Biol. Cell (2005) **97**, 75–86 (Printed in Great Britain)

Review

RNA localization in yeast: moving towards a mechanism

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Abstract

RNA localization is a widely utilized strategy employed by cells to spatially restrict protein function. In *Saccharomyces cerevisiae* asymmetric sorting of mRNA to the bud has been reported for at least 24 mRNAs. The mechanism by which the mRNAs are trafficked to the bud, illustrated by ASH1 mRNA, involves recognition of *cis*-acting localization elements present in the mRNA by the RNA-binding protein, She2p. The She2p/mRNA complex subsequently associates with the myosin motor protein, Myo4p, through an adapter, She3p. This ribonucleoprotein complex is transported to the distal tip of the bud along polarized actin cables. While the mechanism by which ASH1 mRNA is anchored at the bud tip is unknown, current data point to a role for translation in this process, and the rate of translation of Ash1p during the transport phase is regulated by the *cis*-acting localization elements. Subcellular sorting of mRNA in yeast is not limited to the bud; certain mRNAs corresponding to nuclear-encoded mitochondrial proteins are specifically sorted to the proximity of mitochondria. Analogous to ASH1 mRNA localization, mitochondrial sorting requires *cis*-acting elements present in the mRNA, though *trans*-acting factors involved with this process remain to be identified. This review aims to discuss mechanistic details of mRNA localization in *S. cerevisiae*.

Introduction

Both eukaryotic and prokaryotic cells display polarized growth in response to external (cell–cell contacts, chemical gradients or matrix) and/or internal stimuli. This phenomenon is vital for differentiation during development as well as cell motility. Cellular polarity requires that specific proteins be spatially restricted to distinct subcellular locations. RNA localization is one mechanism which results in the intracellular and intercellular sorting of specific proteins. RNA localization is used by a variety of organisms [yeast, insects (*Drosophila*), amphibians (*Xenopus*) and mammals (rodents)] and cell types (fibroblasts, neurons, oocytes and embryos) for establishing polarity (Bashirullah et al., 1998; Kloc et al., 2002). In this review our discussions will be limited to the role of mRNA localization in establishing polarity in *Saccharomyces cerevisiae* since this themed issue contains reviews concerning the role of mRNA localization in other organisms and cell types.

Budding yeast is an extremely valuable unicellular organism for investigating polarized growth. Yeast cells can establish polarity in response to external and internal cues (Casamayor and Snyder, 2002). Haploid yeast cells exist as two mating types: a or α. Each of these cell types secretes a distinct mating pheromone that directs cells of the opposite mating-type to arrest in G1 of the cell cycle and stimulates polarized cell growth towards the source of the opposite mating pheromone. Ultimately, the two polarized cell types fuse to form an a/α diploid cell.

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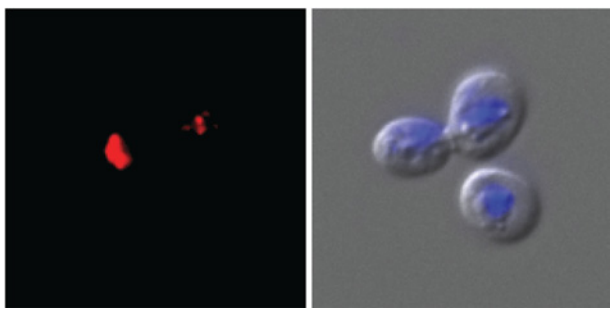
Key words: anchoring, *ASH1*, polarization, RNP, transport.
Abbreviations used: ER, endoplasmic reticulum; Lat-A, latrunculin A; MTS, mitochondrial targeting sequence; ORF, open reading frame; mRNP, mRNA ribonucleoprotein particle; UTR, untranslated region.

Figure 1 | Intracellular distribution of ASH1 mRNA

DNA oligonucleotide probes (red) complementary to ASH1 mRNA were used for *in situ* hybridization (left-hand panel). ASH1 mRNA is localized at distal tip of anaphase cells. Also shown (right-hand panel) is the corresponding DAPI (blue)/Nomarski merged image.



During vegetative growth, specific polarized budding patterns are determined with respect to ploidy (Casamayor and Snyder, 2002). Haploid cells display an axial budding pattern in which the bud germinates at the pole adjacent to the previous bud site. Diploid cells display a bipolar budding pattern in which the bud will germinate at either pole in the mother cell, and the daughter cell forms a bud opposite to the birth pole.

Haploid yeast cells are also polarized with respect to regulation of gene expression. Interconversion between a and α mating types is regulated by the asymmetric expression of the HO endonuclease (Herskowitz, 1988; Nasmyth, 1983). Mother cells, but not daughter cells, express the HO endonuclease (Herskowitz, 1988; Nasmyth, 1983). Asymmetric expression of *HO* allows mother cells to switch mating type from a to α or vice versa. Mating type switching permits mother cells to form a/α diploid cells through mating with their progeny.

