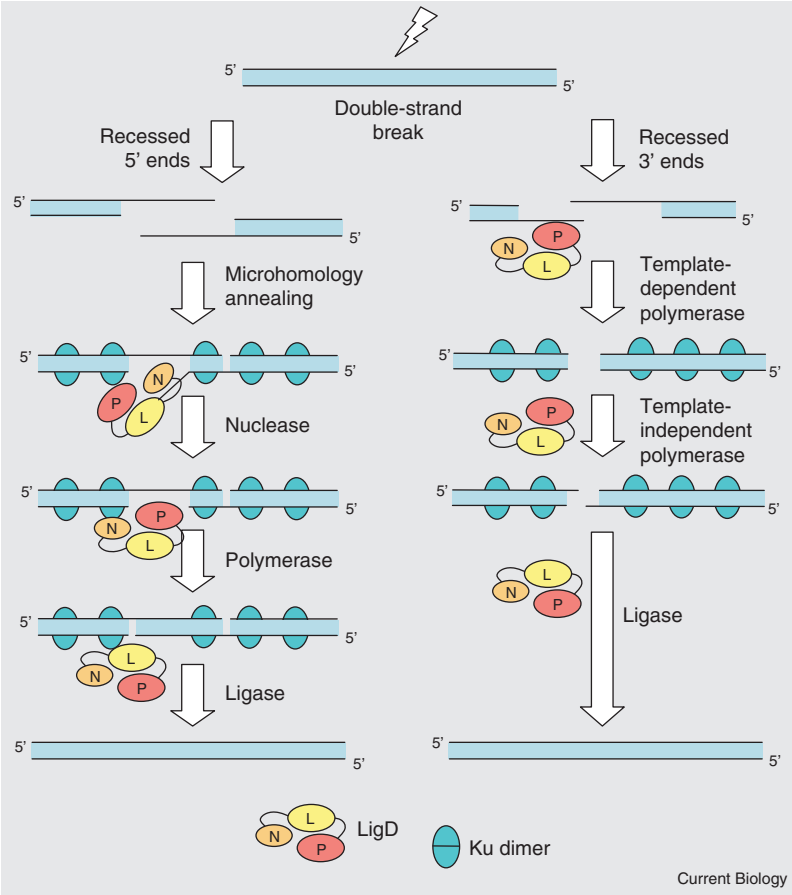

Figure 2. A model for non-homologous end joining in bacteria.

The model is based on biochemical analysis of *M. tuberculosis* LigD protein (left, processing 5′ recessed ends [5]) and *P. aeruginosa* LigD (right, processing 3′ recessed ends [8]). The repair of blunt ends appears to be accomplished by template-independent single-nucleotide addition, followed by ligation (shown as lower parts of 3′ recessed end processing reactions).

break repair by homologous recombination. As noted by Weller *et al*. [5], NHEJ may offer a particular advantage to bacterial species that spend at least part of their life cycle either as spores, like *B. subtilis*, or in stationary phase, like *M. tuberculosis*, where homologous recombination may not be possible.

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Nuclear Envelope: Nuclear Pore Complexity

A new study shows that the filamentous fungus, Aspergillus nidulans, which has a closed mitosis, does not maintain a continuous permeability barrier during mitosis. This work challenges current views of the differences between closed and open mitosis and has implications for understanding mitotic specific changes in the nuclear pore complex and Ran GTPase system in lower eukaryotes.

Shelley Sazer

The presence of a lipid bilayer called the nuclear envelope that keeps the genetic material separate from the rest of the cell distinguishes eukaryotes from prokaryotes. Nuclear pore complexes (NPCs) traverse the nuclear envelope and form channels that allow the diffusion of small molecules and the selective transport of larger molecules between the nucleus and the cytoplasm (reviewed in [1]). The asymmetric distribution of the GTP-bound form of the Ran GTPase across the nuclear envelope is essential for regulation of nucleocytoplasmic

transport and other aspects of mitosis (reviewed in [2]. Plants and animals, so-called 'higher' eukaryotes, undergo an 'open'mitosis (Figure 1), in which the nuclear envelope and the NPC break down as cells enter mitosis and then reform after chromosome segregation in a Ran-GTPase-dependent manner. Any interphase-specific accumulation of soluble proteins in the nucleus would thereby be lost, with critical consequences for the execution and regulation of mitosis-specific processes.

