Septum Formation and Cytokinesis in Ascomycete Fungi

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I. Introduction

Cell division is a fundamental cellular process that is essential for proliferation of unicellular as well as multicellular organisms. It reflects the final stage of the cell cycle, during which a cell is physically divided into two daughter cells that contain a full set of chromosomes and other cellular organelles. Cytokinesis can be divided into several general steps that apply to most eukaryotic cells (Barr and Gruneberg 2007; Eggert et al. 2006): (1) the selection of the future **cell division plane** based on spatial as well as temporal cues, (2) the **assembly of a cortical actomyosin ring (CAR)** at this site, and (3) its **constriction coupled with membrane invagination**. In general, the formation of the CAR and its subsequent constriction is tightly coupled to the cell cycle to ensure that cell separation does not occur prior to chromosome segregation. (4) The formation of an extracellular cell wall, the septum, composed of glucans, chitin, and other polysaccharides in fungi further requires coordination of CAR constriction with secretion of cell wall biosynthetic and remodeling enzymes to build the extracellular septum. (5) This **primary septum** is covered by additional layers of cell wall material that form the **secondary septum** and is finally degraded by secreted hydrolytic enzymes in the unicellular yeasts and during sexual development of filament-forming molds to allow detachment of the two cells.

Several overviews have recently summarized the mechanistic principles underlying cell polarization and division in budding yeast and fission yeast, two unicellular fungi that consis-

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tently serve as conceptual framework for the analysis of fungal as well as higher eukaryotic cell biology (Pollard and Wu 2010; Bi and Park 2012; Weiss 2012; Martin and Arkowitz 2014). However, the vast majority of fungi forms mycelial colonies that consist of networks of branched hyphe. These cells grow by tip growth and are compartmentalized by septum that partition cellular environments within the hypha. In contrast to the unicellular yeasts, not every nuclear division triggers the formation of a new septum in mycelial fungi, and thus hyphal compartments are generally multinucleate. Moreover, a small septal pore is retained in higher fungi to enable intercellular communication and transport of cytoplasm and organelles between adjacent hyphal compartments. This controlled segmentation of hyphal units through septal cross walls in a multicellular mycelium is the basis for the morphological complexity achieved by the fungi during vegetative growth, differentiation, and infection processes and is thus a prerequisite for the evolutionary success of the fungal kingdom (Gull 1978; Pringle and Taylor 2002; Blackwell 2011).

The phylum *Ascomycota* comprises three major subphyla: ***Taphrinomycotina***, ***Saccharomycotina***, and ***Pezizomycotina*** (McLaughlin et al. 2009; Stajich et al. 2009). The subphylum of the *Pezizomycotina* contains over 90% of the *Ascomycota* species. Almost all species of this clade generate multinuclear hyphae that are compartmentalized by septa and include the model molds *Aspergillus nidulans* and *Neurospora crassa*. The *Saccharomycotina* contain the industrial yeasts and parasitic *Canidida spec.* species, dimorphic fungi that can switch between yeast and mycelial states (Sudbery 2011). Most members of the *Saccharomycotina* are unicellular, but this group also includes filamentous forms, such as *Ashbya gossypii*. This species is very closely related to *Saccharomyces cerevisiae*, and more than 90% of the genes are highly conserved in the two fungi (Dietrich et al. 2004; Wendland and Walther 2005), but the two species have developed dramatically different growth forms: constitutive multinucleate tip growth in *A. gossypii* versus unicellular growth in *S. cerevisiae*. The third subphylum includes the *Schizosaccharomycetes* and other early diverging lineages and is primarily represented by *Schizosacchomyces pombe*. The monophyletic origin of this subphylum is still under debate, but most recent data support the monophyly of the taxon (James et al. 2006). Both filaments and yeasts are found in this subphylum, suggesting that both morphologies are ancestral in *Ascomycota*.

Despite the importance of septum for hyphae, proliferation, and differentiation in filamentous fungi, our understanding of septum formation and its regulation is highly fragmentary (Harris 2001; Seiler and Justa-Schuch 2010; Mourino-Perez and Riquelme 2013). In this review, we will focus on recent progress that confirms the use of conserved molecular modules during cell division in the unicellular yeasts and during septum formation in the filamentous fungi. However, it is also becoming apparent that proper placement and regulated formation and function of septa in the different phylogenetic groups of *Ascomycota* requires significant rewiring and species-specific adaptation of these conserved modules (Gu et al. 2015).

II. Spatial Cues: Mechanisms Specifying the Position of the Division Plane

A. Positioning the Cell Division Plane in Unicellular Yeasts

The regulatory pathways that control the spatial aspects to place the future cell division plane are poorly conserved among different eukaryotic organisms despite the high importance of a tight coordination of cytokinesis with chromosome and organelle segregation. For example, the two model yeasts *S. cerevisiae* and *S. pombe* have developed fundamentally distinct mechanisms to control the spatial aspects for placing the future cell division plane (Fig. 1a, b). The bud-site selection system of budding yeast relies on cortical cues of the previous cell division cycle in order to redirect the dividing nucleus to the bud neck, while the position

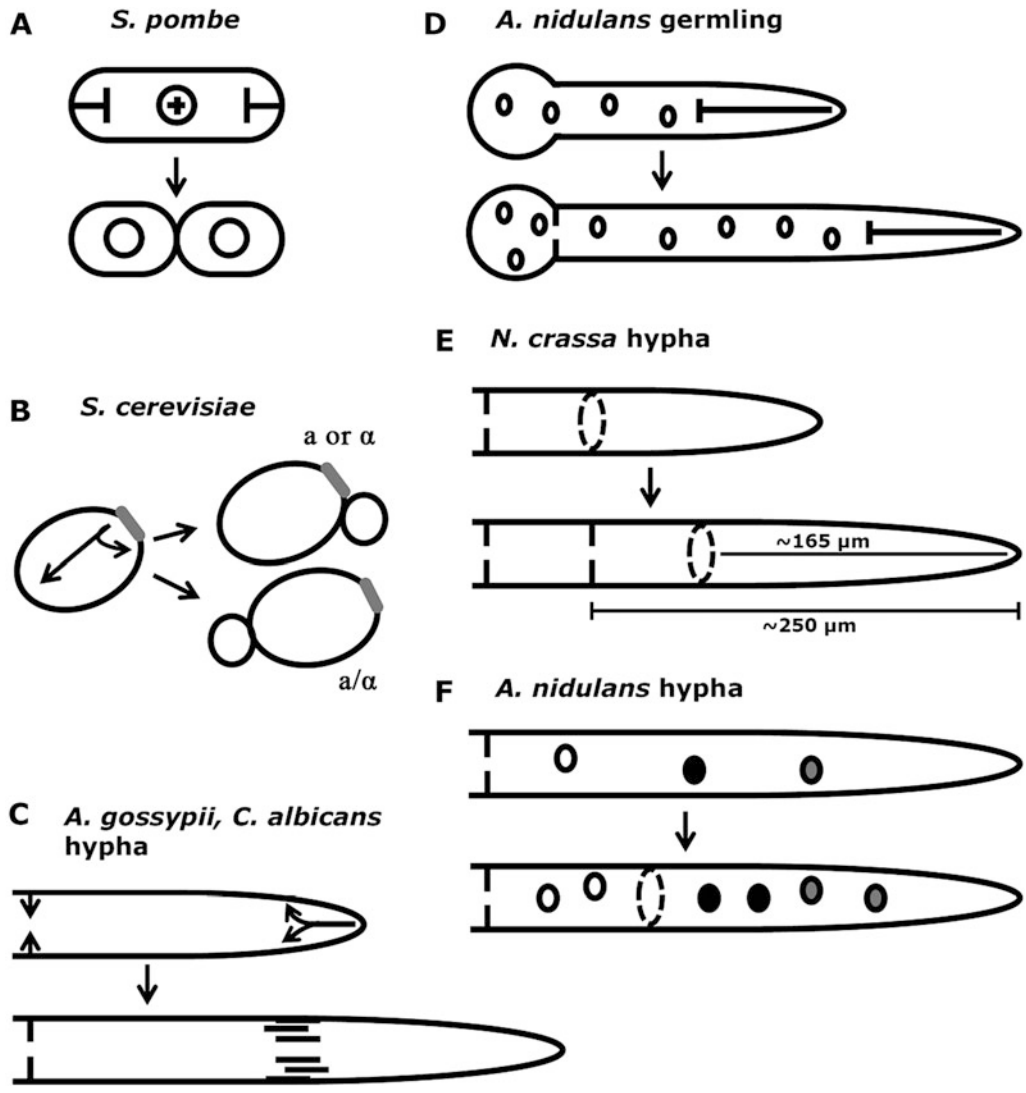

Fig. 1 Diverse types of spatial signals regulate septum placement and cell division plane. (**a**) The site of cell division in fission yeast is chosen based on a positive signal provided by the position of the pre-mitotic nucleus and negative signals from the two cell ends. (**b**) A transient cortical landmark at the bud scar functions as a spatial memory from one cell cycle to the next in budding yeast. *a* or *α* cells divide axially, such that dividing cells form their buds immediately adjacent to the site of the previous cell separation. *a/α* cells exhibit a bipolar budding pattern and place the new bud either proximal or distal to the site of the previous cell division. (**c**) Cortical cues deposited at the hyphal tip may trigger septum position in the filament-forming *Saccharomycotina* species *A. gossypii* and *C. albicans*. The cue for landmark deposition at the tip has been proposed to be reduced tip elongation as a consequence of septum formation in the subapical region of the hypha. (**d**) A tip-high inhibitory gradient has been proposed in *A. nidulans* to restrict septum formation in growing germlings until a certain cell size is reached. (**e**) A similar tip-high inhibitory signal has been proposed for mature *N. crassa* hyphae, where the new septum is placed when the apical cell reaches a critical size of approximately 250 µm. (**f**) Unlike *S. pombe*, where the position of the pre-mitotic nucleus serves as positive signal, the majority of septa in *A. nidulans* are formed at a position corresponding to a location in between two pairs of previously mitotic nuclei

of the fission yeast nucleus itself specifies the future plane of cell division (Chang and Peter 2003). Both mechanisms are dissected at the molecular level, and the reader is referred to recent reviews for details (Laporte et al. 2010; Pollard and Wu 2010; Bi and Park 2012; Weiss 2012).

