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REVIEW

Polarisome assembly mediates actin remodeling during polarized yeast and fungal growth

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ABSTRACT

Dynamic assembly and remodeling of actin is critical for many cellular processes during development and stress adaptation. In filamentous fungi and budding yeast, actin cables align in a polarized manner along the mother-to-daughter cell axis, and are essential for the establishment and maintenance of polarity; moreover, they rapidly remodel in response to environmental cues to achieve an optimal system response. A formin at the tip region within a macromolecular complex, called the polarisome, is responsible for driving actin cable polymerization during polarity establishment. This polarisome undergoes dynamic assembly through spatial and temporally regulated interactions between its components. Understanding this process is important to comprehend the tuneable activities of the formin-centered nucleation core, which are regulated through divergent molecular interactions and assembly modes within the polarisome. In this Review, we focus on how intrinsically disordered regions (IDRs) orchestrate the condensation of the polarisome components and the dynamic assembly of the complex. In addition, we address how these components are dynamically distributed in and out of the assembly zone, thereby regulating polarized growth. We also discuss the potential mechanical feedback mechanisms by which the force-induced actin polymerization at the tip of the budding yeast regulates the assembly and function of the polarisome.

KEY WORDS: Actin cytoskeleton, Fungal polarisome, Macromolecular assembly, Phase separation

Introduction

In the fungi kingdom, polarized cell growth is a dominant behavior underlying various cellular processes, such as budding, for vegetative cell growth, or the formation of cellular projections, which is required for the mating process (Pruyne and Bretscher, 2000). Research on polarized cell growth in *Saccharomyces cerevisiae* has shed light on the molecular basis of the underlying hierarchical regulatory processes, in which different proteins participate in a spatially and temporally regulated manner. Here, we review insights into the mechanism and regulation of cell polarity, mainly stemming from studies in the yeast *S. cerevisiae*, where conserved pathways, and in particular the polarisome complex, discussed below, are shared among a variety of fungi species, including budding yeast and filamentous fungi, such as *Candida albicans*. The central factor for the establishment of cell polarity, the small GTPase Cdc42, is conserved among different yeast species, sharing 80% similarity with human Cdc42 (Chiou et al., 2017; Drubin, 1991). In *S. cerevisiae*, Cdc42 is activated through a positive-feedback loop that involves the p21-activated kinase (PAK)-bud emergence protein 1 (Bem1)-guanine nucleotide exchange factor (GEF) complex to achieve local accumulation of membrane-bound GTP-Cdc42 at the site destined to be polarized (Chiou et al., 2017; Howell et al., 2009). To support polarized cell growth processes, such as budding in yeast, factors including chitin synthase II (Chs2) need to be transported and exchanged effectively at the polarized zone (Foltman et al., 2018; VerPlank and Li, 2005). To that end, active GTP-Cdc42 recruits and concentrates effectors that organize the polarized actin cable tracks for myosin V-mediated long-distance transportation of cargo-containing vesicles towards the cell tip, as well as of the exocytosis machinery, which facilitates the release of the cargo at the incipient bud site (Bi and Park, 2012; Howell and Lew, 2012; Johnston et al., 1991; Pruyne et al., 1998). In budding yeast, actin cables consist of unbranched actin filaments (F-actin), organized in bundles that polymerize from the apex of the cell tip or the bud neck (Imamura et al., 1997; Yang and Pon, 2002). The actin nucleation factors (NFs), formins Bni1 and Bnr1, are downstream effectors of GTP-Cdc42, and nucleate two arrays of actin cables from the bud tip and bud neck, respectively, during the budding process (Bi and Park, 2012; Evangelista et al., 2003). Bni1 exhibits a dynamic localization pattern following bud emergence, whereas Bnr1 is stably localized to the bud neck region (Buttery et al., 2007). *bni1*Δ cells show more substantial defects in polarity and bud emergence than *bnr1*Δ cells, suggesting that Bni1 is the major formin involved in these processes (Chen et al., 2012; Imamura et al., 1997; Vallen et al., 2000). Bni1-mediated actin-cable assembly is also crucial for hyphal growth in filamentous fungi, such as *C. albicans* (Sudbery, 2011; Xie et al., 2020). Hyphal development and progressive elongation of filamentous fungi are highly associated with their pathogenicity during tissue invasion in mammals and plants, such as seen with *C. albicans* and *Ashbya gossypii* (Desai, 2018; Kohli et al., 2008; Noble et al., 2017; Sudbery, 2011). Hyphae formation is mainly driven by the assembly of the polarized actin cable and exocytosis (Steinberg, 2007; Xie et al., 2020). During subsequent maintenance of filamentous fungal growth, secretion vesicles are deposited at a region close to the hyphae tip that is rich in membrane compartments, named the Spitzenkörper (Jones and Sudbery, 2010). This structure serves as a vesicle supply center to support the hyphae shape and growth direction (Crampin et al., 2005). Directional bud tip expansion requires effective vesicular transport between the Spitzenkörper and the hyphae tip, which depends on actin cables (Taheri-Talesh et al., 2012). Disruption of the actin cytoskeleton can lead to the disappearance of the Spitzenkörper and the mislocalization of chitin synthase I at the apex, which affects cell wall synthesis during hyphae development (Sánchez-León et al., 2011). The integrity of the Spitzenkörper relies on the polarisome (Crampin et al., 2005), which is involved in assembling the actin cable and thus may guide Spitzenkörper vesicles to the hyphal tip.

The polarisome is a macromolecular complex that is comprises the core members Spa2, Bni1, Bud6, Aip5, and Pea2 (Fujiwara

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et al., 1998; Glomb et al., 2019; Sheu et al., 1998; Shih et al., 2005; Tcheperegine et al., 2005; Xie et al., 2019). Rab GTPase-activating proteins (GAPs) Msb3 and Msb4 have also been suggested to be polarisome members, given their direct interactions with Spa2 (Li et al., 2013), although the role of Msb3 and Msb4 in polarisome assembly is still not known. The local concentration and compositional stoichiometry of polarisome components undergo dynamic changes to tune actin polymerization during cell polarization. The polarisome complex is involved in multiple cellular activities, but the molecular mechanisms by which the dynamic ensemble of the aforementioned components is achieved, in order to support its different functions, remain unclear. Intriguingly, the polarisome complex is assembled as a dense structure and compartmentalized at the bud tip during budding yeast polarity establishment, without having a physical barrier between the polarisome and cytosol, and the packing and localization of the different polarisome complex components are dynamically tuned along with the cell cycle (Arkowitz and Lowe, 1997; Chao et al., 2014; Glomb et al., 2019; Ozaki-Kuroda et al., 2001; Segal et al., 2000; Tcheperegine et al., 2005; Xie et al., 2019). Currently, our knowledge about how the molecular assembly and disassembly of polarisome complex are regulated by the physicochemical properties of surrounding environment, such as actin treadmilling, molecular crowding or cytoplasmic rheology, is still limited. Mathematical modeling has proposed that stochastic spatial dynamics trigger the localized clustering of actin and two polarisome members, Spa2 and Bni1 (Lawson et al., 2013). The recruitment of Bni1 by Spa2 to the membrane is also suggested to provide positive feedback to guide the polarized localization of Spa2 (Lawson et al., 2013). It is unclear whether this spatial stochastic model could also be applied to explain the dynamic assembly of other components into the polarisome (Chen et al., 2012; Tu et al., 2012; Xie et al., 2019).

