Nuclear anarchy: asynchronous mitosis in multinucleated fungal hyphae

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Multinucleated cells are found in diverse contexts and include filamentous fungi, developing insect embryos, skeletal muscle and metastasizing tumor cells. Some multinucleated cells such as those in muscles arise from cell fusion events, but many are formed through specialized cell cycles in which nuclear and cell division are uncoupled. Recent work in the fungus *Ashbya gossypii* illustrates how unique spatial and temporal regulation of conserved cell cycle regulators directs mitosis in multinucleated cells.

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Modes of nuclear division in multinucleated fungal cells

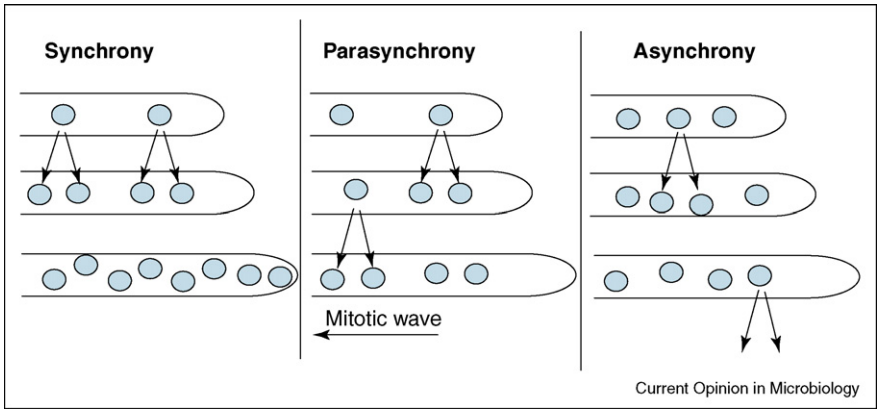
A variety of mitotic patterns have been observed in multinucleated, filamentous fungi. The most commonly described modes of nuclear division are asynchronous, parasynchronous and synchronous, and these vary between organisms and environmental conditions (Figure 1). Nuclei in *Neuropora crassa* and *Ashbya gossypii* divide asynchronously such that nuclei residing in the same cytoplasm appear to behave independently of their neighbors [1,2•,3•,4,5]. This type of nuclear division might enable cells to spatially restrict mitoses within a shared cytoplasm, potentially facilitating local responses to nutrients or other environmental stimuli. Additionally, with asynchronous division, cells disperse energy investment for nuclear replication across time and space. Asynchrony might also buffer cells from dramatic changes in the nucleocytoplasmic ratio that would occur if all nuclei divided at the same time.

By contrast, synchronous or parasynchronous (a wave of division which flows linearly across the cell) nuclear divisions produce swift and dramatic doubling of nuclei (Figure 1). Synchronous divisions occur in the tip compartments of vegetative hyphae of *Ceratocystis fagacearum* (oak wilt causing fungus) [6]. In *Aspergillus nidulans*, a parasynchronous wave of nuclear division spreads from the tip across the leading hyphal compartment in about 20 min. These waves can travel over a distance up to 700 μm and can involve 60–100 nuclei [7–9]. Interestingly, in *A. nidulans* this wave only travels backward from the hyphal tip. Synchrony breaks down between hyphal compartments that are divided by septae. This parasynchronous wave in *A. nidulans* is disturbed in poor media and nuclei can then start dividing asynchronously [7,8]. Similarly, *Fusarium oxysporum* produces waves of nuclear division, although these emanate from the midpoint of a central compartment rather than the tip compartment and radiate both apically and basally in the cell [6]. These coordinated nuclear division cycles yield uniform and spatially homogenous responses to a stimulus, such as a cell size cue or an environmental signal. Furthermore, such synchrony in nuclear division might also contribute to the function of developmental or morphogenesis programs, although these possibilities have not been formally tested in any of these fungal systems. Here, I consider some of the unique challenges faced in regulating nuclear division in syncytial fungal cells, and support this discussion with recent work in the fungus *Ashbya gossypii*. I highlight cell biological problems pertaining to multinucleated division that await investigation and that are broadly applicable to understanding how large cells compartmentalize and organize signaling.