Asymmetric expression of *HO* is regulated by Ash1p, a DNA-binding protein that is specifically sorted to daughter cell nuclei where it represses transcription of *HO* (Bobola et al., 1996; Jansen et al., 1996; Maxon and Herskowitz, 2001; Sil and Herskowitz, 1996). ASH1 mRNA localizes to the bud tip during anaphase of the cell cycle, resulting in the sorting of Ash1p to daughter cell nuclei (Figure 1) (Chartrand et al., 2002; Long et al., 1997; Takizawa et al., 1997). ASH1 mRNA localization is the sole mechanism for delivering Ash1p to daughter cell nuclei, since in the absence of ASH1 mRNA localization, Ash1p is symmetrically distributed between mother and daughter nuclei (Bobola et al., 1996; Chartrand et al., 2002; Jansen et al., 1996; Long et al., 1997; Sil and Herskowitz, 1996; Takizawa et al., 1997). Symmetric sorting of Ash1p results in complete repression of *HO* transcription, leading to the absence of mating type switching (Jansen et al., 1996).

mRNA localization in *S. cerevisiae* is not confined to ASH1 mRNA. At least 23 additional mRNAs are localized to daughter cells (Shepard et al., 2003; Takizawa et al., 2000). Additionally, an emerging phenomenon in budding yeast is mRNA sorting to mitochondria dependent on the 3′-untranslated region (UTR) (Corral-Debrinski et al., 2000; Marc et al., 2002; Margeot et al., 2002; Sylvestre et al., 2003a; Sylvestre et al., 2003b). This review will highlight advances in each of these areas, paying particular attention to details for ASH1 mRNA localization since this process provides the most in-depth understanding of an active transport and anchoring mechanism for mRNA localization.

The ASH1 mRNA transport freeway: the actin cytoskeleton

Higher eukaryotes utilize both microtubules and microfilaments for polarized growth. Long distance transport is often accomplished using microtubules, whereas short-range transport and anchoring often utilize microfilaments. Budding yeast rely solely on the actin cytoskeleton for generating polarity. In yeast, filamentous actin (F-actin) is present as cables, bundles of actin filaments that run through the cell parallel to the growth axis, and in cortical actin patches, dynamic structures found at invaginations of the plasma membrane.

Actin cables serve as the highway for delivering molecular components (e.g. secretory vesicles, vacuole membrane, peroxisomes and Golgi cisternae) to buds (Pruyne and Bretscher, 2000a; Pruyne and Bretscher, 2000b). Depolymerization of actin cables

3′-Untranslated region (3′-UTR): The region of mRNA that is 3′ to the open reading frame.

using cable-defective mutants *act1-a133*, *tmp1Δ* or *pfy1-p111* or the actin depolymerizing drug latrunculin A (Lat-A) results in the delocalization of ASH1 mRNA (Table 1) (Long et al., 1997; Takizawa et al., 1997). The importance of the actin cytoskeleton in ASH1 mRNA localization is further underscored by the isolation of *SHE5* in a mutant selection designed to isolate genes defective for asymmetric expression of the *HO* promoter as well as the observations that ASH1 mRNA is delocalized and the actin cytoskeleton misorganized in strains defective for components of the exocytic apparatus (Aronov and Gerst, 2004; Jansen et al., 1996). ASH1 mRNA is mislocalized to the bud neck in *she5* cells, and cloning of *SHE5* revealed that it is identical to *BNI1*, a formin that stimulates actin cable assembly (Evangelista et al., 2003; Long et al., 1997; Takizawa et al., 1997). Additionally, the observation that ASH1 mRNA is mislocalized in *bud6*/*aip3* cells, further substantiates the role of actin filaments in the localization of ASH1 mRNA (Beach and Bloom, 2001; Beach et al., 1999). Bni1p-induced actin cable assembly is thought to be stimulated by interaction with Bud6p/Aip3p (Amberg et al., 1997; Evangelista et al., 2002; Evangelista et al., 2003).

While actin cables have a firmly established role in ASH1 mRNA localization, the role of filamentous cortical actin patches and the actin cap structure has remained elusive. A limited amount of evidence suggests that cortical actin structures may be involved in anchoring ASH1 mRNA at the bud tip. Actin patches are sensitive to depolymerization by Lat-A, and ASH1 mRNA localization is defective in cells treated with Lat-A (Takizawa et al., 1997). Mislocalization of ASH1 mRNA could result from the depolymerization of actin cables, actin patches, or both. Furthermore, the cortical actin cap structure contains Bni1p and Bud6p as well as numerous additional proteins (Pruyne and Bretscher, 2000a; Pruyne and Bretscher, 2000b). The intracellular distribution of this cap complex is reminiscent of localized ASH1 mRNA. Based on the similar distribution of Bni1p, Bud6p and ASH1 mRNA, it has been hypothesized that a Bud6p/Bni1p complex is a component of a molecular scaffold that functions to anchor ASH1 mRNA (Beach and Bloom, 2001; Beach et al., 1999). However, given that Bni1p and Bud6p also function to stimulate actin cable assembly, it is possible that the molecular scaffold responsible for anchoring ASH1 mRNA may not be properly polarized/positioned at the bud tip in strains defective for these genes. While it is tantalizing to speculate that the cortical actin cap structure directly participates in ASH1 mRNA anchoring, identification and characterization of specific components for the ASH1 mRNA anchoring complex remain elusive.