The cytoplasm and nucleoplasm are both extremely crowded environments packed with macromolecules, such as nucleic acid and proteins (Ellis, 2001). In recent decades, studies have shown that molecular condensation, driven by liquid-liquid phase separation (LLPS), can be a driving mechanism for cellular compartmentalization (Box 1). This creates membraneless compartments that show a dynamic exchange of components between the dense (crowded) and dilute (less crowded) phases, which results in changes in characteristics of the cellular environment, such as the rheology and local concentration of the functional molecules, affecting a variety of cellular processes (Box 2). Increasing evidence demonstrates that LLPS-tuned biochemical activities can affect the nucleation of the cytoskeleton through different signal transduction pathways. For example, during T-cell signaling, LLPS-driven weak multivalent interactions between the linker for activation of T cells (LAT), the transmembrane receptor nephrin, the actin effector non-catalytic region of tyrosine kinase (Nck) and the Arp2/3 activator neural Wiskott–Aldrich Syndrome protein (N-WASP), increases the dwell time of these proteins on the plasma membrane and thus enhances Arp2/3-mediated actin polymerization in a stoichiometry-dependent manner (Case et al., 2019b). Conversely, a compartment formed by LLPS that is mediated by the scaffolding factor spindle-defective protein-5 (SPD-5) can recruit microtubule polymerase to induce microtubule assembly in *C. elegans* (Woodruff et al., 2017). In addition, targeting protein for Xklp2 (TPX2) phase separates into a co-condensate with tubulin on pre-existing microtubules to mediate branched microtubule nucleation (King and Petry, 2020).

In this Review, we will discuss a LLPS-driven mechanism for the molecular condensation of polarisome proteins through inter- and intra-protein interactions using interactive and flexible intrinsically disordered regions (IDRs). In addition, we will address how molecular condensation of the polarisome complex might regulate actin cable polymerization, and propose that this actin assembly may provide mechanical feedback to control the assembly states of polarisome components. Furthermore, we believe the molecular condensation principle could guide future studies on the dynamic functions of the polarisome complex during signaling events.

Box 1. What is liquid–liquid phase separation?

Certain macromolecules can undergo liquid–liquid phase separation (LLPS) within the crowded cytoplasm and nucleoplasm; they condense into a dense phase and exhibit liquid-like physical properties, coexisting with a dilute phase (Franzmann and Alberti, 2019a). The dense phase represents the frequently observed membraneless compartments within the cells, such as the nucleolus (Banani et al., 2017; Shin and Brangwynne, 2017). Importantly, the formation of these membraneless compartments depends on the concentration and characteristics of certain macromolecules, which can be modified by signaling events or environmental conditions, including temperature, salt type, pH and the volume excluded by other macromolecules (Banani et al., 2017; Franzmann and Alberti, 2019a). One commonly regarded model to understand the dynamics of macromolecules within membraneless compartments is the concept of scaffolds and clients (Banani et al., 2017; Franzmann and Alberti, 2019a). Scaffold molecules drive phase separation, whereas client molecules partition into condensates formed by the scaffolds. Generally, there are two types of networks formed by protein–protein or protein–nucleic-acid interactions that have been identified to promote LLPS. One is characterized by multiple folded domains (e.g. SH3 domains in the non-catalytic region of the tyrosine kinase Nck), which interact with short linear motifs in other proteins [e.g. proline-rich motifs in neural Wiskott–Aldrich syndrome protein (N-WASP)] (Banani et al., 2017; Case et al., 2019b; Franzmann and Alberti, 2019a; Li et al., 2012). The protein–nucleic-acid interaction is characterized by the presence of IDRs with multiple distinctive short linear interaction motifs (Banani et al., 2017; Boeynaems et al., 2018; Franzmann and Alberti, 2019a; Mitrea et al., 2016; Nott et al., 2015). The primary sequence of IDRs usually determines the phase separation behavior of the resulting condensates, such as their dense phase concentration, rheological properties, viscoelastic properties and surface tension (Franzmann and Alberti, 2019a). The common feature of protein–protein and protei–nucleic-acid interactions is the presence of multivalence, where the interaction within the macromolecular assembly occurs through a ‘sticker’ and ‘spacer’ framework (Choi et al., 2020). Generally speaking, the binding domains or motifs are termed as ‘sticker’, whereas the linkers interspersed in between are termed as ‘spacers’. Interestingly, IDRs could function as both ‘stickers’ and ‘spacers’, depending on the properties of their amino acid or nucleic acid sequences (Wang et al., 2018). For example, charged residues within IDRs would act as a ‘sticker’ by interacting with oppositely charged biopolymers (protein or nucleic acid) and thus facilitating LLPS, in a process termed as complex coacervation (Choi et al., 2020; Franzmann and Alberti, 2019a). Conversely, any IDRs not undergoing direct interactions represent flexible regions within proteins and thus serve as ‘spacers’ that facilitate the dynamic exchange of proteins between the dense and dilute phase (Boeynaems et al., 2018). Thus, it is important to determine the physical-chemical properties of IDRs when studying LLPS-mediated macromolecular assembly.

Polarisome components and their interactions

Spa2 was initially identified as a component of the 12S polarisome multiprotein complex (named as the result of a velocity sedimentation experiment using the budding yeast cell lysates) and is considered to be the scaffolding protein in the polarisome complex, which also contains the polarity regulatory factors Bud6, Pea2 and actin (Sheu et al., 1998). However, further genetic or biochemical experiments in

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Box 2. Why are membraneless compartments important?