Molecular mechanisms governing mitotic synchrony or asynchrony

Early insight into the molecular basis of synchronous mitosis came from the mammalian cell fusion experiments of Rao and Johnson [10], which gave the first hints that a diffusible signal could stimulate mitosis and lead to synchronization of nuclei sharing a common cytoplasm. Subsequent work in many systems identified that the maturation or mitosis promoting factor (MPF) composed of a cyclin-dependent kinase (CDK) and a cyclin was a key activator of mitosis [11]. The synchrony in mammalian cells observed by Rao and Johnson [10] has been explained by the fact that the MPF complex could be passed among nuclei residing in the same cellular space, leading to coordinated division. Additionally, animal cells undergo an open mitosis (in which the nuclear envelope disassembles for mitosis), further enhancing the potential mixing of mitosis promoting signals among nuclei in a common cytoplasm [12]. Basidiomycete fungi are thought

Figure 1



Patterns of nuclear division in multinucleated fungi. In synchronous division, all nuclei divide simultaneously. In parasynchronous division, a mitosis is initiated in one spot and then a wave of mitoses travel down the hyphae linearly so that nuclei divide sequentially, almost as dominos fall in a line. In asynchronous division, nuclei divide independently of neighbors, giving an apparent random spatial and temporal pattern to mitosis.

to undergo a more open, animal cell-like mitosis and, interestingly, in *Ustilago maydis* nuclear envelope breakdown depends upon nuclear migration [13]. By contrast, the ascomycete fungi, including *Podospora anserina*, *A. nidulans* and *N. crassa*, and the model budding and fission yeasts all undergo some form of a closed mitosis whereby the nuclear envelope does not completely breakdown [14–18].

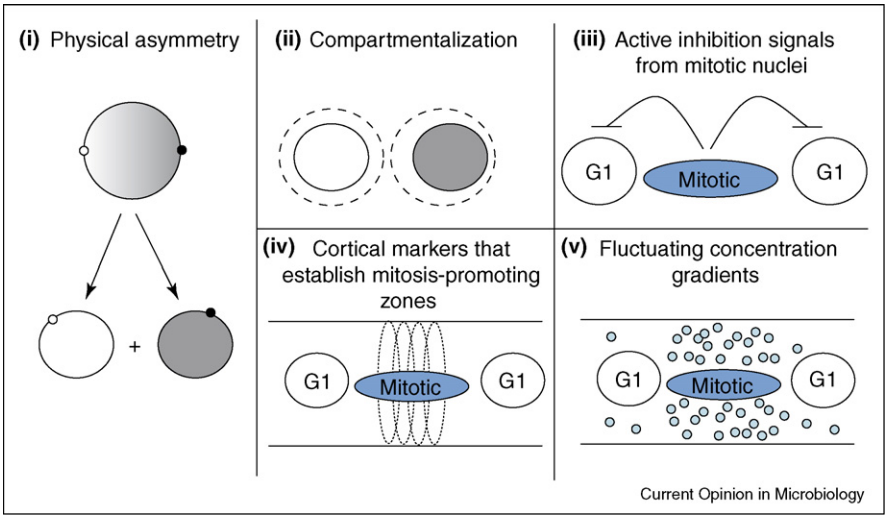
Synchronous mitoses are commonly observed in multinucleated fungi in which the nuclear envelope stays intact during division, potentially because of selective transport of cell cycle control factors across nuclear pores. Notably, in *A. nidulans* the permeability of nuclear pore complexes (NPCs) increases in mitosis in part to facilitate the construction of the mitotic spindle from cytoplasmic pools of tubulin [19•,20•,21•]. This transient opening of pores might also increase the signaling connectivity among nuclei and contribute to the observed mitotic parasynchrony in these cells. Similarly, budding yeast cells remodel their NPC to alter nuclear transport specifically during mitosis [22•]. Furthermore, in budding and fission yeasts, MPF, as well as other cell cycle regulators, actively shuttles in and out of the nucleus [23–25]. Thus, even if nuclear permeability does not dramatically change across the division cycle in all ascomycetes, there is potentially the capacity for control factors to diffuse among nuclei because of their partial residence in the common cytoplasm. Although the shuttling of cell cycle regulators in and out of and between neighboring nuclei is a plausible explanation for the mitotic synchrony in some fungi, this has not been formally tested in any of the species undergoing synchronous mitosis. Furthermore, the basis for unidirectional parasynchronous waves of mitoses flowing from hyphal tips remains an intriguing cell biological mystery.