ASH1 mRNA molecular zipcodes: *cis*-acting localization elements

RNAs destined for localization need to be identified from the general population of unlocalized mRNAs. In concert with *trans*-acting factors, the 3′-UTR of most localized mRNAs contains *cis*-acting elements required for targeting the mRNAs to particular regions of the cytoplasm (Bashirullah et al., 1998; Kloc et al., 2002). Analogously, ASH1 mRNA contains a *cis*-acting element (E3) extending from the end of the open reading frame (ORF) into the 3′-UTR (Chartrand et al., 1999; Gonzalez et al., 1999; Long et al., 1997; Takizawa et al., 1997). Detailed characterization of this zipcode has revealed that structure, not sequence, is critical for mRNA localization activity (Chartrand et al., 1999; Gonzalez et al., 1999). Besides the E3 element, the ASH1 ORF contains three additional zipcodes: E1, E2A and E2B (Chartrand et al., 2002; Chartrand et al., 1999; Gonzalez et al., 1999). Sequence comparison of the four ASH1 zipcodes reveals no obvious homologies, implying that a shared structure might be the driving force behind these mRNA localization elements. While each of the individual ASH1 *cis*-acting elements can localize a heterologous reporter mRNA to daughter cells, the presence of multiple *cis*-acting elements increases the quality and efficiency of ASH1 mRNA localization (Chartrand et al., 2002).

Formin: A family of proteins involved in cell polarization and cytokinesis that function to nucleate actin filament assembly.

***trans*-Acting factor:** A protein necessary for localization of a particular mRNA through association with a *cis*-acting element.

***cis*-Acting localization element/zipcode:** An RNA sequence or structure sufficient for delivering RNA to the site of localization.

Open reading frame: The region of mRNA that is translated into a polypeptide by ribosomes.

Table 1 | *trans*-Acting factors involved in ASH1 mRNA localization

Common protein name	Alternative name	Proposed function	Intracellular location	Selected references
ASH1 mRNA transport				
Myo4p	She1p	Type V myosin that transports mRNA and ER to daughter cells	Cytoplasm/asymmetrically sorted to daughter cells dependent on associated mRNA	Bertrand et al., 1998; Estrada et al., 2003; Gonsalvez et al., 2003; Jansen et al., 1996; Kruse et al., 2002
She2p		RNA-binding protein that interacts with ASH1 zipcode elements	Cytoplasm and nucleus/symmetrically distributed between mother and daughter cells	Bohl et al., 2000; Gonsalvez et al., 2003; Kruse et al., 2002; Long et al., 2000
She3p		Adapter that links Myo4p and She2p/ASH1 mRNA; also required for ER inheritance	Cytoplasm/asymmetrically sorted to daughter cells dependent on associated mRNA	Bohl et al., 2000; Estrada et al., 2003; Gonsalvez et al., 2003; Long et al., 2000; Takizawa et al., 2000
ASH1 mRNA anchoring				
Khd1p	Hek2p	RNA-binding protein involved in anchoring and translational regulation of ASH1 mRNA	Cytoplasm/co-localizes with ASH1 mRNA in daughter cells	Irie et al., 2002; Tadauchi et al., 2001
Loc1p		RNA-binding protein involved with ASH1 mRNA localization and ribosome biogenesis	Nucleus/nucleolus	Harnpicharnchai et al., 2001; Huh et al., 2003; Long et al., 2001
Puf5p	Htr1p/Mpt5p/Uth4p	RNA-binding protein apparently involved in anchoring and possibly involved in translational regulation of ASH1 mRNA	Cytoplasm/symmetrically distributed between mother and daughter cells	Irie et al., 2002
Puf6p		RNA-binding protein that associates with UUGU sequence elements and has a role in ASH1 mRNA translational regulation and localization	Predominantly nuclear	Gu et al., 2004
Scp160p		RNA-binding protein apparently involved in anchoring and possibly involved in translational regulation of ASH1 mRNA	ER	Frey et al., 2001; Irie et al., 2002
Actin cytoskeleton				
Act1p		Actin cables; actin patches	Cytoplasm	Long et al., 1997; Takizawa et al., 1997
Bud6p	Aip3p	Possible regulator of Bni1p	Cytoplasm; cortical actin cap	Beach et al., 1999; Evangelista et al., 2003
Pfy1p	Prf1p	Monomeric actin-binding protein profilin enhances formin-stimulated actin nucleation	Cytoplasm; cortical actin cap	Long et al., 1997; Evangelista et al., 2003
Bni1p	She5p	Formin that acts to nucleate actin filaments	Cytoplasm; cortical actin cap	Long et al., 1997; Evangelista et al., 2003
Tpm1p		Stablizes actin filaments	Cytoplasm actin filaments	Long et al., 1997; Evangelista et al., 2002
Accessory factors				
She4p		Regulator of motor domain of class I and V myosins	Cytoplasm/symmetrically distributed between mother and daughter cells	Toi et al., 2003; Wesche et al., 2003

The locasome connects ASH1 mRNA to the actin cytoskeleton

Apart from *SHE5*, the original mutant selection for genes defective in asymmetric expression of *HO* identified four additional genes: *SHE1*, *SHE2*, *SHE3* and *SHE4* (Jansen et al., 1996). While no discernable RNA-binding motifs can be identified in any of the She proteins, She2p directly and specifically interacts *in vitro* with each of the ASH1 *cis*-acting elements, although with apparently weak affinity (Bohl et al., 2000; Darzacq et al., 2003; Long et al., 2000). She2p RNA-binding activity apparently resides within the first 70 amino acids of the protein, since a deletion of this region (ΔN70) abolishes the ability of She2p to associate with ASH1 mRNA (Kruse et al., 2002). A cluster of arginine residues within this region (R43, R44, R52 and R63) as well as asparagine residue 36 (N36) are important for She2p RNA-binding activity (Gonsalvez et al., 2003). The RNA recoginition motif (RRM), the arginine rich motif (ARM), the RGG box and the double-stranded RNA-binding motif (dsRBD) all contain arginine residues critical for RNA binding activity. However, it is unknown whether any of the arginine residues in She2p directly contact ASH1 mRNA or whether they play a more indirect role in mRNA association. The role of N36 in mRNA association may involve homo-dimerization of She2p since N36 is located in a region of She2p predicted to form a leucine zipper motif (Gonsalvez et al., 2003). Definitive insight into She2p RNA association awaits structural analysis of She2p in complex with ASH1 mRNA.

