Dancing genomes: fungal nuclear positioning

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Abstract

The many different mechanisms that fungi use to transmit and share genetic material are mediated by a broad range of chromosome and nuclear dynamics. The mechanics underlying nuclear migration are well integrated into detailed models, in which the forces supplied by plus- and minus-end-directed microtubule motors position and move the nucleus in a cell. Although we know much about how cells move nuclei, we know much less about why the cell invests in so many different nuclear 'dances'. Here, we briefly survey the available models for the mechanics of nuclear migration in fungi and then focus on examples of how fungal cells use these nuclear dances — the movement of intact nuclei in and between cells — to control the integrity, ploidy and assortment of specific genomes or individual chromosomes.

Different fungal species propagate as haploids, diploids, dikaryons, multinucleates, polyploids or aneuploids, and some fungal life cycles include not just one or two but several of these different states. Each of these genome conditions has different challenges and requirements for moving nuclei around the cell, and this movement must be developmentally regulated. Molecular analysis of a range of fungal organisms (TABLE 1) has revealed both similarities to and important differences from the paradigms that have been established in the model yeast <u>Saccharomyces cerevisiae</u>. Here, we discuss how whole nuclei move during normal cell division, how nuclei maintain or alter the ploidy of one or all chromosomes and how different nuclei interact in multinucleate cells. We also discuss some of the additional functions of nuclear movement and our emerging understanding of how nuclear positioning might

contribute to the preservation of an 'immortal' template chromosome in stem cell-like nuclei. The specific nuclear dynamics discussed here are examples from fungi, but many of them resemble processes that occur in mammalian cells, including cancer cells and stem cells. Thus, in this Review, we survey diverse fungal systems and many different areas of cell biology in order to highlight open questions about the interplay between nuclear content and nuclear migration.

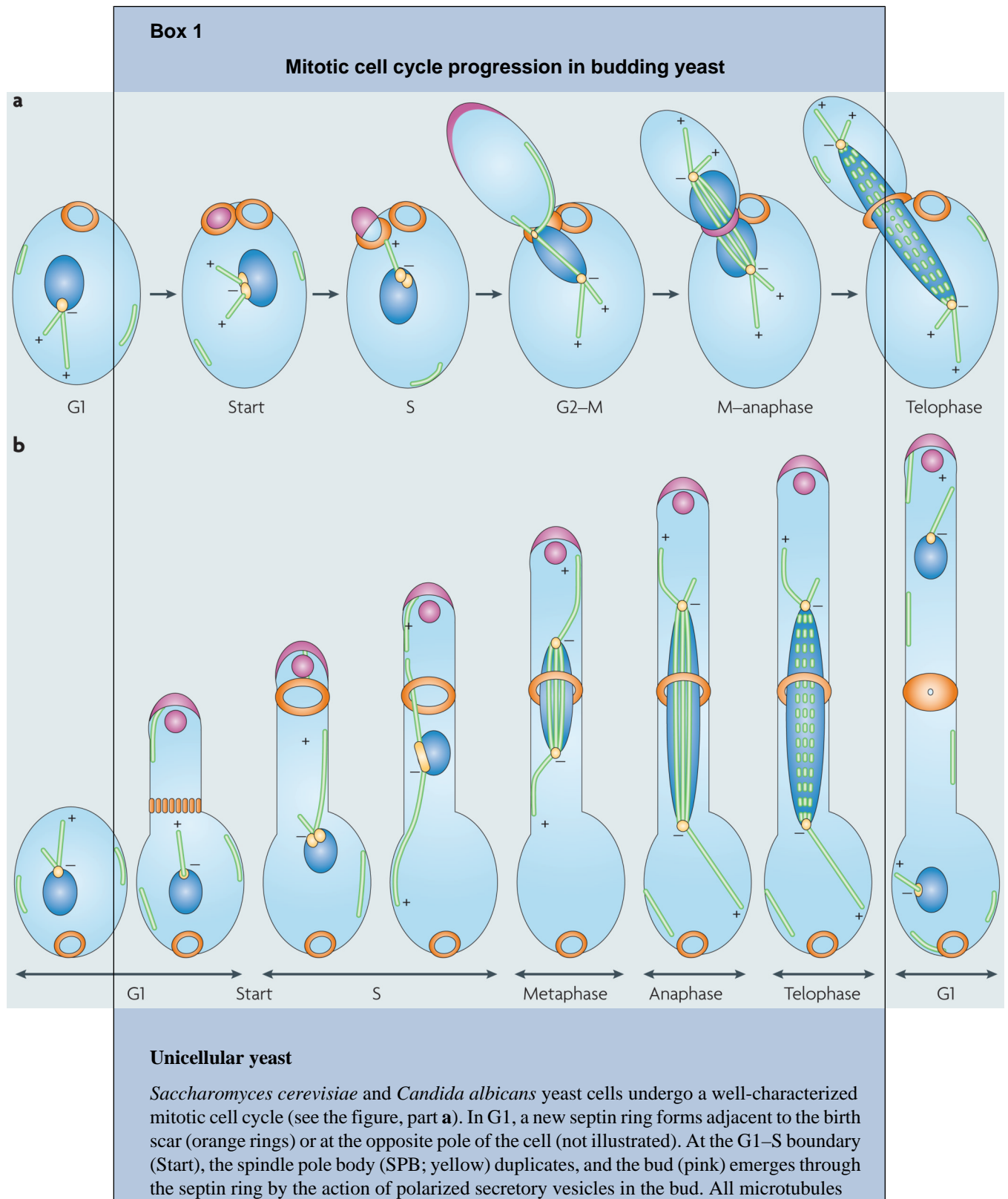## Nuclear dances: moving intact nuclei

The paradigms for nuclear migration have emerged from studies in both the budding yeast *S. cerevisiae* and the multinucleate filamentous fungus <u>*Aspergillus nidulans*</u>. These have been exhaustively reviewed elsewhere, and we do not attempt to review this vast literature here. Instead, we provide a brief overview of the general mechanics that underlie nuclear migration. Summaries of mitotic cell cycle progression in yeast and dimorphic yeast cells and in filamentous fungi are provided in BOXES 1,2,3.

Microtubules (MTs) and microtubule-organizing centres (MTOCs) have a central and conserved role in moving nuclei. Kinetochore MTs in the nucleus segregate chromosomes by pulling them towards the poles. Interpolar MTs facilitate nuclear pole separation by pushing against each other. Outside the nucleus, astral MTs emanate from the MTOC to orientate the mitotic spindle across the mother–daughter cell junction through interactions with the cell cortex and to promote pole separation. In some organisms, such as *S. cerevisiae*, all MTs originate from spindle pole bodies (SPBs) (FIG. 1). In other organisms (for example, the fission yeast <u>*Schizosaccharomyces pombe*</u> (BOX 2) and the basidiomycete <u>*Ustilago maydis*</u> (BOX 3)), MTOCs arise at extra-SPB positions during interphase[1] or during mating, when mating-specific MTOCs form at the tips of mating projections[2]. In *S. pombe*, MT dynamics, along with plus-end tracking (+TIP) proteins, which localize to the plus ends of MTs, have central roles in nuclear positioning. The cylindrical shape, small size and symmetrical division plane of fission yeast all facilitate a simple mechanism of nuclear positioning that relies on MT dynamics. In this system, parallel arrays of MTs interact with the cortex and exert pushing forces on the nucleus[1,3]. However, in most systems, particularly in large filamentous fungal hyphae, MT dynamics alone are not sufficient for nuclear positioning during interphase, and nuclear migration is also powered by molecular motors.

