RNA granules *in vivo* was shown to be perturbed by kinesin-1 gain-of-function and loss-of-function experiments, demonstrating a role for kinesin in transport of RNA in neurons (Kanai et al., 2004). Similarly, dominant-negative experiments showed a role for kinesin-1 in *shank1* mRNA transport in rat neurons (Falley et al., 2009). The links between RNA localization, spatially restricted translation, and synaptic plasticity are exciting (reviewed in Wang et al., 2010), and the underlying molecular mechanisms, once elucidated, promise new insights into regulation of neuronal function.

In oocytes, RNA localization can specify both cell and developmental polarity, and can provide the basis for patterning of the embryo. Localization of *oskar* mRNA during *Drosophila* oogenesis (Figure 1B) is necessary for both posterior patterning during embryogenesis and germline specification (Ephrussi et al., 1991). In oocytes lacking KHC, *oskar* mRNA fails to localize to the posterior pole, suggesting that localization of *oskar* RNA requires kinesin-1 motor activity (Brendza et al., 2000). However, KLC appears to be dispensable for kinesin-1-dependent transport of *oskar* mRNA (Palacios and St. Johnston, 2002), suggesting that a non-canonical kinesin-based transport mechanism may operate to localize *oskar* mRNA. Thus, kinesin could localize *oskar* RNA indirectly; kinesin-1 is required for cytoplasmic flows that could mediate the accumulation of *oskar* mRNA at the posterior pole (Palacios and St. Johnston, 2002), or *oskar* mRNA could be linked to an organelle localized by kinesin-1. However, recent results indicate that *oskar* mRNA may be linked to kinesin-1 by a novel KLC-like protein (Loiseau et al., 2010), potentially explaining the independence of *oskar* RNA transport from canonical KLC.

Studies in *Xenopus* oocytes have revealed a direct role for kinesin molecular motors in RNA localization. During oogenesis, Vg1 mRNA is transported to the vegetal pole of the oocyte, where it is critical for patterning during embryogenesis (Melton, 1987; Birsoy et al., 2006). Kinesin-1 and kinesin-2 are components of the Vg1 mRNP

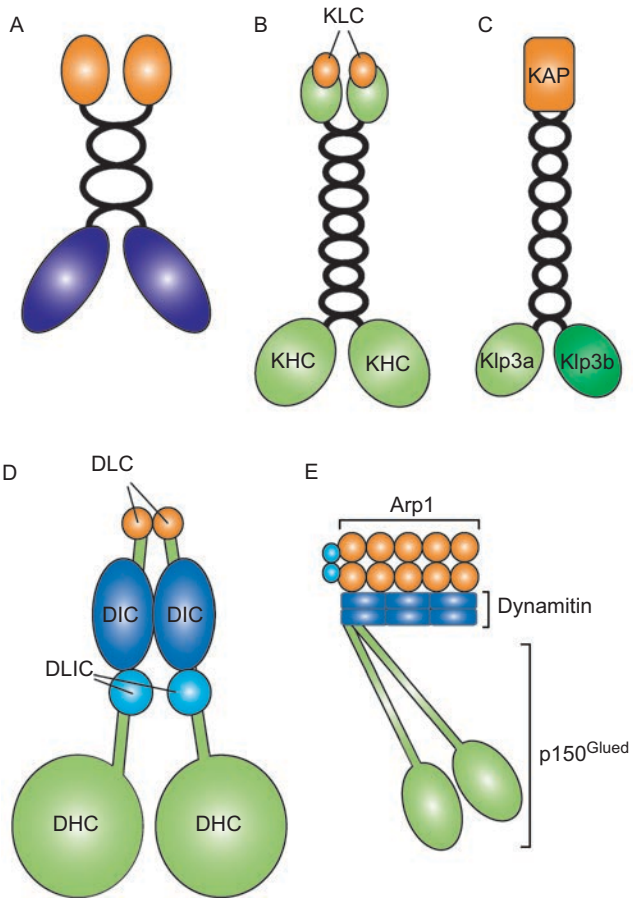

Figure 2. Molecular motors. Cargo domains are labeled in orange, microtubule binding domains in green, and actin-binding domains in purple. Some subunits have been omitted for clarity. (A) Dimeric myosin-V. (B) Heterotetrameric kinesin-1. Kinesin heavy chains (KHC) and kinesin light chains (KLC) are indicated. (C) Heterotrimeric kinesin-2. The motor domain-containing heavy chains, Klp3a and Klp3b are indicated, as is the cargo-binding kinesin-associated protein (KAP). (D) Cytoplasmic dynein. Dynein heavy chains (DHC), dynein light–intermediate chains (DLIC), dynein intermediate Chains (DIC), and dynein light chains (DLC) are indicated. (E) The multi-subunit dynactin complex. p150^{Glued}, dynamitin and Arp1 subunits are indicated.

