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Orientation and Function of the Nuclear-Centrosomal Axis During Cell Migration

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Abstract

A hallmark of polarity in most migrating cells is the orientation of the nuclear-centrosome (NC) axis relative to the front-back axis. Here, we review “effector functions” associated with the NC axis during cell migration. We highlight recent research that has demonstrated that the orientation of the NC axis depends upon the coordinated, but separate positioning of the nucleus and centrosome. We stress the importance of environmental factors such as cell-cell contacts and substrate topology for NC axis orientation. Finally, we summarize tests of the importance of this axis for cell migration and disease.

Introduction

The intimate association of the centrosome and nucleus has been noted by cell biologists since the discovery of the centriole by Van Beneden [1]. Interestingly, Van Beneden postulated that an axis of cellular polarity can be identified by drawing a line through the center of the nucleus and the centrosome [1,2] (Fig 1A). This NC axis aligns with morphological polarity in many cell types suggesting that it contributes to the generation and/or maintenance of cell polarity [3].

The concept that the NC axis plays an important role in cell polarity is well developed in the field of cell migration. The NC axis and the front-back cellular axis are often aligned during migration, so that the centrosome lies between the nucleus and the leading edge [4]. This anterior NC axis orientation (aka “centrosome reorientation”) is observed in many cell types migrating on two dimensional (2D) substrata [4] and in neurons migrating in 3D substrata [5] (Table 1). However, NC axis orientation is not always anterior; chemotaxing neutrophils and T cells exhibit posterior orientation and some cells change their NC axis orientation depending on environmental conditions (Table 1 and see below).

The mechanism of anterior NC axis orientation has been extensively studied and we first consider functions associated with anterior NC axis orientation as well as how they contribute to cell migration. We then examine factors involved in the establishment of anterior NC axis orientation in migratory cells. We focus on recent studies that show that the positioning of both the nucleus and the centrosome contribute to NC axis orientation. The elucidation of pathways controlling NC axis orientation has allowed tests of the role of NC

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axis orientation in cell migration and we describe recent results from these studies that support a role for NC axis orientation in cell migration.

NC axis effector functions during migration

Anterior NC axis orientation is thought to contribute to cell migration through localizing centrosome-mediated microtubule (MT) nucleation and organelle positioning towards the front of the cell (Fig. 1B)[3,6,7]. These activities can be considered “effectors” of the NC axis. The movement of the nucleus is also dictated by NC axis orientation, identifying a third effector of this axis (Fig. 1B).

MT nucleation and radial projection as an NC axis effector

The centrosome nucleates most MTs in migrating cells [8]. MT minus-ends are anchored at the centrosome while plus-ends extend radially and explore the cytoplasm through dynamic instability [9]. Centrosomal MT nucleation is typically symmetrical [10,11]; however, due to the proximity of the centrosome to the nucleus, dynamic MTs grow away from the nucleus freely, but are impeded in their growth around the nucleus. Therefore, anterior NC axis orientation biases MT extension towards the cell front (Figure 1B). Dynamic MTs have direct activities in targeting focal adhesions for disassembly [12-14], regulating Rho and Rac GTPase signaling [15,16], and serve as “precursors” for the effector functions described below.

MT-based vesicular trafficking as an NC axis effector

The centrosome positions two organelles central to MT-based vesicular trafficking: the Golgi apparatus and the perinuclear endocytic recycling compartment (Figure 1C). Both organelles are maintained in close proximity to the centrosome by dynein [6,17-19]. Exocytosis of model cargoes from these organelles is polarized towards the leading edge during migration through a MT-dependent mechanism [20,21]. The exocyst complex is also localized at the leading edge and is important for the migration of various cell types [22-24].

Recent studies of migrating *Drosophila* border cells revealed polarized recycling of receptor tyrosine kinases important for the migration of these cells [25,26]. Polarized recycling of integrins, the main adhesive receptor in migrating cells, has also been implicated in cell migration [27-29]. Focal adhesion disassembly is a clathrin- and dynamin-dependent process, which supports the idea that integrins are recycled during migration [12,14,28]. Trafficking of Golgi cargoes may also be important for cell migration; inhibition of protein kinase D (PKD), which regulates Golgi trafficking, impedes fibroblast migration [30].

