Nuclear migration: **Cortical anchors for cytoplasmic dynein**

Kerry Bloom

Nuclear migration in yeast provides a model system for studying how a cell polarizes the actin and microtubule cytoskeletons toward sites of cell growth. Recent findings indicate that cortical anchors are necessary for directing microtubule-based processes.

Address: Department of Biology, University of North Carolina at Chapel Hill, North Carolina, USA.
E-mail: kbloom@email.unc.edu

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The nucleus is not just a passive body that rolls around at random inside the sack of a eukaryotic cell. Controlled nuclear movements are important in a number of contexts — for example, during very early *Drosophila* development, where they play a key role in establishing oocyte polarity, and in the yeast *Saccharomyces cerevisiae*, where they are required during budding. Nuclear migration in budding yeast was first proposed by Hartwell *et al.* [1], more than a quarter of a century ago now, to be under genetic control by "the same pathway as bud emergence and subsequent to it on this pathway". How prescient they were. Nuclear migration in budding yeast is indeed under genetic control, and dependent on many of the same proteins required for bud-site selection. With new results on a protein known as Num1, the mechanism of nuclear migration is becoming increasingly clear.

It is justice that Num1 receives this attention, as this is the protein defective in one of the first mutants identified in the nuclear migration pathway — Num1 is named for nuclear migration [2]. The *NUM1* gene encodes a complex, 313 kDa protein which has pleckstrin homology domains, twelve near-identical 64 residue repeats and putative Ca^{2+}-binding domains. The *num1* mutant was isolated independently in two other genetic screens: as *rvs272* [3], for the reduced viability upon starvation that the mutant exhibits; and as *pac12* [4], as the mutant cells perish in the absence of Cin8, a kinesin motor protein of the BimC class. Recent studies [5,6] indicate that Num1 is the cortical anchor for the motor protein dynein, and provide a critical link in understanding the basis of nuclear migration in yeast.

The nuclear migration pathway

In budding yeast, the microtubule organizing center — known as the spindle pole body — is embedded in the nuclear envelope, where it nucleates spindle microtubules from the inner spindle plaque and astral cytoplasmic microtubules from the outer plaque. Astral microtubules, together with microtubule-based motor proteins, the actin cytoskeleton and cell-polarity determinants, orchestrate nuclear movements to and through the neck of budded cells (Figure 1). To understand this process fully we must consider nuclear dynamics, polarity of the actin cytoskeleton and microtubule dynamics. There are two major movements in nuclear migration during yeast budding: one is the pre-anaphase alignment of the nucleus along the mother–bud axis and positioning at the neck, and the second is the post-anaphase propulsion of the daughter nucleus through the neck of budded cells.

Alignment and positioning

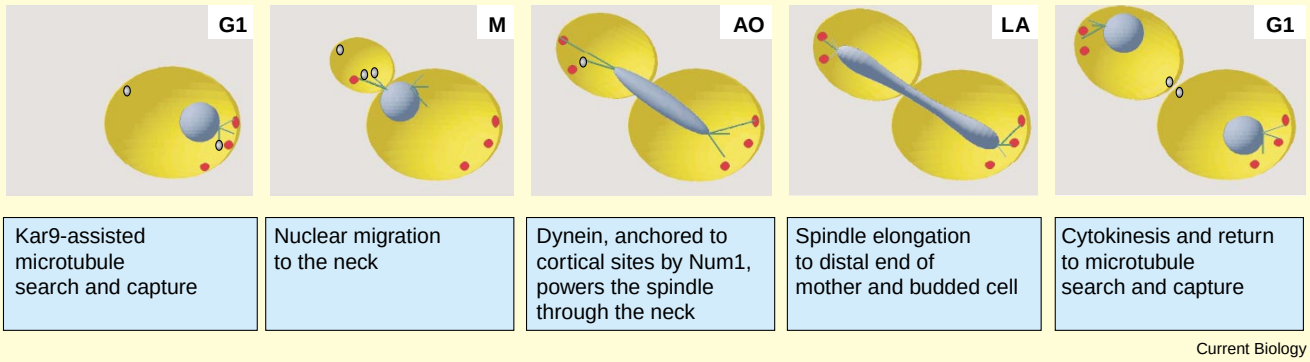
The alignment of the yeast cell nucleus along the mother–bud axis, and its movement to the neck, requires an intact actin cytoskeleton. Filamentous (F) actin provides the spatial cues that direct the nucleus toward the bud and sites of polarized growth. This mechanism is mediated through a protein known as Kar9. Kar9 was identified by virtue of the karyogamy defect exhibited by *kar9* mutant cells [7] — the delay in migration and fusion of the nuclei of mating mutant cells. Kar9 is capable of binding microtubules via Bim1 [8,9], a homologue of the mammalian microtubule-associated protein EB1, and actin via the type V myosin Myo2 [10]. Kar9 thus provides a critical link between the actin and microtubule cytoskeletons.