and are required for vegetal RNA localization (Betley et al., 2004; Yoon and Mowry, 2004; Messitt et al., 2008). Like kinesin-1, kinesin-2 (Figure 2C) transports cargos to the plus ends of microtubules, but unlike kinesin-1, kinesin-2 is a heterotrimeric motor protein containing two non-identical motor subunits and a single non-motor accessory protein (Cole et al., 1993; Wedaman et al., 1996). In *Xenopus* oocytes, interfering with function of either kinesin-1 or kinesin-2 abolishes vegetal RNA localization, indicating that both motors are involved in transport of Vg1 RNA (Messitt et al., 2008). Moreover, interaction between kinesin-1 and kinesin-2 was shown to be RNA-dependent, suggesting that both motors are simultaneously bound to the Vg1 RNP. Although it is unclear why both kinesin motors are necessary, cross-rescue experiments in which kinesin-1 overexpression could rescue reduction in kinesin-2 activity (and vice versa) suggest that kinesin motors are limiting for vegetal RNA localization (Messitt et al., 2008).

Dynein-dependent RNA transport

The dynein family of molecular motors contains two classes, the axonemal dyneins and cytoplasmic dynein. Axonemal dyneins coordinate the beating of flagella and cilia, leaving the cytoplasmic dynein motor responsible for transport of cargos to the minus end of microtubules and several mitotic functions (reviewed in Karki and Holzbaur, 1999). Cytoplasmic dynein (Figure 2D) is a multi-protein complex that consists of a catalytic homodimeric heavy chain and additional non-catalytic subunits, which appear to be dispensible for dynein mobility but may function to adapt different cargos and regulate dynein function (reviewed in Kardon and Vale, 2009). Distinct from the dynein complex is the cargo adapter complex dynactin (Figure 2E). Dynactin was first identified as an activator of dynein-dependent transport of cargos (Gill et al., 1991) and is now known to be required for almost all cellular functions of dynein (reviewed in Schroer, 2004). The dynactin complex contains at least 11 subunits and acts to target dynein to subcellular locations, affect dynein processivity, and adapt dynein to cargo (reviewed in Schroer, 2004). In addition to its established role in regulating dynein function, dynactin may also be involved in coordinating transport of cargos bound by both kinesin and dynein molecular motors, as discussed below.

Dynein was first shown to play a role in RNA localization in *Drosophila* embryos (Figure 1E) where transport of the pair-rule mRNAs such as *wingless*, *hairy*, and *ftz* to the apical cytoplasm is responsible for coordinating segmentation of the embryo (Bullock and Ish-Horowicz, 2001; Wilkie and Davis, 2001; Bullock et al., 2004). Dynein associates with these transcripts through the adapter proteins BicaudalD (BicD) and Egalitarian (Egl) (Bullock and Ish-Horowicz, 2001; Dienstbier et al., 2009). Dynein is also responsible for localization of *bicoid* and *gurken* mRNAs during *Drosophila* oogenesis (Duncan and Warrior, 2002; Januschke et al., 2002). Localization of these mRNAs is critical for patterning the embryo along both the anterior–posterior and dorsal–ventral axes (Figure 1B; reviewed in Minakhina and Steward, 2005). Localization of *gurken* mRNA to the dorsal–anterior cytoplasm of the oocyte relies on dynein in a two-step process where RNP particles are first transported toward the anterior cortex and subsequently relocalize dorsally (MacDougall et al., 2003). Recent results suggest that the second step in this process, transport to the anterodorsal cortex, may rely on a trapping or anchoring mechanism that is distinct from dynein-dependent anterior-directed transport (Lan et al., 2010). For *bicoid* RNA, tracking of transport in live oocytes has further defined a role for dynein in continually transporting *bicoid* mRNA. Disruption of dynein function results in delocalization of *bicoid* mRNA, suggesting that dynein acts to continuously maintain localization to the anterior pole (Weil et al., 2006). Recent high-resolution electron microscopy studies confirmed an association between dynein and *bicoid* RNA (Weil et al., 2010), and taken together, these data strongly support a role for dynein in transporting *bicoid* mRNA to the minus ends of microtubules in the *Drosophila* oocyte. In *Xenopus* oocytes, dynein is responsible for a highly directional step in the vegetal RNA transport pathway (Gagnon et al., unpublished). In this case, unidirectional transport by dynein precedes bidirectional kinesin-dependent transport and provides a directional cue for polarized RNA transport. Potential mechanisms for coordinating the opposing functions of different classes of molecular motors to yield net directional transport of cargo are discussed below.

