Stuck in the middle: Rac, adhesion, and cytokinesis

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Rho family small GTPases (Rac, RhoA, and Cdc42) function at the core of cytokinesis, the physical division of one cell into two. In this issue, Bastos et al. (2012. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201204107) identify a new role for Rac inhibition: to release cell adhesion at the division plane and allow efficient constriction of the contractile ring. They show that the GTPase-activating protein, CYK4, suppresses equatorial cell substrate adhesion by inhibiting Rac and therefore its effectors ARFGEF7 and PAK1/2.

To ensure that cell division occurs at the correct time and place, spatially regulated signaling mediated by the mitotic spindle directs the assembly and constriction of an actomyosin contractile ring at the site of cytokinesis. Critical for cytokinesis is centralspindlin, a conserved tetramer consisting of a dimeric Rho family GTPase-activating protein (GAP), CYK4 (also called MgcRacGAP in mammals, CYK-4 in *Caenorhabditis elegans*, and RacGAP50C in *Drosophila melanogaster*), constitutively bound to a kinesin-6 dimer (Mklp1 in mammals, ZEN-4 in *C. elegans*, and Pavarotti in *Drosophila*). Centralspindlin localizes to the spindle midzone (or central spindle) in anaphase and to the midbody (or Fleming body) late in cytokinesis. At the midzone, centralspindlin contributes to the organization of antiparallel microtubule bundles and the recruitment of other players (Green et al., 2012). One controversial aspect of centralspindlin function is the role of CYK4 GAP activity during cytokinesis, as different studies have concluded that it inhibits RhoA (Jantsch-Plunger et al., 2000; Miller and Bement, 2009) or Rac signaling (Canman et al., 2008), activates RhoA signaling indirectly (Loria et al., 2012), or is dispensable for cell division altogether (Goldstein et al., 2005; Yamada et al., 2006).

Much of the controversy regarding CYK4 function has arisen because the main targets of its GAP activity in vitro, Rac and Cdc42, do not play an essential positive role during cytokinesis. When CYK4 was first identified in human male germ cells, it was shown biochemically to specifically stimulate the GTPase activity of Rac and, to a lesser extent, Cdc42, but not RhoA, and was thus termed MgcRacGAP (Touré et al., 1998). This increased activity toward Rac and Cdc42 relative to RhoA was also later reported for recombinant worm and mammalian CYK4 (Jantsch-Plunger et al., 2000; Kawashima et al., 2000). However, as RhoA is the only Rho family GTPase essential for cytokinesis during mitosis, it was assumed that CYK4 acts on RhoA in vivo (Jantsch-Plunger et al., 2000). It has also been postulated that Aurora B phosphorylation of S387 in the human CYK4 GAP domain active site can functionally convert the specificity from Rac/Cdc42 to RhoA during cytokinesis (Minoshima et al., 2003). This model has remained contentious, though, because of the lack of conservation of S387 in CYK4 across species.

Bastos et al. (in this issue) now address this controversy by demonstrating that, consistent with work performed in *Drosophila* and *C. elegans*, mammalian Rac plays a negative role in cytokinesis, and CYK4 is required to "release this brake" for cytokinesis to proceed. They first began their study with a comprehensive in vitro analysis of CYK4 GAP activity. By testing a recombinant human CYK4 GAP domain against a panel of small GTPases, they confirmed that the GAP activity is specific for Rac and Cdc42 but shows very little detectable activity for RhoA. They then tested native protein by assaying the activity of centralspindlin immunoprecipitated at different stages of the

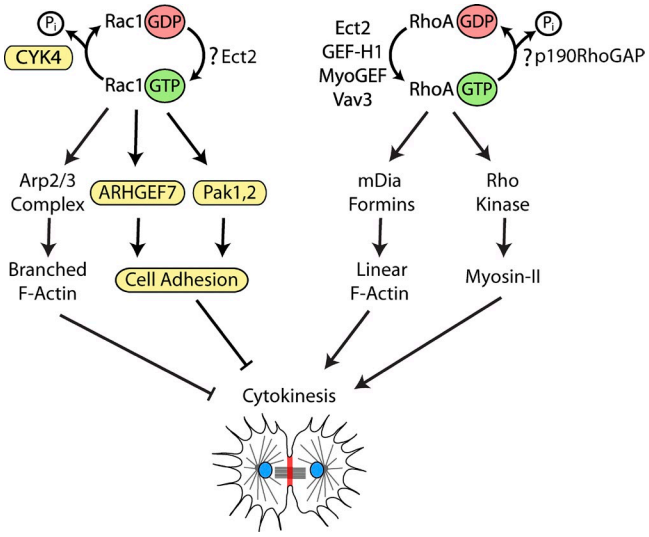

Figure 1. **Rac and RhoA signaling pathways during cytokinesis.** Components of the Rac pathway revealed by Bastos et al. (2012) are shown in yellow. mDia, mammalian diaphanous; MyoGEF, myosin-interacting guanine nucleotide exchange factor.

