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SPOTLIGHT

Cell cycle pacemaker keeps adhesion in step with division

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Adherent cells round up before division but it is unclear how detachment is regulated by the cell cycle. In this issue, Jones et al. (2018. *J. Cell Biol.* https://doi.org/10.1083/jcb.201802088) find the kinase CDK1 maintains adhesion during interphase by phosphorylating integrin adhesome proteins, including the formin FMNL2, and loss of this function of CDK1 activity in G2 triggers adhesion disassembly.

The actomyosin cytoskeleton is used by cells for various processes that require mechanical force, such as migration and division. Every process is driven by a distinct actomyosin structure. Although the basic contractile machinery is common to all actomyosin structures, each has a unique subset of accessory proteins that define its internal organization and links it with other cellular structures, such as the plasma membrane, the nucleus, or cell adhesion sites (Zaidel-Bar et al., 2015). How specific actomyosin networks are assembled or disassembled at the correct time and place is an area of intense research. Multiple actomyosin structures can coexist in the cell simultaneously. However, some cytoskeletal organizations are incompatible with others. In this issue, Jones et al. address the ill-fitting combination of basal actomyosin stress fibers with the cortical organization that is required for mitosis.

It has long been observed that adherent cells disassemble their basal stress fibers and most of their cell matrix adhesion complexes before they round up in preparation for cell division. Cell rounding is important to make space for the mitotic spindle and for correct spindle alignment (Luxenburg et al., 2011). Not allowing cells to disassemble their matrix adhesions inhibits cell rounding and results in severe defects in cytokinesis and multinucleated cells. Why is the disassembly of cell matrix adhesions and stress fibers necessary? First of all, strong cell matrix adhesion physically opposes the detachment of the cell membrane necessary for cell rounding. Second, protein components of stress fibers may be needed in the cortex to increase its contractility. Finally, it is important for the mitotic cortex to start out as an isotropic, or uniform, network upon which signals emanating from the spindle will direct the assembly of the cytokinetic ring.

Jones et al. (2018) examined the size and distribution of cell matrix adhesion complexes and the actin cytoskeleton in synchronized HeLa and U2OS cells. They found small peripheral adhesions and circumferential actin in G1 phase, large adhesions and massive stress fibers throughout the cell in S phase, and then a return to small peripheral adhesions and actin in G2 phase, in anticipation of M phase. These observations are consistent with recent traction force measurements performed on RPE1 cells expressing a cell cycle reporter, showing high mechanical traction energies in S phase and low traction energies in G2 (Vianay et al., 2018).

The question then becomes what is regulating the disassembly of adhesion complexes and stress fibers during G2 and how is this disassembly coordinated with cell cycle progression? Based on their previous mass spectrometry studies, Jones et al. (2018) had a candidate regulator: CDK1, the cyclin-dependent serine/threonine kinase that in complex with cyclins is a major driver of the cell cycle. Phosphoproteomics revealed that many integrin adhesion complex proteins are potential substrates of CDK1 and inhibition of CDK1 resulted in a loss of actin stress fibers and the existence of only small adhesions in the cell periphery, reminiscent of the phenotype of cells in G2 (Robertson et al., 2015). In the current study, Jones et al. (2018) went on to show that cyclin A2 is the cyclin working with CDK1 to promote large integrin adhesion complexes and stress fibers and they used more precise proteomics to identify CDK1 substrates in interphase cells. Among the 26 substrates identified were several unconventional myosins and actin regulators, including WDR1, PLS3, WASF2, and FMNL2.

Jones et al. (2018) followed up on the formin FMNL2, an actin nucleator and elongation-promoting factor, demonstrating both in vitro and in cells that it is phosphorylated by CDK1 on serine residue 1016. Importantly, a phosphomimetic FMNL2-S1016E can partially rescue the cell adhesion phenotype in CDK1 knockdown cells, demonstrating that phosphorylation of FMNL2 contributes to formation of central adhesion complexes and stress fibers downstream of CDK1, but that CDK1 has other targets during S phase. Interestingly, FMNL2 phosphorylation levels do not increase during S phase, but they go down in G2. Thus, it appears that cyclin A2–CDK1

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activity is required constitutively for central adhesions and stress fibers to appear and remain during S phase, and their disassembly in G2 is the result of CDK1 inactivation. CDK1 is well known to be inactivated in G2 by Wee1-dependent phosphorylation, as part of the G2/M checkpoint (reviewed in Hégarat et al., 2016). However, Jones et al. (2018) found that an increase in cyclin B1 expression in G2 is essential for CDK1 inactivation, since RNAi of cyclin B1 prevents adhesion disassembly in G2, just as inhibition of Wee1 does. It remains unclear how cyclin B1 regulates Wee1 activity toward CDK1.

A recent study of forces exerted by epithelial cells in a monolayer throughout the cell cycle has revealed that 3 h before mitosis, during the G2 phase, cells pull less on their neighbors, presumably by modulating their cell–cell junctions (Uroz et al., 2018). It will be worthwhile to study whether CDK1 inactivation also plays a role in down-regulating tension at cell–cell junctions. Of note, the formin FMNL2 and its paralog FMNL3 have both been shown to contribute to actin polymerization and stability of adherens junctions (Grikscheit et al., 2015; Rao and Zaidel-Bar, 2016).

Finally, it should be pointed out that although classical integrin adhesion complexes are being disassembled during G2, it was recently reported that the integrins themselves remain in place. These plaque-less integrin contacts connect mitotic cells to the underlying matrix throughout mitosis and guide the respreading of daughter cells (Dix et al., 2018). It will be interesting to test whether the CDK1-induced disassembly of adhesion sites is distinct from the disassembly that occurs, for instance, at the trailing edge of migrating cells and figure out how cells balance the loss of most of their adhesion complex components with the retention of sufficient adhesion so micro-environmental knowledge is retained.

Returning to the question of how specific actomyosin structures are assembled and disassembled at specific times and locations within the cell and how this regulation is coordinated with the cell cycle, we must acknowledge that the cell came up with a very elegant solution: the cell cycle pacemaker kinase (CDK1) is moonlighting during interphase to regulate integrin adhesion complexes and stress fibers. It will be exciting to find out what other nonmitotic activities CDK1 engages in during interphase.

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