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Review

The early impact of genetics on our understanding of cell cycle regulation in *Aspergillus nidulans*

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Abstract

The application of genetic analysis was crucial to the rapid progress that has been made in cell cycle research. Ron Morris, one of the first to apply genetics to cell cycle research, developed *Aspergillus nidulans* into an important model system for the analysis of many aspects of cell biology. Within the area of cell cycle research, Ron's laboratory is noted for development of novel cell biological and molecular genetic approaches as well as seminal insights regarding the regulation of mitosis, checkpoint regulation of the cell cycle, and the role of microtubule-based motors in chromosome segregation. In this special edition of FGB dedicated to Ron Morris, and in light of the recent progress in fungal genomics, we review the outstanding contributions his work made to our understanding of mitotic regulation. Indeed, his efforts have provided many mutants and experimental tools along with the conceptual framework for current and future studies of mitosis in *A. nidulans*.

1. Introduction

Mitosis lies at the heart of cell biology and has been the subject of investigation for over 100 years. Many regulatory mechanisms and mechanical steps of mitosis have been revealed, and genetic analysis of mitosis in fungi has been a major contributor to the progression of our knowledge. Morris' original publication of mitotic mutants of *Aspergillus nidulans* was the first published genetic analysis specifically targeted at nuclear division (Morris, 1976a,b). The work differentiated itself from contemporary genetic analyses of the cell division cycle in yeasts (Hartwell et al., 1974; Nurse, 1975) in its focus on nuclei. Whereas the *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* studies used landmarks related to cell growth (bud size and cell size, respectively) to distinguish cell cycle mutants from temperature sensitive lethal mutations in general, Morris (1976a,b) directly examined nuclei to identify mutations affecting nuclear division, morphology, and distribution. The vast majority of subsequent work on mitosis in *A. nidulans* stems directly or indirectly from that initial, insightful study. Similarly, the majority of *A. nidulans* cell cycle researchers benefited directly or indirectly from the mentorship of Ron Morris. In honor of this special issue of FGB dedicated to Ron Morris, and in light of recent progress in fungal genomics, we review the significant contributions his pioneering research made in providing a framework for current and future understanding of the regulation of mitosis.

2. The experimental paradigm

Mitosis (Fig. 1) is the process by which the genetic information in DNA is divided equally to two daughter nuclei to ensure continuation of normal growth and development of all eukaryotic cells. By the early 1970s, microscopic studies, stretching back well over 100 years, had established the conserved nature of the mitotic process from fungi through to humans. However, nothing was understood, even at a rudimentary level, about how this most amazing of cell functions is regulated. Truly remarkable progress has subsequently been made and our current level of understanding of the

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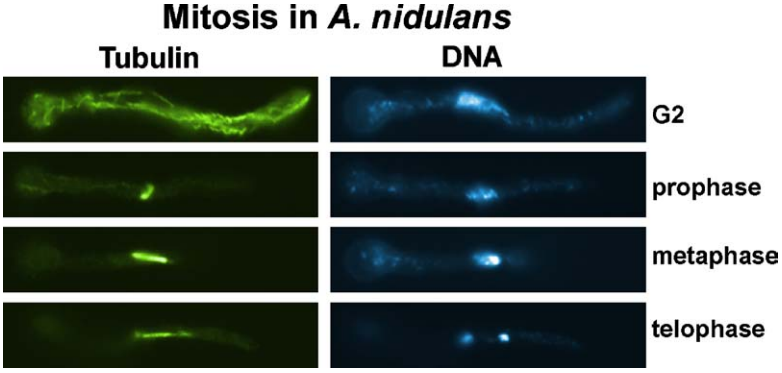

Fig. 1. *Aspergillus nidulans* germlings processed to reveal microtubule architecture and DNA condensation during transition through mitosis from G2 to telophase.

mitotic process, and of the proteins involved in its regulation, have advanced to a level that even allows mathematical modeling (Novak et al., 1999).

How did we progress from cytological images of mitosis to sophisticated understanding of the regulation of the process at a biochemical level? Quantum leaps in knowledge came from biochemical analyses of highly synchronized cell cycle systems and from genetic approaches using model genetic systems including yeasts and *A. nidulans*. When Leland Hartwell, Paul Nurse, and Ron Morris independently had the foresight to utilize genetics to study mitosis, rapid advances in understanding the regulation of mitosis were forthcoming.

Although biochemical studies from the Morris lab identified some of the key components, tubulins and histones for example, required for mitosis in *A. nidulans*, (Felden et al., 1976; Sheir-Neiss et al., 1976), more significant advances regarding its regulation were derived subsequent to the isolation of conditional mutations in cell cycle specific genes and the genetic cloning of such genes.