Many 'lower' eukaryotes, including yeasts and other fungi, undergo a 'closed' mitosis (Figure 1), in which chromosome segregation takes place within the confines of the nuclear envelope, which remains intact throughout the entire cell cycle [3]. Between these two extremes lie a continuum of mechanisms in which the envelope becomes porous, either transiently to allow the mitotic spindle access to the chromosomes [3], or completely at late stages of mitosis, as in organisms such as *Caenorhabditis elegans* [4] and some filamentous fungi [3].

The difference between open and closed forms of mitosis has important consequences for the regulatory and structural changes that take place at this critical stage of the cell cycle. But is closed mitosis fundamentally different from open mitosis? There has been speculation that, although the nuclear envelope remains intact during closed mitosis, it may undergo a change in permeability at mitosis. In the fission yeast, *Schizosaccharomyces pombe,* and the budding yeast, *Saccharomyces cerevisiae,* there is no evidence so far that soluble proteins, including the Ran GTPase and its regulators, equilibrate across the nuclear envelope at mitosis.

A study published recently in *Current Biology* [5] now provides evidence for a transient change in nuclear envelope permeability in the filamentous fungus, *Aspergillus nidulans*, which undergoes closed mitosis. This study shows that, at mitosis, NPC proteins, including SONBn^{Nup98} and SONA^{Gle2}, lose their association with the pore and that this reorganization correlates with changes in the localization of several transport cargo proteins and RanGAP, the GTPase activating protein of Ran.

The Nuclear Pore Complex

In a monumental undertaking, the constituents of the budding yeast NPC were identified, characterized and localized within the structure by Rout and colleagues in 2000 [6]. The animal cell nuclear pore complex consists of similar proteins in roughly the same arrangement and structure (reviewed in [2,7]).

Our understanding of the NPC as a static structure changed significantly when it was shown that, in animal cells, some constituents cycle on and off the pore during interphase (reviewed in [7,8]). A more recent paper [9] reported a range of residence times amongst 19 components of the animal cell NPC, spanning more than three orders of magnitude. Proteins of the Nup107–Nup160 complex, known to form the core of the nuclear pore and to nucleate NPC assembly [10,11], have long residence times at the pore, whereas for more peripheral proteins, including Nup98, the residence times are much shorter.

The NPC undergoes functional changes brought about by phosphorylation [11] and structural rearrangements in response to passage through specific stages of the cell cycle (reviewed in [12]), to changes in the Ran GTPase system [13] or to external growth conditions [14]. The nuclear pore also has functions unrelated to nucleocytoplasmic transport (reviewed in [7]).

The Osmani laboratory [15] has previously shown a genetic interaction between nuclear pore proteins, SONBn^{Nup98} and SONA^{Gle2}, and the two cell-cycle kinases required for mitotic entry in *A. nidulans*, NIMA and the NIMX^{cdc2}–NIME$^{cyclin\ B}$ complex called Cdk1. These two kinases, and the tubulin subunits of the spindle microtubules [16], relocalize to the nucleus at mitosis in this filamentous fungus, which undergoes a closed mitosis. This group has also shown that NIMA phosphorylates SONBn^{Nup98} and SONA^{Gle2} at mitosis [15]. They now report [5] that GFP-tagged versions of SONBn^{Nup98} and four other NPC components dissociate from the NPC at mitosis, while other components remain in place [15].

In starfish oocytes, partial disassembly of the NPC by loss of peripheral components early in mitosis changes the permeability barrier of the intact nuclear envelope [17]. In *A. nidulans*, the dramatic reorganization of the NPC at mitosis allows RanGAP to relocalize from the cytoplasm to nucleus, where it may dissipate the Ran–GTP gradient. Together, these changes in NPC composition and the Ran GTPase system alter the intracellular localization of both nuclear and cytoplasmic proteins.

The Ran GTPase System

One critical regulatory system compartmentalized by the nuclear envelope is the Ran GTPase (reviewed in [3]). Ran's guanine nucleotide exchange factor (GEF), which catalyzes conversion of Ran from its GDP- to GTP-bound form, is an exclusively nuclear, chromatin-associated protein. RanGAP, which stimulates the low intrinsic GTPase activity of Ran, converting it from its GTP- to GDP-bound state, is cytoplasmic.

This asymmetric distribution of its regulatory proteins results in an enrichment of Ran in its GTP-bound form inside of the nucleus. The Ran–GTP gradient across the nuclear envelope is essential for nucleocytoplasmic transport. Cargoes destined for the nuclear interior form stable complexes with their transport carriers in the cytoplasm and are dissociated from these carriers in the nucleus. The opposite is true for nuclear protein export, in which cargo forms a stable complex with its carrier inside of the nucleus in the presence of Ran–GTP but dissociates when the GTP is hydrolyzed in the cytoplasm by RanGAP.