Myosin-based RNA transport

The myosin molecular motor superfamily represents a diverse set of genes containing 20 structurally and functionally distinct classes (reviewed in Krendel and Mooseker, 2005). Most myosin motors contain an N-terminal motor domain used for actin binding and ATP hydrolysis, a neck domain required for light chain attachment, and a C-terminal tail domain for cargo binding (reviewed in Rayment and Holden, 1994). Myosin motors (Figure 2A) operate on the actin microfilament cytoskeleton and are best known to control muscle contraction (reviewed in Adelstein and Eisenberg, 1980). More recently, essential non-muscle functions for myosin have been described in cell adhesion, cell motility, signal transduction, and cargo transport (reviewed in Bridgman, 2009; Vicente-Manzanares et al., 2009).

A class V myosin motor, Myo4p, is required for transport of mRNAs in the budding yeast *Saccharomyces cerevisiae*. One such mRNA, *ASH1* mRNA, provides arguably the best-understood mechanism of mRNA localization (Figure 1D). Ash1p is a transcription factor that represses mating type switching in the daughter cell (but not the mother) by blocking expression of HO endonuclease (reviewed in Gonsalvez et al., 2005). Polarized expression of Ash1p is restricted to the daughter cell of the budding yeast by active localization of *ASH1* mRNA (Long

et al., 1997). *ASH1* mRNA localization requires She2p, an RNA-binding protein, that binds to She3p, which then recruits the Myo4p (Jansen et al., 1996; Long et al., 1997, 2000; Böhl et al., 2000). This motor-containing RNP complex transports on the actin cytoskeletal network to the daughter cell (reviewed in Gonsalvez et al., 2005).

Myosin-based transport is also implicated in localization of β-actin mRNA to the leading edge of vertebrate fibroblasts, where early studies demonstrated a requirement for microfilaments (Sundell and Singer, 1991). Both knockout of myosin II-B and inhibition of myosin ATPase activity (Latham et al., 2001) disrupt localization of β-actin to the leading edge of mouse embryonic fibroblasts, providing evidence of a direct role for myosin motors. Myosin motors are also involved in transport of *oskar* mRNA in *Drosophila* oocytes, as Myosin-V has been shown to be necessary for short-range transport at the posterior pole of the oocyte (Krauss et al., 2009). Intriguingly, Myosin-V appears to interact with KHC, suggesting interplay between microtubule- and microfilament-based motors.

Coordination between motors

Many cargos are transported bidirectionally in cells, yet ultimately result in polarized transport. How bidirectional transport, presumably controlled by differential motor activity, is coordinated during transport of cargos remains poorly understood. It is evident that dynein and kinesin activities are often tightly coupled, as disruption of either motor in many systems decreases transport in both directions (Waterman-Storer et al., 1997; Martin et al., 1999; Deacon et al., 2003; Ling et al., 2004; Ally et al., 2009; Uchida et al., 2009). Some evidence also hints that dynactin may be able to bridge interactions between dynein and kinesin motors (reviewed in Kardon and Vale, 2009). Dynactin can interact with kinesin-2 and appears to increase processivity of kinesin-2 through microtubule binding (Deacon et al., 2003; Berezuk and Schroer, 2007). An interaction between dynactin and kinesin-5 has also been reported, though it is unclear whether this represents motor coordination or simply transport of kinesin-5 by dynein (Blangy et al., 1997; Uteng et al., 2008).

