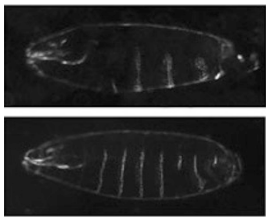
C3PO meets R2D2



RNA interference (RNAi) is triggered by double-stranded RNA (dsRNA) and mediated by the RISC complex, a key component of which is Argonaute (Ago). In *Drosophila melanogaster*, RNAi is mediated by AGO2, which is loaded with small interfering RNA (siRNA) by a RISC loading complex that contains DCR2 and R2D2. Liu and colleagues reconstituted long dsRNA-initiated and duplex siRNA-initiated RISC activity using recombinant DCR2–R2D2 and AGO2 proteins. Given that the recombinant system produces less RISC activity than *Drosophila* S2 cell extracts, the authors used this reconstitution system to purify new RISC-enhancing activities and thus isolated C3PO (component 3 promoter of RISC). C3PO is a multimeric complex of the conserved proteins Translin and Trax. In a Translin mutant, dsRNA-initiated RISC activity is reduced, a defect that is rescued by recombinant C3PO complex. In addition, the segmentation phenotype generated by *ftz* siRNA during embryogenesis was not observed in the translin mutant, implying defective RNAi. The authors then followed RISC activation *in vitro*. The transition to active RISC, which requires cleavage and removal of the strand not involved in targeting RISC to a cognate transcript (the passenger strand) was diminished in C3PO's absence. In addition, passenger strand cleavage products persisted on RISC without C3PO, implicating the complex in passenger strand removal. The authors show that C3PO is an endonuclease, and mutagenesis of predicted catalytic residues (based on conservation and structural modeling) revealed that the endonucleolytic activity of C3PO correlates with its role in RISC activation. The findings implicate a new endonuclease component in *Drosophila* RISC activation. Whether similar activities will be found associated with other Argonautes and across species remains to be determined. (*Science* **325**, 750–753, 2009) *SL*

Variation from a quartet

To evade the host immune response, some pathogens periodically change their cell surface antigens, a process known as antigenic variation (Av). The bacterial pathogen *Neisseria gonorrhoeae* has three different diversification systems, one of which involves its pilin locus, *pilE*, whose product is the major component of the bacterial pilus. Pilin variation involves a type of DNA recombination reaction known as gene conversion in which genetic information is unidirectionally donated from one of many silent pilin genes to *pilE*. Transposon mutagenesis data showed that a specific DNA element upstream of *pilE* is required for pilin Av. Cahoon and Seifert have recently screened for mutants that are unable to undergo pilin Av, and they found that single mutations within 12 GC base pairs upstream of *pilE* were inhibitory. This GC-rich region was shown to form a structure called a guanine quartet (G4). Mutations that block pilin Av also inhibit G4 formation. Replacing the *pilE* G4 sequence with other G4-forming sequences, or inverting the *pilE* sequence, prevents pilin Av. To demonstrate that the G4 sequence formed *in vivo*, cells were plated on media containing a G4-binding compound, NMM. This resulted in decreased Av. They also found that the GC-rich region has a higher density of single-strand nicks than does the surrounding DNA, and that this is dependent on G4 formation. The authors speculate that the G4 is formed when the DNA strands are separated during replication, but it remains unclear how the homologous recombination machinery exploits this structure to promote recombination from the silent pilin loci. (*Science* **325**, 764–767, 2009) *AKE*

Written by Inês Chen, Angela K. Eggleston, Maria Hodges & Sabbi Lall

Double take

In eukaryotic cells, membrane proteins are sent to the endoplasmic recticulum (ER) during or shortly after synthesis. Most membrane proteins possess a signal-recognition peptide that ensures co-translational targeting to the ER. However, a small but very important group that includes the SNARE proteins and the Bcl2 family members use a post-translational pathway. This group, called the tail-anchored proteins, has a single C-terminal transmembrane domain (TMD) that holds the targeting information. In yeast, this domain interacts with a highly conserved ATPase called Get3 (Asna1 or TRC40 in mammals) to form a complex that is then targeted to the ER. Mateja *et al.* have now solved the crystal structures of Get3 in both nucleotide-free and nucleotide-bound forms, revealing the likely mechanism for Get3 binding to substrates. Both structures are symmetrical homodimers composed of ATPase and α-helical subodomains. In the nucleotide-free or 'open' state, the α-helical subdomains have a space between them of at least 20 Å. The charged surface of this gap suggests that it may have a function other than binding substrates' TMDs. The nucleotide-bound state, however, undergoes a conformational change that brings the α-helical domains together, creating a solvent-exposed hydrophobic groove that spans both monomers. Extensive mutagenesis of the residues within this groove showed that these hydrophobic amino acids are indeed needed for proper function of this pathway. (*Nature*, advance online publication, doi:10.1038/nature08319, 12 August 2009) *MH*

The tau of degradation

Cells can regulate the levels of intracellular proteins using two different systems for protein processing and degradation, the proteasome and the lysosome/autophagy system. In the latter, substrates are targeted to the lysosome via three pathways: macroautophagy, microautophagy and chaperone-mediated autophagy (CMA). When protein degradation systems are disturbed, abnormal protein may accumulate and form aggregates, which could contribute to a number of neurodegenerative diseases. In particular, the lysosomal system has been shown to be altered in samples from patients with Alzheimer's disease. Fragments of tau, a neuronal microtubule-associated protein, can form abnormal aggregates and fibrils, found in patients with Alzheimer's and other diseases. How these pathogenic fragments are formed and which cellular system normally clears tau in neuronal cells are still not clear. Now, Yipeng Wang and colleagues have used a mouse neuroblastoma cell model to find that aggregates of a tau domain carrying a mutation implicated in dementia and Parkinson's disease can be efficiently degraded via the macroautophagy pathway. Interestingly, a fragment of this mutant tau, originating from a cytosolic proteolytic cleavage, is targeted to lysosomes through interaction with CMA components, but instead of being translocated to the lysosomal lumen this polypeptide remains anchored to the lysosomal membrane. Further processing of this fragment by lysosomal proteases generates pathogenic polypeptides that form complexes on the lysosomal surface, inhibiting the translocation of other CMA substrates. Thus, the lysosome contributes to production of toxic forms of mutant tau, which in turn disrupt lysosomal functions and potentially affect the processing and clearing of other aggregation-prone proteins, in a 'snowball' effect that may have a role in neurodegenerative diseases. (*Hum. Mol. Genet.* published online, doi:10.1093/hmg/ddp367, 4 August 2009) *IC*