S. cerevisiae

Work from a number of laboratories has shown that the dynein heavy chain (<u>Dyn1</u>) has a key role in *S. cerevisiae* nuclear positioning, as it provides the main force that pulls the nucleus, through astral MTs. For example, *S. cerevisiae dyn1* mutants exhibit abnormal nuclear localization and elevated levels of binucleate cells[4]. During G2 and early M phase in the cell cycle, the entire mitotic spindle, including the duplicated chromosomes, undergoes a series of oscillations across the mother–bud neck. This requires Dyn1 and interactions between astral MTs and actin cables, mediated by the MT-associated proteins karyogamy protein 9 (<u>Kar9</u>) and <u>Bim1</u>, which tether the *S. cerevisiae* type v myosin <u>Myo2</u> to the plus ends of MTs[4]. In the absence of Kar9 or the spindle polarity determinants <u>Bni1</u> and bud site selection protein 6 (<u>Bud6</u>), dynein-mediated nuclear movement occurs earlier and more rapidly than in wild-type cells, indicating that Bud6 and Bni1 normally attenuate dynein activity[5], although they probably do so through independent mechanisms[6]. Similarly, in response to double-strand breaks, the mitosis entry checkpoint protein 1 (<u>Mec1</u>)-dependent checkpoint signals through <u>Rad52</u> and checkpoint kinase 1 (<u>Chk1</u>) to repress dynein-dependent nuclear migration through the bud neck[7]. This balance of forces ensures that, in *S. cerevisiae*, the mitotic spindle is properly orientated to span the mother–bud neck and that replicated chromosomes are accurately delivered to both mother and daughter cells. One key question that remains to be resolved is how mitotic checkpoints help cells to determine when both nuclei have been

properly positioned. This process involves the asymmetrical association of proteins such as Kar9 and Dyn1 with the SPB that localizes to daughter cells[8,9].

Box 1

Mitotic cell cycle progression in budding yeast

a

G1 Start S G2–M M–anaphase Telophase

b

G1 Start S Metaphase Anaphase Telophase G1

Unicellular yeast

Saccharomyces cerevisiae and *Candida albicans* yeast cells undergo a well-characterized mitotic cell cycle (see the figure, part **a**). In G1, a new septin ring forms adjacent to the birth scar (orange rings) or at the opposite pole of the cell (not illustrated). At the G1–S boundary (Start), the spindle pole body (SPB; yellow) duplicates, and the bud (pink) emerges through the septin ring by the action of polarized secretory vesicles in the bud. All microtubules

(MTs; green) seem to nucleate at the SPBs, and mitosis is closed (the nuclear envelope does not break down). In *C. albicans*, free microtubules are evident along the cell cortex. The duplicated SPB forms a short spindle, and astral MTs communicate with the septin ring to orientate the spindle with the mother–bud axis. Spindle oscillations across the bud neck are mediated by dynein acting on the astral MTs. Growth of the bud is mediated by secretory vesicles, which are organized into a crescent-shaped polarisome (pink). During anaphase, spindle elongation forces are sufficient to deliver the sister chromatids into the two daughter nuclei (blue). Polarized growth is re-orientated to the septin ring, the ring contracts, and it is filled with chitin-rich cell wall material before cell separation. Yeast and pseudohyphal cells have similar dynamics, with the main difference being that pseudohyphae spend more time in G2 and, thus, the buds become longer than buds in yeast cells before the onset of anaphase, and cells often do not separate completely, remaining attached in chains of elongated budded cells.

Unicellular hyphae in multimorphic budding yeast

When grown under conditions that induce hyphal growth (see the figure, part **b**), a *C. albicans* germ tube evaginates through a band of septin proteins (orange). Polarized growth is driven by a polarisome (pink cresent) in addition to a Spitzenkörper (pink circle), which is a vesicle-organizing centre that drives the highly polarized growth of hyphal tips. Septin ring formation occurs ~10–20 μm from the basal cell at the time of SPB duplication[88], and the ring is laid down just as the tip passes this future site of septation (the presumptum). Nuclei with short spindles migrate to the presumptum by oscillatory movements that are mediated by contacts between astral MTs and the cell cortex and that are primarily dynein dependent. Anaphase occurs across the septin ring, and spindle elongation forces deliver the daughter nuclei into the two compartments (the basal cell and the hyphal tip cell). Other, slower forces move the mother nucleus back into the basal cell. Dissolution of the spindle is followed by contraction of the septin ring and accumulation of cell wall material at the septum, but the cells remain attached to one another.

+, microtubule plus end; −, microtubule minus end.

Box 2

Mitotic cell cycle progression in fission yeast
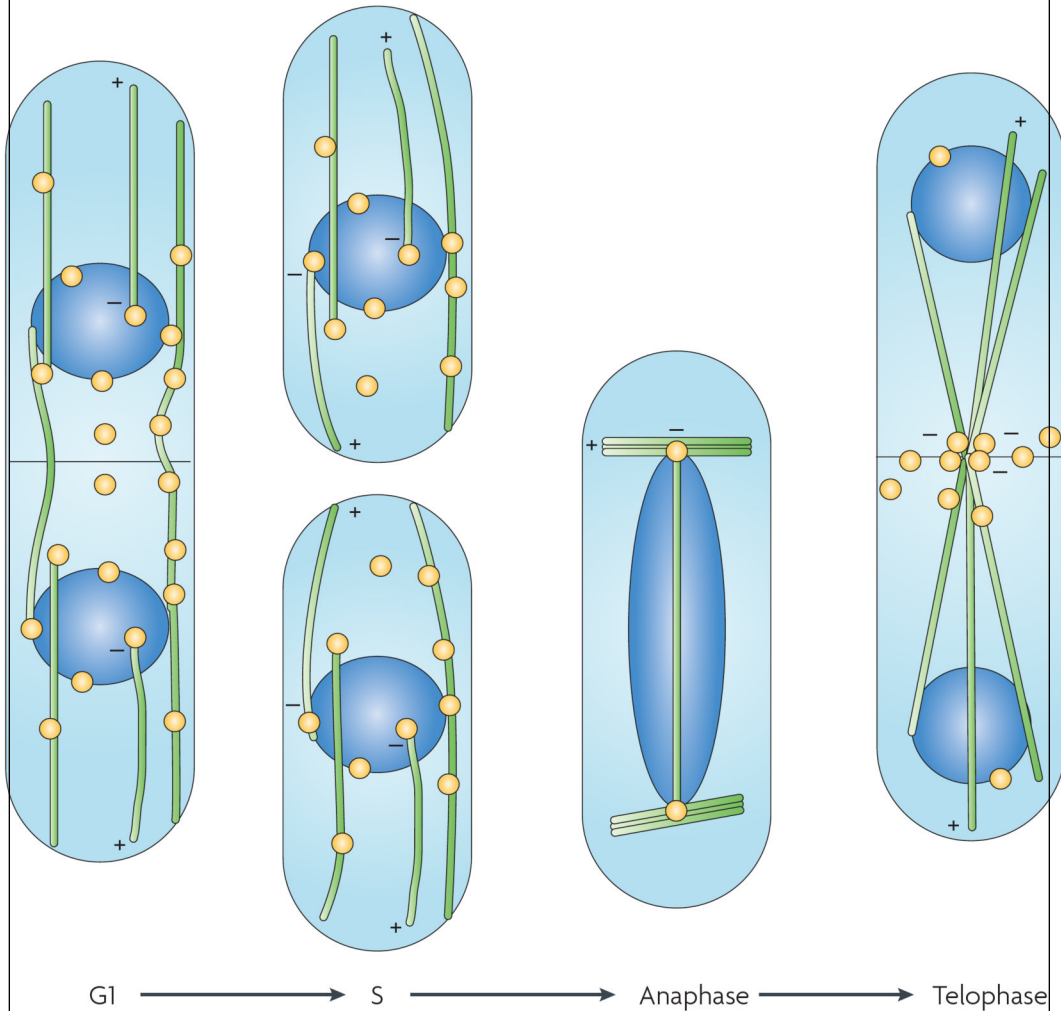
In *Schizosaccharomyces* pombe cells (see the figure), G1 is very short-lived, and thus cytokinesis and cell separation are accomplished during S phase of the next cell cycle. Microtubule-organizing centres (MTOCs; yellow), primarily at the nuclear periphery, nucleate microtubules (MTs; green) in interphase, in addition to the spindle pole bodies, which nucleate the mitotic spindle. Before cell division, equatorial MTOCs nucleate MTs from the future site of septation. In general, MT minus ends (−) are located towards the centre of the cell and the plus ends (+) are located towards the tips.

