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Arrested Detachment: A DEPDC1B-Mediated De-adhesion Mitotic Checkpoint

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Mitotic cell rounding is accompanied by changes in the actin cytoskeleton, de-adhesion, and an increase in cortical rigidity. In this issue, Marchesi et al. (2014) describe an adhesion-dependent mitotic checkpoint and identify DEPDC1B as the factor responsible for coordinating de-adhesion with the ability of cells to enter mitosis.

One of the most dramatic changes in cell shape occurs during cell division. At the onset of mitosis, flat interphase cells become spherical in a matter of minutes. Mitotic cell rounding has been observed for several years, and it is believed to play a role in spindle orientation, chromosome orientation, and positioning of the cleavage furrow (Cramer and Mitchison, 1997; Sanger et al., 1984; Théry et al., 2005). Mitotic cell rounding is accompanied by changes in the actin cytoskeleton. During interphase, actin is predominantly organized into stress fibers that extend across the cytoplasm, anchored at one or both sides by focal adhesions (FAs). Upon entry into mitosis, focal adhesions and stress fibers disassemble, and actin relocalizes primarily to the cell cortex. This disassembly of FAs, or "de-adhesion," is a key event in the transition from flat to round cells and needs to be tightly coordinated with the cell cycle. The existence of an adhesion-dependent checkpoint has been a matter of speculation for many years. In this issue of *Developmental Cell*, Marchesi and colleagues characterize the role of DEPDC1B, a protein that accumulates during G2, in coordinating de-adhesion and cell-cycle progression at mitotic entry (Marchesi et al., 2014).

Marchesi et al. found that silencing DEPDC1B expression induced a delay in mitotic entry, specifically at the transition from G2 to M. DEPDC1B knockdown (KD) cells displayed FAs that were bigger and took longer to disassemble, suggesting that the mitotic delay could be caused by defects in de-adhesion. These effects could be rescued by conditions that weakened adhesion, such as vinculin KD, and phenocopied by conditions that promoted adhesion, confirming that adhesion was the key element regulating the mitotic delay. Overall, this can be considered a checkpoint since it is coordinated with the cell cycle and ensures the fidelity of the process. However, this adhesion-mediated checkpoint is "milder" than a G2/M arrest induced by DNA-damaging agents, and it delays rather than arrests cell-cycle progression.

Phenotypically, DEPDC1B KD cells are flatter and more motile and often fail to detach and become rounded. They also show a delay in the activation of mitosis-promoting factors, such as CDK1, and in the disassembly of the nuclear envelope, suggesting that the adhesion checkpoint acts upstream of these processes. In an elegant experiment, the authors show that silencing DEPDC1B had no effect in cells that had been adapted to grow in suspension, confirming that when no de-adhesion is needed, the function of DEPDC1B is dispensable.

It has been shown previously that the transition from flat interphase to round mitotic cells is a carefully choreographed process that is regulated by the small GTPase RhoA through its effector Rho kinase (ROCK) (Maddox and Burridge, 2003). RhoA activity is high during interphase and also during cell rounding, but it is not required for de-adhesion (Maddox and Burridge, 2003). These results suggest RhoA may need to be inactivated for cells to detach. Indeed, in DEPDC1B KD cells, both RhoA activity and Myosin light chain 2 (MLC2) phosphorylation levels are increased. Since MLC2 is one of the main targets of ROCK, these results suggest that DEPCD1B functions as a negative regulator of the RhoA/ROCK signaling pathway. Consistent with this hypothesis, the DEPDC1B mitotic delay can be rescued by silencing RhoA (but not other GTPases) or by inhibiting ROCK.

