Septum formation in *Aspergillus nidulans*

Steven D Harris

Filamentous fungi form multicellular hyphae that are partitioned by septa. In *A. nidulans*, septum formation requires the assembly of a septal band following the completion of mitosis. Recent observations show that this band is a dynamic structure composed of actin, a septin and a formin. In addition, assembly is dependent upon a conserved protein kinase cascade that regulates mitotic exit and septation in yeast. Hyphal differentiation may reflect the regulation of this cascade by cyclin-dependent kinase activity. In this review, the dynamics and regulation underlying the assembly of the septal band are discussed.

Addresses
Plant Science Initiative, N234 Beadle Center, University of Nebraska, Lincoln, Nebraska 68588-0660, USA; email: sharri1@unlnotes.unl.edu

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Abbreviations

cdk	cyclin-dependent kinase
MEN	mitotic exit network
SIN	septation initiation network

Introduction

Filamentous fungi display an astounding degree of morphological complexity that is associated with the development of structures specialized for vegetative growth, reproduction and infection. Regardless of cell shape or form, these structures are supported by the basic unit of fungal growth, the hypha. Fungal hyphae are highly polarized structures composed of individual cells that often differ in their fates. The partitioning of hyphae by septa is an essential feature of hyphal differentiation [1]. Despite this, relatively little is known about the molecular mechanisms underlying septation in filamentous fungi.

Recently, the genetic tractability of the fungus *Aspergillus nidulans* has been exploited to identify and characterize genes required for septum formation. These studies, which are summarized in this review, have shown that *A. nidulans* is a powerful complement to the model yeasts for understanding the basic process of cytokinesis. At the same time, they have also revealed potentially novel regulatory mechanisms that reflect the unique multicellular organization of fungal hyphae [2]. In particular, they have shown that the formation of the first septum does not occur until hyphae (that is, pre-divisional hyphae) reach a certain cell size [3]. During this time, unlike yeast cells, multiple rounds of nuclear division occur in the absence of cytokinesis. Once the size threshold is attained, septum formation is triggered by the completion of the next round of mitosis. Thereafter, each parasynchronous round of mitosis in the hyphal tip cell is followed by a wave of septation in its basal region.

The septal band

Actins

An early cytological study detected a contracting septal band in filamentous fungi that were undergoing septum formation [4]. This investigation also highlighted the differences between fungal septation and the phragmoplast-mediated cell division process characteristic of plants. Specifically, whereas plant cells assemble an equatorial array of microtubules and actin filaments known as the phragmoplast, the presence of a constricting actin ring in the septal band of several fungi [5-7], including *A. nidulans* [8], suggested that fungal septation resembles cytokinesis in animal cells. More recent studies have characterized the temporal and spatial requirements for actin ring formation at septation sites in *A. nidulans* [8]. The actin ring forms prior to the appearance of the septum, and subsequently contracts coincident with the deposition of septal wall material. Both the formation and contraction of the actin ring are dependent upon the presence of intact microtubules. Combined with the observation that the septation site is specified by nuclear position in *A. nidulans* [3], these results are consistent with a model in which signals emanating from mitotic spindles orchestrate both the assembly and the dynamics of the septal band (Figure 1). Additional studies using labelled components of the band are needed to determine how its formation is coordinated with mitosis.

Septins

The septins are a conserved family of eukaryotic proteins that appear to function as morphogenetic scaffolds at sites of cell division and polarized growth. The role of septins during cytokinesis in yeast, flies and humans is well-documented [9]. The use of PCR-based cloning and genomics has revealed that *A. nidulans* possesses five members of the septin family (*aspA–E*), each belonging to a specific orthologous group [10•]. Although the function of the other *A. nidulans* septins during septum formation has not yet been determined, mutation and localization experiments suggest an important role for the septin AspB (P Westfall, M Momany, personal communication; Figure 1). Following the completion of mitosis, AspB forms a single ring overlapping the actin ring at the septation site. Coincident with the contraction of the actin ring and deposition of septal wall material, AspB splits into two rings that flank the new septum. Subsequently, the AspB ring located on the apical side of septum persists, whereas the basal ring is lost. By specifically marking the apical side of the completed septum (or the basal end of the new hyphal cell), AspB may help propagate the intrinsic polarity of *A. nidulans* hyphae. Much like the *Saccharomyces cerevisiae* septins, it may do so by localizing specific cell-cycle regulators and/or morphological landmarks [11,12]. The asymmetry of AspB localization was exploited to show that the multiple septation events within a tip cell proceed in a parasynchronous

