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Editorial overview: Catalysis and regulation: Structural features guiding enzyme catalysed processes

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Enzymes are macromolecular catalysts designed to carry out reactions in biological settings under the mild conditions of the cell. They do their chemistry at outstanding rate accelerations and in a highly regulated manner, ensuring catalysis is carried out with temporal and spatial control. They extend their catalytic capability with high specificity and selectivity on a wide range of substrates including small molecules, lipids, carbohydrates, proteins and nucleic acids. Structural biology provides unprecedented views of the molecular architecture of enzymes giving essential insights into the mechanism by which they carry out their chemistry. In this issue of Current Opinion in Structural Biology a wide range of enzymes are discussed with emphasis on their structural features and how their chemistry is regulated.

Ronnenbaum and Lamb [1] provide an overview of the structures of nonribosomal peptide synthetases (NRPSs) involved in the synthesis of siderophores, iron-chelating molecules needed by bacteria to acquire iron from their environments. NRPSs are large multidomain and multifunctional enzymes. A module consists of different domains that enable the adenylation, thiolation and condensation chemistries to occur in order for the addition of a single amino acid to the growing peptide chain to build up the siderophore structure. Structures of different modules in various stages of the adenylation and thiolation chemistry reveal different orientations of the domains in each of the modules and suggest that the enzymes carry out their chemistries in an assembly line manner with multiple steps occurring on the enzyme concurrently. Further diversity of the peptides produced by NRPSs are achieved by accessory enzymes which act to modify specific amino acids within the peptide.

The current state of structural knowledge around the human methyltransferome, a vast family of enzymes that methylate a broad range of substrates including DNA, RNA and proteins is reviewed by Fenwick and Ealick [2]. Emphasis is placed on the structural diversity of these enzymes. Broadly speaking these enzymes can be grouped into S-adenosyl methionine (SAM) dependent and independent enzymes. Structures of methyltransferases involved in epigenetic based control show regulation through conformational changes brought about by histone modifications including methylation and ubiquitinylation. This invokes a reading mechanism prior to a writing mechanism. Reading can occur through domains on the methyltransferase or through auxiliary reader proteins that are recruited to the assembly. Methyltransferases involved in post transcriptional modifications to RNA show the importance of accessory proteins that activate SAM binding and nucleic acid substrate recognition.

Low and Howell [3] review the state of structural knowledge for the bacterial synthase dependent exopolysaccharide biosynthesis and translocation machinery. This molecular machine is involved in the synthesis of components of the bacterial extracellular biofilm matrix and transfer of the polymer to the exterior of Gram-negative bacteria. Crystal structures of the glucosyltransferase/bis-(3'-5')-cyclic-dimeric guanosine monophosphate regulatory protein complex in the presence of this secondary messenger reveal how ligand binding activates transferase activity for synthesis of the growing polymer and how this is coupled to polymer translocation across the inner membrane. Further structures of a range of polysaccharide modification enzymes localised in the periplasm are detailed and a discussion of the beta barrel component that move the polysaccharide across the outer membrane are reviewed. Finally, a tantalising model of the full transenvelope complex for cellulose biosynthesis is presented based on X-ray crystal structures, small angle X-ray scattering data and negative stain electron microscopy results of the various components making up the assembly.

Caveney, Li and Strynadka [4] review structures of enzymes involved in bacterial cell wall biogenesis with emphasis on peptidoglycan (PG) biosynthesis and modification involving the attachment of wall teichoic acid in Gram-positive bacteria. The PG biosynthesis pathway involves the transfer of the uridine diphosphate-MurNAc pentapeptide to a C_{55}-P lipid carrier by the membrane protein MraY followed by the addition of GlcNAc to the lipid by the membrane tethered glucosyltransferase, MurG. The product is then flipped to the periplasm by the membrane bound flippase, MurJ. The review also discusses subsequent steps in PG biosynthesis, namely glycopolymerisation and transpeptidation through the action of membrane anchored penicillin binding proteins. Finally, PG modification with wall teichoic acid polymers is detailed through a sequential set of enzymes including wall teichoic acid biosynthesis, polymerisation, glycosylation, export to the periplasm and attachment to the peptidoglycan substrate. The elucidation of structures of many of the enzymes involved in peptidoglycan biosynthesis provides an important platform for future structure guided drug design aimed at developing novel antibiotics.

Along a similar vein of bacterial drug design targets Kahler *et al.* [5] review a series of virulence enzyme targets involved in protein folding, peptidoglycan biosynthesis and lipopolysaccharide modification. Oxidoreductases, namely disulphide exchange (DsB) enzymes are described including progress towards structure guided inhibitor design. Polyprolyl isomerases (MIPs) are also reviewed, the structures of which have been determined for the enzyme from a series of different bacteria, and progress towards development of small molecule inhibitors detailed. The Mur pathway of enzymes involved in peptidoglycan biosynthesis are structurally described as well as progress and challenges faced in terms of transition state inhibitor design. Finally, two new membrane bound enzyme structures are reviewed, involved in lipopolysaccharide modification, namely phosphoethanolamine addition by EptA and 4-amino-4-deoxy-L-arabinose addition by ArnT.

Routh and Sankaranarayanan [6] give an overview of the ubiquitously found DTD-like fold involved in catalysis by RNA-protein complexes, specifically through proofreading functions during translation. DTD-like fold domains are found in proteins that associate with tRNA synthetases. In the case of D-aminoacyl-tRNA deacylase (DTD), which exhibits chiral proofreading activity by removing mischarged D-amino acids from tRNA-synthetases, the active site contains a chiral selectivity filter consisting of a Gly-*cis*Pro for this enantioselectivity. The structural rigidity of this filter assists in rejecting L-chiral substrates as opposed to selecting D-chiral substrates. Fascinatingly, the fold makes use of protein main chain atoms (not side chains) and the terminal 2'-OH of the RNA for substrate discrimination and catalysis suggesting that the fold functions at the RNA-protein interface.