How does DEPDC1B modulate RhoA activity? Although DEPC1B has a

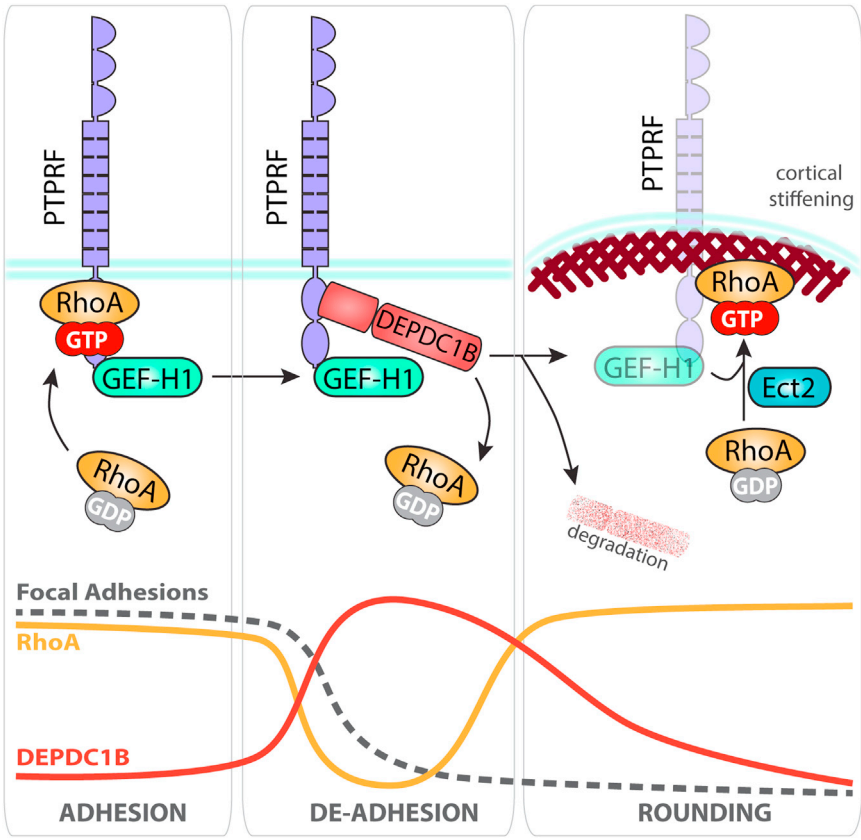

Figure 1. A DEPDC1B-Mediated De-adhesion Checkpoint

During interphase, cells are firmly attached to the extracellular matrix through focal adhesions (FAs). Assembly of FAs and stress fibers require the activity of RhoA. RhoA activation is mediated (at least in part) by GEF-H1, which is recruited to FAs by the transmembrane phosphatase PTPRF. DEPDC1B accumulates during G2 and competes with RhoA for PTPRF binding. This results in a displacement of RhoA from the complex and subsequent inactivation. During mitotic rounding, RhoA is activated by Ect2 to promote the polymerization of cortical actin, which imparts rigidity to the cell and functions in positioning the mitotic spindle and the cleavage furrow. When cells enter mitosis, DEPDC1B is slowly degraded by the proteasome, potentially allowing PTPRF to recruit RhoA, which could then be activated by GEF-H1.

RhoGAP domain, it lacks a critical arginine residue and is therefore inactive. Therefore, to understand the molecular mechanisms that regulate DEPDC1B-mediated de-adhesion, the authors performed a two-hybrid screen and found that DEPC1B interacts with protein tyrosine phosphatase, receptor type, F (PTPRF). PTPRF is a transmembrane receptor protein tyrosine phosphatase involved in the regulation of adhesion and migration that localizes to FAs (Serra-Pagès et al., 1995). Silencing PTPRF expression had no effect on mitotic entry, but it rescued the mitotic delay induced by DEPDC1B KD. This function did not require the phosphatase domain since phosphatase inhibitors had no effect on mitotic entry either in control cells or in DEPDC1B KD cells. Interestingly, a proteomic analysis revealed that PTPRF interacts with several components of the RhoA signaling pathway, including RhoA itself, and several RhoA-specific guanine nucleotide-exchange factors (GEFs). Among the GEFs identified, GEF-H1 is of particular importance because it is known to activate RhoA at FAs in response to mechanical stress downstream of integrins (Guilluy et al., 2011). Silencing GEF-H1 in DEPDC1B KD cells rescued the mitotic delay, whereas overexpressing it in control cells phenocopied DEPDC1B silencing. By analyzing the components of the PTPRF signaling complex, the authors showed that DEPDC1B competes with RhoA for binding to PTPRF. Thus, when cells express DEPDC1B, RhoA is displaced from PTPRF and fails to be activated by GEF-H1, leading to de-adhesion. Once de-adhesion is completed and mitotic rounding starts, generation of cortical rigidity requires high RhoA activity. It is not clear whether PTPRF and GEF-H1 contribute to RhoA activation at this stage, but since DEPDC1B is degraded when cells enter mitosis, RhoA could potentially reassociate with PTPRF and become reactivated by GEF-H1.

Some of these findings were corroborated at the organism level in zebrafish. Silencing the DEPDC1B ortholog in zebrafish (*depdc1b*) using morpholinos induced severe mitotic defects, possibly at the G2/M transition. These defects were rescued by expressing the human DEPDC1B or by silencing the RhoA or PTPRF orthologs in zebrafish, suggesting that the pathway is conserved.

