In vitro reconstitution of an mRNA-transport complex reveals mechanisms of assembly and motor activation

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The assembly and composition of ribonucleic acid (RNA)-transporting particles for asymmetric messenger RNA (mRNA) localization is not well understood. During mitosis of budding yeast, the Swi5p-dependent HO expression (SHE) complex transports a set of mRNAs into the daughter cell. We recombinantly reconstituted the core SHE complex and assessed its properties. The cytoplasmic precomplex contains only one motor and is unable to support continuous transport. However, a defined interaction with a second, RNA-bound precomplex after its nuclear export dimerizes the motor and activates processive RNA transport. The run length observed in vitro is compatible with long-distance transport in vivo. Surprisingly, SHE complexes that either contain or lack RNA cargo show similar motility properties, demonstrating that the RNA-binding protein and not its cargo activates motility. We further show that SHE complexes have a defined size but multimerize into variable particles upon binding of RNAs with multiple localization elements. Based on these findings, we provide an estimate of number, size, and composition of such multimeric SHE particles in the cell.

Introduction

mRNA localization by motor-containing transport particles is a widespread mechanism to control gene expression on a spatial and temporal level (Martin and Ephrussi, 2009; Jansen and Niessing, 2012). In higher eukaryotes, such complexes consist of dozens of factors that interact in a largely unknown fashion, resulting in RNA–protein complexes that are actively transported (Marchand et al., 2012). Because of their great complexity, only little information about principles of complex assembly and regulation are available. To date, the most detailed information has been obtained for the yeast Saccharomyces cerevisiae (Heym and Niessing, 2012).

During mitotic cell division of S. cerevisiae, ASH1 (asymmetric synthesis of HO 1) mRNA is actively transported to the daughter cell (Long et al., 1997; Takizawa et al., 1997). Localized translation of the protein Ash1p leads to repression of mating type switching exclusively in the daughter cell (Bobola et al., 1996; Sil and Herskowitz, 1996). The Swi5p-dependent HO expression (SHE) complex mediates the active transport of ASH1 mRNA and ~30 other transcripts (Takizawa et al., 2000; Shepard et al., 2003; Aronov et al., 2007; Oeffinger et al., 2007; Hogan et al., 2008). In contrast to localizing particles in higher eukaryotes, the core SHE-transport complex consists of only a few components: the RNA-binding proteins She2p and She3p and the type-V myosin Myo4p (Jansen et al., 1996; Böhl et al., 2000; Chung and Takizawa, 2010). Assembly of the SHE complex occurs already cotranscriptionally with the binding of She2p to nascent mRNAs (Shen et al., 2010; Müller et al., 2013). This article is distributed under the terms of an Attribution-Noncommercial-Share Alike-No Mirror Sites license for the first six months after the publication date (see http://www.rupress.org/terms). After six months it is available under a Creative Commons License (Attribution-Noncommercial-Share Alike 3.0 Unported license, as described at http://creativecommons.org/licenses/by-nc-sa/3.0/)

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After nuclear export, this subcomplex further assembles with She3p and the type-V myosin Myo4p into the mature transport complex (Böhl et al., 2000; Long et al., 2000; Kruse et al., 2002). In this complex, She2p and She3p bind synergistically with high specificity to zip code elements in localizing mRNAs, ensuring the selective transport of target mRNAs (Müller et al., 2011).

Type-V myosins are usually dimeric, which allows for processive movement along actin filaments (Trybus, 2008). In contrast, Myo4p is monomeric and nonprocessive in isolation (Reck-Peterson et al., 2001; Dunn et al., 2007; Heuck et al., 2007; Hodges et al., 2008). Nevertheless, in vivo, the SHE complex supports sustained transport of mRNAs over several micrometers (Bertrand et al., 1998; Beach et al., 1999; Lange et al., 2008).

The crystal structure of She2p revealed a dimeric assembly (Niessing et al., 2004). In solution, two She2p dimers interact to form a tetramer that is required for the recruitment of multiple Myo4p motors (Chung and Takizawa, 2010; Krementssova et al., 2011) and mRNA localization in vivo (Müller et al., 2009). Although one study suggested recruitment of three or more Myo4p motors for processive movement (Dunn et al., 2007; Chung and Takizawa, 2010), a second study reported dimerization of Myo4p in complex with a mutated version of She2p by a mechanism that is not understood to date (Krementssova et al., 2011). It also remains unclear whether the SHE complex forms well-defined or variable molecular arrangements. A major limitation toward such an understanding is that the role of cargo mRNA for mRNP assembly and motor function is not well understood. Furthermore, all previous studies assumed that She3p is a monomer (Dunn et al., 2007; Heuck et al., 2007; Hodges et al., 2008; Bookwalter et al., 2009; Chung and Takizawa, 2010; Heuck et al., 2010; Krementssova et al., 2011), despite the lack of clear experimental evidence.

To date, the most advanced studies on mRNA-transport complexes either work with particles purified from organisms (Chung and Takizawa, 2010; Amrut-Nayak and Bullock, 2012) or involve partial complexes that lack RNA cargo and have been assembled without knowledge of stoichiometric requirements (Krementssova et al., 2011). Studying mRNP assembly of mature RNA-containing complexes under well-defined conditions in vitro would yield stoichiometric ratios and molecular masses of such complexes. It would resolve the question of whether such a complex has a defined molecular mass and, if not, which components control its size. Even more importantly, these insights would explain what effect cargo RNA has on the assembly and activity of a transport complex. To date, these questions remain largely unresolved.

We present a complete stoichiometric in vitro reconstitution of a functional mRNA-transport complex. Determination of size and stoichiometry of the SHE complex revealed that two Myo4p motors associate with one RNA-bound She2p tetramer via two She3p dimers to form a defined complex of almost 1 MD in size. This reconstituted complex processively transports RNA along actin filaments in vitro with a run length that is consistent with long-distance transport in vivo. Whereas the absence of RNA does not greatly alter complex motility, She2p is essential for activation of motility. When investigating the effect of cargo RNA with multiple zip code elements, we observed clustering of SHE complexes into large assemblies. These multimeric particles show motilities very similar to single zip code RNA-containing particles. By combining known cellular copy numbers of SHE factors and our results on complex assembly, we provide an estimate on the number, molecular mass, and composition of SHE particles in the cell.

