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RNP LOCALIZATION AND TRANSPORT IN YEAST

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■ **Abstract** The localization of mRNAs is used by various types of polarized cells to locally translate specific proteins, which restricts their distribution to a particular sub-region of the cytoplasm. This mechanism of protein sorting is involved in major biological processes such as asymmetric cell division, oogenesis, cellular motility, and synapse formation. With the finding of localized mRNAs in the yeast *Saccharomyces cerevisiae*, it is now possible to benefit from the powerful yeast laboratory tools to explore the molecular basis of RNA localization. Because mRNA transport and localization in yeast share many features with RNA localization in higher eukaryotes, including the formation of a large ribonucleoprotein (RNP) localization complex, the requirement of a polarized cytoskeleton and molecular motors, and the role of nuclear RNA-binding proteins in cytoplasmic localization, the yeast can be used as a paradigm for unraveling the molecular aspects of this process. This review summarizes the current knowledge on RNP transport and localization in yeast.

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INTRODUCTION

Establishing and maintaining cellular polarity requires the asymmetric segregation of specific proteins. A classic mechanism for segregating proteins relies on targeting sequences contained within the amino acid sequence of the protein. Alternatively, the localization of specific mRNAs to the site of action of the protein is another mechanism employed by cells for intracellular sorting of proteins.

At least three mechanisms have been identified for localizing mRNAs: (*a*) direct transport of the mRNA, (*b*) stabilization of the mRNA at the site of localization, and (*c*) trapping the mRNA at the site of localization (see review by Lipshitz & Smibert 2000). In the first pathway, mRNAs destined for localization are packaged into ribonucleoprotein particles that are directly transported and anchored at the site of localization. Transport of the ribonucleoprotein (RNP) particles is dependent on motor proteins and cytoskeletal filaments. In a second pathway, localized mRNAs are preferentially stabilized at the localization site and degraded at all other cellular positions. In a third pathway, localized mRNAs are freely diffusible throughout the cell but upon reaching the site of localization are trapped at this position within the cell.

In *Drosophila*, *Xenopus*, and zebrafish, mRNA localization is responsible for the segregation of cell-fate determinants between daughter cells during development (Bashirullah et al. 1998). However, localization of mRNA is not restricted to development. In polarized somatic cells of higher vertebrates such as fibroblasts and neurons, mRNA localization is involved in the intracellular sorting of specific proteins within these cell types (Bassell et al. 1999). The yeast *Saccharomyces cerevisiae* also localizes specific mRNAs to asymmetrically segregate proteins. The genetic and cell biological approaches available in yeast have allowed the identification and characterization of various factors involved in mRNA localization at a pace unrivaled by other model systems. Consequently, yeast is an important route

toward understanding the molecular basis of mRNA transport and localization in higher eukaryotes.

This review highlights mRNA localization in *S. cerevisiae*, focusing on the interplay between *cis*- and *trans*-acting localization factors. To date, three localized mRNAs have been identified in *S. cerevisiae*: *ASH1*, *IST2*, and *ATM1*.

LOCALIZED RNA IN YEAST: ROLES AND FUNCTIONS

ASH1

ASH1 was identified in mutant selections designed to identify cell-type-specific regulators of *HO* expression, which is involved in mating-type switching in yeast (Bobola et al. 1996, Sil & Herskowitz 1996). In wild-type yeast cells, the HO endonuclease is expressed exclusively in mother cells. This results in mating-type switching in mother cells, whereas daughter cells maintain their original mating type. However, in *ash1* cells, *HO* is active in both mother cells and daughter cells, demonstrating that Ash1p is a cell-type-specific regulator of *HO* expression. By immunofluorescence, Ash1p was observed exclusively in daughter cell nuclei where it functions as a transcriptional repressor of *HO* expression, restricting *HO* expression to mother cell nuclei (Bobola et al. 1996, Sil & Herskowitz 1996). It was observed by fluorescent in situ hybridization (FISH) (Figure 1) that during anaphase, *ASH1* mRNA localizes to the distal tip of daughter cells, which results in the asymmetric segregation of Ash1p (Long et al. 1997, Takizawa et al. 1997).