One model of cargo trafficking proposes that both motors can be present during transport and that their functions are regulated at the level of motor engagement (reviewed in Welte, 2004; Gross et al., 2007). This regulation can occur by modulating the on/off rate of motor to the cytoskeleton, or via binding partners or post-translational modification of motors. Indeed, transport of axonal prion protein vesicles involves stably bound motors of opposing directionality to both stationary and motile vesicles, suggesting that regulation of motor function, not simply motor association, may dictate transport directionality (Encalada et al., 2011). An alternative hypothesis, the "tug of war," states that motors fight over cargos, and the strongest force wins. One set of modeling studies has produced complex and processive transport behaviors from a tug of war mechanism, without the need to invoke regulation of motor activity, by emphasizing the quick resolution of the non-productive tug of war state (Müller et al., 2008, 2010; reviewed in Welte and Gross, 2008). The tug of war model is supported by experiments in which high-resolution analysis of vesicle transport demonstrated that the stoichiometric ratio of kinesin-2 and dynein can dictate vesicle directionality (Hendricks et al., 2010).

Transport of RNA may involve similar mechanisms to modulate transport directionality. Studies of *hairy* mRNA transport in the *Drosophila* embryo suggest that the localization signals on the RNA itself bias transport by recruiting extra motor molecules (Bullock et al., 2006). In these studies, live cell particle tracking demonstrated that the *hairy* localization element dictates a bias toward long runs in the minus-end direction, whereas non-localized mRNAs exhibit short and non-productive bidirectional runs (Bullock et al., 2006). This effect also requires the proteins now known to directly link RNA transcripts to the dynein motor; more of these factors are recruited to localized RNAs than to non-localized RNAs (Bullock et al., 2006; Dienstbier et al., 2009). A different model has emerged from studies of *oskar* mRNA localization. *Oskar* mRNP particle tracking in live *Drosophila* oocytes revealed that the vast majority of *oskar* transcripts move toward the plus ends of microtubules, consistent with the established role of kinesin-1 in *oskar* mRNA transport (Brendza et al., 2000; Zimyanin et al., 2008). However, only a slight bias in overall particle directionality toward the posterior of the oocyte was detected, possibly because microtubule orientation in the fly oocyte is only weakly biased toward the posterior (Zimyanin et al., 2008). Importantly, this directionality bias was reversed in mutants known to affect localization of *oskar* mRNA, suggesting that factors that bind *oskar* mRNA could promote kinesin recruitment by blocking access of the dynein motor complex (Zimyanin et al., 2008). Future studies in diverse systems promise to test whether the models presented above are widely applicable to directional RNA transport. Ultimately, single-molecule analysis of cargo transport may be necessary to elucidate the mechanisms of motor coordination (reviewed in Holzbaur and Goldman, 2010; Veigel and Schmidt, 2011).

Linking RNA cargos to motors

Although the molecular links between *ASH1* mRNA and the myosin-V motor, Myo4p (Jansen et al., 1996; Long et al., 1997, 2000; Böhl et al., 2000), and between dynein and the *Drosophila* pair-rule mRNAs (Bullock and Ish-Horowicz, 2001; Navarro et al., 2004; Dienstbier et al., 2009) are best understood, remarkably little evidence is available in most systems to directly connect the factors involved in RNA recognition and binding to molecular motors. A general model suggests that RNA-binding proteins recognize localization elements, usually in the 3′ UTR, and interact with binding adapters to

connect molecular motors to localized RNAs (reviewed in St. Johnston, 2005). Although the identities of the binding factors and adapters remain largely unknown, the *ASH1* and pair-rule mRNAs may provide important clues for other systems. These examples illustrate the unique and complex interactions that are necessary in order to assemble an mRNP that is fully competent to be localized.