The ability of neighboring nuclei to communicate when immersed in the same cytoplasm complicates any potential molecular explanations of mitotic asynchrony. Various scenarios could explain autonomous nuclear behavior that would bypass potential connectivity among nuclei in the same cytoplasm (Figure 2). (i) Parental nuclei could have a physical asymmetry that makes the two new-born sister nuclei have different fates. Such an asymmetry could be in the distribution of nuclear pore complexes (NPCs) that might lead to different rates of entry of cell cycle factors into progeny nuclei, different aged spindle pole bodies (SPBs, these are thought to be conservatively duplicated in fungi [26–28]) that recruit control factors with different affinities, or different concentrations of transcription factors that regulate the timing of entry of a nucleus into the cell cycle [29]. (ii) Nuclei could be insulated from one another by endomembrane systems or by localized translation which could restrict the localization of either the message or the protein to the area of a specific nucleus. (iii) Mitotic nuclei could emit 'anti-sync' factors such as CDK inhibitors that limit the progression of nuclei neighboring a mitotic nucleus. (iv) Cortical markers that have discrete spatial distributions might create zones that promote or inhibit mitosis so that the position of a nucleus in the hyphae relative to these markers directs its mitotic fate [30]. (v) Stochastic spatial fluctuations in the concentration of key cell cycle control factors combined with high thresholds for commitment to cell cycle events could lead to nuclei dividing apparently randomly on the basis of local enrichment or depletion of mitotic regulators. Several of these models have begun to be systematically tested using the highly tractable ascomycete *A. gossypii* [2•,30].

Asynchronous mitosis in *A. gossypii*

A. gossypii has a simple life cycle in the laboratory, in which uninucleated, haploid spores germinate and give

Figure 2



Models for asynchronous mitoses. These five models are discussed in the text as possible mechanisms that might lead to mitotic asynchrony. **(i)** Maternal nuclei have a physical asymmetry which translates into unequal progeny that then replicates with different rates. **(ii)** Neighboring nuclei might be compartmentalized, therefore blocking diffusion and communication. **(iii)** Mitotic nuclei might actively secrete inhibitors to prevent simultaneous division of neighbors. **(iv)** The position of the nuclei relative to cortical markers might direct mitotic fate. **(v)** Local enrichment of mitotic signals due to stochastic fluctuations in the concentrations of such signals might lead to randomly placed mitoses. The large circles represent nuclei and the small circles in panel (i) represent SPBs.

