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RNA asymmetric distribution and daughter/mother differentiation in yeast

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Abstract

Active transport and localized translation of the *ASH1* mRNA at the bud tip of the budding yeast *Saccharomyces cerevisiae* is an essential process that is required for the regulation of the mating type switching. *ASH1* mRNA localization has been extensively studied over the past few years and the core components of the translocation machinery have been identified. It is composed of four localization elements (zipcodes), within the *ASH1* mRNA, and at least three proteins, She1p/Myo4p, She2p and She3p. Whereas the movement of the RNA can be attributed to direct interaction with myosin, the regulation of the RNA expression is less well understood. Recent insights have revealed a role for translation that might have a key function in the regulation of Ash1 protein sorting.

Introduction

Modulation of gene expression occurs at various steps, from initiation of transcription up to the final delivery of an active protein to its correct location within the cell. One powerful mechanism for control of gene expression involves localized translation of a specific mRNA at the place where the protein is needed. This cytoplasmic aspect of gene expression is a well-documented mechanism for regulating local protein expression in higher eukaryotes and patterning during embryonic development [1]. For instance, during *Drosophila* oogenesis, the future axes of the embryo are predetermined maternally by the specific localization of specific mRNAs to the two poles of the oocyte. Later in development, pair–rule mRNAs are actively transported and anchored to the cortex of the syncytium, preventing these morphogenes from diffusing along the embryo before cellularization occurs. Somatic cells use mRNA localization to locally produce proteins in the subcellular location where they are needed. For example, β-actin mRNA is localized to the leading edge of fibroblasts, where it is utilized for rapid responses to extracellular signals [2]. These examples confirm that specific mRNA subcellular localization generates the cellular protein asymmetry that is required for diverse cellular functions.

In the yeast *Saccharomyces cerevisiae*, *ASH1* mRNA localization is used to control mating type switching. The localized translation of the transcriptional repressor, Ash1p, to the daughter cell specifies different patterns of gene expression between mother and daughter

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cells. Here, the most comprehensively understood mechanism of mRNA localization, that of *ASH1* in budding yeast, will be discussed.

Budding yeast mating type switching

The budding yeast *S. cerevisiae* alternates between a diploid growing state and, under conditions of nutrient deprivation, a haploid growing state. The return to a diploid state is accomplished through the mating of two haploid cells of the opposite mating type (‘a’ or ‘α’). Mating type switching is unique to the mother cell. The daughter never switches, and thus mother and daughter cells are necessarily of opposite mating types. This ensures that an isolated spore will be able to form diploids cells through mating between its descendents. Mating type switching results from asymmetric (mother-specific) expression of the *HO* endonuclease. *HO* initiates a genomic rearrangement of the MAT locus, resulting in the conversion of an ‘a’ cell to an ‘α’ or vice–versa (Figure 1). The basis of asymmetric *HO* transcription comes from the restriction of the transcriptional repressor Ash1p to the daughter cell [3-5]; a result of the asymmetric distribution of its mRNA.

ASH1 mRNA is packaged in a mRNP, the ‘locasome’, which is actively transported to the bud, ensuring the exclusive translation of Ash1p in the daughter cell [6]. *ASH1* mRNA localization near to the daughter nucleus is the sole determinant of Ash1p sorting [7]. Ash1p asymmetry might not be restricted to mating type switching; it might also be required for pseudohyphal growth [8, 9]. Ash1p is restricted to the pseudohyphal cell-nucleus but the dependence of pseudohyphal growth on restricted expression of Ash1p has not been proven, thus far [9]. Together with daughter cell-specific *ASH1* mRNA localization, the cell cycle-regulated transcription in late anaphase [4, 10] has a crucial role in mating type switching control. Ash1p also interacts with the promoters of*SGA1* and *PCL1*, which are implicated in sporulation control and cell cycle control, respectively [11]. Interestingly, Pcl1p is one of the cyclins associated with the Pho85 kinase complex, which has recently been demonstrated to phosphorylate Ash1p, regulating its stability [12]. In a *PHO85* strain, Ash1p is stabilized so that its activity persists in the daughter cell nucleus during the cell cycle. This aberrantly represses mating type switching when the cell enters the next bud cycle [12].