### Results

**She3p oligomerizes via its N terminus**

Previous studies showed that isolated Myo4p is a monomer (Dunn et al., 2007; Heuck et al., 2007; Hodges et al., 2008) and She2p is a tetramer in solution (Müller et al., 2009; Chung and Takizawa, 2010). The oligomeric state of She3p has not been addressed so far. We used bacterially expressed N- and C-terminal fragments of She3p and baculovirus-expressed full-length She3p to investigate its oligomeric state by chemical cross-linking with 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide (EDC) and N-hydroxysuccinimide (NHS; Fig. S1, A and B). This experiment revealed oligomerization of She3p via its N-terminal half (amino acids 1–234; Fig. S1 B). In contrast, the C terminus of She3p (amino acids 274–425) was not cross-linked. Also, the C terminus of bacterially expressed monomeric Myo4p (Myo4p-C) failed to show cross-linking (Fig. S1 A). Although the exact determination of oligomeric states by cross-linking has to be considered with caution, the migration of the main cross-linking product of She3p in SDS-PAGE suggests the formation of a She3p dimer.

**One She3p dimer and one Myo4p monomer form a stable complex**

For efficient cargo transport, the monomeric Myo4p requires oligomerization (Chung and Takizawa, 2010; Krementssova et al., 2011). In the cytoplasm, Myo4p forms a stable, high affinity complex with She3p (Heuck et al., 2007; Hodges et al., 2008). Hence, a She3p dimer might provide a way to promote such an oligomerization of Myo4p.

To test this possibility, we determined the stoichiometry and the molecular mass of the She3p:Myo4p-C complex. Isothermal titration calorimetry experiments indicated a stoichiometric ratio of two She3p molecules to one Myo4p-C molecule (Fig. 1 A). A titration by size-exclusion chromatography (SEC) confirmed this result (Fig. S1 C). Quantitative mass spectrometry with heavy isotope-labeled peptides yielded a stoichiometric ratio of 2.56 ± 0.11 for She3p:Myo4p-C (Fig. 1 B). These experiments indicate a stoichiometric ratio of 2:1 for the She3p:Myo4p-C complex and suggest a She3p dimer or multimers thereof in the co-complex.

In line with previous studies (Dunn et al., 2007; Heuck et al., 2007; Hodges et al., 2008), molecular mass determination by static light scattering (SLS) confirmed that recombinant Myo4p-C is a monomer (Fig. 1 C and Table 1). SLS with the complex of She3p and Myo4p-C yielded a molecular mass of 160.2 ± 3.6 kD (Fig. 1 D and Table 1) and suggests a complex of one She3p dimer and one Myo4p monomer. Thus, oligomerization of Myo4p does not occur at this step of assembly.
Figure 1. **Stoichiometries and molecular masses of SHE subcomplexes.** (A) Isothermal titration calorimetry with She3p titrated into Myo4p revealed a stoichiometry of 1.93 ± 0.20 (mean and standard deviation of two experiments) for the She3p:Myo4p complex. (B) Quantitative mass spectrometry with heavy isotope-labeled peptides showed a stoichiometry of 2.56 ± 0.11 (mean and standard deviation of two independent experiments) for the She3p:Myo4p complex. The amount of She3p was normalized with respect to Myo4p. (C) SLS profile of Myo4p shows that this protein is monomeric. RI, refractive index; RALS, right angle light scattering; MM, fitted molecular mass. (D) SLS profile of the She3p:Myo4p complex. Note that a slight excess of Myo4p was used to ensure saturation of the complex. (E) SLS profile of the ASH1-E3-77-tRNA:She2p complex.
One She2p tetramer binds two zip code RNAs

In the nucleus, She2p preassembles with ASH1 mRNA (Kruse et al., 2002; Shen et al., 2010; Müller et al., 2011). Efficient mRNA binding and localization require the formation of She2p tetramers (Müller et al., 2009; Chung and Takizawa, 2010).

As different stoichiometric ratios have been suggested for She2p RNA complexes (Niessing et al., 2004; Olivier et al., 2005), we determined the molecular mass of bacterially expressed She2p in complex with a 77-nucleotide ASH1-E3 zip code RNA fused to tRNA (ASH1-E3-77-tRNA) by SLS. The tRNA tag was used to produce large amounts of stable RNA (Ponchon and Dardel, 2007). The measured molecular mass suggests that one She2p tetramer binds two ASH1-E3 RNAs (Fig. 1 E and Table 1), which is in agreement with a previous study (Niessing et al., 2004). We also observed complex formation between two zip code elements and one She2p tetramer with a 51-nucleotide-long ASH1-E3 RNA lacking the tRNA tag (Fig. S1 D) and with the IST2 zip code RNA fused to tRNA (Fig. S1 E). In summary, the data indicate that the ASH1-E3 and the IST2 zip code elements bind the She2p tetramer with the same stoichiometry.

Assembly of the SHE core complex induces Myo4p dimerization

When the RNA:She2p subcomplex reaches the cytoplasm, it associates with the single-headed subcomplex of She3p and Myo4p. We reasoned that Myo4p oligomerization is likely to occur during this final step of assembly. To determine the protein stoichiometries in the SHE core complex, we performed quantitative mass spectrometry with heavy isotope-labeled peptides. The stoichiometric ratio between She3p and Myo4p-C was 2.12 in a SHE core complex reconstituted with ASH1-E3-51 RNA and 2.23 in a SHE core complex reconstituted with ASH1-E3-77-tRNA (Fig. 2 A). Thus, the stoichiometry between She3p and Myo4p-C in the SHE core complex is the same as in the She3p:Myo4p-C subcomplex (compare with Fig. 1, A and B).

We performed electrophoretic mobility shift assays (EMSAs) to investigate the stoichiometry of the assembly between the RNA:She2p and the She3p:Myo4p-C subcomplex (Fig. 2 B). A constant amount of 63-nM unlabeled ASH1-E3-77:She2p (2:4) subcomplex was titrated with increasing amounts of the She3p:Myo4p-C (2:1) subcomplex. In a control experiment,