Cells contain both membrane-bounded and membraneless compartments to separate or recruit specific biomolecules and execute distinct biological functions. The formation of membraneless compartments on demand allows cells to create flexible and heterogenous assemblies of macromolecular complexes in response to cellular and environmental signals. There are several functional types of membraneless compartments based on their cellular localization. Cytoplasmic membraneless condensates concentrate biomolecules locally in response to signal transduction to accelerate biochemical reactions (Hernández-Vega et al., 2017; Woodruff et al., 2017) or to adapt to environmental stress (Buchan et al., 2008; Kroschwald et al., 2018; Riback et al., 2017; Xie et al., 2019). When cells are stressed and enter a quiescent state, immiscible condensates store the functional macromolecules; once the stress is released, these molecules are dissolved into the dilute phase and can be utilized for various cellular functions (Franzmann et al., 2018; Marco et al., 2018; Xie et al., 2019). If these biomolecular condensates turn into gel or solid states, this will lead to a delay in or irreversible dissolving of functional macromolecules, which can be detrimental for the cell, resulting in dysfunctional or disease states, such as the pathological fibrillization of RNA-binding proteins, such as superoxide dismutase 1 (SOD1) (Grabocka and Bar-Sagi, 2016; Mateju et al., 2017; Molliex et al., 2015). Another example is nuclear phase separation, which generates a discrete nuclear organization by concentrating the transcription apparatus, which is important for the regulation of gene expression (Boija et al., 2018; Cai et al., 2019; Gibson et al., 2019; Sabari et al., 2018; Strom and Brangwynne, 2019). Finally, condensates of membrane-associated proteins can trigger various signaling pathways and cellular processes, such as immune response activation and actin nucleation by clustering the receptor and actin regulatory proteins (Case et al., 2019a,b; Du and Chen, 2018; Huang et al., 2019; Kalappurakkal et al., 2019). These examples demonstrate that cells can benefit from the dynamic formation of membraneless organelles in different biological contexts.

yeast have identified additional polarisome members that directly associate with Spa2, including Msb3, Msb4, Bni1 and actin-interacting protein 5 (Aip5) (Fujiwara et al., 1998; Glomb et al., 2019; Shih et al., 2005; Tcheperegine et al., 2005; Xie et al., 2019). The original 12S sedimentation coefficient (~300 kDa in size, assuming a globular conformation) (Sheu et al., 1998) is not sufficiently large to account for the full assembly of all members at the known minimum composition of the complex, including the Bni1 dimer (440 kDa) (Xu et al., 2004), the Bud6 dimer (160 kDa) (Park et al., 2015; Tu et al., 2012), the Spa2 dimer (326 kDa) (Zheng et al., 2020), Pea2 (48 kDa) (Sheu et al., 1998) and the Aip5 dimer (272 kDa) (Xie et al., 2019), as well as possibly three or four actin monomers (G-actin) bound to Bud6 and Aip5 (>120 kDa). This discrepancy suggests that the polarisome complex might have tunable inter- and intra-molecular interactions with a different constituent stoichiometry, which result in the changes in partition and retention rates for each member that entail differential dynamics across the different areas of the growing tip (Case et al., 2019a,b; Choi et al., 2020; Huang et al., 2019), and is consistent with their dynamic localization between the cytoplasm and polarized tip (Xie et al., 2019). Instead of using the 12S sedimentation coefficient, a Spa2-centric approach to define the polarisome as a macromolecular complex might reflect better its different functional states. The core members of the polarisome complex might be determined based on their known physical interaction with Spa2 (Fig. 1A) and the list of the current polarisome components may need to be updated as new high-affinity Spa2-binding partners are identified.

Spa2 has three conserved domains, the Spa2 homolog domain I (SHD-I), SHD-II and SHD-V (Foltman et al., 2018). SHD-II plays an important role in localizing Spa2 and its homolog protein Sph1 to the sites of polarized growth in budding yeast (Arkowitz and Lowe, 1997). The SHD-I of Spa2 binds to Msb3 and Msb4, which then bind to the Rab GTPase Sec4 to facilitate exocyst-mediated cargo deposition to the incipient bud (Gao et al., 2003; Tcheperegine et al., 2005). Finally, the SHD-V of Spa2 has been shown to interact with the formin Bni1, which is crucial for the establishment of the actin cable tracks (Evangelista et al., 1997; Fujiwara et al., 1998; Liu et al., 2012). Thus, by interacting with both GAPs and a formin, Spa2 physically connects the polarized actin cable tracks with vesicle deposition at the polarized zone (Bi and Park, 2012; Howell and Lew, 2012; Tcheperegine et al., 2005). Spa2 coordinates multiple pathways during polarized growth; it is important for the polarized localization of mitogen-activated protein kinase 1 (Mpk1) and mitogen-activated protein kinase kinase 1 (Mkk1), both members of the MAPK pathway involved in the maintenance of cell wall integrity (CWI) in budding yeast (Levin, 2005; van Drogen and Peter, 2002). The tethering between the polarisome and the endoplasmic reticulum (ER), which is mediated by the interaction between the polarisome component Pea2 and Epo1, an ER-membrane-interacting protein, at the growing tip, might facilitate the timely supply of membrane and cell wall materials (Chao et al., 2014; Neller et al., 2015). During cytokinesis, Bni1, rather than Cdc42, is critical for the assembly of the actomyosin ring (AR) upon its activation by the Rho-type GTPase Rho1 (Tolliday et al., 2002; Vallen et al., 2000). Spa2 is recruited to the AR by the F-BAR-domain protein Cdc15 in fission yeast (McDonald et al., 2015) and the F-BAR-domain protein Hof1 in budding yeast (Foltman et al., 2018). By directly interacting with Chs2, Spa2 promotes the incorporation of Chs2 into the AR and septum formation (Foltman et al., 2018). In summary, the polarisome complex regulates diverse cellular processes that result in polarized cell growth, including polarized actin cable formation, exocytosis, the CWI pathway and AR formation, as well as maintaining faithful ER segregation (Fig. 1A).

All components of the budding yeast polarisome exhibit concentrated yet dynamic localization patterns that differ between each stage of the cell cycle (Arkowitz and Lowe, 1997; Glomb et al., 2019; Haarer et al., 2007; Moseley and Goode, 2006; Neller et al., 2015; Ozaki-Kuroda et al., 2001; Sheu et al., 1998; Xie et al., 2019) (Fig. 1B). During bud formation in G1, the GTP-Cdc42 effector Gic2 recruits Bud6, Bni1 and Spa2 to the incipient bud site to polymerize F-actin (Chen et al., 2012; Jaquenoud and Peter, 2000; Liu et al., 2012). Afterwards, during the G2-M transition, the localization of core polarisome components, including Spa2, Bni1, Aip5 and Bud6, transforms from a confined foci into a crescent shape, which might be caused by the tunable exchange of polarisome members in and out of the dense phase (Box 1) at the tip (Chiou et al., 2017; Glomb et al., 2019; Kohli et al., 2008; van Drogen and Peter, 2002; Xie et al., 2019). Prior to cytokinesis, the polarisome proteins translocate from the crescent shape at the tip to the septin ring that is formed at the neck of the dividing cells and assist in AR formation as mentioned above (Bi and Park, 2012; Kadota et al., 2004; Tolliday et al., 2002; Vallen et al., 2000) (Fig. 1B). The polarisome also plays an essential role in cell mating; accordingly, *spa2*Δ deletion mutants show a drastic decrease in mating performance (Arkowitz and Lowe, 1997; Gehrung and Snyder, 1990). A haploid yeast cell senses the mating pheromone from its partner, generating a mating projection, termed a shmoo, toward the source; Cdc42 concentrates at the shmoo tip and subsequently recruits polarisome components (Gehrung and Snyder, 1990; Lawson et al., 2013; Segall, 1993) (Fig. 1B). *spa2*Δ cells fail to align the two shmoos of the mating partners due