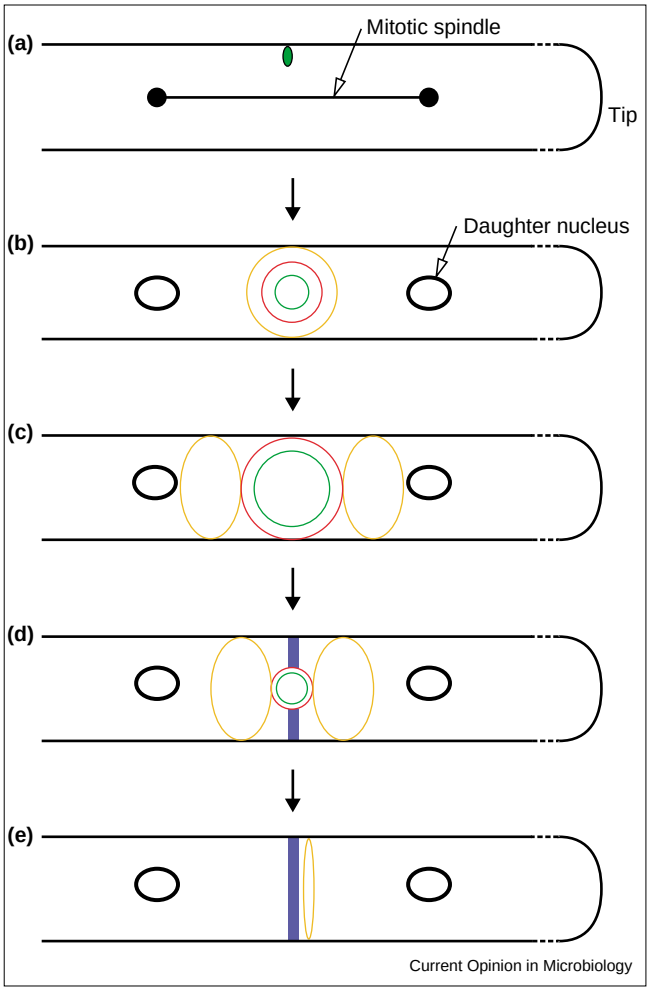
apical-to-basal wave. Given that mitosis occurs in the same pattern [13], this observation strengthens the notion that mitotic signals direct the assembly of the septal band.

Formins

Temperature shift mutations in the *sepA*, *sepD*, *sepG* and *sepH* genes block septum formation at restrictive temperatures [14]. Temperature shift experiments demonstrate that the block caused by these mutations occurs late in septation, and is reversible. Molecular characterization of *sepA* revealed that it encodes a member of the conserved formin homology (FH) domain family of proteins [15]. Several metazoan and fungal formins play an integral role in organizing the actin cytoskeleton at cell division sites [16]. Indeed, SepA is required following mitosis for assembly of the actin ring at septation sites [15]. The use of a functional SepA–green fluorescent protein (GFP) fusion has shown that SepA first appears as a cortical dot at the presumptive septation site (KE Sharpless, SD Harris, unpublished data; Figure 1). Thereafter, it forms a ring that co-localizes with actin and ultimately constricts as the septal wall material is deposited. Because SepA rings collapse in hyphae treated with cytochalasin A, their assembly most likely requires the presence of an intact actin ring. Similarly, the formation of AspB rings also depends upon the integrity of the actin cytoskeleton and requires functional SepA (P Westfall, M Momany, personal communication). Although the role of AspB in mediating the assembly of SepA and actin rings is not yet known, these observations suggest that all three components may function in a mutually dependent manner to form the septal band (Figure 1).

It has been established that mitotic signals direct the assembly of the septal band [3,8]. However, if each mitotic nucleus triggered the formation of a septum, hyphal cells would be uninucleate. The multinucleate nature of hyphal cells suggests that septal band assembly in response to mitotic signals is restricted to specific cortical regions. These regions presumably contain morphological landmarks that identify them as potential septation sites. The septins could conceivably be a component of the landmark, as they are in *S. cerevisiae* [11]. Alternatively, all cortical regions may be competent to undergo septum formation, but the assembly of a septal band at one site might block the ability of adjacent mitotic nuclei to trigger the same event.

Figure 1



Schematic model depicting the assembly and dynamics of the septal band. A hyphal segment is drawn as parallel black lines oriented such that the tip is to the right. **(a)** In response to signals emanating from the mitotic spindle, SepA localizes to the septation site as a cortical dot (green). Although not shown, it remains possible that small patches of actin and/or the septin AspB may co-localize with SepA. **(b)** The septal band, which is composed of co-localized actin (red), AspB (orange) and SepA (green) rings, assembles. The daughter nuclei have undergone mitotic exit. **(c)** The AspB ring splits into two rings that flank the actin and SepA rings. As has been proposed in *S. cerevisiae* [24], splitting of the septin (AspB) ring may trigger constriction of the actin ring. **(d)** The actin and SepA rings constrict as septal wall material (blue) is deposited. **(e)** Following completion of septum formation, the actin, SepA and basal AspB rings disappear, whereas the apical AspB ring persists.

