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# Mitotic entry elucidated with bacterial toxin toolbox

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The transition from $G_2$ phase into mitosis is driven by the kinase activity of the Cdk1/cyclin B complex. Cdk1 becomes activated in late $G_2$ phase by mitotic kinases, including Aurora A and mitotic Cdc25 phosphatases. About 10 y ago, Jackman and coworkers identified the centrosomes as a scaffold for the activation of Cdk1-cyclinB,[1] and Hirota et al. demonstrated that Cdk1 activation is induced by Aurora A, which itself becomes activated by phosphorylation during late $G_2$ phase at the centrosomes.[2] Aurora A activation is crucial for the centrosomal recruitment and activation of Cdk1/cyclin B, and therefore for the mitotic entry.[2] Although the role of the centrosomes as a scaffold for the spatiotemporally controlled activation of the mitotic kinases to trigger $G_2$/M transition was established, the upstream regulators controlling their centrosomal recruitment and activation remained unknown. However, the findings of Zhao et al.,[3] that p21-activated kinase (PAK), a downstream effector of Rac and Cdc42 family members of Rho-GTPases, catalyzes the centrosomal activation of Aurora A, suggested a central function of Rho-GTPases in the control of mitotic entry.

Rho-GTPases are central "molecular switches" that interact in their GTP-bound forms with multiple effector molecules to regulate various cellular functions, including reorganization of the actin cytoskeleton, migration, or cytokinesis.[4] As important hubs of cellular organization, Rho-GTPases are also the specific intracellular substrates for bacterial protein toxins, such as toxin B (TcdB) from *Clostridium difficile*.[5] TcdB is very efficiently taken up into the cytosol of target cells, where its catalytic domain specifically glucosylates Rho, Rac, and Cdc42. This modification prevents the interaction of the GTPases with their effector molecules and inhibits the signaling through these Rho-GTPases in living cells.[5] Narumyia and coworkers used TcdB as molecular scalpel to establish regulatory functions of Rho-GTPases for centrosomal PAK activation in late $G_2$ phase.[6] Treatment of synchronized HeLa cells with TcdB in the $G_2$ phase prevented the centrosomal activation of PAK, Aurora A, and Cdk1 and delayed mitotic entry.[6] Experiments with *Clostridium botulinum* C3 toxin, a selective inhibitor of Rho-mediated signaling, excluded a role of Rho for these effects.[6] The question whether Rac, Cdc42, or both control the centrosomal recruitment and activation of the mitotic kinases remained, however, open.

Published in this volume of *Cell Cycle*, May and coworkers tackled this question by extending the work by Ando et al. In cleverly designed experiments they used a variant of *Clostridium difficile* toxin B that selectively inhibits Rac activity in living cells.[7] Treatment of synchronized HeLa cells in $G_2$ phase of the cell cycle with this specific Rac-inhibiting toxin prevented the activation of PAK, Aurora A, and Cdk1/cyclinB at the $G_2$/M border and delayed entry of cells into mitosis for 2 h. This observation indicates Rac—but not Cdc42 or Rho—as the missing player in this process.[7] Where is Rac's mitotic point of entry? By analyzing isolated centrosomes, the authors demonstrated that Rac1—but not Cdc42 or Rho—is associated with the centrosomes during $G_2$ phase.[7] This centrosome-associated Rac1 specifically recruits PAK to the centrosomes in the late $G_2$ phase,[7] suggesting that the activation of the mitotic kinases and thereby the entry of cells into mitosis is regulated by a Rac1/Pak2-dependent pathway. The study not only answered but also raised further questions about the mechanism of Pak activation at the centrosomes. How does Rac associates and dissociates in time from the centrosomes? Inactivating the Rac/PAK connection only delays but does not abrogate mitotic entry. Which "rescue" mechanism kicks in, and why does it take 2 h for doing so?

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