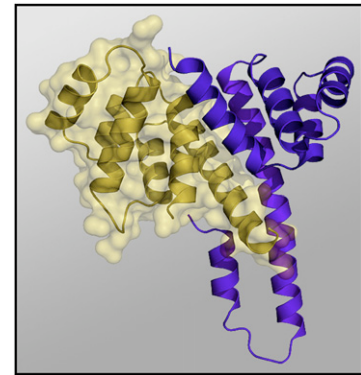


This issue's Cell Biology Select highlights recent studies that improve our understanding of how switches and tuners regulate cellular processes. Two studies encompassing very different processes—programmed cell death and plant lateral root formation—both reveal regulatory switches that work independently of enzyme action. Three other studies present new insights into how cell motility, signal transduction, and periodic gene expression may be tuned.

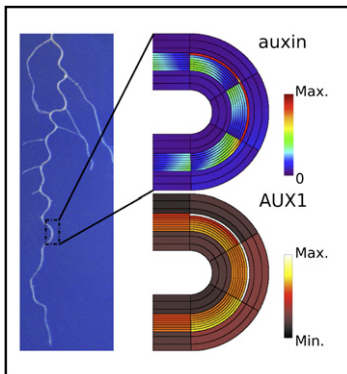
## A Fas-ter Way to Switch On Death

Programmed cell death or apoptosis can be initiated by the formation and clustering of death-inducing signaling complexes (DISCs) induced by Fas ligand. Certain DISCs are formed by Fas receptor (Fas), FADD (Fas-associated death domain), and caspase 8, and the interactions between the death domain of Fas and FADD are pivotal triggers of DISC formation and apoptosis. Scott et al. (2009) now present the 2.7 Å crystal structure of the human Fas-FADD death domain complex. From this structure, the authors surmise that conformational changes in Fas and weak binding interactions between Fas and FADD are the central elements of the molecular switch that triggers DISC formation. The authors observe that the Fas-FADD death domain complex in solution exists in a tetrameric arrangement consisting of four FADD death domains and four Fas death domains. FADD-bound Fas death domains undergo structural rearrangements to adopt an “open” conformation. This open Fas conformation creates new interaction surfaces crucial for FADD binding and Fas-Fas interactions within the tetrameric complex. Importantly, Fas forms weak binding interactions with FADD in the death domain complex. These weak interactions may prevent spurious activation of DISC formation in the absence of Fas ligand. The authors propose that Fas normally oscillates between unstable open and closed conformations. However, upon clustering of Fas induced by Fas ligand during apoptosis initiation, Fas-Fas interactions would promote stabilization of the open conformation, establishing permissive binding sites for FADD. Indeed, Scott et al. find that the expression in cultured cells of a mutant form of Fas prone to adopting the open conformation results in a higher rate of cell death even in the absence of Fas ligand. The investigators also point out that interactions among multiple Fas-FADD complexes would stabilize weak Fas-FADD binding and further promote clustered DISC formation. This model of DISC formation presents a switch mechanism for signaling receptor activation that is dependent only upon conformational changes and does not require enzymatic activity.

F.L. Scott et al. (2009). *Nature*. Published online December 31, 2008. 10.1038/nature07606.



Structure of the primary Fas (blue)-FADD (yellow) death domain complex. Image courtesy of S. Riedl and R. Schwarzenbacher.



Lateral roots emerge from the outside of curves on an *Arabidopsis* root (left). Model simulations (right) show auxin and AUX1 accumulation toward the outside of the root curve. Image courtesy of V.A. Grieneisen.