By contrast, the nuclear migration that is involved in karyogamy (the fusion of haploid nuclei following conjugation between gametes) seems to be powered primarily by interactions between cytoplasmic MTs that extend from the SPBs to interconnect the fusing nuclei[10]. Depolymerization of SPB-bound MTs pulls the nuclei together, and these nuclei then fuse near one edge of the SPBs. a careful study of this nuclear congression found that karyogamy is mediated by the interaction of MT plus ends rather than by MT sliding and extensive overlap[11]. The prevailing model is that during karyogamy MT plus-end interactions from

oppositely orientated MTOCs provide the force to move nuclei through the cytoplasm[11]. Mutations causing defects in karyogamy also provide powerful genetic tools for performing cytoduction[12].

Filamentous fungi—Most filamentous fungi have multinucleate hyphal compartments. Thus, in expanding mycelia, daughter nuclei remain in the same compartment after mitosis (which is not spatially and temporally coordinated with cell division; see REF. 13 for an exception). However, the nuclei are evenly distributed along the hyphal compartments, which balances the cytoplasmic volume and contents that are sampled by each nucleus. MTs and their motors have central roles in positioning nuclei in hyphae, and this has been reviewed in detail elsewhere[14]. The role of kinesins in nuclear positioning has been elusive, potentially because of functional redundancy among the conventional kinesins; for example, in *A. nidulans* there are at least 11 different forms. There is some, limited, evidence in *A. nidulans* for a role for kinesins in destabilizing MTs and localizing dynein to promote nuclear migration, but on the whole much remains to be discovered about the role of kinesins in nuclear migration in filamentous fungi[15,16].
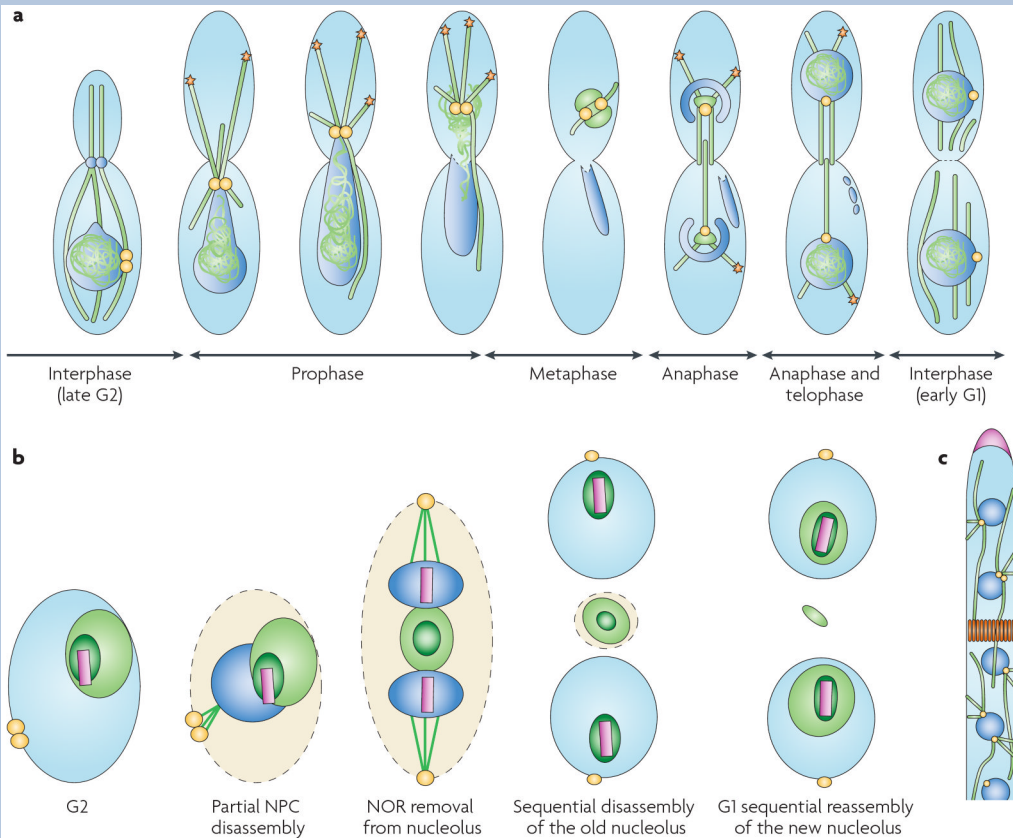
By contrast, dynein is central to nuclear migration in many filamentous fungi. Mutations in dynein components, such as the classic *nud* alleles of *A. nidulans* and the *ropy* alleles of *Neurospora crassa*, have revealed that dynein has a broad range of functions. In *Ashbya gossypii, N. crassa* and *A. nidulans* dynein mutants, the nuclei are no longer evenly spaced, leading to clumping of nuclei in regions of the mycelia[17–19]. In *A. gossypii*, the loss of dynein leads to the clustering of all the nuclei at hyphal tips, leaving vast areas (tens of microns) of cytosol devoid of nuclei[17] (BOX 3). By contrast, in *A. nidulans* dynein mutants, all of the nuclei clump at the germ bubble by the ascospore (the area of the hypha that is farthest from the hyphal tip)[17,19]. In these systems, dynein ensures the balanced distribution and even spacing of nuclei throughout the hyphal tube. However, it is not known whether this is accomplished directly, by dynein acting as a conventional motor, or whether it is an indirect effect of dynein on MT dynamics[14]. In *U. maydis*, between three and six small, motile, γ-tubulin-containing MTOCs nucleate MTs at the boundary of mother and budding daughter cells, and dynein-based transport of MTs and MTOCs is required to polarize the MT cytoskeleton[20].

Candida albicans

The human pathogen *Candida albicans*[21] can form yeast, pseudohyphae or true hyphae, depending on the environmental conditions[22,23]. The nuclear dynamics in *C. albicans* yeast and pseudohyphae resemble those in *S. cerevisiae*: the nuclei cross the mother–bud neck, and the elongated mitotic spindle oscillates across the neck in a dynein-dependent manner[24,25] (FIG. 2). Spindle elongation is sufficient to deliver a single nucleus to each daughter cell. The nuclear dynamics in *C. albicans* true hyphae are different: the nucleus migrates ~10 μm into the growing germ tube to the presumptum, which is the site of germ tube division and septum formation[24]. The spindles undergo dynein-dependent oscillatory movements that cross the presumptum (Supplementary information S1 (movie)). In the absence of dynein in both hyphae and yeast (FIG. 2c), the velocity of nuclear movement is reduced such that mitosis ensues in the mother cell. However, a spindle checkpoint mediated by Bub2 prevents premature cytokinesis, such that a post-mitotic daughter nucleus often succeeds in eventually reaching the bud. The slower movement of nuclei in dynein mutants is probably mediated by MT plus-end dynamics[25].

