

# Nuclear envelope-associated dynein cooperates with Eg5 to drive prophase centrosome separation

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**E**g5 (kinesin-5) is a highly conserved microtubule motor protein, essential for centrosome separation and bipolar spindle assembly in human cells. Using an “in vitro” evolution approach, we generated human cancer cells that can grow in the complete absence of Eg5 activity. Characterization of these Eg5-independent cells (EICs) led to the identification of a novel pathway for prophase centrosome separation, which depends on nuclear envelope (NE)-associated dynein. Here, we discuss our recent findings and elaborate on the mechanism by which dynein drives centrosome separation.

During mitosis, the duplicated chromosomes are segregated to the two new daughter cells. This segregation of chromosomes is mediated by the bipolar spindle, a highly dynamic structure composed of microtubules (MTs) and many associated proteins. In mammalian cells, bipolar spindle assembly is in large part dictated by the centrosomes. The two centrosomes separate to opposite sides of the nucleus at the beginning of mitosis, in most cells in prophase. Directly after nuclear envelope breakdown (NEB), MTs emanating from the centrosomes can interact with the chromosomes and the bipolar spindle is formed.<sup>1</sup> The highly conserved kinesin Eg5 (kinesin-5), is one of the main drivers of centrosome separation.<sup>2</sup> Eg5 can slide antiparallel MTs apart, thereby driving centrosome separation. Inhibition of Eg5-activity in mammalian cells blocks centrosome separation in both prophase and prometaphase and

cells arrest in mitosis with a characteristic monopolar spindle.<sup>3-6</sup>

## NE-Dynein can Drive Prophase Centrosome Separation

To identify additional pathways involved in centrosome separation, we designed an “in vitro” evolution approach. By treating cells with increasing doses of the Eg5 inhibitor S-trityl-L-cysteine (STLC<sup>7</sup>), we generated cells that grow in the complete absence of Eg5-activity.<sup>8</sup> Careful characterization of these Eg5-independent cells (EICs) showed that they undergo a relative normal cell division, with functional bipolar spindles and, strikingly, these EICs performed normal centrosome separation in prophase. We used these cells as a tool to study Eg5-independent mechanisms for centrosome separation. We found that kinesin-12/Kif15 (also known as Hklp2) promotes bipolar spindle assembly in these cells. Kif15 was previously identified to act together with Eg5 in bipolar spindle assembly.<sup>9,10</sup> Under normal conditions, Kif15-activity is not sufficient for bipolar spindle assembly in the absence of Eg5-activity. However, overexpression of Kif15 can fully compensate for the loss of Eg5-activity in prometaphase.<sup>9</sup> This function of Kif15 depends on its interaction with TPX2, a Ran-regulated microtubule-binding protein.<sup>9,10</sup> But Kif15 cannot take over all the functions of Eg5 since TPX2 is nuclear during interphase whereas Kif15 is present in the cytoplasm. This physical separation of Kif15 and TPX2 before NEB makes it impossible for Kif15 to drive centrosome separation in prophase. Indeed,

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depletion of Kif15 in normal cells and in EICs did not result in reduced centrosome separation in prophase.<sup>8,9</sup> Using the EICs, we instead identified a novel pathway that drives prophase centrosome separation involving the minus-end-directed motor dynein. Depletion of selected pools of dynein showed that nuclear envelope (NE)-associated dynein is responsible for prophase centrosome separation in the EICs. Interestingly, we found that NE-dynein is also involved in centrosome separation in normal cells, although in most cell types this function of NE-dynein is masked by the dominant activity of Eg5.

### **Mechanism of NE-Dynein-Dependent Centrosome Separation**

How can dynein, anchored to the NE, drive prophase centrosome separation? Results from various studies indicate that Eg5 generates an outward force specifically on the centrosome pairs, most likely by crosslinking overlapping MTs from both centrosomes in an antiparallel orientation and subsequently sliding them apart.<sup>5,11</sup> However, it was shown over a decade ago that centrosomes move largely independently of each other during prophase, indicating that the mechanism of prophase centrosome movement is, at least partially, intrinsic to each centrosome.<sup>12</sup> This suggests that the forces that drive prophase centrosome movement not only derive from the Eg5-driven antiparallel MT-sliding, but that additional forces that act on individual centrosomes also contribute to centrosome separation, independent of the MT-overlap.

