A Tense Time for the Nuclear Envelope

Minireview

John D. Aitchison[1] and Michael P. Rout[2,3]
[1]Institute for Systems Biology
1441 North 34th Street
Seattle, Washington
[2]The Rockefeller University
1230 York Avenue
New York, New York

When many cells divide, the nuclear envelope poses a problem: the spindle microtubules can't access the chromosomes. Two recent papers in *Cell* describe how the spindle solves this problem by literally pulling open the nucleus at the beginning of mitosis.

Eukaryotic cells have not opted for the simple life. Rather than the carefree open protoplasm of their prokaryotic cousins, they have complicated their lives with elaborate skeletal and membranous structures. The most obvious such membranous structure is the nuclear envelope, which serves to segregate the genetic material from the rest of the cell. But when cells need to divide, this double membrane poses a significant problem: the microtubules of the mitotic spindle, which are organized in the cytoplasm by the centrosome, can't access the DNA to mediate chromosome segregation. It turns out, as described in two papers in a recent issue of *Cell* (Beaudouin et al., 2002; Salina et al., 2002), that the microtubules take matters into their own hands, and literally rip open the nucleus at the beginning of mitosis so that they can orchestrate the allocation of chromosomes to each daughter cell.

The Breakdown of the Nuclear Envelope at Mitosis

The nuclear envelope consists of three membranous domains. The outer nuclear membrane is continuous with the membranes of the endoplasmic reticulum (ER), while the inner nuclear membrane faces the nucleoplasm and chromatin, and is the attachment site for a fibrous support structure termed the nuclear lamina. The pore membrane is found in numerous annular connections joining the inner and outer nuclear membranes; contained within these annuli are the nuclear pore complexes (NPCs), multiprotein superstructures that mediate the exchange of materials between the nucleoplasm and cytoplasm.

After the repair, growth, and duplication of cellular resources, a dynastically minded eukaryotic cell will divide itself into two identical daughter cells to continue the line. This process of mitosis begins at prophase, with the condensation of the cell's chromosomes (present as joined pairs of replicated chromatids) and the formation of a mitotic spindle, needed to correctly segregate the daughter chromatids. During prometaphase, the spindle microtubules attach to the chromatids via their kinetochores, and the nuclear envelope breaks down. The spindle is now free to perform: it aligns the chromo-somes during metaphase, and separates the daughter chromatids at anaphase. At telophase, the nucleus begins to reform, with the disassembly of the spindle, the decondensation of the chromatids, and the reassembly of the nuclear envelope. Finally, at cytokinesis, the formation of the two daughter cells is completed by the equal partitioning of the cytoplasm.

Certainly, metazoan mitosis relies on the dynamic nature of the nuclear envelope. Nuclear envelope breakdown (NEBD) involves the depolymerization of the lamina, the fragmentation and removal of the nuclear membranes from the chromatin, and the disassembly of the NPCs. The lamina in metazoans is composed of the intermediate filament-like proteins, called lamins, that connect with the NPCs and inner nuclear membrane to form a network underlying the nuclear envelope and extending into the nuclear interior. Here, the lamina can help to organize chromatin into functional domains and provide structure to the nucleus (Liu et al., 2000; Wilson et al., 2001). The lamina, and by extension, chromatin, are attached to integral inner nuclear membrane proteins which, along with the integral pore membrane proteins, define the unique composition of the nuclear membranes (Worman and Courvalin, 2000).

There is a large body of evidence that many nuclear envelope-associated proteins are reversibly phosphorylated during mitosis, concomitant with their dramatic redistribution away from the vicinity of the nucleus. Initially, it was thought that these phosphorylation events promote NEBD, leading to the dispersal of the inner nuclear membrane into a discrete population of vesicles (Vigers and Lohka, 1991), but recent work has indicated that the nuclear envelope is not fated to vesiculate. Rather, mitosis involves the redistribution of the nuclear envelope membrane proteins into the ER. Although the ER-nuclear envelope membrane system is continuous, all membrane proteins do not normally freely diffuse within it. Instead, once synthesized, inner nuclear membrane proteins diffuse from the ER through the pores to the inner nuclear membrane where they become trapped, presumably by their interactions with the lamina, chromatin, and each other (Worman and Courvalin, 2000). Similarly, pore membrane proteins are likely to be retained there by interactions with the NPC. Thus, it is proposed that phosphorylation of proteins within the lamina and NPCs during mitosis causes these structures to disassemble and disperse (Collas and Courvalin, 2000; Ellenberg et al., 1997). Phosphorylation also detaches the nuclear envelope membrane proteins from their chromatin and lamina anchor points, allowing them to redistribute back to the ER. So while the nuclear envelope and ER remain as a permanent system throughout the cell cycle, each component loses its identity as their defining protein markers intermingle. However, with vesiculation now an unlikely chief mechanism for NEBD, how else might the nuclear envelope rupture?