Analysis of *ASH1* mRNA localization in budding yeast has provided a detailed molecular view of the links between motors, the cytoskeleton, and cargo (Figure 3A). Initially, an RNA-binding protein, She2p, recognizes *ASH1* mRNA through sequence elements in both the coding region and the *ASH1* 3′ UTR and recruits the motor protein Myo4p via the adapter protein She3p (Jansen et al., 1996; Long et al., 1997, 2000; Böhl et al., 2000). Myo4p subsequently transports the complex on the actin cytoskeleton to the bud tip of the daughter cell. When purified from yeast extracts, Myo4p is a non-processive monomeric motor (Reck-Peterson et al., 2001; Dunn et al., 2007; Heuck et al., 2007), initially raising questions about its ability to transport *ASH1* mRNA to the bud tip. However, Myo4p can form larger multi-motor complexes and engage in processive transport *in vivo* (Dunn et al., 2007; Heuck et al., 2007).

Several models have emerged to address how Myo4p forms these larger complexes that allow processive transport. Experimental dimerization of Myo4p results in more stable cargo binding and increased cargo transport (Heuck et al., 2007), supporting a model in which the adapter protein She3p may link multiple motors to a single RNA cargo to increase efficiency of transport. Extensive mapping of the Myo4p domains required for interaction with She3p revealed a crucial region in the middle of the Myo4p amino acid sequence, alternatively termed the rod or linker domain, which is necessary for She3p binding *in vitro* and correct localization of *ASH1* mRNA *in vivo* (Hodges et al., 2008; Bookwalter et al., 2009; Heuck et al., 2010). However, some controversies remain over the requirement for the C-terminal globular tail of Myo4p in binding of She3p and proper localization of *ASH1* mRNA (Bookwalter et al., 2009; Heuck et al., 2010). She3p binding to Myo4p could inhibit Myo4p self-dimerization, but an alternate model has been proposed in which She3p does not act to dimerize Myo4p. Instead, multiple copies of the Myo4p/She3p/She2p/*ASH1* mRNP could be formed into larger particles, which would contain multiple motors to promote processivity (Bookwalter et al., 2009). A third model posits that since She2p binds *ASH1* mRNA as a tetramer, it could act to bring multiple copies of Myo4p to a single RNA cargo (Chung and Takizawa, 2010). Finally, the *ASH1* RNA contains multiple localization elements that could act to multimerize Myo4p (reviewed in Gonsalvez et al., 2005). In the absence of She2, artificially tethering increasing numbers of Myo4p/She3p to *ASH1* RNA improves the efficiency of localization to the bud tip, reinforcing the