Ash1p also functions in pseudohyphal growth (Chandarlapaty & Errede 1998, Pan & Heitman 2000). In response to nitrogen limitation, *S. cerevisiae* displays a dimorphic transition to a filamentous pseudohyphal morphology. In *ash1* cells, pseudohyphal growth is not observed, whereas overexpression of *ASH1* stimulates pseudohyphal growth. However, it remains to be determined if *ASH1* mRNA localization is required for pseudohyphal differentiation.

IST2

In a genome-wide screen to identify additional mRNAs that preferentially interact with the *trans*-acting factors required for *ASH1* mRNA localization, 11 mRNAs, including *IST2*, were found to be associated with these RNA localization factors (Takizawa et al. 2000). From these 11 mRNAs, only *IST2* mRNA was localized to daughter cells. In contrast to *ASH1* mRNA, *IST2* mRNA was localized to daughter cells throughout the cell cycle. *IST2* mRNA localization apparently functions to segregate Ist2p to the plasma membrane of medium- and large-budded cells. Ist2p, which has similarities to calcium and sodium channel proteins (Mannhaupt et al. 1994), has been reported to increase the sodium tolerance of yeast, but it remains to be determined why Ist2p is specifically segregated to daughter cells.

ATM1

ATM1 is a nuclear gene encoding a mitochondrial ATP-binding cassette (ABC) transporter (Leighton & Schatz 1995). Through biochemical fractionation, *ATM1* mRNA was observed to preferentially associate with mitochondria (Corral-Debrinski et al. 2000). Using a green fluorescent protein (GFP) reporter assay to monitor mRNA in living yeast cells, two regions of *ATM1* mRNA were found to be sufficient to localize a heterologous reporter mRNA to mitochondria. *Trans*-acting factors required for *ATM1* mRNA localization remain to be identified. These observations demonstrate that RNA localization is involved in targeting nuclear-encoded proteins to mitochondria.

CIS-ACTING ELEMENTS THAT TARGET AN RNA TO ITS SPECIFIC LOCATION

Specific *cis*-acting element(s), known as zipcodes (Kislauskis & Singer 1992), must be present within the sequence of a localized mRNA in order to target this mRNA to a specific region in the cell cytoplasm. Usually present in the 3′ untranslated region (3′UTR) of the mRNA, the localization element(s) is recognized by the cell localization machinery.

ASH1 mRNA

To identify *cis*-acting sequences responsible for the localization of the *ASH1* mRNA, fragments of *ASH1* were inserted into a reporter mRNA, and the cytoplasmic distribution of these chimeric mRNAs was determined by FISH (Chartrand et al. 1999, Gonzalez et al. 1999). By this method, four *ASH1* localization elements, each sufficient to localize a reporter mRNA to the bud of dividing yeast cells, have been identified: three within the coding sequence (elements E1, E2A, and E2B) and one overlapping the stop codon (E3). Element E3 includes the last 15 nucleotides of the coding sequence and the first 100 nucleotides of the 3′UTR. Interestingly, in most known localized mRNAs, the localization elements are located in the 3′UTR of the RNA, whereas all the *ASH1* localization elements are located within or in part of the coding sequence. This unusual disposition of localization elements suggests an interaction between the RNA localization machinery and the translation machinery during transport of this mRNA.

A detailed mutagenesis analysis of the four elements reveals that although they perform a similar function, these elements have distinct sequences predicted to fold into different stem-loop structures (Chartrand et al. 1999, Gonzalez et al. 1999). These mutagenesis experiments also demonstrated that secondary and tertiary structures are important for recognition by the *trans*-acting factors involved in *ASH1* mRNA localization. Because all four elements appear to interact with the same RNA-binding protein, She2p (see below), it is likely that a structural motif shared between these four elements is recognized by She2p. In addition to She2p, RNA localization driven by each of these elements is dependent on She1p, She3p,

She4p, and She5p (see below) but is independent of Ash1p (Long et al. 1997, Takizawa et al. 1997).

IST2 mRNA

Cis-acting localization elements for this RNA remain to be determined.

ATM1 mRNA

Two independent *cis*-acting elements in the *ATM1* mRNA have been shown to localize a reporter mRNA to mitochondria: one within the first 48 nucleotides of the N-terminal mitochondria-targeting sequence (mts) and one in the 3′UTR (Corral-Debrinsky et al. 2000). Interestingly, the localization function of the mts element appears to be translation independent, suggesting that the mts could perform two roles: mRNA localization as a *cis*-acting RNA element and protein translocation into mitochondria as a peptide signal sequence.