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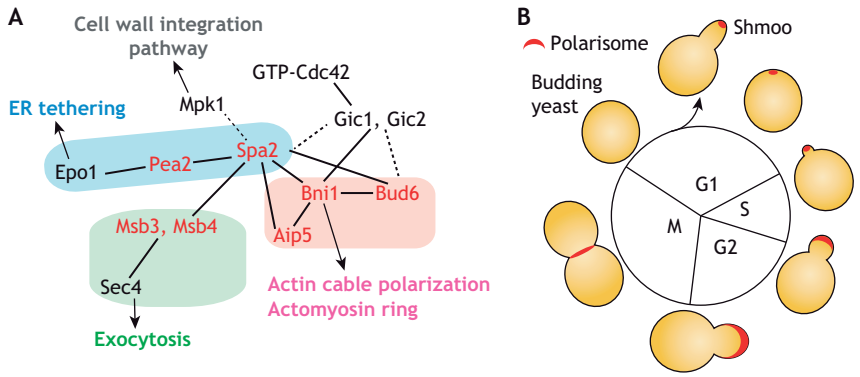

Fig. 1. The polarisome in *Saccharomyces cerevisiae*. (A) The network of interactions between the core components of the polarisome. The polarisome components identified to date include the scaffold protein Spa2, the actin nucleation factor (NF) Bni1, the nucleation-promoting factors (NPFs) Bud6 and Aip5, Pea2, and the GTPase-activating proteins (GAPs) Msb3 and Msb4 (highlighted in red). Several pathways are coordinated by Spa2 and regulated by different polarisome components. During polarized growth, Spa2 activates and regulates Mpk1, which is involved in the cell wall integrity pathway. In addition to Spa2, Epo1 interacts with Pea2 and controls the tethering of cortical ER to the polarized tip. Spa2 also interacts with Msb3 and Msb4, which regulate the Rab GTPase Sec4 during exocytosis. Spa2 binds to the NF Bni1 and the NPFs Bud6 and Aip5 to regulate polarized actin cable assembly and formation of the actomyosin ring at the bud neck. The solid lines indicate physical interactions, and the dashed lines represent genetic interactions. (B) Polarisome localization throughout cell cycle progression. Upon receiving the polarization signal from GTP-Cdc42, the effectors Gic1 and Gic2 activate polarisome components, which then localize to the future bud tip in late G1. During S phase, a small bud starts to grow and the polarisome continues to concentrate at the polarized tip. Upon continued bud emergence during the G2 phase, the polarisome spreads out under the cortical region of the bud. In M phase, during cytokinesis, the polarisome translocates and concentrates at the bud neck region. When the budding yeast cell senses the mating pheromone from its partner, it forms a specialized projection termed a shmoo, to which the polarisome localizes to guide the membrane protrusion in the direction of its mating partner and promote fusion.

to their depolarized actin cables and, therefore, they cannot tether the exocytic vesicles to the exocyst complex efficiently at the fusion site (Ghose and Lew, 2020; Lawson et al., 2013). Filamentous fungi growth also requires the transport of exocytic vesicles along actin cables toward the site of polarization (Riquelme et al., 2018, 2014). Overall, polarized protrusions of both yeast and filamentous fungi require the insertion and expansion of the membrane at the leading edge, coordinated by the interaction-driven condensation of polarisome proteins at the bud tip and polarized assembly of actin cables.

Polarisome-mediated actin polymerization

In *S. cerevisiae*, an appropriate assembly of the polarisome complex at the bud tip determines polarized actin cable polymerization. The scaffold protein Spa2 is crucial for the maintenance of a low local concentration of polarisome members, such as Bni1 and Aip5 (~100 nM), at a nascent polarity site; this allows the initiation of an actin-nucleation center that guides polarized actin cable track assembly (Buttery et al., 2007; Fujiwara et al., 1998; Glomb et al., 2019; Xie et al., 2019). Owing to the loss of interaction with Spa2, *spa2*Δ mutant cells are no longer able to recruit Aip5 and Bni1 to the budding tip (Fujiwara et al., 1998; Glomb et al., 2019; Ozaki-Kuroda et al., 2001; Sheu et al., 1998; Xie et al., 2019). Furthermore, *bni1*Δ*79-988* mutant cells, which lack the N-terminal Spa2-binding domain in Bni1, are synthetic lethal when Spa2 is also deleted, suggesting that Spa2 plays additional roles besides binding to Bni1 (Liu et al., 2012). Spa2 possibly also regulates the function of Bud6 and Aip5, the two polarisome-resident nucleation-promoting factors (NPFs), both of which synergistically promote the activation of the NF Bni1, which localizes to the barbed end of F-actin to initiate polymerization (Glomb et al., 2019; Moseley et al., 2004; Sheu et al., 1998; Xie et al., 2020, 2019). Apart from the Spa2-regulated localization of polarisome proteins, physical interactions between other polarisome members could also influence their localization. For example, *bni1*Δ deletion mutants display reduced tip localization of Aip5–GFP and Bud6–GFP (Segal et al., 2000; Xie et al., 2019). This interdependency between polarisome members with regard to their localization is also observed at the tip region of hyphae of the fungus *Ashbya gossypii* (Kohli et al., 2008), suggesting that the polarisome has a similar role in the polarized growth of filamentous fungi. Overall, the macromolecular complex, resulting from inter- and intra-molecular interactions between scaffold and clients, appears to be an ideal system for polarisome members to be condensed at the bud tip where they can regulate polarized cell growth.

Polarized actin polymerization during tip growth is achieved by concentrating the polarisome complex at the tip. In fact, the condensation of the polarisome complex at the cell tip changes dynamically through the cell cycle; this is spatial and temporally modulated by the different multivalent states in the complex, which involve orchestrating heterogeneous interactions between the folded domain and the unfolded IDRs of different polarisome components (Fig. 2A,B). These interaction motifs, which are embedded in the folded domain and unfolded IDRs, serve as ‘stickers’, whereas the region without any interaction motifs that are present within the IDRs of polarisome components can serve as ‘spacers’ (Choi et al., 2020) (Box 1) (Fig. 2A,B). Such multi-domain interactions create tunable protein interactions upon changes of the physical-chemical properties of the proteins, such as post-translational modification or cellular environment changes seen after signal transduction, as occurs during stress adaptation (Choi et al., 2020; Franzmann and Alberti, 2019b; Riback et al., 2017; Xie et al., 2019; Yoo et al., 2019), and these regulate a complex assembly mechanism of the polarisome (Gibson et al., 2019; Guillen-Boixet et al., 2020; Miao et al., 2016, 2018). The folded SHD-V domain of Spa2 in budding yeast [1306–1466 amino acid (aa)] directly interacts with the IDR of Bni1 (826–987 aa) (Fujiwara et al., 1998). Moreover, Spa2 SHD-V is also critical for the maintenance of a tip-localized pool of the NPF Aip5 through direct binding to the IDR of Aip5 (1–271 aa) (Glomb et al., 2019; Xie et al., 2019). The NPFs Bud6 and Aip5 promote the actin-nucleating activity of Bni1 without affecting F-actin elongation induced by profilin (Graziano et al., 2011; Moseley and Goode, 2005; Moseley et al., 2004; Xie et al., 2020, 2019). The Bni1 C-terminus (1767–1953 aa; Bni1-C) is an IDR that associates, with strong affinity, with the core domain of Bud6 (550–688 aa) and