Box 3

Mitosis in filamentous fungi

a

| Interphase (late G2) | Prophase | Metaphase | Anaphase | Anaphase and telophase | Interphase (early G1) |

b

| G2 | Partial NPC disassembly | NOR removal from nucleolus | Sequential disassembly of the old nucleolus | G1 sequential reassembly of the new nucleolus |

c

Ustilago maydis

In the basidomycete corn smut fungus, *Ustilago maydis*, yeast growth (see the figure, part **a**) is accompanied by microtubule organizing centre (MTOC)-mediated nucleation of microtubules (MTs; green) from the bud neck region (blue circles). The plus-end localization of dynein (orange stars) mediates spindle elongation and is essential for retrograde endosome motility. In hyphae, MTs nucleate near the central nucleus (dark blue), or the central pair of nuclei in dikaryotic cells[89]. Nuclear envelope breakdown begins with extension of the nuclear envelope. In prophase, dynein in the bud cortex pulls on MTs and spindle pole bodies (SPBs; yellow) that are attached to condensed chromosomes. The nuclear envelope eventually breaks at the SPB, and the chromosomes congregate with the SPB outside the old nuclear envelope. Once the chromosomes are separated in anaphase, a new nuclear envelope forms around the daughter chromosomes.

Aspergillus nidulans

In the most apical hyphal compartment, *Aspergillus nidulans* nuclei divide in a parasynchronous wave, and SPBs of post-mitotic nuclei nucleate cytosolic MTs[90,91]. The basal compartments, which are separated from the apical compartment by a septum that is established by septin rings, contain nuclei that are arrested in interphase until a side branch emerges, at which time the nuclei re-enter the division cycle. Cytosolic MTs are present at interphase and generally disappear during mitosis, except in the hyphal tip[92]. *A. nidulans* nucleolar proteins are separated from the ribosomal DNA that makes up the nucleolar

organizing region (NOR) and are expelled from daughter nuclei to the cytoplasm between them (see the figure, part **b**). Unlike most nuclear reorganizations, this process can occur even when spindle attachment is abrogated[93]. Nuclear DNA in hyphae undergoes a partially open mitosis, in which nuclear pore complexes (NPCs) disassemble, making the nucleus more permeable during mitosis. In addition, on the initiation of mitosis, DNA (dark blue) condenses and nucleolar proteins (dark green) are exported from the nucleus, whereas the NOR (pink) is retained. Following mitosis, nucleolar proteins are imported through reassembled NPCs in the nuclear membrane to form new nuclei.

Ashbya gossypii

This ascomycete is thought to nucleate MTs exclusively from nuclear-membrane-associated SPBs. Short astral MTs associate with the cortex, and longer cytosolic microtubules extend for tens of microns through the cytosol. The nuclei divide asynchronously, and therefore in the figure (part **c**), individual nuclei are depicted in different states of SPB duplication and separation[94,95].

C. albicans pseudohyphal cells that lack dynein exhibit a distinctive nuclear dance: the nuclei move into the daughter cell before mitosis and divide there (FIG. 2d). In the absence of dynein, there is no oscillation across the mother–bud neck. Importantly, in pseudohyphae there is no evident checkpoint-mediated delay of cytokinesis when the entire mitotic spindle is in the bud; the result is the formation of a dikaryotic daughter cell and an anucleate mother cell. In subsequent divisions, the binucleate cells continue to grow and divide with multiple nuclei (K. Finley, personal communication). a similar multinuclear phenotype is seen in a subpopulation of mutants with cell cycle defects, such as those lacking forkhead transcription factor 2 (Fkh2)[26] or the cell cycle kinase Dbf2[27]. Interestingly, in some of the cells that lack Fkh2 the spindles seem to be in synchronous stages of the cell cycle, whereas in other multinucleate cells mitotic spindles coexist with duplicated SPBs, suggesting that different stages of the cell cycle can coexist in the same cytosol (E. Bensen, personal communication). Similarly, in *S. pombe*, nuclear alignment and the plane of division is determined by the nuclear oscillations that occur just before mitosis, and defects in MT attachment to the nucleus lead to misaligned spindles and the mis-segregation of nuclei[28,29]. Thus, defects in nuclear movement, like defects in cell cycle progression, can result in multinucleate cells.

Dancing partners: genome integrity

MTs and their motors are not simply a generic 'mass transit system' in which astral MTs move nuclei. With each turn of the cell cycle there is another layer of choreography within each migrating nucleus, as the chromosomes move in the process of attaching to kinetochore MTs. Recent work indicates that there is an intimate association between the molecular machines that move the DNA and the amount and content of the DNA that is transported. Controlled MT dynamics combined with specific MT motors ensure that each individual chromosome is correctly tethered to, and later appropriately released by, its kinetochore MTs.

S. cerevisiae

In *S. cerevisiae*, cells are normally maintained as haploids or diploids, although crosses can be manipulated to yield triploid and tetraploid strains. Although deviations in normal ploidy can occur through endoreplication, non-disjunction or mating of non-haploid strains (reviewed in REF. 30), most tetraploidy is assumed to arise unintentionally, as a by-product of defects such as a failure of cytokinesis. Cytokinesis can fail as a consequence of subtle defects in the segregation of a single pair of chromosomes or because of wholesale defects in mitotic processes (reviewed in REFS 31,32). In mammalian cells, such '4n' cells usually undergo

apoptosis. However, when they do divide, these cells exhibit high levels of genome instability, resulting in the extensive aneuploidy that is typical of cancer cells (reviewed in [REF. 30]).

In *S. cerevisiae*, mitotically dividing tetraploids exhibit high levels of genome instability, including the accumulation of point mutations during S phase at a twofold higher rate than that in diploid cells[33]. A genome-wide screen for ploidy-specific lethality revealed that only a small number of genes are necessary to maintain tetraploidy[33]. These genes fall into three main functional groups: genes encoding components of the mitotic spindle, including the SPB; genes that are necessary for the establishment of chromosome cohesion, including several kinetochore components; and genes that are required for homologous recombination[33]. This suggests that the presence of extra chromosome copies causes cells to expend more energy on whole-chromosome dynamics and maintaining the integrity of the DNA. It has been proposed that extra stress is placed on tetraploid cells, because MTs do not increase in length despite the higher number of chromosomes on a tetraploid mitotic spindle[33].

When unstressed and salt-stressed haploid, diploid and tetraploid *S. cerevisiae* strains were followed for ~1,800 doublings, all cultures converged to diploidy, suggesting that the diploid state has a selective advantage in these conditions[34]. Furthermore, diploids had an advantage over haploids when serial cultures of mutator (*msh2*Δ) strains were propagated, presumably owing to the ability of the diploid state to buffer deleterious mutations[35]. Despite the instability of cells with increased ploidy, many industrial *S. cerevisiae* strains are polyploid[36]. It is likely that the fitness of a strain with increased ploidy depends on the specific chromosomes that are amplified.

Aneuploidy is assumed to arise from defects in the associations between individual kinetochores and their kinetochore MTs. aneuploidy was studied in haploid yeast strains that each carried an extra copy of one or more chromosomes[37]. These disomic (haploid + 1) aneuploids shared several phenotypes, including sensitivity to conditions that interfere with protein folding, protein synthesis and cell cycle progression. In addition, the transcription level of ~400 genes was altered in almost all of the aneuploid strains, suggesting that, irrespective of the chromosome involved, disomic haploids exhibit growth defects and changes in transcription due to the presence of extra chromosomes[37]. Similarly, trisomic mouse cell lines (diploid + 1) exhibited reduced proliferation and cellular fitness[38]. an important open question is: why do aneuploid cancer cells exhibit increased proliferative capacity[39], but aneuploid *S. cerevisiae* and mouse cells exhibit reduced fitness[37]? It is likely that specific aneuploidies provide fitness advantages under specific stress conditions[40,41].