The *ASH1* mRNA transport machinery (the locasome)

ASH1 mRNA localization is mediated by different *cis* elements within the mRNA, termed ‘zipcodes’, that are recognized by *trans*-acting factors that mediate the directed movement to the bud tip (place of translation). *ASH1* mRNA contains 4 *cis*-acting zipcodes, termed E1, E2a, E2b and E3 (Figure 2a) [13-16]. These zipcodes act synergistically, ensuring efficient transport. Each is able to direct an mRNA to the bud but all four together ensure that this happens more frequently [13, 14, 17]. The *ASH1* zipcodes are contained within the coding sequence of the mRNA, with one of them overlapping the stop codon (Figure 2a). The sequence homology between the four elements is weak and no common sequence has yet been identified. Three of the four elements are entirely located in the coding region; this could introduce constraints in the evolution of such elements, making it difficult to identify clearly defined sequence/structure motifs. Additional functions of these elements, aside from their common role in localization, cannot be excluded.

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Each of the four localization elements is recognized by She2p, an RNA-binding protein, containing no known RNA-binding motifs [18, 19]. She2p interaction with the E3 zipcode *in vitro* can be demonstrated by UV cross-linking [13]. However, She2p *in vitro* binding to the full-length *ASH1* mRNA is weak, showing a K_d in the micromolar range, indicating that an additional protein could be required for efficient binding *in vivo* (Hüttelmaier and Singer, unpublished). She2p shuttles from the nucleus to the cytoplasm in an RNA-dependent manner, indicating that newly transcribed *ASH1* mRNA is predetermined for a cytoplasmic location before its nuclear export [20] (Figure 2c). Mutants in *SHE2* were initially identified in a genetic screen for genes affecting *HO* expression [21]. This screen also identified two other genes that are directly involved in*ASH1* mRNA localization: *SHE1* and *SHE3*. She1p, also called Myo4p, is a type V unconventional myosin, providing the motor activity of the locasome along the actin network [4, 13, 14, 17, 21, 22]. Motion driven from a single myosin V would be inefficient, due to the poor processivity of this type of motor. Redundancy of ASH1 mRNA zipcodes is therefore needed to provide a persistent motion of the cargo [23]. This is consistent with the presence of four localization elements in *ASH1* mRNA, each presumably binding one myosin molecule (Figure 2a). The link between the RNA-binding protein She2p and the motor She1p/Myo4p is mediated by She3p [18, 24]. She1p/Myo4p and She3p are exclusively cytoplasmic proteins, and the process by which these two proteins recognize the newly exported mRNP containing She2p is yet to be determined. *ASH1* mRNA, together with the trimeric complex She1p/Myo4p–She2p–She3p, initiates formation of the locasome — the RNA localization particle [18, 24]. Each of these components is absolutely necessary for *ASH1* mRNA localization; their individual deletions result in the delocalization of *ASH1* mRNA [13, 14]. The kinetics of the transport of the locasome to the bud tip has been demonstrated *in vivo* by tagging the mRNA with GFP reporters making ASH1 mRNA localization a powerful tool by which to study RNA movements *in vivo* [17, 25, 26]. (Figure 2).

Accessory factors involved in locasome targeting

She1p/Myo4p, She2p and She3p are core components of the locasome but additional proteins have been identified that affect *ASH1* mRNA localization (Table 1). These proteins play an accessory role in the trafficking process and their deletion seems to affect general steps, such as cell polarization, motor-driven motility and translation [6].

Two additional *SHE* genes (*SHE4* and *SHE5*) were identified in the initial screen and, subsequently, were shown to affect *ASH1* mRNA localization [13, 14, 17, 21, 26, 27]. *SHE4* mutants show additional defects in endocytosis and actin polarization [28, 29]. She4p binds to the motor domains of unconventional myosins and enhances their binding to microfilaments [30, 31]. Interestingly, She4p interacts with She1p in a two-hybrid assay [31]. It has been proposed that She4p is required for the structural integrity or the regulation of the motor domain of unconventional myosins [31].

She5p/Bni1p is a formin that has recently been shown to promote nucleation of barbed-end actin polymerization [32, 33]. Deletion of *SHE5/BNI1* leads to the accumulation of *ASH1* mRNA at the bud neck [13], consistent with a defect in promoting polymerization of actin fibers at the bud tip. Bud6p binds to She5p/Bni1p and is also required for actin filament

organization to the bud growth pole [34, 35]. Consistent with the *SHE5* phenotype, in a *BUD6* strain, the mRNA shows a directed motorized motion in the mother cell but mRNAs observed in the daughter cell are freely diffusing [26, 27].

