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REVIEW

Messenger RNA transport in the opportunistic fungal pathogen *Candida albicans*

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Abstract *Candida albicans*, a common commensal fungus, can cause disease in immunocompromised hosts ranging from mild mucosal infections to severe bloodstream infections with high mortality rates. The ability of *C. albicans* cells to switch between a budding yeast form and an elongated hyphal form is linked to pathogenicity in animal models. Hyphal-specific proteins such as cell-surface adhesins and secreted hydrolases facilitate tissue invasion and host cell damage, but the specific mechanisms leading to asymmetric protein localization in hyphae remain poorly understood. In many eukaryotes, directional cytoplasmic transport of messenger RNAs that encode asymmetrically localized proteins allows efficient local translation at the site of protein function. Over the past two decades, detailed mechanisms for polarized mRNA transport have been elucidated in the budding yeast *Saccharomyces cerevisiae* and the filamentous fungus *Ustilago maydis*. This review highlights recent studies of RNA-binding proteins in *C. albicans* that have revealed intriguing similarities to and differences from known fungal mRNA transport systems. I also discuss outstanding questions that will need to be answered to reach an in-depth understanding of *C. albicans* mRNA transport mechanisms and the roles of asymmetric mRNA localization in polarized growth, hyphal function, and virulence of this opportunistic pathogen.

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Background

The opportunistic pathogen *Candida albicans* lives commensally in the majority of humans. Under different circumstances such as immune system suppression, however, this normally benign fungus can cause disease in human hosts ranging from mild mucosal infections to severe disseminated infections, which have a mortality rate of up to ~35% (Wisplinghoff et al. 2004). In mammalian hosts and in vitro, *C. albicans* cells exhibit different morphologies including ovoid budding yeast and elongated hyphae (Sudbery 2011). The ability of this fungus to switch between yeast-form cells and filamentous hyphal growth is linked to its pathogenicity in animal models (Lo et al. 1997; Saville et al. 2003). Many proteins and pathways are implicated in promoting hyphal growth and function through transcriptional, post-transcriptional, and post-translational mechanisms (Kadosh 2016; Lu et al. 2014; Sudbery 2011; Verma-Gaur and Traven 2016). Large-scale changes in gene expression upon hyphal induction include upregulation of hyphal-specific cell-surface adhesins, which promote attachment to host cells, and secretion of lipases and proteases, which facilitate host-tissue invasion and damage (de Groot et al. 2013; Schaller et al. 2005). Specific mechanisms leading to the asymmetric hyphal localization of proteins such as adhesins, hydrolases, and proteins that direct polarized hyphal growth, however, remain poorly understood.

In eukaryotic processes from neuronal signaling to *Drosophila* embryogenesis, directional mRNA transport allows efficient local translation at the site of protein function

(Holt and Bullock 2009). RNA transport is also linked to polarized growth and differentiation in fungi (Zarnack and Feldbrugge 2007). In the ascomycete yeast *Saccharomyces cerevisiae*, transport of the *ASH1* mRNA along actin cables to the bud tip is driven by a complex including RNA-binding proteins She3 and She2 and the Myo4 myosin motor (Gonsalvez et al. 2005). Bud-tip *ASH1* mRNA localization allows daughter cell-specific expression of the Ash1 transcription factor, which prevents mating-type switching (Jansen et al. 1996; Sil and Herskowitz 1996). The *Ustilago maydis* RNA-binding protein Rrm4, which mediates long-distance transport of mRNAs along microtubules, is required for polarized growth and virulence of this filamentous basidiomycete fungus (Becht et al. 2006). Rrm4 transport of the septin *CDC3* mRNA on the surface of endosomes allows proper assembly of septin filaments at growth poles (Baumann et al. 2014), whereas Rrm4 transport of endochitinase *CTS1* mRNA facilitates unconventional secretion and predominantly unipolar localization of the Cts1 protein (Koepke et al. 2011). Models of apical tip growth of filamentous fungi involve trafficking of secretory vesicles from a sub-apical vesicular structure, termed a Spitzenkörper, to the plasma membrane at the site of polarized growth (Riquelme 2013). The presence of ribosomes in the Spitzenkörper of filamentous fungi (Grove and Bracker 1970) suggests local translation of proteins at the tip may influence hyphal processes. These findings raise the question of whether polarized mRNA transport plays a role in *C. albicans* hyphal growth and function by driving asymmetric localization of hyphal proteins.