TRANS-ACTING FACTORS INVOLVED IN RNA LOCALIZATION

A genetic selection for cell-type-specific regulators of *HO* expression identified five genes, *SHE1* to *5*, essential for asymmetric *HO* expression (Jansen et al. 1996). A mutation in any of these genes results in the repression of *HO* expression in both cell types (Bobola et al. 1996), and *ASH1* mRNA is symmetrically distributed between mother and daughter cells (Long et al. 1997, Takizawa et al. 1997). Numerous studies have now demonstrated that She1p, She2p, and She3p are components of a ribonucleoprotein particle that directly transports *ASH1* and *IST2* mRNA to daughter cells. These observations are described below and are summarized in Table 1.

Myo4p/She1p

SHE1 is identical to *MYO4*, which codes for a type V unconventional myosin that localizes to daughter cells (Haarer et al. 1994, Jansen et al. 1996). By immunoprecipitation, Myo4p associates with *ASH1* mRNA, which is dependent on She2p and She3p (Münchow et al. 1999, Takizawa & Vale 2000). Furthermore, it was observed that Myo4p colocalizes with *ASH1* mRNA-containing particles (Bertrand et al. 1998, Takizawa & Vale 2000), and in living yeast cells, Myo4p directly transports *ASH1* mRNA to daughter cells (Bertrand et al. 1998, Beach et al. 1999). Because Myo4p does not contain intrinsic RNA-binding activity, accessory proteins are necessary to interface the myosin with *ASH1* mRNA localization elements.

She3p

She3p is a novel protein with no significant homology to any known proteins. She3p colocalizes with Myo4p and *ASH1* mRNA at the bud tip (Münchow et al. 1999). Furthermore, by two-hybrid, three-hybrid, and coimmunoprecipitation experiments,

TABLE 1 *Trans*-acting factors involved in *ASH1* mRNA localization

Name	Function	Role in *ASH1* localization	Interactions	Location in the cell
She1p/ Myo4p	Type V unconventional myosin	Molecular motor	She3p	Bud tip
She2p	RNA-binding protein	Binds *ASH1* mRNA localization elements	She3p	Cytoplasm/ bud tip
She3p	Myosin-binding protein	Bridges She2p with the the myosin She1p	She1p She2p	Bud tip
She4	Unknown	Stabilizes the actin cytoskeleton (?)	Unknown	Cytoplasm
She5p/ Bni1p	Scaffold protein	Involved in the polarization of the actin cytoskeleton	Cdc42p, Bud6p EF1α, Pfy1p	Bud tip
Loc1p	RNA-binding protein	Binds *ASH1* localization elements; efficiency factor	Unknown	Nucleus

the N-terminal half of the She3p was found to interact with the C-terminal tail of Myo4p (Münchow et al. 1999, Böhl et al. 2000, Long et al. 2000). It is hypothesized that the interaction between Myo4p and She3p occurs through coiled-coil domains located in both proteins (Böhl et al. 2000). By immunoprecipitation and three-hybrid experiments, She3p association with *ASH1* mRNA was observed to be dependent on She2p (Münchow et al. 1999, Long et al. 2000, Takizawa & Vale 2000). These results suggested that the Myo4p-She3p complex interacts with the *ASH1* mRNA *cis*-acting RNA localization elements through She2p.

She2p

She2p is also a novel protein with no homology to known proteins. Two-hybrid analysis and experiments using recombinant She2p and She3p demonstrate that She2p directly interacts with She3p through a domain in the C terminus of She3p (Böhl et al. 2000, Long et al. 2000). These results support the hypothesis that She2p functions to interface the Myo4p-She3p complex to *ASH1* mRNA (Böhl et al. 2000, Long et al. 2000). The hypothesis implies that She2p could be the RNA-binding protein required for *ASH1* mRNA localization. By electrophoretic gel mobility shift (EMSA) and UV cross-linking assays, it was observed that purified recombinant She2p could directly and specifically bind each of the *ASH1* *cis*-acting localization elements (Böhl et al. 2000, Long et al. 2000). It is not known whether the simultaneous interaction of She2p with each of the *cis*-acting elements is additive or cooperative. The RNA-binding and She3p interaction domains of She2p remain to be identified.

The function of She2p in *ASH1* mRNA localization is apparently restricted to bridge the Myo4p-She3p complex to *cis*-acting localization elements. When the

Myo4p-She3p complex was artificially tethered to a heterologous reporter mRNA, Myo4p-She3p-dependent RNA localization was observed to be independent of She2p (Long et al. 2000).