## Throwing Auxin a Curve

In plants, the growth hormone auxin directs the formation of lateral roots along the primary root. Laskowski et al. (2009) use experiments and computational modeling to show that root curvature in the plant *Arabidopsis thaliana* controls lateral root initiation by modulating the distribution of auxin. They also propose that a balance between auxin importers (AUX1) and efflux carriers (PIN) contributes to the spacing of lateral roots. There is a tendency for lateral roots to arise on the outer (convex) side of the curved primary root. The authors find that lateral roots preferentially form in the curved regions of the differentiation zone in primary roots that are induced to curve by mechanical bending or by inverting the developing plant seedlings for varying time periods. Confocal imaging of roots expressing a fluorescent protein responsive to auxin revealed high levels of auxin in the root vasculature on the outside of the curve. To determine how curvature may direct the accumulation of auxin, the authors developed a computational model for auxin dynamics in the root that takes into account auxin mobility (efflux and influx due to PIN and AUX1 expression, respectively) as well as physical characteristics of the root system. Model simulations show that root bending, which alters the size and shape of cells in the curved region, is sufficient to alter auxin distribution. This results in a high auxin concentration that is necessary for specifying lateral root formation in the outer half of the bend. Computational modeling further suggests that accumulation of AUX1 in response to auxin stabilizes and amplifies the higher auxin concentration at the curve in a positive feedback loop. Consistent with this, the authors find that AUX1 proteins accumulate in the outer part of the root curve in a pattern similar to that of auxin. Modeling also predicts that downregulation of PIN proteins in regions adjacent

to the curve where auxin levels are lower would suppress nearby lateral root initiation and so would help to determine lateral root spacing. These results highlight the complex developmental patterning that results from a simple modulation of tissue shape.

M. Laskowski et al. (2009). *PLoS Biol.* 6, 2721–2735.

## A Septin Corset Drives T Cells Forward

As T lymphocytes move through tissues in search of foreign antigens, they assume a distinct amoeboid morphology consisting of a compact cell body trailed by a pinched structure known as a uropod. Tooley et al. (2009) now identify a role for septins, a family of

cytoskeletal proteins, in modulating the shape and motility of mouse T lymphocytes. Migrating mouse T cells immunostained for septins exhibited an annular septin network in the midzone of the T cell cortex. Confocal microscopy further showed that the septin filaments formed dense arrays that resemble a corset around the midsection of the cell. Depletion of *septin 7* in T cells with short hairpin RNAs resulted in abnormal T cell morphology and behavior. The leading edges of *septin 7*-deficient T cells exhibited excessive membrane blebbing and aberrant protrusion formation. Meanwhile, the trailing edges of these cells formed uropods that were nearly twice as long as those of wild-type cells. Although the actomyosin structures of these septin-deficient cells seem to be normal, time-lapse movies revealed that these cells have motility defects both in terms of velocity and net displacement. Strikingly, both cultured and primary T cells depleted of septins were able to migrate through very small artificial membrane pores during transmigration assays more efficiently than wild-type cells, suggesting that cells lacking septins also lose cortical rigidity. Taken together, these data suggest that septins play a role in maintaining cell cortical structure to promote efficient T cell migration and to restrict movement to within tissue boundaries. Tooley and colleagues propose that the septin network acts as a stable framework that helps to focus motile forces generated by dynamic actomyosin filament rearrangements, thus effectively “tuning” T cell motility. Given that altered septin expression has been observed in certain human and mouse tumors, it is possible that misregulation of septin expression may contribute to the ability of cancer cells to invade tissues during metastasis.

A.J. Tooley et al. (2009). *Nat. Cell Biol.* **11**, 17–26.