She3-mediated mRNA transport in *C. albicans*

Similar to its *S. cerevisiae* ortholog ScAsh1,[1] *C. albicans* Ash1 (CaAsh1) localizes asymmetrically to the daughter cell nucleus during budding growth (Inglis and Johnson 2002). Localization of CaAsh1 to apical nuclei in hyphae, combined with hyphal formation defects and lower virulence of cells lacking CaAsh1 (Inglis and Johnson 2002), suggests that She3-mediated mRNA transport might play a role in *C. albicans* hyphal differentiation. *CaASH1* mRNA also localizes asymmetrically, concentrating at the bud tip during budding growth and the apical tip cell during hyphal growth (Elson et al. 2009). In the absence of CaShe3, however, Ca*ASH1* mRNA is detected throughout the cell and CaAsh1 is found in mother, daughter, and hyphal nuclei, supporting a role for CaShe3 in asymmetric mRNA and protein localization in *C. albicans* (Elson et al. 2009).

[1] For clarity, the names of orthologous proteins in different fungi are preceded by the first letter of the genus and species.

CaShe3 binds to Ca*ASH1* mRNA and at least 39 additional mRNAs, only one of which has an ortholog transported by ScShe3 in *S. cerevisiae* (Elson et al. 2009). CaShe3 binds to nine of these mRNAs specifically in hyphal cells, including *SAP5*, which encodes a secreted aspartic protease (Elson et al. 2009). In addition to *ASH1*, thirteen of the CaShe3-bound mRNAs are visible over background in fluorescence in situ hybridization experiments; these mRNAs localize to the bud and/or hyphal tip only in the presence of CaShe3, supporting a role for She3 in polarized mRNA transport in *C. albicans* (Elson et al. 2009). The impact of Ca*SHE3* deletion on the localization of most of the proteins encoded by CaShe3-bound mRNAs is unknown; however, the hyphal tip-focused gradient of CaMss4p, a 1-phosphatidylinositol-4-phosphate 5-kinase required for hyphal formation, is maintained in the absence of CaShe3, as it is the gradient of its product, $PI(4,5)P_2$ (Vernay et al. 2012). This result indicates that, as for multiple ScShe3-transported mRNAs (Aronov et al. 2007; Shepard et al. 2003), additional mechanisms can promote asymmetric localization of proteins encoded by CaShe3-bound mRNAs in the absence of CaShe3.

Phenotypes of *C. albicans* cells lacking CaShe3 suggest that CaShe3-based transport impacts hyphal growth and function without being absolutely required for hyphal formation or virulence. In broth culture, *C. albicans she3Δ/Δ* cells treated with serum have abnormal hyphal morphology and on solid medium under embedded conditions *she3Δ/Δ* cells show defects in extended hyphal growth (Elson et al. 2009). The absence of CaShe3 decreases epithelial cell damage by *C. albicans* in vitro, but does not affect endothelial cell damage in vitro or virulence in a murine model of disseminated infection (Elson et al. 2009). Thus, CaShe3 mRNA transport may impact localization of a subset of proteins that influence specific hyphal functions.

Cells with homozygous deletion of genes encoding CaShe3-transported mRNAs display a wide variety of filamentation phenotypes, from wildtype growth to severe defects in hyphal extension, few of which precisely mirror *she3Δ/Δ* hyphal growth defects (Elson et al. 2009). In cases such as that of CaMss4 described above, proper protein localization may not depend solely on mRNA localization. In *S. cerevisiae*, the absence of the ScShe3 complex RNA-binding protein She2 does not affect the bud-tip localization of the majority of proteins encoded by She2-bound and transported mRNAs (Aronov et al. 2007; Shepard et al. 2003). Similar to ScAsh1 localization to the daughter cell nucleus, however, ScSro7 bud-tip localization does depend on She2, leading to the hypothesis that mRNA transport may play a greater role in localization of proteins that are not targeted to membranes by other mechanisms such as lipidation or secretion (Aronov et al. 2007).