C. albicans

In contrast to *S. cerevisiae, C. albicans* is much more tolerant of aneuploidies and translocations. Whole-chromosome aneuploidies arise frequently following transformation of laboratory strains[42–44], with trisomy being much more prevalent than monosomy. aneuploidy, including whole-chromosome and segmental amplifications, has been detected in 50% of *C. albicans* strains that have elevated resistance to fluconazole[41], the most commonly used antifungal drug. One specific segmental aneuploidy, isochromosome 5l, in which 2 copies of the left arm of chromosome 5 flank the centromere, accounts for at least 20% of these aneuploidies[41]. The elevated copy number of two genes on isochromosome 5l, one near the telomere and one near the centromere, is responsible for most of the resistance that is seen in strains carrying this isochromosome[45].

The high level of tolerance for aneuploidy and translocations could be because *C. albicans* does not undergo the conventional meiotic pairing that requires alignment of homologous chromosomes. alternatively, this tolerance may stem from the flexibility in spindle length between yeast, pseudohyphal and hyphal *C. albicans* cells[24]. This flexibility would allow *C.*

albicans to accommodate extra chromosomes by adjusting its spindle geometry to the changing needs of a dynamic genome in a manner that does not occur in *S. cerevisiae*. Such genome plasticity is far from unique and has been seen in many other fungi. among the human pathogens, *Candida glabrata* undergoes rapid changes in karyotype during bloodstream infections[46,47], and *Cryptococcus neoformans*, a basidiomycete, also exhibits aneuploidy[48]. In addition, supernumerary chromosomes (or dispensable 'B' chromosomes) have arisen in specific fungal isolates and seem to have been acquired by horizontal transmission (for examples, see [REFS 49–51]). In some cases, supernumerary chromosomes are associated with virulence factors and confer pathogenic properties on an otherwise benign fungus.

The ability of cancer cells and certain fungal pathogens to adapt to the aneuploid state is striking. On the basis of work in budding yeast, it is likely that this occurs at least partially through adaptive pressure on the MTs and MT motors that move chromosomes and nuclei. Thus, multimorphic fungi hold promise for studying the basic mechanisms that facilitate aneuploidy and, by implication, tumorigenesis and pathogenesis. It is becoming clear that MTs and MT motors are not passive shuttles of chromosomes and nuclei but instead respond to changes in the environment and to the genotypic alterations that arise as a consequence of changes in ploidy or, as we discuss below, when two different genotypes share a cell.

Dikaryons: a nuclear two-step

Although we have a clear mechanistic understanding of nuclear migration in mating and vegetative cells for a range of model ascomycetes and a few basidiomycetes with yeast forms, there is a paucity of information about the regulation of nuclear dynamics in many basidiomycete mushrooms. Dikaryons are cells in which two haploid nuclei, one from each parent cell, share a single cytoplasm for a period of time without undergoing nuclear fusion or meiosis[52]. The dikaryon stage dominates the life cycles of many basidiomycetes, such as mushrooms. By contrast, filamentous ascomycetes, such as aspergilli or *Podospora anserina*, produce transient dikaryons as part of their sexual cycle. Dikaryons also form following mating in *S. cerevisiae* karyogamy (*kar*) mutants[53,54]. The receipt of an unknown environmental signal ends the dikaryon stage and triggers rapid karyogamy, meiosis and fruiting-body development. In many systems, dikaryons emerge from a heterogeneous mixture of nuclei in the large syncytia that form when the parental mycelia fuse. The process of dikaryon formation involves nuclear migration and sorting of the nuclei by genotype to ensure that each dikaryon contains a balance of each parental genome (FIG. 3).

When hyphae of compatible mating types fuse, the nuclei rapidly migrate (for example, up to 2–3 mm per hour in *Schizophyllum commune*[55] and a remarkable 4 cm per hour in *Coprinellus congregatus*[56]) to the distal reaches of the partner mycelium[56,57]. unlike yeast, filamentous fungi that form dikaryons do not use pheromones to recognize mates extracellularly, as the hyphae will fuse (a process termed anastomosis) regardless of mating type[52]. nuclei do not initiate migration when cell fusion is with the same mating type, so the rapid increase in motility is triggered by the coexistence of compatible nuclei, as defined by their mating-type loci[52]. Presumably, there are regulators of MT motors and MT organization that are controlled (directly or indirectly) by the mating-type loci and that lead to the induction of nuclear migration, but these regulators have not been identified.

On arrival at the hyphal tips, nuclei of opposite mating types pair through MT associations, but the basis for detecting the opposite mate for such pairing is unknown. One possibility is that the pheromones and pheromone receptors that are encoded by the mating-type locus are spatially limited to either the nuclear membrane[58] or to the cell cortex surrounding the nuclei. In the filamentous ascomycete *P. anserina*, genetic evidence points to autonomous nuclear signals, such as nuclear-membrane markers, that sort the nuclei so that each dikaryon contains

two different parental nuclei[59]. Remarkably, in multiparental matings, in which there are multiple competing genotypes in a single cell, the most genetically diverse nuclei will form dikaryon pairs[60].

In some fungal species, nuclear pairing initiates the formation of a clamp or crozier cell; this is a specialized projection that connects two adjacent hyphal cells and facilitates the segregation of two daughter nuclei, one of each mating type, into distinct cellular compartments (FIG. 3). In species that form clamp cells, one nucleus divides in the clamp cell and the other divides in the main hypha, but the nuclei have spindles of different lengths, which helps to ensure sorting of the daughter nuclei into different cells. In _Coprinopsis cinerea_, the genotypes alternate positions along the hypha so that in one cell a nucleus with one mating type enters the clamp cell and in the adjacent cell the nucleus with the other mating type enters the clamp cell (FIG. 3). This produces a perfect alternating pattern of parental genotypes along the dikaryotic hypha[61]. However, clamp cell formation is not essential for stable and accurate dikaryon formation and is not seen in all species that can form dikaryons[62]. In species that do not form clamp cells, different spindle lengths, different spindle elongation rates or simply a small enough starting distance between the two nuclei ensure that the spindles overlap in anaphase, enabling a 'two-step' swap of different sister nuclei. This is seen in _S. pombe_ dikaryons, which can be forced to form using conditional cell cycle mutants such that, after mitosis, the sister nuclei (that is, nuclei originating from the same mother nucleus) are systematically segregated to different cells[63]. Thus, spindle elongation-based sorting mechanisms occur in the absence of a clamp cell and during otherwise normal vegetative growth, even in a yeast[63].

Dikaryons are neither true haploids nor true diploids, and this unique state of ploidy seems to lend them some adaptive power. Mutations can be more readily expressed phenotypically in dikaryons than in homokaryotic, haploid mycelia[64]. In laboratory selection experiments with _S. commune_[64], the biparental nuclei in the dikaryon underwent co-adaptation as a result of compensatory changes. Some basidiomycetes form stable heterokaryons, in which multiple nuclei from each mating partner are maintained in a multinuclear compartment. Intriguingly, the balance of parental genotypes in the heterokaryons deviates from a strict 1/1 ratio depending on environmental conditions[65]. Work in _Heterobasidion parviporum_ suggests that individual nuclei are under selection[65]. In addition, nuclei with different genotypes or different epigenetic states also differ in their rates of replication and migration, further adding to the intriguing connections between genotype, ploidy and nuclear migration that are seen in filamentous fungal cells[65,66].

It has long been known that the nuclei in a dikaryon can communicate: exchange of genetic material and somatic recombination occurs between genotypes[60,64]. Interestingly, the exact position of the two nuclei in a dikaryon influences the specific genes that are expressed in these nuclei. For example, in _S. commune_ dikaryons, when the nuclei are close together (<2 microns apart) a different set of hydrophobin-encoding genes is expressed than when the nuclei are further apart[67]. Thus, the precise positions of the biparental nuclei in the dikaryon can specify the transcriptional programme of the cell. Intriguingly, a similar proximity effect has been seen in _S. pombe_ cells. The nuclei in induced multinucleate fission yeast are different sizes, depending on their proximity to other nuclei. This indicates that nuclei sharing a single cell may be able to locally sense and respond to the presence of the other nuclei in the same cell[68].

