CELL DIVISION

Battle at the p15 promoter

The company we keep often affects our behaviour, and the same is true of the oncoprotein Myc: in the company of Max, Myc can activate the transcription of genes that generally stimulate proliferation; but Myc can also behave as a trancriptional repressor. The proteins that bring out this side of Myc's personality have remained elusive, but two papers in the April issue of *Nature Cell Biology* identify them.

Miz-1 is a zinc-finger-containing protein that induces cell-cycle arrest and activates the transcription of several genes that are repressed by Myc. Staller and colleagues reasoned that Miz-1 might activate transcription of a cyclin-dependent kinase inhibitor, and used PCR and western blots to identify $p15^{Ink4b}$ as a target of Miz-1. Deletion mapping identified a Miz-1-binding region in the promoter of *Cdkn2b*, the gene that encodes $p15^{Ink4b}$. Co-expression of Myc blocked transactivation of *Cdkn2b* by Miz-1, and co-immunoprecipitations identified a complex of Miz-1, Myc and Max. But how does Myc block the ability of Miz-1 to activate transcription of *Cdkn2b*? The authors wondered whether Myc might prevent Miz-1 from recruiting a co-activator and their speculations proved correct: inactivation of the co-activator protein p300 inhibited Miz-1's ability to activate *Cdkn2b* transcription, and Miz-1 co-immunoprecipitated with p300. Mapping of the p300-binding site on Miz-1 showed that Myc and p300 both bind to overlapping sites on Miz-1, explaining how Myc blocks reruitment of p300 to the *Cdkn2b* promoter.

But does Myc use this mechanism to make cells proliferate? To find out, the authors made chimeras of Myc and Mad-1 that have Myc's transcriptional activator activity, but can't bind Miz-1. Unlike wild-type Myc, the chimeras couldn't block accumulation of $p15^{Ink4b}$, and couldn't transform $p53^{-/-}$ cells. Myc's oncogenic abilities threfore depend on its ability to block *Cdkn2b* transcription.

Transforming growth factor-β (TGF-β) can also induce expression of $p15^{Ink4b}$ by activating Smad transcription factors, but why this response is blocked by overexpression of c-Myc has remained a mystery. Joan Seoane and colleagues now explain why. Treatment of keratinocytes with TGF-β downregulated Myc and decreased levels of the Myc–Miz-1 complex. To see whether this upregulates transcription of *Cdkn2b*, they created a dominant-negative form of Miz-1, Miz-dZF, that binds Myc but not the *Cdkn2b* promoter. Overexpression of Miz-dZF increased expression from a *Cdkn2b* reporter contruct, but not as well as treatment with TGF-β. So TGF-β must activate transcription of *Cdkn2b*, as well as relieving repression of its transcription by Myc. Reasoning that Smads were the most likely candidates for this bipartite effect of TGF-β, the authors located a Smad-binding region in the *Cdkn2b* promoter, and immunoprecipitated a Smad-containing complex from the region. Forced expression of Myc didn't prevent formation of this complex, but it did block its ability to transactivate *Cdkn2b*, so Myc's ability to repress transcription dominates the ability of Smads to activate it. The authors identified a ternary complex of Myc, Miz-1 and Smad4, so Myc and Smads don't battle it out by binding to the same site on Miz. The most likely explanation is that Myc's ability to represss transcription — at least of *Cdkn2b* — is due to its ability to block the interaction between Miz-1 and the co-activator p300. Whether this is a general mechanism by which Myc represses transcription is an exciting possibility.

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PROTEIN DEGRADATION

Water, water everywhere …

Cells in water-transporting tissues, such as the kidney and the airways of the lung, achieve a remarkable feat — they survive large fluctuations in osmolarity without either exploding or shrivelling up. To do this, the cell recruits the help of water channel proteins — the aquaporins (AQPs) — which allow extremely high water permeability at the plasma membrane. When cells are under hypertonic stress, the expression of AQP1 is induced. Little is known about the role that AQP1 protein stability or post-translational modifications might have during this process. Reporting in *Proceedings of the National Academy of Sciences*, Leitch and colleagues now describe how expression of AQP1 is upregulated under hypertonic stress, not simply by inducing its expression, but also by preventing its degradation.

Many proteins in the cell are targeted for degradation by a post-translational modification — multi-ubiquitylation — which recruits the proteasome, sending proteins to their death. This pathway, although known to regulate numerous mammalian cytosolic and membrane receptor proteins, has been implicated for only a few membrane transport proteins.

The first hint that the ubiquitylation–proteasome pathway might be important for degrading AQP1 came when the authors looked at the effects of proteasome inhibitors on AQP1 levels. Inhibiting the proteasome, they found, increased AQP1 expression. This led them to wonder whether AQP1 is ubiquitylated. To address this, they precipitated AQP1 with antibodies and then looked for the presence of ubiquitin — a test that confirmed their suspicions.

So if ubiquitylation regulates the levels of AQP1 in the cell, what happens when cells are exposed to hypertonic stress? Leitch and co-workers found that ubiquitylation of AQP1 actually decreased during hypertonic stress. This suggests that one way the cell might react to stress is to hang on to any AQP1 that it already has, by preventing its degradation. To test this idea, the authors used metabolic labelling to follow the fate of AQP1 in different conditions. And, consistent with this model, they found that the half-life of AQP1 increases markedly under conditions of hypertonic stress compared with normal conditions.

The conclusion, say the authors, is that this mechanism "functions to facilitate protein induction at a time when the general pressure on the cell is to reduce protein synthesis". But how general a mechanism is this for the induction of proteins that are required during stress? Understanding how AQP1 and the other members of the aquaporin family are regulated is imperative, particularly in light of pathophysiological conditions in which their expression is altered.

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