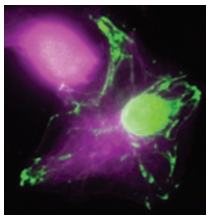
Type III effectors in action



Gram-negative pathogens such as *Salmonella enterica* use specialized machinery called the type 3 secretion system to inject effector proteins into the eukaryotic host cell. These proteins provoke changes in cellular signaling, allowing the bacteria to invade the host cells and to replicate within vacuoles. There are over 60 effector proteins secreted by *Salmonella*, but their specific activities within the cells can be difficult to investigate: fusion of effector proteins to green fluorescent protein (GFP) disturb their secretion, whereas expression of the effector protein directly in the host cell cytoplasm does not reproduce the conditions during infection. Now Van Engelenburg and Palmer have developed a method to label and track *Salmonella* effectors in real time within infected host cells. The authors used a split-GFP approach, where an incomplete, nonfluorescent fragment of GFP (containing only 10 of the 11 β-strands that form the GFP β-barrel) is expressed in the host cells. A short, 13-residue sequence corresponding to the 11th β-strand of GFP is then fused to the effector protein being studied. Only when and where these two pieces come together can a fluorescence signal be observed. The technique was initially validated for the well-characterized effector PipB2, known to bind directly to kinesin-1. The tag did not affect its function or localization, and fluorescence could be observed with the expected kinetics. Two other effectors were similarly studied: tagged SteA seemed to localize to the *Salmonella*-containing vacuole and associated membrane tubules and partially co-localized with Golgi-derived membranes. SteC tagging resulted in fluorescence on the vacuole and in its actin-containing filamentous protrusions. The authors discuss the potential limitations of this approach, which might not be ideal for every effector protein, but the work also indicates the possibility of tagging bacterial proteins that enter host cells via other systems. (*Nat. Methods* doi: 10.1038/nmeth.1437, published online 14 March 2010) *IC*

Moonlighting Dicer

Cells undergoing apoptosis show a number of well-characterized changes, including the fragmentation of nuclear DNA. In fact, labeling of free 3′-OH DNA ends is commonly used to detect apoptotic cells *in situ*, in an assay dubbed TUNEL (TdT-mediated dUTP nick end labeling). A number of different DNases have been implicated in apoptotic DNA fragmentation. In mammals, the process seems to be initiated by DNA fragmentation factor (DFF40), whose activity is controlled by caspases; several nucleases then degrade the DNA further. In contrast, *Caenorhabditis elegans* lacks a DFF40 ortholog, and now Xue and colleagues show that an unexpected nuclease performs that function in worms. The ribonuclease Dicer plays a key role in the biogenesis of small RNAs, processing double-stranded RNAs into shorter duplexes of 21–25 base pairs. The authors show that *C. elegans dcr-1* is required for the initiation of apoptotic DNA degradation, whereas genes encoding other factors involved in small RNA biogenesis are not. The role of *dcr-1* in DNA degradation was dependent on the caspase CED-3, and activated CED-3 can cleave DCR-1 at its C terminus *in vitro*, generating a smaller polypeptide that lacks dsRNA-dicing activity but can nick plasmid DNA and generate 3′-OH ends that are detectable by TUNEL. In addition to uncovering Dicer's 'night job' during apoptosis in *C. elegans*, this intriguing work also points to our still incomplete knowledge of caspase substrates. (*Science* doi: 10.1126/science.1182374, published online 11 March 2010) *IC*

Written by Inês Chen, Angela K. Eggleston & Arianne Heinrichs

A dynamic peroxisomal pore

The way proteins are translocated across the peroxisomal membrane is poorly understood. Though the existence of transient pores in the membrane had been proposed, their identification had remained elusive until now. In a new study, Erdmann and colleagues tagged peroxisomal matrix protein receptor Pex5p and isolated three Pex5p-containing complexes from yeast peroxisomal membranes. These three complexes represented various transient complexes in the import pathway, two of which showed ion-channel activity. By eliminating three components of the translocation machinery, thereby preventing the association of the docking complex with the export machinery and the interaction of an alternative peroxisomal matrix protein import pathway, the authors got a more stable, better-defined pore complex, with more homogeneous gating activity and a surprisingly small estimated pore size (~0.6 nm). Attempts to open the peroxisomal channel by providing either synthetic or natural cargoes were unsuccessful, but when the reconstituted pore complex was incubated with purified Pex5p–cargo complex, the channel conductance increased, corresponding to a pore of ~2.8 nm, which is larger than that of protein-conducting channels of mitochondria and chloroplasts. The pore size could be further increased to ~9 nm by incubating the pore complex with Fox1p cargo, which is known to form high-order oligomers. Further analyses indicated that the peroxisomal pore complex consists of a single highly dynamic channel, in contrast to protein import pores of other organelles that have been reported to comprise multiple coupled pores in one complex. Understanding the molecular basis underlying the dynamic nature of the peroxisomal import pore will be an important goal of future studies. (*Nat. Cell Biol.* **12**, 273–277, 2010) *AH*

A polymerase triad in NER

UV light causes DNA lesions that are reversed by a process known as nucleotide excision repair (NER). The early steps of NER involve assembly of repair proteins at the damage site, single-strand incisions on both sides of the lesion and removal of the DNA between the incisions; this results in a gap of about 30 nucleotides. *In vitro* studies suggested that the normal replicative DNA polymerases, δ and ε, were used in gap filling, although some data indicated that an error-prone polymerase, κ, also played a role. Work by Ogi, Lehman and colleagues now shows that there are two modes of gap filling in human fibroblasts. UV damage leads to ubiquitination of PCNA, a protein clamp that encircles DNA and helps recruit replicative polymerases to a 3′-OH end. Ubiquitinated PCNA interacts with pol κ, and the absence of pol κ reduces NER repair synthesis by about half. Pol δ recruitment to sites of NER is independent of PCNA ubiquitination, but its depletion also reduces repair synthesis by 50%. Levels of synthesis following depletion of both pol δ and pol κ indicate they act in the same pathway. The remaining repair synthesis is carried out by pol ε via recruitment by an alternative clamp loader complex, CTF18-RFC. The data suggest that, in simple cases where dual incisions are made to leave a gap, pol ε carries out rapid synthesis. If, however, the nature of the lesion or the chromatin environment is such that the 3′ incision is delayed, a displacement type of synthesis is required. Thus, pol δ is recruited by the classical clamp loader RFC1-RFC to start repair synthesis, which is completed by pol κ recruited by the damage-modified PCNA. (*Mol Cell* **37**, 714–727, 2010) *AKE*