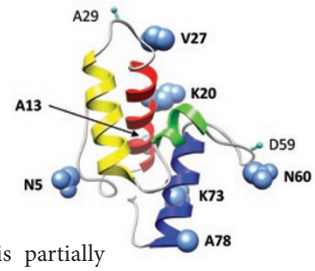
Sweet perturbations



Glycosylation—the attachment of polysaccharide chains or glycans to proteins—is one of the most common and important protein modifications and occurs in the endoplasmic reticulum while the polypeptide is still being biosynthesized and is partially unfolded. This suggests that glycosylation plays a role in protein folding and stability, but investigating this using natively glycosylated proteins is challenging because these proteins are usually large, multisubunit and/or membrane associated. Most studies thus focus on defined, chemically synthesized glycopeptides. In an effort to develop general rules to define and predict glycosylation-induced effects on protein folding and structure, Chen *et al.* used protein semisynthesis to create variants of the bacterial immunity protein Im7 that were site-specifically modified with the chitobiose disaccharide (GlcNAc-GlcNAc). Im7 is ideal for studying this modification because it is a small, globular, 87-amino-acid protein whose folding pathway is well defined. Likewise, the chitobiose disaccharide was previously shown to serve as a proxy for larger glycans. The authors created seven Im7 variants glycosylated at solvent-exposed sites and studied the kinetic and thermodynamic consequences of these modifications. Glycans located in the center of α-helices negatively affected folding, whereas one located in the tight loop between two helices increased the overall rate of folding. Finally, glycosylation at the ends of α-helices and in larger loops seemed to have only a minimal effect. These results provide insight into why there is an increased probability of finding glycosylation sites between regions of secondary structure. The introduction of a glycan can thus have an effect on protein folding that critically depends on the exact position of the modification. (*Proc. Natl. Acad. Sci. USA* doi:10.1073/pnas.1015356107, published online 9 December 2010) *BK*

Inhibiting factor

RNA polymerase (RNAP) is tightly regulated by transcription factors such as the bacterial protein GreA or its *Thermus thermophilus* homolog Gfh1, which inhibit transcription initiation and elongation. Previously solved structures of RNAP in the absence of transcription factors show a crab's-claw appearance, with four separate modules—the core, shelf, clamp and jaw-lobe—that are able to move relative to each other. Between the two central modules, the core and the shelf, lies the entry point of nucleotide triphosphates (NTPs) into the catalytic site. When Gfh1 is added to RNAP, transcription is inhibited in a process whose exact mechanism has not been clear. Now Yokoyama and colleagues have revealed the structure of Gfh1 bound to RNAP in the presence of DNA and RNA. In the structure, the four-module architecture of RNAP is retained, but the two central modules are rotated into a 'ratcheted' orientation by 7° relative to their positions in the previous transcription elongation complex or apo holoenzyme structures. This has the effect of opening up the nucleic acid binding channel. At the same time, the N-terminal coiled coil of Gfh1 blocks the NTP entry channel between the two central modules, with the tip of the coiled coil located within the phosphate-binding site. This structure shows that Gfh1 works by stopping NTP from binding to RNAP and holding RNAP in a ratcheted state, which perhaps might be useful for other transcription steps. (*Nature* doi:10.1038/nature09573, published online 1 December 2010) *MH*

Written by Maria Hodges, Boyana Konforti, Sabbi Lall & Steve Mason

FERM-ly bound

Tumor suppressors regulate a variety of processes in cells in order to restrain cancer progression and metastasis. The adhesion molecule tumor suppressor in lung cancer 1 (TSLC1) shows lost or diminished expression in many metastatic tumors. Through the 4.1 binding motif in its cytoplasmic tail (a hallmark of the protein 4.1 family), TSLC1 interacts with the FERM domain–containing tumor suppressor DAL-1, which controls cell adhesion. The significance and molecular details of this interaction are still not clear. By solving the X-ray crystal structure of the FERM domain of DAL-1 complexed with a peptide from TSLC1, Hallberg and colleagues have uncovered the basis for the interaction between DAL-1 and TSLC1, providing the first crystal structure of 4.1 superfamily members in complex. The structure shows a peptide derived from TSLC1 binding in a conserved hydrophobic pocket in the C-lobe of the trilobed DAL-1 FERM domain. The overall structure of DAL-1 is not altered by the binding of TSLC1, but local conformational changes stabilize the interaction. Surface plasmon resonance analysis of TSLC1 binding to DAL-1 revealed both fast and slow reactions, likely representing initial binding and the resulting conformational changes, respectively. The TSLC1–DAL-1 structure has features similar to those due to interactions in other FERM domain–containing proteins and helps to resolve earlier controversy over domain interactions in 4.1 superfamily members. Although the full significance of interactions in FERM domain–containing proteins for tumor progression remains to be revealed, the model provided by this cocrystal will be integral to efforts to understand how tumor cells escape their local environment. (*J. Biol. Chem.* doi:10.1074/jbc.M110.174011, published online 3 December 2010) *SM*

Formin does the twist

Cytoskeletal remodeling is central to many biological processes; for example, cell protrusion depends upon the polymerization and disassembly of actin filaments, processes that are regulated by a number of factors, including those in the formin family. Formins are key to actin nucleation and carry two conserved formin homology domains, FH1 and FH2. Although the formin mouse protein diaphanous homolog 1 (mDia1) was previously observed tracking processively in cells, about half of mDia1 remains stationary. In addition, the relationship between formin movement and conformational changes relative to the growing end of the filament where actin is being added is unclear. Because actin filaments consist of two strands wrapped in a long-pitched double helix, it has been predicted that formin would rotate around the strands. Watanabe and colleagues have now monitored formin movement relative to the double helical strand of actin. In this setup, actin is labeled with tetramethylrhodamine (TMR) at low density while a protein formed by the glutathione-S-transferase fusion of the FH2 domains of mDia1 is immobilized. Using fluorescence polarization, the orientation of the TMR-actin filament was monitored and the resulting oscillations were found to be consistent with a process wherein mDia1 rotates around the actin filament as the latter elongates, with the length of the filament corresponding to its pitch. The authors then tested whether actin-bound nucleotides or profilin, which promotes ATP-actin elongation, could affect this rotation. They found that the distance over which mDia1 rotation occurs persists in ADP- or ATP-actin, as does the presence of profilin, suggesting that rotation is an intrinsic property of this formin. Whether this rotation can be modulated and whether it occurs in the cell remain questions for future study. (*Science* doi:10.1126/science.1197692, published online 9 December 2010) *SL*