Coping with loss: Cell adaptation to cytoskeleton disruption

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Summary

Unravelling the role of cytoskeleton regulators may be complicated by adaptations to experimental manipulations. In this issue of *Developmental Cell*, Cerikan et al. (2016) reveal how acute effects of *DOCK6* RhoGEF depletion on RAC1 and CDC42 activation are reversed over time by compensatory mechanisms that re-establish cellular homeostasis.

Targeted genetic disruption has become an essential component of the molecular toolkit that enables researchers to characterize their genes of interest. However, the choice of approaches raises important questions: Is permanent disruption or transient depletion (for example, by RNA interference) more informative of the “true” function of a gene under study? Similarly, is transient depletion a valid approach to model genetic disruptions observed in human diseases or pathologies? In this issue of *Developmental Cell*, Cerikan et. al. (2016) identify a compensatory mechanism that explains why acute depletion of the *DOCK6* guanine nucleotide exchange factor (GEF) rapidly leads to cytoskeleton collapse due to reduced RAC1 and CDC42 activity, and yet sustained depletion or complete loss of *DOCK6* do not. These observations clarify how mutation of both *DOCK6* alleles in fibroblasts from Adams-Oliver Syndrome (AOS) patients has relatively mild effects on cytoskeletal structures and cell morphology (Shaheen et al., 2011) compared to the profound alterations and mitotic defects induced by small interfering RNA (siRNA)-mediated *DOCK6* knockdown (Cerikan et al., 2016). This study also demonstrates that, by examining the differences in responses to acute versus permanent gene depletion, the molecular basis for adaptations that re-establish homeostasis can be identified.

Rho GTPases are regulators of cytoskeleton organisation and cell morphology (Sadok and Marshall, 2014). Their function as molecular switches upstream of signaling pathways that regulate actin dynamics is dependent on cycling between active GTP-bound and inactive GDP-bound states. Switching between these active and inactive states is regulated by the actions of GEFs, which stimulate GDP release to allow GTP binding, and GTPase-activating proteins (GAPs), which catalyse GTP hydrolysis. Mutations of Rho GTPases, GEFs or GAPs may have significant consequences on cytoskeletal structures and cell morphology, and may contribute to human genetic pathologies or diseases. Adams-Oliver Syndrome (AOS) is a condition characterized by abnormal skin development and limb malformations (Lehman et al., 2016), with four associated autosomal dominant and two recessive gene mutations contributing to AOS etiology. Interestingly, dominant *ARHGAP31* mutations lead to gain-of-function of RAC1/CDC42 GAP activity (Southgate et al., 2011), while recessive *DOCK6* mutations result in loss-of-function of RAC1/CDC42 GEF activity (Shaheen et al., 2011). The presence of two mechanisms that reduce RAC1/CDC42 activity in AOS is a striking indication of their importance in skin and limb development.

Cerikan et. al. sought to characterize the function of DOCK6 to further elucidate the role of RAC1 and CDC42 regulation in AOS (Cerikan et al. 2016). Acute *DOCK6* depletion using RNAi induced cytoskeletal collapse in HeLa cells, which could be rescued through expression of RNAi-resistant *DOCK6*, or constitutively-active RAC1. In addition, *DOCK6* depleted cells had significant mitotic defects that could be reversed with active CDC42. Furthermore, RAC1 and CDC42 activity were reduced and RHOA activity was increased in *DOCK6* silenced cells, while inhibition of the RHOA effector ROCK kinases prevented cell rounding,

indicating a role for RHOA activation in response to RAC1/CDC42 inhibition (Sander et al., 1999) in the morphological changes induced by *DOCK6* depletion (Figure 1).

