N Nuclear Migration: From Fungi to the Mammalian Brain

N. Ronald Morris, M.D.

Department of Pharmacology, University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, New Jersey 08854

Textbooks represent the animal cell nucleus as a sort of cellular Jabba the Hutt, torpidly enthroned in the center of the cell. In fact nothing could be farther from the truth. The nucleus more closely resembles Luke Skywalker, the hero of Star Wars, in its ability to move about in its cellular universe. Instances of nuclear motility are found throughout biology. Indeed, nuclear migration appears to be required for the proper growth and development of essentially all eukaryotes. Some well known examples, i.e., those in textbooks, are the congression of male and female pronuclei during fertilization, the movement of nuclei to the egg cortex during embryogenesis in Drosophila melanogaster, and during karyogamy and the migration of the daughter nucleus into the bud in Saccharomyces cerevisiae. Innumerable other nuclear motility events have been described in animals, plants, insects, algae, and fungi. However, until relatively recently, little was known about the mechanism of nuclear migration, except that it required microtubules (MTs). A recent excellent recent review focuses particularly on how MTs exert forces on nuclei (Reinsch and Gonczy, 1998). The focus of the present review will be on the contribution of genetic systems to our understanding of nuclear migration. The early work on the genetics of nuclear migration came from three “simple” organisms, the yeast S. cerevisiae, and two filamentous fungi, A. nidulans and A. fumigatus, and possibly man. Nuclear migration has been studied in relation to karyogamy and migration of the daughter nucleus into the bud in budding yeast; in relation to the migration of nuclei through the mycelium in the filamentous fungi; during migration to the cortex in fly development; and during cell specific migrations in worm development. Similarities between the NUDF nuclear migration protein of A. nidulans and LIN51, a protein required for neuronal migration in the brain, have led us to suggest that nuclear migration is also a feature of brain development.

Nuclear Migration in Yeast

Our knowledge of nuclear migration in yeast comes from genetic and morphological studies of mating and mitosis in S. cerevisiae. Mating yeast respond to each other by forming pear-shaped, stem-end-opposed cells termed shmooes. In the first stage of mating, astral MTs emanating from the spindle pole body (SPB), a microtubule organizing center embedded in the nuclear envelope, are oriented to the shmoo tip. This occurs by a random search and capture mechanism that requires Kar3p kinesin and Kip3p, which is a MT orientation protein whose position at the shmoo tip depends on cell polarization proteins (actin, Bni1p, Spa2p, Pea2p, and Bud6p; Lee et al., 1999; Miller et al., 1999a). Direct observations of nuclear movements during karyogamy show that they closely track the growth and shortening of the shmoo tip MT bundle (Maddox et al., 1999), raising the possibility that MT dynamics provides the motive force that moves the nucleus. As the nuclei move toward the shmoo tips, the tips fuse, and the shmoo tip MT bundles fuse to form an intranuclear bundle, which progressively shortens to mediate nuclear congression (Maddox et al., 1999). These nuclear movements are affected by mutations in Kar3p, which is a microtubule-dependent motor that also depolymerizes MTs, and other proteins that influence astral MT dynamics (Cin1p, Cin2p, and Cin4p). See review of karyogamy by Rose, 1996. Whether Kar3p functions primarily as a motor or as a mediator of MT dynamics (or both) is unclear.