At the inception of the genetic studies of mitotic regulation in *A. nidulans*, Morris identified specific questions regarding mitotic regulation which he hoped a genetic approach would help to address.

First:

What are the chemical events causing;

Chromosome condensation,

Formation of the mitotic spindle,

Separation of chromosomes at anaphase,

Depolymerization of the mitotic spindle during mitotic exit,

Chromosome decondensation during mitotic exit.

Second:

How are the following aspects of mitosis synchronized with each other;

Chromosome condensation and spindle formation,

Chromosome decondensation and spindle depolymerization,

DNA segregation and cytokinesis,

DNA replication and DNA segregation during mitosis.

Answers to these questions needed to be obtained before mitosis could be considered a process which was fully understood and subject to manipulation and control. Such understanding of mitosis has both intellectual interest and is also of practical importance for our understanding and treatment of cell cycle regulated diseases such a cancer.

The cytological studies of mitosis in many systems, including filamentous fungi (Fig. 1), laid the foundations for the genetic studies of mitosis undertaken by Morris. Of at least equal importance were the pioneering studies of Guido Pontecorvo and Etta Kafer on the genetics of *A. nidulans* that made possible the establishment of *A. nidulans* as a model genetic system (Kafer, 1977; Pontecorvo et al., 1953). For example, Morris realized the utility of both sexual and parasexual cycles available for genetic analysis in *A. nidulans*. Eight well-marked linkage groups had been defined representing the eight chromosomes of *A. nidulans* and haploid, diploid and herterokaryotic vegetative states had been described further enhancing *A. nidulans* as a genetic system. Perhaps the icing on the cake was the fact that *A. nidulans* had been shown to be homothallic, meaning that mutants could be directly tested for genetic complementation without the encumbrances of genetic mating types.

After deciding on *A. nidulans* as his experimental genetic system, Morris had to next decide what a cell cycle specific mutation would look like in *A. nidulans*. He correctly assumed that genetic defects in cell cycle progression would result in lethality necessitating isolation of conditional mutations. Studies by Robinow and Caten (1969) had established aceto-orcein staining as a means to visualize DNA and its compaction during mitosis and acid-fuchin to stain microtubules to visualize spindles during mitosis. As shown in Fig. 1, both the microtubule architecture and chromosomes undergo dramatic changes in *A. nidulans* during mitosis and Morris set upon the idea of scoring the number of hyphal tips containing mitotic spindles and condensed

chromatin to determine the spindle mitotic index and the chromosome mitotic index to allow classification of a library of temperature sensitive mutants. Mutations causing a decrease in the mitotic indices at restrictive temperature where termed nim mutants for never in mitosis. Those with an increased mitotic index were termed bim mutants for blocked in mitosis. Additional analysis scored the distance between nuclei to confirm normal cell growth could occur but not entry into or progression through mitosis. Such criteria, coupled to genetic analysis, identified 9 *bim* mutants in 6 genes (two alleles of *bimA*, *bimB*, and *bimD*) and 26 nim mutants in 23 genes (four alleles of *nimA*). Additionally, mutants affecting septation (sep mutants) and the even distribution of nuclei within the cell (nud mutants) were also identified. These mutants laid the foundation for research that has provided significant contributions to our understanding of cell cycle regulation (Osmani and Ye, 1996), septation (Harris, 2001), and nuclear movements (Morris, 2000) within cells. This review focuses on the early insights provided by the Morris lab on the regulation of mitosis.

2.1. The utility of conditional, cell cycle-specific mutants

The genetic screen completed by Morris to uncover cell cycle specific genes was clearly not saturating, with the majority of genes identified only by a single mutation. For example, of the 26 mutations causing the nim phenotype only the *nimA* gene was found mutated more than once. Even so, some generalities regarding mitotic regulation could be gleaned from this genetic screen. Analysis of the nim mutants and bim mutants indicated that chromosome condensation and spindle formation were regulated in a coordinated way. All nim mutants blocked both the process of DNA condensation and spindle formation. Similarly, the bim mutants all caused an increase in both the spindle mitotic index and the chromosome mitotic index further demonstrating these two aspects of mitosis are coordinately regulated. Morris concluded that "these processes would appear to be tightly coupled to each other and to be under common genetic control." These genetic insights were of significance as they were influential in formulating subsequent concepts to explain how the cell cycle was regulated. Morris also hypothesized that some of the nim mutants would be found to be defective in some biochemical process necessary for both chromosome condensation and spindle assembly, and we now know of course this expectation has been fulfilled in the most spectacular manner.