Nuclear movement as an NC axis effector

A newly recognized NC axis effector function is nuclear movement. In most migrating cells, the centrosome tracks the cell center and so will reposition towards a new protrusion [31,32]. This also causes movement of the nucleus towards the new protrusion due to the close juxtaposition of the nucleus and centrosome. Migrating neurons provide a notable exception to coincident movement of the centrosome and nucleus: they move their centrosome forward in an extended leading process, followed by a separate movement of the nucleus toward the centrosome, a “two-stroke” mode of migration [33].

Centrosomal and nuclear movements in migrating neurons require dynein and its regulator, LIS1 [34,35]. Dynein localizes to a cytoplasmic swelling ahead of the centrosome in the leading process, suggesting that dynein may move the centrosome by pulling on MTs. Dynein also localizes around the nucleus and may be responsible for moving the nucleus

towards the centrosome [34,36]. Although little separation occurs between the centrosome and nucleus in migrating fibroblasts, a similar dynein mediated movement of the nucleus may keep the nucleus close to the centrosome [37]. Supporting this idea, dynein is required for fibroblast migration (even after NC axis orientation) and inhibiting dynein causes the nucleus to lag behind the centrosome [38].

In neurons, dynein co-immunoprecipitates with the outer nuclear envelope proteins, nesprin-1 and nesprin-2, suggesting that these proteins may couple dynein to the nucleus to move it along MTs [36]. Nesprins and their interacting partners, the inner nuclear membrane SUN proteins, form the “linker of cytoskeleton and nucleoskeleton (LINC) complex” that gives the nucleus the capability to interact with actin, MTs, and intermediate filaments [39]. Nesprin-1 and -2 double knockout mice die perinatally and have defective neuronal migration and brain development underscoring the importance of nuclear movement during neuronal migration [36].

Myosin II is also implicated in nuclear movement in migrating neurons [34,40-42]. Myosin II accumulates behind the nucleus and contraction of actomyosin at this site contributes to nuclear and cell body movement. Interestingly, myosin II activation during nuclear movements may be controlled in part by centrosomal activities. The polarity protein, Par6 α localizes to the centrosome and is required for centrosome positioning during glial-guided neuronal migration [43]. Additionally, Par6 α reduces myosin II activation by enhancing myosin light chain phosphatase activity [40]. This intriguing result suggests that centrosomal Par6 α may create asymmetric myosin activation around the nucleus with lower myosin II activity ahead of the nucleus. For additional consideration of neuronal migration, we direct the reader to recent reviews [5,33,44].

Cdc42 signaling during anterior NC axis orientation

Stimulation of fibroblast, endothelial cell and astrocyte migration by wounding 2D monolayers triggers anterior NC axis orientation. Early studies using fixed cells suggested that axis orientation correlated with the onset of migration [45-47]. More recently, direct imaging of the centrosome revealed that productive protrusion and migration of fibroblasts occurred only after anterior NC axis orientation was established [48].

The mechanisms for establishing anterior NC axis orientation before cell migration have been extensively studied in wounded monolayers of fibroblasts and astrocytes. Results from these systems show that NC axis orientation depends upon the GTPase, Cdc42, which regulates separate pathways controlling the positioning of the centrosome and the nucleus. During anterior NC axis orientation in fibroblasts, the centrosome is kept in the cell center while the nucleus moves rearward [48]. Active Cdc42 stimulates the effectors, Par6 and myotonic dystrophy related kinase Cdc42-binding kinase (MRCK), to control centrosome and nuclear positions, respectively [48,49]. MRCK stimulates myosin II phosphorylation and the attachment of the nucleus to rearward moving actin cables, while Par6 interacts with protein kinase C ζ (PKC ζ) to maintain the centrosome near the cell center by a MT- and dynein-dependent mechanism that involves another polarity protein, Par3 [48,50,51].

A similar Cdc42-dependent signaling pathway controls anterior NC axis orientation in astrocytes. After wounding, integrin signaling stimulates Cdc42 activation and accumulation at the leading edge where it activates Par-6 and PKC ζ , both of which are required for NC axis orientation [52]. It is unknown if Cdc42 controls positioning of both the centrosome and nucleus in astrocytes as it does in fibroblasts. Recent reviews discuss the evolutionarily conserved role of Cdc42 in regulating these events [53,54].

MTs and dynein control centrosome positioning during anterior NC axis orientation

Fibroblasts and astrocytes both require dynein for anterior NC axis orientation (Figure 2A and B) [49,52]. In fibroblasts, dynein is required for centrosome centration, but not nuclear movement, and localizes to cortical sites along with MTs and Par3 [48,51]. Interestingly, Par3 associates specifically with one of the two dynein light intermediate chains (LIC2), which is also required for centrosome centration [51]. Both Par3 and LIC2 contribute to increased MT pausing at sites of cell-cell contact, supporting a model where Par3 acts as a cortical dynein receptor for tethering MTs (Figure 2A). Additional factors may contribute to this MT tethering as the interaction between Par3 and dynein did not appear to be direct.