The dikaryotic state ensures that an organism is poised for meiosis whenever the proper environmental cues are sensed and also confers functional and fitness benefits on the cell. Dikaryon formation requires the regulation of nuclear migration when there are multiple cohabiting genotypes. The variation in the length and alignment of spindles may be the basis of alternating genotypes, but it does not explain how opposite mates find each other and pair

in large syncytia (for example, in mushrooms). There are still many open questions to be answered, including how nuclei recognize one another as 'different' in a common cytoplasm, and how this information is converted to the signals that regulate the migration, positioning and sorting of the genotypes into dikaryons. Studying these questions in model filamentous fungi will probably reveal new lines of communication between the genome and MT motor machinery.

Reasons to dance: harnessing nuclear migration

Nuclear movement in fungi is not only used in specialized sexual cells or to position nuclei relative to the plane of cell division, but it also contributes to other basic nuclear functions in vegetative cells. In *N. crassa* hyphae, chromatin structure responds to the direction of nuclear migration, thereby establishing intranuclear polarity and often mirroring the hyphal axis of polarity[69]. This suggests that chromatin organization either responds to the external MT-based forces that are encountered by a migrating nucleus or, conversely, influences the direction of nuclear migration. notably, polarization of histone localization and modification is also seen in migrating uninucleate mammalian cells in tissue culture, suggesting that signals coordinating nuclear or cell migration with chromatin organization may be evolutionarily conserved[70].

U. maydis makes use of dynein-dependent nuclear migration for the mechanical process of tearing open the nuclear envelope[71] (BOX 3). This basidiomycete undergoes 'open' mitosis[71,72], meaning that the nuclear envelope breaks down with each cell cycle. This is unlike the closed mitosis in most ascomycetes, such as budding yeast, in which the nuclear envelope remains intact and mitosis occurs in the nucleus[73], or the partially open mitosis in *A. nidulans*, in which the envelope remains intact but the nuclear pores open to allow non-selective transport[74] (BOX 3). In animal cells, open mitosis is achieved by fragmenting the nuclear membrane and disassembling the nuclear lamina. By contrast, *U. maydis* uses the energy from MT motors to rip apart the nuclear membrane and enable cytoplasmic MTs to form the kinetochore attachments that are necessary for chromosome segregation[71]. Presumably, this is an evolutionary intermediate or at least an alternative solution to mixing the nucleoplasm and cytosol.

Nuclear oscillations might also protect genome integrity in budding and fission yeast. a dicentric *S. cerevisiae* strain undergoing chromosome breaks due to the attempted segregation of the two centromeres can be rescued by DNA repair mechanisms. These repair processes are accompanied by MT-dependent, dynein-independent nuclear oscillations[75]. Similarly, the prophase zygote in *S. pombe*, which exists for a short period following conjugation and before meiosis, undergoes dramatic chromosomal DNA oscillations, termed 'horse tail' movements[76]. These occur through dynein-dependent movement of astral MTs that are bound to SPBs which are, in turn, attached to telomeres from the time of conjugation to the end of meiosis I[76]. The movement has been proposed to assist in the alignment of homologous chromosomes before meiotic recombination[77]. Recent work presented a mechanism for these oscillations that may be applicable to nuclear migration in many systems. In an elegant marriage of *in vivo* microscopy and mathematical modelling, vogel *et al.* provide evidence for the collective self-organization of dynein motors, during horse tail movements, into an asymmetrical distribution on MTs. The distribution changes as a consequence of mechanical forces on the MTs and thus generates the oscillations[78]. Therefore, nuclear movements seem to facilitate other DNA transactions as well as chromosome segregation in these model yeasts.

Nuclear migration is also required for the assembly of an appressorium, which is a specialized extension of a germ tube that uses turgor pressure to penetrate rigid plant cell walls. In the filamentous ascomycete *Magnaporthe grisea* (rice blast fungus), the appressorium ultimately builds up to 8 MPa of turgor to cross the plant cuticle[79]. This process requires nuclear migration

and the completion of one cycle of mitosis in a germ tube. Intriguingly, after the single mitosis, one nucleus migrates back into the spore body and, from there, triggers an autophagy programme that is essential for appressorium development. It has been proposed that checkpoints monitor the completion of mitosis and couple it to the autophagy program. This checkpoint signal may be 'carried' back to the spore by the migrating nucleus to trigger autophagy and thereby enable appressorium formation. Thus, nuclear migration might be used as a messenger system to ensure linear progression through a developmental programme that is necessary for plant infection.

The perpetual dance: the immortal strand

As early as 1968, evidence existed for intriguing positional differences between nuclei of different ages that shared the cytoplasm of *A. nidulans* vegetative hyphae. Rosenberger and Kessel incubated germinating conidia in radioactive adenine to label the DNA of the 'founding' nucleus in the spore[80]. They then analysed where the labelled nuclei were found along the hypha. after multiple rounds of mitosis with random segregation of chromatids, the radioactivity should have been dispersed between the nuclei. Instead, these early pulse–chase studies indicated that the two nuclei proximal to the hyphal tip contained the labelled 'old' DNA, and the more basal nuclei contained the 'new' DNA. To establish this gradient of genome age along a hypha, non-random sister chromatid segregation must be coupled with nuclear segregation that can position nuclei on the basis of the presence of the template strand.

A few years later, John Cairns proposed his 'immortal strand' hypothesis, in which non-random segregation of old and new DNA occurs to protect the 'original' genome in the asymmetric division of stem cells[81]. In self-renewing division cycles, the asymmetric distribution of chromosomes on the basis of DNA age enables the original or template genome (and, perhaps, any imprinting) to be preserved without the risks of replication errors. Interestingly, in *Armillaria gallica*, a wood rot honey mushroom that is clonal and can live for more than 1,000 years, there is extremely low sequence variation between nuclei that are separated by hundreds of metres in individuals found in nature[82,83]. Such stability in the sequence is puzzling and unexpected in nuclei that are physically far apart and presumably separated by many rounds of nuclear division. One mechanism to preserve the original genotype throughout the extensive mycelia is through a repository of stem-cell-like nuclei, which mitigate variation over time and space.

Recent evidence from mammalian stem cells supports the idea that self-renewing stem cells can retain their 'template' DNA. Experiments in both adult muscle stem cells ('satellite' cells) and mouse embryonic stem cells showed that non-random chromosome segregation occurs[84,85]. Thus, the most apical tip zone of some fungal hyphae potentially contains a self-renewing pool of nuclei that is selectively retained in this position. alternatively, in *A. gallica* there might be repositories of 'stem nuclei' throughout the mycelia. An exciting possibility is that the molecular mechanisms underlying this process may be conserved from fungi to mammalian stem cells. Although asymmetrical segregation is not widespread in eukaryotic cells (for example, it is not seen in *S. cerevisiae*[86]), many potential mechanisms resulting in asymmetrical chromatid segregation[87] can be envisioned. Accordingly, this might be an ancient molecular process that is used in hyphal development. *A. nidulans, A. gallica* and perhaps other filamentous fungi can be powerful, genetically tractable models for identifying possible conserved mechanisms of selective template DNA maintenance and of transmission of template age into spatial coordinates in the cell.