CaShe3-mediated mRNA transport may also influence hyphal formation and function through mechanisms beyond allowing local translation of asymmetrically localized proteins. Directional mRNA transport might ensure widespread localization of a protein in both the mother cell and hypha. In wildtype *S. cerevisiae* cells, ScIst2 protein is found at the plasma membrane of the mother cell and the bud. In the absence of transport of the *IST2* mRNA to the bud tip by the She complex, however, ScIst2 is restricted to the mother cell (Juschke et al. 2004; Takizawa et al. 2000). In addition, coupling of directional mRNA transport with membrane trafficking might influence assembly of the encoded proteins into functional complexes, as seen with endosomal transport of a septin mRNA in *U. maydis* (Baumann et al. 2014). Studies to determine the localization of proteins encoded by CaShe3-transported mRNAs in the presence and absence of CaShe3 should help elucidate cellular mechanisms underlying the *C. albicans she3Δ/Δ* growth and virulence defects.

Despite the presence of She3-mediated mRNA transport pathways in both *S. cerevisiae* and *C. albicans*, differences between the two systems are highlighted not only by the transport of different mRNAs, but also by the absence of orthologs of the Type V myosin Myo4 and RNA-binding protein She2 in *C. albicans*. The sole type V myosin in *C. albicans*, CaMyo2, is essential for hyphal growth (Woo et al. 2003); CaMyo2 is 53% identical to ScMyo4 and 60% identical to ScMyo2, which copurifies with over 50 mRNAs in *S. cerevisiae* (Casolari et al. 2012). Therefore, the CaMyo2 motor may drive mRNA transport by CaShe3 in *C. albicans*. ScShe3 binds to Myo4 via the evolutionarily conserved She3 N-terminal coiled-coil domain, whereas the poorly conserved C-terminus directs binding to Sc*ASH1* mRNA and She2 (Bohl et al. 2000; Long et al. 2000). The C-termini of the She3 proteins in both fungi lack a classical RNA-binding domain, but many proteins without canonical RNA-binding domains can bind to RNA (Beckmann et al. 2016). Therefore, CaShe3 may mediate mRNA transport on its own. Alternatively, CaShe3 mRNA transport complexes may contain as-yet unidentified RNA-binding proteins.

SR-like RNA-binding protein 1: candidate CaShe3 collaborator

Recent data indicate that the *C. albicans* SR-like RNA-binding protein Slr1, which has no apparent *S. cerevisiae* ortholog, may be a candidate to aid in mRNA transport to the hyphal tip (Ariyachet et al. 2017). Although Slr1 is a predominantly nuclear phosphoprotein, a fraction of Slr1 copurifies with 80S ribosomes and polysomes, suggesting a cytoplasmic role for Slr1. Serine-to-alanine mutations in six SR/RS dipeptides at the C-terminus of Slr1

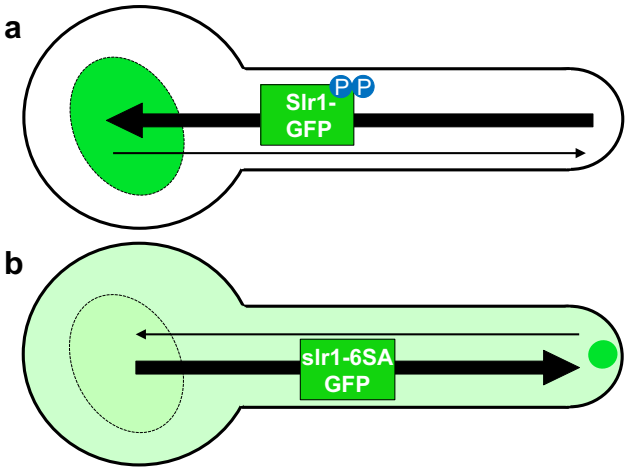

Fig. 1 Model for phosphorylation control of Slr1 nucleocytoplasmic transport in *C. albicans*. **a** Wildtype Slr1-GFP is predominantly nuclear due to phosphorylation of SR motifs favoring nuclear import over export. **b** Slr1-6SA-GFP accumulates in the cytoplasm and at the hyphal tip because it cannot be phosphorylated. *Arrow line* width reflects relative speed of transport

block phosphorylation of this slr1-6SA mutant protein and increase its cytoplasmic localization compared to wildtype Slr1. Intriguingly, in hyphal cells the slr1-6SA-GFP mutant protein is often detected in a focus at the hyphal tip (Ariyachet et al. 2017). The slr1-6SA-GFP hyphal tip focus partially overlaps with the Spitzenkörper (Ariyachet et al. 2017) and is reminiscent of the concentration of Ca*ASH1* and other CaShe3-transported mRNAs at the hyphal tip (Elson et al. 2009).