She2p is not excluded from the nucleus and hence could identify localization substrates in the nucleus prior to export to the cytoplasm. In support of this hypothesis, She2p-ΔN70 (first 70 amino acids of the protein deleted) accumulates in the nucleus, suggesting that She2p may require mRNA association for nuclear export (Kruse et al., 2002). However, experiments using She2p mutants N36S and R63K, which are specifically defective for ASH1 mRNA association, do not support the assertion that nuclear export of She2p is dependent on associated mRNA (Gonsalvez et al., 2003). Resolution of these apparently contradictory results will require further investigation into She2p nuclear function and/or export.

She1p is identical to Myo4p, a type V barbed-end-directed myosin motor (Haarer et al., 1994; Jansen et al., 1996). Analysis of ASH1 mRNA-dependent transport in living yeast revealed that Myo4p is capable of directly transporting mRNA localization substrates along polarized actin cables (Beach et al., 1999; Bertrand et al., 1998; Munchow et al., 1999). Since Myo4p has been described as a non-processive motor, it has been suggested that the presence of multiple and redundant *cis*-acting localization elements provides continual movement of the ASH1 mRNA ribonucleoprotein particle (mRNP) (Darzacq et al., 2003; Reck-Peterson et al., 2001). Further analysis of localization elements in other asymmetrically sorted mRNAs will shed light on this hypothesis. The motor domain of Myo4p is capable of associating with the conserved UCS (UNC-45/CRO1/SHE4) domain-containing protein She4p, and this protein has been proposed to regulate myosin activity, possibly by assisting in the proper folding of motor domains (Jansen et al., 1996; Toi et al., 2003; Wesche et al., 2003).

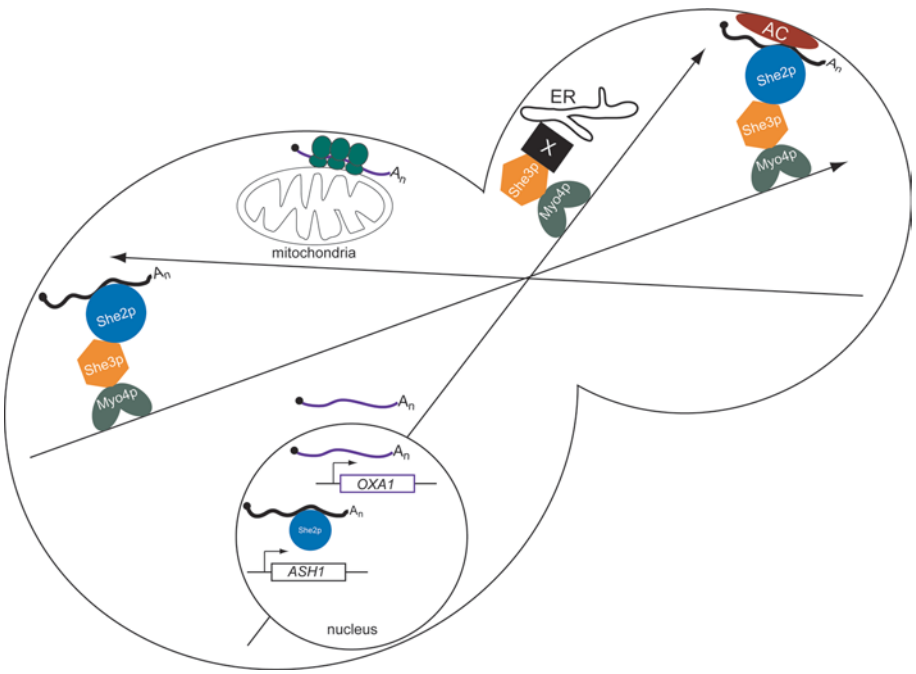
Myo4p is also capable of associating with an N-terminal domain of She3p, while a C-terminal domain of She3p is able to associate with She2p (Bohl et al., 2000; Long et al., 2000). Since ASH1 mRNA associates with Myo4p and She3p dependent on She2p, She3p has been proposed to act as an adapter, linking the She2p/ASH1 mRNA complex to the actin cytoskeleton through Myo4p (Bohl et al., 2000; Long et al., 2000; Munchow et al., 1999; Takizawa and Vale, 2000). The proposed ability of these molecular interactions to occur simultaneously lead to the heterotrimeric Myo4p/She3p/She2p model for ASH1 mRNA localization, also referred to as the locasome (Figure 2) (Bohl et al., 2000; Long et al., 2000; Takizawa and Vale, 2000). According to this model, She2p binds the ASH1 *cis*-acting localization elements, and subsequently associates with She3p and Myo4p forming the heterotrimeic complex. The heterotrimeric complex then localizes the mRNA to daughter cells on polarized actin cables. This model has gained more credence through the demonstration

Locasome: A complex of proteins that participates directly in mRNA localization.

mRNA ribonucleoprotein particle (mRNP): A complex of RNA and associated proteins.