Briefly, **budding yeast cells** divide in two precise spatial patterns (Chant and Herskowitz 1991). *a* or α cells divide axially, such that dividing cells form their buds immediately adjacent to the site of the previous cell separation. *a*/α cells exhibit a bipolar budding pattern and place the new bud either proximal or distal to the site of the previous cell division. The axial budding pattern depends on a transient cortical landmark consisting of the **Bud3p-Bud4p complex** that binds to the transmembrane glycoprotein Axl2p (Gao et al. 2007; Kang et al. 2012; Wu et al. 2015). The localization of these proteins acts as a spatial memory from one cell cycle to the next. An Axl2p containing spot localizes to the cell cortex at the time of bud emergence and serves together with the encircling septin ring as a landmark to recruit Bud3p and Bud4p. This Bud3p-Bud4p-Axl2 complex matures at the mother-bud neck into a double ring, and one ring is passed to each daughter cell, marking the previous site of attachment. This localization of the landmark complex at the mother-bud neck depends on the integrity of the septin collar; thus the Bud3p-Bud4p complex rings are likely assembling through the direct interaction with the septins (Wloka et al. 2011; Eluere et al. 2012; Kang et al. 2013). In the next round of budding, these cortical marker proteins recruit a **Ras-related GTPase module** (consisting of the GTPase Rsr1p/Bud1p, its GTPase-activating protein (GAP) Bud2p, and guanine nucleotide exchange factor (GEF) Bud5p) to the future bud site, which organizes the morphogenetic machinery toward the site of growth. At this site a new septin ring and Axl2p spot is formed generating a cyclic pattern of interdependence between the septins and the axial landmark proteins throughout the cell cycle.

The **fission yeast** *S. pombe* grows by elongation at its two ends and divides by medial fission, generating two roughly equally sized daughter cells. In contrast to budding yeast, in which the division site is solely determined by cortical cues, the division site in fission yeast is chosen based on a positive signal provided by the position of the pre-mitotic nucleus and negative signals from the two cell ends. Microtubules control the positioning of the nucleus at the cell center through opposing pushing forces generated by the dynamic instability of the microtubule system (Daga and Chang 2005; Tran et al. 2001). Furthermore, the bipolar longitudinal orientation of the microtubule system marks the cell ends by transporting the Tea1-Tea4 polarity complex to the tips, where it is tethered to the cortex through the prenylated protein Mod5 (Snaith et al. 2005; Snaith and Sawin 2003). This complex recruits the Dyrk family kinase Pom1 and other, yet undefined factors, to the cell ends (Bähler et al. 1998; Tatebe et al. 2005). A key factor that integrates both types of spatial signals is the anillin-related but *S. pombe*-specific protein Mid1. Mid1 reads the nuclear localization by shuttling between the nucleus and the adjacent cell cortex, where it forms a series of ca. 50 cortical dots that later in the cell cycle assemble and recruit CAR components (Almonacid et al. 2009; Celton-Morizur et al. 2006; Padte et al. 2006). The restriction of these interphase nodes to the cell center depends on Pom1-dependent and other inhibitory signals that are generated from both cell poles (Celton-Morizur et al. 2006; Huang et al. 2007; Padte et al. 2006; Rincon et al. 2014). In addition, Pom1 also phosphorylates the F-BAR protein Cdc15, a central component of the CAR, to inhibit CAR assembly at cell ends (Ullal et al. 2015).

B. Septum Placement in Filamentous Ascomycete Fungi

In contrast to the in-depth understanding of spatial signals present in the two unicellular yeast models, we have only very limited data about the presence and nature of positional cues that regulate septum placement in **filamentous ascomycete fungi**. It has been proposed that cortical cues deposited at the hyphal tip may trigger septum position in the filament-forming *Saccharomycotina* species

Ashbya gossypii (Kaufmann and Philippsen 2009; Fig. 1c). Although attractive, this hypothesis is primarily based on the observation that cortical marker proteins homologous to the *S. cerevisiae* Bud3p-Bud4p-septin landmark complex and associated proteins, such as the F-BAR scaffold protein Hof1, a central organizer of the CAR in both yeast models, are deposited at the hyphal tip in *A. gossypii* and *Candida albicans* at the time when tip growth slows as a consequence of septum formation in the subapical region of the hypha (Sudbery 2001; Wightman et al. 2004; Knechtle et al. 2003; DeMay et al. 2009; Helfer and Gladfelter 2006; Gonzalez-Novo et al. 2008; Kaufmann and Philippsen 2009). Moreover, effective progression through the cell cycle seems to require repositioning of migrating nuclei to these preselected sites for efficient initiation of septation (Alberti-Segui et al. 2001; Finley and Berman 2005; Helfer and Gladfelter 2006; Finley et al. 2008). Nuclear position relative to the imprinted septation sites may thus be a consequence of morphogenetic markers placed at the incipient bud site in unicellular or the tip growth in filamentous species of the *Saccharomycotina* clade. Clearly, this hypothesis needs further experimental support, especially because the mechanism(s) by which the bud-septin complex is deposited at the tip to mark future septation sites has not been addressed at the molecular level.

No changes in the rate of tip extension are observed during mitosis, septum formation, and branch initiation in ***A. nidulans*** and *N. crassa* hyphae (Horio and Oakley 2005; Jackson 2001; Riquelme and Bartnicki-Garcia 2004; Riquelme et al. 2003; Sampson et al. 2003). Thus, no growth rate-dependent septum to tip signal can exist in these species of the *Pezizomycotina* clade to mark future septation sites analogous to that proposed for the filamentous *Saccharomycotina* species. An alternative explanation may be an inhibitory gradient originating from the tip that overrides positive yet still undefined signals generated from the nuclei. Such a tip-high inhibitory gradient has been proposed in *A. nidulans* to restrict septum formation in growing germlings until a certain cell size is reached (Kaminskyj 2000; Wolkow et al. 1996; Harris 2001) (Fig. 1d). Although the nature of this proposed gradient is currently unknown, homologs of the *S. pombe* Tea1-Tea4 system may represent an attractive possibility (Fischer et al. 2008; Higashitsuji et al. 2009; Konzack et al. 2005; Takeshita et al. 2008, 2014; Takeshita and Fischer 2011). *A. nidulans* TeaA and TeaR, functional homologs of the *S. pombe* cell-end marker Tea1 and its membrane anchor Mod5, localize in an interdependent manner to the hyphal tip and colocalize there with the formin SepA (Takeshita et al. 2008). Deletion of either TeaA or TeaR results in wavy and meandering growth, indicating that the apical localization of these cell-end markers is required for stabilizing the axis of growth polarity. Interestingly, TeaC, the homolog of *S. pombe* Tea4, localizes to septa in addition to its presence at the hyphal tip (Higashitsuji et al. 2009). Overexpression of TeaC does not affect apical tip extension rates but represses septation and generates almost aseptate strains, while deletion of *teaC* results in increased septation, consistent with the hypothesis of positioning factors that inhibit septum formation in the tip region to regulate compartment size in growing hyphae. This view is consistent with recent data obtained for ***N. crassa*** that also support some kind of size-sensing mechanism for septum placement (Delgado-Alvarez et al. 2014; Fig. 1e). When mature *N. crassa* tip cells reach a critical size of ca. 250 μm, a new septum is initiated approximately 165 μm distal of the tip. However, unlike *S. pombe*, where the position of the pre-mitotic nucleus serves as additional positive signal, the majority of septa in *A. nidulans* are formed at a position corresponding to a location in between two pairs of previously mitotic nuclei (Fig. 1f; Shen et al. 2014), and the precise involvement of nuclear behavior in septum placement remains unclear (also see Sect. III-C).