Concluding remarks

The study of nuclear and chromosome dynamics in diverse fungal systems provides ample evidence that there is tremendous variety in how and why cells move nuclei. The nuclear

'dances' that are powered by MTs and MT motors ensure the clearance of the division plane and the segregation of euploid genomes. In addition, fungi exploit the MT-based migration of nuclei and individual chromosomes for diverse purposes that range from sorting nuclei by genotype or age to facilitating recombination and generating genome alterations such as aneuploidy. Nuclear movements respond to the genome content, or its expression, as well as to nuclear position. The diverse fungal models that we have discussed are powerful tools for studying the molecular mechanisms that underlie the generation of genetic diversity during mitotic and meiotic divisions, the maintenance of low levels of diversity in stem cells and the generation of polyploidy and aneuploidy in tumour cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Glossary

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Dikaryon	A hyphal cell in which two compatible nuclei are maintained without karyogamy (nuclear fusion). Ascomycete dikaryons can produce crozier cells, whereas basidiomycete dikaryons can produce clamp connections for the formation of the dikaryotic state.
Aneuploid	A cell with an abnormal number of chromosomes; for example, in a diploid organism, the lack of one copy of a chromosome (monosomy) or the presence of an extra copy of a chromosome (triploidy).
Microtubule-organizing centre	(MTOC). A structure that nucleates and often retains a connection to microtubules. MTOCs at the centrosome (or centriole or spindle pole body) organize the mitotic (and meiotic) spindle apparatus.
Spindle pole body	In yeast cells, the microtubule-organizing centre that functions like a centrosome and is usually associated with the nuclear membrane for part or all of the cell cycle.
Dynein	A minus-end-directed microtubule motor protein that transports cellular cargo along microtubules. In fungi, dynein is a key motor protein that is responsible for nuclear movement through interactions with astral microtubules.
Cytoduction	The production of a cell with a mixed cytoplasm but only one of the two parental nuclei. In *Saccharomyces cerevisiae*, cytoduction is accomplished by mating cells with defects in nuclear fusion (karyogamy).
Supernumerary chromosomes	Small extra chromosomes that are generally dispensable for normal cell functions but that, in some cases, are required for pathogenicity and thus are 'conditionally dispensable'.
Dicentric	A chromosome that has two functional centromeres. When they are tethered to opposite poles of the mitotic spindle the chromosome will break during mitosis.
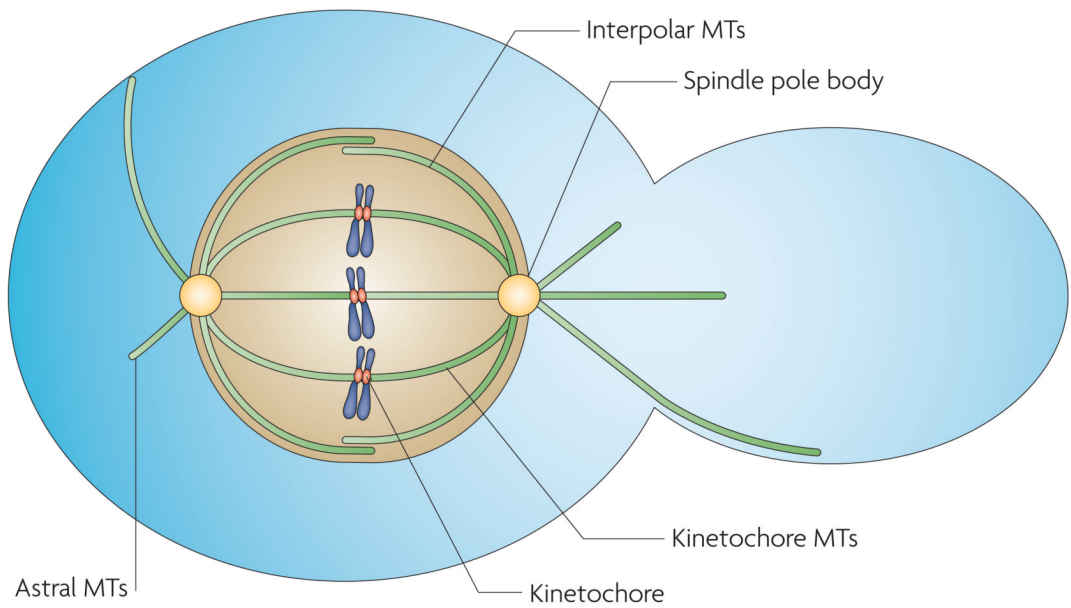
Figure 1. Microtubules and nuclear movement
In *Saccharomyces cerevisiae*, all microtubules (MTs) originate from the spindle pole body. Kinetochore MTs are required for chromosome segregation, and interpolar MTs are required for nuclear pole separation. Astral MTs interact with the cell cortex and septin ring at the bud neck to orientate the mitotic spindle across the mother–daughter cell junction.
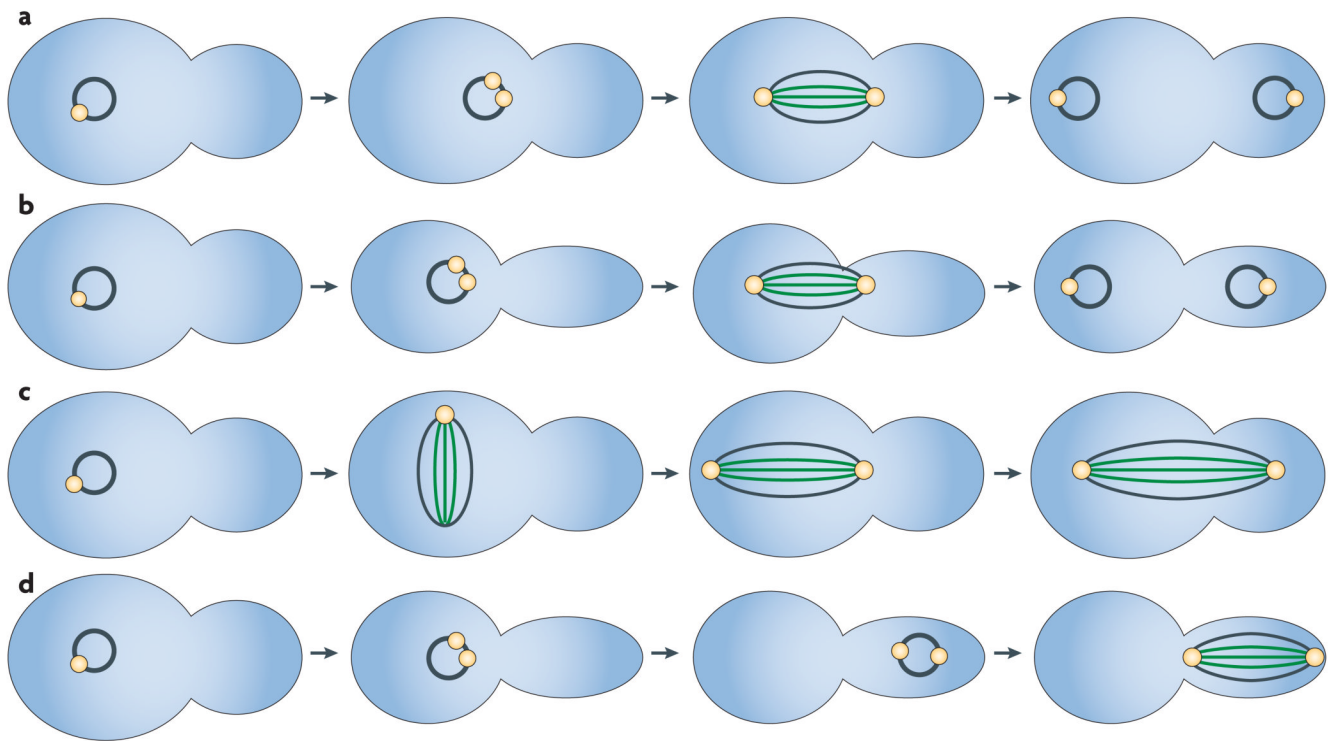
Figure 2. Mitotic dynamics in *Candida albicans*
a,b | In wild-type yeast (part **a**) and pseudohyphal (part **b**) cells, the nuclei are aligned by the mitotic spindle (spindle pole bodies are in yellow, kinetochore microtubules are in green and interpolar microtubules are in black) and divide across the mother–bud neck. **c** | In yeast cells lacking the dynein heavy chain, the nuclei divide in the mother cell and a checkpoint delays cell cycle progression until the nucleus enters the daughter cell. **d** | In pseudohyphal cells lacking dynein, nuclei migrate into the daughter cell and divide there. There is no checkpoint to ensure that the mother nucleus returns to the mother cell. In this schematic, the details of microtubule attachment to chromosomes and of inter-microtubule interactions are not shown.
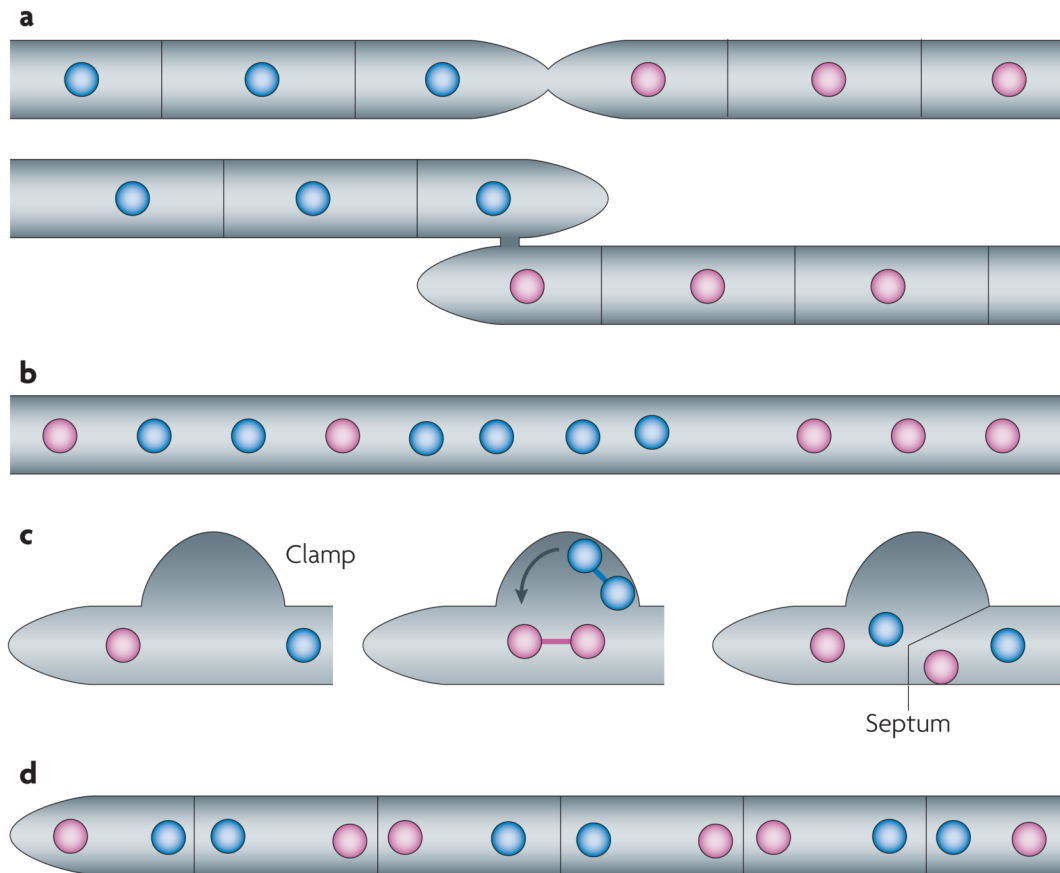
Figure 3. Overview of dikaryon formation