Figure 2 | Differential routes for mRNA localization in yeast and transport of ER to daughter cells

Once transcribed, bud-tip-localized mRNAs, typified by ASH1 mRNA, may be identified by She2p in the nucleus, thereby marking them as targets for mRNA localization. In the cytoplasm, the She2p/mRNA complex associates with She3p and Myo4p forming a heterotrimeric transport complex. Subsequently, the transport complex delivers the mRNA cargoes to daughter cells on polarized actin cables. The mRNA cargoes are retained at the bud tip by an anchoring complex (AC) that may contain components of the actin cap structure, translational machinery as well as Khd1p. Myo4p and She3p deliver a second cargo, ER, to daughter cells. mRNAs such as OXA1 that are found associated with mitochondria are likely to be targeted by a diffusion/entrapment mechanism.



that the Myo4p/She2p interaction is dependent on She3p (Gavin et al., 2002; G.B. Gonsalvez and R.M. Long, unpublished results).

While the heterotrimeric model is consistent with the experimental observations cited above, the model does not satisfactorily explain some of the phenotypes observed for ASH1 mRNA localization. If the Myo4p/She3p/She2p heterotrimeric complex assembles in a 1:1:1 stiochiometry, a reasonable prediction is that these three proteins should co-localize. Both Myo4p and She3p are asymmetrically sorted to daughter cells dependent on She2p, yet She2p itself is not asymmetrically distributed even though it is expressed at levels very similar to Myo4p and She3p (Bohl et al., 2000; Gonsalvez et al., 2003; Kruse et al., 2002; Munchow et al., 1999; G.B. Gonsalvez and R.M. Long, unpublished results). Furthermore, the asymmetric sorting of Myo4p and She3p to daughter cells is responsive to mRNA cargo. Under experimental conditions which allow for the formation of the heterotrimeric complex in the absence of mRNA cargo, Myo4p and She3p are symmetrically distributed between mother and daughter cells (Gonsalvez et al., 2003; Kruse et al., 2002). Surprisingly, Myo4p and She3p asymmetric sorting can be restored when a heterologous mRNA is artificially tethered to She3p (Kruse et al., 2002; Long et al., 2000). Thus, it is possible that upon mRNA binding certain signalling events occur that mediate the proper localization of the Myo4p/She3p complex. The nature of this signalling event is not yet known, and it is not clear whether the loss of sorting observed in the absence of mRNA association is due to a defect in the active transport of the Myo4p/She3p complex or whether it is due to a defect in anchoring of the complex at the bud tip. In addition to Myo4p, She3p and She2p, the locasome model needs to be further refined to integrate the function of accessory factors Khd1p,

Table 2 | Localized yeast mRNAs

+, strong mRNA localization activity; +/−, weak or partial mRNA localization activity; A, protein asymmetrically sorted to daughter cells; S, protein symmetrically distributed between mother and daughter cells; MIM, mitochondrial inner membrane; M, mitochondria; MT, mitochondrial matrix; N.D., not determined.

		cis-Acting element			
Gene	RNA localization	Full†	3′-UTR	Protein distribution	Selected References
ASH1	daughter cell	+	+	A	Long et al., 1997; Takizawa et al., 1997
*EGT2**	daughter cell	+	+/−	A	Shepard et al., 2003; Takizawa et al., 2000
MMR1	daughter cell	+	-	A	Shepard et al., 2003; Takizawa et al., 2000
SRL1	daughter cell	+	-	A	Shepard et al., 2003; Takizawa et al., 2000
*TPO1**	daughter cell	+	-	A	Shepard et al., 2003; Takizawa et al., 2000
*WSC2**	daughter cell	+	-	A	Shepard et al., 2003; Takizawa et al., 2000
*YJL051c**	daughter cell	+	N.D.	A	Shepard et al., 2003; Takizawa et al., 2000
*YML072c**	daughter cell	+	-	A	Shepard et al., 2003
*YNL087w**	daughter cell	+	N.D.	A	Shepard et al., 2003
BRO1	daughter cell	+	N.D.	S	Shepard et al., 2003
CLB2	daughter cell	+	+/−	S	Shepard et al., 2003
CPS1	daughter cell	+	N.D.	S	Shepard et al., 2003
DNM1	daughter cell	+	N.D.	S	Shepard et al., 2003
ERG2	daughter cell	+	+/−	S	Shepard et al., 2003; Takizawa et al., 2000
*IST2**	daughter cell	+	-	S	Juschke et al., 2004; Takizawa et al., 2000
KSS1	daughter cell	+/−	N.D.	S	Shepard et al., 2003
LCB1	daughter cell	+/−	N.D.	S	Shepard et al., 2003
MET4	daughter cell	+/−	N.D.	S	Shepard et al., 2003
MID2	daughter cell	+	-	S	Shepard et al., 2003
MTL1	daughter cell	+/−	N.D.	S	Shepard et al., 2003
YGR046w	daughter cell	+	-	S	Shepard et al., 2003; Takizawa et al., 2000
YLR434c	daughter cell	+	N.D.	S	Shepard et al., 2003
YMR171c	daughter cell	+	-	S	Shepard et al., 2003
YPL066c	daughter cell	+/−	N.D.	S	Shepard et al., 2003
ATM1	mitochondria	+	+	MIM	Corral-Debrinski et al., 2000
ATP2	mitochondria	+	+	M	Margeot et al., 2002
AFG1	mitochondria	N.D.	+	M	Marc et al., 2002
COQ1	mitochondria	N.D.	+	MIM	Marc et al., 2002
MRF1	mitochondria	N.D.	+	M	Marc et al., 2002
OXA1	mitochondria	N.D.	+	MIM	Sylvestre et al., 2003a
COX10	mitochondria	N.D.	N.D.	MIM	Corral-Debrinski et al., 2000
TIM44	mitochondria	N.D.	N.D.	MT	Corral-Debrinski et al., 2000

*Plasma membrane-associated proteins.
†Full is defined as *cis*-acting element residing in the 5′-UTR, ORF and/or 3′-UTR.