Table 1. Analysis of complex assemblies by SLS

<table>
<thead>
<tr>
<th>Sample</th>
<th>MM exp. (kD)</th>
<th>MM calc. (kD)</th>
<th>Copy no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myo4p-C</td>
<td>54.9 ± 1.1</td>
<td>56.7</td>
<td>1</td>
</tr>
<tr>
<td>She3p/Myo4p-C</td>
<td>160.2 ± 3.6</td>
<td>153.2</td>
<td>2/1</td>
</tr>
<tr>
<td>ASH1-E3-51/She2p</td>
<td>139.2 ± 8.0</td>
<td>147.2</td>
<td>2/4</td>
</tr>
<tr>
<td>ASH1-E3-77-tRNA/She2p</td>
<td>196.3 ± 5.1</td>
<td>211.8</td>
<td>2/4</td>
</tr>
<tr>
<td>IST2-tRNA/She2p</td>
<td>176.6 ± 2.6</td>
<td>202.1</td>
<td>2/4</td>
</tr>
<tr>
<td>ASH1-E3-77-tRNA/She2p/She3p/Myo4p-C</td>
<td>502.8 ± 9.8</td>
<td>518.2</td>
<td>2/4/4/2</td>
</tr>
</tbody>
</table>

Molecular masses from experimental SLS analysis (MM exp.) compared with calculated molecular masses (MM calc.) for complexes with the indicated copy number of proteins and RNAs. Data represent the means and standard deviation of at least three experiments with varying sample concentrations. Calculated theoretical molecular masses of the monomers are as follows: 56.7 kD (Myo4-C), 48.2 kD (She3p), 28.7 kD (She2p), 16.3 kD (ASH1-E3-51), 48.7 kD (ASH1-E3-77-tRNA), and 43.7 kD (IST2-tRNA).
She2p on its own was unable to induce a mobility shift (Fig. 2 B, lane 1), whereas high concentrations of She3p:Myo4p-C in the absence of She2p resulted in a strong shift to high-molecular mass complexes (Fig. 2 B, lane 9, asterisk). We observed similar unspecific RNA binding of She3p in the absence of She2p in a previous study (Müller et al., 2011). At a ratio of two She3p:Myo4p-C subcomplexes to one RNA-bound She2p tetramer, a single band appeared (Fig. 2 B, lane 6, double arrow), indicating the formation of a homogeneous, stoichiometric complex. At a lower titration concentration, at which ASH1-E3-77:She2p was not yet saturated with She3p:Myo4p-C, an additional band emerged, indicating formation of a smaller complex (Fig. 2 B, lanes 3–5, single arrow). It is likely that this complex consists of a She2p tetramer that is bound by only one She3p:Myo4p-C subcomplex, leaving one binding site of She2p free. In contrast, when She3p:Myo4p-C was added in excess, a higher molecular mass band appeared at the size of the unspecific RNA binding observed with She3p:Myo4p-C alone (Fig. 2 B, lanes 7–9, asterisk). Control EMSAs suggest that this increase in size is not simply caused by concentration-dependent aggregation but rather reflects unspecific binding of excess She3p:Myo4p-C subcomplexes to the RNA in the SHE core complex (Fig. S2). In summary, the EMSA analyses suggest that two She3p:Myo4p-C subcomplexes associate with one RNA-bound She2p tetramer to form a SHE core complex with defined size. This complex between ASH1-E3-77, She2p, She3p, and Myo4p has a stoichiometric ratio of 1:2:2:1 and most likely consists of two ASH1-E3-77 RNAs, a She2p tetramer, two She3p dimers, and two Myo4p monomers.

To validate the EMSA titration, we performed SEC experiments. In the first experiment, equimolar amounts of She2p and She3p were incubated with concentrations of ASH1-E3-51 RNA and Myo4p slightly exceeding the determined stoichiometric ratio. This resulted in a homogeneous complex of all the components but without excess of ASH1-E3:She2p (Fig. S3 A). In contrast, if a twofold molar excess of She2p over She3p was used, a large amount of ASH1-E3:She2p was separated from the quaternary SHE core complex (Fig. S3 B). In agreement with the EMSA analysis, these experiments indicate that the SHE core complex is saturated at equimolar amounts of She2p and She3p.

To determine the copy numbers of its components, we measured the molecular mass of the SHE core complex by SLS. We used again the RNA-tagged ASH1-E3-77 RNA for reconstitution. For a SHE core complex consisting of ASH1-E3-77-rRNA, She2p, She3p, and Myo4p-C, a molecular mass of 502.8 ± 9.8 kD was measured (Fig. 2 C and Table 1). This result is consistent with our stoichiometry determination and suggests binding of two She3p:Myo4p-C subcomplexes to one RNA-bound She2p tetramer.

In summary, stoichiometry and molecular mass analyses indicate that the She2p tetramer provides a platform for binding of two zip code RNAs and two She3p:Myo4p-C subcomplexes. These components assemble into a defined SHE core complex of ~500 kD. Considering full-length Myo4p bound by six light chains, a single SHE complex has a molecular mass of 835 kD plus its bound mRNA.

RNAs with two zip code elements induce clustering of SHE core complexes

So far, we characterized the assembly of SHE core complexes with RNAs containing single zip code elements. However, transported mRNAs, such as ASH1, WSC2, or DNM1 mRNA, contain more than one zip code element (Chartrand et al., 1999; Gonzalez et al., 1999; Jambhekar et al., 2005). Because She2p has two binding sites for zip code elements, multi–zip code RNAs and SHE complexes could potentially multimerize into large particles of variable size.

To test this possibility, we designed an RNA construct, in which two ASH1-E3 elements were fused to the tRNA tag via a three-way junction from the Twort intron (Fig. 3 B; Lescoute and Westhof, 2006). This RNA was reconstituted with SHE core complexes and analyzed by dynamic light scattering and sucrose density gradient centrifugation. Compared with a SHE core complex with a single ASH1-E3 zip code (Fig. 3 A), this complex showed a marked increase in the hydrodynamic radius as well as faster sedimentation (Fig. 3 B). When assuming an idealized globular particle shape, this increase in hydrodynamic radius translates into an >100-fold increase of its volume, indicating that a large number of SHE core complexes are cross-linked into one particle. The broad distribution of these complexes in the sucrose gradient (Fig. 3 B, fractions 2–5) further indicates that a range of multimers with variable particle sizes was formed. This behavior is well explained by the association of two bivalent interaction partners. As a control, a tRNA fused to a three-way junction with one ASH1-E3 zip code and one HIV-1 trans-activation response element (TAR) stem loop was used (Fig. 3 C). This RNA behaved similar to ASH1-E3-tRNA (Fig. 3 A). The HIV-1 TAR stem loop fused to tRNA also failed to induce complex formation (Fig. 3 D). In summary, these experiments demonstrate that an RNA containing at least two zip code elements is able to multimerize SHE core complexes. This might provide a way to incorporate several mRNAs and multiple motors into one particle.

