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Mixing and matching nuclear envelope remodeling and spindle assembly strategies in the evolution of mitosis

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In eukaryotes, cellular genome is enclosed inside a membrane-bound organelle called the nucleus. The nucleus compartmentalizes genome replication, repair and expression, keeping these activities separated from protein synthesis and other metabolic processes. Each proliferative division, the duplicated chromosomes must be equipartitioned between the daughter cells and this requires precise coordination between assembly of the microtubule-based mitotic spindle and nuclear remodeling. Here we review a surprising variety of strategies used by modern eukaryotes to manage these processes and discuss possible mechanisms that might have led to the emergence of this diversity in evolution.

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Introduction

The nucleus is delimited by the membranous nuclear envelope (NE) containing two apposed lipid bilayers. Of these, the outer nuclear membrane (ONM) is continuous with the endoplasmic reticulum (ER) and the inner nuclear membrane (INM) faces the intranuclear space and organizes an underlying protein meshwork of the nuclear lamina. The two membranes connect at the nuclear pores, communication channels between the nucleus and the cytoplasm decorated by the nuclear pores complexes (NPCs). The symmetrical NPC core is formed by nucleoporins that share common evolutionary roots with membrane-bending vesicle coat proteins. Peripheral Phe-Gly (FG)-repeat nucleoporins residing at the pore aperture ensure selectivity of nucleocytoplasmic transport along with the Ran GTPase system and other soluble factors. Remarkable conservation of most NPC components suggests that the last eukaryotic common ancestor (LECA) already had a functional NE (for reviews see [1–3]).

A number of other proteins and protein complexes contribute to NE function. LINC (linkers of nucleoskeleton and cytoskeleton) complexes made of an INM-anchored SUN and the ONM-bound KASH proteins couple the chromatin to cytoplasmic cytoskeletal arrays and stabilize connections between the two membranes under mechanical load (for review see [4]). The SUN proteins also interact with other INM proteins and the nuclear lamina, which in turn organize chromatin at the nuclear periphery and supports NE structure and function (for review see [5]). Although functionality of the lamina is evolutionarily conserved, its molecular composition can vary substantially between species. Intermediate filaments lamins form the lamina meshwork in metazoans and proteins with similar structural motifs and functions have been recently discovered in other eukaryotic supergroups including protozoans, excavates and plants [6–8]. In organisms that lack lamin-like proteins altogether, lamina functions are likely performed by INM constituents, in particular the LEM-domain proteins. Yeast LEM proteins span the INM twice, with both the chromatin-interacting N-terminal LEM (or, rather its helix-extended loop-helix version) and the C-terminal MSC (MAN1–Src1p–C-terminal) domains facing the nucleoplasm. Proteins with similar architecture are found in all eukaryotic supergroups, indicating their ancient origin. The LEM proteins in yeast support NE integrity, organize chromatin at the nuclear periphery and regulate gene expression [9–14].

Eukaryotic chromosomes are segregated by a bipolar cytoskeletal array called the mitotic spindle. This structure containing microtubules, microtubule motors and other associated proteins, usually assembles at mitotic entry. Various spindle assembly mechanisms observed in different species can be categorized as either intranuclear or cytoplasmic. Intranuclear assembly — which often but not always accompanies a fully closed mitosis — implies that the spindle forms inside an intact nucleus. This requires that the mitotic microtubule organizing centers (MTOCs) are anchored at the inner side of the NE and that the tubulin dimers and microtubule-associated proteins are actively transported from the cytoplasm into the nucleus (for reviews see [15,16]). Conversely, cytoplasmic spindle assembly relies on extranuclear microtubule nucleation and does not explicitly require delivery of the spindle constituents into the nucleus. This mechanism largely — but again not always — necessitates at least

partial NE breakdown to enable access of spindle microtubules to the chromosomes.

The plethora of mitotic programs found in modern eukaryotes can be seen as a combination of these two spindle assembly strategies with distinct modes of NE remodeling (Figure 1) [17,18]. Here we examine natural variability in mitotic NE management focusing on the evolution of NE disassembly. Molecular mechanisms driving NE reformation after exit from mitosis have been recently discussed elsewhere [19,20].