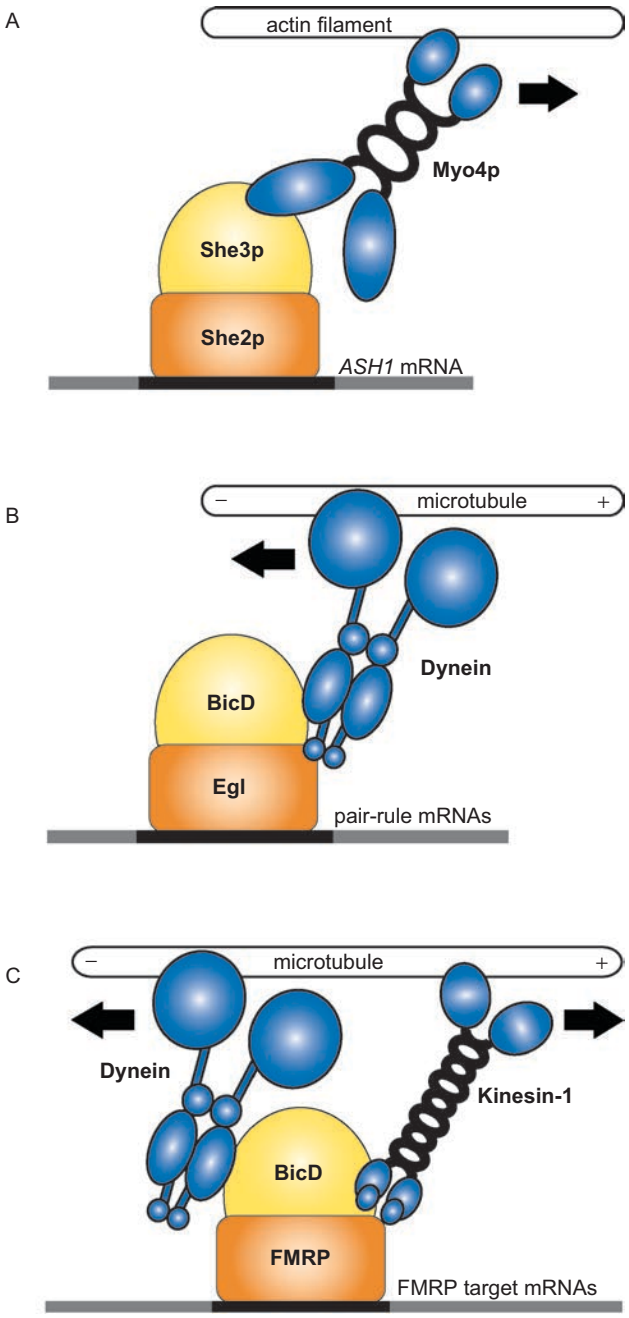

Figure 3. Links between localized RNAs and molecular motors. (A) The *ASH1* mRNA localization complex in budding yeast. Elements in the *ASH1* mRNA are recognized by the RNA-binding protein She2p. She3p functions as an adapter by binding both Myo4p and She2p to connect *ASH1* mRNA to the myosin motor for transport to the barbed ends of actin microfilaments. (B) Localization of pair-rule mRNAs in *Drosophila*. Egalitarian (Egl) recognizes RNA motifs in the 3′ UTR of pair-rule mRNAs and is bound by BicaudalD (BicD). Both Egl and BicD interact with the dynein motor, via the DLC and DIC, respectively. Dynein acts to transport the RNP cargo to the minus ends of microtubules. (C) Suggested interactions with FMRP target RNAs. RNAs recognized by the RNA-binding protein FMRP may be coupled to dynein and kinesin motors either directly or indirectly through the adapter protein BicD, for transport on microtubules. Dynactin is omitted from this figure for simplicity.

hypothesis that multiple Myo4p motors act together to achieve processive transport of mRNA cargoes to the bud tip (Chung and Takizawa, 2010). Although the details of this molecular link between cargo and motor are yet to be resolved, further biochemical studies in yeast promise to tackle these questions. The requirement for myosin in transport of mRNAs in vertebrate cells (Latham et al., 2001) and, more recently, in *Drosophila* oocytes (Krauss et al., 2009) suggests that actin microfilament-based transport mechanisms are widespread in eukaryotes.

The molecular linkage between dynein and RNA cargos was first revealed by genetic evidence demonstrating a requirement for the binding partners BicD and Egl in mediating transport of pair-rule RNAs in *Drosophila* embryos (Mach and Lehmann, 1997; Bullock and Ish-Horowicz, 2001). Both factors are also recruited to exogenously added RNAs before transport and remain bound during transport, suggesting a direct role for BicD and Egl in coupling cargo to the dynein motor (Bullock and Ish-Horowicz, 2001). Egl also directly interacts with dynein light chain (DLC), suggesting a direct molecular link to the dynein complex (Navarro et al., 2004), though it is not clear how DLC could simultaneously bind both Egl and dynein intermediate chain, as crystallographic data suggest that they bind the same domain of DLC (Williams et al., 2007). Until recently, it remained unknown how BicD and Egl were connected to localized RNA, as neither factor displayed obvious RNA-binding domains. The molecular mechanism (Figure 3B) has now been elucidated through the work of Bullock and coworkers who conclusively demonstrated that Egl directly and specifically binds localized mRNAs via a non-canonical RNA-binding domain and recruits the dynein transport complex via a direct interaction with BicD (Dienstbier et al., 2009). Further experiments will likely clarify the roles of the dynactin complex and DLC in pair-rule mRNA localization.