C. Signal Integration by Anillin-Type Landmark Proteins

Despite the poor conservation of spatial cues, **anillin-type landmark proteins** coordinate these spatial signals and are thus critical for organizing the future site of cell division in all fungi that have been analyzed to date. Also,

vertebrate anillin is among the first proteins that are recruited to the cleavage site of dividing cells (Cabernard et al. 2010; Piekny and Glotzer 2008), suggesting conserved functions of this class of poorly defined proteins in orchestrating cell division plate. In animals, anillin functions as scaffold for the **Rho GTPase RhoA** and its regulators ECT2/Pbl and RacGAP50C at the cleavage furrow (D'Avino et al. 2008; Gregory et al. 2008; Hickson and O'Farrell 2008). Also Bud4p and the fission yeast homolog, the Mid1-related protein Mid2, interact with specific Rho GTPase modules and are recruited to the incipient separation site in a septin-dependent manner. In budding yeast, the **axial landmark Bud3p, Bud4p, and Axl2p** assemble into a protein complex at mitosis. Septin filaments first recruit Bud4p and Bud3p, which interact through their C-termini, to the bud neck (Gao et al. 2007; Kang et al. 2012, 2013; Wu et al. 2015). Bud3p has weak GEF activity toward the Rho GTPase Cdc42p and can activate Cdc42p in vivo (Kang et al. 2014), which may be one important aspect for organizing the morphogenetic machinery. In fission yeast septin rings are involved primarily in cell-cell separation after the septum has formed. The fission yeast anillin Mid2 localizes as a ring in the middle of the cell after anaphase in a septin- and actin-dependent manner and influences septin ring organization (Tasto et al. 2003; Berlin et al. 2003). The GEF Gef3 interacts with $Rho3^{GTP}$ in vitro and functions as activator of Rho4 in vivo. Gef3 co-localizes and physically interacts with septins and Mid2 and requires septins and Mid2 for its localization (Munoz et al. 2014; Wang et al. 2015). Together these data support that Gef3 interacts with the septin complex and activates one or several Rho GTPases as a Rho GEF for septation in fission yeast. Similar modules, consisting of the Rho GTPase Rho4/RHO-4, its GEF Bud3/BUD-3, and the anillin Bud4/BUD-4, are required for septum formation in the filamentous ascomycetes *A. nidulans* and *N. crassa* (Justa-Schuch et al. 2010; Si et al. 2010, 2012; Rasmussen and Glass 2005). Thus, a general function of anillins may be the organization of a Rho GTPase-GAP-GEF module at the future cytokinetic site (D'Avino 2009; Zhang and Maddox 2010; Seiler and Justa-Schuch 2010).

The septins in budding yeast bud4Δ cells fail to form a double ring during cytokinesis, while overexpression of Bud4p causes extra septin structures (Wloka et al. 2011; Eluere et al. 2012; Kang et al. 2013), suggesting a positive feedback in the organization of septin collar and axial landmark complex. A positive feedback for the cortical recruitment of the anillin-RHO-4 GTPase module was also described for *N. crassa* (Justa-Schuch et al. 2010), where localization of BUD-3 depends on BUD-4, whose localizations in turn lead to the recruitment and activation of RHO-4. However, the stable cortex association of BUD-3 and BUD-4 also requires RHO-4. This feedback also allows the stable accumulation of the BUD-3-BUD-4-RHO-4 complex at presumptive septation sites prior to the initiation of septum formation.

Recent structural data of animal anillin and *S. pombe* Mid1 defined several functional regions of these two proteins despite their poor conservation at the sequence level (Fig. 2). The N-terminal regions of both proteins bind to multiple components of the CAR machinery, and Mid1 and animal anillin have functional exchangeable N-terminal domains (D'Avino 2009; Piekny and Maddox 2010; Watanabe et al. 2010; El Amine et al. 2013; Sun et al. 2015). This region can bind active myosin and can bundle actin filaments (Field and Alberts 1995; Straight et al. 2003). The central region of anillin carries an anillin-homology domain, which contains a coiled-coil region that—in animal anillin—harbors a well-defined RhoA-binding domain, followed by a membrane-binding C2 domain that forms β-sandwich structure. Moreover, the Mid1 C2 domain allows dimerization. The C-terminus of anillin possesses a PH domain that binds septins and phospholipids (Liu and Young 2012; Oegema et al. 2000; Sun et al. 2015). Although both animal anillin and Mid1 attach to the plasma membrane via the same core elements of the C2 domain and the conserved PH domains, the PH domain of Mid1 is not essential for membrane anchorage (Sun et al. 2015; Lee and Wu 2012; Paoletti and Chang 2000; Liu and Young 2012).

Most fungal anillin-like proteins cluster phylogenetically with *S. pombe* Mid2, which promotes cell separation as a late-acting septin-dependent scaffold, but does not influence cell

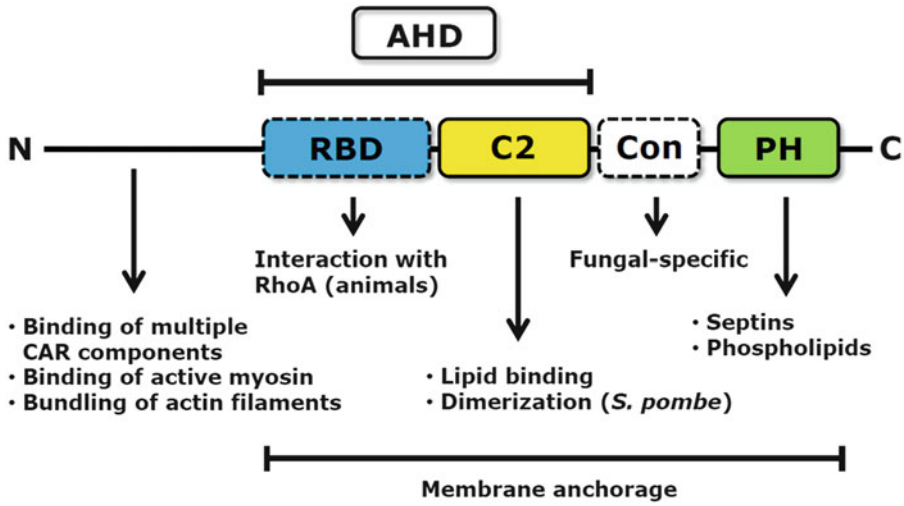

Fig. 2 Schematic representation of functional regions of fungal and animal anillins. *AHD* anillin-homology domain, *RBD* RhoA-binding domain, *C2* membrane-binding module, *CON* connector domain, *PH* Pleckstrin homology domain. For detailed explanation, refer to the text

division plate positioning. Mid1 and Mid2 arouse by gene duplication followed by functional divergence (a duplication that is specific for the genus *Schizosaccharomyces*), suggesting that the ancestral and conserved function of fungal anillin-related proteins is to scaffold CAR complexes in a septin-dependent manner (Gu and Oliferenko 2015; Gu et al. 2015; Moseley 2015). This is also supported by the recent finding that Mid1 of the close relative *Schizosaccharomyces japonicus* serves as an interphase cortical anchor for type II myosin, and the medial assembly of the CAR in mitotic *S. japonicus* cells relies on the cortical anchor protein Cdc15 instead of Mid1, which is regulated by a tip-localized Pom1 gradient (Gu and Oliferenko 2015; Gu et al. 2015; Moseley 2015).

III. Temporal Cue(s): Coordination of Cell Cycle Progression and Septation Initiation

A. Coordination of Mitotic Exit and Initiation of Septation in Unicellular Yeasts

The localization of the anillin-related landmark proteins Mid1 and Bud4p provides the spatial cue for septum placement in the two model yeasts. In addition, a GTPase-coupled kinase cascade—known as **mitotic exit network (MEN)** and **septation initiation network (SIN)** in budding and fission yeast, respectively—mediates the strict temporal coordination of cell cycle progression and cell division in the two unicellular fungi (Fig. 3a, b; Meitinger et al. 2012, Johnson et al. 2012). At the end of mitosis, the **spindle pole body (SPB)**-associated ras superfamily GTPase Spg1 is activated by the polo kinase Plo1 **in fission yeast.** This is achieved by phosphorylation, and thereby inhibition of its bipartite GTPase-activating protein (GAP) Cdc16-Byr4 results in recruitment of the STE kinase Cdc7 to activated Spg1 at the SPBs. Two additional SPB-associated kinases Sid1 and Sid2, with their respective regulatory subunits Cdc14 and Mob1, are part of the **SIN.** Localization studies suggest a hierarchical order of Cdc7-Sid1-Sid2, although biochemical evidence for a linear cascade is lacking (Johnson et al. 2012). Phosphorylation of the Cdc14 phosphatase Clp1 by the nuclear Dbf2p-related (NDR) effector kinase Sid2 promotes mitotic exit by counteracting the function of the cyclin kinase Cdc2 (Chen et al. 2008a). However, the SIN is not required for controlling mitotic exit, and SIN inactivation generates multinucleated