Four papers published from the Morris lab further demonstrated the utility of conditional cell cycle specific mutants to help study the regulation of mitosis. Many of the nim mutants were found to be readily reversible (Bergen and Morris, 1983; Bergen et al., 1984; Oakley and Morris, 1983), as was the *bimE7* mutant (Morris, 1976a). Perhaps one of the most influential of these papers, which inspired and guided a lot of subsequent research on the initiation of mitosis, was that of Oakley and Morris demonstrating the high reversibility of the *nimA5* mutation (Oakley and Morris, 1983) from interphase into mitosis. By growing *nimA5* strains for a period at the restrictive temperature before subsequent return to the permissive temperature, Oakley and Morris were able to generate very synchronous mitoses with 90% of cells in mitosis within 7.5 min of return to permissive temperature. This was of great practical significance as it allowed generation of large cultures synchronously traversing mitosis and subsequent biochemical analysis of mitosis in *A. nidulans* has made use of this tool. More significantly, the results demonstrated *nimA* function was required specifically for the initiation of mitosis. This fact was instrumental in *nimA* being the first cell cycle specific gene to be cloned from *A. nidulans* (Osmani et al., 1987). Subsequent work revealed *nimA* encodes a protein kinase with a key role in regulating mitosis (see below).

One other highly reversible nim mutant, *nimT23*, was also instrumental in analysis of the kinetics of the nuclear division cycle of *A. nidulans* and in demonstrating that the fungus has clearly defined G1, S, G2, and M phases during its cell cycle (Bergen and Morris, 1983). By imposing the *nimT23* arrest in G2 in combination with reversible arrest in S-phase with hydroxyurea (HU) Bergen and Morris defined the times of the various phases of the cell cycle. The analysis also determined that asexual conidia are arrested in G0 and enter the cell cycle at G1. These findings were of fundamental importance for subsequent studies of the cell cycle in *A. nidulans* and also established HU as a tool to reversibly arrest cells in S-phase, a tool still commonly used by other researchers.

The reversibility of many of the nim mutants also allowed an analysis of which phase of the cell cycle (G1, S, or G2) each nim mutant arrested. Using the reciprocal shift method, various nim mutants could be defined as being required either in G1, S, or G2 phase (Bergen et al., 1984). Five mutants were found to arrest in S-phase and nine arrested in G2 phase. This information was of fundamental importance to researchers interested in a particular phase of the cell cycle.

Somewhat surprisingly, none of the nim mutants were found to arrest in G1. Cells arrested in G1 were subsequently generated in *A. nidulans* by reverse genetics to isolate and mutate $nimX^{cdc2}$, which encodes the Cdc2 protein kinase (now known as cyclin dependent kinase 1 or CDK1) in *A. nidulans* (Osmani et al., 1994). NIMX, as in yeast, is required both during G1 and G2. At the G1 arrest of $nimX^{cdc2}$ mutants, DNA morphology of arrested cells is atypical because of over-replication of mitochondrial DNA caused by a lack of $nimX^{cdc2}$

function. Such G1 arrested cells may have been discarded by Morris during his cell cycle specific mutant screen or maybe G1 mutants were not identified because the screen was not saturating.

The genetic studies outlined above utilizing the cell cycle specific nim and bim mutants set the stage for further discovery with the advent of a transformation system for *A. nidulans* (Ballance et al., 1983; Tilburn et al., 1983) to allow molecular identification of some of these genes.

2.2. The major role played by reversible protein phosphorylation to regulate mitosis

The first cell cycle gene cloned from *A. nidulans* was the *nimA* gene. As mentioned above, *nimA* first attracted attention because conditional mutations within this gene could reversibly arrest cells in late G2 for an extended period of time whilst still retaining the ability to rapidly enter mitosis. Pioneering work from cell fusion experiments (Johnson and Rao, 1970; Rao and Johnson, 1970) and injection of mitotic extracts into *Xenopus* oocytes had indicated a conserved cytoplasmic factor, termed maturation promoting factor (MPF), was responsible for triggering mitosis (Gerhart et al., 1984; Wasserman and Smith, 1978). Because *nimA* function was required to trigger mitosis in *A. nidulans* it was hoped that identifying NIMA at a biochemical level would help lead to a molecular understanding of MPF. It is now known that MPF consists of CDK1/cyclin B (Nurse, 1990) and not NIMA, but the cloning and identification of NIMA as a protein kinase was still of significance to our understanding of how mitosis is regulated.

nimA was cloned by complementation of the temperature sensitivity caused by the *nimA5* mutation. With the gene in hand it was possible to follow the level of *nimA* mRNA expression through the cell cycle demonstrating its expression to be under cell cycle regulation. This regulation ensures maximal mRNA levels during G2 and mitosis with much reduced levels during G1 and S-phase. It was proposed that controlled expression of *nimA* led to maximal levels of the NIMA gene product which, when reaching a critical level, would promote cells into mitosis. As *nimA* mRNA also dramatically declined during mitotic exit, removal of NIMA was proposed to be important for exit from mitosis (Osmani et al., 1987). Both proposals concerning the role of regulating NIMA protein levels in regulation of NIMA function have proven correct, although, other levels of regulation exist (Ye et al., 1995).