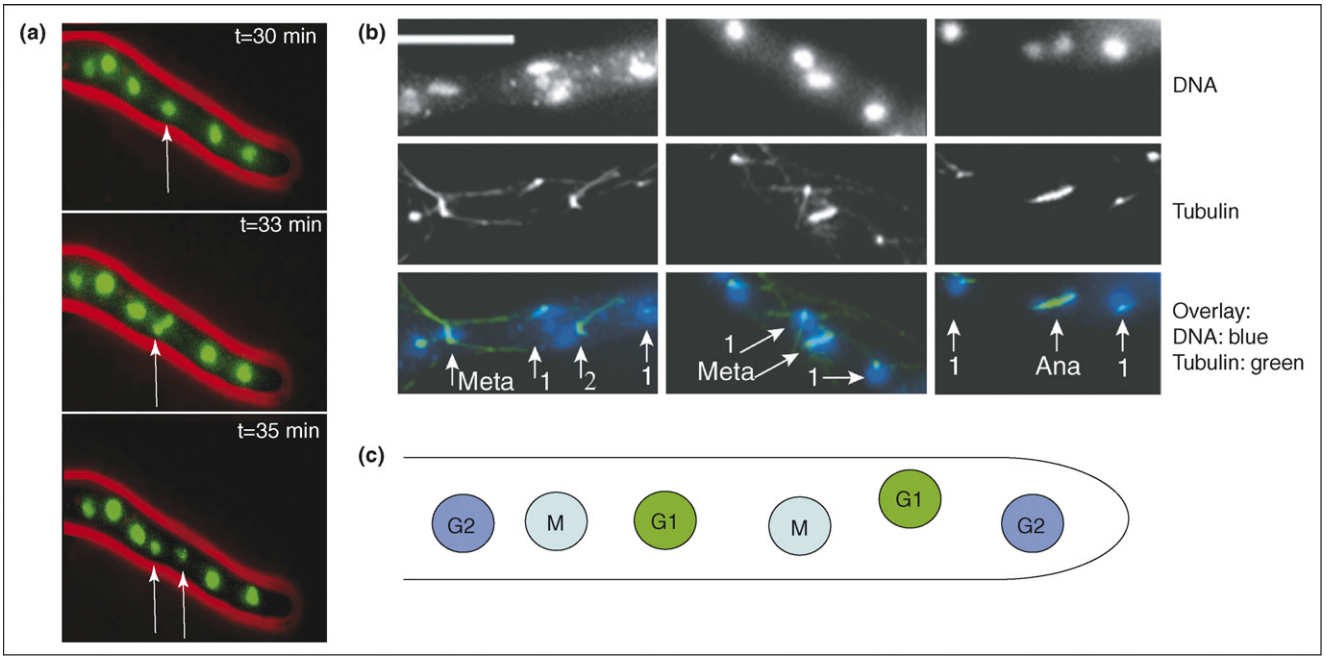
rise to multinucleated vegetative hyphae, which grow exclusively in a polarized manner [31,32]. Upon starvation, asexual haploid spores are produced in asci formed from within the hyphae. *A. gossypii* has never been found in a yeast form, which is remarkable given that over 90% of *A. gossypii* genes are homologous and syntenic compared with the budding yeast *Saccharomyces cerevisiae* [33]. This relatively close evolutionary relationship between budding yeast and *A. gossypii* has greatly facilitated the use of molecular genetic and cell biological techniques to study cell cycle control in this fungus.

In vivo time-lapse analysis of nuclei labeled with histone–green fluorescent protein (GFP) fusion proteins and observations of tubulin in spindles and SPBs demonstrated that nuclei divide asynchronously in *A. gossypii* hyphae (Figure 3, [2•]). The nuclear division cycle of individual nuclei in *A. gossypii* varies from as little as 45 min to over 200 min, with no apparent spatial pattern that correlates with individual division times. Nuclei that are produced from the same mitosis event generally have different division times, thus suggesting that the cell cycle clock timing is not inherited. Nuclei that are as close as 1–2 μm are readily found in different nuclear division cycle stages on the basis of spindle or SPB appearance. These nuclei can be synchronized reasonably well by incubation in the microtubule poison nocodazole, but asynchrony returns to the system within a single nuclear division cycle suggesting that asynchrony is fundamental to the cycle and not simply a matter of a starting state [2•].

Several approaches have been taken to determine whether asynchrony in *A. gossypii* is the result of nuclear intrinsic or extrinsic signals that differ either among nuclei or regions of hyphae. Septin proteins, which assemble into ring structures in the tips and at regular intervals along the hyphae, were selected as candidates for extrinsic controllers of asynchrony. Passage through or near septins was predicted to stimulate mitoses because in budding yeast the septins contribute to negative regulation of the wee1 homologue, Swe1p. Thus, in hyphal cells septins might establish zones with limited amounts of this division inhibitor and would then promote mitosis locally. Supporting this idea, mitoses are more commonly observed near assembled septins in *A. gossypii* (AS Gladfelter, unpublished; [30]). In cells lacking septins, however, nuclei still divide asynchronously but division is more frequent in spaces between septins [30]. Thus, it seems that nuclear extrinsic signaling from septins cannot be the major cause of asynchrony. Although not essential for asynchrony, the septins are potentially important for spatially coordinating the nuclear division cycle with external nutrient availability [30].