Interestingly, the cytoskeletal defects following acute *DOCK6* depletion were reversed 120 hours after RNAi treatment, despite continued DOCK6 protein reduction. To address the possibility that cells might overcome long-term *DOCK6* depletion, stable *DOCK6* knockout (KO) cells were established using CRISPR/Cas9 (Wright et al., 2016). *DOCK6* KO cells, as well as fibroblasts from AOS patients, had shorter actin filaments and higher cytoplasmic G-actin, but did not undergo cytoskeletal collapse and were free of the mitotic defects observed in transiently *DOCK6* depleted cells. *DOCK6* KO cells also had CDC42, RAC1 or RHOA activity comparable to control cells, suggesting that long-term *DOCK6* depletion, or mutation in AOS patients, may lead to adaptions in Rho GTPase activity.

To identify factors that might re-equilibrate Rho GTPase activity, gene expression in fibroblasts from healthy controls or AOS patients were compared to *DOCK6* KO and control cell lines. The small ubiquitin-like modifier ISG15 was reduced in AOS fibroblasts and *DOCK6* KO cells relative to matched controls, and was also progressively reduced following *DOCK6* RNAi treatment. Silencing *ISG15* expression in *DOCK6* depleted cells prevented cytoskeleton alterations and normalized active RAC1, CDC42 and RhoA levels, while overexpressing *ISG15* in *DOCK6* depleted cells induced cytoskeleton defects, suggesting that *ISG15* downregulation compensates for reduced *DOCK6*.

Given that DOCK6 depletion was associated with cytoskeleton alterations and increased G-actin levels, one possibility was that cytoplasmic G-actin sequestered the myocardin-related transcription factor A/serum response factor (MRTF-A/SRF)

transcription complex in the cytoplasm, blocking nuclear translocation and subsequent *ISG15* gene transcription (Hermann et al., 2016). Consistent with this hypothesis, MRTF-A distribution was more cytoplasmic and synthetic promoter-reporter assays revealed lower MRTF-A/SRF transcriptional activity in *DOCK6* KO cells compared to control cells.

To determine whether free or conjugated ISG15 mediated the suppression of *DOCK6* depletion induced effects, the E2 ligase enzyme *UbCH8* was knocked down along with *DOCK6*. Like *ISG15* knockdown, *UbCH8* depletion reversed cytoskeletal and morphological alterations. In agreement with previous studies (Zhao et al., 2005), the scaffolding protein IQGAP1 (Watanabe et al., 2015) was confirmed as an ISGylated protein, which was reduced in *DOCK6* KO cells. *IQGAP1* depletion in *DOCK6* KO cells caused cells to round up, while ectopic *IQGAP1* expression reversed the cytoskeletal collapse induced by *DOCK6* RNAi. These results are consistent with reduced ISG15 expression in *DOCK6* KO cells promoting interactions between IQGAP1 and activated CDC42 and RAC1, resulting in stabilization of their active GTP-bound state. It is additionally possible that reduced ISG15 promotes the RAC1 and CDC42 effector function of IQGAP1 in regulating cytoskeleton organization (Watanabe et al., 2015).

The ability of cells to adapt to environmental or genetic stressors by adjusting the outputs of complex cell signalling networks is an important attribute to maintain cell integrity and to promote survival. The approach described by Cerikan et. al. provides a platform for addressing adaptive mechanisms by comparing acute knockdown against stable deletion of a target gene. These findings also likely reflect the adaptive changes that naturally occur in cells harbouring potentially deleterious mutations. By implication, the use of acute depletion to model chronic loss-of-

function, or gene knockout to characterize acute functions, demands rigorous validation.