Marchesi et al. thus describe an adhesion-mediated checkpoint, supporting a model in which both GEF-H1 and RhoA are recruited to FAs by PTPRF in interphase cells, where GEF-H1 activates RhoA and promotes adhesion (Figure 1). As cells progress into mitosis, expression of DEPDC1B inhibits RhoA signaling and stimulates de-adhesion and cell rounding. These findings raise some questions for future investigations. Regulation of FA disassembly during mitosis has been previously shown to require the function of another small GTPase, Rap1 (Dao et al., 2009). Rap1 activity follows a pattern similar to that of RhoA: it is active in interphase, and activity decreases during de-adhesion and increases again in mitosis. It is not known how Rap1 regulation is coordinated with the cell cycle or whether it acts in concert with RhoA during de-adhesion. Is Rap1 activity regulated by DEPDC1B? If so, does it function upstream or downstream of RhoA? There is some evidence of potential crosstalk between Rap1 and RhoA outside mitosis. It would be interesting to determine whether such crosstalk also exists during mitosis. It would also be interesting to characterize this system in cancer cells, where this "adhesion-dependent" checkpoint may be subverted to favor cell-cycle progression.

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HORMA Domains at the Heart of Meiotic Chromosome Dynamics

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HORMA domain proteins are required for the careful orchestration of chromosomal organization during meiosis. Kim et al. (2014) and Silva et al. (2014) now provide structural and functional insights into the roles of *C. elegans* HORMA proteins, revealing parallels to the function of the HORMA protein MAD2 in mitotic checkpoint signaling.

Fidelity of chromosome segregation is crucial to the homeostasis of eukaryotic organisms. During meiosis, this process is characterized by extensive reorganization of chromosome structure and behavior, allowing pairing and synapsis of homologous chromosomes. Although these are essential preconditions for faithful chromosome segregation during meiosis, how these events are achieved and coordinated with cell-cycle progression is poorly understood. The evolutionarily conserved HORMA domain-containing proteins (named after the Hop/Rev7/Mad2 proteins; Aravind and Koonin, 1998) play conserved roles in chromosome segregation. Two new studies in this issue of *Developmental Cell* provide insights into the events and mechanisms by which HORMA proteins regulate chromatin dynamics during meiosis in *C. elegans* (Kim et al., 2014; Silva et al., 2014), highlighting similarities with the functions of the mammalian HORMA domain-containing protein Mad2 in mitosis.

Mad2 is a crucial effector of mitotic checkpoint signaling, and studies of this protein have established a paradigm for how HORMA domain proteins function. Mad2 establishes an inhibitory mitotic checkpoint complex (MCC), which binds and inhibits the APC/C activator Cdc20 to halt cell-cycle progression in the presence of unattached kinetochores (Mapelli and Musacchio, 2007). Unattached kinetochores catalyze the formation of a "closed" active state of the Mad2 HORMA domain (C-Mad2), which binds and locks a motif of Cdc20 using a "safety belt" topological domain. Repositioning of this safety belt converts Mad2 to an "open," inactive (O-Mad2) state, releasing Cdc20. The switching between the two states is essential for proper mitotic checkpoint signaling.

The meiosis-specific *C. elegans* HORMA domain proteins, HIM-3, HTP-1, HTP-2, and HTP-3, have both overlapping and divergent roles regulating chromosome cohesion, DNA break formation and recombination, checkpoint control, and chromosome pairing and synapsis (Muniyappa et al., 2014; Subramanian and Hochwagen, 2014), which is potentially due to their divergent C-terminal regions. How these proteins organize themselves to accomplish these roles is unknown, and Kim et al. (2014) determined the crystal structure of HIM-3 to provide molecular insights into this question.

This crystal structure revealed that the C-terminal region of HIM-3 has a topology similar to C-Mad2. However, the safety belt of HIM-3 binds a motif from its own extended C terminus, leading the authors to dub this motif a "closure motif." Similar closure motifs are also present in the C termini of HTP-1, HTP-2, and HTP-3, with HTP-3 harboring six motifs. By combining biochemical and structural analyses, the authors revealed a molecular network of HORMA-closure motif interactions and showed that HTP-3 recruits HIM-3 and HTP-1/HTP-2, whereas the closure motifs in HIM-3 interact with the HORMA domains of HTP-1 and HTP-2. Collectively, these interactions define a hierarchical assembly model in which HTP-3 is the most chromosome-proximal component, HIM-3 is the intermediate component, and HTP-1 and HTP-2 are the most peripheral components. Elegant in vivo dissection of this HORMA domain assembly network (using several closure motif mutants) demonstrated differential effects of the HORMA proteins on chromosomal functions, with HTP-3 showing the most pleiotropic effects, as expected from its inferred biochemical role at the basis of this assembly (Figure 1).