Mitotic NE dynamics in cells with intranuclear spindle assembly

During fully closed mitosis, the NE remains functionally intact, which may limit the availability of cytoplasmic MTOCs for spindle nucleation. Some organisms, such as the excavate *Trypanosoma brucei* solve this problem by building specialized mitotic MTOCs at the inner side of the NE (Figure 1a; see [21] for review). Yet, many other organisms have invented a way to use the same MTOCs to organize cytoplasmic microtubules in interphase and nuclear microtubules in mitosis. The budding yeast *Saccharomyces cerevisiae* permanently anchors its spindle pole bodies (SPBs) within the plane of the NE, so that microtubules can be nucleated at both nucleoplasmic and cytoplasmic sides throughout the cell cycle. Each cell cycle, the SPB duplicates and the daughter SPB is inserted alongside the mother (for review see [22]). Another model Ascomycete, the fission yeast *Schizosaccharomyces pombe* (*S. pombe*), keeps the SPBs at the cytoplasmic side of the NE in interphase and relocates them into the NE for the duration of mitosis, necessitating spatially and temporally confined NE opening, or fenestration (Figure 1b and [23]). Mechanisms underlying MTOC placement within the NE and their regulation by the cell cycle machinery have been extensively discussed [15,24]. While specific requirements may differ between systems, SPB insertion and anchorage overall rely on functional interactions between the transmembrane SPB component Ndc1/Cut11 (incidentally, also required for NPC anchorage) and NE components such as LINCs, the membrane shapers reticulon and Yop1, along with TMEM33 and Brr6 proteins that may aid membrane bending required for insertion by promoting specific lipid composition of the NE [25–32].

Intranuclear assembly of the mitotic spindle does not necessarily mean that the nucleus will remain intact for the duration of mitosis. The fission yeast *Schizosaccharomyces japonicus* (*S. japonicus*), a relative of *S. pombe* [33], starts mitosis in a manner very similar to its sister species but breaks the NE at the nuclear equator in anaphase (Figure 1c and [11,34]). Curiously, the NE remains fully functional up until the moment it breaks, as assessed by fully assembled NPCs and active nucleocytoplasmic transport [35]. Although the mechanism driving the NE breakage remains unknown, it appears to be controlled by the cell cycle machinery and further fine-tuned by interplay between the LEM-domain proteins Lem2 and Man1 and the mitotic spindle [11,12].

Another Ascomycete that assembles the mitotic spindle from NE-embedded SPBs, *Aspergillus nidulans* (*A. nidulans*) also breaks the nuclear membrane in late anaphase [36]. In addition, it loses nucleocytoplasmic compartmentalization in early mitosis, downstream of mitotic CDK and NIMA kinase signaling. In a manner reminiscent of early events in nuclear envelope breakdown (NEBD) in metazoans (see below), the peripheral nucleoporins disperse from the NPCs, disrupting nuclear transport selectivity and presumably allowing tubulin, microtubule-associated proteins and other mitotic regulators to access the nucleoplasm (Figure 1d and [37–39]).

Thus, cells building intranuclear spindles tend to maintain an intact nuclear membrane at least until anaphase. The nucleocytoplasmic transport may be abolished and the membrane may rupture later in mitosis but spindle assembly and kinetochore attachment proceed within the confines of the NE.

Mitotic NE dynamics in cells with extranuclear spindle assembly

This form of spindle assembly is found throughout the eukaryotic domain. At its simplest, the spindle remains cytoplasmic and chromosomes are segregated within an intact NE. In the core dinoflagellate group an extranuclear spindle passes through NE invaginations and makes indirect contacts with the chromosomes attached to the inner side of the NE (Figure 1e). It appears that the kinetochore-like structures may be integrated into the NE [40–42]. If and how accurate chromosome segregation is ensured in cells where microtubules do not establish direct contacts with chromosomes remain unknown.

The flagellated green algae *Chlamydomonas reinhardtii* assembles a cytoplasmic spindle from the pair of polar organizing centers that form around cortex-associated basal bodies. The bulk of the NE remains unbroken but large polar fenestrae form to allow microtubule access to the chromosomes (Figure 1f; for review see [43]). Similar strategy is utilized by the excavate *Giardia intestinalis* that divides its both diploid nuclei simultaneously by basal-body anchored spindles passing through large NE openings [44].

In fact, NE opening at the spindle poles allowing access of microtubules to mitotic chromosomes is widely spread in nature. Basidiomycetous budding yeasts *Ustilago maydis* and *Cryptococcus neoformans* undergo unusual mitoses as compared to their Ascomycete counterparts. In interphase *U. maydis* cells, SPBs lie on the outer side of the nucleus located in the mother cell. As cells enter mitosis, the NPCs disassemble and the nuclear membrane