In astrocytes, the polarity protein discs large 1 (Dlg1), the scaffolding protein guanylate-kinase-associated protein (GKAP), and dynein are recruited to the leading protrusion where together they are required for anterior NC axis orientation (Figure 2B). Dynein, Dlg1 and GKAP contribute to the polarization of MTs towards the ventral plasma membrane near the leading edge [55]. These results imply that in astrocytes, the ventral cortex of the leading process is the site at which forces are exerted for NC axis orientation.

Fibroblasts and astrocytes use many of the same factors for NC axis orientation, yet employ them at different cellular sites. These differences may reflect the astrocyte's longer leading process (astrocytes: 50-100 μm ; fibroblasts: 20-30 μm) and slower NC axis orientation (astrocytes: 6hr; fibroblasts: 2hr) [49,52,56]. Additionally, in fibroblasts anterior NC axis orientation is separable from the physical wounding of the monolayer by removing serum factors; NC axis orientation can be triggered by the addition of the specific serum factor lysophosphatidic acid, which induces axis orientation in the absence of cell migration [49]. Such a separation has not been achieved in astrocytes, so results in this system may reflect processes involved in both NC axis orientation and cell migration. Interestingly, fibroblasts stimulated with serum to induce both axis orientation and cell migration accumulate dynein and its regulators, dynactin and LIS1, at their leading edge [38]. It is tempting to speculate that this accumulation may reflect a site of MT tethering that becomes important during migration.

Actin and myosin II control nuclear position during anterior NC axis orientation

In fibroblasts, rearward nuclear movement during NC axis orientation occurs by myosin II-dependent retrograde flow of actin cables, which engage the nucleus through a novel structure known as transmembrane actin-associated nuclear (TAN) lines [50]. TAN lines are arrays of nesprin-2Giant (nesprin-2G) and SUN2 [50]. Nesprin-2G interacts with dorsal actin cables in the cytoplasm and SUN2 in the perinuclear space while SUN2 interacts with nuclear lamins and/or chromatin in the nucleoplasm [39]. TAN line formation and nuclear movement are actin- and myosin II-dependent [48,50]. TAN line-mediated nuclear movement requires A-type lamins for productive force transmission from actin retrograde flow to the nucleus [57].

Nuclear movement in astrocytes is observed upon calcium-stimulated junctional formation on micropatterned substrata [58,59]. In this system actin flow, but not myosin, is important for the positioning of both the nucleus and centrosome towards the cell rear, away from the cell center. This nuclear movement is independent of nesprins and instead depends upon intermediate filaments, which are proposed to couple actin flow to the nucleus.

Cell-cell junctions and anterior NC axis orientation

Cell-cell junctions play an important role in NC axis orientation in migrating cells. An initial study revealed that E-cadherin containing cell junctions are required for rearward nuclear positioning in kidney epithelial cells grown on micropatterned substrates [60]. In astrocytes, the positions of both the nucleus and centrosome depend upon N-cadherin, while anterior NC axis orientation depends upon the geometry of N-cadherin cell-cell contacts [58]. Disruption of anterior NC axis orientation is observed following N-cadherin inhibition in vascular smooth muscle cells, astrocytes, epithelial cells and endothelial cells [58,61]. The role of junctional molecules in NC axis orientation may ensure that cells move away from each other when stimulated to migrate [60].

An analogous process may function during cell division. Spindle centrosomes are positioned on the side of the nucleus opposite the cleavage furrow generating anterior NC axis orientation in the daughter cells that may contribute to their migration away from each other [62,63]. Cell division determined NC axis orientation may be important in asymmetric stem cell divisions where a mother cell is maintained as a stem cell within a specific niche while the daughter cell, destined to differentiate, migrates away [64]. Specific anchoring mechanisms act on the mother cell centrosome to maintain its orientation towards supporting niche cells and may contribute to immobility of the mother stem cell [65].