During mitosis a much larger set of proteins moves the nucleus to the bud neck and into the bud, including three kinesins (Kar3p, Kip3p, and Kip2p) and cytoplasmic dynein (CD) plus dynactin (Eshel et al., 1993; Li et al., 1993; Cottingham et al., 1999; Kahana et al., 1998; Miller et al., 1998). There is substantial functional overlap among CD, Kar3p, and Kip3p. Deletion of any one of them has little effect, but deletion of more than one has an increasingly severe, deleterious effect on both nuclear positioning and cell viability. This is not necessarily related to loss of their motor functions because, like Kar3p and Kip3p, deletion of CD heavy chain causes an increase in astral MT length that is intensified by deletion of Kip3p. Since their loss increases MT length (i.e., stability), these proteins must normally act to destabilize MTs in vivo. In contrast, deletion of Kip2p increases astral MT length, reverses the MT length abnormalities caused by deletion of the CD heavy chain, and Kip3p and Kip3p, and suppresses the effects of these deletions on...
nuclear positioning. Loss of Bim1p, which also affects MT stability, has similar effects (Schwartz et al., 1997; Tirnauer et al., 1999). These observations and other related pieces of genetic and pharmacological evidence suggest that astral MT dynamics play a significant role in nuclear positioning. Time-lapse fluorescence microscopy studies of GFP-tagged MTs showed that nuclear movements during mitosis, as well as karyogamy, mirror the growing and shrinking rate of astral MTs attached to the cell cortex, consistent with the idea that dynamic MTs interacting with the cell cortex mediate nuclear movement (Carminati and Stearns, 1997; Shaw et al., 1997; Maddox et al., 1999). Kar9p, which during mitosis orients astral MTs to the bud tip (Miller et al., 1999a), and Num1p, which is associated with the mother cell cortex, are also required for nuclear positioning, apparently by providing targets for MT capture by the cortex (Farkasovsky and Kuntzel, 1995). A II of the motors involved in S. cerevisiae nuclear movements are probably now known, but how they are targeted to specific locations in the cell, how their activities are regulated and coordinated, and how they actually generate force are still incompletely understood.

Nuclear Migration in Filamentous Fungi

In comparison to the short range migrations seen in yeast, nuclear migration in the filamentous fungi can be a long-range process, sometimes very long-range, as some fungal colonies are miles in diameter (Smith et al., 1992). As the fungal colony grows, the nuclei migrate through the cytoplasm toward the advancing hyphal tip. Early observers noted that nuclei appeared to be pulled from a point on the cell surface, which is now known to be the SPB. Observations on living fungi show that the nuclei move after mitosis, then migrate in the same direction, but at different rates, towards the hyphal tip, resulting in a relatively even distribution along the mycelium (Suelmann et al., 1997). Differential laser ablation of spindle versus astral MTs in Fusarium solani and Nectria haematococca revealed that a tractive force on the SPB MTs separates the nuclear masses during anaphase B of mitosis (Aist et al., 1991). The pulling force that moves interphase nuclei through the fungal cytoplasm is thought to be a continuation of this process. Laser tweezer experiments showed that nuclei of N. haematococca are anchored in place during interphase and that this, like the anaphase B force, depends on CD (Inoue et al., 1998). Unlike the situation in yeast, deletion of the CD heavy chain causes a decrease in the number and length of the SPB MTs in Nectria. Because the astral MTs are decreased in the absence of CD, one cannot in this case conclude that the anchoring force is related to CD motor activity.

As in yeast, genetic studies have identified many of the proteins required for nuclear migration in the filamentous fungi (Morris, 1975; Plamann et al., 1994; Xiang et al., 1994, 1999; Bruno et al., 1996; Inoue et al., 1998; Minke et al., 1999a). The first nuclear migration mutants were a byproduct of a mitotic mutant search in A. nidulans 25 years ago (Morris, 1975). These were termed nud (for nuclear distribution) mutations. In N. crassa similar mutations were termed roopy (ro) because the hyphae resemble intertwined rope strands. Phenotypically, the nud and ro mutants are characterized by a strikingly uneven distribution of nuclei along the mycelium (Fig. 1). Both grow slowly, branch excessively, and sporulate poorly. The A. nidulans apsA (which encodes a protein similar to Saccharomyces cerevisiae NUM1p) and apsB mutants, initially identified as sporulation defective, also affect nuclear distribution (Clutterbuck, 1994; Fischer and Timberlake, 1995). Many of the nud and ro genes encode subunits of CD or of dynactin (Table I), including the heavy, intermediate, and light chains of CD and the p150<sup>glued</sup> and A R P1 subunits of dynactin (Xiang et al., 1994; Plamann et al., 1994; Robb et al., 1995; Tinsley et al., 1996; Eckewith et al., 1998; Xiang et al., 1999; Xiang and Morris, unpublished data). Other nud and ro genes encode proteins that are not known components of CD or dynactin, but may be required for the integrity, localization (e.g., ro-10) or activity of CD or dynactin. Of particular interest is the nudF gene of A. nidulans, which encodes a protein similar to LIS1, a protein required for human brain development (Xiang et al., 1995a; see below).