The cloning of *nimA* from *A. nidulans* required significant technical advances. Improvements in the transformation procedure to allow cloning by complementation, library construction and a process to retrieve the complementing plasmids are but three of these advances. Of equal importance to these technical advances from the Morris laboratory was the construction of an inducible expression system based upon the *alcA* regulatable promoter (Waring et al., 1989) generated by Richard Waring and Greg May while in the Morris lab. This regulatable expression system was key to the next advance regarding the role of *nimA* in mitotic regulation. The *alcA* inducible plasmids have subsequently been very instrumental in allowing many more insightful experiments.

As *nimA* expression reached a maximum during mitosis, the critical experiment was to experimentally induce *nimA* to ask if there was a causal relationship between *nimA* expression and mitosis. The timely generation of the *alcA* inducible expression system (Waring et al., 1989) made these experiments possible. Ectopic expression of *nimA* was found to profoundly affect both the microtubule architecture of the cell and also compaction of chromosomes, even in cells arrested in S-phase with HU (Osmani et al., 1988b).

Sequence analysis of *nimA* revealed it contained the hallmarks of all protein kinase catalytic domains strongly suggesting *nimA* encoded a protein kinase, which was subsequently confirmed (Osmani et al., 1991). This finding further supported the idea that the important trigger for the initiation of mitosis involved protein phosphorylation. Seminal work in *S. pombe* had revealed a phosphorylation cascade involved in regulating mitosis (Russell and Nurse, 1987) but induction of *nimA* out of cell cycle phase to promote mitotic events helped to firmly establish protein phosphorylation as a keystone of mitotic regulation.

Further studies from the Morris lab helped to solidify protein phosphorylation, and dephosphorylation, as essential for mitotic regulation. These insights came from the identification of *bimG* as a protein phosphatase by Doonan and Morris (1989). Logic would predict that, if protein phosphorylation was important for initiation of mitosis, then dephosphorylation of the proteins phosphorylated to promote mitosis would be important for mitotic exit. Lack of such a phosphatase would prevent normal mitotic exit and thus the bim phenotype.

Biochemical analysis and immunostaining of *bimG11* mutants indicated it arrested mitotic progression and greatly increased the phosphoprotein content of cells at restrictive temperature as revealed by reactivity to the phospho-specific MPM-2 antibody, which was previously shown to react with mitotic specific phosphoproteins in *A. nidulans* (Engle et al., 1988). Cloning of *bimG11* by complementation of its heat sensitivity revealed it encoded a protein with very high homology to type 1 protein phosphatase of mammalian cells. The identity of *bimG* was confirmed by showing a decrease in type 1 phosphatase activity in *bimG11* strains and the ability of rabbit muscle type 1 phosphatase to complement the *bimG11* allele (Doonan et al., 1991).

These studies utilizing *nimA* and *bimG* alleles and their encoded proteins demonstrated convincingly that protein phosphorylation is essential for the initiation of mitosis and that protein dephosphorylation was equally important for exit from mitosis.

2.3. Checkpoint control: a conceptual breakthrough in our understanding of how the cell cycle is regulated

Early studies of cell cycle regulation revealed that there are many dependence relationships within the cell cycle. For instance, arrest of DNA replication using inhibitors of DNA replication prevents the initiation of mitosis. Much thought had gone into how cells ensure cell cycle events occur in the correct order at the correct time and how downstream events are stopped when upstream events are not completed. Early work from the Morris lab provided key clues to how this regulation worked and, along with insights from other systems, helped formalize the concept of checkpoint regulation as explained in the landmark review of Lee Hartwell and Ted Weinert in 1989 (Hartwell and Weinert, 1989).

During the early 1970s two potential regulatory mechanisms had begun to surface as likely ways cells ensured order of cell cycle events (Hartwell et al., 1974). First, the dependent pathway model suggested that each cell cycle stage supplied an essential component of the next stage, in a manner analogous to a biochemical pathway. In fact, early genetic studies of the cell cycle were completely consistent with such a mechanism to regulate cell cycle events. A mutation in S-phase would prevent downstream events and so the dependent pathway model seemed to be supported by genetic analysis of the yeast cell cycle (Hartwell et al., 1974).