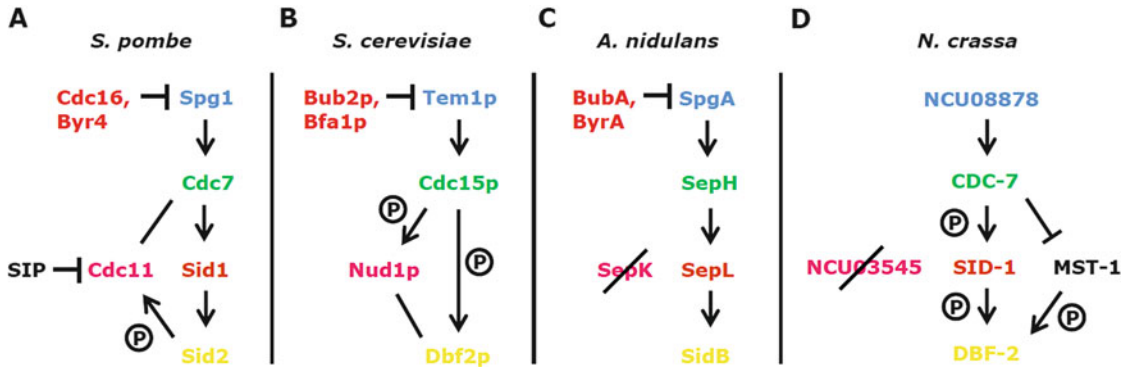

Fig. 3 Composition and regulation of the septation initiation network. (**a**) A GTPase-coupled, tripartite kinase cascade regulates initiation of septation in *S. pombe*. SPB-association of the effector kinase Sid2 depends on the two upstream kinases Sid1 and Cdc7, yet biochemical proof of a stepwise phosphorylation and activation of the three kinases is lacking. Positive feedback is provided by Sid2-dependent phosphorylation of the scaffold Cdc11, which is counteracted by the septation-inhibitory phosphatase complex SIP. (**b**) The related mitotic exit network (MEN) of budding yeast lacks a homolog of the fission yeast kinase Sid1, and the effector kinase Dbf2p is directly phosphorylated by the Cdc7 homolog Cdc15p. Recruitment of Dbf2p to SPBs and MEN activation also requires phosphorylation of the MEN scaffold Nud1p by Cdc15p. (**c**) A tripartite kinase cascade homologous to the fission yeast SIN also regulates septum formation in *A. nidulans*. However, SPB association of the SIN kinases through the scaffold SepK is not critical for septum formation, and SidB activation likely occurs in the cytosol. (**d**) The *N. crassa* SIN acts as cascade of three kinases, in which CDC-7 promotes the activity of SID-1, which in turn activates DBF-2 through hydrophobic motif phosphorylation. An additional kinase, MST-1, acts in parallel to SID-1 and is negatively regulated by interaction with CDC-7. This may allow fine-tuning of the SIN by generating an incoherent type 4 feedforward loop through the two, parallel functioning, but oppositely regulated CDC-7/SID-1 and CDC-7/MST-1 complexes that together control DBF-2 activity. SPB-association of these SIN components is not required for septum formation

cells, while overexpression of positive-acting SIN components causes the formation of multiple septum (Fankhauser and Simanis 1994; Ohkura et al. 1995; Schmidt et al. 1997). In both scenarios cell division is uncoupled from nuclear division. The assembly and subsequent constriction of the CAR that triggers septum formation requires the relocation of the active Sid2-Mob1 kinase complex, yet not the other SIN components, from the SPBs to the cell cortex (Roberts-Galbraith and Gould 2008; Hachet and Simanis 2008). The mechanisms that target the Sid2-Mob1 complex to the cell cortex are yet elusive.

The related **mitotic exit network (MEN) of budding yeast** has a similar composition and functions in an analogous manner (Meitinger et al. 2012). However, several significant differences exist (Fig. 3a, b). First, the MEN lacks a homolog of the fission yeast kinase Sid1, and the effector kinase Dbf2p is directly phosphorylated by the Cdc7 homolog Cdc15p (Mah et al. 2001). The Dbf2p phosphosites identified in this study correspond to sites highly conserved in other fungal NDR kinases (Hou et al. 2004; Jansen et al. 2006; Maerz et al. 2012), suggesting that Dbf2p activation involves activation segment autophosphorylation and phosphorylation of its C-terminal hydrophobic motif through Cdc15p. Second, recruitment of the Dbf2p-Mob1p complex to SPBs (and thus MEN activation) requires phosphorylation of the MEN scaffold protein Nud1p by upstream kinase Cdc15p (Mah et al. 2001; Rock et al. 2013). This differs from the positive feedback mechanism of the fission yeast SIN assembly at the SPB, in which the scaffold Cdc11 is phosphorylated by the effector kinase Sid2 to promote enhanced interaction with the upstream kinase Cdc7 (Feoktistova et al. 2012). Third, budding yeast MEN strictly controls mitotic exit, and consequently *MEN* mutants arrest at a late mitotic state with a CAR that has formed, but is unable to constrict (Yoshida et al. 2006), while *S. pombe SIN* mutants produce multinucleate cells.

B. The Septation Initiation Network in the *Pezizomycotina* Fungi

The MEN also controls mitotic spindle orientation and nuclear segregation in yeast cells of ***C. albicans***. Depletion of the MEN effector kinase Dbf2 results in growth arrest after the assembly of the actomyosin ring, indicating that **MEN activity is also essential for CAR contraction**, but not for CAR assembly (Gonzalez-Novo et al. 2009). Consequently septum formation is also inhibited in *C. albicans* filaments. As in yeast cells, Calcofluor White-stainable material still accumulates at presumptive septation sites, suggesting that CAR assembly is still possible and constriction is blocked, but this has not been addressed experimentally. However, not all *C. albicans MEN* mutant share identical defects, arguing against a simple and linear signal cascade and supporting distinct functions of individual MEN components. For example, unlike Tem1p-depleted *S. cerevisiae* cells, which arrest as large-budded cells as any other *MEN* mutant in budding yeast, GTPase-depleted *C. albicans* forms filaments that originate from large-budded yeast cells, suggesting that the GTPase Tem1 has an important, MEN-independent function for filament induction (Milne et al. 2014). The hyphae generated in a Tem1-depleted strain are binucleated and arrested in telophase with an elongated spindle, indicating that the MEN operates normally in these filaments and that MEN deficiency blocks mitotic exit after one round of nuclear division.

In contrast, the ***A. gossypii* MEN** has been suggested to function as linear GTPase-coupled bipartite kinase cascade, an assumption that was based on common mutant defects of deleting any MEN component (Finlayson et al. 2011). However, in contrast to *S. cerevisiae* or *C. albicans*, where the MEN controls mitotic exit at the anaphase stage, *A. gossypii MEN* mutants are enriched for metaphase nuclei, and thus the MEN seems to function earlier in the cell cycle of this organism. Also, cell cycle control is not absolute, and the multinuclear status of the hyphal compartments is maintained. As in *C. albicans*, the *A. gossypii* MEN is also essential for septum formation, and *MEN* mutants form aseptate hyphae (Finlayson et al. 2011).

In summary, a common denominator for the MEN in the three *Saccharomycotina* species seems to be a central role in CAR constriction but not CAR assembly. The regulation of the MEN and a mechanistic basis for its importance during septum formation in the filamentous context has not been addressed.

The **SIN in the *Pezizomycotina*** models *A. nidulans* and *N. crassa* functions as three-leveled kinase cascade that is activated by an upstream GTPase module as described for fission yeast. Deletion of any positive-acting SIN component in either fungus results in aseptate strains with no CAR formed, while no involvement in cell cycle control has been described (Bruno et al. 2001; Kim et al. 2006, 2009; Heilig et al. 2013; Maerz et al. 2009). All SIN components localize to the SPBs and—with the exception of the scaffold proteins and the upstream kinase—to constricting septa (Heilig et al. 2013; Kim et al. 2009). The *N. crassa* SIN components accumulate at the cell cortex several minutes prior to initiation of septum constriction, arguing for their involvement in CAR assembly, while CDC-7 is recruited to the forming septum only after septum constriction had started (Heilig et al. 2013, 2014). Similarly, the SidB-MobA module of *A. nidulans* associates with constricting septa (Kim et al. 2006, 2009), while SepH does neither associate with forming nor mature septum (De Souza et al. 2014).

Although the function of the SIN as linear acting GTPase-coupled kinase device has originally been assumed for both fungi (e.g., Harris 2001; Seiler and Justa-Schuch 2010), recent data suggest a more complicated network of kinases (Fig. 3c, d). The ***N. crassa* SIN** acts as cascade of three kinases, in which CDC-7 promotes the activity of SID-1, which in turn activates DBF-2 through hydrophobic motif phosphorylation (Heilig et al. 2013). However, an additional kinase, MST-1, acts in parallel to SID-1 and is negatively regulated by interaction with CDC-7 (Heilig et al. 2014). A possible function of MST-1 in fine-tuning the SIN may be achieved in the generation of an incoherent type 4 feedforward loop through the two, parallel functioning, but oppositely regulated CDC-7/SID-1 and CDC-7/MST-1 complexes that together control DBF-2 activity. This rarely found regulatory motif

may allow for adaptive and thus robust SIN activity (Rodrigo and Elena 2011). This hypothesis is supported by the phenotypic characteristics of *N. crassa SIN* mutants, which revealed less severe septation defects of *sid-1* and *mst-1* mutants compared to strains deficient for *cdc-7* or *dbf-2* (Maerz et al. 2009; Heilig et al. 2013, 2014). The biochemical data obtained in this study also imply that the CDC-7/MST-1 complex may function independently of the GTPase SPG-1 (Heilig et al. 2014), a speculation that requires further exploration.

Interestingly, MST-1 is also essential for sexual development (Heilig et al. 2014), and mutants in the *Sordaria macrospora* homolog SmSTK24 was recently found to interact with the STRIPAK (striatin-associated phosphatase and kinase) complex (Bloemendal et al. 2012; Frey et al. 2015). Although the *N. crassa* STRIPAK complex does not localize to septa and has primarily been associated with nuclear-cytoplasmic distribution of the cell wall integrity MAP kinase MAK-1 (Dettmann et al. 2013), several *S. macrospora STRIPAK* mutants form aseptate ascogonia (although vegetative septation is normal (Bloemendal et al. 2010). It is tempting to predict the involvement of the STRIPAK complex at an early stage of sexual development in the *Pezizomycotina* fungi. Intriguingly, the *S. pombe* STRIPAK complex has also been associated with regulating the SIN. This SIN-inhibitory phosphatase (SIP) complex counteracts the positive feedback generated by Sid2-dependent phosphorylation of Cdc11 by dephosphorylating the scaffold (Singh et al. 2011).