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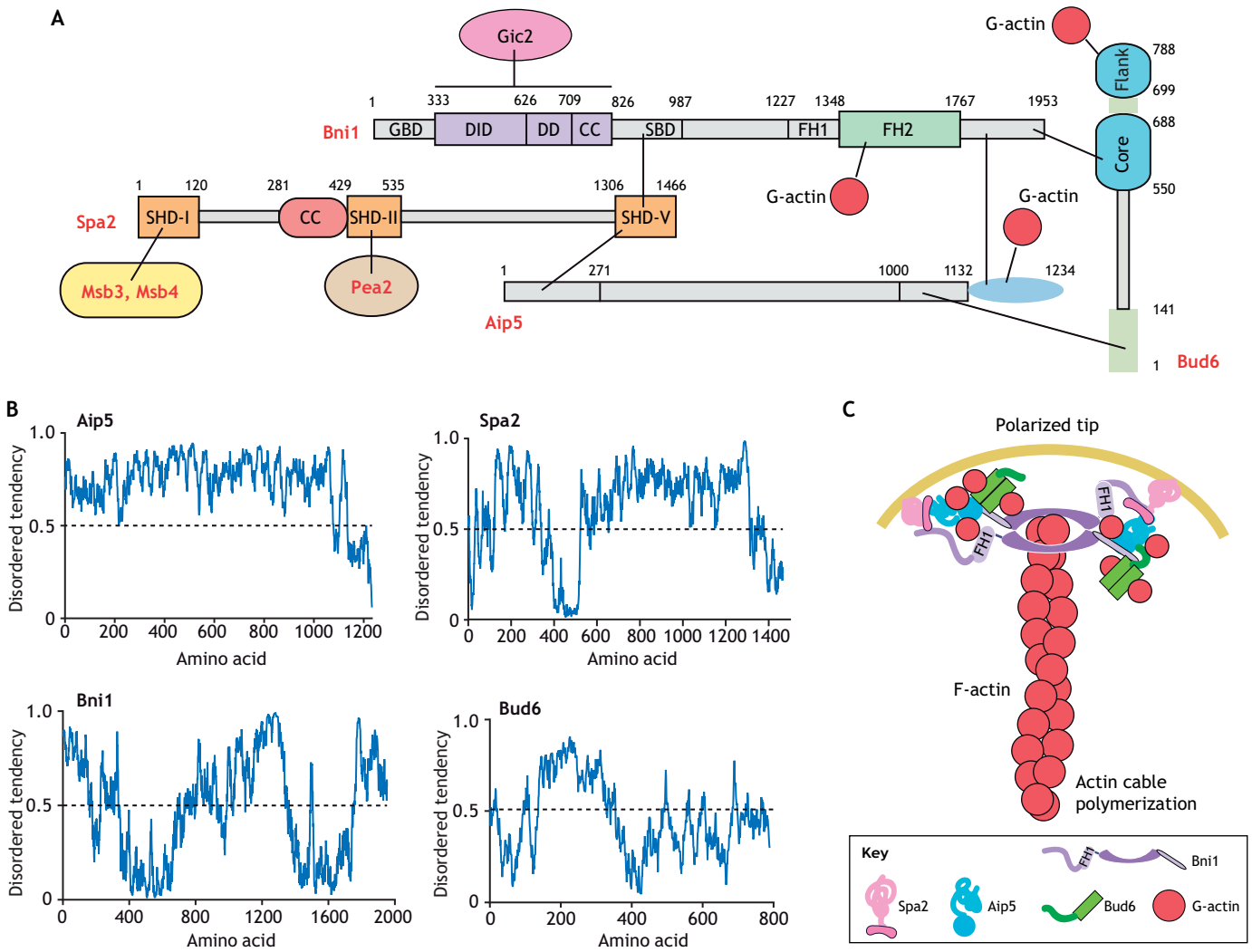

Fig. 2. Domain organization and interaction networks within the polarisome complex. (A) Characterized functional domains of individual polarisome proteins and their interaction map. Several interaction pairs are formed by polarisome components and are important for actin assembly: the scaffold protein Spa2 utilizes the SHD-V domain (1306–1466 aa) to interact with both the N-terminus of Aip5 (1–271 aa) and the SBD domain (826–987 aa) of Bni1. The C-terminus IDR of Bni1 (1767–1953 aa) is able to interact with both the C-terminal domain of Aip5 (1132–1234 aa) and the Bud6 core domain (550–688 aa). The IDR of Aip5 (1000–1131 aa) interacts with Bud6 (1–141 aa). G-actin can interact with the FH2 domain (1348–1767 aa) of Bni1, the flank domain of Bud6 (699–788 aa) and the C-terminal domain of Aip5 (1110–1234 aa). (B) Prediction of IDRs that are involved in actin cable assembly, as determined by IUPred2A (https://iupred2a.elte.hu/), from Aip5, Spa2, Bni1 and Bud6 of *S. cerevisiae*. These IDRs suggest the flexible regions that likely serve as 'spacers' if no 'sticker' is embedded. (C) Schematic illustration of polarisome-regulated actin cable assembly at the polarized tip of the budding yeast, modified from Xie et al. (2019) where it was published under a CC-BY 4.0 license. Bni1 is able to nucleate actin assembly through its FH2 domain, and its adjacent C-terminal IDR region can bind to both the NPFs Bud6 and Aip5. The dimeric Bud6 and Aip5 directly associate with G-actin to promote Bni1-mediated actin nucleation. Thus, G-actin can be quickly delivered to the Bni1, which enhances actin nucleation. Spa2 directly interacts with Bni1, Bud6 and Aip5, which is important to increase the local concentration of nucleation factors and NPFs. GBD, GTPase-binding domain; DID, diaphanous inhibitory domain; DD, dimerization domain; CC, coiled-coil domain; SBD, Spa2-binding domain; FH, formin homology domain; SHD, Spa2 homology domain; G-actin, monomeric (globular) actin; F-actin, filamentous actin.

the folded C-terminus of Aip5 (1110–1234 aa, Aip5-C), forming an NF–NPF core that mediates actin nucleation at the bud tip (Park et al., 2015; Tu et al., 2012; Xie et al., 2019). Furthermore, both the formin homology 1 (FH1) and FH2 domains of Bni1 enhance the physical interaction between Aip5-C and Bni1-C (Xie et al., 2019). In addition, yeast two-hybrid-based interaction analysis and pulldown assays have also revealed a physical interaction between Aip5 (1000–1131 aa) and Bud6 (1–141 aa) (Glomb et al., 2019). Consistent with this, the polarization of Aip5 at the tip was significantly attenuated in both *bni1*Δ and *bud6*Δ mutant yeast cells (Glomb et al., 2019; Xie et al., 2019). This association between Bud6, Aip5 and Bni1 maintains a balanced supply of G-actin to the formin and so regulates actin nucleation (Xie et al., 2020) (Fig. 2C).