In addition to the *SHE* gene mutations, four additional genes have been shown to affect*ASH1* mRNA localization: *LOC1*, *KHD1*, *SCP160* and *PUF5*. Deletion of each of these four genes affects *ASH1* mRNA localization to a lesser extent than the *SHE* mutants, which might explain why they were not identified in the original genetic screens. Loc1p was isolated by the three-hybrid system, due to its ability to bind to the E3 and E1 zipcodes. In a *LOC1* strain the ASH1 mRNA is not correctly localized and restricted Ash1p localization to the daughter cell nucleus is strongly affected [36]. Loc1p is an exclusively nuclear protein and so might have a role in early *ASH1* mRNP formation. Interestingly, Loc1p has been identified as part of the 66S pre-rRNA complex and has been shown to affect 60S rRNA processing [37]. It is actually unclear whether the *ASH1*mRNA delocalized phenotype is direct or if it involves a translational defect.

KHD1, *SCP160* and *PUF5* were identified in a systematic survey of yeast RNA-binding proteins that affect *ASH1* mRNA localization [38]. Of these three proteins, only Khd1p co-localizes with the *ASH1* mRNA locasome, indicating a direct role in mRNA localization. Interestingly, in a *KHD1* , *SCP160* or *PUF5* strain, the strongest effect on*ASH1* mRNA localization is seen at the bud tip, meaning that the function of these genes might be more specific for anchoring of the mRNA to its translational location [38] (Table 1 and following sections).

Translation and localization

Sequential deletions of the four zipcodes of *ASH1* mRNA progressively affect its correct localization, therefore, demonstrating that they all contribute in targeting the mRNA to the bud tip [7]. The number of zipcodes seems to be crucial for efficient *ASH1* mRNA localization but their position is not. When inserted in the 3'UTR of a nonlocalizing mRNA, each one of these four elements is able to direct bud localization [7]. The shift to the 3'UTR of the four ASH1 zipcodes did not affect mRNA localization but it did affect Ash1p asymmetric localization. This demonstrates that the presence of localization elements within the coding region of *ASH1* mRNA provide translational silencing while it is transported to the bud tip. Chartrand *et al.* [7] proposed that a reduced translation of the*ASH1* mRNA could balance the slow localization of *ASH1* mRNA [17, 26]. Another interesting link between *ASH1* mRNA translation and localization is the observation that, upon cycloheximide treatment, *ASH1* mRNA is not properly localized to the bud tip [20]. Mutation of the *ASH1* methionine initiation codon or insertion of a stop codon in the middle of the *ASH1* coding sequence affects anchoring to the bud tip but doesn't affect mRNA transport [15]. This phenotype cannot be rescued by the expression of the wild type Ash1p in *trans*, demonstrating that the C-terminus of Ash1p is implicated in anchoring as a *cis*-acting element. This finding introduces the interesting possibility that anchoring to a currently uncharacterized complex could be mediated co-translationally, via the nascent Ash1 polypeptide [15].

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Recently, the KH domain containing protein Khd1p, has been reported to affect *ASH1*mRNA anchoring to the distal bud tip. Interestingly, overexpression of Khd1p reduces the cellular amounts of Ash1p, possibly due to a decrease in translation [38]. Khd1p was found in association with different translation initiation complexes in a proteomic survey [39]. Khd1p has been proposed to bind to the 5' region of *ASH1* mRNA and so co-localize with the locasome [38], suggesting that Khd1 could silence *ASH1* mRNA translation during transport. Khd1p could also have a more specific role at the bud tip, enabling the specific retention of *ASH1* mRNA to the tip. The deletion of *KHD1* [38] and the abolition of translation of the Ash1p C-terminus [15] appear to produce a similar phenotype on mRNA anchoring; we suggest that these two factors could be involved in the same process.

Puf5p belongs to a highly conserved family of RNA binding proteins with a well-characterized role in protein translation regulation [40]. Puf5p is a translational regulator of the *HO* endonuclease, functioning through binding to the 3'UTR of its mRNA [41]. Scp160p is known to interact with membrane-bound polysomes [42]. The role of *SCP160* on *ASH1* mRNA could be more general, as this protein was recently shown to interact with 69 mRNAs with diverse functions, among which, *ASH1* mRNA was not found [43]. Aside from their general role in translation, the link between *ASH1* mRNA localization and the function of these proteins remains unclear.