Taken together, these results support a working model (Fig. 1) where Slr1 shuttles between the nucleus and hyphal tip and phosphorylation at the hyphal tip facilitates release of Slr1 from mRNA–protein complexes, allowing rapid return to the nucleus (Fig. 1a). The activity of multiple RNA-binding proteins involved in mRNA transport in *S. cerevisiae* is similarly modulated by phosphorylation. SR-like protein ScNpl3 phosphorylation decreases ScNpl3 affinity for mRNA and increases its nuclear localization and its affinity for the nuclear import receptor ScMtr10 (Gilbert et al. 2001; Yun and Fu 2000). ScKhd1 and ScPuf6 bind to Sc*ASH1* mRNA and repress translation until the mRNA is released by phosphorylation of both proteins at the bud tip (Deng et al. 2008; Paquin et al. 2007).

According to the Slr1 transport model, S-to-A mutations that block phosphorylation of Slr1 slow mRNA release and cause the mutant slr1-6SA protein to accumulate at the hyphal tip (Fig. 1b). In cells that lack CaShe3, however, hyphal tip localization of slr1-6SA-GFP decreases ~fourfold (Ariyachet et al. 2017), suggesting that Slr1 may travel to the tip in part with CaShe3 transport complexes. In addition, both Sc*ASH1* mRNA

in *S. cerevisiae* (Beach and Bloom 2001) and slr1-6SA-GFP in *C. albicans* (Ariyachet et al. 2017) appear in foci at the bud neck in large-budded cells. Future experiments to test binding of Slr1 to CaShe3 and CaShe3-transported mRNAs, as well as to determine the localization of these mRNAs in the absence of Slr1, will help define whether wildtype Slr1 plays a role in CaShe3-mediated mRNA transport in *C. albicans*.

Homozygous deletion of *SLR1* reduces *C. albicans* hyphal growth and epithelial and endothelial cell damage in vitro. In addition, *slr1Δ/Δ* cells are less virulent than wildtype or *slr1Δ/slr1Δ* + *SLR1* reconstituted cells in a murine model of disseminated infection (Ariyachet et al. 2013). These stronger phenotypes for *SLR1* compared to Ca*SHE3* deletion suggest that Slr1 functions in processes beyond any role it may have in CaShe3-mediated mRNA transport. The similar generation times and hyphal formation of cells expressing Slr1-GFP and slr1-6SA-GFP indicates that the steady-state shift of slr1-6SA-GFP toward the cytoplasm and hyphal tip does not severely impact its functions (Ariyachet et al. 2017). Since slr1-6SA-GFP still localizes to the hyphal tip of some cells in the absence of CaShe3 (Ariyachet et al. 2017), identification of mRNAs bound to slr1-6SA-GFP in *she3Δ/Δ* cells may reveal additional candidate targets for polarized mRNA transport in *C. albicans* hyphae.

Sec2: linking membranes to *C. albicans* mRNA transport

In *S. cerevisiae*, the She3 complex transports mRNAs that encode several bud-tip localized polarity and secretion (POL) factors, including the small GTPase ScSec4 (Aronov et al. 2007). This Rab family protein mediates post-Golgi secretory vesicle binding to the plasma membrane and is activated by the guanyl nucleotide exchange factor ScSec2 (Salminen and Novick 1987; Walch-Solimena et al. 1997). The *C. albicans* Sec2 and Sec4 orthologs localize to the Spitzenkörper in hyphal cells and CaSec2 protein lacking the C-terminal 168 residues does not support hyphal formation (Bishop et al. 2010; Jones and Sudbery 2010). Although CaSec2, like CaShe3, does not contain a classical RNA-binding domain, it binds to and co-localizes with its cognate Ca*SEC2* mRNA in *C. albicans* hyphae (Caballero-Lima et al. 2014). Neither CaShe3 nor the CaShe3-bound mRNA *SAP5* copurifies with CaSec2, indicating that CaSec2 and CaShe3 are likely present in distinct mRNA transport complexes (Caballero-Lima et al. 2014).