C. Coordination of Nuclear Behavior and Septum Formation in *Pezizomycotina* Fungi

CAR assembly and septum formation are clearly controlled through nuclear position and cell cycle progression in *A. nidulans* (Harris et al. 1994; Wolkow et al. 1996). This may potentially also apply to *N. crassa*, although the connection between nuclear cycle and septum positioning is blurred by its nuclear asynchrony (Plamann et al. 1994; Minke et al. 1999; Seiler and Justa-Schuch 2010). The recent finding that circadian rhythms can synchronize mitosis via the *N. crassa* homolog of the WEE-1 kinase (Hong et al. 2014) might provide a platform for a more detailed analysis. Septum placement was originally proposed to depend on the position of mitotic nuclei in *A. nidulans* (Momany and Hamer 1997; Bruno et al. 2001; Harris 2001), but detailed life imaging recently revealed that the majority of septa formed at a position corresponding to a location in between two pairs of previously mitotic nuclei (Shen et al. 2014; Fig. 1c). Moreover, septum formation still occurs when the microtubule cytoskeleton was destroyed, indicating that initiation and completion of septation does not require microtubules.

Although we have currently no mechanistic understanding concerning the coordination of nuclear behavior and septation, a number of candidate proteins/pathways have been analyzed, the most obvious being the SIN. Although all components of the SIN localize to SPBs in *A. nidulans* and *N. crassa*, no involvement in controlling cell cycle progression has been observed in either species during vegetative growth (Bruno et al. 2001; Kim et al. 2009; Heilig et al. 2013, 2014). Moreover, in contrast to the yeasts, where SPB association of the SIN/MEN components is essential for activation of the NDR effector kinase and the subsequent recruitment of the NDR kinase-Mob1 complex to the cell cortex for cytokinesis (Morrell et al. 2004; Rosenberg et al. 2006), SIN activation and septum formation in *A. nidulans* and *N. crassa* does not require SPB association (Kim et al. 2009; Heilig and Seiler, unpublished). This is consistent with the observation that the *A. nidulans* Polo kinase PlkA is not a central regulator of septum formation and has only a minor function in cell cycle control (Bachewich et al. 2005; Mogilevsky et al. 2012). Consequently, activation of the SIN may not be mediated through PlkA as established for the MEN/SIN through the budding and fission yeast Polo kinases Cdc5p and Plo1, respectively (Ohkura et al. 1995; Song et al. 2000). Finally, the two scaffold proteins SnaD and SepK (structural homologs of Sid4 and Cdc11 in *S. pombe*, respectively) that are necessary to anchor SIN components to the spindle pole body in *A. nidulans* are not critical for septum formation, and mutations in either scaffold only result in

delayed septation (Kim et al. 2009). Thus, SPB association of the SIN might modulate temporal aspects of septum formation, but details of a mechanistic involvement of the SIN in coordinating nuclear behavior with septation during vegetative hyphal growth remain obscure.

Interestingly, it was recently shown that SepH concentrates in the basal region of the apical cells as cytosolic foci in addition to its association with mitotic SPB in *A. nidulans* (De Souza et al. 2014). Cytosolic SepH foci were particularly prominent in periods preceding septation and resulted in a preferential association of SepH with the SPB of mitotic nuclei, which were located distal from the tip. Moreover, SepH associated with SPB in a biphasic manner first during mitosis and again during the period of septum formation, suggesting that the predominant-basal activation of the SIN [if SPB-association can serve as marker for SIN activity as described for fission yeast; (Johnson et al. 2012)] might help restricting SIN activity to the basal region of the tip cell to promote asymmetric septation in *A. nidulans*. These cytosolic foci were also observed for the *N. crassa* homolog CDC-7 (Heilig et al. 2013, 2014) but not for any of the other SIN kinases or respective regulatory subunits in *N. crassa* (Heilig et al. 2013, 2014; Dettmann et al. 2012) or the SidB-MobA module in *A. nidulans* (Kim et al. 2006, 2009). CDC-7 kinase activity is not required for cytosolic clustering of the protein and its association with SPBs in *N. crassa*, and CDC-7 foci were more abundant when a kinase dead version of CDC-7 was localized (Heilig et al. 2014). No asymmetric localization of CDC-7 was noticed in *N. crassa*, but only mature hyphae were analyzed, in which fast cytosolic flow rates likely had mixed any potential gradient of CDC-7 clusters. A potential function of these cytosolic CDC-7 speckles might be suggested by the co-purification of the **microtubule-organizing center (MTOC)** component NCU12004 (homolog of *A. nidulans* ApsB and *S. pombe* Mto1) with CDC-7 yet with none of the other SIN components in *N. crassa* (Heilig et al. 2014). MT nucleation occurs primarily at centrosomes/SPBs, but more diverse types of cytosolic MTOC also exist. The mechanisms for the generation of this MTOC diversity are poorly understood, but it is essential for functional MT organization in fungi and higher eukaryotes. Mto1/ApsB is central for cytosolic MTOC assembly and function (Samejima et al. 2010; Lynch et al. 2014), and ApsB is recruited to the septal pore through a peroxisome-dependent pathway to form septum-associated MTOCs (Veith et al. 2005; Zekert et al. 2010). Intriguingly, CDC-7 localization around the mature septal pore in *N. crassa* (Heilig et al. 2014) displays striking similarities with the sepal pore localization of ApsB and γ-tubulin in *A. nidulans* (Zekert et al. 2010). Future analysis should therefore consider the speculation that these cytosolic clusters might represent non-SPB-associated MTOCs.

Other candidate proteins that are involved in coordinating nuclear behavior with septum biology in *A. nidulans* are the cyclin kinase $NimX^{Cdk1}$ and the NEK-family kinase NimA. Septum formation depends on a threshold level of activity of the $NimX^{Cdk1}$ (Harris 2001; Harris and Kraus 1998; Kraus and Harris 2001). How $NimX^{Cdk1}$ coordinates nuclear division with septum in *A. nidulans* remains unresolved. NimA functions as central regulator of multiple stage-specific aspects of mitosis. Key functions of NimA are chromatin condensation through histone H3 phosphorylation and the partial opening of the nuclear envelope though disassembly of nuclear pore complex (De Souza et al. 2000, 2004). NimA also has interphase-specific functions such as regulating microtubule dynamics and thus tip growth and was shown to localize at the tip growth and microtubule plus ends in a EB1-dependent manner (Govindaraghavan et al. 2014). Important in the context of septum formation is the recruitment of NimA to the cell cortex prior to the initiation of septum constriction (Shen et al. 2014). Intriguingly, the fission yeast NimA homolog Fin1 is activated for mitotic commitment by phosphorylation through the NDR kinase Sid2 (Grallert et al. 2012; not to be confused with Plo1-dependent activation of the SIN at mitotic exit). In G2 phase, the Sid2-Mob1 complex acts independently of other SIN components such as the scaffold Cdc11 to control the timing of mitotic entry, suggesting that Sid2 activation does not occur at the SPBs. Although highly speculative, activation and/or recruitment of

NimA by SidB to cortical sites (primed by unknown cues) might be involved in triggering initiation of septum constriction. Moreover, in contrast to other protein machinery required for septum formation in *A. nidulans* such as type II myosin MyoB (Hill et al. 2015), NimA is maintained at the septal pore after septum constriction has terminated (Shen et al. 2014). It is thus possible that NimA at this position controls the regulated closure of septal pores in a cell cycle-dependent manner in order to restrict leakage of nuclear proteins into subapical compartments during the parasynchronous mitoses of the nuclei present in the apical cell.

IV. Assembly and Regulation of the Contractile Actomyosin Ring (CAR)

A. CAR Assembly in Budding and Fission Yeast

Significant advances have allowed establishing the approximate spatiotemporal sequence of events during assembly and constriction of the CAR that drives cytokinesis in the two yeasts (Bi and Park 2012; Pollard and Wu 2010). In fission yeast, two independent but synergistic pathways driven by the anillin-related landmark protein Mid1 and the SIN control assembly of the CAR (Pollard and Wu 2010, Bathe and Chang 2010, Johnson et al. 2012; Fig. 4a). Interestingly, both pathways are controlled by the **Polo kinase Plo1**. This central cell cycle kinase is therefore exerting spatial control through regulating Mid1 localization and is also providing the temporal cue through regulation of SIN activity. Cortical, Mid1-containing dots recruit class II myosin, the formin Cdc12, and other components to form medial nodes that allow F-actin nucleation early in mitosis. Search-capture-pull-release interactions between myosin and f-actin from distinct nodes form a highly organized ring (Wu et al. 2006; Vavylonis et al. 2008; Laporte et al. 2011; Lee and Wu 2012; Saha and Pollard 2012). Also, actin filaments assembled by the formin Cdc12 at nonmedial sites are transported to the division site in a myosin-dependent manner and are integrated into the maturing ring (Huang