The function of She2p in yeast cells is apparently not limited to *ASH1* and *IST2* mRNA localization. By immunofluorescence, She2p was observed to be uniformly distributed between mother and daughter cells (Böhl et al. 2000). However, when *ASH1* mRNA is overexpressed, She2p accumulates at the bud tip (Böhl et al. 2000). Furthermore, She2p can preferentially associates with at least 10 unlocalized mRNAs (Takizawa et al. 2000). However, the function of She2p with these other mRNAs remains to be determined.

A Nuclear Factor Involved in RNA Localization: Loc1p

In higher eukaryotic organisms, heterogeneous nuclear ribonucleoproteins (hnRNP) RNA-binding proteins such as Squid (Lall et al. 1999) and hnRNP A2 (Hoeck et al. 1998) have been shown to function in mRNA localization. These proteins shuttle between the nucleus and cytoplasm, suggesting a role for a nuclear component in cytoplasmic mRNA localization.

In yeast, one *trans*-acting factor, Loc1p, was found to be a nuclear protein (Long et al. 2001). Loc1p is a very basic (pI $\sim$ 10), double-stranded RNA-binding protein identified in a three-hybrid screen for RNA-binding proteins specific for *ASH1 cis*-acting localization elements. Purified recombinant Loc1p was found to interact only with double-stranded RNA (Long et al. 2001). In *loc1* cells, *ASH1* mRNA is predominantly delocalized, and in those cases, Ash1p is symmetrically distributed between mother and daughter nuclei. Loc1p is a nuclear protein, but in contrast to the proteins Squid and hnRNP A2, Loc1p does not shuttle between the nucleus and the cytoplasm. One possible function of Loc1p might be to interface the *ASH1* mRNA with a nucleo-cytoplasmic shuttling protein (perhaps She2p), which is required for *ASH1* mRNA localization. Another possibility is that localized mRNA could be tagged by Loc1p in the nucleus after its synthesis in order to be recognized by the cytoplasmic localization machinery. Further studies are ongoing to determine if Loc1p interacts with nucleo-cytoplasmic shuttling proteins.

ROLE OF THE ACTIN CYTOSKELETON AND ASSOCIATED PROTEINS

The Actin Cytoskeleton in Yeast

Transport of mRNA by a molecular motor along the cytoskeleton is the main mechanism by which polarized cells localize mRNAs (Lipshitz & Smibert 2000). The finding that a myosin motor (Myo4p) is involved in the localization of the *ASH1* and *IST2* mRNAs points to a role for actin cytoskeleton being the track that directs mRNA to the localization site.

The yeast actin cytoskeleton is primarily organized in a network of F-actin cables that run parallel to the longitudinal axis of the cell. These cables are highly

dynamic and polarized, with their barbed ends directed toward the growing bud during anaphase (Pruyne & Bretscher 2000). The polarized growth of the actin cytoskeleton is responsible for the formation of the bud during mitosis (Adams & Pringle 1984), formation of the mating shmoo (Gehrung & Snyder 1990), polarized secretion (Finger & Novick 1998), and mitochondrial inheritance (for review, see Drubin et al. 1993, Botstein et al. 1997).

Using a temperature-sensitive mutant allele *act1-133*, which affects the myosin-binding site on actin, Long et al. (1997) showed that *ASH1* mRNA is delocalized at the non-permissive temperature. Mutations in actin-associated proteins such as tropomyosin (*tpm1*) and profilin (*pfy1*) also affect *ASH1* mRNA localization (Long et al. 1997). Because all these mutants affect the formation of actin cables that extend from the mother cell to the bud, these observations strongly suggest that the integrity of the actin cytoskeleton is essential for the proper transport of this mRNA. In contrast, the localization of *ASH1* mRNA was not affected in a tubulin (*tub2*) mutant strain, which is defective in the formation of the astral microtubule, suggesting that the anaphase microtubule network is not required for mRNA localization (Long et al. 1997).