The resolved crystal structures of Bud6 (PDB: 4WYB) and Aip5 (PDB: 6ABR) revealed that both their C-termini interact to form homodimers, which could bind two actin monomers at a time, increasing the local concentration G-actin for Bni1-mediated nucleation around the actin barbed-end (Park et al., 2015; Tu et al., 2012; Xie et al., 2020, 2019). In addition, Bni1 also binds to Rho GTPase with its N-terminal regulatory region for potential activation (Dong et al., 2003; Drees et al., 2001; Evangelista et al., 1997; Kohno et al., 1996; Logan et al., 2010; Tolliday et al., 2002). It is still not known how the polarisome coordinates the stoichiometry and activities of Aip5 and Bud6 to orchestrate the dynamic delivery of G-actin to Bni1 during cell cycle progression. Future structural studies of the interactions between the functional

domains of the polarisome components involved in actin nucleation, by co-crystallization or cryo-electron microscopy, might help to unravel the mechanisms underlying polarisome-mediated actin assembly.

In general, the aforementioned protein interactions between NF, NPFs and G-actin might generate diverse combinations that could lead to different actin nucleation and cable assembly capabilities, resulting in different local concentrations and thereby different compositional stoichiometry at the bud tip during cell cycle progression or under different environmental stimuli. In the following section, we highlight the importance of the IDRs of several polarisome components and discuss LLPS as the underlying mechanism that drives polarisome assembly to initiate actin polymerization during signal transductions.

Coordination between polarisome condensation and actin polymerization

The formation of membraneless compartments by LLPS (Box 1) is driven by weak multivalent interactions that increase the molecular connectivity in the cytoplasm, nucleoplasm or at the cell membrane (Case et al., 2019b; Franzmann et al., 2018; Hernández-Vega et al., 2017; Shin et al., 2018; Su et al., 2016). The characterization of the rheological properties of an ensemble of biomolecules and their surrounding environment is important to understand molecule dynamics and macromolecular assembly (Delarue et al., 2018; Golkaram and Loos, 2019). Taking advantage of a recently developed technique that uses genetically encoded multimeric nanoparticles (GEMs), it was observed that the polarized growing zone of fungi is a crowded environment packed with macromolecules, resulting in a low local diffusivity for cytoplasmic biomolecules (Delarue et al., 2018; McLaughlin et al., 2020); this might favor the interaction of each macromolecular component with others and the formation of a functional complex that persists over a long period of time (Grimaldo et al., 2019). In *S. cerevisiae*, the polarisome components display local condensation behavior at the polarized tip (dense phase), which shows a dynamic exchange of polarisome proteins with the cytosolic pool (dilute phase), such as described above during cell cycle progression. Fluorescence recovery after photobleaching (FRAP) experiments have also revealed a dynamic behavior for Spa2, Bni1 and Aip5 (Jones and Sudbery, 2010; Lawson et al., 2013; van Drogen and Peter, 2002; Xie et al., 2019). For instance, Aip5 molecules undergo strong multivalent interactions with each other that lead to amorphous condensates *in vitro*; the addition of Spa2 generates spherical liquid droplets and prevents the formation of glass-like amorphous condensates by creating a fluid biomolecular compartment, which we characterized by using a FRAP-based assay (Xie et al., 2019). When we examined Aip5 behavior *in vivo*, its polarized localization at the tip was shown to be highly dynamic and liquid-like in the presence of Spa2, and the condensates dissolved quickly upon addition of 1,6 hexanediol (Xie et al., 2019), an aliphatic alcohol molecule that disrupts weak hydrophobic interactions (Kroschwald et al., 2015; Nair et al., 2019). Moreover, the modulation of LLPS of Aip5 by Spa2 can also be observed *in vivo* under stress conditions as Spa2 prevents the formation of Aip5 aggregates as a means of adaptation to stress (Xie et al., 2019). The phase-separation behavior of Aip5–Spa2, which is mainly controlled by their IDRs, presents the basis for a better understanding of the assembly mechanisms of the polarisome and its biochemical activities, such as mediating actin cable polymerization from the polarized region.

In light of the above-mentioned tunable condensation of polarisome components, these proteins are less likely to freely diffuse, given their multiple interaction partners at the growing polarized zone. First, the protein–protein interactions mediated by the C-termini of Bni1, Bud6 and Aip5 are crucial for the establishment of the macromolecular complex; all these folded domains form homodimers *in vitro* that are able to assemble a minimum NF–NPF complex as the nucleation core in order to drive actin nucleation (Tu et al., 2012; Xie et al., 2020, 2019; Xu et al., 2004). In addition, IDR-mediated multivalent interactions could regulate the flexible assembly of the complex and accommodate different stoichiometries of the NF–NPF core components (Moseley and Goode, 2005; Xie et al., 2020, 2019), as well as their equilibrium between the dilute and dense phases of the polarized tip region; this might allow the fine-tuning of formin-mediated actin nucleation as a regulatory mechanism for N-WASP-mediated Arp2/3 complex (Case et al., 2019b; Su et al., 2016). Thus, we speculate that the polarisome may make use of molecular condensation to increase the local concentration of NFs and NPFs, and to allow for quick actin cable polymerization. In support of this notion, *in vitro* biochemical experiments have shown that higher protein concentrations of the NF Bni1 and the NPF Aip5 in the reaction result in a higher actin nucleation activity (Pruyne et al., 2002; Sagot et al., 2002; Xie et al., 2019). At the polarized tip in *S. cerevisiae*, we observed an ∼1.5-fold enrichment of both Bni1 and Aip5 compared to that seen in the cytoplasm away from the tip (Xie et al., 2019), which suggests that a locally increased concentration of this NF–NPF pair could also correspond to higher actin nucleation activity *in vivo*. Future studies are needed to obtain a more comprehensive understanding of actin assembly mediated by the intricate mechanism of assembly of the polarisome.

