Report

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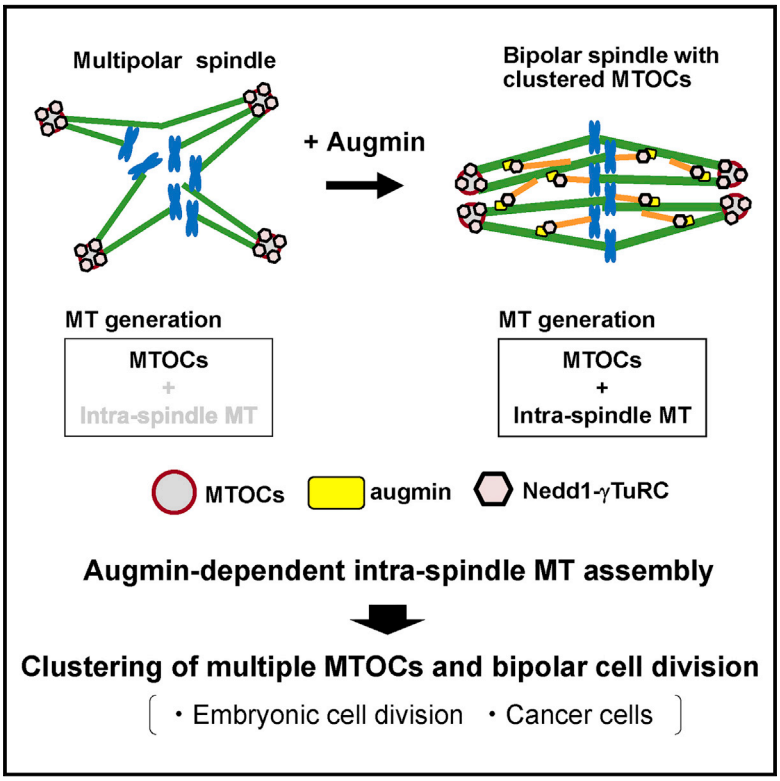
Intra-spindle Microtubule Assembly Regulates Clustering of Microtubule-Organizing Centers during Early Mouse Development

Graphical Abstract



Highlights

- An augmin knockout (KO) mouse is used to examine microtubule nucleation
- Augmin is essential for embryonic cell division during mouse development
- Live imaging of synchronized embryos reveals augmin-dependent clustering of MTOCs
- Intra-spindle MT assembly, but not augmin itself, is required for MTOC clustering

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In Brief

Microtubule-dependent microtubule nucleation is mediated by the augmin complex. Watanabe et al. analyze an augmin subunit knockout mouse and find that, in the knockout embryo, intra-spindle microtubules are reduced and MTOCs are not properly clustered, preventing bipolar spindle assembly.

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Intra-spindle Microtubule Assembly Regulates Clustering of Microtubule-Organizing Centers during Early Mouse Development

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SUMMARY

Errors during cell division in oocytes and early embryos are linked to birth defects in mammals. Bipolar spindle assembly in early mouse embryos is unique in that three or more acentriolar microtubule-organizing centers (MTOCs) are initially formed and are then clustered into two spindle poles. Using a knockout mouse and live imaging of spindles in embryos, we demonstrate that MTOC clustering during the blastocyst stage requires augmin, a critical complex for MT-dependent MT nucleation within the spindle. Functional analyses in cultured cells with artificially increased numbers of centrosomes indicate that the lack of intra-spindle MT nucleation, but not loss of augmin per se or overall reduction of spindle MTs, is the cause of clustering failure. These data suggest that onset of mitosis with three or more MTOCs is turned into a typical bipolar division through augmin-dependent intra-spindle MT assembly.

INTRODUCTION

The mitotic spindle, a microtubule (MT)-based structure, is critical for equal chromosome segregation into two daughter cells. In mammals, mechanisms of spindle MT generation and organization have been studied mostly in cell lines, which often have a pair of centrosomes as the major MT generation sites and determinants of spindle bipolarity (Reber and Hyman, 2015; Walczak and Heald, 2008). However, spindle MTs are additionally nucleated around chromosomes and from existing MTs. These MTs also play a critical role in efficient spindle assembly, chromosome segregation, and cytokinesis. In contrast, the in vivo spindle formation process in mammals has been less studied.

The mouse early embryo is a notable in vivo system, in which an atypical mechanism is employed for spindle assembly (Clift and Schuh, 2013; Courtois et al., 2012). In the first several divisions after fertilization, centrioles/centrosomes are absent, and instead three or more acentriolar MTOCs (MT organizing centers) are formed during the prophase. Upon nuclear envelope breakdown (NEBD), MTOCs dynamically change their positions in the cell and are clustered to form two spindle poles. Thus, the critical step toward spindle bipolarity establishment is MTOC clustering during the prometaphase. However, its mechanism has been poorly characterized. Since errors in chromosome segregation and/or cell division during embryonic development are directly linked to birth defects, it is important to understand the mechanism underlying spindle formation at this particular stage.

Augmin is a conserved, eight-subunit protein complex originally identified in *Drosophila* cells (Goshima et al., 2008). In every animal and plant cell type studied so far, augmin is required for MT-dependent MT nucleation (i.e., MT amplification) during cell division (Du et al., 2011; Hayward et al., 2014; Lawo et al., 2009; Meireles et al., 2009; Nakaoka et al., 2012; Petry et al., 2011; Uehara et al., 2009; reviewed by Sánchez-Huertas and Lüders, 2015). Augmin functions by localizing the γ-tubulin ring complex (γ-TuRC), a potent MT nucleator, to the existing MT (Goshima et al., 2007). A failure in intra-spindle MT amplification results in the formation of an insufficient number of kinetochore MTs or anaphase central spindle MTs (Uehara and Goshima, 2010; Uehara et al., 2009). Here, we examine the physiological function of augmin during mammalian development using an augmin-deficient mouse by knocking out a core subunit of augmin, HAUS6 (also called Aug6/Dgt6/FAM29A).

RESULTS

HAUS6 Is Essential for Early Embryonic Cell Division

We disrupted the gene encoding the HAUS6 subunit of murine augmin (Figures S1A–S1C). Heterozygous deficient mice (*Haus6*$^{+/-}$) were born healthy and fertile. However, all homozygous-deficient mice (*Haus6*$^{-/-}$; hereafter called KO mice) from the *Haus6*$^{+/-}$ intercrossing died before birth. The knockout (KO) embryos were identified during embryonic day 2.5 (E2.5), but not at post-implantation stages, suggesting that the development of KO mice was affected at early stages (Table 1).

During the blastocyst stage, KO embryos were often disorganized, in which mitotic defects were frequently observed (Figures 1A–1C). Spindle-localized γ-tubulin was reduced, consistent with the RNAi phenotype in mouse cell lines (Figures 1C, insets,