Another approach to study the role of the yeast cytoskeleton in mRNA localization is to use drugs that specifically depolymerize either the actin or the microtubule cytoskeleton. In the presence of latrunculin A, an actin-depolymerizing drug, anaphase yeast cells fail to localize the *ASH1* mRNA (Takizawa et al. 1997). However, the addition of nocodazole, a microtubule-depolymerizing drug, to a yeast culture does not result in any defect in *ASH1* mRNA localization (Takizawa et al. 1997). Altogether, these results show that the transport of the *ASH1* mRNA to the bud tip of budding yeasts requires an intact actin cytoskeleton.

Other proteins found to be essential for the localization of the *ASH1* mRNA are also involved in the formation and polarization of the actin cytoskeleton:

She4p

SHE4 is among the five *SHE* genes essential for the proper localization of the *ASH1* mRNA (Jansen et al. 1996). *SHE4* was also identified in a screen for yeast endocytosis mutants (Wendland et al. 1996). Although its specific function is not known (*SHE4* is a non-essential gene), the actin cytoskeleton is altered in *she4* cells, suggesting that She4p plays a role in actin polarization (Wendland et al. 1996). This result suggests that She4p functions indirectly in the localization of *ASH1* mRNA and indicates that She4p is probably not a direct member of the mRNA localization machinery.

Bni1/She5p

Bni1p is a member of the formin family of proteins, which is defined by the presence of the proline-rich formin (FH) homology domains (Frazier et al. 1997). These proteins play an important role in polarization of the actin cytoskeleton and affect processes like cytokinesis, establishment of cell polarity, and polarized growth. A

bni1 strain is viable but shows defects in the organization of the actin cytoskeleton (Evangelista et al. 1997). Bni1p localizes at the site of bud growth, where the actin cytoskeleton is actively reorganized (Ozaki-Kuroda et al. 2001). One possible role of Bni1p is to act as a scaffold to recruit different factors involved in actin polymerization (Frazier et al. 1997). The formin domains of Bni1p have been shown to interact with profilin, an actin-binding protein implicated in actin polymerization (Imamura et al. 1997). Bni1p also interacts with EF1αp and Bud6p/Aip3p, which bind to and bundle actin filaments (Evangelista et al. 1997, Umikawa et al. 1998), and Rho1p and Cdc42, both Rho-related GTPase involved in the regulation of actin polymerization (Evangelista et al. 1997).

Because of its role in the actin cytoskeleton organization, Bni1p probably functions indirectly in the localization of *ASH1* mRNA. However, some data suggest that it could play a role in the anchoring of this mRNA at the bud tip (Beach et al. 1999; see below). Unlike the other *she* mutants, cells containing a *SHE5* mutation accumulate *ASH1* mRNA at the bud neck between the mother and the daughter cells (Long et al. 1997, Takizawa et al. 1997). This particular phenotype may reflect a disruption of actin polarity in the bud.

ANCHORING THE RNA AT THE LOCALIZATION SITE

After the transport of mRNA to its localization site, it must be anchored there in order to avoid diffusion throughout the cytoplasm. In the case of the *ASH1* mRNA, a tight localization of this mRNA is observed at the bud tip during late-anaphase (Figure 1) (Long et al. 1997). *IST2* mRNA also localizes tightly at the bud tip (Takizawa et al. 2000). Several lines of evidence point to a role of various redundant factors involved in the anchoring process of mRNA in yeast.

Role of mRNA Translation in Anchoring

A potential factor involved in the anchoring of the *ASH1* mRNA could be the translated protein itself, Ash1p. Because Ash1p is translated at the bud tip and is a nucleic acid–binding protein, it is reasonable to suggest that it may be involved in the anchoring of its own mRNA at the bud tip. Such a case is known in *Drosophila*, where the Oskar protein is involved in the anchoring of its own messenger RNA at the posterior pole of the *Drosophila* oocyte (Rongo et al. 1995). Indeed, the insertion of a stop codon after the initiation codon results in a less tightly localized *ASH1* mRNA at the bud tip (Gonzalez et al. 1999). Moreover, the presence of a stop codon in the middle of the coding sequence also displays a partially localized phenotype, suggesting that the C-terminal half of the protein could be involved in the anchoring of the mRNA. Interestingly, the C-terminal half of Ash1p contains a zinc-finger domain involved in DNA binding (Bobola et al. 1996, Sil & Herskowitz 1996). Because zinc fingers are also known to bind RNA (Burd & Dreyfuss 1994), one model could be that when the Ash1p is translated at the bud tip, it may bind

and anchor its own mRNA at the cell cortex. Another possibility could be that the translation of the mRNA is needed to reveal a *cis*-acting element involved in the anchoring of the RNA (Gonzalez et al. 1999).