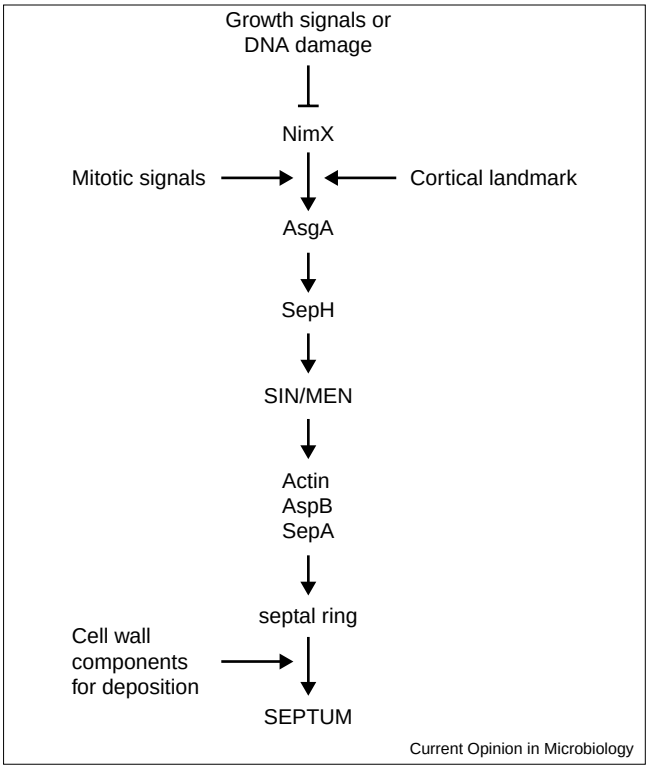
Regulation of septum formation

Cyclin-dependent kinase

Although the nature of the growth signals that regulate septum formation is not yet known, they appear to delay septum formation in pre-divisional hyphae in a glucose-dependent manner [17•]. Genetic observations suggest that the ultimate target of these signals is NimX [18], the sole mitotic cyclin-dependent kinase (cdk) in *A. nidulans* [19]. In particular, the control of septum formation by cell size is abolished by mutations that subvert the regulatory mechanisms that maintain NimX in an inactive, tyrosine-phosphorylated state [17•,18]. It is important to note that this regulation is functionally independent of nuclear division. Although it is not clear how NimX independently regulates nuclear division and septum formation, one possible mechanism could be the existence of a septation-specific cyclin that targets NimX to septation sites.

The uncoupling of septum formation from nuclear division in pre-divisional hyphae has revealed a potentially novel effect of DNA damage on cytokinesis. In particular, the formation of the first septum is inhibited when hyphae are chronically exposed to sublethal levels of DNA damage (that

Figure 2



Regulation of septum formation by growth signals or DNA damage is mediated by the cdk NimX and components of the SIN/MEN. NimX is proposed to work in conjunction with mitotic signals and a cortical landmark to activate the SIN/MEN via AsgA and SepH. The SIN/MEN recruits components of the septal ring to the septation site. The septal ring subsequently directs localized cell wall deposition, resulting in the formation of a septum. Because growth signals or DNA damage are thought to delay the activation of NimX, signaling through the SIN/MEN would be curtailed and septum formation would not occur.

is, levels that do not block nuclear division; [18,20]). This response, which is somewhat analogous to the inhibition of cell division caused by activation of the SOS pathway in prokaryotes [21], is dependent upon DNA damage checkpoint signals and the ability to maintain NimX in an inactive state [18,20]. Because the formation of the first septum is a key step in hyphal differentiation [1,2], the purpose of this response may be to prevent cellularization of hyphae containing damaged chromosomes that have escaped repair.

