Structure watch

OGT–SUBSTRATE INTERACTIONS

In eukaryotes, metabolic status can be detected and transduced to downstream signalling pathways by *O*-GlcNAc transferase (OGT). This detects glucose levels by sensing the levels of UDP-GlcNAc, which it then uses to modify downstream targets, including insulin signalling proteins and transcription factors regulating gluconeogenesis, with *O*-GlcNAc.

OGT is made up of a series of tetratricopeptide repeats (TPR; which are thought to mediate protein–protein interactions) and a catalytic region that had not been structurally resolved. Lazarus *et al.* report two structures of OGT that provide insight into substrate binding and the enzyme's catalytic mechanism. First, they resolved a structure of OGT–UDP that includes the catalytic region. This region comprises an amino-terminal and a carboxy-terminal domain that are separated by an intermediate domain, which is present only in metazoans and has a unique fold. Next, they obtained a structure of OGT–UDP with peptide. The structure revealed that OGT shows preference for Ser or Thr that are flanked by residues which enforce an extended conformation of the amino acid. The OGT substrates bind in an ordered manner to a cleft between the TPR domain and the catalytic region, access to which is controlled by the TPR domain through an unknown molecular mechanism.

ORIGINAL RESEARCH PAPER Lazarus, M. B. *et al.* Structure of human *O*-GlcNAc transferase and its complex with a peptide substrate. *Nature* **469**, 564–567 (2011)

CLATHRIN UNCOATING BY HSC70

Clathrin has a triskelion (three-leg) structure that oligomerizes into lattice-like coats, which drive vesicle formation at the membrane. After the vesicle has separated from the membrane, the clathrin coat is disassembled in a tightly regulated process that is mediated by heat shock cognate 70 (HSC70), the most abundant member of the heat shock protein 70 (HSP70) family of chaperones. Now, Böcking *et al.* track the disassembly of clathrin by HSC70 at a single-molecule level. They propose that HSC70 destabilizes the clathrin lattice by trapping transient local conformational fluctuations for long enough to allow fluctuations at other vertices to destabilize the entire lattice.

To allow single-molecule analysis, they developed a system of immobilized fluorescently labelled clathrin-lattice structures that they treated with fluorescently labelled HSC70, allowing real-time analysis of individual complexes through total internal reflection fluorescence microscopy. They found that uncoating requires accumulation of a critical level of one HSC70 per two clathrin trimers. Importantly, their single-molecule analysis method allowed them to conclude that disassembly is all-or-nothing, never partial. Moreover, disassembly depends on the specific binding of HSC70 to a target sequence within clathrin, which facilitates the trapping of the destabilizing conformational fluctuation. The authors propose that this mechanism may apply to all HSP70 family members.

ORIGINAL RESEARCH PAPER Böcking, T. *et al.* Single-molecule analysis of a molecular disassemblase reveals the mechanims of Hsc70-driven clathrin uncoating. *Nature Struct. Mol. Biol.* 30 Jan 2011 (doi:10.1038/nsmb.1985)