Trans-Acting Factors Involved in Anchoring

Other *trans*-acting factors can also be involved in the anchoring of the *ASH1* mRNA. Obvious candidates are proteins present at the bud tip. These proteins are generally involved in bud site selection and polarized cell growth (Chant 1999), but some could potentially serve as anchors for messenger RNAs localized at the bud tip (Pruyne & Bretscher 2000). Several of these factors may have been identified by Beach et al. (1999) using a reporter RNA containing a single *ASH1* localization element to follow the dynamics of localization and anchoring in living yeasts. They observed that in *bni1/she5* and *bud6/aip3* strains the reporter RNA was still asymmetrically distributed to the bud but had lost its cortical localization. Because both Bni1p/She5p and Bud6p/Aip3p are proteins localized to the cortex of the bud tip (Amberg et al. 1997, Ozaki-Kuroda et al. 2001), it is possible that these proteins are involved, directly or indirectly, in the anchoring of the localized *ASH1* mRNA. However, the endogenous *ASH1* mRNA appears not to be affected by a *bud6* mutation (R.M. Long, unpublished observation; R.P. Jansen, personal communication), suggesting that redundant factors are involved in the anchoring of the full-length mRNA.

MECHANISM OF RNA TRANSPORT AND LOCALIZATION

Kinetic Aspects of RNA Localization

Since mRNA localization is a dynamic process, the kinetic and temporal aspects of this mechanism remain to be revealed. Questions such as how fast the mRNA is transported, how long the process takes, and when it is translated need to be answered. In the case of the *ASH1* mRNA, for instance, the timing of mRNA transcription, localization, and translation must be carefully regulated in order to avoid contamination of Ash1p into the mother's nucleus before cytokinesis.

Some of these questions were approached by the development of a new technique for the visualization of mRNA in living yeast cells. GFP was fused to an RNA-binding protein (MS2 RNA-binding domain) and coexpressed with a reporter mRNA containing RNA motifs (MS2-binding sites) recognized by this protein (Bertrand et al. 1998, Beach et al. 1999). A similar system was also developed with the U1A RNA-binding protein (Takizawa & Vale 2000, Brodsky & Silver 2000). When an *ASH1* localization element is present in the reporter mRNA, a single bright particle consisting of the GFP-MS2 fusion protein and the reporter mRNA is seen localized at the bud tip of budding yeasts. The formation of this particle requires an intact localization element (Chartrand et al. 1999) and the proteins She2 and She3 (Bertrand et al. 1998), which suggests that it contains components of the localization machinery of the *ASH1* mRNA.

Using video microscopy, it was possible to follow the movement of this particle from the mother cell cytoplasm to the bud tip (Bertrand et al. 1998). The particle moved at velocities that varied between 200 and 440 nm/s, which is consistent with the velocity expected from a myosin V motor, e.g., Myo4p, which is reported to move between 200 and 400 nm/s (Cheney et al. 1993). Overall, it took 128 s for the particle to be transported over a net linear distance of 4.4 μm, from the mother cell cytoplasm to the bud tip. When the GFP-MS2 construct was expressed with the reporter mRNA in a strain deleted of the *MYO4/SHE1* gene, the particle exhibited diffusion only within the cytoplasm of the mother cell (Bertrand et al. 1998). Altogether, these results indicate that Myo4p directly transports the *ASH1* particle to the bud tip. Moreover, it shows that the *ASH1* mRNA can be transported and localized within 2 min after its export from the nucleus.

The Locasome: A Localization Machine

The transport of an mRNA to its site of localization usually requires the assembly of a multiprotein complex, the localization machine, on the RNA localization element(s). The resulting RNP complex, called a locasome (Bertrand et al. 1998, Bassell et al. 1999), consists minimally of a protein motor, an RNA-binding protein, and the localizing mRNA (Long et al. 2000). The formation of a localization complex appears to be a characteristic of several localized mRNAs in various organisms. It has been observed as particles or granules in different systems: in vivo using fluorophore-labeled mRNA (Ainger et al. 1993) or GFP-labeled localization *trans*-acting factors (Wang & Hazelrigg 1994, Kohrmann et al. 1999) or in vitro by biochemical purification (Wilhelm et al. 2000).