Introduction of a temperature-sensitive nudF mutation into a strain carrying a CD heavy chain deletion causes no more inhibition of nuclear migration or growth than the CD deletion alone. Therefore, NUDF protein must lie on the same functional pathway as CD. It presumably acts upstream of CD, since the growth inhibition caused by deletion of NUDF can also be suppressed by mutations in the CD heavy chain (Willins et al., 1997), and therefore it may
be an upstream activator of CD function. Physical interactions of the yeast (Pac1p) and human (LIS1) homologues ofNUDF with CD have recently been reported in support of this idea (Faulkner, N., and R. Vallee. 1999. American Society for Cell Biology (ASCB). 65. [Abstr.]; Geiser, J., J. Kahana, P. Silver and M. Hoyt. 1999. ASCB. 114. [Abstr.]). As in yeast, however, the effects of CD andNUDF on nuclear migration also involve MTs. The growth inhibition caused by deletion of the CD heavy chain orNUDF can be suppressed by destabilizing MTs, indicating thatNUDF and CD normally decrease MT stability in vivo (Willins et al., 1995). Recently, we have purified theNUDF protein and demonstrated that it directly inhibits thepolymerization of MAP free tubulin in vitro (Ahn, C., J. Kahana, P. Silver and M. Hoyt. 1999. ASCB. 114. [Abstr.]). Thus, the in vivo effect ofNUDF on MT stability may reflect either a direct effect on MT dynamics or an indirect effect mediated via loss of CD activity, or both. That thegrowth defect of an A. nidulans strain doubly mutant for both CD andNUDF is no more severe than that caused by either parental mutation could be due to the fact that there may be a limit to the extent to which MTs can be sta-
bilized, such that loss of eitherNUDF or CD function causes maximal hyperstabilization.

Immunostaining showed the CD heavy chain to be concentrated at the hyphal tip in bothA. nidulans andN. crassa (Xiang et al., 1995b; Minke et al., 1999b). This could reflect an abundance ofCD-containing vesicles near the tip (see Seiler et al., 1999), but it is also consistent with a model in which tip-anchored CD pulls the nucleus toward the tip by migrating on astral MTs toward the SPB (Xiang et al., 1995b). A modification of this model suggested that CD on SPB MTs links nuclei together in a chain pulled toward the tip as described above (Plamann et al., 1994), and a third model suggested that the CD is anchored to the cortex at intervals along the mycelium (Efimov and Morris, 1998). However, CD has not been detected either onMTs between nuclei or on the lateral cell wall. Observations ofGFP-tagged CD andNUDF in living cells shows comet-like structures that migrate toward, and become more concentrated at, the tip (Xiang, X., D. Winkelmann, and N.R. Morris, unpublished data; also, see http://www2.umdnj.edu/rmlabweb/moventer.html#two). The comets appear to be at the ends of advancing MTs. Whether this localization of CD andNUDF at the tips ofMTs influences MT stability or is even related to nuclear migration still remains to be determined. Direct observational studies similar to those that have been done in yeast are needed to determine the relationship between CD,NUDF, and MT dynamics and nuclear migration inA. nidulans and other filamentous fungi.