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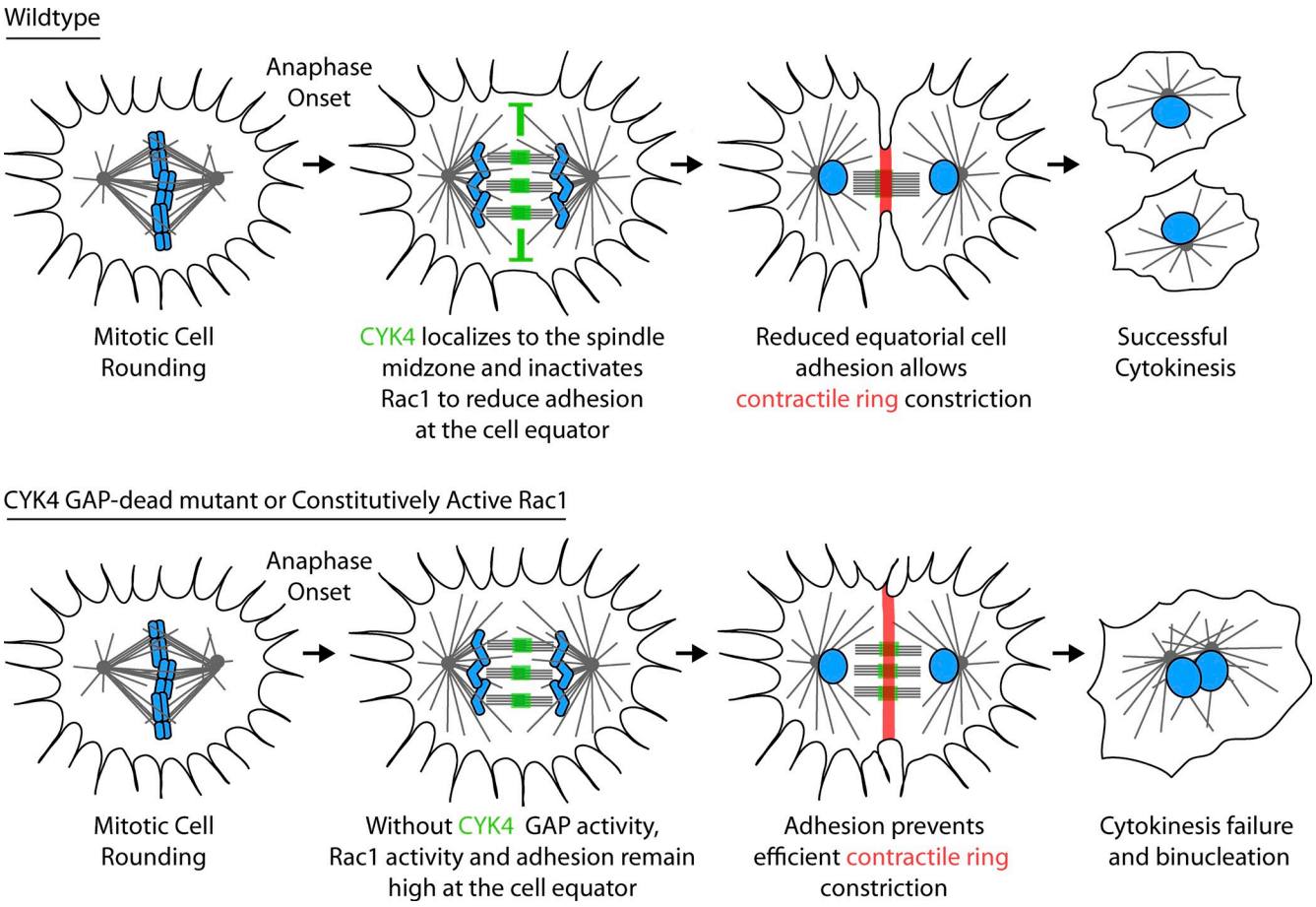

Figure 2. **Adhesion and cytokinesis.** Schematic depicting why Rac-mediated activation of cell adhesion within the division plane inhibits cytokinesis.