a | Hyphae of many types of filamentous fungi can fuse regardless of mating type. **b** | If fusion occurs between mates with compatible mating loci (blue and pink nuclei), rapid nuclear migration and exchange between the two parental mycelia is initiated. It is unknown how microtubules (MTs) and MT-based motors are regulated during this process, but there is no migration if noncompatible mates fuse. **c** | The nuclei migrate until they reach the distal tip of a hypha and then pair with a nucleus from the other parent. In some cells, a specialized polarized cell structure forms, called a clamp, crozier or hook cell (depending on the species). Some dikaryons form without a clamp. The clamp cell is a side projection of the hypha, and one nucleus migrates up into this projection while the opposite mating-type nucleus remains in the hypha. Both nuclei divide synchronously. Owing to the placement of the septa and the subsequent fusion of the clamp cell back to the main hypha, one daughter nucleus from each mitosis ends up in each cell of the hyphal tube. **d** | This intricate process produces a dikaryotic hypha: it is compartmentalized such that two parental nuclei share the same cytosol. The dikaryon state can persist for extended periods and there can be exchange of genetic material between nuclei.

Table 1

Selected model fungi

Organism	Phylum	Environment	Utility and areas of investigation	Genome sequence
Saccharomyces cerevisiae	Ascomycete	Ubiquitous	Baker's and brewer's yeast; premier eukaryotic model organism used to study a broad range of molecular, cell-biological and genetic questions	1996 (REF. 96)
Candida albicans	Ascomycete	Ubiquitous	Human commensal and opportunistic pathogen with budding yeast and hyphal forms; studies focus on drug resistance, biofilm formation and morphogenesis, as the ability to switch to the hyphal form seems to be important for virulence	2004 (REF. 97)
Schizosaccharomyces pombe	Ascomycete	Ubiquitous	Fission yeast with three chromosomes that are detectable by light microscopy; used to study many aspects of cell cycle progression and the general biology of eukaryotes	2002 (REF. 98)
Neurospora crassa	Ascomycete	Tropical and subtropical; found on dead plant matter following fires	Classic haploid genetic model; used to study circadian rhythms, epigenetics, gene silencing, polarized growth and cell development	2003 (REF. 99)
Ashbya gossypii	Ascomycete	Associated with insects and plants; potential symbiote with both	Filamentous, multinucleate growth with haploid nuclei that divide asynchronously in a common cytoplasm; high genome similarity to *S. cerevisiae* and highly tractable for molecular genetics; used as a model system for the cell cycle and morphogenesis and in industry to produce riboflavin (vitamin B2)	2004 (REF. 100)
Ustilago maydis	Basidiomycete	Corn smut pathogen	Model organism readily amenable to gene replacement; used to study DNA recombination and repair, and cytoskeleton dynamics; biotrophic organism requiring plant tissue for growth and development	2006 (REF. 101)
Podospora anserina	Ascomycete	Herbivore dung	Classic genetic model with no asexual spores; used to study cytoplasmic inheritance, differentiation and cell death, ageing, multicellularity and epigenetics; close relative of *N. crassa*	2008 (REF. 102)
Schizophyllum commune	Basidiomycete	Widespread	Split-gill fungus that causes white rot; >20,000 different compatible mating types found worldwide	Incomplete[*]
Coprinopsis cinerea	Basidiomycete	Widespread	Inky-cap mushroom with a sexual cycle that is completed in ~2 weeks in the laboratory	Draft sequence annotated[‡]
Heterobasidion parviporum	Basidiomycete	North American and European conifer forests	Root rot fungus and important pathogen of conifers; model system for evolutionary genetics	NA
Magnaporthe grisea	Ascomycete	Widespread	Rice blast fungus that also infects grasses; model system for understanding plant–fungal-pathogen interactions	2005 (REF. 103)
Armillaria gallica	Basidiomycete	Hardwood forests	A 'honey mushroom', one of the oldest and largest living organisms; important model system for evolutionary genetics	NA

[*] Entrez Genome Project *S. commune* entry (see Online links box).

[‡] Broad Institute *C. cinerea* genome project (see Further Information).