### Nuclear Migration in Flies and Worms

Studies of nuclear migration in the developing D. melanogaster embryo have provided strong evidence that CD and microtubule-dependent forces exerted on the centrosome are responsible for nuclear migration. During early embryosogenesis nuclei move to the egg cortex. If nuclear division is inhibited, either by the GNU (giant nucleus) mutation or by inhibition of DNA synthesis (Freeman et al., 1986; Raff and Glover, 1989), the centrosomes continue to replicate, pull free from the nondividing nucleus, and progress to the egg cortex. Thus, a tractive force on the centrosome is responsible for this nuclear migration. Evidence for a tractive force on the spindle poles also comes from a mutation inDrosophilaKLP3A kinesin, which disrupts the interdigitation of central spindle microtubules in spermatocyte central spindles, but does not affect spindle elongation during anaphase B (Williams et al., 1997). In bothD. melanogaster andC. elegans, interference with CD function causes dislocation of centrosomes from the nucleus (Gonzcz et al., 1999; Robinson et al., 1999). It also causes failure of centrosome separation and causes abnormal spindle orientation both in the fertilized single cell worm embryo and the coenocytic fly embryo after nuclear migration to the cortex (Robinson et al., 1999). CD is concentrated at the periphery of the male and female pronuclei in the worm egg, leading to the suggestion that the centrosome attaches to the nucleus by an interaction between the astral MTs of the centrosome and the perinuclearCD. It also has been suggested that migration of centrosomal astral MTs on perinuclearCD is responsible for centrosome separation (Skop and White, 1998; Gonzcz et al., 1999). UNC-84, a transmembrane protein required for certain specific nuclear migrations and for nuclear anchor-
ing during development in C. elegans, localizes to the nu-
clear envelope and has been proposed to anchor CD and
the centrosome to the nucleus in the worm (Malone et al.,
1999). The klarsicht gene product (marbles renamed),
which is required for nuclear migration in the fly eye, also
has a perinuclear distribution and has been suggested to
interact with CD (Mosley-Bishop et al., 1999).

Nuclear Migration and Neuronal Migration

We have hypothesized that neuronal migration in the hu-
man brain is mediated by a mechanism similar to that re-
ponsible for the long range nuclear migration seen in fila-
mentous fungi (Morriss et al., 1998a). Dosage insufficien-
ty provides a model to connect LIS1-mediated neuronal
accession AAC83820).

The Journal of Cell Biology, Volume 148, 2000 1100

A mutation of

vides a model to connect LIS1-mediated neuronal
accession AAC83820).

Nuclear Migration and Neuronal Migration

We have hypothesized that neuronal migration in the hu-
man brain is mediated by a mechanism similar to that re-
ponsible for the long range nuclear migration seen in fila-
mentous fungi (Morriss et al., 1998a). Dosage insufficien-
ty provides a model to connect LIS1-mediated neuronal
accession AAC83820). LIS1 is a homodimer, NUDF is also a

NUDF nuclear migration protein and LIS1 have led us to

NUDF, LIS1 affects MT stability, but whereas NUDF de-

Many features are common to nuclear migration in lower

and higher eukaryotes. In both, nuclei are pulled

around by an attached organelle: in the fungi by the SPB

and in higher eukaryotes by the centrosome. In all cases,

MTs, CD, and dynactin are involved, but in S. cerevisiae,
kinesin proteins also play a role. Whether the involvement of

the kinesins in nuclear migration is general or specifi-
cally related to the peculiarities of yeast bud nucleation

remains to be determined. The effects of the motor proteins

on MT stability and nuclear movement in the fungi are of
great interest because they invoke the question of whether

nuclear migration is mediated by motor activity and/or by

effects on MT dynamics. Whether NUDF and LIS1 have a

A

B

C

D

E

many conserved mechanism, what that mechanism may be, and

whether and how they affect CD, dynactin, and/or MTs,

remain as fascinating topics for future investigation.

My thanks to Dr. S. Hirotsune for permission to cite his unpublished data

and to Drs. Xin Xiang and V. V. Adimir E. Fimov for critical reading and comments

on the manuscript. My sincere apologies to those whose work could not be

mentioned because of space limitations.

This work was supported by a grant GM 52309 from the National Insti-
tutes of Health.

Submitted: 10 January 2000

Revised: 17 February 2000

A accepted: 18 February 2000

References


dence for the existence, structural basis and function of astral forces during


cytoskeletal dynein light chain is required for nuclear migration and for


translocation: role of cellular elongation and axonal outgrowth in the acous-

Bruno, K. S., J. H. Tinley, P. F. M. Inke, and M. Plamann. 1996. Genetic interac-
tions among cytoskeletal dynein, dynactin, and nuclear distribution mutants of


yeast through dynein-dependent interactions with the cell cortex. J. Cell


Cottingham, P. R., and M. A. Hoyt. 1997. Mitotic spindle positioning in Sacca-
romyces cerevisiae is accomplished by antagonistically acting microtubule motors. Cell. 130:1041–1051.