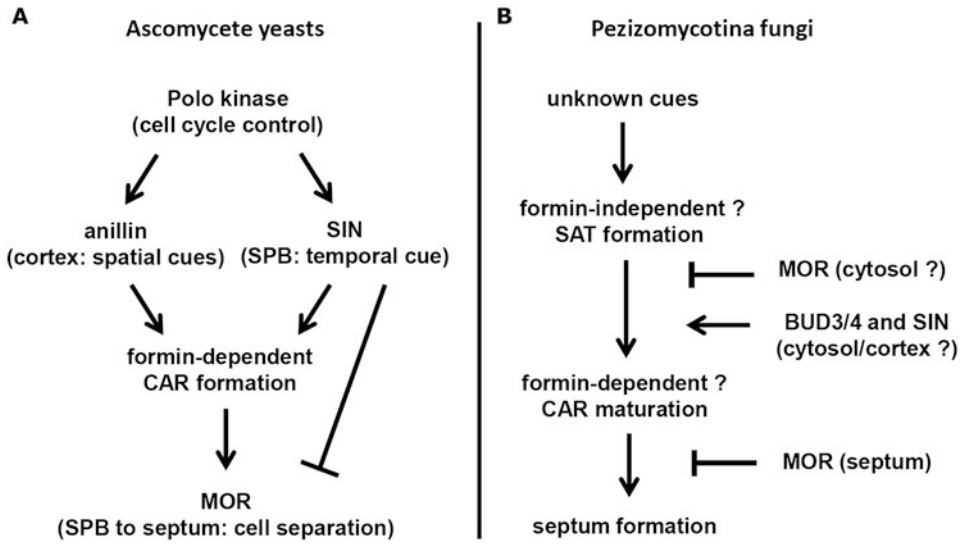

Fig. 4 Spatiotemporal sequence of events that control assembly and constriction of the CAR. (**a**) Two independent, but synergistic pathways driven by anillin-related landmark proteins and the SIN/MEN control assembly and/or constriction of the CAR in budding and fission yeast. SIN activity also inhibits the MOR network, which is required for timely cell separation. (**b**) Unknown cues initiate the formation of the septal actomyosin tangle SAT in the *Pezizomycotina* fungi. SAT initiation, CAR formation, and CAR constriction are regulated at various levels by the anillin-Rho GTPase complex and the interplay of the SIN and MOR kinase networks

et al. 2012). However, Mid1 nodes are not essential for CAR assembly, and *mid1* mutants produce misplaced septa in the anaphase. This indicates the presence of a second, SIN-dependent pathway that is responsible for CAR assembly in late mitosis (Huang et al. 2008; Hachet and Simanis 2008; Roberts-Galbraith and Gould 2008). Because *sin* mutants form only a transient CAR that is instable, these data suggest that the primary task of Mid1 is providing positional information, while the SIN is required for CAR maturation. Moreover, CAR constriction also requires SIN activity.

The maturation of the CAR from cortical nodes requires phosphorylation of several independent targets by the terminal SIN kinase Sid2. First, the recruitment of the F-BAR scaffold protein Cdc15 to the medial region of the cell is indirectly controlled by the SIN (Clifford et al. 2008; Hachet and Simanis 2008; Roberts-Galbraith et al. 2010). Interphase Cdc15 is phosphorylated at multiple sites that induces a closed conformation of the protein and inhibits its assembly at the division site. Activation of the Cdc14 phosphatase Clp1 by the SIN effector kinase Sid2 allows association of Clp1 with Mid1 and dephosphorylation of Cdc15. This induces an open conformation and oligomerization of Cdc15 that activates its scaffold function. Second, the SIN directly activates the formin Cdc12 through Sid-2-dependent phosphorylation of a novel Cdc12 domain that controls F-actin bundling and therefore a central function of the formin during CAR maturation (Bohnert et al. 2013). Importantly, this novel Cdc12 function should not be confused with the central function of formins in F-actin nucleation, which is controlled through dimerization of the FH2 domains (Xu et al. 2004). Although is it clear that the SIN is also important during septum constriction, the mechanism that triggers initiation of CAR constriction and the specific functions of the SIN during constriction are yet unknown (Johnson et al. 2012).

The molecular composition of the budding yeast CAR is very similar to that of fission yeast although the CAR components accumulate over longer time periods (Luo et al. 2004; Shannon and Li 2000; Wloka and Bi 2012; Balasubramanian et al. 2004; Bi et al. 1998; Lippincott and Li 1998). One important difference between the two yeasts is that the CAR assembles at the bud neck only after the septin collar has formed in response to the Bud3p-Bud4p-Axl2p landmark described earlier (Gladfelter et al. 2001; McMurray and Thorner 2009). As discussed before, the future septation site in the filament-forming *Saccharomycotina* species *A. gossypii* and *C. albicans* may be marked at the hyphal tip as response of the reduced growth rate triggered by subapical septum formation. This apical landmark consists of *Ashbya gossypii* Bud3 or the *C. albicans* Bud4 homolog Int1 and various septins in both fungi (Wendland 2003; DeMay et al. 2009; Gale et al. 2001). This allows recruitment of proteins required for CAR assembly, such as the F-BAR protein Hof1 (homolog of *S. pombe* Cdc15), type II myosin, and the IQGAP protein Cyk1 to form cortical filaments or "bars" within 5–10 μm of the hyphal tip, which are subsequently transformed into a subapical cortical ring as tip growth proceeds (DeMay et al. 2009; Helfer and Gladfelter 2006; Kaufmann and Philippsen 2009).

B. CAR Assembly in the *Pezizomycotina*

The CAR is also a key constituent of the cytokinetic machinery required for septum formation in the ***Pezizomycotina*** fungi (Seiler and Justa-Schuch 2010; Berepiki et al. 2011). The dynamics of the actin cytoskeleton during septum formation has recently been addressed using Lifeact (a marker for labeling f-actin in living cells; Riedl et al. 2008) in *N. crassa* (Delgado-Alvarez et al. 2010, 2014; Berepiki et al. 2010). A prominent tangle of actin filaments, the septal actomyosin tangle (SAT), occurs 3–4 min prior to the formin BNI-1 and the anillin BUD-4 at sites of future septum formation. Thus, formin- and anillin-dependent f-actin nucleation and organization seems to be of minor importance for SAT formation, while the appearance of BUD-4 and BNI-1 coincides with maturation of the CAR from the SAT (Fig. 4b). Another striking observation is that the SAT may primarily be generated by transferring existing filaments from established subapical septa to

the future site of septation. It is currently unclear if this transfer is based on treadmilling of filaments or on myosin-dependent transport of f-actin.

Moreover, a cortical double ring of patches consisting of components of the Arp2/3 complex, fimbrin and coronin, appear after the coalescence of Lifeact-GFP-labeled cables into a sharp ring, a few seconds before the membrane contraction begins and disappear after CAR contraction has terminated (Delgado-Alvarez et al. 2010, 2014; Echauri-Espinosa et al. 2012; Upadhyay and Shaw 2008). These patches are likely part of the endocytosis machinery that may contribute to membrane remodeling and recycling of supernumerary cell wall enzymes.

Several models are currently discussed that describe the transition from cortical Mid1-containing nodes into the mature CAR in fission yeast (Bathe and Chang 2010; Pollard and Wu 2010; Laporte et al. 2011), a process that seems to correspond to the SAT-CAR transition described for *N. crassa*. These models are non-exclusive and primarily differ in the number and distribution of f-actin nucleating formin assemblies and how the de novo generated filaments coalesce into a stable ring. A recent study also reported that pre-existing f-actin filaments can be recruited in a myosin-dependent manner to cortical actin nodes (Huang et al. 2012). This is consistent with the results obtained in *N. crassa* and analogous findings in animal cells (Chen et al. 2008b; Zhou and Wang 2008), supporting the hypothesis that de novo formin-dependent f-actin assembly at the division site and transport of pre-assembled filaments can contribute to CAR assembly in all eukaryotes. However, the relative contribution of the two actin populations may vary in the different systems.

C. Crosstalk Between the SIN and the MOR Pathways for CAR Regulation

Another fundamental mechanism by which the fission yeast SIN promotes CAR maturation is the inhibition of a competing polarity pathway called the **MORphogenesis network**, which is required for actin organization at cell ends during polarized growth (Gupta and McCollum 2011; Ray et al. 2010). The MOR (called RAM in budding yeast) represents a second Dbf2-related kinase network with an organization similar to that of the SIN (Nelson et al. 2003; Kanai et al. 2005; Das et al. 2009). To promote tip growth, actin is confined to the cell ends where it is required for cell wall deposition. As cells enter mitosis, actin relocates to site of cell division to form the CAR. Since, both processes involve restructuring of the actin cytoskeleton, coordination is presumably important to keep competing actin polarity programs from interfering with each other. Thus, mutual antagonistic function of the SIN and MORphogenesis network is required to coordinate cell growth and division. Inhibition of the MOR is achieved by the SIN effector Sid2, which phosphorylates the MOR kinase Nak1 and the Nak1-associated protein Sog2 in order to block interaction of Nak1-Sog2 with the scaffold protein Mor2 (Gupta et al. 2013). Moreover, the upstream SIN components Cdc7 und Sid1 control and enhance MOR activity in the subsequent interphase after cell division by an unknown mechanism (Kanai et al. 2005).