Puf5p, Puf6p and Scp160p that have been implicated in anchoring ASH1 mRNA at the bud tip (Gu et al., 2004; Irie et al., 2002).

An expanding list of molecular cargoes for Myo4p, She3p and She2p

In addition to ASH1 mRNA, a genome-wide analysis revealed that at least 23 additional mRNAs also associate with Myo4p, She3p and She2p (Table 2) (Shepard et al., 2003; Takizawa et al., 2000). These mRNAs localize to the bud tip of daughter cells in a She2p-dependent manner, suggesting that their mechanism of localization is identical to that of ASH1 mRNA (Shepard et al., 2003; Takizawa et al., 2000). From these twenty-three mRNAs, only eight of the corresponding proteins are asymmetrically

distributed to daughter cells (Shepard et al., 2003; Takizawa et al., 2000). Unlike ASH1 mRNA localization, which is absolutely required for asymmetric Ash1p sorting to daughter cells, these other eight proteins are asymmetrically sorted to daughter cells in the absence of mRNA localization, suggesting that yeast has acquired redundant mechanisms to ensure protein polarization (Shepard et al., 2003).

In contrast to ASH1 mRNA localization, which results in the asymmetric segregation of Ash1p, the asymmetric localization of mRNA does not always result in asymmetric sorting of the corresponding protein. IST2 mRNA is localized to daughter cells dependent on the Myo4p/She3p/She2p localization pathway. When over-expressed, Ist2p, a polytopic membrane protein, is asymmetrically distributed in the plasma membrane of daughter cells; however, when expressed at endogenous levels, Ist2p is symmetrically distributed between the mother and daughter cells (Juschke et al., 2004; Takizawa et al., 2000). Furthermore, in the absence of mRNA localization, Ist2p is almost exclusively confined to the plasma membrane of mother cells (Juschke et al., 2004). Consequently, it appears that asymmetric mRNA localization represents a novel pathway, independent of the classical secretory pathway, to ensure that Ist2p will be incorporated in the plasma membrane of daughter cells (Juschke et al., 2004). This novel mechanism may not be unique to Ist2p since seven of the Myo4p/She3p/She2p-associated mRNAs code for proteins symmetrically distributed in membranous structures (Shepard et al., 2003; Takizawa et al., 2000). Asymmetric sorting of mRNAs coding for symmetrically distributed proteins may be a strategy to ensure maternal deposition of a 'start-up' mRNA package to the daughter cell (Shepard et al., 2003).

Identification of a battery of mRNA cargoes promises to contribute to the understanding of the molecular determinants for *cis*-acting localization elements. Analogous to ASH1 mRNA, all of the mRNA localization substrates contain at least a single *cis*-acting localization element within the ORF, and three of the mRNAs have an additional element in the 3′-UTR (Shepard et al., 2003; Takizawa et al., 2000). Daughter cell localization of all these substrates is dependent on She2p, suggesting that a common motif may be present within the *cis*-acting localization elements (Shepard et al., 2003; Takizawa et al., 2000). Minimally, 30 daughter-cell-specific *cis*-acting localization elements are present in yeast, of which only four have been extensively analysed. Characterization of the remaining elements, followed by computational comparisons between the various elements, may reveal homologies in sequence and/or structure not yet obvious from analysis of ASH1 mRNA.

Recently it was demonstrated that, in addition to mRNA cargoes, She3p and Myo4p are also involved in transporting cortical endoplasmic reticulum (ER) to daughter cells (Estrada et al., 2003). In biochemical fractionations, Myo4p and She3p co-purify with other ER components (Estrada et al., 2003). In the absence of Myo4p and She3p a defect in the sorting of cortical ER to daughter cells was observed, and the ER inheritance defect in *myo4* and *she3* cells is apparently direct, since inheritance of vacuoles, mitochondria and early or late Golgi is normal in these cells (Estrada et al., 2003). However, ER inheritance is normal in cells devoid of She2p, suggesting that an mRNA cargo is not required for Myo4p/She3p-dependent ER inheritance (Estrada et al., 2003). Several important questions are raised by this study. Although mRNA localization is not required for cortical ER sorting, is it still possible that both cargoes are transported to daughter cells on the same transport particle? In this regard, it will be interesting to determine whether the ER fractions that contain She3p and Myo4p also contain She2p and/or any mRNA localization substrates. Alternatively, is it possible that there are separate pools of the Myo4p/She3p complex *in vivo* that mediate the localization of mRNA and cortical ER, separately? If so, what is the mechanism that regulates assembly of a specific cargo with the Myo4p/She3p complex?