Transport of *ASH1* and pair-rule mRNAs by Myo4p and dynein, respectively, exemplifies molecular mechanisms for which factors have been identified that specifically recognize a localized mRNA and attach it to a molecular motor for transport. Less clear are the molecular links between motors and RNA cargos in other systems. One potential player is FMRP, an RNA-binding protein extensively implicated in mRNA localization in *Drosophila* and mammalian neurons (Figure 3C). Knockdown of dynein heavy chain (DHC) and KHC in *Drosophila*-cultured cells decreases FMRP RNP granule motility, suggesting that FRMP-bound cargoes are transported by both classes of microtubule-based motors (Ling et al., 2004). Although FMRP interacts with a large number of RNAs (reviewed in Penagarikano et al., 2007), evidence that it is directly responsible for RNA transport rather than aspects of RNA metabolism remains uncertain. Recently, more sensitive and quantitative imaging methods, including single particle tracking in live cells, have revealed a connection between FMRP and RNA trafficking in mammalian neurons (Dictenberg et al., 2008). As predicted, FMRP interacts biochemically with kinesin and DHC (Ling et al., 2004; Dictenberg et al., 2008), perhaps directly in the case of kinesin (Davidovic et al., 2007). Complicating matters, however, FMRP appears to have differing mechanisms of action in different systems. RNAi knockdown of KLC, the canonical cargo adapter, has no effect on FMRP transport in *Drosophila* S2 cells (Ling et al., 2004), suggesting that KLC is dispensable and that another cargo adapter connects FMRP cargoes to the kinesin motor. In contrast, FMRP interacts with KLC in mammalian tissue culture cells, and a dominant-negative KLC significantly reduces FMRP transport rates in neurons (Dictenberg et al., 2008). *Drosophila* FMRP also interacts with BicD, potentially linking it to dynein for transport (Bianco et al., 2010). As BicD can interact with both kinesin and dynein subunits, and in some cases may regulate transport by influencing motor function (Larsen et al., 2008), it represents a likely candidate as an adapter that can modulate interplay between motors of opposing directionality. Studies of FMRP have given us many insights and illuminated new potential mechanisms for assembly of localizing RNPs. Combined with work in other systems, we are beginning to put together a more complete picture of the different possibilities for linking motors and RNAs.

Staufen is a double-strand RNA-binding protein first identified in *Drosophila* oocytes as necessary for transport of both *bicoid* mRNA to the anterior pole and *oskar* mRNA to the posterior (St. Johnston et al., 1991), and subsequently implicated in RNA transport in a variety of systems. In *Xenopus* oocytes, disruption of Staufen function through dominant-negative approaches abolishes Vg1 mRNA localization (Yoon and Mowry, 2004), and evidence that kinesin-1 co-sediments and co-immunoprecipitates with Staufen in *Xenopus* oocytes led to the hypothesis that Staufen recruits kinesin-1 to the Vg1 mRNP in the cytoplasm, where it associates with microtubules to direct transport to the vegetal pole (Yoon and Mowry, 2004; Messitt et al., 2008). In mammalian cells, Staufen has been shown to play a role in dendritic RNA transport (Tang et al., 2001; Vessey et al., 2008) and both dynein intermediate chain and KHC are components of Staufen-containing complexes (Villacé et al., 2004). However, evidence of a direct role for Staufen in coupling motors with cargo RNPs remains elusive, and high-resolution imaging approaches have revealed that in *Drosophila* oocytes, Staufen co-localizes with *oskar* mRNA only at late stages of transport (Mhlanga et al., 2009). Thus, Staufen is perhaps more directly involved in RNA anchoring and translation. Further investigations into endogenous interactions between RNA-binding proteins such as FMRP and Staufen, motor subunits, and target RNAs may yet clarify these mechanisms of action.

Microtubule organization and polarized transport

The selective linking of cargo to molecular motors is not the only level of control for directed transport of mRNPs

along microtubules. The organization and reorganization of the microtubule network also plays an active role in RNA localization, and many factors are known to mediate microtubule dynamics. Microtubule-associated proteins (MAPs) are thought to be the primary modulators of microtubule growth and stability (reviewed in Hirokawa, 1994). Additionally, microtubules can be altered by post-translational modifications, including phosphorylation, acetylation, tyrosination, polyglutamylation, and polyglycylation (reviewed in Redeker, 2010). Although the functional consequences of tubulin modifications are largely unknown, in some cases they have been shown to alter microtubule function (reviewed in Westermann and Weber, 2003) and can be spatially restricted to subcellular regions (Janke and Kneussel, 2010). Indeed, a genome-wide screen for RNAs that localize to pseudopodial protrusions in mouse fibroblasts revealed a population of RNAs associated with the plus ends of detyrosinated microtubules (Mili et al., 2008). Modifications to microtubules could also modulate interactions with molecular motors, providing a signal for transport, or could preferentially stabilize a subpopulation of microtubules.