The dramatic loss of nuclear compartmentalization in open mitosis dissipates the Ran–GTP gradient across the nuclear

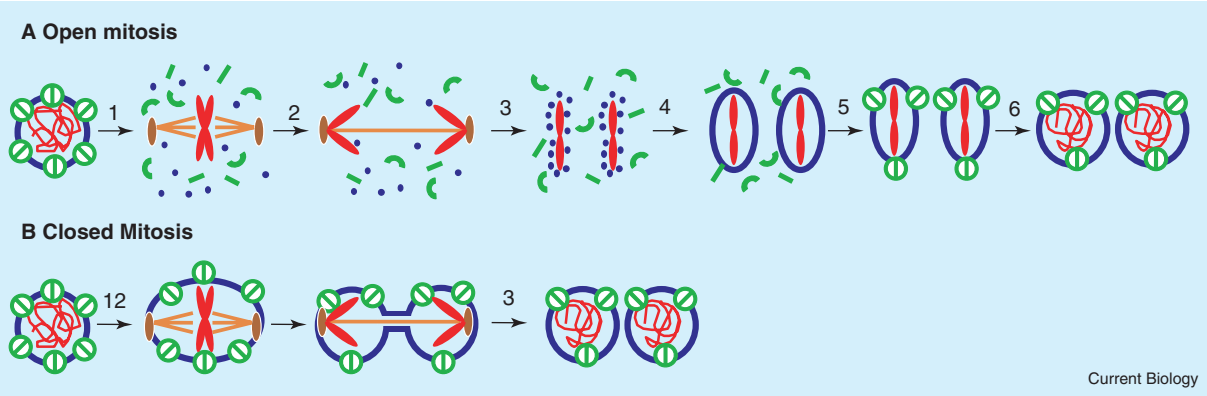

Figure 1. Mitosis-specific nuclear envelope changes in the open mitosis of higher eukaryotes and closed mitosis of lower eukaryotes.

(A) Nuclear envelope breakdown and reformation in the open mitosis of *Xenopus laevis* extracts. (1) In interphase, the nuclear envelope (blue), which is penetrated by nuclear pore complexes (green), surrounds the decondensed chromatin (red). At the onset of mitosis, the nuclear envelope breaks down and nuclear pore complexes disassemble. The chromosomes condense and the mitotic spindle (orange) is nucleated from the centrosomes (brown). (2) The mitotic spindle separates the duplicated sets of chromosomes. (3) Nuclear envelope vesicles are targeted to the condensed chromosomes after telophase. (4) The vesicles fuse to form a continuous membrane around the chromosomes. (5) Nuclear pore complexes are assembled in the nuclear envelope. (6) The chromosomes decondense and the nucleus expands. (B) Nuclear division in the closed mitosis of yeast. (1) In interphase, the nuclear envelope (blue), which is penetrated by nuclear pore complexes (green), surrounds the decondensed chromatin (red). At the onset of mitosis the chromosomes condense and the mitotic spindle (orange) is nucleated from the spindle pole bodies (yeast centrosome equivalents; brown) which are embedded in the nuclear envelope. (2) The mitotic spindle separates the duplicated sets of chromosomes and the nucleus changes from oblong to dumbbell shaped. (3) The two daughter nuclei separate from one another and the chromosomes decondense.

envelope, but a Ran–GTP gradient is established immediately surrounding the condensed mitotic chromosomes (reviewed in [3]). The establishment of this mitotic gradient depends on the chromatin association of RanGEF and the presence of a soluble pool of RanGAP.

The chromosome-based gradient is important for several mitosis-specific processes in higher eukaryotes, including mitotic spindle formation, kinetochore microtubule association and spindle checkpoint function (reviewed in [3]). Is it possible that dissipation of the Ran–GTP gradient across the nuclear envelope is also necessary in organisms that undergo closed mitosis?

De Souza *et al*. [5] report that, in the closed mitosis of *A. nidulans*, the composition of the NPC changes and RanGAP, which is confined to the cytoplasm in interphase, enters the nucleus [1]. The mitosis-specific entry of RanGAP to the nucleus may convert nuclear Ran–GTP to Ran–GDP, except in the immediate vicinity of the chromosomes. This situation resembles, in many respects, the changes that occur during the open mitosis of higher eukaryotes. It will be important to determine whether a chromosome-based Ran–GTP gradient is established at mitosis in *A. nidulans* and in other lower eukaryotes that undergo a closed mitosis.

