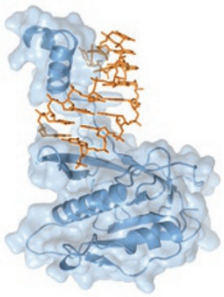
CRISPR cleavage



In many eubacteria and most archaea, CRISPRs, clusters of regularly interspaced short palindromic repeats, protect cells from bacteriophages and conjugative plasmids. A CRISPR is an array made up of direct repeats separated by unique spacers. The repeats are preceded or followed by a set of CRISPR-associated (*cas*) genes. CRISPR functions as an inherited adaptive immune system, and it is the spacers that provide the specificity and express CRISPR RNAs (crRNAs)—small RNAs—that target invasive nucleic acids. CRISPR function requires three steps: the acquisition of new spacer sequences; the expression and processing of crRNAs; and CRISPR interference. Now Doudna and colleagues have identified the endonuclease (Csy4) required for CRISPR RNA processing in *Pseudonomas aeruginosa*. The crystal structure of Csy4 bound to RNA shows that the C-terminal domain mediates most of the interactions with RNA. Csy4 makes sequence-specific interactions in the major groove of the crRNA stem-loop. Together with electrostatic contacts to the phosphate backbone, Csy4 recognizes the hairpin element of the CRISPR repeat sequence and cleaves immediately downstream of it using conserved serine and histidine residues in the active site. Phylogenetic analysis of CRISPR loci suggests that CRISPR repeat sequences and structures have coevolved with the *cas* genes. The specifics of the RNA-protein interaction described here suggest how Csy4 could discriminate crRNAs from other cellular RNAs. Finally, the authors propose that the ability of Csy4 to form a tight complex with the cleaved crRNA product points to a role for Csy4 in the CRISPR pathway beyond pre-crRNA cleavage. (*Science* **329**, 1355–1358, 2010) *BK*

Seeing the invisible

The folded, native state of a protein is generally the lowest-energy conformation. To reach this state, some proteins adopt rare, transient, metastable intermediate conformations (I), which are difficult to characterize at atomic resolution. Working from the concept that these transient, 'invisible' forms will lead to broadening of the NMR signals from the detectable forms, Kay and colleagues have devised an approach to determine the 'invisible' I conformation of a 71-residue phosphopeptide binding module, the FF domain, whose native structure shows a four-helix bundle. Using NMR relaxation methods that are sensitive to ms time-scale dynamics, chemical shifts and residual dipolar couplings (RDCs) were measured, allowing determination of the structure of the I conformer using a modified CS-Rosetta program. Similar to native structure, the I state has four helices (H1–H4), although H3 is expanded to include a sequence forming a loop in the native state, and H4 is not well defined. The I core is well ordered, but the termini and the loop following H3 are more dynamic compared to the native state. The predicted RDCs of helices H1–H3 are consistent with the experimental data, whereas discrepancies with the measured values for H4 are consistent with its dynamic nature in I. The orientations of H1, H2 and H4 in I are similar to those in the native structure, but the expanded H3 makes a variety of non-native contacts. These data provide further evidence that disrupting such interactions is what renders the I-to-native conversion rate limiting. H4 was known to stabilize the native FF domain, but its partially disordered conformation in I suggests it might not contribute to the stability of the latter. Indeed, H4 truncation resulted in chemical shifts that were very similar to those of the I state. This is a powerful approach that should not be limited to folding studies; in fact, the ability to access these 'invisible' structures could be used to probe in molecular detail questions such as how ligands bind proteins or the nature of enzymatic transition states. (*Science* **329**,1312–1315, 2010) *AKE*

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Gp96 playing duo

Gp96, also known as Grp94, is a member of the Hsp90 chaperone family that resides in the mammalian endoplasmic reticulum (ER) and is essential for the folding and function of multiple Toll-like receptors (TLRs). In contrast to its cytoplasmic relative, which functions alongside several known cochaperones, gp96 is thought to work alone. In fact, Hsp90 regions involved in cochaperone interaction are not conserved in gp96. On the other hand, structural information for gp96 implies that additional factors might be necessary for its activity. Now Li and colleagues show that the ER protein canopy3 (CNPY3) works together with gp96 to facilitate the folding and maturation of TLR9 and several other TLRs, with the exception of TLR3. It was previously known that CNPY3 was involved in TLR regulation, but this new work provides cellular and biochemical data that support the role of CNPY3 as a cochaperone for gp96. More specifically, the authors show that gp96 and CYNP3 interact in cells in a nucleotide-dependent manner and can form a ternary complex with TLR9. The gp96–CNPY3 interaction is functionally relevant, as mutations on either partner that disrupt the interaction also reduced levels of folded TLR9. The interaction of gp96 with TLR9 was dependent on CNPY3, whereas the association of CNPY3 with TLR9 was less efficient without gp96. Altogether, these data suggest a model whereby CNPY3 and gp96 form a complex that interacts with and promotes the folding of nascent TLR9 within the ER, and they provide evidence that, similarly to cytoplasmic Hsp90, gp96 works with client-specific cochaperones. (*Nat. Commun.* doi:10.1038/ncomms1070, published online 21 Sep 2010) *IC*

Sensing the good fat

Fish oil contains eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), two omega-3 fatty acids with a number of health benefits that include reduction of blood triglyceride levels and anti-inflammatory effects. Olefsky and colleagues now report that GPR120 functions as a receptor for omega-3 fatty acids in proinflammatory macrophages and mature adipocytes. The authors showed that DHA and a synthetic small molecule repressed the ability of lipopolysaccharide to induce inflammatory responses via Toll-like receptor 4 (TLR4). Knocking down GPR120 with siRNA abrogated this effect, suggesting that the anti-inflammatory properties of DHA and the synthetic small molecule were mediated by GPR120. Additional experiments revealed that β-arrestin2 was involved, as treatment of cells with DHA led to a migration of β-arrestin2 from the cytoplasm to the plasma membrane, where GPR120 resides. Because GPR120 was known to be expressed in adipocytes, the authors tested whether it might also play a role in glucose transport. Treatment of adipocytes with DHA resulted in the migration of the glucose transporter type 4 (GLUT4) to the plasma membrane and an increase in the intracellular glucose concentration. When omega-3 fatty acids were fed to mice on a high-fat diet, wild-type mice, but not GPR120 KO mice, showed increased sensitivity to insulin relative to animals that only received the high fat diet. This suggests that GPR120-selective agonists might find clinical use for diseases that are linked to insulin resistance. (*Cell* **142**, 687–698, 2010) *JMF*