HeLa cell cycle and showed that endogenous centralspindlin also acts as a GAP for Rac and Cdc42 but not RhoA (at least in vitro). Using mass spectrometric analysis, they were unable to identify any phosphorylation sites within the CYK4 GAP domain, although previously identified phosphorylation sites outside this domain were found. They also showed that the CYK4 GAP specificity was independent of the mitotic kinases Cdk1, Aurora B, and Plk1. Lastly, the CYK4^{S387D} mutation previously suggested to mimic phosphorylation by Aurora B did not upregulate RhoA GTPase activity but rather inhibited all in vitro CYK4 GAP activity, refuting the model that Aurora B phosphorylation alters CYK4 specificity (Minoshima et al., 2003).

The authors then focused on identifying potential targets of these GTPases via pull-downs of recombinantly expressed Rac, RhoA, and Cdc42. They found that each GTPase likely regulates a functionally distinct set of proteins: proteins pulled down with Cdc42 are known to act in cell motility, those binding RhoA contribute to contractile ring formation and ingression, and those binding Rac act in cell adhesion. As CYK4 negatively regulates Rac, they hypothesized that limiting cell adhesion may be important for division. To support this model, they showed that depletion of Rac binding partners implicated in cell adhesion (ARHGEF7, PAK1, and PAK2) suppresses the cytokinesis failure induced by expression of either constitutively active Rac or GAP-dead CYK4 (Fig. 1). Furthermore, they found that cells expressing GAP-dead CYK4 had increased cell substrate adhesion during cytokinesis as well as aberrant equatorial adhesion fibers, indicating that CYK4 is important for inhibiting Rac-dependent adhesion at the division plane (Fig. 2).

An inhibitory role for CYK4 as a Rac GAP during cytokinesis has also been demonstrated genetically in both *Drosophila* (D'Avino et al., 2004) and *C. elegans* (Canman et al., 2008). Depleting or mutating Rac in the fly eye dramatically suppresses the rough eye phenotype of CYK4 GAP dead–expressing mutants, presumably caused by suppressing cell division failure. In *C. elegans*, depletion of Rac or Rac effectors that activate the Arp2/3 complex (a nucleator of branched actin filaments) also suppresses the cytokinesis defect of GAP-dead CYK4 mutant embryos (Canman et al., 2008). Thus, Rac inhibition at the cell equator may suppress multiple downstream pathways within the same cell or may control different pathways across species.

The inability of CYK4 to affect RhoA GTP hydrolysis under any condition tested seems to challenge models in which CYK4 acts directly on RhoA but certainly does not preclude an indirect effect. Cross talk among Rho GTPases is well documented in other cellular contexts (Burridge, 1999; Machacek et al., 2009). In motile cells, Rac activity can indirectly affect RhoA activity (Sander et al., 1999), and this could also occur during cytokinesis. Indeed, expression of GAP-dead CYK4 in

dividing *Xenopus laevis* embryos defocuses the zone of active RhoA in the division plane (Miller and Bement, 2009). Therefore, CYK4 down-regulation of Rac may be indirectly shaping the zone of RhoA activity at the cell equator in vivo.

Nevertheless, this newly identified role for CYK4 as a Rac GAP controlling equatorial adhesion disassembly lends further support to a model in which Rac inactivation in the division plane is critical for cytokinesis. The use of an in vivo fluorescence resonance energy transfer activity sensor revealed that active Rac is lower in the division plane than in the polar regions of dividing cells (Yoshizaki et al., 2003). This reduction in equatorial Rac activity was not observed in cells expressing GAP-dead CYK4 (Yoshizaki et al., 2004). Together, these data suggest that CYK4 functions to inhibit Rac activity at the cell equator.

In summary, the Barr laboratory has helped to clarify some of the contentious issues surrounding CYK4 in Rho family GTPase regulation during cytokinesis. Their model, in which equatorial Rac inactivation reduces adhesion at the cell equator (Fig. 2), reveals fresh links among known cytokinetic regulators and effectors and implicates a novel role for ARFGEF7 and PAK1/2 inhibition in cell division (Fig. 1).

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References

Bastos, R.N., X. Penate, M. Bates, D. Hammond, and F.A. Barr. 2012. CYK4 inhibits Rac1-dependent PAK1 and ARHGEF7 effector pathways during cytokinesis. *J. Cell Biol.* 198:865–880.