Furthermore, it is still not known what are the underlying mechanisms that allow the continuous re-establishment of the dynamic equilibrium of the polarisome components during cell cycle progression. In *S. cerevisiae*, the concentration of the polarisome components at the bud tip guides polarized actin cable assembly (Buttery et al., 2007; Fujiwara et al., 1998; Glomb et al., 2019; Moseley et al., 2004; Shih et al., 2005; Xie et al., 2019) (Fig. 3A). Conversely, polarisome-mediated polymerization of actin cables provide, in return, a mechanical pulling force that adjusts the confinement and localization pattern of the polarisome complex. Owing to its F-actin-barbed end localization, the location of Bni1 tracks actin cable polymerization and elongation, and thus moves towards the mother cell from the bud tip, opposite to the direction of myosin II-dependent transport (Buttery et al., 2007). A similar process of actin-cable-mediated retrograde transport was also observed for the formin For3 in *Schizosaccharomyces pombe* (Martin and Chang, 2006). Moreover, actin depolymerization induced by latrunculin A (LatA) results in the formation of Bni1 speckles next to the tip cortex (Buttery et al., 2007; Xie et al., 2019) (Fig. 3A), suggesting that the migration of Bni1 towards the cytoplasm is mediated by actin polymerization. Strikingly, without F-actin polymerization in LatA-treated cells, Bni1 appears to be further condensed (Xie et al., 2019). The increase in fluorescence intensity of the Bni1 foci at the bud tip might not simply be due to a decrease in the distance between Bni1 speckles; instead, it could reflect an increase of the cellular fraction of Bni1 that localizes to the foci, as a concurrent decrease in the cytoplasmic signal of Bni1 was also observed (Buttery et al., 2007; Xie et al., 2019). Not only Bni1, but also other polarisome members, such as Spa2, Bud6 and Aip5, are concentrated at the bud tip and colocalize with each other in small foci after LatA treatment (Fig. 3A) (Xie et al., 2019). Similarly, *Candida albicans* Spa2 also shows a response to actin depolymerization by rapidly packing into condensed foci at the hyphae tip (Jones and Sudbery, 2010). Therefore, without the continued treadmilling of F-actin that allows associated proteins to move away from the bud tip, the exchange equilibrium between cytosolic and tip pools might be re-established by the loss of pulling

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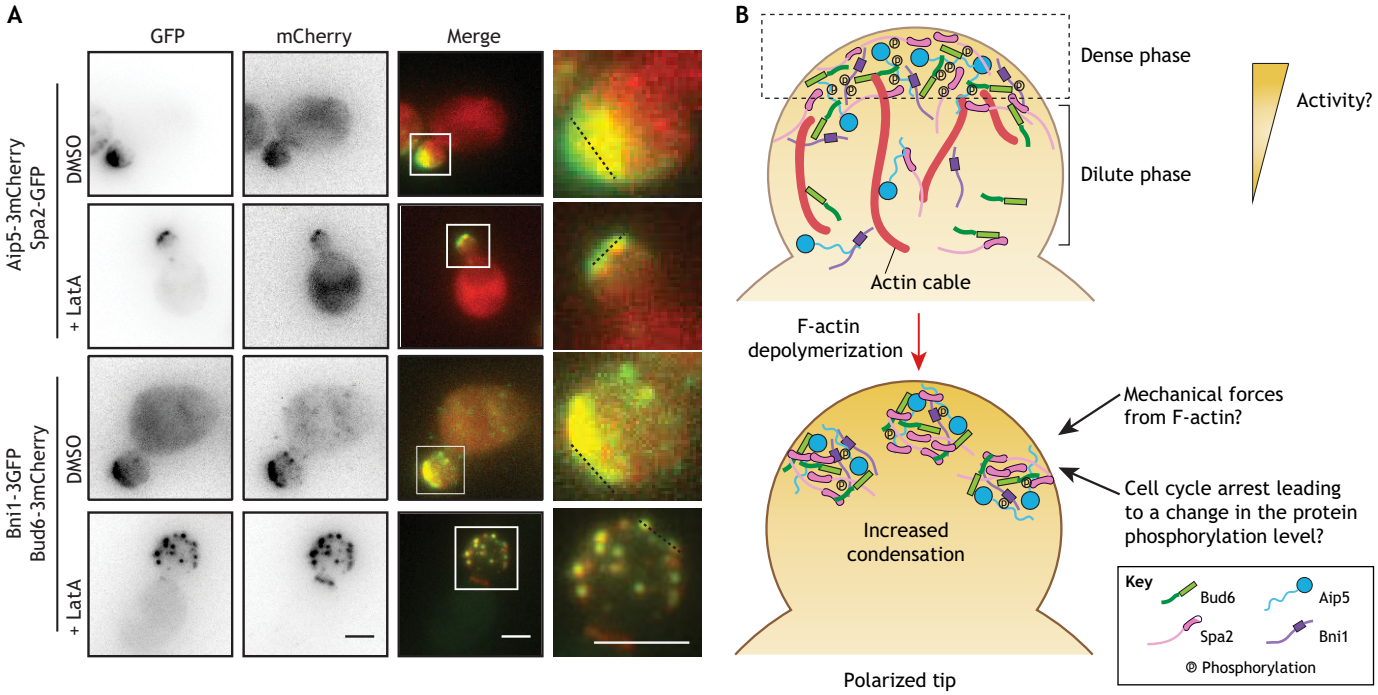

Fig. 3. Polarisome condensation is modulated by actin dynamics. (A) Dense colocalization of polarisome proteins upon actin cable depolymerization. Representative maximum *Z*-projection images of fluorescently tagged fusion proteins (Aip5–3mCherry; Spa2–GFP; Bni1–3GFP and Bud6–3mCherry) at the bud tip, in the absence or presence of 10 µM latrunculin A (LatA) for 30 min, which is sufficient to depolymerize all actin cables. Scale bars: 2 µm. This panel has been adapted from Xie et al. (2019) where it was published under a CC-BY 4.0 license. (B) Model of polarisome condensation at the polarized tip with or without polymerizing actin filaments in *S. cerevisiae*. During normal polarized growth, the concentration of polarisome components at the tip can be considered as a dense phase; this results from liquid–liquid phase separation (LLPS), where components can be dynamically exchanged with the cytosolic dilute phase. When filamentous actin (F-actin) is depolymerized, the condensation level of the polarisome increases, with more compact interaction between its components, which results in bright speckles at the bud tip as shown in A. This could be due to the reduced mechanical force upon F-actin depolymerization or cell cycle arrest, which may lead to a change in the phosphorylation of polarisome components and thus modify their interactions.

forces, which results in enhanced confinement of complex members at the tip (Fig. 3B) and thereby changes in the stoichiometry of components that are driving factors for LLPS and complex assembly (Case et al., 2019a,b; Choi et al., 2020; Huang et al., 2019). In contrast to Bni1, the other three components, Aip5, Spa2 and Bud6, are not known to associate directly with the actin barbed end (Paul and Pollard, 2009). Therefore, the condensation of these polarisome components after actin depolymerization might be derived from the constant confinement force, which results from the intermolecular interactions within the polarisome complex. Otherwise, the force generated by F-actin elongation that pulls Bni1 into the cytoplasm can counteract the confinement force and thereby tune the connectivity and structural tensegrity of the cortex-associated complex (Fig. 3B).