To investigate whether intrinsic differences in protein content among nuclei could be the root of asynchrony, a G1 and a mitotic cyclin were localized in *A. gossypii* hyphae. Notably, neither of these cyclins seemed to vary dramatically between nuclei so that both were present regardless of cell cycle stage [2•]. Further investigation of the mitotic cyclin behavior demonstrated that cyclin expressed from one nucleus could be found in neighboring nuclei, thus

Figure 3



Mitosis in *A. gossypii*. **(a)** Cells expressing a histone–GFP fusion protein were observed by *in vivo* time-lapse microscopy, and mitoses were monitored over time. The three frames depict a single nucleus (arrows) dividing, while the neighboring nuclei on either side do not divide. **(b)** *A. gossypii* cells in which the mitotic spindles have been visualized using an anti-tubulin antibody. These images show that neighboring nuclei appear to be in different cell cycle stages on the basis of the appearance of SPBs and spindles. Bar is 10 μm. Keys: 1, nuclei with a single SPB; 2, nuclei with a duplicated SPB; Meta, metaphase nuclei; Ana, anaphase nuclei. **(c)** Scheme summarizing the *A. gossypii* nuclear division cycle in which nuclei in hyphae are all cycling independently and direct neighbors are in different cell cycle stages [2].

suggesting that at least this central regulator was probably freely diffusing and exchanged between nearby nuclei [2•]. Similarly, if the nuclear sequestration or insulation of MPF were the basis for asynchrony, one would expect that increasing the amount of MPF in cytoplasm should enhance synchrony [2•]. When, however, the mitotic cyclin pool was displaced from the nuclei to the cytoplasm with the addition of nuclear export signals (NESs), levels of asynchrony were unchanged in the hyphae. These combined results suggest that at least central cell cycle controllers such as cyclins are neither restricted to some nuclei nor highly variable in their levels among nuclei to produce the capricious division times observed in *A. gossypiii*. Likewise, in *N. crassa*, where nuclei also divide asynchronously, there is strong genetic evidence for exchange of at least some proteins among nuclei within heterokaryons [34]. Thus, we predict that nuclear asynchrony in filamentous fungi is not simply due to complete nuclear insulation or impermeable barriers between nuclei but has alternative mechanistic foundations awaiting discovery.

Conclusions and perspectives

Nuclear asynchrony observed in multinucleated fungal cells such as *N. crassa* and *A. gossypii* leaves us with tantalizing questions and there are still many experiments to be performed to distinguish among possible mechanisms of asynchrony (Figure 2). Although initial experiments in *A. gossypii* indicate that cyclin proteins are shared among nuclei, future work needs to address whether some key cell cycle factors are asymmetrically distributed among nuclei. In both *N. crassa* and the basidiomycete *Schizophyllum commune*, there are some provocative, although indirect, data that hint at possible mechanisms of nuclear independence. *N. crassa supersuppressor (ssu)* mutants, which suppress amber codon mutants (mutations which lead to premature translation termination), act nuclear autonomously such that they cannot suppress neighboring nuclei sharing the cytoplasm of a heterokaryon [35]. Recent work has demonstrated that these suppressors code for tRNAs, thus suggesting that the translation machinery might be spatially restricted and not shared between nuclei [35,36]. Such spatial control of translation could generate asymmetries in some proteins in different nuclei. In *Schizophyllum commune*, it has been suggested that the spatial position of nuclei in a dikaryon regulates expression of some genes. Cells in which the two nuclei are very closely positioned (2 μm apart) have different patterns of gene expression than cells where the two nuclei are positioned farther apart (8 μm apart), yet still residing in the same cytoplasm. Thus, in *S. commune* there might be both nuclear communication and compartmentalization depending upon nuclear dynamics and positioning in this basidiomycete [37].

Knowledge of the mechanistic basis of asynchronous mitoses will be generally applicable to understanding how large cells insulate and spatially organize signaling pathways. Furthermore, understanding the basis for asynchrony will ideally also be informative for mathematical biology because very little is known about how uncoupled oscillators, such as dividing asynchronous nuclei, could be maintained in a common space. Future work in both *A. gossypii* and *N. crassa* to generate large scale, automated pedigrees of dividing nuclei in living cells will ideally yield quantitative information about populations of asynchronous nuclei and complex spatial patterns of division. With these data, quantitative modeling of asynchrony in fungi has the potential to bring insights into both mathematical and cell biological problems.

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