Reconstituted SHE complexes move processively along actin in vitro

The incorporation of two Myo4p motors suggests that reconstituted SHE complexes indeed move processively along actin filaments. To test this assumption, we performed in vitro single-particle motility assays. We copurified full-length, FLAG-tagged Myo4p with She3p, She4p, calmodulin (Cmd1p), and myosin light chain (Mlc1p) after baculovirus expression and used this preparation for reconstitution with bacterially expressed GFP-She2p. As cargo RNA, we used a fragment of the ASH1 mRNA containing one E3 zip code element (ASH1-3’; Table S5). Particles were visualized via GFP and total internal reflection fluorescence (TIRF) microscopy (TIRFM).

ASH1-3’ RNA–containing particles were transported with a mean velocity of 0.74 ± 0.20 µm/s (mean and standard deviation) and run length of 2.13 ± 0.89 µm along actin filaments (Fig. 4, A, E, and F; and Video 1). The observed velocity is only moderately higher than published velocities of SHE particles in yeast cells (200–440 nm/s [Bertrand et al., 1998], 546 nm/s [Lange et al., 2008], and 630 nm/s [Beach et al., 1999]). Thus,
RNA cargo is dispensable for processive particle transport

To test the requirement of RNA cargo for processive movement, particles lacking RNA were targeted for TIRFM motility assays by visualizing GFP-labeled She2p. The mean velocity centered around 0.65 ± 0.15 µm/s, whereas the determined mean run length was 2.48 ± 1.02 µm (Fig. 4, B, E, our data are in good agreement with in vivo events. For a subset of these motile particles, photobleaching experiments were performed. As an internal control, we also assessed the bleaching step profiles of She2-GFP protein alone. The results indicate that motile particles with a single zip code element indeed contain only up to four GFP fluorophores and thus a single SHE complex (Fig. S4, A–C).
The presence of RNA contaminations could bind to the She2p:She3p complex and potentially promote motility. To exclude this possibility, we performed TIRFM motility assays with particles containing the GFP-She2p mutant (N36S, R63K), which is defective and F; and Video 2). A comparison of these values with motilities in presence of RNA (Fig. 4, A and F) suggests that RNA cargo does not activate processivity.

Figure 4. Single-particle motility assays with different SHE complexes visualized by TIRFM. (A) Single-particle motility of SHE complexes in the presence of ASH1-3' RNA containing one E3 zip code element. 281 processive runs were analyzed. (B) Single-particle motility assay of the SHE complex in the absence of zip code RNA. 292 processive runs were analyzed. The presence of zip code RNA does not greatly change the motility properties of the SHE complex. (C) Single-particle motility assay of SHE complex with the RNA binding–deficient She2p (N36S, R63K) mutant confirms that the RNA cargo is dispensable for processive movement. 283 processive runs were analyzed. (D) Single-particle motility assays with RNA containing two zip code elements fail to show enhanced processivity. Mean run length and velocity of a tRNA fusion construct with two ASH1-3' zip code elements are even slightly reduced compared with particles with only a single zip code RNA. (E) Box plots representing data distributions for run lengths and velocities of each experiment, except for ASH1-3' and IST2 RNA together. Here, the number of particles is too low for valid statistical analyses. Outliers (open circles, for details see Materials and methods) were determined to confirm the homogeneity in the collected data. Only a small number (n < 28) represent outliers; thus, all the events herein analyzed were considered representative for the respective experimental condition. The bold black lines show the median, the boxed regions indicate the first and third quartiles, and the whiskers above and below show the limits for outliers. (F) Table summarizing results from the different single-particle motility assays. The mean values for run length (left) and velocity (right) are derived from single-exponential and Gaussian fits to the distributions, respectively. All data derive from TIRFM motility assays, in which the GFP from the GFP-She2p fusion protein was visualized (as indicated by the boxed molecule name in the complex descriptions), unless stated otherwise. Besides the motor and SHE proteins, particles were assembled in the presence of calmodulin, myosin light chain, and the myosin chaperone She4p.

and F; and Video 2). A comparison of these values with motilities in presence of RNA (Fig. 4, A and F) suggests that RNA cargo does not activate processivity.

A potential limitation of this interpretation is that residual RNA contaminations from the insect cell purification might still be present in the supposedly RNA-free protein preparations. Thus, such RNA contaminations could bind to the She2p:She3p complex and potentially promote motility. To exclude this possibility, we performed TIRFM motility assays with particles containing the GFP-She2p mutant (N36S, R63K), which is defective
in binding to RNA (Du et al., 2008). Corresponding SHE complexes moved with a mean velocity of 0.62 ± 0.20 μm/s and run length of 2.02 ± 0.69 μm along actin filaments (Fig. 4, C, E, and F; and Video 3). As these values are comparable to wild-type particles, it confirms that RNA per se is dispensable for the processive movement of SHE complexes.

She2p:She3p interaction is essential for motility
A She2p mutant lacking its protruding helix E (She2p [ΔhE]) fails to interact with She3p (Müller et al., 2011). If the interaction between She2p and She3p rather than the cargo RNA is essential for motility, SHE particles containing the She2p (ΔhE) mutant should not move processively along actin filaments. To test this, we reconstituted GFP-She2p (ΔhE)—containing particles and traced their motility via GFP and TIRFM. Indeed, for these complexes, no motility on F-actin was observed under otherwise identical experimental conditions (Fig. 4 F and Video 4). This experiment confirms that the interaction between She2p and She3p is essential for SHE complex motility.

SHE complexes with a two zip code RNA show no increased processivity
ASH1 mRNA has four zip code elements. In vitro, already, two zip code elements multimerize SHE complexes into large particles (Fig. 3). To find out what effect multiple zip codes per RNA have on particle movement, we performed TIRFM motility assays with the 2×ASH1-E3-77-tRNA construct (Fig. 3 B) and GFP-She2p—containing particles. 2×ASH1-E3-77-tRNA particles were on average brighter than observed with a single zip code RNA (Video 5; compare with Video 1). As expected, the here-performed photobleaching experiments show that particles containing 2×ASH1-E3-77-tRNA show twice as many fluorescence intensity populations than particles with ASH1-3′ (Fig. S4, D and E). Along those lines, in photobleaching experiments, over 65% of the 2×ASH1-E3-77-tRNA particles analyzed showed more steps in photobleaching than the majority of particles with a single zip code RNA (30 processive runs were analyzed). The mean velocity of particles with 2×ASH1-E3-77-tRNA was 0.54 ± 0.13 μm/s, and the run length was 1.93 ± 0.68 μm (Fig. 4, D–F). These motility parameters are in close range of particles bound to RNA with a single zip code element (Fig. 4 F). In summary, these data suggest that particles with 2×ASH1-E3-77-tRNA contain oligomerized SHE complexes and that these multimeric particles move with velocities and mean run lengths that are indistinguishable from particles with a single SHE complex.