Tip growth and septum formation occurs simultaneously in filamentous fungi, which requires differential regulation between both NDR kinase pathways (Fig. 4). The localization of *N. crassa* SIN components at the time of SAT to CAR transition suggests the involvement of the SIN in the maturation of a functional CAR (Heilig et al. 2014). A key function of the MOR network in molds seems to inhibit septation initiation (Maerz and Seiler 2010). *N. crassa* MOR mutants produce hyperseptated hyphae, while hyperactivation of the MOR resulted in a reduced septation index (Yarden et al. 1992; Ziv et al. 2009; Seiler and Plamann 2003; Seiler et al. 2006; Maerz et al. 2009). Because the *N. crassa* MOR components do not associate with SPBs (Maerz et al. 2012; Dettmann et al. 2012; Heilig et al. 2014), inhibition of septation initiation by the MOR likely occurs in the cytosol. A second, presumably late function of the MOR during septation might be indicated by the recruitment of all MOR components to the forming septum after initiation of CAR constricting has started (i.e., 2–3 min later than cortex recruitment of the SIN kinase DBF-2; Heilig et al. 2014, Maerz et al. 2012). Such a late function of the MOR is also supported by the analysis of conditional mutants in the MOR effector kinase COT-1, which produce thickened septa and altered septum when grown at restrictive conditions

(Gorovits et al. 2000). These data suggest multiple functions of the MOR in regulating septation initiation and biogenesis of the septum wall in molds.

In line with the opposite functions of the SIN and the MOR during septation, multilevel cross talk between both networks has recently been determined in *N. crassa* (Heilig et al. 2014). The SIN kinase MST-1 regulates the MOR and the SIN both in an enzyme-dependent and -independent manner. MST-1 phosphorylates and activates both the MOR and SIN effector kinases, COT-1 and DBF-2, respectively, by hydrophobic motif phosphorylation. In addition, heterodimerization of the two germinal center kinase MST-1 and the MOR kinase POD-6 inactivates both kinases. An analogous mutually inhibitory interaction occurs between the two NDR kinases DBF-2 and COT-1, which can also form heterodimers. A putative mechanism for kinase inactivation is based on the observation that NDR kinase heterodimerization requires the same protein regions that are required for interaction with their activating MOB protein subunits. Moreover, the kinase-kinase and kinase-MOB interactions were found to be mutually exclusive, and kinase heterodimerization therefore resulted in the displacement of the regulatory subunit and consequently kinase inactivation (Heilig and Seiler; unpublished data).

V. Cell Wall Biogenesis and Cell Division

A. Cell Wall Biogenesis

CAR constriction is coupled with membrane invagination and secretion of membrane-bound cell wall biosynthetic enzymes that build the extracellular septum. Simultaneously, endocytic recycling of supernumerous components (enzymes, membrane, etc.) accompanies septum development. The major enzymes involved in cell wall formation are **chitin and glucan synthases** (Lesage and Bussey 2006; Free 2013). **Chitin** (linear chains of β-1,4-linked *N*-acetylglucosamine molecules) is synthesized by a multifamily group of enzymes that can be grouped into seven classes of chitin synthases (ChS; Riquelme and Bartnicki-García 2008). Most *Pezizomycotina* fungi have single genes for each of the seven reported classes of ChS, while yeasts have a more restricted set of ChS genes. Class II ChS functions as the major enzyme for synthesizing the primary septum in budding yeast (Chs2p; Schmidt et al. 2002) and in *C. albicans* (Chs1; Munro et al. 2001; Walker et al. 2013), while the other ChS in the *Saccharomycotina* are involved in the biosynthesis of the general cell wall and in repair functions (Lesage and Bussey 2006). Although the three-layered septum structure is also described for fission yeast, its primary septum is mainly composed of **β-1,3-glucan** (Humbel et al. 2001; Sugawara et al. 2003). The secondary septum in both yeast models and in *C. albicans* is built by several layers of β-1,3-glucans and mannoproteins (Lesage and Bussey 2006; Humbel et al. 2001; Sugawara et al. 2003).

Despite considerable effort in the cell wall analysis in the *Pezizomycotina* fungi (Free 2013; Latge and Beauvais 2014), the composition and biosynthesis of their septal walls is still poorly understood. Chitin has been shown to be the major component of the *N. crassa* septum (Hunsley and Gooday 1974). The specific localization of CHS-2 (class II) at the constricting rim of the developing septum (in addition to its localization at the tip growth) in *N. crassa* is consistent with a general function of class II ChS in primary septum formation (Fajardo-Somera et al. 2015). The remaining six *N. crassa* ChS localize along the entire septal plate, suggesting a function of these enzymes in remodeling the primary septum and/or synthesizing additional layers that form the secondary septum (Fajardo-Somera et al. 2015).

Regulation of primary septum formation is best understood in budding yeast. The MEN promotes cytokinesis by influencing multiple pathways involved in CAR constriction and septum formation. For instance, the MEN is involved in targeting the chitin synthase **Chs2p** to the bud neck (Meitinger et al. 2010) and also directly regulates the late cytokinetic components Hof1p and Inn1p, two PCH proteins which are homologs of the *S. pombe* Cdc15 (Sanchez-Diaz et al. 2008; Nishihama et al.

2009; Meitinger et al. 2010, 2011). Chs2p is held in an inactive state in the endoplasmatic reticulum by phosphorylation through Cdc28p/CDK1 that blocks its interaction with the COPII component Sec24p and sorting into COPII vesicles (Chuang and Schekman 1996; Zhang et al. 2006; Meitinger et al. 2010; Teh et al. 2009; Jakobsen et al. 2013), while MEN-controlled dephosphorylation by Cdc14p allows Chs2p to enter the secretory pathway (Chin et al. 2012). Subsequent inactivation of Chs2p is achieved through phosphorylation by the MEN effector kinase Dbf2p (Oh et al. 2012).

The second major component of the fungal cell wall is β-1,3-glucan. Its synthesis is catalyzed by membrane-bound β-1,3-glucan synthase complexes, which consist of the catalytic FKS and regulatory Rho1 GTPase subunit (Mazur and Baginsky 1996; Beauvais et al. 2001). Both yeasts have multiple, partially redundant **FKS genes**, and glucan synthesis is essential for viability. This is also the case for the dimorphic *Saccharomycotina* species *C. albicans* (Munro 2013). Most *Pezizomycotina* fungi have only a single FKS gene. Although all components of the β-1,3-glucan synthase complex as well as putative regulators localize to sites of polar growth at hyphal tips, emerging branches and along septa in *N. crassa* and other molds (Vogt and Seiler 2008; Verdin et al. 2009; Richthammer et al. 2012; Sanchez-Leon and Riquelme 2015), β-1,3-glucan synthesis is not essential in *A. fumigatus* (Dichtl et al. 2015), suggesting compensatory functions by other cell wall components in the *Pezizomycotina* fungi.

B. Septum Formation and Cell Division During Fungal Development

Premature activation of the fission yeast MOR (e.g., by misregulation of the SIN) results in inappropriate septum degradation and consequently cell lysis (Gupta et al. 2014). Degradation of the primary septum to trigger cell separation is initiated by RAM-dependent activation of the **transcription factor Ace2p** that controls the expression of Cts1p and Eng1p, the major cell wall-degrading chitinase and glucanase, respectively (Dohrmann et al. 1992; Colman-Lerner et al. 2001; Weiss et al. 2002). *C. albicans* Ace2 mutants also display cell separation defects (Kelly et al. 2004), and, consequently, transcription factor function is inhibited by the filament-inducing transcription factor Efg1 in order to inhibit cell separation after septum formation in hyphae (Wang et al. 2009; Saputo et al. 2014). Budding yeast Ace2p has a paralog, Swi5p, a transcription factor that is primarily involved in cell cycle regulation. Therefore it is currently unclear, if the *Pezizomycotina* fungi have a functional homolog of budding yeast Ace2p. In addition, we have no data that indicate transcriptional regulation by the MOR during tip growth in these fungi, and thus it remains open, if transcriptional regulation impacts vegetative septum formation in the *Pezizomycotina* clade.

Asexual sporulation (conidium) in the *Pezizomycotina* fungi involves the formation of conidia, formed on specialized structures called conidiophores. This is, in principle, achieved by two distinct sporulation patterns exemplified by *A. nidulans* and *N. crassa*, respectively (Park and Yu 2012). During basipetal sporulation the spore forms at the base of a chain and pushes the older cells of the conidial chain away from the spore-forming region, while the acropetal sporulation pattern refers to the fact that the most recently formed spore is at the tip of a chain of spores. Thus, the machinery required for septum formation and cell division must be targeted accordingly. Conidiation in molds is analogous to cell separation in the unicellular yeasts and requires full cell separation through a multilayered cell wall, which is followed by the digestion of the primary cell wall material between two completely formed secondary septum to release mature spores (Springer and Yanofsky 1989; Adams et al. 1998). Consequently, all *A. nidulans* and *N. crassa* mutants of currently characterized proteins required for septum formation in vegetative hyphae are also aconidiate (Rasmussen and Glass 2005; Maerz et al. 2009; Justa-Schuch et al. 2010; Dvash et al. 2010; Heilig et al. 2013; Bruno et al. 2001; Kim et al. 2006, 2009; Si et al. 2010). We have very limited mechanistic insights how the cell division machinery is reprogrammed

during the two basic developmental cell division patterns observed in molds. It has recently been shown that the *A. nidulans* homolog of the budding yeast axial bud-site landmark component Axl2p has no obvious role during vegetative growth (Si et al. 2012). **Axl2** is specifically required for the regulation of phialide morphogenesis during conidium, where it appears to promote the recruitment of septins, and Axl2 mutants fail to produce the long chains of conidium. Consistently, Axl2 specifically localized to the phialide-spore junction, implying Axl2 as landmark for reorientation of the division pattern from acropetal growth during phialide formation to basipetal growth during sporulation (Si et al. 2012).