In living yeast, using the GFP-MS2 fusion protein and the *lacZ-ASH1* mRNA system, the *ASH1* locasome is seen as a single large particle in the cell cytoplasm (see above; Bertrand et al. 1998, Beach et al. 1999). This phenotype is possibly created by the aggregation of multiple small particles into a single large one. This particle was found by in situ hybridization to contain the *ASH1* mRNA, and its formation requires the presence of intact *SHE2* and *SHE3* genes (Bertrand et al. 1998). Moreover, the proteins She1p, She2p, and She3p coimmunoprecipitated with the GFP-MS2/*lacZ-ASH1* mRNA complex (W. Gu & R.H. Singer, unpublished observations), suggesting that this particle contains the main *trans*-acting factors involved in *ASH1* mRNA localization. Because the same *trans*-acting factors are involved in the localization of the *IST2* mRNA (Takizawa et al. 2000), a single locasome could possibly contain different mRNAs.

From all the genetic, cell biology, and biochemical studies discussed above on the various *cis*- and *trans*-acting factors involved in *ASH1* mRNA localization, we propose the following model for the assembly of the *ASH1* mRNA locasome in the yeast cytoplasm (Figure 2). As shown previously, (1) the localization elements in the *ASH1* mRNA sequence are bound by the She2 protein. (2) She2p interacts with the C-terminal domain of She3p. (3) The N-terminal coiled-coil domain of She3p then associates with a similar coiled-coil domain at the C-terminal end of the She1p myosin. (4) Together, this complex interacts

with the actin cytoskeleton via the myosin. Although this model is based on the core components of the localization machinery (She1p, She2p, and She3p), other unidentified *trans*-acting factors would also be expected to be part of this complex, e.g., factors involved in the translation or the stability of the mRNA.

A general working model for the localization of the *ASH1* mRNA in yeast can also be proposed (Figure 3), where this mRNA is first recognized in the nucleus by nuclear proteins, such as Loc1p, which act as tags for the localization machinery (1). Once in the cytoplasm, the localization machinery completes its assembly on the tagged mRNA and forms the locasome (3). The locasome is transported along the actin cytoskeleton up to the bud tip (5) where it becomes anchored (7). Once localized, the mRNA is translated (8), and the Ash1 protein is synthesized at the bud tip juxtanuclearly and then is imported into the daughter cell nucleus.

PERSPECTIVES

Remarkable progress has been made in the characterization of the molecular process behind RNA localization in yeast. Although several features of this process are specific to yeast, the broad outlines of the mechanism are certainly conserved across the eukaryotic kingdom: for instance, the role of nuclear factors, the formation of a locasome by the localization machinery, and the importance of the cytoskeleton and associated proteins in this process. These aspects are also present in RNA localization pathways in other biological systems. However, several aspects of this process are still poorly understood: for example, the role of nuclear factors in RNA localization, the mechanism of mRNA anchoring, and the assembly of the various *trans*-acting factors and the mRNA into a locasome. Filling in the details of the mRNA localization mechanism will occupy a new generation of investigators over the next years.

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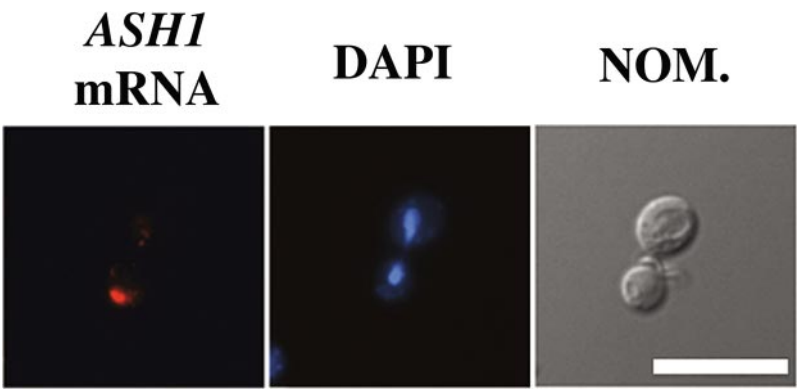

Figure 1 Fluorescent in situ hybridization on a late-anaphase budding yeast to detect the *ASH1* mRNA. Note the tight crescent localization of the mRNA at the bud tip. DAPI: DNA staining. Size bar: 10 μm.