SHE complexes can simultaneously transport two RNA species
In vivo transport of ASH1 and IST2 mRNA to the bud tip is sometimes accomplished by the same particle (Lange et al., 2008). To find out whether our reconstituted particles can recapitulate this feature, we fluorescently labeled ASH1-E3-77 and the IST2 zip code element with Cy3.5 and Atto488 fluorophores, respectively. Motility assays revealed that some of the motile particles indeed contained both fluorophores (Fig. 4 F and Video 6), indicating that two different RNAs can be transported by the same reconstituted SHE complex particle. Because fluorophores were directly attached to the RNA, this experiment also serves as direct proof that the reconstituted particles indeed transport RNA cargo.

Discussion
Here, we fully reconstituted a functional mRNA-transport complex including all recombinantly expressed core proteins together with RNA in their correct stoichiometries. We show that the individual components assemble with fixed copy numbers to form a defined complex with a molecular mass of ~835 kD plus bound mRNA. This complex processively transports RNA containing a single zip code element with physiological run length and velocity in vitro. We further show that in the presence of RNAs with more than one zip code element, several SHE complexes undergo higher-order assembly into large, polydisperse particles. This finding resolves several previously contradictory findings on Myo4p multimerization (Dunn et al., 2007; Heuck et al., 2007; Bookwalter et al., 2009; Chung and Takizawa, 2010; Kremensova et al., 2011).

Previous assessments of She3p:Myo4p complexes assumed a monomeric She3p (Dunn et al., 2007; Hodges et al., 2008). Our chemical cross-linking experiments suggest that She3p dimerizes via its N terminus, where a predicted coiled-coil region of >100 amino acids could potentially mediate dimerization. In this respect, She3p is reminiscent of organelle adapter proteins of type-V myosins, which often contain coiled coils and dimerize (Li and Nebenführ, 2008).

Myo4p forms a constitutive complex with She3p (Dunn et al., 2007; Heuck et al., 2007; Hodges et al., 2008). One study suggested that this subcomplex contains only one myosin (Hodges et al., 2008), whereas another study showed that artificial dimerization of Myo4p stabilizes its interaction with She3p, suggesting dimerization of Myo4p in this subcomplex (Heuck et al., 2007). In a third study, chemical cross-linking suggested a complex of one Myo4p and one She3p (Dunn et al., 2007). Here, we used quantitative interaction studies to show that one She3p dimer binds to one Myo4p monomer, forming a single-headed motor complex (Fig. 5 B). This finding suggests that the She3p:Myo4p subcomplex alone is unable to support processive movement. Indeed, we found that SHE complexes with the She2p (ΔhE) mutant, in which She2p and the She3p:Myo4p subcomplex fail to interact, do not show any processive movement (Video 4).

She2p assembles into a tetramer with two large, continuous RNA-binding surfaces on opposite sides (Müller et al., 2009). Because different stoichiometric ratios for RNA:She2p complexes have been suggested for different zip code elements (Niessing et al., 2004; Olivier et al., 2005), we measured the molecular mass of She2p in complex with ASH1-E3 zip code RNA. Our results suggest binding of two zip code elements to one She2p tetramer (Fig. 5 A), which is consistent with the stoichiometry suggested by us in an earlier study (Niessing et al., 2004).

When the subcomplex of ASH1 mRNA and She2p reaches the cytoplasm, it interacts with the preassembled She3p:Myo4p
subcomplex to form the mature transport complex (Kruse et al., 2002; Müller et al., 2011). Our analyses of the size and stoichiometry suggest that one RNA-bound She2p tetramer recruits two She3p:Myo4p subcomplexes (Fig. 5 C). Thus, the She2p tetramer provides a scaffold for dimerization of Myo4p. This result is consistent with the previous finding that a single transport complex purified from yeast cells contains at least two Myo4p molecules (Chung and Takizawa, 2010).

Another previous study showed that active mRNA transport is achieved in ΔSHE2 cells, when She3p is fused to an MS2 coat protein and ASH1 mRNA is fused to MS2-target stem loops in its 3′UTR (Kruse et al., 2002). The authors concluded that the only function of She2p might be the tethering of ASH1 mRNA to the motor complex. However, the work presented here as well as previous studies (Müller et al., 2011; Sladewski et al., 2013) indicates that She2p plays a more central role. The mechanistic requirement of defined stoichiometries provided here (Fig. 5, A–D) suggests a potential rationale for the observation by Kruse et al. (2002). Because MS2 coat proteins form dimers, it is likely that individual Myo4p:She3p-MS2 complexes are dimerized by the MS2 coat protein. As a result, processive movement could potentially be activated in a manner, which is reminiscent of the activation by She2p.

Our in vitro reconstitution experiments suggested equimolar concentrations of myosin and RNA in the SHE particles. Thus, we also performed single particle motility assays with equimolar concentrations of myosin and RNA (Fig. 4). The observed mean run length of particles with single zip code RNAs was 2.13 µm, which is significantly higher than observed in a concurrent study (0.9–1.4 µm; Sladewski et al., 2013). In summary, our observation suggests that in vivo, some SHE particles are likely capable of moving the entire distance to the bud tip (>2–4 µm, depending on the size of the bud cell; Lange et al., 2008) with one continuous run.

ASH1-3′ RNA-containing particles had a mean velocity of 0.74 µm/s in TIRFM experiments, which is also in good agreement with previous actin gliding assays (0.45–0.65 µm/s; Dunn et al., 2007) and in vivo observations of localizing particles (0.55 µm/s [Lange et al., 2008], 0.20–0.44 µm/s [Bertrand et al., 1998], and 0.63 µm/s [Beach et al., 1999]). To date, the effect of RNA cargo binding on particle motility remains unclear. To elucidate this issue, we used particles that were either assembled in the absence of RNA or assembled with the RNA binding–deficient She2p (N365, R635) mutant. Both types of experiments yielded similar run lengths (2.48 and 2.02 µm, respectively) and slightly decreased velocities (0.65 and 0.62 µm/s, respectively) when compared with ASH1-3′–loaded particles (run length: 2.13 µm; velocity: 0.74 µm/s). Also, a comparison of the motile events per F-actin length and acquisition time for the different reconstituted SHE complexes did not show any positive effect of RNA binding on the interaction and subsequent transport of those SHE particles on F-actin (Fig. S5). In summary, we conclude that the rather small differences in run length with or without RNA are not pointing toward an RNA-dependent activation of processive movement.