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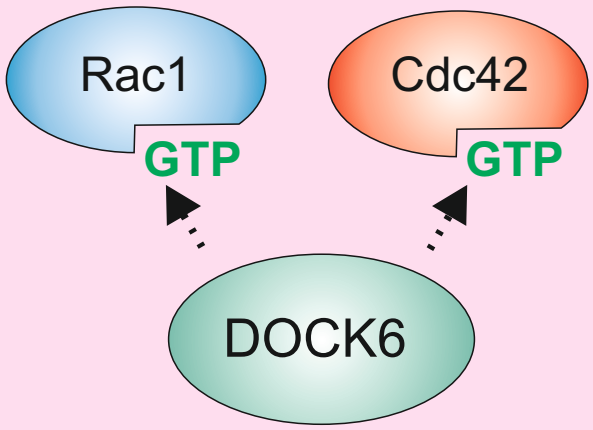
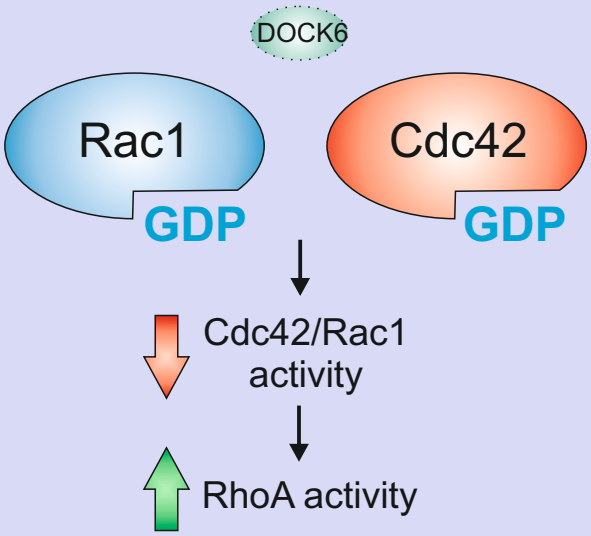
Figure 1. Acute vs sustained DOCK6 depletion/deletion. DOCK6 acts as a RAC1/CDC42 GEF under normal conditions. RNAi silencing of *DOCK6* reduces RAC1/CDC42 activity, inducing cytoskeletal collapse and cell rounding. Cells adjust to continued *DOCK6* loss by suppressing *ISG15* expression, resulting in reduced ISGylation of IQGAP1 and restored CDC42/RAC1 activity.

Basal conditions

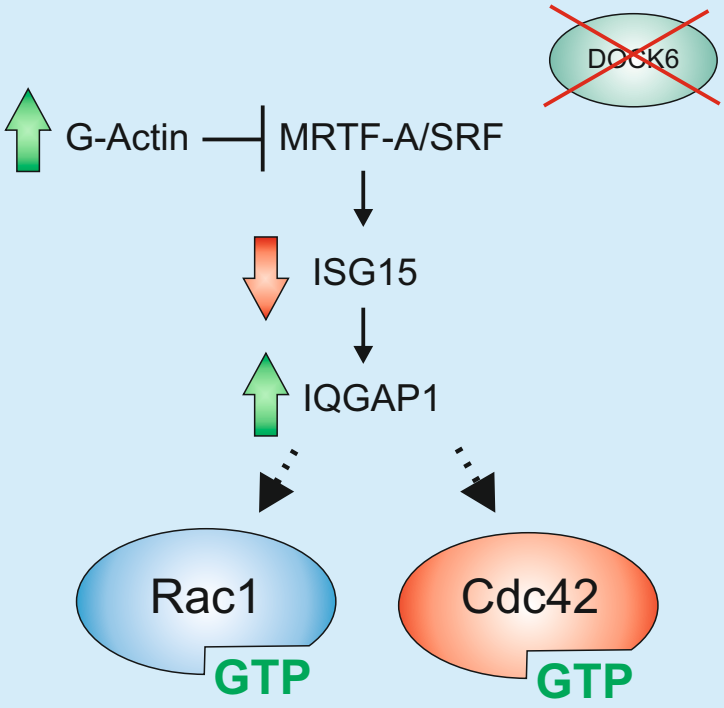


Acute DOCK6 depletion



- Collapse of F-actin, microtubule and intermediate filament cytoskeletons
- Cell rounding and blebbing
- Mitotic defects

Sustained DOCK6 depletion or deletion



- Restoration of F-actin structures
- Cell spreading and growth