Substrate topology and NC axis orientation

NC axis orientation in migrating cells is affected by the topology of the substratum. Migrating fibroblasts orient their NC axis posteriorly on 3D substrata [66,67] (Table 1). The NC axis orientation of BSC1 cells migrating on narrow strips of fibronectin is typically posterior, but switches to an anterior orientation on wider fibronectin strips [68] (Table 1). Collectively migrating cells of the primordium that generates the lateral line during zebrafish development also have a posteriorly oriented NC axis [68,69] (Table 1). Substrate geometry is also important for NC axis orientation in retinal pigment epithelial (RPE1) cells [70] (Table 1).

The mechanisms and functions of posterior NC axis orientation are relatively unexplored. Nonetheless, given that fibroblasts adjust the orientation of the NC axis depending on the mode of migration implies that the requirements for dynamic MTs or vesicle trafficking in the different modes of migration may not be the same. For example, the need for vesicular trafficking may be reduced for cells migrating in 3D given that they do not produce the broad lamellipodia of cells migrating in 2D. Alternatively, they may rely on stable posttranslationally modified MTs, which remain anteriorly oriented in fibroblasts in both 2D [47] and 3D [66] and are known to provide directionally biased vesicular trafficking as well as preferential tracks for a subset of kinesin MT motors[71-73].

Function and dysfunction of NC axis orientation

The analysis of the NC axis function during cell migration has benefited greatly from the identification of separate centrosome and nuclear positioning pathways. Disruption of centrosome positioning through dynein inhibition slows the migration of fibroblasts, astrocytes, and neurons [34,37,38,49,51,52]. Also, depletion of Par3 or expression of the LIC-2-interacting fragment of Par3 reduces fibroblast migration, suggesting that anterior NC axis orientation is important for efficient cell migration [51]. Disruption of nuclear positioning in fibroblasts and U2OS cells by interfering with MRCK inhibits cell migration during wound healing [48,74]. Additionally, disrupting nesprins-2G or SUN2, which control nuclear positioning in fibroblasts, decreases cell migration [50,75]. Given that only nuclear proteins were disrupted in these studies, these results are some of the clearest evidence to

date that proper NC axis orientation plays a key role in cell migration. These experiments are not without caveats; for example, dynein is likely to perform other functions in addition to centrosome positioning during cell migration [37,38].

Another approach used to test the function of NC axis orientation during cell migration is centrosome ablation. Laser ablation of an anterior centrosome in migrating newt eosinophils reduced the speed of migration [76]. Recent studies on U2OS carcinoma and PtK cells have produced conflicting results [77]. While ablating the centrosome reduced cell polarity in both types of cells, there was only a small effect on the speed of PtK cell migration and almost no effect on U2OS cells. Centrosome ablation may not interfere with all centrosome effector functions since MTs have the ability to self-organize into radial arrays in cells lacking centrosomes [77,78].

Further evidence for the functional importance of NC axis orientation comes from the identification of disease-associated mutations in key players in NC axis orientation. For example, LIS1 is mutated in the developmental disorder lissencephaly which results in the malformation of the sulci and gyri of the brain due at least in part to defect neuronal migration [79]. Nesprins are mutated in patients with movement disorders such as Emery-Dreifuss muscular dystrophy (EDMD) and cerebellar ataxia [80,81]. Additionally, *LMNA*, the gene encoding lamin A/C, is mutated in a wide variety of human diseases collectively referred to as the laminopathies including EDMD, dilated cardiomyopathy, familial partial lipodystrophy, and Hutchinson–Gilford progeria syndrome [82]. Interestingly, *LMNA* mutations associated with diseases of striated muscles primarily disrupt nuclear movement while those associated with disease of adipose tissue disrupt centrosome centration in fibroblasts [57]. This study suggests that there may be a connection between disease pathogenesis and the NC axis and that further understanding of the basic cellular activities controlling the NC axis may uncover new insights into these diseases.

Conclusions and perspectives

There is growing evidence of the importance of NC axis orientation in normal cell migration and that disruption of this axis leads to disease. Nonetheless, additional studies are needed to understand the factors governing posterior NC axis orientation in amoeboid cells and fibroblasts migrating in 3D and the role of this “reversed” orientation in migration. The relationship between NC axis orientation and key cellular activities, such as directing membrane traffic and regulating adhesions needs to be better defined. It will also be interesting to address how neurons establish an anterior NC axis orientation prior to migration as the majority of work on the NC axis in migrating neurons has only considered this axis after migration has commenced.

In conclusion, it appears that Van Beneden's insight that the NC axis may be a key to cell polarity has been validated for cell migration. It will be exciting to extend the insights obtained from studying the mechanisms of orienting this axis and its functions in cell migration to other fields of cell biology.