In order to study centrosome movements that occur independent of the MT-overlap, we imaged centrosome movements in cells that contain only a single centrosome and are thus unlikely to form an antiparallel MT-overlap. We observed that single centrosomes moved substantial distances along the NE during prophase.<sup>8</sup> These movements are driven by NE-dynein since depletion of dynein itself or the dynein recruitment factor BICD2, significantly reduced the observed movements.

An important remaining question is: how can a motor that is homogeneously distributed over the NE provide

an asymmetric pulling force on a single centrosome, which is required for the centrosome to undergo net movement? One attractive hypothesis is that the MT aster growing from the centrosome is non-symmetric. Centrosomal MTs will have different lengths and longer MTs can interact with more dynein molecules on the NE. In this way, differences in the length of centrosomal MTs will be translated into differential forces acting on the centrosome from different sides. This would result in a net imbalance of forces acting on the centrosome where the longest MTs direct centrosome movement (**Fig. 1B**). But how could MTs nucleated from the centrosomes, be generated with different lengths? Stochastic changes in MT-length continuously occur because of dynamic instability of MTs. This alone will generate differences in length between different centrosomal MTs. However, stochastic fluctuations in length are unbiased and will likely not generate stable asymmetry in the total pool of centrosomal MTs. Possibly, the oval geometry of the nucleus could lead to differences in MT-stability along the surface of the nucleus. Alternatively, the pulling forces generated by dynein molecules could increase MT-stability and can thereby promote asymmetry of the aster toward the direction of movement. It was shown previously that polymerizing MTs show a dramatic increase in catastrophe frequency when they collide against barriers.<sup>13</sup> In case of two centrosomes that need to separate, MTs nucleated from one centrosome might collide into the dense MT network emanating from the other centrosome, resulting in more frequent catastrophes when growing toward the opposing centrosome. This would result in an asymmetric centrosomal MT-network with longer MTs growing away from the other centrosome. As a consequence, this asymmetric distribution might result in a net outward force leading to separation of the two centrosomes (**Fig. 1B**). A similar mechanism, involving MT length-dependent force generation, has been proposed during aster centering in early frog and fish embryos.<sup>14</sup> Future work should be directed to understand how NE-dynein functions and how it can generate polarized pulling forces on prophase MT-asters that promote centrosome separation.