Burridge, K. 1999. Crosstalk between Rac and Rho. *Science*. 283:2028–2029. http://dx.doi.org/10.1126/science.283.5410.2028

Canman, J.C., L. Lewellyn, K. Laband, S.J. Smerdon, A. Desai, B. Bowerman, and K. Oegema. 2008. Inhibition of Rac by the GAP activity of centralspindlin is essential for cytokinesis. *Science*. 322:1543–1546. http://dx.doi.org/10.1126/science.1163086

D'Avino, P.P., M.S. Savoian, and D.M. Glover. 2004. Mutations in *sticky* lead to defective organization of the contractile ring during cytokinesis and are enhanced by *Rho* and suppressed by *Rac*. *J. Cell Biol.* 166:61–71. http://dx.doi.org/10.1083/jcb.200402157

Goldstein, A.Y., Y.N. Jan, and L. Luo. 2005. Function and regulation of Tumbleweed (RacGAP50C) in neuroblast proliferation and neuronal morphogenesis. *Proc. Natl. Acad. Sci. USA*. 102:3834–3839. http://dx.doi.org/10.1073/pnas.0500748102

Green, R.A., E. Paluch, and K. Oegema. 2012. Cytokinesis in animal cells. *Annu. Rev. Cell Dev. Biol.*

Jantsch-Plunger, V., P. Gönczy, A. Romano, H. Schnabel, D. Hamill, R. Schnabel, A.A. Hyman, and M. Glotzer. 2000. CYK-4: A Rho family GTPase activating protein (GAP) required for central spindle formation and cytokinesis. *J. Cell Biol.* 149:1391–1404. http://dx.doi.org/10.1083/jcb.149.7.1391

Kawashima, T., K. Hirose, T. Satoh, A. Kaneko, Y. Ikeda, Y. Kaziro, T. Nosaka, and T. Kitamura. 2000. MgcRacGAP is involved in the control of growth and differentiation of hematopoietic cells. *Blood*. 96:2116–2124.

Loria, A., K.M. Longhini, and M. Glotzer. 2012. The RhoGAP domain of CYK-4 has an essential role in RhoA activation. *Curr. Biol.* 22:213–219. http://dx.doi.org/10.1016/j.cub.2011.12.019

Machacek, M., L. Hodgson, C. Welch, H. Elliott, O. Pertz, P. Nalbant, A. Abell, G.L. Johnson, K.M. Hahn, and G. Danuser. 2009. Coordination of Rho GTPase activities during cell protrusion. *Nature*. 461:99–103. http://dx.doi.org/10.1038/nature08242

Miller, A.L., and W.M. Bement. 2009. Regulation of cytokinesis by Rho GTPase flux. *Nat. Cell Biol.* 11:71–77. http://dx.doi.org/10.1038/ncb1814

Minoshima, Y., T. Kawashima, K. Hirose, Y. Tonozuka, A. Kawajiri, Y.C. Bao, X. Deng, M. Tatsuka, S. Narumiya, W.S. May Jr., et al. 2003. Phosphorylation by aurora B converts MgcRacGAP to a RhoGAP during cytokinesis. *Dev. Cell*. 4:549–560. http://dx.doi.org/10.1016/S1534-5807(03)00089-3

Sander, E.E., J.P. ten Klooster, S. van Delft, R.A. van der Kammen, and J.G. Collard. 1999. Rac downregulates Rho activity: reciprocal balance between both GTPases determines cellular morphology and migratory behavior. *J. Cell Biol.* 147:1009–1022. http://dx.doi.org/10.1083/jcb.147.5.1009

Touré, A., O. Dorseuil, L. Morin, P. Timmons, B. Jégou, L. Reibel, and G. Gacon. 1998. MgcRacGAP, a new human GTPase-activating protein for Rac and Cdc42 similar to *Drosophila* rotundRacGAP gene product, is expressed in male germ cells. *J. Biol. Chem.* 273:6019–6023. http://dx.doi.org/10.1074/jbc.273.11.6019

Yamada, T., M. Hikida, and T. Kurosaki. 2006. Regulation of cytokinesis by mgcRacGAP in B lymphocytes is independent of GAP activity. *Exp. Cell Res.* 312:3517–3525. http://dx.doi.org/10.1016/j.yexcr.2006.07.026

Yoshizaki, H., Y. Ohba, K. Kurokawa, R.E. Itoh, T. Nakamura, N. Mochizuki, K. Nagashima, and M. Matsuda. 2003. Activity of Rho-family GTPases during cell division as visualized with FRET-based probes. *J. Cell Biol.* 162:223–232. http://dx.doi.org/10.1083/jcb.200212049

Yoshizaki, H., Y. Ohba, M.C. Parrini, N.G. Dulyaninova, A.R. Bresnick, N. Mochizuki, and M. Matsuda. 2004. Cell type-specific regulation of RhoA activity during cytokinesis. *J. Biol. Chem.* 279:44756–44762. http://dx.doi.org/10.1074/jbc.M402292200