The second model of regulation suggested that there would be no direct causal relationship between events in the cell cycle, rather, some master timing mechanism would impose order on cell cycle events. However, initial genetic studies of the cell cycle found no data to support the master regulator mechanism for cell cycle control.

Two results from the Morris laboratory were instrumental in supporting the master regulatory view of cell cycle regulation. These insights came from experiments aimed at answering the questions posed by Morris about how aspects of the cell cycle were synchronized with each other as outlined previously in Section 2.

If the cell cycle is regulated like a biochemical pathway, then downstream events should not happen without completion of upstream events. However, genetic manipulation of *A. nidulans* allowed mitotic events to occur in the absence of DNA replication in two experimental situations. In the first, as described above, induction of the NIMA kinase promotes mitotic events even in S-phase arrested cells (Osmani et al., 1988b). Second, inactivation of *bimE*, using the *bimE7* mutation, in combination with arrest in S-phase or G2 phase, allowed mitotic events to occur when they normally would not (Osmani et al., 1988a). Thus, using two different experimental approaches *A. nidulans* could be forced into making cell cycle specific events occur out of order.

These data clearly demonstrated that the cell cycle is not regulated like a biochemical pathway, but strongly supported the master regulator concept for how the order of cell cycle events is maintained (Osmani et al., 1988a). In this regulatory paradigm, if an aspect of cell cycle progression fails to occur, a negative signal is generated that inhibits the master regulator responsible for triggering subsequent cell cycle phases. The term checkpoint regulation was coined to name this type of regulation and it is now widely accepted that most events of the cell cycle are coordinated by checkpoint type regulation (Hartwell and Weinert, 1989).

2.4. How to observe the phenotype of a null mutation in an essential gene?

In addition to supplying basic information about how the cell cycle is regulated, the paper demonstrating that mitotic events could occur out of cell cycle phase also describes a very useful technique to observe the terminal phenotype of a null mutation of an essential gene (Osmani et al., 1988a). Upon first submission of this paper, reviewers argued that although the *bimE7* mutation was recessive, Osmani et al. did not know that *bimE7* was a loss of function mutation. Reviewers wanted proof that a null mutation of *bimE* caused the same phenotype as the *bimE7* mutation at restrictive temperature. Of course, at the time this was asking the impossible. How can you see the phenotype of a null allele of an essential gene without using a conditional mutation? This was quite a challenge to say the least. However, the concept of maintaining a null allele in a heterokaryon was hatched and the heterokaryon rescue technique was established and found to work exceptionally well. The heterokaryon rescue technique has been utilized in many subsequent studies and is one of many technical advances to come from the Morris lab to help make *A. nidulans* an exceptional experimental system with which to work.

2.5. Motor proteins and mitosis: proof of a role for mechanochemical enzymes in mitosis and discovery of the kinesin gene superfamily

By the end of the 1980s, little in cell biology was more obviously certain, yet less rigorously demonstrated, than the role of microtubule-based motors in mitosis. The separation of microtubule organizing centers to form the mitotic spindle and the movement of chromosomes during anaphase A and anaphase B was clearly microtubule dependent. Furthermore, the mechanochemical

proteins kinesin and cytoplasmic dynein were conspicuous candidates for mitotic motors. Nevertheless, no functional evidence supported the candidacy of these proteins as mitotic motors. Evidence for cytoplasmic dynein's function was limited to axonal transport, and kinesin was still considered to be a single, mechanochemical protein, which localized to mitotic spindles in some studies but not in others (Hollenbeck, 1989; Leslie et al., 1987; Neighbors et al., 1988; Pfister et al., 1989; Scholey et al., 1985). Of the nine mutants isolated by Morris that caused an increase in the spindle mitotic index at restrictive temperature, *bimC3* was unique as it caused arrest with small "minute" spindles (Morris, 1976b). The subsequent discovery that *bimC* encoded a kinesin-related protein required for mitosis in *A. nidulans* (Enos and Morris, 1990) linked kinesin function with mitosis and further validated Ron Morris' expectation that his cell cycle mutants would contribute to our understanding of the chemical events underlying spindle formation and chromosome movements.