The dissipation of the Ran–GTP gradient across the nuclear envelope in *A. nidulans*, in which the nuclear envelope and NPCs are intact, would be expected to disrupt all aspects of Ran-dependent nucleocytoplasmic transport. However, under these conditions, the intracellular localization of some, but not all, normally compartmentalized proteins is altered at mitosis. This suggests that structural changes in the NPC at mitosis alter the specificity of nucleocytoplasmic transport, but do not make the pore permeable to all substrates. This might be caused by perturbations in the specific interactions between various transport factors and individual components of the NPC, as has previously been shown in other systems (reviewed in [7]).

Conclusions

De Souza *et al*. [5] have discovered mitosis-specific partial disassembly of the NPC in the filamentous fungus, *Aspergillus nidulans* [1], which undergoes a closed mitosis in which the nuclear envelope remains intact. This change in NPC composition changes the permeability of the nuclear envelope at mitosis, blurring the distinction between open and closed mitosis.

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Animal Evolution: The Enigmatic Phylum Placozoa Revisited

A recent report of high levels of genetic variation between strains of *Trichoplax adhaerens* challenges the traditional view that the phylum Placozoa comprises only one species. At the morphological level, placozoans are amongst the simplest extant animals, but molecular evidence suggests that they may have more complex origins.

David J. Miller[1*] and Eldon E. Ball[2]

Often described as the simplest known animal, the unassuming marine placozoan *Trichoplax adhaerens* is one of a handful of 'lower' metazoans that have so far defied being pigeonholed.

The history of *Trichoplax* and its relatives has the elements of a scientific mystery story (summarized in [1]). In 1971, Karl Grell [2] formally described a new Phylum, the Placozoa, to accommodate two species that had been reported a hundred years earlier. These were originally greeted with excitement as 'living fossils' representing the ancestral animal morphology. However, the suggestion that they were, in fact, modified cnidarian larvae prompted a loss of interest for the next fifty years. One of the species upon which Placozoa was founded, *Treptoplax reptans*, has never been seen since its original description, and is assumed not to exist; *T. adhaerens*, on the other hand, appears to be widely distributed and relatively common in warm marine environments [1]. However, other than field surveys [3], all that is known about it is based on aquarium cultures.

Although *T. adhaerens* was until now the sole recognized species in the phylum Placozoa, the levels of molecular heterogeneity reported by Voigt *et al.* [4] reported in a recent issue of *Current Biology* imply that what has previously been considered one species may actually be several. Cryptic molecular diversity thus underlies the apparently uniform morphology of placozoans and, as the majority of the cell biological studies to date have been based on a single isolate from the Red Sea, this study highlights the need for further research on this enigmatic group of animals.

Trichoplax Biology

In culture, individual *Trichoplax* are flat and irregular disc-like animals a few millimeters in diameter (environmental isolates are often smaller) and 10–15 μm thick (Figure 1A). Although molecular studies point to additional cellular complexity (see below), *Trichoplax* has been repeatedly described as comprising just four cell types arranged in three layers — an upper and a lower epithelium separated by the 'fiber cell' layer (Figure 1B). The latter has a syncytial organization and its contractile properties are often assumed to be responsible for the amoeba-like changes in shape. The upper layer consists of monociliated 'cover' cells, whereas two cell types make up the lower epithelium — gland cells, which are non-ciliated and thought to secrete digestive enzymes, and ciliated 'cylinder' cells that may be adhesive and capable of resorbing digestion products [1]. Little is known about the natural diet of *Trichoplax,* although it is assumed to consist of micro-algae and organic detritus. In culture, they have been maintained for years on a diet of *Cryptomonas*, which are more or less dissolved upon contact with the gland cells. The morphology of the cylinder cells indicates that they are responsible for uptake of the dissolved nutrients. *Trichoplax* sometimes elevate their center from the substrate to form one or more digestive bags, and on glass substrates they frequently leave behind an area that is cleared of everything edible.

Is *Trichoplax* Secondarily Simple?

Although it would be hard to imagine a simpler animal than *Trichoplax*, it is unclear whether it had more complex ancestors, or whether its simplicity reflects its humble origins. *Trichoplax* has some of the morphological characteristics that are considered to define higher