A glimpse at toxicity

Emerging evidence suggests that the toxic forms of amyloid-forming proteins are small amyloid oligomers, not amyloid fibrils. These oligomers are transient, making them difficult to characterize, but they have been described as β-sheet rich. In an effort to gain insight into small amyloid oligomers, Eisenberg, Laganowsky and colleagues have identified an oligomer-forming hairpin loop in the amyloid-forming protein αB-crystallin (ABC). A six-residue peptide (G6V), an 11-residue peptide (K11V) and a tandem repeat of K11V (K11V-TR), all derived from the hairpin loop, form amyloid fibrils when agitated at high temperature. Under normal solution conditions, K11V and K11-VTR formed hexamers and trimers, respectively. Like other small amyloid oligomers, these oligomers were toxic and were recognized by the conformational antibody A11. The crystal structures of K11V and K11V-TR reveal six-stranded antiparallel β-barrel cylinders ('cylindrins'), differing from the native ABC structure and the β-sheet steric zipper formed by G6V. The cylindrins share some features of the steric zippers, having backbone-mediated hydrogen bonds and shape-complementary packing interactions at strand interfaces. However, the cylindrin strands are tilted and, when unrolled into a β-sheet, out of register. The β-strands in amyloid fibrils and steric zippers are in register, and a transition from a cylindrin-like oligomer to a fibril would be energetically costly. Although this suggests that cylindrin is off pathway to fibril formation, a β-amyloid sequence could also be modeled with a cylindrin structure, providing additional support for cylindrin-type models of amyloid oligomers. (*Science* **335**, 1228–1231, 2012) *MM*

Senescence-associated TGS

Cellular senescence is associated with the appearance of heterochromatin foci. These foci are presumed to contain proliferation-promoting genes that are transcriptionally repressed by the retinoblastoma (RB1)–E2F complex. Given that Argonaute (AGO) proteins, microRNAs (miRNAs) and other small noncoding RNAs have been implicated in heterochromatin formation and transcriptional gene silencing (TGS), Benhamed *et al.* analyzed the possible involvement of miRNAs and AGO proteins in senescence-associated repression of E2F target genes in human cells. Genome-wide identification of AGO-bound E2F target genes uncovered many genes involved in cell-cycle control, whereas AGO- and heterochromatin-bound miRNAs were positively correlated in senescent cells. AGO2 was found to accumulate in the nucleus of senescent cells, where it cooperates with RB1 to repress E2F target genes, as shown in promoter-reporter and AGO2 knockdown assays. Depletion of AGO2 delayed senescence onset in fibroblasts, whereas AGO2 overexpression induced an abrupt proliferative arrest. Members of the let-7 family of miRNAs were found to interact with both AGO2 and repressive heterochromatin. Promoter-reporter assays with an E2F target gene promoter containing a putative let-7f binding site showed that AGO2 and let-7f cooperate to induce TGS, but promoter binding of let-7f is AGO2 dependent. Together, these data suggest that cellular senescence can trigger miRNA-mediated TGS, which may contribute to tumor suppression by transcriptionally repressing proliferation-promoting genes. (*Nat. Cell Biol.* **14**, 266–275, 2012) *AH*

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Ku gets exclusive

The multifunctional Ku70–Ku80 heterodimer (Ku) mediates DNA double-strand break repair via nonhomologous end joining (NHEJ) and, in *Saccharomyces cerevisiae,* promotes telomere maintenance as a telomerase accessory subunit. Loss of Ku reduces nuclear levels of the telomerase holoenzyme and leads to shortened telomeres. As Ku has separately been shown to bind double-stranded DNA (dsDNA) and telomerase RNA (TLC1), a leading model for Ku's regulation of telomerase has been that Ku recruits telomerase to telomere ends by simultaneously binding TLC1 and dsDNA. Cech and colleagues have now tested that model by attempting to create Ku mutants that would only bind either RNA or dsDNA. Deletion of a large portion of the DNA-binding loop of *S. cerevisiae* Ku80 hindered DNA binding, which correlated with impaired NHEJ and shortened telomeres. Surprisingly, this deletion also impaired TLC1 binding and reduced nuclear TLC1 levels, indicating that Ku binds dsDNA and TLC1 using the same or mutually exclusive binding sites. In support of this idea, TLC1 and dsDNA can compete for Ku *in vitro*, and the authors were unable to detect a ternary complex containing Ku, dsDNA and TLC1. The authors propose a revised model in which Ku first binds TLC1, promoting nuclear retention of the telomerase holoenzyme; because Ku has higher affinity for dsDNA than for TLC1, Ku would then release TLC1 and bind the telomere end. (*Cell* **148**, 922–932, 2012) *SM*

Starvation signal

When carbon sources are low, *Escherichia coli* and other bacteria enter stationary phase: they stop dividing and activate a transcriptional program to protect the cells from stress and promote long-term survival. The master regulator of this process is the sigma factor RpoS (also known as σ^S). Under normal conditions, RpoS is translated but is immediately degraded by the AAA+ protease ClpXP in a process dependent on the adaptor protein SprE. When cells are starved for carbon, RpoS is stabilized; conversely, upon addition of glucose (nutrient upshift), RpoS is quickly degraded. Silhavy and colleagues have now used nutrient upshifts with different carbon sources and the power of genetics to identify the molecular signals that control RpoS stability. They found that carbon metabolism is necessary to trigger RpoS degradation. Furthermore, functional glycolysis or the TCA cycle is required, pointing to the energy-transducing molecules produced by these pathways: ATP and NADH. To determine which molecule is involved, the authors used carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) to disrupt the proton motive force, thereby blocking oxidative phosphorylation. When succinate was provided as a carbon source, the CCCP-treated cells could not produce ATP from glycolysis or from oxidative phosphorylation, and RpoS was not degraded. Importantly, ClpXP was still active under those conditions. These results indicate that RpoS degradation is sensitive to ATP levels. This conclusion was further supported by data under conditions in which ATP biosynthesis was defective. Finally, the authors reconstituted the system *in vitro*, using purified RpoS, SprE and ClpXP, and observed RpoS stabilization under low ATP levels. Interestingly, another ClpXP substrate tested did not show a similar sensitivity to ATP concentrations. Exactly how ATP controls RpoS proteolysis by ClpXP will likely be revealed in future biochemical and structural work. (*Genes Dev.* **26**, 548–553, 2012) *IC*