mRNAs actively transported to the daughter cell; a growing family

The function of the locasome is not restricted to the transport of *ASH1* mRNA to the daughter cell bud tip. Immunoprecipitation experiments using the core locasome proteins (She1p–3p), followed by DNA microarray technology [44, 45] and validation in a living cell assay [45], identified a total of 22 mRNAs. All these mRNAs show a preferential localization to the bud in a *SHE2*-dependent manner, implying that their transport machinery is identical to the *ASH1* locasome [45]. As is the case for *ASH1* mRNA, all of these mRNAs contained localization elements within their coding sequence and for only three of them did the 3' UTR confer specific localization to a reporter mRNA, thus, demonstrating that at least some of them contain additional signals. Nevertheless, localization element redundancy is not a rule; *IST2* mRNA seems to contain only one element [20]. Ist2p encodes an ion transporter that is restricted to the daughter cell membrane by the septin ring acting as a diffusion barrier [44]. In addition, eleven of the newly identified localized mRNAs code for proteins that are not localized to the daughter cell; moreover, eight additional mRNAs code for proteins that are able to localize to the daughter cell independently of their mRNA localization, indicating that mRNA localization is not a key determinant for the subcellular distribution of these 19 proteins. None of the 22 known localized mRNAs, nor the locasome components (she1–3), were essential in yeast. One hypothesis to explain the significance of these observations is that mRNA localization to the daughter cell is a dynamic event, ensuring enhanced distribution of mRNAs between mother and daughter cells. This could ensure the ability of the daughter cell to respond to environmental situations independently of transcription. Consistent with this, a fraction of mRNAs that are localized to the daughter cell code for proteins related to environmental sensing.

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Conclusions

mRNA localization in *S. cerevisiae* has proven to be a complex system, involving more steps than just those for moving the RNA. Additional considerations must include the translational regulation of the mRNA during transport and anchoring at its final destination; the molecular mechanisms of these two crucial processes are yet to be fully elucidated. Clearly, the locasome has a role in this regulation, by sequestering the RNA away from the translational components. However, the mechanism by which it is disassembled at the bud tip, thereby enabling translation, remains elusive. Recent developments have also proved that generation of cell fate asymmetry could be one of several functions of mRNA localization in yeast. The discovery of localized mRNAs, not involved in protein asymmetry, and vice-versa, suggests that there might be other roles for mRNA localization.

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Abbreviations

5'/3' UTR	5'/3' untranslated regions of a mRNA
DAPI	4',6-diamidino-2-phenylindole
FISH	fluorescent *in situ* hybridization
***K*d**	dissociation constant
mRNP	messenger RNA ribonucleoprotein complex

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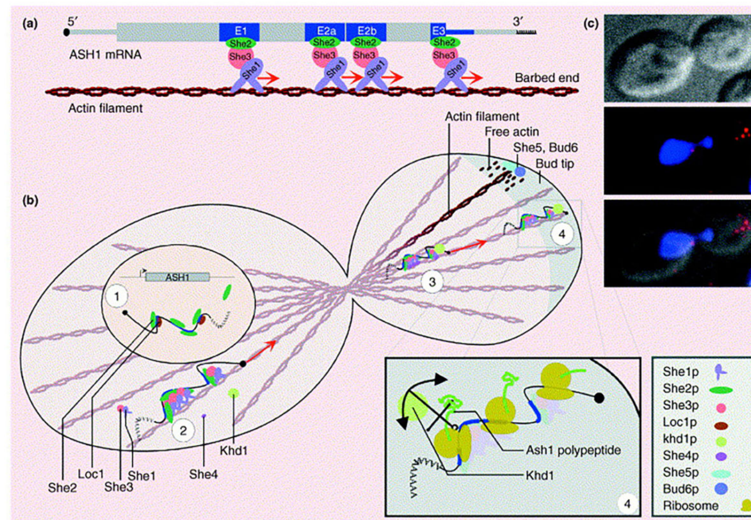

Figure 1.

Mating type switching in the yeast *S. cerevisiae*. The mating type of a cell is defined by the expression of 'a'- or 'α'- specific gene(s) from the active MAT locus. These specific factors are encoded by the transcriptionally silenced HMLa and HMRα loci. Switching to the opposite mating type occurs by gene conversion at the active MAT locus. The template used for this conversion is issued from the silenced HMLa or HMRα locus of the opposite type. *HO* endonuclease cuts the active MAT locus, initiating this replacement. In daughter cells, the transcriptional repressor Ash1p inhibits the expression of *HO*, thereby repressing mating type switching (see text for details).

