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UNC-83 coordinates kinesin-1 and dynein activities at the nuclear envelope during nuclear migration

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ABSTRACT

Nuclei migrate during many events, including fertilization, establishment of polarity, differentiation, and cell division. The *Caenorhabditis elegans* KASH protein UNC-83 localizes to the outer nuclear membrane where it recruits kinesin-1 to provide the major motor activity required for nuclear migration in embryonic hyp7 cells. Here we show that UNC-83 also recruits two dynein-regulating complexes to the cytoplasmic face of the nucleus that play a regulatory role. One consists of the NudE homolog NUD-2 and the NudF/Lis1/Pac1 homolog LIS-1, and the other includes dynein light chain DLC-1, the BicaudalD homolog BICD-1, and the Egalitarian homologue EGAL-1. Genetic disruption of any member of these two complexes caused nuclear migration defects that were enhanced in some double mutant animals, suggesting that BICD-1 and EGAL-1 function in parallel to NUD-2. Dynein heavy chain mutant animals also had a nuclear migration defect, suggesting these complexes function through dynein. Deletion analysis indicated that independent domains of UNC-83 interact with kinesin and dynein. These data suggest a model where UNC-83 acts as the cargo-specific adaptor between the outer nuclear membrane and the microtubule motors kinesin-1 and dynein. Kinesin-1 functions as the major force generator during nuclear migration, while dynein is involved in regulation of bidirectional transport of the nucleus.

Introduction

A wide variety of cell and developmental processes, including establishment of polarity, fertilization, cell division, and cell migration, depend on actively positioning the nucleus to a specific location within the cell (Burke and Roux, 2009; Starr, 2007, 2009). A failure in proper nuclear positioning leads to a wide variety of developmental defects and diseases. For example, the nuclear anchorage genes Syne-1, 2 (Nesprin-1, 2) have been linked to the pathogenesis of an autosomal recessive cerebellar ataxia, Emry–Dreifuss muscular dystrophy, and the premature aging disease Hutchinson–Gilford progeria (Gros-Louis et al., 2007; Kandert et al., 2007; Zhang et al., 2007). In addition, a nuclear migration defect in the developing brain causes lissencephaly, a severe mental retardation disease (Morris et al., 1998). Despite the importance of nuclear migration in development and disease, a detailed mechanistic understanding of nuclear migration remains unclear in most cases.

The connection between the cytoskeleton and the nuclear envelope is essential for moving the nucleus. Microtubules, actin filaments, and intermediate filaments have all been implicated in nuclear positioning (Starr, 2009). Microtubules are required for nuclei to migrate and remain evenly spaced in *Aspergillus* during hyphal growth (Xiang and Fischer, 2004). Dynein and many of its associated regulatory proteins, including Lis1 and NudE, were discovered to play an important role in this nuclear migration (Efimov and Morris, 2000; Xiang et al., 1995), although it is still unknown how and where dynein is acting to move the nucleus. Dynein is also required for nuclear migration in radially migrating neurons (Tsai et al., 2007; Zhang et al., 2009). During migration, the centrosome moves at a constant rate towards the leading edge of the cell while the nucleus moves in a salutatory manner behind it. Dynein attached to the nuclear envelope may be responsible for providing a pulling force on centrosomal microtubules to help move the nucleus (Tsai et al., 2007; Zhang et al., 2009). Thus, in different nuclear migration events, dynein plays different roles in multiple cellular locations and the exact role of dynein in most examples remains unknown.

The cytoskeleton is linked to the nuclear envelope by the SUN (Sad1 and UNC-84) and KASH (Klarsicht, ANC-1, and Syne Homology) families of proteins (Starr, 2009; Wilhelmsen et al., 2006). SUN proteins are targeted to the inner nuclear membrane and recruit KASH proteins to the outer nuclear membrane through a direct interaction between the SUN and KASH domains in the perinuclear space (Crisp et al., 2006; McGee et al., 2006; Padmakumar et al., 2005). The cytoplasmic domains of KASH proteins are then free to interact with the cytoskeleton and perform a variety of functions, including nuclear positioning (Starr, 2009; Wilhelmsen et al., 2006). In the nuclear envelope bridging model of how these proteins function, SUN and KASH proteins span both membranes of the nuclear envelope and

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transfer forces from the cytoskeleton to the nuclear lamina (Starr, 2009). For example, the *Caenorhabditis elegans* KASH protein ZYG-12 and SUN protein SUN-1 function during pronuclear migration by coupling the centrosome to the nuclear envelope. An interaction between ZGY-12 and dynein mediates attachment of centrosomes to the nuclear envelope, which is essential during pronuclear migration and nuclear positioning in the gonad (Malone et al., 2003; Minn et al., 2009; Zhou et al., 2009).