Enos and Morris' (1990) seminal paper reporting that a temperature sensitive lethal mutation in the *bimC* kinesin-blocked mitosis had several impacts. First, the work functionally linked the motor protein kinesin to the successful completion of mitosis. This was the nail in the coffin that buried any doubts over the role of kinesin-like motors in mitosis. Second, as the motor domains of BIMC, *Drosophila* kinesin, and the yeast KAR3 kinesin (the only kinesin sequences published at that time) were clearly related, the isolation of the BIMC kinesin confirmed the expectation that kinesins were widely conserved. Third, the defect in *bimC* mutants was in spindle formation: SPBs could duplicate but not separate and bipolar spindles never formed. Therefore, Enos and Morris provided what is now the generally accepted view that BIMC-like kinesins "could form cross-bridges between interdigitating spindle microtubules and, as a plus end motor, push the SPBs apart." Without BIMC, force was lacking for bipolar spindle formation, resulting in a protracted endomitosis and the eventual accumulation of polyploid nuclei. Thus BIMC became the founding member of a branch of the kinesin gene family, members of which have subsequently been shown to also be involved in bipolar spindle formation (Moore and Endow, 1996).

Enos and Morris (1990) also supplied insight into the relationship between the structure and function of kinesin-related proteins. They noted that the similarities in kinesin sequences did not extend into the non-motor domains, and suggested that these differences could underlie the different roles these kinesins appeared to play in the cell. For example, in contrast to BIMC, KAR3 is not required for mitosis but is required for nuclear fusion. Also, *bimC* mutants were normal for nuclear migration and polar growth, both of which were microtubule-dependent and presumed to require MT-dependent transport. These results suggested that individual organisms contained multiple kinesin-related genes, each encoding a kinesin-related protein with specialized, MT-motor function. Subsequent work from the Morris lab identified a second *A. nidulans* kinesin-related gene (Enos et al., 1991) involved in mitosis, *klpA*, and, with the help of the Rose lab, demonstrated that *klpA* was functionally related to its closest relative, yeast KAR3 (Enos et al., 1991; O'Connell et al., 1993). This paper also included the first demonstration that presumably orthologous kinesins were functionally interchangeable.

Enos and Morris recognized that the phenotype of *bimC* mutants suggested the existence of a cell cycle checkpoint monitoring spindle function. *bimC* mutants accumulate abnormal, mono-polar spindle asters which persist significantly longer than do mitotic spindles in wild type cells, which disassemble during mitotic exit. Thus, spindle MT disassembly was delayed in *bimC* mutants, and they suggested that a checkpoint which monitors spindle function might exist. The existence and nature of such a checkpoint has been demonstrated in other organisms (Hoyt et al., 1991; Li and Murray, 1991) and subsequently in *A. nidulans* (Efimov and Morris, 1998).

3. Conclusions: looking back to see the way forward

This retrospective of Ron Morris' research on mitosis has provided an opportunity to pause for a moment and consider where we have been and where we are going. In our review of Ron's early work on mitosis, we have taken an admittedly nostalgic and clearly humbling look back to the contributions of an inspirational leader. And now we must look to the future and contemplate what lies ahead. This is the time to ask important and often hard questions, just as Ron asked such questions of his colleagues in his investigation for the secrets of mitosis. What important contributions remain to be made by the continued investigation of mitosis in *A. nidulans*? What approaches should those of us who take up the challenge use? How will we find the new talent and manpower that is essential to the success of these future endeavors and inspire them in a manner worthy of our professional heritage? As in many areas of human endeavor, we find that reviewing the past points the way into the future.

3.1. Aspergillus nidulans remains an important experimental organism for the investigation of mitosis

Aspergillus nidulans remains in a unique position to contribute to fundamental discovery in the areas of mitotic mechanics and mitotic control. Continued study of mitosis in *A. nidulans* is important to the general cell cycle community, for *A. nidulans* is an important model