Nevertheless, it is still unclear how these mechanical forces balance the elastic confinement and flexible assembly of the polarisome complex during diverse signaling events, which occurs through the tuning of the viscoelastic properties of the assembled complex. In contrast, depolymerization of actin cables can lead to activation of Mpk1, and consequently the CWI pathway, which triggers cell cycle arrest at G2 (Harrison et al., 2001). Because cell cycle kinases and phosphatases regulate dynamic phosphorylation and dephosphorylation events throughout the cell cycle, these proteins could have important roles in regulating the electrostatic landscape or conformation of the IDRs, thereby contributing to fine-tuning of the molecular condensation (Aumiller and Keating, 2016; Iakoucheva et al., 2004; Miao et al., 2016, 2018; Wang et al., 2014). It remains unknown whether the perturbation of the cell cycle progression by the CWI pathway, induced by actin depolymerization, could be an additional regulatory step for the condensation of polarisome components. In *S. cerevisiae*, cell cycle events are controlled by a cyclin-dependent kinase 1 (CDK1), whose periodic activation is driven by different cyclin–CDK complexes (Donaldson, 2000; Loog and Morgan, 2005). Global analysis of Cdk1 substrates has revealed that polarisome components (e.g. Spa2, Bni1 and Bud6) are highly phosphorylated in their IDRs during cell cycle progression (Holt et al., 2009; Loog and Morgan, 2005) (Fig. 3B). High levels of phosphorylation of the *C. albicans* Spa2 IDR by CDK1 is essential to maintain the localization of this protein at the hyphae tip, preventing an inappropriate septal localization during hyphae growth (Wang et al., 2016). Several actin-binding proteins, including Bni1, are highly phosphorylated by Clb2–Cdk1, whereas phosphorylation levels induced by Clb5–Cdk1 are reduced (Miao et al., 2016); however, it is still unclear how this difference in phosphorylation level might regulate their biochemical activities. It is possible that the phosphorylation of polarisome components regulates their conformation and their inter- or intra-molecular interactions, and therefore, the condensation of the polarisome components at different stages of the cell cycle. Recent studies that aimed to understand the role of LLPS in the regulation of stress granule formation have shown that phosphorylation of the IDR of G3BP1 (a core component of stress granules) can regulate its saturation concentration in the condensate (Guillen-Boixet et al., 2020; Yang et al., 2020). Phosphorylation levels determine the net negative charge of the protein and, therefore, can modulate the electrostatic interaction within the condensate (Guillen-Boixet et al., 2020; Yang

et al., 2020). Currently, it is technically challenging to address the effects of phosphorylation on polarisome components and their assembly *in vivo*, as it occurs rapidly at diverse sites. *In vitro* reconstitution of the complex might provide an exciting feasible alternative to dissect the detailed mechanism of how cyclin-dependent phosphorylation influences the dynamic assembly of the polarisome complex. The mechanistic studies of the abovementioned mechanoregulation and phosphoregulation of polarisome assembly are worthy of future research to better understand actin remodeling during signal transductions.

Conclusions and perspective

It has been three decades since Spa2 was identified at the cell tip and found to be involved in shmoo formation in *S. cerevisiae* and hyphae growth of filamentous fungi (Snyder, 1989). Great efforts have been made to identify Spa2-associated proteins and their function in the establishment and maintenance of polarity in fungi. However, many questions remain with regard to the spatio-temporal regulation of polarisome assembly, as well as how changes in actin nucleation and cell polarity at a given stage of the cell cycle can affect the assembly process. In *S. cerevisiae*, Spa2 acts as the scaffold protein for the polarisome complex and helps to establish the nucleation-core unit Bni1–Bud6–Aip5 near the polarized cap. We are now beginning to understand that polarisome components undergo LLPS to form a condensed macromolecular complex at the polarized tip; this is achieved through their high-affinity domains, creating 'stickers', and their interspersed IDRs, serving as 'spacers', in the macromolecular polarisome complex. Moreover, the molecular condensation of NFs and NPFs at the cell cortex appears to maintain the appropriate actin nucleation activity for polarized cell growth. Concurrently, mechanical forces generated by actin treadmilling regulate the motility and equilibrium of polarisome proteins in and out of the biomolecular condensates, in a feedback manner. Further studies are required to understand how a change in material properties of the macromolecular polarisome complex, such as elasticity and connectivity, could affect specific activities of its components, such as actin nucleation. Another question is how Cdk1-mediated phosphorylation could modulate the dynamic condensation of the polarisome complex to facilitate its function in actin cable assembly at different cell cycle stages (Holt et al., 2009; Miao et al., 2016, 2013). Owing to the low abundance of several polarisome members (e.g. Bni1 and Aip5; ∼100 nM) and their fast-moving behavior in the cytoplasm, we require high-resolution fluorescence microscopy with a sensitive detection system to study their cooperative dynamics and function in actin remodeling *in vivo*. Moreover, integrated strategies are needed to quantitatively study spatio-temporal regulation of polarisome assembly, such as liquid cell transmission electron microscopy, which can be used to visualize the dynamic assembly of protein complexes in solution at subnanometer resolution (Liao and Zheng, 2016), *in vitro* minimum component reconstitution and coarse-grained mathematical modeling (Choi et al., 2020; Franzmann et al., 2018; Harmon et al., 2017; Xu et al., 1999). The expression of full-length constructs or purification of polarisome components might also allow a systematic titration of their stoichiometry to better characterize the molecular condensation of the polarisome and its rate of actin polymerization.

Furthermore, polarisome proteins are conserved among various pathogenic fungi species and most of them are predicted to have high ratios of IDRs (Table S1). It is likely that the formation of the polarisome complex in different fungi species shares conserved mechanisms with regard to the dynamic macromolecular assembly driven by molecular condensation. Nevertheless, the specific consequences depend on the recruitment and capacity for multivalent interactions (Box 1) by the scaffold protein Spa2. Moreover, we still do not understand the mechanisms of polarisome assembly regulated by the Spitzenkörper and the master polarization regulator, the Cdc42 protein cluster, which both drive bud growth throughout the cell cycle. Further quantitative analysis of data obtained by mass spectrometry and cell imaging is required to understand how polarisome components move in and out of the polarisome complex and Cdc42 clusters during polarity establishment and maintenance. We anticipate that such fundamental studies of the dynamics of the macromolecular polarisome complex will help us understand the mechanisms by which fungal polarity is triggered and established by different signaling events.

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Competing interests

The authors declare no competing or financial interests.

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Supplementary information

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