Septation initiation network/mitotic exit network

The mechanisms by which active NimX cdk complexes control the timing of septum formation are not clear. In both model yeasts, a GTPase-regulated protein kinase cascade (called the septation initiation network, SIN, or the mitotic exit network, MEN) integrates cdk signals to coordinate cytokinesis with nuclear division [22]. One possibility is that NimX regulates components of a similar pathway (Figure 2). Recent molecular characterization of the *sepH* gene has shown that it encodes an orthologue of the *S. pombe* protein kinase Cdc7 [23••], a pivotal component of the SIN/MEN. Results from temperature shift experiments reveal a post-mitotic function for SepH during septum formation. Moreover, multiple components of the septal band, including actin, the septin AspB and the formin SepA fail to localize to septation sites in *sepH* mutants (P Westfall, M Momany, personal communication; KE Sharpless, SD Harris, unpublished data). These observations are consistent with the notion that SepH is required for the assembly of the septal band in response to mitotic signals (Figure 2). In contrast, in the model yeasts, although the SIN/MEN directs the deposition of septal wall material, it is not required for actin-ring formation [24,25]. Indeed, the presumptive role of the SIN/MEN in *A. nidulans* septal band assembly may be a common feature of cytokinesis in organisms that must complete mitosis before forming an actomyosin ring.

In *S. pombe*, the SIN/MEN is regulated by a Ras superfamily GTPase, Spg1, which, in its active GTP-bound state, controls the localization of the Cdc7 protein kinase [26]. Similarly, in *S. cerevisiae*, the Cdc7 orthologue Cdc15 is activated by the GTPase Tem1 [27]. An *A. nidulans* orthologue of the Spg1/Tem1 GTPases, AsgA, has recently been identified in an expressed sequence tag (EST) database [23••]. Although its function has not yet been determined, it could conceivably serve as a focal point for the regulation of septum formation by NimX (Figure 2). For example, NimX could promote septum formation via the SIN/MEN by regulating the AsgA GTPase module such that AsgA is converted to its active state. Moreover, if AsgA localized to spindle poles and its exchange factor to septation sites, this would provide a mechanism for integrating spatial information with the mitotic signals that regulate septation. An analogous mechanism underlies the regulation of Tem1 activity in *S. cerevisiae* [28]. Because the *sepD1* and *sepG1* mutations are phenotypically indistinguishable from *sepH* mutants, the genes affected by these mutations are ideal candidates to encode AsgA or its putative exchange factor.

Conclusions and future directions

The past year has brought several exciting observations that have yielded insight into the mechanisms of cytokinesis in *A. nidulans*. Preliminary characterization of the structure and dynamics of the septal band has been achieved (P Westfall and M Momany, personal communication; KE Sharpless, SD Harris, unpublished data). A conserved protein kinase cascade likely to play a role in coordinating septal band assembly with nuclear division has been identified [23••]. However, there are still many issues to be addressed. First and foremost, the inventory of gene products required for septum formation is clearly incomplete. There are surely additional components of the septal band and of the regulatory networks integrating septation with nuclear division and hyphal growth. It should be possible to identify these components by exploiting the genetic tractability of *A. nidulans*. At the same time, conserved components will almost certainly be harvested from the *A. nidulans* EST and genomic sequence databases. Second, the relationship between components of the septal band and their regulators has not been fully clarified. Does the

interaction between a mitotic spindle and the cell cortex determine whether or not a septum will form at a given site? Do the components of the septal band assemble in a mutually dependent manner? How is the formation of the septal band directed by the SIN/MEN? Third, the nature of the signal transduction pathways that block septum formation in response to growth signals or genomic insults has yet to be fully elucidated. How do these pathways modulate NimX activity? Is the ultimate effect of these signals to curtail signaling through the SIN/MEN? The resolution of these issues will undoubtedly demonstrate the utility of *A. nidulans* as a model for understanding the process of cytokinesis in multicellular organisms.

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Now in press

The work referred to in the text as (P Westfall, M Momany, personal communication) and (KE Sharpless, SD Harris, unpublished data) is now in press:

29. •• Westfall PJ, Momany M: The ***Aspergillus nidulans*** septin AspB plays pre- and post-mitotic roles in septum, branch, and conidiophore development. *Mol Biol Cell* 2001, in press.
The authors use antisera directed against AspB to describe its localization pattern at septation sites and branch sites, and during conidiophore development. In addition, they show that a temperature-sensitive mutation in the *aspB* gene causes the formation of irregular septa. Their observations suggest that the septin ring may function as a scaffold to facilitate assembly of the septal band and localized deposition of the septal wall material.

30. •• Sharpless KE, Harris SD: Functional characterization and localization of the ***Aspergilus nidulans*** formin SepA. *Mol Biol Cell* 2001, in press.
The authors show that a functional SepA–GFP fusion protein displays dynamic localization at both septation sites and hyphal tips. They demonstrate that this localization pattern is dependent upon the integrity of the actin cytoskeleton, and is also mediated by the FH3 domain located in the amino terminus of SepA. Their observations suggest that SepA promotes the recruitment of actin to septation sites and functions interdependently with the actin cytoskeleton to modulate constriction of the septal band.