Polarized subpopulations of microtubules are likely to play important roles in directional RNA transport. For example, a subpopulation of microtubules with plus ends oriented toward the vegetal cortex emerges prior to localization of Vg1 mRNA to the vegetal cortex in *Xenopus* oocytes (Messitt et al., 2008). Transport by plus end-directed kinesin motors is preceded by a dynein-dependent step in the vegetal RNA transport pathway (Gagnon et al., Submitted), and it is possible that the establishment of this subpopulation of microtubules triggers vegetal localization of Vg1 mRNA (Messitt et al., 2008). In addition, during the middle stages (7–10) of *Drosophila* oogenesis, microtubules are polarized with minus ends at the anterior and plus ends at the posterior (Clark et al., 1994), resulting in dynein-dependent transport of *gurken* RNA to the anterior pole (MacDougall et al., 2003). A distinct network of microtubules associated with the oocyte nucleus may then mediate the second step of *gurken* mRNA localization, during which *gurken* mRNA is enriched at the dorsoanterior pole of the oocyte (MacDougall et al., 2003). Recent work has dissected these two steps and refined the model, suggesting that an anchoring or trapping mechanism mediates the second step of localization (Delanoue et al., 2007; Jaramillo et al., 2008; Lan et al., 2010). Thus, both Vg1 mRNA in *Xenopus* oocytes and *gurken* mRNA in *Drosophila* oocytes localize in multi-step processes dependent on discrete and polarized microtubule arrays that emerge during development. These examples point to organization of the cytoskeleton as an active regulator in spatial and temporal control of RNA transport.

Molecular links between RNA transport and anchoring

A key feature of most RNA transport pathways is a requirement for anchoring of the RNA at its final destination, but little is known about this critical step in the RNA transport pathway. Perhaps surprisingly, molecular motors appear to have a role in this process as well. Anchoring of pair-rule transcripts in the *Drosophila* embryo requires microtubules, but not actin, and involves a novel transition in dynein activity from RNA transport to static anchor (Delanoue and Davis, 2005). Transport of *ftz* mRNA by dynein requires the co-factors BicD and Egl (Bullock and Ish-Horowicz, 2001). Interestingly, dynein is also required for anchoring *ftz* mRNA (Delanoue and Davis, 2005). After transport, microinjection of function-blocking antibodies specific for BicD and Egl no longer interferes with anchoring, suggesting that a transition in the mechanism by which dynein and cargo interact with each other. Further support for this is provided by results showing that an ATPase inhibitor that eliminates dynein-dependent transport has no effect on its role as a static anchor (Delanoue and Davis, 2005). An analogous situation has been observed in *gurken* anchoring in the *Drosophila* oocyte, where the RNA-binding protein Squid is proposed to mediate the transition between active transport and anchoring by dynein (Delanoue et al., 2007). Since most proposed anchoring mechanisms posit a handoff from the transport machinery to the anchoring machinery, these data reveal that both cargo transport and anchoring can use the same molecular motor. Interestingly, a myosin motor may play a role in anchoring *oskar* mRNA at the posterior pole. Oocytes with null mutations in the myosin-V motor fail to accumulate *oskar* mRNA at the posterior cortex; instead, *oskar* mRNA often remains near but not tightly associated with the cortex (Krauss et al., 2009), suggesting that myosin-V could mediate anchoring of *oskar* mRNA. The next challenge lies in determining if these functions are truly universal or if they are specific to individual systems.

Conclusions and perspectives

The simple concept of a single motor protein being responsible for transport of cargo is increasingly being replaced with more complex models in which multiple motor proteins are bound to a single cargo enhancing processivity and directional transport (reviewed in Holzbaur and Goldman, 2010). This new model is likely applicable to RNA localization, as accumulating evidence points to roles for multiple molecular motors, regulation of motor activity, and stoichiometry in mediating RNA transport. It is possible that large, highly polarized cells require coordinated motor activities to ensure cargo delivery to defined cytoplasmic domains. Although much has been discovered about the motors and other factors that control RNA localization, many open questions remain. Further detailed analyses of the processes of recognition, complex assembly, directional transport, and anchoring of localized RNAs will require complex and integrative biochemical, cell biological, and imaging approaches—no easy task—but promise invaluable insight into the critical events in the establishment and maintenance of cellular asymmetry via the spatial regulation of gene expression.

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Declaration of interest

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