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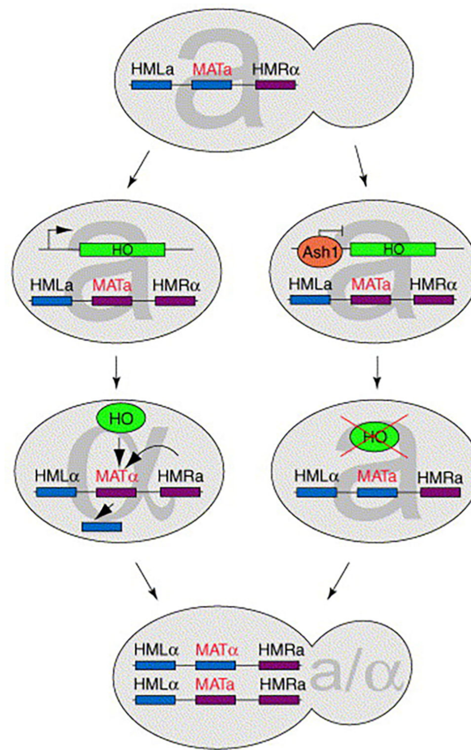

Figure 2.

Ash1 mRNA localization. **(a)** Cartoon of the *Ash1* mRNA core locasome transported on an actin filament. E1, E2a, E2b and E3 zipcodes are recognized by the She1–3p complex, providing the motor activity. **(b)**Schematic view of the different steps of *ASH1* mRNA bud tip localization (see also Table1). 1 – Nuclear events: *ASH1* transcription in late anaphase, association of *ASH1* mRNA with She2p, interaction with Loc1p. 2 – Early cytoplasmic events: Assembly of the locasome by association of the newly exported *ASH1*mRNA-She2p RNP with She3p and She1p/Myo4p, recruitment to the actin network and interaction with Khd1p and She4p. 3 – Transport of the locasome to the bud tip. She1p/Myo4 provides the motor activity. Role of She4p as a translocation co-factor. 4 – Anchoring and translation at the bud tip: the mechanism of anchoring is still not understood; nevertheless it involves Khd1p and the nascent Ash1p polypeptide. Puf5p and Scp160p are not represented because their role remains largely unknown. **(c)** Upper image: phase image of a yeast budding cell in early anaphase. Middle image: fluorescent *in situ* hybridization (FISH) of*ASH1* mRNA using 6 CY3-labeled probes in red and DAPI staining in blue. Lower image: overlay of the two previous images.

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Table 1

Proteins involved in ASH1 mRNA localization.

Protein	Alternative names	Function	Role in ASH1 mRNA localization	Locasome association	Subcellular localization	phenotype bud/bud tip	References
She1p	Myo4p	Unconventional myosin V type motor	Locasome molecular motor	Yes	C, bud enriched	Abolished	13, 14, 17•, 21, 22.
She2p		RNA-binding	Binds *ASH1*mRNA zipcodes	Yes	N/C, bud enriched	Abolished	13, 18, 19, 20•, 21, 22.
She3p		Myosin binding protein	Molecular link in between She2p and She1p	Yes	C, bud enriched	Abolished	13, 18, 19, 21, 22, 24.
She4p	Dim1p	Endocytosis Enhance association of Myo3p and Myo5p to the actin network Interacts with She1p	Enhance association of She1p to the actin network?	Yes	C, bud enriched	nd/nd bud neck	13, 21, 28, 29.
She5p	Bni1p, Ppf3p	Actin filament polymerization at the bud tip	Polarization and growth of the actin network	No	C, bud tip	nd/nd bud neck/bud enriched	13, 22, 26, 33.
		Bud growth Establishment of cell polarity	Interacts with Bud6p (two-hybrid and synthetic lethality)				
Bud6p	Aip3p	Actin filament organization at the bud tip	Polarization and growth of the actin network	No	C, bud tip	nd/nd bud enriched	26, 27, 34, 35.
		Bud growth Establishment of cell polarity	Interacts with She5p (two-hybrid and synthetic lethality)				
Loc1p		RNA binding rRNA processing	Binds *ASH1*zipcodes, general translation defect?	Yes (nucleus)	No	nd/13	36, 37.
Khd1p	Hek2p	RNA binding, KH	Involved in mRNA bud tip anchoring,	Yes	C	93/40	38••, 39.

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Protein	Alternative names	Function	Role in ASH1 mRNA localization	Locasome association	Subcellular localization	phenotype bud/bud tip	References
		domain	translational repression				
Puf5p	Mpt5p, Htr1p, Uth4p	Translational regulation mRNA stability	Involved in mRNA bud tip anchoring, Translational regulation?	No	nd	83/22	38••, 40.
Scp160p		RNA binding, KH domain ER polysomes binding	Involved in mRNA bud tip anchoring, Translational regulation?	No	C	84/23	38••, 42.

C, Cytoplasmic; N, Nuclear; No, nucleolar; deletion phenotype is shown as: percentage of cells showing daughter cell localization of *ASH1* mRNA/percentage of cells showing daughter cell bud tip localization of *ASH1* mRNA. The wild type strain shows a phenotype of 99/87 [38••].