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Research highlights

1. The N-C axis is a central polarity axis defined by drawing a line through the centers of the nucleus and centrosome.
2. Anterior N-C axis orientation such that the centrosome is positioned between the nucleus and leading edge is observed in many, but not all migrating cells.
3. Separate cellular mechanisms control the positioning of the centrosome and nucleus which together are required for anterior N-C axis orientation in migrating cells.
4. Environmental factors, such as the topology of the substratum and the presence of adjacent cells and cell-cell junctions can affect the orientation of the N-C axis.
5. Cell migration depends upon the effector functions of the N-C axis: MT nucleation and radial positioning; Golgi and ERC positioning; and nuclear movement.

Figure 1. The nuclear-centrosomal axis and its effectors

A) A reproduction of Van Beneden's drawing of the nuclear-centrosomal axis (from [2]). **B)** Effector functions of the nuclear-centrosomal axis. ERC is the perinuclear endocytic recycling compartment.

Figure 2. Dynein cortical interactions involved in centrosome positioning

A) In fibroblasts the LIC2 pool of dynein associates with Par3 at lateral sites in the lamella [51]. Whether the association between LIC2-dynein and Par3 is direct is unknown. **B)** In astrocytes, dynein associates with Dlg1 at ventral sites in the lamella through GKAP [55]. Whether the association between dynein and GKAP and GKAP and Dlg1 are direct is unknown.

Figure 3. Nuclear movements during centrosome orientation

A) In fibroblasts, the nucleus is moved rearward by retrograde flow of dorsal actin cables. A novel nuclear membrane structure, TAN lines, composed of outer nuclear membrane nesprin-2G and inner nuclear membrane SUN2, mediates the connection between the nucleus and actin cable [50]. Lamin A/C anchors the TAN lines to allow force to be transmitted to the nucleus [57]. **B)** In astrocytes, the nucleus is moved rearward by retrograde flow of actin that is also dependent on intermediate filaments [59]. Whether there are direct molecular connections between intermediate filaments and actin or the nucleus is unknown.

Table 1
NC axis orientation diversity in migrating cells

Orientation	Cell type	Source	Migration environment	Reference
Anterior	Astrocyte	Rat	2D glass	[52]
	Dictyostelium discoideum	Dictyostelium chemotaxing towards cAMP gradients	2D agar plates	[83]
	Endothelial cells	Bovine carotid arterial endothelium cells, porcine thoracic aorta cells	2D glass	[45,84]
	Eosinophils	Newt eosinophils	2D glass	[76]
	Epithelial cells	RPE1, BSC-1	2D glass, 2D micropatterned substrates	[32,70,85-87]
	Fibroblasts	NIH3T3, COS7, CHO, NRK, mouse and chicken embryonic fibroblasts, human fibroblasts	2D glass	[46,47,67, 88-90]
	Macrophages	Mouse peritoneal macrophages	2D glass	[91,92]
	Neuronal precursors	Rat cortical pyramidal, neuronal precursors, mouse cerebellar granule cells, rat and mouse SVZA primary olfactory bulb precursors	3D matrigel 3D brain slices	[34,42,43, 93]
	Osteosarcoma cells	U2OS, SaOS2	2D glass	[89,94,95]
Posterior	Dictyostelium discoideum	Dictyostelium chemotaxing (random migration)	2D agar plates	[83]
	Dictyostelium discoideum	Dictyostelium chemotaxing (aggregation)	2D agar plates	[83]
	Epithelial cells	PtK, BSC-1	2D glass, 1D micropatterned strips of fibronectin,	[32,68,96]
	Mesenchyme	Sea urchin primary	Developing embryo	[97]
	Lymphocytes	Human peripheral blood T cells, mouse metastatic mouse T-lymphoma cells	2D glass, 3D collagen gels, 3D Matrigel, 3D invasion through monolayers of 10T1/2 fibroblast-like cells	[92,98,99]
	Natural killer cells	Cloned mouse natural killer cells	2D glass	[100]
Variable	Sensory primordium	Zebrafish lateral line	Live developing zebrafish embryos	[68]
	Fibroblasts	Chicken embryonic fibroblasts	3D collagen gels, 1D photolithographic etched grooves in glass	[67]
	Neuronal precursors	Zebrafish neurons of the tegmental hindbrain nuclei	Live developing zebrafish embryos	[101]
	Neutrophils	Human peripheral blood polymorphonuclear leukocytes	2D glass	[102]