The interplay between translation and mRNA localization

The relationship between mRNA localization and translational regulation is well documented in *Drosophila* (Johnstone and Lasko, 2001; Lipshitz and Smibert, 2000). However, detailed investigations into this aspect of ASH1 mRNA physiology are in their infancy. Restricting mating type switching to the mother cell requires that ASH1 mRNA not be translated until it reaches the daughter cell. Individually the ASH1 localization elements are sufficient and redundant for mRNA localization activity (Chartrand et al., 2002; Chartrand et al., 1999;

Gonzalez et al., 1999). However, simultaneously all four elements apparently have a second function to maintain transcripts in a translationally-quiescent state by reducing the rate of translation for ASH1 mRNA *en route* to daughter cells, effectively preventing Ash1p production in the mother cell (Chartrand et al., 2002). The lower rate of ASH1 mRNA translation cannot be attributed to the association of the Myo4p/She3p/She2p complex since the rate of translation is not altered in the absence of She2p (Chartrand et al., 2002). Consequently, reducing the kinetics of translation could be an intrinsic property of the *cis*-acting localization elements, or some other accessory factor that associates with the elements. In any event, such a mechanism would not require translational repression in itself. Rather, the stem-loop structures present within the ASH1 *cis*-acting localization elements, either alone or in combination with accessory factors, could effectively reduce the rate of translation such that Ash1p is not synthesized prior to its arrival at the bud tip. This model predicts that if Myo4p-dependent transport of ASH1 mRNA is reduced, but not completely eliminated, Ash1p should be symmetrically distributed between mother and daughter cells. This assertion remains to be investigated.

While it is intuitively obvious that translation of ASH1 mRNA should be regulated during transport, evidence is emerging suggesting that, once at the daughter cell, translation of ASH1 mRNA may be linked with anchoring. Interfering with translation of ASH1 mRNA either by removal of the start codon, insertion of a pre-mature translational stop codon or by incubation of the cells with cycloheximide affects the ability of ASH1 mRNA to be anchored at the bud tip (Gonzalez et al., 1999; Irie et al., 2002; Kruse et al., 2002). There is additional evidence for Puf6p, Khd1p, Loc1p, Puf5p and Scp160p supporting a link between translation and ASH1 mRNA localization (Gu et al., 2004; Harnpicharnchai et al., 2001; Irie et al., 2002; Long et al., 2001).

Puf6p is a novel member of the PUF (Pumilio/FBF) family of RNA-binding proteins and co-purifies with She2p (Gu et al., 2004). While predominantly a nuclear protein, a fraction of Puf6p co-localizes with the ASH1 mRNA at the distal bud tip. Consistent with this observation Puf6p is associated with ASH1 mRNA *in vivo* and *in vitro* dependent on UUGU sequence elements present in the E3 localization element, and *puf6*Δ cells show a dramatic decrease in Ash1p sorting as well as ASH1 mRNA localization. The function of Puf6p in ASH1 mRNA localization may be related to its ability to partially repress translation of ASH1 mRNA.

KHD1 mutant cells are defective for anchoring of ASH1 mRNA (Irie et al., 2002). Khd1p is an RNA-binding protein that interacts with the E1 ASH1 *cis*-acting localization element and colocalizes with ASH1 mRNA at the bud tip (Irie et al., 2002). Over-expression of Khd1p lowers the expression of Ash1p suggesting that Khd1p inhibits translation of Ash1p through its interaction with ASH1 mRNA (Irie et al., 2002). Alternatively, Khd1p over-expression may sequester factors that positively affect translation of ASH1 mRNA.

Loc1p, an exclusively nuclear protein and enriched in the nucleolus, was identified as an RNA-binding protein that is required for efficient ASH1 mRNA localization and Ash1p sorting (Huh et al., 2003; Long et al., 2001). Surprisingly, Loc1p was also shown to be associated with a 66 S pre-ribosome and required for efficient 60 S ribosome biogenesis (Harnpicharnchai et al., 2001). Additionally, *loc1*Δ cells apparently contain increased levels of large subunit precursor rRNAs as examined by a functional genomics approach analysing non-coding RNA processing (Peng et al., 2003). Currently it is unclear whether the ASH1 mRNA localization and ribosome biogenesis phenotypes associated with *loc1*Δ cells are mutually exclusive or somehow related. It is attractive to speculate that loss of 60 S ribosomes in *loc1*Δ cells leads to a global translational defect, which, in turn, affects ASH1 mRNA localization/anchoring.

Puf5p and Scp160p are RNA-binding proteins, and cells deficient for either of these proteins display subtle alterations in ASH1 mRNA anchoring (Irie et al., 2002; Tadauchi et al., 2001). Puf5p binds to the 3′-UTR of HO mRNA and represses translation, but it is unknown whether Puf5p has any affect on ASH1 mRNA translation (Tadauchi et al., 2001). More recently, Puf5p was observed to associate with mRNAs encoding nuclear and cytoplasmic proteins involved in regulating polarized cell growth (Gerber et al., 2004). Consequently, the *puf5* phenotype for ASH1 mRNA anchoring may be an indirect effect by altering the expression of one or more proteins required for establishing polarity. Although Scp160p associates with polysomes, its role in ASH1

mRNA localization is likely to be indirect as it does not co-localize or associate with ASH1 mRNA (Frey et al., 2001; Irie et al., 2002; Lang and Fridovich-Keil, 2000). Scp160p co-localizes with ER membranes, further suggesting that ASH1 mRNA transport and/or anchoring may somehow be connected to the ER (Frey et al., 2001).