New Approaches—New Discoveries

While the fate of the nuclear envelope during mitosis has been studied in some detail for over two decades,

[3]Correspondence: rout@rockvax.rockefeller.edu

only now, with the advent of new technologies and markers of the distinct membrane components, are we beginning to converge on a model that satisfies the numerous observations made over the years. The two reports in *Cell* employ state-of-the-art fluorescence microscopy to reevaluate how cells break down their nuclear envelopes at mitosis. Ellenberg's group (Beaudouin et al., 2002) uses live cell imaging of numerous green fluorescent protein (GFP)-tagged protein markers and novel applications of imaging technologies, while Burke's group (Salina et al., 2002) uses more classical immunofluorescence microscopy methods and deconvolution techniques combined with a thorough analysis of marker proteins. Both methodologies, of course, come with their own strengths and weaknesses. Live cell imaging allows cellular markers to be followed in real time as the processes of mitosis and NEBD unfold; however, overexpression of the fluorescent proteins could lead to artifacts in morphological changes of the nuclear envelope (Ellenberg et al., 1997; Georgatos, 2001). The alternate approach of immunofluorescence microscopy does not allow the temporal progression of processes to be followed in individual cells, and fixation can lead to changes in apparent morphology. However, a wider range of markers can be studied at their normal cellular levels and in a wide range of cell types. The complementarity of these two approaches is underscored by the similarity of many of the two groups' conclusions; remarkably, both arrive at roughly the same novel model for NEBD.

The Morphology of NEBD—A Role for the Nascent Mitotic Spindle

In order to arrive at this new model, both groups carefully followed the behavior of various nuclear-envelope-associated markers at the onset of mitosis. Despite its high order of structural organization, the nuclear envelope is a surprisingly dynamic structure. Both groups showed that in dividing cells, after the beginning of prophase, the nuclear envelope specifically develops a pair of deep invaginations. Nestled within these hollows are the newly duplicated centrosomes, separate but connected to each other by early spindle microtubules that line a furrow in the nuclear envelope (Beaudouin et al., 2002; Salina et al., 2002). Examination by electron microscopy also showed that the invaginations develop numerous microtubule-containing projections (Salina et al., 2002). Both immunofluorescence and live fluorescence imaging showed that these invaginations contain lamins, inner nuclear membrane markers, and nucleoporins. The association of microtubules and centrosomes with the nuclear envelope has been known for some time and folds of this sort were reported long ago (Robbins and Gonatas, 1964), but their functional relevance remained obscure. Georgatos et al. (1997) detected microtubules and centrosomes within the invaginations, which led them to suggest that NEBD occurs when microtubules push on the nuclear envelope until they finally puncture it. The detailed studies by the Ellenberg and Burke groups, however, show that the nuclear envelope does not rupture at the invaginations, but rather appears to be pulled apart at sites distal to them. Indeed, careful measurements of the spatial and temporal order of events demonstrate that NEBD starts with the formation of 1 to 3 holes spanning both mem-

branes of the nuclear envelope; while the integrity of the nuclear envelope/ER lumen is maintained, the integrity of the nucleus itself is disrupted. These holes lead to the catastrophic flooding of the nuclear volume with cytoplasmic components, as detected by the rush of large fluorescent dextran molecules into the nucleus. Hole formation is coincident with loss of the lamina and NPCs, and with a rapid decrease in nuclear volume, due to an acceleration in chromatin condensation. As prophase continues, it appears that the invaginations expand at the expense of the rest of the nuclear envelope, such that some 30% of the nuclear envelope is drawn into the membrane folds surrounding the centrosomes, resulting in a huge hole in the nuclear envelope (Salina et al., 2002).

