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Review

Spindle orientation: What if it goes wrong?

Dan T. Bergstralh[a,b,*], Daniel St Johnston[a,b]

[a] The Gurdon Institute, Tennis Court Road, Cambridge CB2 1QN, United Kingdom
[b] The Department of Genetics, University of Cambridge, Tennis Court Road, Cambridge CB2 1QN, United Kingdom

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ABSTRACT

The angle of cell division is critical in at least two contexts. It can determine cell fate, as it does in developing neural tissue. It can also dictate tissue architecture, as it does in many epithelia. One way to ensure the correct angle of cell division is through controlled orientation of the spindle at metaphase. What happens when that control is lost? Ongoing work suggests that the consequence of metaphase spindle misorientation may be significant, but multiple mechanisms exist to protect the cell and the tissue. We speculate that one such mechanism involves a recently identified anaphase activity for two of the key players at metaphase: NuMA (Mud, LIN-5) and dynein.

Contents

1. Introduction

Evidence from multiple organisms demonstrates that the angle of division is central to cell fate in neural tissues and to the formation of epithelia [1]. A complex machinery exists to orient the metaphase spindle in a variety of cell types and organisms [2]. In this review we will consider the consequence of its failure.

As defects in both cell fate and tissue organization are implicated in tumor development, one possibility that must be considered is that spindle misorientation contributes to cancer. This suggestion has been widely discussed in the literature – reviews include (but are not limited to) [3–5]. The article by Pease and Tirnauer in 2011 provides an excellent account of work up until that time, with particular attention to mammalian carcinomas. We will pick up from there, with further attention given to recent advances in vertebrate models and to evidence accumulating in non-mammalian systems.

Our interpretation of this evidence suggests that in most contexts the angle of division is too important to be entrusted to metaphase spindle orientation alone. The organism relies on multiple mechanisms to protect itself from misoriented divisions and ensure the integrity of tissues as they develop.

1.1. How are spindles oriented at metaphase?

Work in *Caenorhabditis elegans*, *Drosophila*, and cultured mammalian cells has identified a canonical spindle orientation machinery that operates during metaphase. This machinery exerts a pulling force between factors localized at the cell cortex and astral microtubules, and thereby pulls indirectly on spindle poles to bring them into orientation [6]. While the list of core factors is slowly expanding, at least four appear to be necessary in most, if not all,

* Corresponding author at: The Gurdon Institute, Tennis Court Road, Cambridge CB2 1QN, United Kingdom. Tel.: +44 01223 334 113.
E-mail addresses: db476@cam.ac.uk (D.T. Bergstralh), d.stjohnston@gurdon.cam.ac.uk (D. St Johnston).

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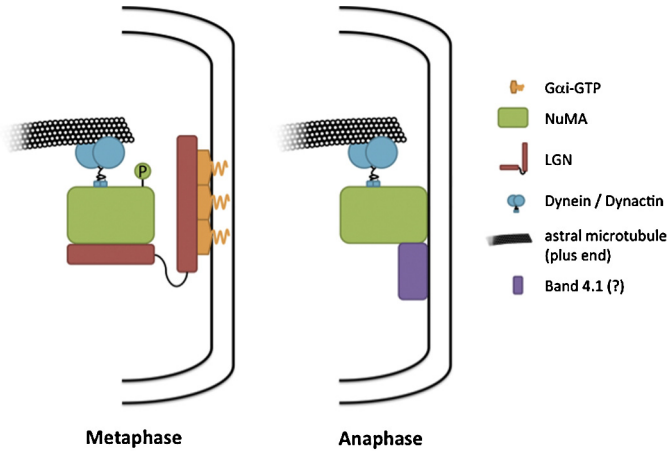

Fig. 1. NuMA/Mud and dynein/dynactin exert pulling from the cortex at both metaphase and anaphase. During metaphase, NuMA is maintained at the cortex by LGN, which is in turn anchored by Gαi. Phosphorylation by CDK1 (not shown) prevents it from localizing at the cortex without LGN. At anaphase NuMA is dephosphorylated and can bind to the cortex independently of LGN. This may or may not occur through interaction with Band 4.1 protein(s).

contexts. In *Drosophila* they are called Gαi (GOA-1 and GPA-16 in *C. elegans*), Pins (LGN or GPSM2 in vertebrates, GPR1/2 in *C. elegans*), Mud (vertebrate NuMA, LIN-5 in *C. elegans*), and dynein/dynactin.

The identification of these molecules and their functions has been reviewed elsewhere (recently in [7,8]). A brief overview of the complex follows: The G-protein subunit Gαi, which is myristoylated, binds to the plasma membrane. This may depend on the guanine exchange factor Ric-8, though its role in different tissues is not yet clear. Gαi-GDP serves as a cortical anchor for Pins, binding to its C-terminal GoLoco motifs. Pins in turn serves as a dock for Mud, to which it binds *via* N-terminal tetricopeptide repeats. Mud binds to the dynein/dynactin complex, which provides the minus-end directed motor activity that generates the pulling force (Fig. 1).