Whereas, the *S. cerevisiae* POL factor mRNAs found in ScShe3 complexes are co-transported with cortical endoplasmic reticulum (Aronov et al. 2007), *CaSEC2* mRNA and CaSec2 copurify with secretory vesicles. The membrane association of Sec2 protein and mRNA implies that they may be co-transported, as it is seen for the septin UmCdc3 and its mRNA on endosomes in *U. maydis* hyphae (Baumann et al. 2014). A phosphomimetic mutation replacing serine 584 with glutamate (S584E) in CaSec2 both decreases co-localization of Ca*SEC2* mRNA with CaSec2 at the hyphal tip and abrogates crosslinking of poly(A) RNA to CaSec2 (Caballero-Lima et al. 2014). These results suggest that phosphorylation helps release CaSec2 from its cognate mRNA. The percentage of cells with hyphal tip localization of sec2-S584E mutant protein is lower for wildtype CaSec2 (Caballero-Lima et al. 2014). This difference might result in part from mRNA delocalization, but it could also be due to lower mRNA or protein stability, or disruption of CaSec2 interactions with other proteins at the hyphal tip.

Candida albicans cells expressing phosphomimetic sec2-S584E display normal hyphal growth whereas a non-phosphorylatable sec2-S584A protein can only be expressed in the presence of wildtype CaSec2, even in budding cells (Bishop et al. 2010). These phenotypes indicate the importance of S584 phosphorylation for budding and filamentous growth. The decreased binding and co-localization of sec2-S584E protein and mRNA at the hyphal tip, therefore, suggest that mechanisms in addition to polarized Ca*SEC2* mRNA localization likely drive CaSec2 hyphal tip localization and function, similar to ScShe3-independent localization of ScSec4 protein at the bud tip (Aronov et al. 2007).

Perspectives

Our detailed understanding of *S. cerevisiae* and *U. maydis* model systems for polarized fungal mRNA transport includes many facets that have yet to be explored in *C. albicans* (Fig. 2).

1. Are there cis-acting sequences in *C. albicans* mRNAs that are recognized by CaShe3 or another protein in the CaShe3 complex for directional mRNA transport?

Given the absence of She2 and low conservation of the She3 C-terminus in *C. albicans* (Muller et al. 2011), such cis-acting sequences or structures likely diverge from those recognized by the She3 complex in *S. cerevisiae*.

2. In the absence of She2, how are CaShe3-transported mRNAs moved from the nucleus to the cytoplasm? Does CaShe3 transit through the nucleus or is another mRNA-binding protein involved in linking transcription to transport?

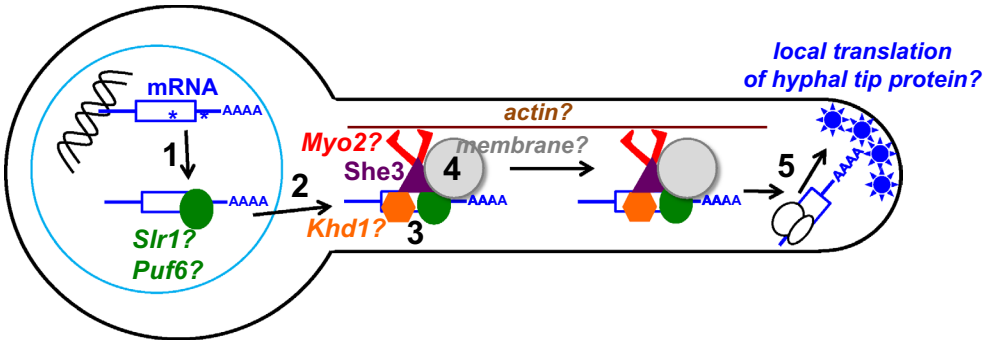

Fig. 2 Outstanding questions about polarized mRNA transport in *C. albicans* hyphae. *1* Are there cis-acting sequences or structures (*asterisk*) on mRNAs that direct localization? *2* What protein(s) help export asymmetrically localized mRNAs from the nucleus? *3* Do RNA-binding proteins repress translation of mRNAs during transport? *4* Are mRNA transport complexes associated with membranes? CaSec2 protein and mRNA associate with secretory vesicles, but CaShe3 is not part of this complex (Caballero-Lima et al. 2014). *5* What proteins depend on directional mRNA transport for their asymmetric localization in hyphae? *Italics* and *question marks* indicate proteins and structures hypothesized to play a role in CaShe3-mediated mRNA transport

In *S. cerevisiae*, She2 binds to *ScASH1* mRNA co-transcriptionally and the complex transits through the nucleolus prior to nuclear export (Du et al. 2008; Shen et al. 2010). Although putative CaShe3 complex protein Slr1-GFP is found throughout the nucleus, brighter foci suggest concentration of the protein in unidentified subnuclear regions (Ariyachet et al. 2017), raising the question of whether Slr1 might be found in the nucleolus and help export CaShe3 complex mRNAs.