Initially seen as a discrete spot in mother cells, Kar9 facilitates microtubule penetration into the bud; when in the bud, Kar9 can 'capture' the plus-ends of microtubules and promote nuclear movement to the bud neck (Figure 1). Nuclear migration to a Kar9 spot has recently been visualized in live cells [11] and is associated with microtubule shortening. It has been proposed that the kinesin Kip3, by stimulating plus-end microtubule disassembly, provides the motive force for this step in nuclear migration. Furthermore, the Kar9 spot is anchored at the bud tip, via proteins Bni1 and Bud6 [11,12]. Bni1 and Bud6 have been proposed to provide a cortical scaffold for a variety of processes, including nuclear migration, RNA localization and now Num1 localization (see below). The forces produced by Kar9 and associated proteins are not sufficient for nuclear translocation through the neck, though overproduction of Kar9 does lead to premature migration of the nucleus through the neck [9].

Nuclear translocation during anaphase

Efficient nuclear translocation through the bud neck requires cytoplasmic dynein [13,14]. Dynein is responsible for pronounced spindle oscillations at the neck of a

Figure 1



Num1 anchors cytoplasmic dynein and contributes to spindle elongation in anaphase. The nucleus (blue sphere) is propelled by astral microtubules (green lines) pushing against the cell periphery (G1). Cytoplasmic dynein fused to GFP (not shown) decorates the astral microtubule lattice. Migration to the neck of budded cells is facilitated by Kar9 (gray spheres in G1 and M phase cells), which serves as a linker between actin and microtubule cytoskeletons (see text). Num1 (red sphere) is present in the cortex of unbudded cells, and appears in the bud of medium to large budded cells (M phase and anaphase onset, AO). Num1 is a cortical protein that binds tubulin and dynein (AO and late anaphase, LA). If dynein is immobilized by Num1, the minus-end directed translocation of microtubules by dynein would result in movement of the spindle pole and nucleus to cortical sites (late anaphase). Upon spindle disassembly, astral microtubule growth propels the nucleus for the next cycle.

budded cell, and contributes to the forces required to pull the nucleus and chromatin DNA through the aperture between mother cell and bud [15]. Dynein is also required for the prominent microtubule sliding that is seen at this stage of the cell cycle [16]. Dynein is symmetrically distributed along the length of both mother and daughter cell microtubules, and is unlikely to provide directional cues itself. The challenge in the field has been to understand how dynein generates any motive force, and in particular how it is responsible for microtubule sliding along the cortex.

There are numerous protein candidates that anchor dynein to cortical (or other) sites. The most notable include the intermediate and light chains of the dynein complex itself, and components of the dynein-associated dynactin complex — in particular, Nip100 (p150), Jnm1 and Act5 (Arp1). Unfortunately for these models, the dynein heavy chain, Dhc1/Dyn1, localizes to the cytoplasmic microtubules, and despite the effort of several laboratories, there is no evidence for the localization of dynein to the cortex in yeast. Similarly, several dynactin components have been localized to the spindle pole body [17,18]. Dynactin at the pole may mediate interactions between dynein and components at the neck, but this does not help us understand dynein's role in microtubule sliding along the cortex.

Cortical anchors for dynein

Num1, like many of the proteins involved in nuclear migration, contributes to the efficiency of nuclear migration but is not required for cell viability. It has taken careful inspection in live cells, protein localization using green fluorescent protein (GFP) fusions and studies of protein–protein interactions to reveal the role of Num1 in nuclear migration. The Num1 protein is initially localized in the cortex of the mother cell [19]. But using Num1–GFP fusions, two groups [5,6] have recently observed Num1 accumulation in the bud of large budded cells. Num1–GFP first appears in medium-sized buds as stationary cortical spots [5]. The sessile nature of Num1, as well as its predominance in the mother cortex, distinguishes it from other proteins implicated in nuclear migration and cell polarity, including Bud6, Bni1 and Kar9.