Targeting mRNA to mitochondria

Localized mRNA in yeast is not an exclusive phenomenon of daughter cells. A number of mRNAs corresponding to nuclear-encoded mitochondrial proteins are targeted to the vicinity of mitochondria (Table 2) (Corral-Debrinski et al., 2000; Marc et al., 2002; Margeot et al., 2002; Sylvestre et al., 2003a; Sylvestre et al., 2003b). mRNA targeting to the mitochondria is thought to increase the efficiency of mitochondrial protein import and is apparently important for mitochondrial function, since yeast cells defective for this process are unable to grow on a non-fermentable carbon source (Margeot et al., 2002). It is hypothesized that the ability of a particular mRNA to associate with mitochondria is related to its ancestry. The mRNAs associated with mitochondria are mainly of prokaryotic origin whereas mRNAs of eukaryotic origin are dispersed in the cytoplasm (Sylvestre et al., 2003b).

Among the mRNAs associated with mitochondria-associated polysomes, ATM1, ATP2 and OXA1 have been studied in depth (Corral-Debrinski et al., 2000; Margeot et al., 2002; Sylvestre et al., 2003a). ATM1 contains two *cis*-acting mitochondrial targeting sequences (MTSs) (Corral-Debrinski et al., 2000). Each of these elements is sufficient to target a heterologous reporter mRNA to mitochondria (Corral-Debrinski et al., 2000). The first element is 48 nucleotides in length and corresponds to the N-terminal 16 amino acids of the protein (Corral-Debrinski et al., 2000). This region of Atm1p also contains a portion of the classical MTS; however, targeting mRNA to mitochondria by this *cis*-acting element is independent of MTS translation (Corral-Debrinski et al., 2000). The second ATM1 *cis*-acting localization element is 265 nt in length and is located in the 3′-UTR (Corral-Debrinski et al., 2000). Analogous to ATM1 mRNA, the ATP2 mRNA also contains two mitochondrial *cis*-acting elements (Margeot et al., 2002). Each of the ATP2 elements is sufficient to target a reporter mRNA to mitochondria (Margeot et al., 2002). The first element corresponds to the N-terminal 35 amino acids of the protein, and the second 100-nt targeting signal in located in 3′-UTR (Margeot et al., 2002). Substitution of the ATP2 3′-UTR with the ADH1 3′-UTR resulted in cells that exhibit respiratory dysfunction, demonstrating the functional significance of mitochondrial-targeting through the 3′-UTR (Margeot et al., 2002). Analysis of OXA1 mRNA targeting to the mitochondria revealed that the functionality of the *cis*-acting element in the 3′-UTR is conserved between the yeast and human genes, although there are no discernable sequence or structural motifs in common (Sylvestre et al., 2003a). The initial identification and characterization of the individual yeast *cis*-acting mitochondrial localization elements has not led to the identification any common sequence and/or structure shared between these elements.

To date, no *trans*-acting factors involved with mitochondrial mRNA targeting have been identified. The ASH1 mRNA localization factors, Myo4p, She2p and She3p, are apparently not involved in mRNA targeting to mitochondria since these three proteins are not associated with any of the mRNAs targeted to mitochondria, and cells devoid of these proteins exhibit no respiratory defects (Shepard et al., 2003; Takizawa et al., 2000; R.M. Long, unpublished results). It is reasonable to hypothesize that mitochondrial mRNA targeting occurs through a diffusion-entrapment mechanism. In this model, the mRNP complex simply diffuses to mitochondria and upon interacting with mitochondrial associated factors the mRNP complex is captured by mitochondria. Alternatively, a directed-transport model similar in mechanism to ASH1 mRNA localization, but utilizing unique factors, is also possible. Insights into the precise mechanism of delivery and anchoring of mRNA with mitochondria are anticipated in the future.

Conclusions

Since the discovery of ASH1 mRNA localization in *S. cerevisiae*, considerable progress has been made in identifying *cis*- and *trans*-acting factors required for this process and characterizing the molecular interactions between the various mRNA localization factors. Although these observations provide the foundation for the heterotrimeric/locasome model for ASH1 mRNA localization, the current rendition of

this model does not satisfactorily explain all aspects of ASH1 mRNA localization.

The ASH1 mRNA-localization pathway is composed of a transport component and an anchoring component. Several *trans*-acting factors have been specifically implicated in anchoring ASH1 mRNA, but the molecular mechanism by which these factors ensure proper anchoring remains to be deciphered. Superimposed on the transport and anchoring aspects of ASH1 mRNA localization is translational regulation. These processes need to be strictly co-ordinated to ensure that Ash1p protein is asymmetrically distributed. The first insights into the choreography between transport, anchoring and translational regulation are only beginning to emerge.

Originally mRNA localization in yeast was perceived as being an exclusive route for asymmetrically sorting proteins to daughter cells. Although poorly understood mechanistically, recent developments demonstrate that yeast utilize mRNA localization in a variety of protein sorting pathways. Furthermore, through Myo4p and She3p, the mRNA localization pathway to daughter cells is connected to daughter-cell inheritance of cortical ER. Analogous to the other type V myosin in yeast, Myo2p, it remains to be determined how the Myo4p/She3p complex can select/associate with the appropriate cargoes. Since the mechanisms for many fundamental biological processes are shared between yeast and multicellular organisms, it is anticipated that advances in the understanding of mRNA localization in yeast will contribute to the understanding of this process in higher eukaryotes.

Acknowledgements

We thank the National Institutes of Health (GM60392 to R.M.L.), the Pew Scholars Program in the Biomedical Sciences and the March of Dimes Basil O'Connor Starter Scholar Research Award for research funding.

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Received 31 March 2004; accepted 1 April 2004

Published on the Internet 20 December 2004, DOI 10.1042/BC20040066