The current state of knowledge on bacterial DNA replication machinery is discussed by Xu and Dixon [7]. Much of the insights are guided by recent single particle cryo-EM and crystallographic structures of subcomplexes in different states of replication and are challenging the current textbook view of the replisome. CryoEM structures of the *Escherichia coli* DNA polymerase III core-clamp-τ_C complexes in polymerisation and proofreading modes show a different interaction network of individual components which is thought to stabilise the entire complex. Differences in these complexes also provide insights into the editing functions which are further informed by single molecule biochemical studies. In addition, structures of replication termination complexes provide an overview of the mechanism of termination of DNA replication.

Zhao *et al.* [8] give an overview of histone acylation, with a focus on crotonylation and β-hydroxybutyrylation writer enzymes as well as the eraser enzymes. Through comparisons of a number of acylation writer enzymes differences in modes of substrate binding and specificity are detailed. Structures of different classes of histone deacetylases provide insight into site preference for the eraser function and, in the case of de-β-hydroxybutyrylation, show the mechanism of chiral selectivity. Finally, an overview is discussed on acylation reader domains as well as some updates on non-histone acylation and the regulation of these modifications.

Eukaryotic mRNA modification is covered by Fisher and Beal [9] with a focus on methylation and deamination at

internal sites in mRNAs. The structures of methyltransferases with different nucleotide base site specificity are compared emphasising the architectural similarity in the core structures of these enzymes, despite different mechanisms of catalysis, depending on whether methylation occurs to nucleophilic or non-nucleophilic atoms of the bases. The known structures of deaminases show a similar core and utilise a Zn^{2+} ion for catalysis. The structure of a deaminase that carries out adenosine to inosine RNA editing (ADAR2) in the presence of double stranded RNA shows the features that provide nucleotide base specificity and facilitate base flipping.

Tsai and Nussinov [10] review the role of allostery in RAF kinase dimerisation based activation. The role of allosteric driver residues in facilitating dimerisation and activation by stabilising the dimeric state of RAF and destabilising its monomeric state are detailed. A structural interpretation of oncogenic RAF mutants that result in active monomeric BRAF states are also discussed. The authors describe a unified theory of RAF activation based on concepts including the relative population of inactive versus active states, the role of phosphorylation, mutation and allosteric binding in additively altering relative populations and the coupling of structural features with regulatory and catalytic features to correctly position the catalytic motifs for activity. An overview of the poly (ADP-ribose) polymerase (PARP) family focusing on the regulation and catalysis of PARP enzymes is presented by Langelier *et al.* [11]. The structural architecture of PARPs are discussed. Comparisons of PARP-1 bound to a DNA double stranded break and to a DNA single stranded break suggest a unified model for PARP-1 detection of DNA damage. A model for a reverse allostery mechanism, by which NAD^+ binding affects PARP interaction with DNA is presented. Finally, an overview of Tankyrases, PARP enzymes that modify proteins with poly(ADP-ribose) is discussed. The modular nature of Tankyrases, containing ankyrin repeat regions that function as interaction domains for other proteins (such as axin), and sterile alpha motif domains regulating catalysis and polymer formation, have led to a proposal that Tankyrases may play a scaffolding role in signaling complexes. However, the link between oligomerisation and catalytic activity is, as yet, not understood.

Enzymes are the cellular machines that sustain life and maintain a healthy and balanced cellular system. Their unique structures drive their specific function. In this context, structural biology continues to play an essential role in understanding these unique molecular entities.

References

1. Ronnebaum TA, Lamb AL: **Nonribosomal peptides for iron acquisition: pyochelin biosynthesis as a case study**. *Curr Opin Struct Biol* 2018, **53**:1-11.
2. Fenwick MK, Ealick SE: **Towards the structural characterization of the human methyltransferome**. *Curr Opin Struct Biol* 2018, **53**:12-21.
3. Low KE, Howell PL: **Gram-negative synthase-dependent exopolysaccharide biosynthetic machines**. *Curr Opin Struct Biol* 2018, **53**:32-44.
4. Caveney NA, Li FK, Strynadka NC: **Enzyme structures of the bacterial peptidoglycan and wall teichoic acid biogenesis pathways**. *Curr Opin Struct Biol* 2018, **53**:45-58.
5. Kahler CM, Sarkar-Tyson M, Kibble EA, Stubbs KA, Vrielink A: **Enzyme targets for drug design of new anti-virulence therapeutics**. *Curr Opin Struct Biol* 2018, **53**:140-150.
6. Routh SB, Sankaranarayanan R: **Enzyme action at RNA-protein interface in DTD-like fold**. *Curr Opin Struct Biol* 2018, **53**:107-114.
7. Xu ZQ, Dixon NE: **Bacterial replisomes**. *Curr Opin Struct Biol* 2018, **53**:159-168.
8. Zhao S, Zhang X, Li H: **Beyond histone acetylation-writing and erasing histone acylations**. *Curr Opin Struct Biol* 2018, **53**:169-177.
9. Fisher AJ, Beal PA: **Structural basis for eukaryotic mRNA modification**. *Curr Opin Struct Biol* 2018, **53**:59-68.
10. Tsai CJ, Nussinov R: **Allosteric activation of RAF in the MAPK signaling pathway**. *Curr Opin Struct Biol* 2018, **53**:100-106.
11. Langelier M-F, Eisemann T, Riccio AA, Pascal JM: **PARP family enzymes: regulation and catalysis of the poly(ADP-ribose) posttranslational modification**. *Curr Opin Struct Biol* 2018, **53**.