Num1 is thus well positioned to be a cortical anchor for cytoplasmic dynein. Direct evidence supporting this idea has come from analysis of microtubule sliding [16]. Microtubule sliding along the cortex, as visualized with tubulin–GFP, is abrogated in the absence of functional Num1 [5]. Genetic interactions confirm that *num1* mutants behave as if they are missing dynein function [6]. In particular, *num1 dynein* double mutants behave like either single mutant, and conversely the double mutants *num1 kar9*, *num1 bni1* or *num1 kip3* behave, respectively, like *dynein kar9*, *dynein bni1* or *dynein kip3* [6,12,15,20]. These results place Num1 on the dynein pathway of nuclear migration.

What then is the specific evidence that Num1 provides a cortical anchor for dynein? Direct physical interactions between Num1 and components of the dynein complex were examined by co-immunoprecipitation experiments [6]. Num1 was found to co-immunoprecipitate with the dynein intermediate chain Pac11, and with the alpha tubulin Tub3. Furthermore, Num1 co-immunoprecipitates with Bni1 and Kar9. The interactions with Pac11 and

Tub3 indicate that Num1 may indeed provide the anchor for dynein in the cortex. The functional significance of Num1's apparent interactions with Kar9 and Bni1 is less obvious; consistent with this finding, however, is the observation that Num1–GFP localization is dependent on *BNI1*, in particular Num1 was seen to relocalize from the tip of the bud to the neck in *bni1* mutants.

These observations support the proposed roles for Bud6 and Bni1 as components of a general cortical scaffold, which perhaps now should include Num1 as a specific effector for dynein. The relocalization of Num1 from the tip of the bud to the neck in *bni*1 mutants mirrors the similar relocalization of Bud6 in *bni1* cells [21]. Loss of Bni1 is accompanied by increased microtubule interactions with the neck region [21]. Thus, microtubule interactions are dependent upon Bni1 anchoring Bud6 and Num1 to the bud tip. The default position for Bud6 and Num1 in *bni1* cells may be the neck, which is possibly indicative of secondary anchors at this site.

The second indication that Num1 has a role in anchoring dynein is the loss of dynein-dependent spindle oscillations in *num1* mutants. In wild-type cells arrested with the DNA synthesis inhibitor hydroxyurea, the nucleus becomes closely apposed to the neck, whereas it is displaced from the neck in hydroxyurea-treated *num1* cells [3]. Similarly, nuclei are displaced from the neck in dynein mutants treated with hydroxyurea [14]. While there may be secondary anchors at the neck for dynein, these data are consistent with the idea that Num1 and dynein contribute to dynein-dependent nuclear motility prior to the onset of anaphase.

How does an anchor embedded in the cortex facilitate microtubule sliding along the cortex? The inferred dynein localization to microtubules has to be cautiously interpreted, given that the observations are based on GFP fusion proteins and the attached GFP could disrupt dynein's normal interactions. The interactions between dynein and Num1 may be very transient, with dynein generating its power stroke while engaged with Num1. The problem is the relatively sparse distribution of Num1 in the bud, and moreover its prominence at the tip. Much of the reported microtubule sliding can be seen along the bud cortex, indicating either that active Num1 is more widely distributed in the bud, but generally below the limits of detection, or that a few contact sites in the cortex suffice to anchor the motor.

Num1's localization at the bud tip as well as in the mother cell indicates that it might have two functional states: a Bni1-dependent state at the bud tip, and a Bni1-independent state in the mother cell. The tip-localized Num1 could serve as the anchor for dynein and facilitate spindle elongation. The mother-cell-localized Num1 may be inactive until anaphase onset, or perhaps in a different (Bni1-independent) conformation and serve a different role in unbudded cells.

Two structural features of Num1 suggest that the mother-bound form of the protein may have cortex-binding sites. Firstly, the pleckstrin homology domains may be important for interactions with the membrane lipid phosphatidylinositol-4,5-bisphosphate, and Num1 has been shown to physically interact with phospholipase C [22]. And secondly, the twelve 64-residue repeats, which resemble the partially conserved tetratricopeptide repeats (TPRs) found in members of the anaphase-promoting complex, suggest that Num1 may be part of a larger multisubunit complex. At least one of these repeats is required for exogenous Num1 to suppress the nuclear migration defect of *num1* mutants [19], and while the repeat number varies in different yeast strains, ranging from 1–24 copies, all variants contain at least one repeat.

While the mechanism of action of Num1 remains elusive, the discovery that this protein is localized in the yeast cell bud, and careful examination of the *num1* mutant phenotype, have revealed an important player in nuclear positioning and migration to the bud. The field can now turn its attention to how cytoplasmic dynein, bound along dynamically growing and shortening microtubules, gets 'captured' by the Num1 anchor and powers the nucleus toward its destiny in the bud.

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