## The Long and Short of ERK Activity

The ERK mitogen-activated protein kinase (MAPK) pathway can induce different signaling outputs depending on whether ERK activity is transient or sustained. Hanafusa et al. (2009) now uncover evidence suggesting that the duration of ERK activity affects mesoderm differentiation in embryos of the frog *Xenopus laevis*. The authors further propose that the proteins Sprouty 2 (XSpry2) and Fos (XFos) may modulate and sense the duration of ERK activity, respectively, during dorsal-ventral patterning in the *Xenopus* embryo. They find that the pan-mesodermal gene *Xbra* is activated by transient ERK activity, whereas the dorsal mesodermal gene *Chd* is activated by sustained ERK activity. In the early *Xenopus* gastrula, sustained ERK activation occurs in the dorsal region but is inhibited in the ventral marginal zone. XSpry2 expression effectively shuts off fibroblast growth factor-induced ERK activity in embryos within 30 minutes, whereas expression of a dominant-negative form of XSpry2 maintains prolonged ERK activation. Depletion of XSpry2 expression in the ventral marginal zone by antisense morpholino oligonucleotides resulted in the ectopic expression of dorsal mesodermal genes and the suppression of a ventral mesodermal gene. Interestingly, the authors find that the amount of XFos protein in different regions of the embryo is closely correlated with the amount of ERK activity. Furthermore, XFos overexpression induces ectopic expression of the gene *Chd* in the ventral marginal zone, which is dependent on sustained ERK activity. These data provide the underpinnings of a model in which XSpry2 acts in the ventral marginal zone of the *Xenopus* embryo to tune the duration of ERK activity, which is detected by XFos and translated into appropriate gene expression during dorsal-ventral patterning.

H. Hanafusa et al. (2009). *Nat. Cell Biol.* **11**, 106–109.

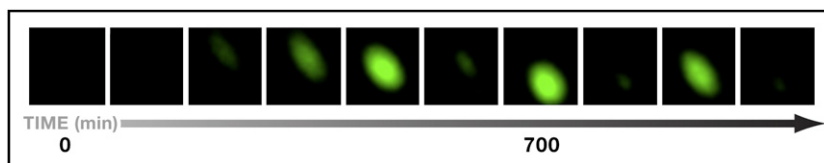
## Design Plans for a Molecular Clock

The periodic induction of gene expression within oscillating circuits forms the basis of central and peripheral circadian clocks. Tigges et al. (2009) now provide insights into the design principles behind these types of circuits by creating a synthetic tunable oscillating circuit in cultured mammalian cells. Unlike previous prokaryotic oscillating synthetic gene networks controlled by transcription, Tigges and colleagues use translational regulation by antisense transcripts to create a circuit with robust oscillatory behavior. Informed by mathematical modeling, the authors assembled an oscillating regulatory circuit containing two interlocking feedback loops that modulate the levels of the tetracycline-dependent transactivator (tTA). In this circuit, tTA expression is controlled by the tTA-specific tetracycline-responsive promoter ( $P_{hCMV-tTA}$ ) in an autoregulated feedback loop. Antisense tTA expression, which blocks tTA protein production, is also indirectly regulated by tTA. Activation of the antisense tTA promoter (the pristinamycin-responsive promoter,  $P_{PIR}$ ) requires the pristinamycin-dependent transactivator (PIT), a protein whose expression is under the control of  $P_{hCMV-tTA}$  and thus also tTA. The authors further use  $P_{hCMV-tTA}$ -driven expression of a variant green fluorescent protein (GFP) with a short protein half-life to monitor tTA protein levels. Cultured Chinese hamster ovary (CHO-K1) cells transfected with equal amounts of plasmids harboring these circuit components exhibited spontaneous self-sustained oscillations in GFP fluorescence monitored by time-lapse microscopy.

Consistent with a correlation between oscillation behavior and gene dosage, Tigges et al. find that the circuit could be tuned according to the concentration of plasmids used to transfect the cells. Transfecting cells with double the amount of plasmids induced oscillations with shorter periods and with reduced amplitude, whereas halving the amount of plasmids transfected increased the oscillation period. Further investigation of the parameters that mediate periodic gene expression in this synthetic circuit may shed light on the regulatory mechanisms underlying oscillatory gene expression networks.

M. Tigges et al. (2009). *Nature* **457**, 309–312.

Marie Z. Bao



A CHO-K1 cell harboring the synthetic circuit exhibits oscillating GFP expression. Image courtesy of M. Tigges.