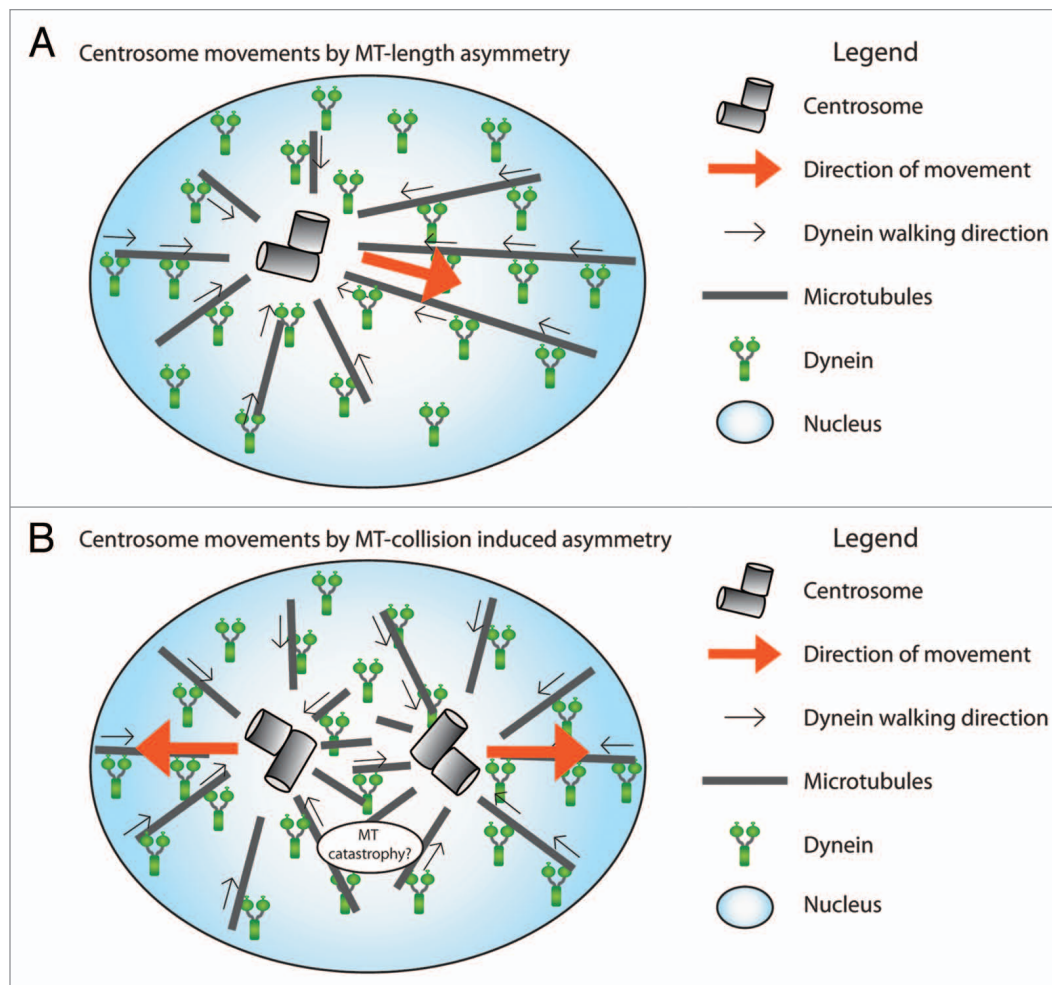
### **Importance of Prophase Centrosome Separation**

Previous studies showed that cells with normal Eg5-activity do not require prophase centrosome separation to build a functional bipolar spindle.<sup>15,16</sup> This raises the question whether prophase centrosomes separation is generally important for cells? In EICs, inhibition of prophase centrosome separation does prevent bipolar spindle assembly. In addition, normal cells with reduced Eg5-activity are more prone to form a monopolar spindle when prophase centrosome separation is inhibited.<sup>8</sup> Together, these results suggest that prophase centrosome separation acts redundant with mechanisms in prometaphase that push centrosomes apart. Such redundancy likely increases robustness in bipolar spindle assembly.

There is also evidence for additional roles for prophase centrosome separation. A recent study showed that cells, separating their centrosomes during prophase show about a 3-fold fewer errors in chromosome segregation and spend shorter time in mitosis compared with their counterparts that fail to separate their centrosomes before NEB.<sup>17</sup> Together, this data indicates that prophase centrosome separation improves the robustness and timely assembly of the bipolar spindle during mitosis, and improves the fidelity of chromosome separation.

### **Future Directions**

Despite the large amount of studies conducted in the last decade to understand centrosome separation, the contribution of different pathways and exact molecular mechanisms are still not fully understood. Due to its essential function in centrosome separation and bipolar spindle assembly, Eg5 has received a lot of attention. Multiple labs showed, using elegant in vitro studies, that Eg5 likely drives centrosome separation by sliding antiparallel MTs apart. In contrast to Eg5, the exact mechanism by which NE-dynein drives prophase centrosome separation remains to be determined. Detailed analysis of the MT network in prophase is required in order to see if symmetry-breaking events of MT growth indeed occur. In addition



**Figure 1.** Model of NE-dynein-mediated centrosome movement. **(A)** When forces act on a single centrosome, movements are likely directed toward the side of the longer MTs, due to the presence of higher number of dynein binding sites. **(B)** When two centrosomes are present, MTs emanating from one centrosome may physically collide with MTs from the other centrosome, resulting in MT catastrophe specifically between the two centrosomes. This results in an asymmetric distribution of dynein pulling forces and shifts the balance of centrosome movement in a net outward direction.

to that, tracking the movement of centrosomal pairs in an Eg5-independent fashion and in vitro reconstitution experiments would also give more insights into dynein-dependent centrosome separation in prophase.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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