What could cause this breakage and the formation of the initial hole? One possibility is that that lamina or NPC disassembly continues to weaken the nuclear envelope until it collapses at a random weak point. If this were the case, the position of the NEBD hole should be random on the nuclear envelope. But the current work shows this is clearly not so—the hole is always distal to the centrosomes. Furthermore, during prophase, lamin and nucleoporin turnover is low, and both the lamina and NPCs are quite stable until *after* NEBD (Salina et al., 2002; Beaudouin et al., 2002). Instead, some remarkable timelapse images provide dramatic evidence for a mechanism in which the nuclear envelope is literally torn open by tension produced by the gathering of the nuclear envelope around centrosomes. This was detected by monitoring lamin-GFP labeled nuclear envelopes in live cells undergoing NEBD (Beaudouin et al., 2002). A grid was bleached onto the surface of the lamina, such that the distortion of the grid pattern revealed the distortion of the nuclear envelope surface. Compression of the grid was observed proximal to the centrosomal hollows and the highest tension was observed distal to the centrosomes, at the eventual site of NEBD. This in turn indicated that a mechanical force imposed on the nuclear envelope caused the distortion and ultimately led to breakage of the envelope. It is likely that local parameters affect where the nuclear envelope will ultimately tear, as particular regions of the nuclear envelope may be more susceptible to distortion and breakage. For example, the lack of chromosome contacts in the region of breakage suggests that this may be one factor, possibly because chromosome attachment strengthens the overlying nuclear envelopes. On the other hand, marker analyses using both antibodies and fluorescent chimeras demonstrate that the overall lamina structure is still intact up to the point of NEBD, suggesting that there are no obvious weak spots on the nuclear envelope. However, partial weakening of the lamina through mitotic lamin phosphorylation may not have been detected by these techniques.

What is the source of this mechanical force? The close association between the invaginations and microtubules suggested to both groups that the microtubules themselves might provide the necessary pull. When cells were treated with the microtubule destabilizing drug nocodazole, NEBD became significantly delayed and inefficient, and the nuclear envelope folds were not present, implicating microtubules as the source of this tension. One possible mechanism might be "treadmilling"

of microtubules, leading to the apparent movement of attached components toward their minus ends, found at the centrosome. However, even after holes are formed and NEBD begins, forces continue to pull on the nuclear envelope, as evidenced by the movement of nuclear envelope fragments toward the centrosomes well into prometaphase. Since this movement occurs with the characteristic "stop and go" behavior of microtubule-dependent motors, it seemed possible that motor proteins might attach to the nuclear envelope and track along the microtubules toward the minus end, gathering the nuclear envelope like a curtain along the way. Indeed, Burke's group confirms earlier observations that dynein (a minus-end-directed, microtubule-based motor) is specifically recruited to the mammalian nuclear envelope in late G2 or early prophase, putting it in the right place at the right time (Busson et al., 1997; Salina et al., 2002). Dynein has also been linked to the nuclear envelope in other organisms; it has been detected at the nuclear envelope of *Drosophila* and *Caenorhabditis* cells, where it is required for centrosome attachment to the nuclear envelope and appears to be involved in nuclear movement (Gönczy et al., 1999; Robinson et al., 1999).

Cytoplasmic dynein is a large complex composed of two heavy chains containing the motor domain, and several intermediate chains, light intermediate chains, and light chains. In some cases, dynein attachment to membranes is mediated by the dynactin complex, which interacts with dynein's intermediate chain and a membrane anchor site. To investigate if a dynein-based motor was involved, Burke's group overexpressed the p62 subunit of the dynactin complex, based on the observation that overexpression of this component disrupts ability of the dynactin/dynein complex to interact with membrane cargo. The results of these experiments were very similar to those where the microtubules were disassembled—NEBD was delayed and the invaginations abolished—leading to the conclusion that dynein is the motor protein complex responsible for generating tension on the nuclear envelope.