ASH1 mRNA and a subset of other localizing RNAs contain more than one zip code element (Takizawa et al., 2000; Shepard et al., 2003; Aronov et al., 2007; Oeffinger et al., 2007; Hogan et al., 2008). Our in vitro experiments showed that an RNA consisting of a tRNA fused to two ASH1-E3 elements cross-links SHE complexes into large particles (Fig. 3). To further test the potential effect of such multimerization events, we performed single particle motility assays with a SHE complex containing such a double zip code RNA. In TIRFM experiments, the mean run length of these particles (1.93 µm) was essentially unchanged from the run length of the single zip code ASH1-3′ RNA (2.13 µm). Also, their mean velocity of 0.54 µm/s provides evidence against an RNA-based activation mechanism (velocity...
of ASH1-3′ particle: 0.74 μm/s) as did the analysis of numbers of motile particles per micrometer actin filament and time (Fig. S5). However, the measured values with the double zip code RNA are again in good agreement with in vivo observations of localizing particles (0.55 μm/s [Lange et al., 2008], 0.20–0.44 μm/s [Bertrand et al., 1998], and 0.63 μm [Beach et al., 1999]).

Concurrent to our study, Sladewski et al. (2013) showed that the mean run lengths of SHE particles become longer with increasing numbers of zip code elements per RNA (multi−zip code RNAs: 1.1–1.5 μm; single zip code RNAs: 0.9–1.3 μm). These experiments were performed at a strong substoichiometric ratio of RNA over myosin (0.035 nM RNA and 25 nM myosin). At much higher concentrations of myosin (250 nM) and RNA (10 nM), run lengths with an eight−zip code element RNA increased to 2.8 μm (1.4 μm for a single zip code; Sladewski et al., 2013). Bleaching and electron microscopy images suggested that a strong underrepresentation of RNA results in particles in which a single multi−zip code RNA is bound to up to four motor complexes and little cross-linking into large particles occurs (Sladewski et al., 2013).

Guided by our in vitro reconstitution study, we performed TIRFM experiments at equimolar conditions of RNA and Myo4p. A calculation of particle numbers per cell also suggests near-stoichiometric conditions in the cell (see following paragraphs of the Discussion: Fig. 5 E and Tables S1 and S2). We found that particles with single zip code elements or even without RNA are already sufficient for processive movement over distances that are close to the ones from the perinuclear regions to the bud tips of dividing yeast cells (Fig. 4). It might well be that the different stoichiometries in SHE complexes of both experiments are responsible for the different observations. In summary, increased run lengths by multi−zip code RNA might be a relevant mechanism in vivo that is perhaps not essential for RNA localization in the cell.

Additional experimental differences between both observations exist as well. We used wild-type She2p and skeletal muscle actin from rabbits. In the concurrent publication, experiments were performed with She2p bearing four point mutations (all cysteines to serines) and yeast actin that had been tropomyosin stabilized (Sladewski et al., 2013). The mutant version of She2p was reported to rescue ASH1 mRNA localization in vivo and thus seems to be at least partially functional (Krementsova et al., 2011). In our hands, thoroughly purified wild-type She2p (see Materials and methods) is monodisperse and well behaved (Müller et al., 2009, 2011). Therefore, we performed all our experiments with wild-type She2p.

In vivo particle-tracking experiments with two differently labeled RNA species showed that different RNA species can be transported in one particle (Lange et al., 2008). By using in vitro–reconstituted SHE particles containing ASH1-E3 and IST2 RNAs that were labeled with Cy3.5 and Atto488 fluorophores, respectively, we recapitulated this observation in vitro. It also supports our in vitro observation that an individual SHE complex is able to bind two independent RNA molecules.

Several localizing transcripts, such as ASH1, WSC2, and DNM1 mRNA, contain two or more zip code elements (Chartrand et al., 1999; Gonzalez et al., 1999; Jambhekar et al., 2005), suggesting multimerization of transport complexes by different RNAs (Fig. 5 D). The observed multimerization by multi−zip code RNAs explains why overexpression of ASH1 mRNA leads to fewer, but larger, particles in the cell (Bertrand et al., 1998; Lange et al., 2008).

Interestingly, higher-order assemblies have also been described for oskar mRNA-transport complexes in Drosophila melanogaster oocytes (Besse et al., 2009). The formation of these large oskar mRNPs is thought to serve two functions: first, the efficient co-transport of several transcripts and second, their translational repression during transport. In analogy, the formation of large SHE particles through RNA-mediated multimerization could serve very similar purposes.

Previous studies determined the number of ASH1 mRNAs present in a single transport particle in vivo as well as the number of ASH1 mRNAs in a dividing yeast cell (Haim et al., 2007; Treck et al., 2012). Using this information, we calculated that between 6 and 37 SHE particles are present in one cell (Fig. 5 E). This is consistent with the number of particles observed by fluorescent in situ hybridization of endogenous ASH1 mRNA (Takizawa et al., 1997; Treck et al., 2012). Based on known copy numbers of core factors per cell (Trek et al., 2012) and the stoichiometry of assembly, we estimated that a maximum of ∼250 SHE complexes with 500 zip code binding sites exist per cell (Table S2).

Interestingly, the low protein levels of She3p limit the number of SHE complexes. We repeatedly observed that excess of She3p induces the formation of unspecific high-molecular complexes with RNA (Fig. 2 B; Müller et al., 2011). It is therefore possible that SHE complexes are limited by the availability of She3p to avoid such unspecific complexes in vivo. On the other hand, the excess of Myo4p over She3p might help to ensure that each transport particle contains two motor molecules and thus is processive.