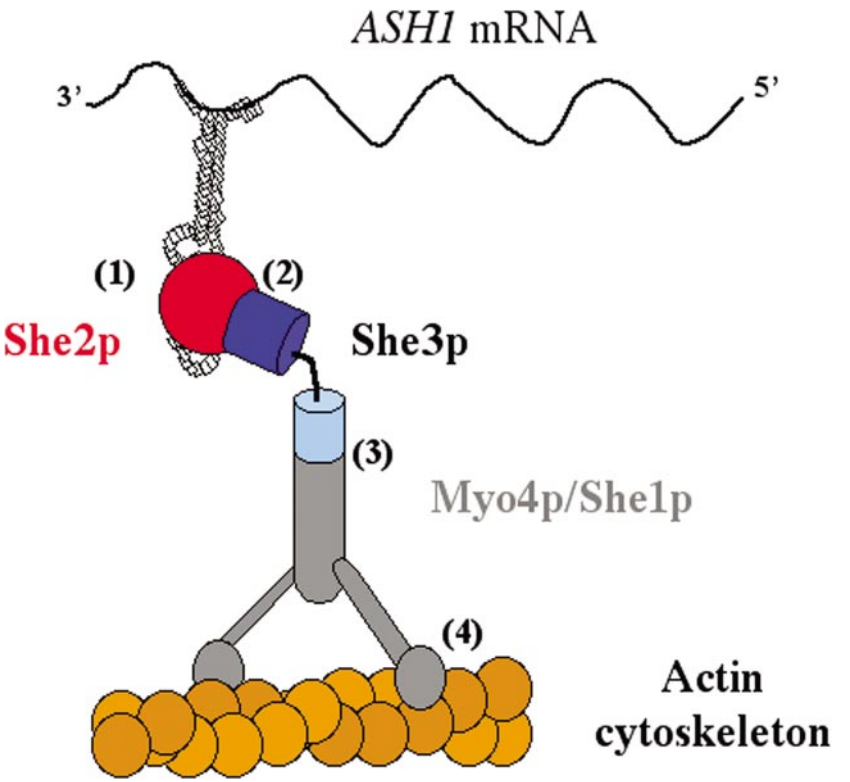

Figure 2 The yeast locasome. Only the elements E3 in the 3′UTR of the *ASH1* mRNA is depicted. A similar complex should also form on the remaining three localization elements. She2p is represented in red, She3p is shown in dark and light blue, and She1p is in gray.

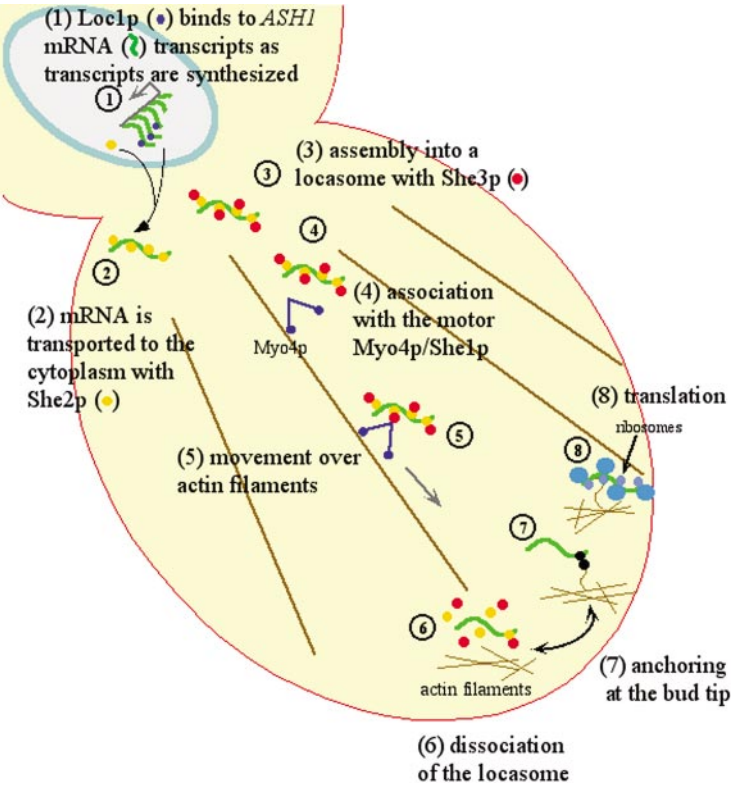

Figure 3 Model of *ASH1* mRNA localization. See text for details.