How does dynein bind specifically to the outer nuclear envelope and how is the force transmitted to inner membrane and lamina so that one membrane doesn't slip against the other? The answers to these questions remain unknown, but the NPCs could potentially provide a solution to both problems. The NPCs provide structural continuity from the outer membrane to the lamina. Furthermore, nucleoporins are the only proteins that distinguish the outer nuclear membrane from the ER, so if dynein were to attach itself to NPCs, then it would be assured of pulling on the correct organelle.

Interestingly, both groups showed that disrupting microtubules or the motor complexes did not shut down NEBD, suggesting that the microtubule-based forces on the envelope are not required, but only facilitate NEBD. One possibility is that in the absence of force generation, lamin phosphorylation still occurs in late prophase, which in turn, leads to lamina disassembly and weakening of the nuclear envelope. The weakened nuclear envelope may then be susceptible to breakage due to normal stochastic tensions. Not exclusive to this suggestion, mitotic phosphorylation of the lamins and NPC components may also disrupt NPCs to the point that they no longer occupy and stabilize the transcisternal

The nucleus, bounded by the nuclear envelope (*gray*), contains NPCs (*large blue disks*), and is continuous with the ER (*gray projections*). The newly-duplicated centrosomes are attached to the microtubules (*green*) and associate with the nuclear envelope.

Dynein motors on the microtubules pull the nuclear envelope towards the centrosomes, creating folds near the centrosomes and stretching the nuclear envelope away from the centrosomes.

The nuclear envelope is eventually pulled open by this motor-generated tension. Disassembly of the lamina (*purple*), NPCs, and inner nuclear membrane structures is initiated by entry of cytoplasm through the opening.

The centrosomes sink into a deep invagination formed from the folds of nuclear envelope gathered by the microtubules.

The tear widens, pulling the nuclear envelope and lamina away from the condensing chromosomes (*orange*). Having been released from their anchoring structures, nuclear membrane proteins (*small blue disks*) are now free to diffuse around the nuclear envelope membranes and out into the ER.

The nuclear envelope remnants surround the centrosomes and continue to dissolve into the ER. Lamina and NPC disassembly completes. The last pieces of nuclear envelope are pulled off the condensed chromosomes, which are now free to align on the forming mitotic spindle.
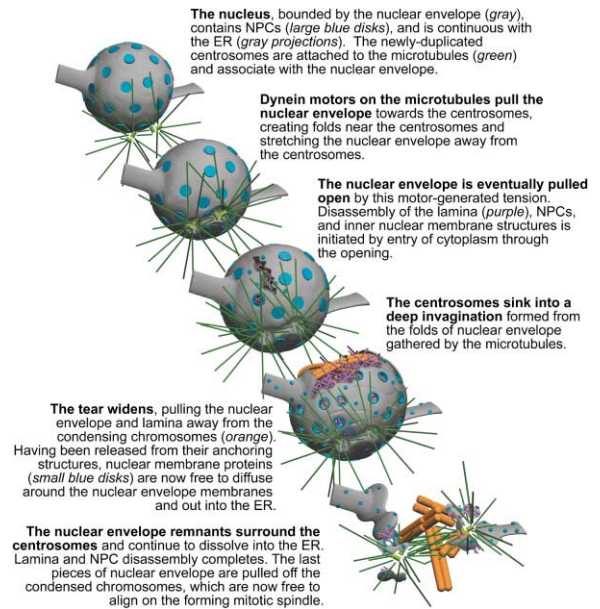
Figure 1. Microtubules Aid Mitotic Nuclear Disassembly

pores. Unstable pores may then expand to generate significant fenestrae in the nuclear envelope analogous to those induced by tension under normal circumstances. Perhaps these fenestrae are where holes begin under normal circumstances and the exact site is determined stochastically at each division. Such a mechanism was recently proposed based on observations of large holes in the nuclear envelope during late prometaphase, like those observed here (Terasaki et al., 2001). But if nuclear envelope breakdown can occur in the absence of microtubules, why go through all the fuss of tearing a hole? Perhaps, as suggested by Ellenberg's group, this mechanism provides a mechanical checkpoint—a warm up of sorts for the big dance of mitosis. Thus, if the centrosomes are not assembled properly and unable to tear the envelope down, it is unlikely that they will perform well in chromosome segregation, which could be disastrous for both daughter cells.