The pathway just described explains how cortical proteins can affect spindle orientation. Recent work also suggests that spindle orientation information may originate from the metaphase plate. In HeLa cells, a chromosome-derived gradient of Ran-GTP feeds back to the cortex to locally inhibit association of LGN and NuMA with the membrane. They are thus concentrated at sites more proximal to the spindle poles [9]. The functional consequence of this activity is not yet known. It may serve to reinforce and maintain spindle alignment once it has been achieved. Another possibility is that it helps promote division along the long axis of the cell, since the chromosomes are farthest from the cortex in this orientation.

Much of our understanding of spindle orientation, as in the case just described, derives from work done in cultured cells. What happens to a single cell when the spindle fails to orient at metaphase?

1.2. A new role for NuMA

The majority of attention given to spindle orientation to date is centered on machinery that operates at metaphase, but the activity of the spindle at this point is only a warm-up for the main event: the segregation of chromosomes. Recent work from four groups demonstrates that elements of the metaphase machinery, namely NuMA and dynein, have an additional role at anaphase [10–13]. The results of these studies are consistent with the following three points: (1) During anaphase, NuMA and dynein localize to two cortical crescents at opposite sides of the cell, along the axis of division. (2) This localization depends on the activity of Cdk1, which is thought to phosphorylate NuMA to restrict its localization prior to anaphase. (3) Anaphase localization of NuMA is independent of LGN and Gαi (Fig. 1).

The studies differ in their details however, and raise several questions.

Firstly, how is NuMA anchored to the cortex during anaphase? It may involve the cytoskeletal protein Band 4.1 [10,11]. In HeLa cells, Band 4.1 and Band 4.1-like 2 provide an anaphase-specific mechanism for localizing NuMA independently of LGN [11]. In mouse keratinocytes, however, the cortical localization of NuMA at anaphase occurs even if both its Band 4.1-binding region and LGN-binding regions are removed [10]. In Cos 7 cells, NuMA associates directly with the lipid membrane during anaphase *via* a newly recognized membrane binding domain [12]. This does not rule out a role for Band 4.1, but suggests at least that it does not provide an anchor.

Secondly, what is the function of NuMA and dynein during anaphase? One attractive possibility is that it may be to help ensure symmetric cell division, in respect to daughter cell size and/or DNA content. Defects in either are associated with tissue disorganization and cancer [14,15].

If the cleavage furrow is not at the center of the cell, there is a risk that cytoplasm and/or chromosomes may be split unevenly during division. Thus unequal chromosome segregation and size asymmetry might be predicted if division occurs along the incorrect axis. The possibility that spindle misorientation promotes these asymmetries can be tested in HeLa cells, which take on a triangular shape when cultured on an L-shaped fibronectin micropattern. In accordance with Hertwig's rule, they divide along their long axis, which is the hypotenuse of the triangle. In the absence of LGN or Gαi, the rule may be disobeyed; neither NuMA nor dynein are recruited to the cortex at metaphase and spindle orientation is randomized [11]. However, cell division in HeLa cells is reliably symmetric regardless of the division axis.

This may be because NuMA and dynein act after metaphase – independently of LGN – to ensure that the spindle is *centered* in the cell even if division is occurring at an incorrect angle [11]. Dynein-dependent centering has been previously illustrated in metaphase-arrested HeLa cells, in which the spindle oscillates relative to the cortex such that neither spindle pole stays too close to the cortex [9]. Kiyomitsu and Cheeseman have now shown that centering continues through anaphase, at which point it is sometimes achieved through asymmetric expansion of the plasma membrane [11]. If one side of the membrane is too close to a spindle pole, the membrane will expand to move away from it. Thus the distance from each pole to the membrane is equalized. In order to work, this mechanism requires the spindle pole at the side that does not expand to stay in place. If that pole is not anchored (presumably by a pulling force generated by localized NuMA and dynein), the spindle moves toward the expanding membrane and size asymmetry is promoted rather than resolved.

Both defective anaphase spindle anchoring and daughter cell asymmetry are observed in cells depleted of LGN, Band 4.1 and Band 4.1-like 2, even if the cells are not plated on an L-pattern ([11] and I. Cheeseman, personal communication). These findings support a model in which Band 4.1 proteins anchor Mud at anaphase to ensure spindle centering.

Data from another cell type complicates the picture. Using mouse keratinocytes, Seldin et al. observed that mechanical stretching of the substrate promotes metaphase spindle orientation along the stretched (long) axis, and this effect depends on the Band 4.1-binding domain of NuMA [10]. Since this domain is dispensable for anaphase localization of NuMA in these cells, this result suggests that Band 4.1 acts during metaphase to promote division along the long axis [10]. While these findings indicate that the relevant activity of Band 4.1 is at metaphase, they do not contradict a role for NuMA and dynein in spindle centering during anaphase.