Using published data on transcript numbers per cell (Miura et al., 2008; Nagalakshmi et al., 2008; Pelechano et al., 2010; Treck et al., 2012), we estimated that ∼140 localizing mRNA molecules with 400–500 zip code elements are present in a dividing yeast cell (Table S1). Strikingly, these copy numbers are in very good agreement with the determined number of binding sites on SHE complexes in the cell. It suggests that the vast majority of zip code elements in the cell are bound by SHE complexes. By combining the aforementioned numbers, we calculate that multimeric SHE particles in the cell have a molecular mass between 8 and 52 MD and contain 4−25 localizing mRNAs and 7–45 SHE complexes (Fig. 5 E; see Materials and methods). Of course, not all RNAs are expressed at the same time, and some transcripts might escape binding by the SHE complex. These values do also not include accessory factor, such as translational repressors. Future work will be required to understand what features of particles with endogenous sizes and heterogeneous RNA composition are responsible for the observed motilities. One potentially important parameter to test is the endogenous actin network that might help SHE particles to move more efficiently to the bud tip.
Materials and methods

Molecular cloning

DNA fragments were produced by PCR and cloned into respective vectors using suitable restriction sites. Detailed information on plasmids, oligonucleotides, and restriction sites are given in Tables S3 and S4. For the construction of plasmid RHP169, CMD1 was amplified by PCR with primers AHO94/AHO95 and cloned into the vector pBDM (Berger et al., 2004) with the restriction sites XhoI–NcoI. FLAG-MYO4 was amplified by PCR with primers AHO49/AHO46 and cloned into the same vector with BamHI–NotI restriction sites. The AvrII restriction site in the MYO4 coding sequence was removed by site-directed mutagenesis using the primers O209/O210, resulting in subplasmid A. SHE2-His6 was produced by PCR using the primers AHO60/O118 and cloned into pBDM with the restriction sites BamHI–XbaI. The SpeI restriction site in the coding sequence of SHE2 was removed by site-directed mutagenesis with the primers O104/O105. SHE4 was produced by PCR with the primers O213/O214 and cloned into the same vector with the restriction sites Smal–NcoI, resulting in subplasmid B. Subplasmid A was digested with Pmel–AvrII, and the resulting insert was ligated into subplasmid B previously linearized with SpeI–NruI. This step resulted in subplasmid C. Pmel–AvrII digestion, and the final restriction site was inserted into subplasmid D previously linearized with Spel–NotI. This final step resulted in plasmid RH169 used for coexpression of all five proteins in insect cells. Point mutations in EGFP-SHE2 (N36S, R63K) were sequentially introduced via site-directed mutagenesis. The first PCR vector amplification, using primers P1/P2, resulted in phosphorylation and elution containing phagemid SHE2 (N36S). The second mutagenesis step with P23/P24 finally yielded plasmid FE#22. For the production of EGFP-SHE2 (Shep), base pairing codes for amino acids 174–183 in She2p were deleted in plasmid p20 by using P25/P26.

Protein expression and purification

She2p, N- and C-terminal fragments of She3p, and Myo4p-C (amino acids 978–1,471) were expressed in bacteria. Full-length She3p was coexpressed with She2p wild type from baculovirus-infected insect cells and purified by multistep procedures (Müller et al., 2011). Insect cells were lysed by sonication in Ni-A1 buffer (20 mM Hepes/NaOH, pH 7.8, 500 mM NaCl, and 20 mM imidazole) and centrifuged at 39,000 g for 20 min. The supernatant was filtered with a 0.45-µm filter before loading on a 5-ml HiTrap FF column (GE Healthcare). The bound protein was first washed with Ni-A2 buffer (20 mM Hepes/NaOH, pH 7.8, 1 M NaCl, and 40 mM imidazole) and second with HepA buffer (20 mM Hepes/NaOH, pH 7.8, 200 mM NaCl). Elution was achieved by supplementing buffer HepA with 300 mM imidazole. Protein passed a 5-ml HiTrap Q FF column (GE Healthcare) before it was loaded on a 5-ml Heparin HP column (HiTrap; GE Healthcare). Here, it was washed with 100% HepB buffer (20 mM Hepes/NaOH, pH 7.8, 1 M NaCl, 2 mM EDTA, and 2 mM DTT) and eluted with HepB buffer. Finally, the protein was loaded on a Superose 6 10/300 GL column (GE Healthcare) in a buffer that contained 20 mM Hepes/NaOH, pH 7.8, 500 mM NaCl, and 2 mM DTT. For motility experiments, the purification of full-length She3p was extended with an additional competition step to get completely rid of She2p wild-type remnants. Hence, She3p bound to the 5-ml HiTrap FF column was incubated with 30 µM She2p (N36S, R63K) for 15 min at 4°C during the purification. For She2p and She3p, the successful removal of nucleic acid contaminants was confirmed by measuring their absorbance at 260/280 nm. She2p and its mutants were expressed in E. coli JM101 cells and purified by gel filtration on Sephacryl S-300 (GE Healthcare) in G buffer (0.2 mM ATP, 0.5 mM DTT, 1 mM CaCl2, 1 mM MgCl2, and 1 mM EGTA) supplemented by 7% (wt/vol) sucrose and protease inhibitors. After sonication, 7 µg/ml yeast Mlc1p, 7 µg/ml yeast Cmd1p, and 2 mM ATP were added, and the lysate was centrifuged twice for 20 min with 39,000 g at 4°C and filtered with a 2.7-µm filter. The cleared lysate was incubated with anti-FLAG M2 affinity gel resin (Sigma-Aldrich) for 1 h at 4°C, and the resin was washed with wash buffer. Bound protein was eluted with 0.15 mg/ml FLAG peptide in wash buffer. The eluate was dialyzed in motility buffer (25 mM imidazole, pH 7.4, 50 mM KCl, 4 mM MgCl2, and 1 mM EGTA) supplemented with 2 mM DTT followed by dialysis in motility buffer supplemented with 2 mM DTT and 50% (vol/vol) glycerol and storage at −20°C. Mass spectrometric analysis showed that She3p, She4p, Cmd1p, and Mlc1p were copurified with full-length Myo4p. All components except for She4p were present in the expected amounts as judged by SDS-PAGE. The preparation was active in actin gliding assays.

RNA preparation

RNAs were produced by chemical synthesis, in vitro transcription, or expression in E. coli (Table S5; Edelmann et al., 2013). In vitro transcribed RNAs were produced with the MEGAshortscript kit (Ambion) and purified by native PAGE or by phenol extraction and isopropanol precipitation. RNAs for rRNA tagging were fused to the E. coli initiator rRNAmet, expressed in E. coli JM101 cells and purified by ion exchange chromatography (Ponchon et al., 2009; Müller et al., 2011). In brief, the harvested bacterial cell pellet was dissolved in a buffer containing 10 mM Tris/HCl, pH 7.4, and 10 mM magnesium acetate. RNA was recovered by phenolic extraction and ethanol precipitation. The resulting pellet was dissolved in 40 mM sodium phosphate buffer, pH 7.0, and loaded on a Mono Q 5/50 GL column (GE Healthcare). After washing with the same buffer containing 450 mM NaCl, RNA was eluted with a shallow gradient over 30 column volumes up to 650 mM NaCl. Finally, RNA was ethanol precipitated.