A New Model and New Questions for NEBD

Together, these data suggest a novel model for the role of microtubule-based motors in the breakdown of the nuclear envelope (Figure 1). As cells exit G2 and enter prophase, the centrosomes duplicate and dynein is specifically recruited to the nuclear envelope, perhaps through an interaction with NPCs. The spindle microtubules emanating in both directions then attach to the nuclear envelope through dynein, which, as a minus-end-directed motor, begins to gather the nuclear envelope toward the centrosomes. The integrity of intact NPCs ensures that the outer and the inner nuclear membranes are pulled together. This activity may also serve to help separate the newly duplicated centrosomes by providing a traction surface as the motors pull on the nuclear envelope. The nuclear envelope begins to accumulate at the centrosomes as folds, while simultaneously experiencing tension at the regions distal to the centrosomes. Plus-end microtubule growth (and perhaps a plus-end-directed motor?) may serve to push

the excess nuclear envelope toward the center of the nucleus, thus forming the observed invaginations containing the centrosomes. The increasing tension in the nuclear envelope eventually tears the nuclear envelope and lamina. The flood of cytoplasmic proteins into the nuclear volume then leads to coordinate rapid phosphorylation and disassembly of the lamina and NPC components, and condensation of the chromosomes. There is also a concomitant rapid expansion of the hole(s) over the nuclear surface, and now that the inner nuclear membrane proteins have been freed of their nucleoplasmic attachment sites, the contents of the nuclear envelope and ER membrane systems intermix. Remnants of the nuclear envelope fragmentation that are attached to the developing spindle continue to be pulled toward the centrosomes, until their final disintegration and the maturation of the spindle at the beginning of metaphase.

One of the major unanswered questions raised by these data is the nature of the motor attachment sites. Dyneins use both dynactin-dependent and -independent strategies to interact with their targets. The data from Burke's group suggest that in the case of NEBD, dynactin is important. If this is so, does this mean that spectrin, the dynactin-interacting component on other target membranes (Karcher et al., 2002), provides the binding site at the nuclear envelope? Or does dynactin interact with other sites on target membranes? It seems that motor proteins primarily play roles in improving the efficiencies of molecular movements over those that would otherwise occur stochastically, and in so doing, have adapted to grab onto whatever specific docking protein they can get their hands on, with little regard for their other functions (Karcher et al., 2002). As discussed above, NPCs are the only markers on the outer nuclear envelope that distinguish it from the ER so the NPC is a good candidate target. If this is the case, how is this interaction mediated, and does this suggest that some NPCs are preferred dynein binding sites? NPCs have long been considered a homogeneous population, but recent data suggest that components previously thought to be permanent constituents actually cycle rapidly on and off the NPCs (Daigle et al., 2001; Dilworth et al., 2001). Perhaps some of these dynamic interactors can define different NPC states, and act as specific regulated targets for cytoskeletal elements or motor proteins. If the NPC is *not* the dynein-docking site, however, perhaps the docking proteins will turn out to specifically define the outer nuclear membrane as distinct from the ER.

Finally, is this proposed mechanism of NEBD likely to hold true for all cell types? Probably not. The extent and timing of NEBD varies between cell types; thus, in the *Drosophila* syncitial blastoderm, the proteins of the nuclear envelope do not completely disperse, and in *Saccharomyces*, the nuclear envelope does not break down at all. Here, the entire process, including spindle formation and chromosome segregation, occurs within the confines of the nucleus (Gant and Wilson, 1997). Finally, in *Caenorhabditis*, NEBD is not coordinated with attachment of the microtubule to the chromosomes, but is delayed until anaphase (Lee et al., 2000). It does seem likely, though, that this model will prove to be a variation on a larger theme, in which dynein or other motors are recruited to the nuclear envelope for other mitosis-spe-

cific movements. Perhaps a price eukaryotes paid for using elaborate higher-order nuclear structures to control gene expression was the need to evolve equally elaborate mechanisms to break down these structures during cell division. Undoubtedly, unraveling the details of these variations to reveal the underlying themes of eukaryotic cell division will require the kinds of studies exemplified by the groups of Burke and Ellenberg.

Selected Reading

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