Table 1
List of *A. nidulans* genes functionally linked to mitosis

Locus	Identification	Description	*S. pombe* ortholog	*S. cerevisiae* ortholog
ankA[a] *(sntA)*	reverse genetics	wee1 kinase, negative regulator of NimX	Wee1	SWE1
bimA[a] *(sepI)*	forward genetics	Blocked in mitosis mutant, APC/C component 3 (APC3)	nuc2	CDC27
bimB[a]	forward genetics	Blocked in mitosis mutant, separation of sister chromatids	cut1	ESP1
bimC[a]	forward genetics	Blocked in mitosis mutant, BimC class kinesin	cut7	KLP1/CIN8
bimD[a]	forward genetics	Blocked in mitosis mutant, DNA metabolism	pds5	PDS5
bimE[a]	forward genetics	Blocked in mitosis mutant, APC/C component 1 (APC1)	cut4	APC1
bimF	forward genetics	Blocked in mitosis mutant		
bimG[a]	forward genetics	Blocked in mitosis mutant, phosphoprotein phosphatase	dis2	GLC7
bimH[a]	reverse genetics	APC/C component 6 (APC6)	cut9	CDC16
bncA	forward genetics	Binucleate conidia		
hfaB	forward genetics	High frequency of aneuploids		
mipA[a]	forward genetics	γ-tubulin	tug1	TUB4
nimA[a]	forward genetics	Never in mitosis mutant, serine/threonine protein kinase	fin1	kin3
nimB	forward genetics	Never in mitosis mutant		
nimC	forward genetics	Never in mitosis mutant		
nimD	forward genetics	Never in mitosis mutant		
nimE[a]	forward genetics	Never in mitosis mutant, cyclin B	cdc13	CLB2
nimF	forward genetics	Never in mitosis mutant		
nimG	forward genetics	Never in mitosis mutant		
nimH	forward genetics	Never in mitosis mutant		
nimI	forward genetics	Never in mitosis mutant		
nimJ	forward genetics	Never in mitosis mutant		
nimK	forward genetics	Never in mitosis mutant		
nimL	forward genetics	Never in mitosis mutant		
nimM	forward genetics	Never in mitosis mutant		
nimN	forward genetics	Never in mitosis mutant		
nimO[a]	forward genetics	Never in mitosis mutant, DNA Replication	dfp1	DBF4
nimP	forward genetics	Never in mitosis mutant		
nimQ[a]	forward genetics	Never in mitosis mutant, DNA Replication	mcm2	MCM2
nimR	forward genetics	Never in mitosis mutant		
nimS	forward genetics	Never in mitosis mutant		
nimT[a]	forward genetics	Never in mitosis mutant, tyrosine phosphatase, positive regulator of NimX	cdc25	MIH1
nimU	forward genetics	Never in mitosis mutant		
nimV	forward genetics	Never in mitosis mutant		
nimW	forward genetics	Never in mitosis mutant		
nimX[a]	reverse genetics	Never in mitosis mutant, protein kinase	cdc2	CDC28
nuvF[a]	forward genetics	Mutagen sensitive, DNA synthesis checkpoint		
sldA	forward genetics	Synthetic Lethal without Dynein, spindle checkpoint	bub1	BUB1
sldB	forward genetics	Synthetic Lethal without Dynein, spindle checkpoint	bub3	BUB3
snoA	forward genetics	Suppressors of nimO		
snoB	forward genetics	Suppressors of nimO		
sntB	forward genetics	Suppressors of nimT		
sntC	forward genetics	Suppressors of nimT		
snxA	forward genetics	Suppressor of nimX		
snxB	forward genetics	Suppressor of nimX		
snxC	forward genetics	Suppressor of nimX		
snxD	forward genetics	Suppressor of nimX		
sonA[a]	forward genetics	Suppressor of nimA1, nucleocytoplasmic transport	rae1	GLE2
sonB	forward genetics	Suppressor of nimA1		
sudA[a]	forward genetics	Suppressor of bimD6, chromosome scaffold protein	psm3	SMC3
sudB	forward genetics	Suppressor of bimD6		
sudC	forward genetics	Suppressor of bimD6		
sudD[a]	forward genetics	Suppressor of bimD6, chromosomal condensation	SPAC10F6.10	RIO1
TinA[a]	forward genetics	Two-hybrid interacting with NimA		
uvsB[a]	forward genetics	UV sensitive, DNA damage checkpoint	rad3	MEC1
uvsD[a]	forward genetics	UV sensitive, DNA damage checkpoint	rad26	

[a] *Gene cloned.*

system. *Aspergillus* differs from yeast significantly in its biology and genomics and has an impressive track record for providing novel findings. NIMA and γ-tubulin are the quintessential examples; discovered in *A. nidulans* but of universal importance to mitotic regulation (Job et al., 2003; O'Connell et al., 2003). Continued

investigation of NIMA's role in *A. nidulans* is clearly critical to our understanding of mitotic control by the widespread NIMA-related kinase family, since *A. nidulans* is the experimental paradigm for NIMA. Similarly, investigations of γ-tubulin in *A. nidulans* continues to produce novel findings relevant to it's role in mitosis (Jung et al., 2001). Continued study of mitosis in *A. nidulans* is also important to the filamentous fungal community, for it is the model mitotic system for this large group of industrially, medically, and ecologically important organisms. This point is underscored by the progress of the fungal genomics initiative (http://www-genome.wi.mit.edu/annotation/fungi/fgi/), which provides a mechanism for rapid translation of the information on mitosis from *A. nidulans* to a growing number of other fungi whose genomes are sequenced. Continued progress on *A. nidulans* mitosis is poised to have significant, widespread impact.