Chemical cross-linking of proteins

In a total volume of 22 µl, 1–10 µg She3p or Myo4p was incubated with 45 mM EDC and 45 mM NHS in a buffer containing 20 mM Hepes/NaOH, pH 7.8, and 500 mM NaCl for 20 min at 25°C. Afterward, the samples were analyzed by SDS-PAGE and Coomassie blue staining.
Analytical SEC.

Analytical SEC was performed at 4°C using a Superose 6 10/300 GL column in HNMD buffer (20 mM Hepes, pH 7.8, 200 mM NaCl, 2 mM MgCl₂, and 2 mM DTT). Fractions were analyzed by SDS-PAGE with Coomassie blue staining and PAGE with GelRed staining (Biotium).

Dynamic light scattering and density gradient centrifugation of SHE core complexes.

In a total volume of 100 µl, the SHE core complex was reconstituted with 30 µM She2p, 30 µM She3p, 15 µM Myo4p-C, and 15 µM zip code RNA (7.5 µM in the case of 2x ASH1-E3-77). Samples were analyzed by dynamic light scattering with a dynamic light scattering instrument (Viscotek 802; Malvern) at 20°C. All samples showed one major species (±5% intensity) with a hydrodynamic radius of 123.9 nm. Volumes of particles were calculated assuming an idealized globular particle sphere (V = 4/3 × τ × π). Using the hydrodynamic radii from dynamic light scattering experiments, the molecular mass of the sample was determined from the light scattering at right angles. Heavy isotope-labeled peptides (SpikeTides_TQL; JPT Peptide Technologies) were dissolved in 40 mM ammonium bicarbonate. In a total volume of 50 µl, the pretreated sample was mixed with 0.5 pmol/µl of each peptide, and the mixture was digested with 0.8 µg trypsin overnight at 37°C. 10 µl thereof (5 pmol of each heavy isoforms labeled peptide) was analyzed by nano–liquid chromatography–electrospray ionization–tandem mass spectrometry. Heavy and native light peptides were identified by search in a homemade database. The ratio of the peaks in the mass spectrometry scan was used to determine the amount of each respective light peptide in the sample. The peptide sequences were as follows: She3p peptide (residues 45–58), VHSHUHDQDLMKT*; and Myo4p peptide (residues 1,445–1,464), YDTSAITQNSNTELAVSKV* (asterisks mark amino acids labeled with the heavy isotopes 15N and 13C). Z marks the cleavable JPT tag.

Single-molecule motility assay.

Single-molecule motility experiments were designed on the basis of previously described conditions (Krementsova et al., 2011).

F-actin preparation.

Fluorescent actin filaments were prepared with 5 µM of purified rabbit G-actin (containing 10% tetramethylrhodamine [TMR]-labeled actin), polymerized in actin buffer (25 mM imidazole, pH 7.4, 25 mM KCl, 4 mM MgCl₂, 1 mM EGTA, and 10 mM DTT) for 1 h at room temperature, and stabilized with equimolar amounts of phalloidin (Sigma-Aldrich).

Flow cell preparation.

oxygen-scavenging system) were not considered. For the photobleaching analysis of processively moving events, in Image (National Institutes of Health), image sequences were background corrected by using a 6 × 6-pixel window. Mean integrated intensities over time were determined for each consecutive frame.

Run lengths and velocities were obtained from a single exponential fit (Miura et al., 2008) and a Gaussian fit $(y = a + b \exp \left[ -\left( (x - x_0) / b \right)^2 \right])$ to the respective distributions. Distances of single displacements were measured by brightest centroid tracking, using MTrackJ for ImageJ. Data plotting and fitting, as well as the statistical analysis, were performed with Igor Pro software (WaveMetrics). Run frequencies were generated by dividing the total number of processive runs by the total F-actin length, time, and final myosin concentration.

Calculation of particle number and size

Our calculation is based on two previous observations in vivo: one transport particle contains between 2.4 and 15.6 (mean: 7.2) ASH1 mRNAs (Haim et al., 2007), and ~88 ASH1 mRNAs are present in a dividing yeast cell (Trcek et al., 2012). From these numbers, we calculated the number of particles per cell by dividing the number of ASH1 mRNAs per cell by the minimal/maximal/mean number of ASH1 mRNAs per particle. We estimated that 142 localizing mRNAs are present per dividing yeast cell (Table S1) and divided this number by the number of particles per cell to obtain the number of mRNAs per particle. For the stoichiometry of the SHE complex determined in this study, we estimated that a maximum of 252 SHE complexes could be formed in one cell (Table S2). This number was derived from the reported protein copy number of 1,010 for She3p (Ghaemmaghami et al., 2003), which is limiting for SHE complex formation. By dividing the number of SHE complexes per cell by the number of particles per cell, we obtained the number of SHE complexes per particle. We determined the molecular mass of the particles by dividing the molecular mass of all SHE complexes and localizing mRNAs in one cell (Table S2) by the number of particles per cell.

Online supplemental material

Fig. S1 shows stoichiometry determination of the SHE subcomplex by SEC and chemical cross-linking. Fig. S2 shows control EMSAs for stoichiometry determination of the mature SHE complex. Fig. S3 shows stoichiometry determination of the mature SHE complex by SEC. Fig. S4 shows results from bleaching experiments. In Fig. S5, motile events per micrometer actin and time from different SHE particles are compared. Video 1 shows single-particle motility of a SHE complex consisting of ASH1-3′ + GFP-She2p + She3p + Myo4p. Video 2 shows single-particle motility of a SHE complex consisting of GFP-She2p + She3p + Myo4p. Video 3 shows single-particle motility of a SHE complex consisting of GFP-2pN365, R63K) + She3p + Myo4p. Video 4 shows single-particle motility of a SHE complex consisting of GFP-2pN365, R63K) + She3p + Myo4p. Video 5 shows single-particle motility of a SHE complex consisting of [Xe E3-77-RNA] + GFP-2p + She3p + Myo4p. Video 6 shows single-particle motility of a SHE complex consisting of Cy3.5-ASH1-3′ + Ato488-IST2 + She2p + She3p + Myo4p. Table S1 shows the mRNAs that have been reported as being transported by the molecular mass of the particles by dividing the molecular mass of all SHE complexes and localizing mRNAs in one cell (Table S2) by the number of particles per cell.

References