All fungal and animal anillin (with the exception of the *S. pombe* landmark Mid1) interact with the septin scaffold. However, the timing of this interaction and its importance for cell division varies between the different fungal groups (Fig. 5). In vegetative *A. nidulans*, *A. fumigatus*, and *N. crassa* hyphae, the septin play only a minor role during septum formation, and the three fungi form proper septa when any of the five septin genes is deleted (Lindsey et al. 2010; Hernandez-Rodriguez et al. 2012; Berepiki and Read 2013; Vargas-Muniz et al. 2015). In contrast, septin deletion strains of both *Aspergilli* result in major defects in conidiophore development. Similarly, *N. crassa* core septin deletion mutants produce chains of unseparated conidium (Berepiki and Read 2013). In summary, these data are consistent with a late-acting function of the septins during cell separation, which—in molds—is suppressed during vegetative growth.

Intriguingly, splice variants of *C. albicans* transcription factor have recently been associated with RAM/MOR-dependent coordination of septin dynamics and regulation of the incorporation of the Sep7 septin into hyphal septal rings in order to avoid cell separation (Calderon-Norena et al. 2015). Thus, septin dynamics during conidial development in molds may also be regulated through NDR kinase signals. Mutants, in which the activity of the MOR effector kinase COT-1 is reduced, result in conidial separation defect (Ziv et al. 2009), indicating that the MOR functions during developmental cell separation in molds as described for vegetative cell separation in the two yeasts. Interestingly, an *A. fumigatus* Ace2 mutant displayed pleiotropic defects that were primarily associated with conidium morphogenesis and resulted in reduced amounts of generated conidial with highly thickened conidial walls (Ejzykowicz et al. 2009). Thus, MOR-dependent transcriptional regulation might be inhibited during vegetative growth to inhibit cell separation but is induced during the developmental program.

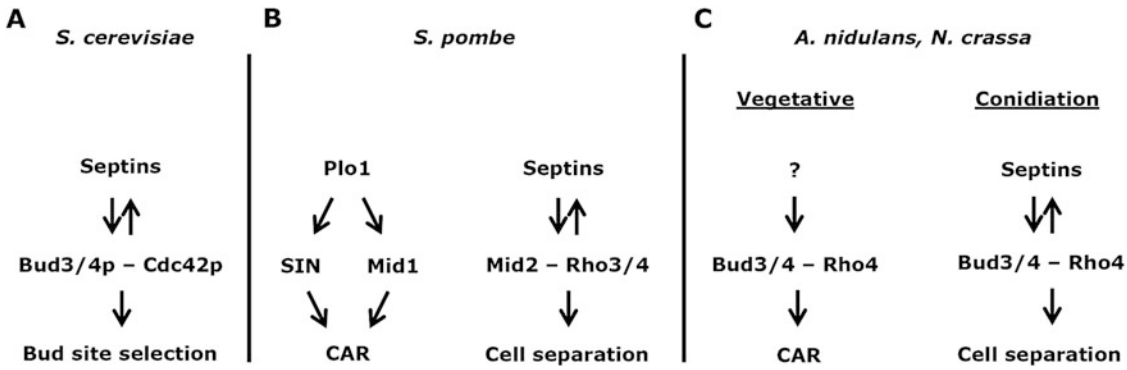

Fig. 5 Putative relationships between anillin-Rho GTPase complexes and the septins. (**a**) The interplay between the septins and the Bud3/4p-Cdc42p complex regulates bud-site selection in budding yeast. (**b**) A similar Mid2-Rho3/4 complex functions at a late stage of cell division to trigger cell separation in fission yeast. In addition, Mid1 has a unique function during septum placement in *S. pombe*. (**c**) The Bud3/4-Rho4 complex is important for CAR formation during vegetative growth of *A. nidulans* and *N. crassa* hyphae. The septins play only a minor role at this stage of the life cycle yet become critical for cell separation during asexual development

Sexual development is also affected in septum mutants. For example, *N. crassa* mutants are female sterile and do not form protoperithecia (Rasmussen and Glass 2005; Maerz et al. 2009; Justa-Schuch et al. 2010; Heilig et al. 2013). Moreover, homozygous crosses of septum strains, in which the female partner has been sheltered by a helper strain, are barren and produce very few ascospores. Interestingly, no septa are formed in ascogenous hyphae in these mutants, indicating multiple developmental defects of theses strains (Rasmussen and Glass 2005; Maerz et al. 2009). In budding yeast and fission yeast, the SIN has recently been shown to be dispensable for progression through meiosis but required for subsequent spore wall formation and ascospore morphology (Krapp et al. 2006; Attner and Amon 2012). Similarly, *N. crassa* SIN mutants produce few but giant ascospores containing all eight nuclei derived from the two meiotic and one mitotic divisions (Raju and Newmeyer 1977; Freitag et al. 2004; Maerz et al. 2009; Heilig et al. 2013). However, these nuclei are then enclosed in a single giant ascospore, supporting an essential function of the SIN during cross wall formation in vegetative cells and during the formation of asco- as well as conidiospores in unicellular and filamentous ascomycetes.

C. Septal Pore-Associated Functions in Filamentous Ascomycete Fungi

The septa of most *Pezizomycotina* species are perforated by simple pores of 350–500 nm in diameter, which allow nuclei, organelles, and cytoplasm to move between compartments (Hunsley and Gooday 1974; Mourino-Perez and Riquelme 2013). However, the mechanisms that terminate CAR constriction and incomplete cell separation are virtually unexplored. The development of certain animal tissues also requires incomplete cytokinesis and the formation of syncytia—a process that is very poorly understood. For instance, in many species, including humans, germ cells remain connected by intercellular bridges, which are required for germ cell development, and their absence results in infertility (Haglund et al. 2011). The robust genetic and cell biological tractability of filamentous fungi thus provides an unparalleled opportunity to determine mechanisms that control complete versus incomplete cytokinesis and the regulated gating of intercellular bridges/septum pores.

The presence of septa is essential for maintaining colony integrity after hyphal injury by rapid plugging of septal pores through peroxisome-derived Woronin bodies (Jedd and Chua 2000; Pieuchot and Jedd 2012). Moreover, the structure and composition of the septum and the connectivity status of septum in filamentous ascomycotina fungi varies within the developing mycelium. This allows age-dependent plugging of interior regions of the colony (e.g., in *N. crassa*: Trinci and Collinge 1973; Hunsley and Gooday 1974) and can establish cellular heterogeneity (e.g., in *A. niger*: de Bekker et al. 2011; Vinck et al. 2011; Wosten et al. 2013). Moreover, dynamic pore closure can be important to separate mitotically active versus inactive compartments (e.g., in *A. nidulans*: Shen et al. 2014) or to compartmentalize communicating regions within the colony (e.g., in *N. crassa*: Dettmann et al. 2014; Jonkers et al. 2014). How pore closure is regulated and which components are involved is poorly understood. Key candidate proteins that are likely involved in this process in varying composition are HEX-1-derived Woronin bodies (Beck et al. 2013), the SOFT protein, which is also involved in cell-cell communication and has recently been identified as scaffold of the cell wall integrity MAP kinase pathway (Fleissner and Glass 2007; Teichert et al. 2014) and a set of septum-associated intrinsically disordered proteins (Lai et al. 2012; Shen et al. 2014). How trafficking of growth-associated factors such as small secretory vesicles toward the tip growth is affected by pore closure is unknown. It also remains possible that more selective transport is still allowed although the pore is closed for bulk transport. In this context, it is also worth noting that a microtubule-organizing center associates with the septum pore in *A. nidulans* (Veith et al. 2005; Zekert et al. 2010; Takeshita and Fischer 2011), which may promote such intercompartmental transport in addition to its implication as component of a speculative cell size-sensing mechanism discussed in Sect. II-B.

VI. Conclusions and Perspectives

Although septum formation is essential for cell proliferation and fungal development, many important questions remain to be addressed. Of particular importance is the identification of signals that determine septum placement in a syncytial compartment. Do specific landmark proteins exist? Are nuclear and cell end-dependent signals involved? What is the function of sMTOCs in this context, and do MTs have any role in sensing of the apical cell size and site selection for septum placement? A second battery of questions involve the regulation of the actin cytoskeleton: how is SAT formation regulated, and what is the relative importance of de novo f-actin nucleation versus assembly of preformed filaments originating from previously formed septum? What is the trigger for CAR constriction and how is CAR constriction terminated? Third, how is polar tip growth and subapical septum formation regulated, if both processes coexist in the filament but depend on the same growth machinery? Forth, why do so many (unrelated) signaling modules associate with the mature septal pore, and how is cell-cell connectivity regulated? Finally, we need to understand how these basic processes are remodeled during fungal development and multicellular differentiation. The answers to these questions will require comparative approaches, and the acknowledgement that yeast and filamentous lifestyles have reused conserved molecular modules in different contexts. In the long term, this will not only improve our understanding of septum formation in vegetative hyphae but also of cell differentiation during ascomycete development and will shed light on the evolutionary consequences of cell compartmentalization when compared to other fungal phyla such as the aseptate zygomycetes or the basidiomycetes that generate highly complex septa.

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