3.2. Research on mitosis in Aspergillus must involve both forward and reverse genetic strategies

A combination of forward and reverse genetics will be necessary to identify the complete set of *A. nidulans* mitotic genes. To date, forward genetic analyses have made the largest contribution. Of the 56 *A. nidulans* genes (Table 1)[2] which have been experimentally linked to nuclear division, more than half were identified in the initial genetic screen by Morris (1976a,b). Among the remaining 27 genes, 17 were identified as suppressors of one of the original 29. These numbers are small by comparison to genes associated with Gene Ontology terms related to "cell cycle" in yeast. For example, there are 510 "cell cycle" genes in *S. cerevisiae* and 706 in *S. pombe*.[3] These genes identify 577 annotated *A. nidulans* ORFS that match a cell cycle gene in at least one of the yeast with a BLAST *E*-value below 10^{-15}.[4] This leads one to ask whether there are cell cycle genes in *A. nidulans* in addition to those already identified by experimentation or bioinformatics. Considering that 577 is only 6% of the *A. nidulans* predicted gene number, whereas 11 and 19% of the G0-annotated genes in *S. cerevisiae* and *S. pombe*, respectively, are associated with cell cycle, we must come to one of two conclusions. Either there remain no undiscovered, novel cell cycle genes in *A. nidulans*, and therefore *A. nidulans* devotes an unexpectedly small fraction of its genome to cell cycle, or there are a considerable number, potentially hundreds, of *A. nidulans* cell cycle genes that remain to be identified. *nimA* provides an argument for the latter, as it was identified by forward genetic analysis in *A. nidulans* and only subsequently linked to cell cycle in yeast and mammals based on sequence homology (Fry and Nigg, 1995; Krien et al., 1998), although it did subsequently turn up in a screen for mitotic mutants in *S. pombe* (Grallert and Hagan, 2002). *sudD*, the founding member of a novel family of protein kinases, is another example of discovery of a cell cycle gene family by forward genetics in *A. nidulans* (Anaya et al., 1998) as is *mipA*, which encodes the founding γ-tubulin (Oakley and Oakley, 1989). It seems most likely that forward and reverse genetic analyses together are needed to identify and define the molecular mechanisms controlling nuclear division in *A. nidulans*, and it is certain that this information will be of considerable interest to the cell cycle and fungal research communities.

Recent technological developments and the genome sequencing of *A. nidulans* have provided a huge boost for future research. For example, the autonomously replicating genomic plasmid library of Osherov et al. (2000) has provided a reliable means to rapidly identify and recover *A. nidulans* genes corresponding to any selectable phenotype. Also, recent application of fusion PCR (Kitazono et al., 2002) to generate deletion constructs and GFP tagging constructs designed utilizing genomic sequence data, and which integrate at high frequency by homologous integration, now enables large scale tagging of proteins and other manipulation of genes in *A. nidulans* at a scale previously unimagined (Osmani, unpublished).

3.3. Large shoes to fill

In this retrospective, we have attempted to convey the magnitude of Ron Morris' impact on the study of mitosis and on the development of that research community. We have documented his fundamental contributions to essentially every aspect of mitosis and numerous technical innovations that remain a staple of mitotic research today. In closing, we wish to give equal recognition to the leadership and friendship Ron has provided to the community for over four decades and the guidance he continues to provide today. We only hope that, in training future *A. nidulans* mitosis

[2] Adapted from the Gene List compiled by John Clutterbuck (http://www.fgsc.net/mirror/www.gla.ac.uk/Acad/IBLS/molgen/aspergillus).

[3] Lists of *S. pombe* and *S. cerevisiae* ORF names with corresponding Gene Ontology terms were downloaded from the Gene Ontology Consortium site (http://www.geneontology.org/).

[4] ORFs corresponding to the complete set of *S. pombe* and *S. cerevisiae* genes were used to identify best hits in the *A. nidulans* genome using MULTIBLAST. Settings were default values, retaining all contig sequence hits with *E* values less than or equal to 10^{-4}. *A. nidulans* annotated ORFS were assigned to the best hit contigs by MULTIBLAST, using the contigs as query to search a database built from the annotated ORFS from the Whitehead Center for Genome research (http://www-genome.wi.mit.edu/annotation/fungi/aspergillus/). Gene Ontology and BLAST search results were combined into tables using Microsoft Access. The number of annotated ORFs with best hit *E* scores below 10^{-15} was determined by query of the tables using Microsoft Access functions. The total number of *A. nidulans* annotated ORFs matching at least one yeast cell cycle gene was determined by query of the tables using Microsoft Access functions.

researchers, we will be able to impart to them some of the inspiration which we have been so fortunate to receive from Ron.

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