In *C. elegans*, hyp7 embryonic hypodermal precursor cells provide an excellent model system for studying the mechanism of nuclear migration. During embryogenesis, left and right groups of dorsal epithelial cells intercalate, and their nuclei migrate contralaterally across the length of the hyp7 cell (Fig. 1A). These cells subsequently fuse, forming the dorsal hypodermal syncytium and the nuclei are positioned laterally (Sulston et al., 1983; Williams-Masson et al., 1998). Hyp7 nuclei are easily scored and used to assay the severity of nuclear migration defects. Mutations in *unc-84* or *unc-83* disrupt hyp7 cell nuclear migration, resulting in nuclei that are mispositioned to the dorsal cord of L1 larvae (Fig. 1) (Horvitz and Sulston, 1980; Malone et al., 1999; McGee et al., 2006;

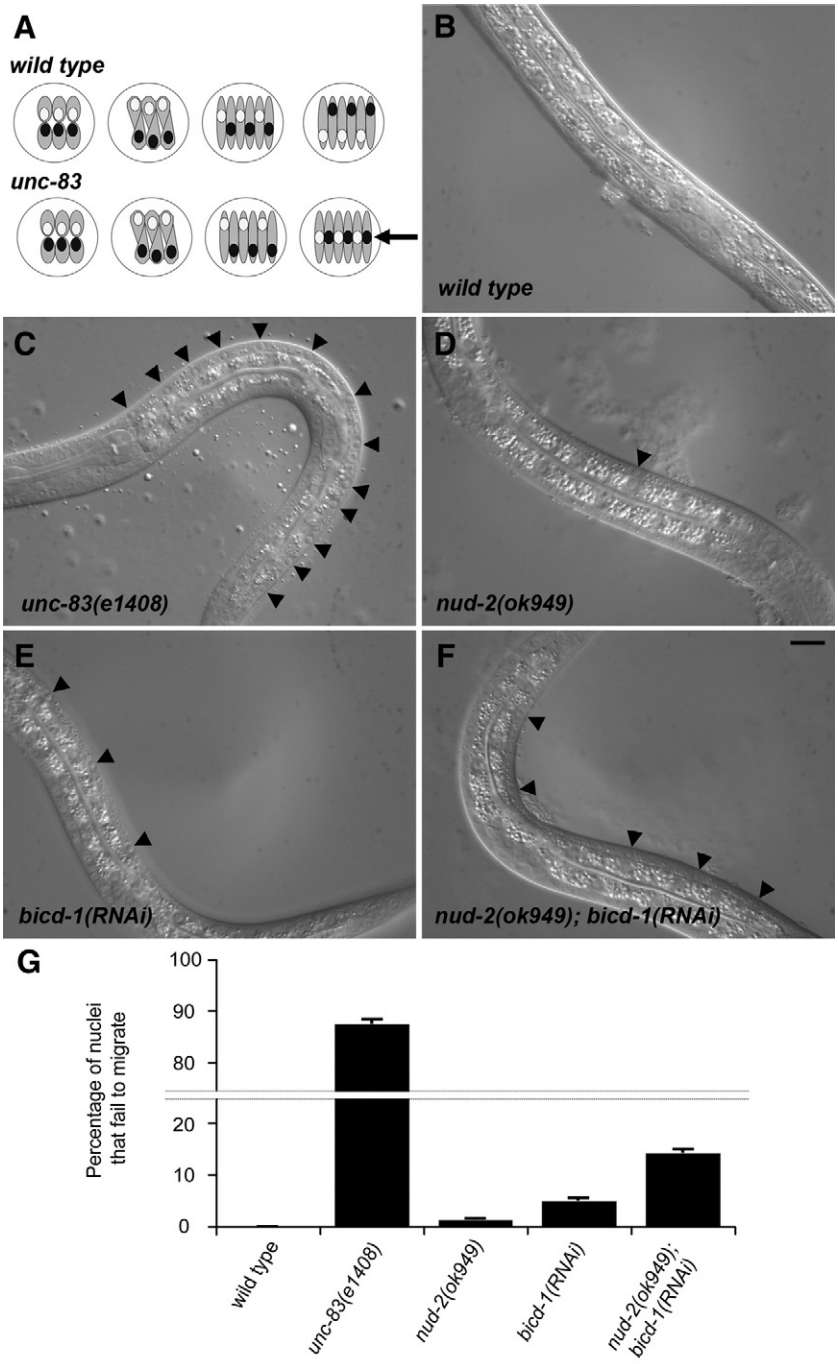

Fig. 1. *nud-2* and *bicd-1* function in hyp7 nuclear migration. (A) A dorsal view of a pre-comma stage embryo illustrating intercalation and nuclear migration of hyp7 precursors in wild-type and *unc-83* embryos. The embryo is white, hyp7 precursors are gray, nuclei that migrate from right to left are white, and nuclei that migrate from left to right are black. Anterior is to the left. Arrow indicates the dorsal cord. (B–F) Lateral view of L1 hermaphrodites. Dorsal is upwards. (B) Wild-type N2 animal showing no nuclei in the dorsal cord. (C) *unc-83(e1408)*. (D) *nud-2(ok949)*. (E) *bicd-1(RNAi)*. (F) *nud-2(ok949);bicd-1(RNAi)*. Black arrowheads mark hyp7 nuclei in the dorsal cord. Scale bar is 10 μm. (G) Quantification of hyp7 nuclear migration phenotypes. Error bars are standard error. See Table 2 for details and statistics.