3. Are mRNAs translationally repressed during transport to the hyphal tip?

Candida albicans orthologs of RNA-binding proteins Khd1 and Puf6 share 22 and 46% identity with ScKhd1 and ScPuf6, respectively. These proteins prevent translation of ScAsh1 during transport to the bud tip and, in their absence, Sc*ASH1* mRNA is transported less efficiently (Paquin and Chartrand 2008). The Ca*ASH1* mRNA contains consensus binding sites for ScKhd1 (CAUU) (Hogan, et al. 2008) and ScPuf6 (UGUU) (Gu et al. 2004), but whether CaKhd1 and CaPuf6 bind to CaShe3-transported mRNAs or are required for hyphal formation or function has not yet been determined.

4. Are CaShe3 and CaShe3-bound mRNAs associated with membranes?

A variety of cellular membranes are linked to mRNA transport in fungi: the ScShe3/She2/Myo4 complex mediates cortical endoplasmic reticulum (ER) transport to daughter cells in *S. cerevisiae* (Aronov et al. 2007; Schmid et al. 2006); a septin mRNA is transported and translated on endosomes in *U. maydis* (Baumann et al. 2014); CaSec2 and its mRNA associate with post-Golgi secretory vesicles (Caballero-Lima et al. 2014). While ER membranes are found throughout *C. albicans* hyphae, Golgi membranes are polarized toward the hyphal tip (Rida et al. 2006). ScMyo2 drives transport of late Golgi membranes to the bud as well as post-Golgi secretion and other organelle trafficking in *S. cerevisiae* (Pruyne et al. 2004). Association of CaShe3 and its mRNA targets with specific membranes could lend insight to functions of mRNA transport in hyphal protein targeting and function.

5. How many proteins beyond CaAsh1 depend on CaShe3 mRNA transport for asymmetric localization?

The CaMss4 protein does not require transport of its mRNA for localization to the hyphal tip or its polarized function (Vernay et al. 2012). The CaCbk1 activator protein CaMob2, which is required for hyphal growth, localizes to the hyphal tip (Gutierrez-Escribano et al. 2011) and CaShe3 binds to the *CaMOB2* mRNA specifically in the hyphae (Elson et al. 2009). Subcellular localization of CaMob2 protein or mRNA in the absence of CaShe3, however, has not been tested.

6. Are there CaShe3-independent mechanisms of mRNA localization beyond Ca*SEC2* transport and do these systems influence hyphal growth or function?

Intriguing work over the past few years has implicated proteins with low-complexity domains in subcellular organization of ribonucleoprotein complexes into non-membranous compartments through liquid–liquid phase separation (Sfakianos et al. 2016). In the filamentous ascomycete *Ashbya gossypii,* the glutamine-rich RNA-binding protein AgWhi3 is unevenly distributed within the hyphae and is required for polarized growth (Lee et al. 2015). AgWhi3

binds to mRNAs that encode polarity factors AgBni1 and AgSpa2, and these mRNAs are found in clusters at the hyphal tip, incipient branch sites, as well as throughout the hypha (Lee et al. 2015). Deletion of a poly-glutamine (polyQ) region in Whi3 decreases *BNI1* and *SPA2* mRNA clustering, most notably at sites of polar growth, reduces the concentration of Whi3 and Bni1 at the hyphal tip, and decreases mycelial branching (Lee et al. 2015). Puf2, an RNA-recognition motif (RRM) and Pumilio-domain-containing protein with relatively low-complexity amino acid composition, is similarly required for clustered *BNI1* and *SPA2* mRNA localization and wildtype mycelial branching (Lee et al. 2015). These findings point to a role for cytoplasmic mRNA clustering by low-complexity RNA-binding proteins in polarized growth of *A. gossypii*.

The *C. albicans* orthologs of Whi3 and Puf2 contain polyQ regions and classical RNA-binding domains, although CaPuf2 lacks the RRM found in AgPuf2. Interestingly, the C-termini of CaShe3 and Slr1 are both marked by low-complexity sequences: CaShe3 with a poly-asparagine region that ScShe3 lacks, and Slr1 with an arginine–glycine-rich region. Future work to understand the roles of such low-complexity regions in *C. albicans* RNA-binding protein function may reveal new mechanisms of mRNA transport in *C. albicans* hyphae and their impact on hyphal growth and function.

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