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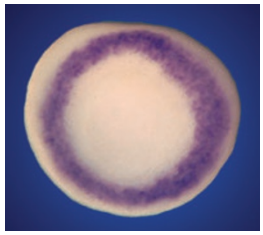
Getting mixed signals



During growth and development, cells must integrate multiple signaling cues. Piccolo and colleagues have now examined integration of two major signaling pathways, pursuing the idea that activity of the RTK/Ras pathway enhances responses to TGF-β pathway stimulation. The authors find that this interaction is integrated at the p53 tumor suppressor. p53 has previously been shown to interact physically with Smad transcription factors, and thus help activate TGF-β target genes. In addition, RTK stimulation is often required for TGF-β to exert its biological effects, but the mechanism of this integration has been unclear. The authors find that in *Xenopus* embryos and human cells, the RTK pathway stimulates p53 phosphorylation at two serines. These residues are required for the role of p53 in integrating the signaling pathways, but not for other processes such as apoptosis. Serine phosphorylation promotes physical association of p53 with Smads and enhances TGF-β target gene responses. The authors determine that the CK1ε kinase mediates this RTK-enhanced p53 phosphorylation. RTK stimulation promotes TGF-β–mediated differentiation of *Xenopus* embryonic cells into mesodermal tissue (such as muscle), and cytostasis (growth arrest) in human cells. This work provides mechanistic insight into how differentiation along specific fates is precisely spatially controlled. In particular, regions of the embryo where FGFs (RTK ligands) are expressed have increased p53 phosphorylation as predicted, and thus should be susceptible to TGF-β–triggered differentiation. In addition, the results intriguingly indicate that RTK signaling expected to stimulate growth can conversely promote activity of a pathway mediating cytostasis in human cells. (*Science*, published online 18 January 2007, doi:10.1126/science.1135961) *SL*

Transcriptional noise

The human immunodeficiency virus (HIV) encodes a small protein, Tat, that activates viral gene expression. However, it is not known how the virus uses only one transcriptional regulator to switch from an active to a silent (latent) phase, in order to hide from the immune system. Recent work from Weinberger and Shenk incorporates single cell expression analysis and kinetic modeling of this activating transcriptional loop. According to their 'feedback resistor' model, the virus behaves like a pinball machine: it produces Tat, which then becomes trapped between the 'bumpers' p300 (a Tat acetyltransferase) and SIRT1 (a Tat deacetylase). Tat fluctuates between acetylated 'on' and deacetylated 'off' states, and the authors propose that Tat acetylation and deacetylation is a minimum requirement for their feedback resistor model. The SIRT1 inhibitor nicotinamide stimulates Tat-mediated transcription, whereas addition of the SIRT1 activator resveratrol reverses nicotinamide's effects. These findings are further supported by the result of a Tat point mutation where the lysine targeted for p300 acetylation is mutated to alanine. The authors' previous observation that HIV transcription is continually active (producing 'transcriptional noise'), combined with additional computational and experimental data, strengthen the proposed feedback resistor model, as the acetylation mutant has reduced residual transcriptional activity compared with the wild-type protein. The authors also make the potentially medically important suggestion that 'blips' of reactivated HIV gene expression in patients on antiretroviral therapy may be attributed to the flavoring agent dihydrocoumarin, a SIRT1 inhibitor. More work will reveal whether these observations can be extrapolated to complex genes or even genomes. (*PLoS Biol.* **5**, e9, 2007) *APD*

Research Highlights written by Alexander P. Dorr, Boyana Konforti, Sabbi Lall and Michelle Montoya.

Controlling ammonium import

Members of the ammonium transporter (Amt) family of proteins facilitate ammonium uptake essential for nitrogen metabolism under ammonium-limiting conditions. The known structure of the AmtB trimer, the prototype for this family, suggests ammonium transport through membrane-spanning hydrophobic channels formed by each AmtB subunit. GlnK, a sensor of intracellular nitrogen levels, has been shown to bind and directly block AmtB transport activity when extracellular levels of ammonium are in excess. Uridylation of a conserved tyrosine on the T-loop of GlnK, which occurs under conditions of nitrogen starvation, disrupts this interaction, which can also be modulated by ATP and 2-oxoglutarate binding. Although the structure of the GlnK trimer is also known, there is no structural information on GlnK-AmtB interactions. Now, new structural data, independently determined by the Stroud and Winkler labs, reveal details of the AmtB-GlnK interaction that suggest how GlnK may be regulating ammonium import. The structures confirm the previously determined 3:3 AmtB/GlnK stoichiometry and reveal a three-fold symmetric complex. In the complex, the GlnK T-loops rearrange into short β-strand hairpins that extend from GlnK and form intimate, stabilizing interactions with the AmtB channel openings. These findings suggest that GlnK inhibits ammonium transport by physically occluding the AmtB channels. Although ATP binding at GlnK subunit interfaces is known to modulate the AmtB-GlnK interaction, three ADP molecules are found in both determined structures. Further structural work will be needed to understand how ATP and 2-oxoglutarate contribute to AmtB regulation. (*Proc. Natl. Acad. Sci. USA* **104**, 42–47, 2007, and *Proc. Natl. Acad. Sci. USA* **104**, 1213–1218, 2007) *MM*

Fine-tuning gene expression

The ability of bacteria to communicate with each other, called 'quorum sensing', allows bacteria to coordinate their behavior. They do so by sensing changes in their numbers and selectively altering gene expression. In the bioluminescent marine bacterium *Vibrio harveyi*, three parallel sensory systems converge to regulate quorum sensing gene expression by controlling the levels of the master transcription regulator LuxR. Tu and Bassler identify five Qrr small regulatory RNAs (sRNAs) that act additively to control quorum sensing. Each sRNA alone is able to repress *luxR* to a certain extent that correlates with the strength of its promoter. Mutational analysis shows that only four of the five sRNAs are required to destabilize the *luxR* messenger RNA. The Qrr sRNAs allow a rapid response to changes in cell density because, unlike proteins, which have to be transcribed and translated, regulatory RNAs need only be transcribed and thus can be rapidly produced; they are likewise rapidly eliminated by degradation. The authors propose a model in which the multiple sRNAs provide precise control over the level of *luxR* mRNA, which can in turn be translated into LuxR protein. (*Genes Dev.* **21**, 221–223, 2007) *BK*