CELL CYCLE

A way out

Exit from mitosis is characterized by low Cdk1 kinase activity and activation of the phosphatase Cdc14. This final stage in the cell cycle is triggered by Tem1, a GTPase located on the spindle pole body that, on entering the daughter cell, is activated and signals the mitotic exit network (MEN) pathway. However, cells must be able to reverse the state of mitotic exit to enter the next cell cycle. Stephen Elledge and colleagues now report, in *Cell*, new insight into how cells 'exit' mitotic exit.

Elledge and co-workers identified 'antagonist of MEN' (*AMN1*) in a genetic screen and set out to establish whether Amn1 is a bona fide negative regulator of MEN. They found that Amn1 overexpression was toxic to MEN mutant strains, and wild-type cells were delayed or arrested in mitotic exit. Amn1 was also required for the nuclear orientation and spindle checkpoints, indicating that *AMN1* is a checkpoint gene and might function coordinately with other checkpoints to control mitotic exit.

Next, the authors showed that *AMN1* expression is cell-cycle regulated and peaks in late M/G1. Moreover, intracellular localization studies using a green fluorescent protein (GFP)-tagged *AMN1* strain showed that high-level expression was daughter-cell specific. The transcription factors responsible for *AMN1* transcription, Swi5 and Ace2, turned out to be dependent on activation by MEN. So, *AMN1* transcription is induced specifically in daughter cells after mitotic exit.

To look for the target of Amn1-mediated MEN inhibition, the Elledge group carried out another genetic screen and isolated *TEM1*. Amn1 binds directly to Tem1, both *in vitro* and *in vivo*, indicating that Amn1 might inhibit MEN through its ability to bind and inhibit Tem1 function. Binding of Tem1 to its target kinase Cdc15 is essential for activation of MEN, and the authors observed that the absence of Amn1 promotes Tem1–Cdc15 association. Conversely, overproduction of Amn1 reduced Tem1–Cdc15 association, suggesting that Cdc15 and Amn1 compete for association with Tem1. When

AMN1 was overexpressed in cells arrested during mitotic exit, levels of Tem1–Cdc15 association were also reduced, indicating that Amn1 can disassemble preformed Tem1–Cdc15 complexes, thereby turning off MEN.

So finally, how is Amn1 itself turned off? The authors have evidence that SCF E3 ubiquitin ligase might be responsible for Amn1 degradation. Moreover, it is possible that Cdk1 phosphorylation targets Amn1 for degradation, which would explain its rapid degradation when cells enter S phase.

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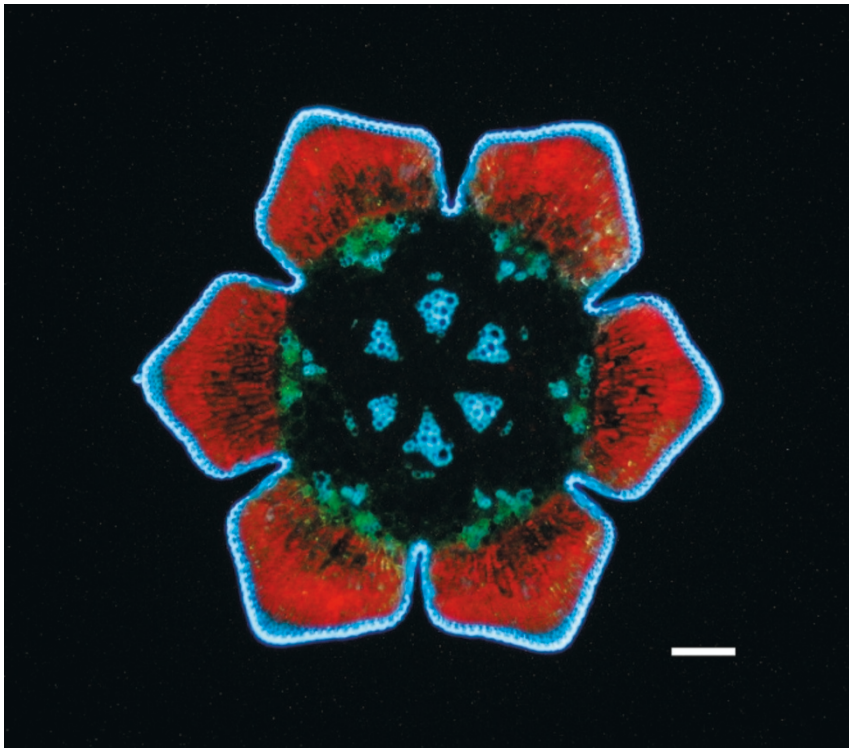
References and links

ORIGINAL RESEARCH PAPER Wang, Y. *et al.* Exit from exit: resetting the cell cycle through Amn1 inhibition of G protein signaling. *Cell* **112**, 697–709 (2003)

CELL OF THE MONTH



This month's winning image was submitted by Sergio Svistoonoff (Institut de recherche pour le développement, Montpellier, France (Sergio.Svistoonoff@mpl.ird.fr)). It shows shoots from *Allocasuarina verticillata* that are expressing green fluorescent protein (GFP) under the control of the 35S promoter.

GFP is expressed in the phloem cells. The chloroplasts of the mesophyll cells fluoresce in red, whereas the lignified cells of the epidermis and the xylem vessels appear in light blue. The image was acquired from transversal shoot sections under blue-UV light using a Leica-DMR microscope, with an epifluorescence filter A (Leica). Bar, 100 µm.

We are pleased to acknowledge the help of our two external advisors, Ariel Ruiz i Altaba (Developmental Genetics Program, New York Medical Center) and Lelio Orci (Department of Morphology, University Medical Center, Geneva).

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