FA1-MS03-O1

Molecular Mechanisms of Yeast Cell Wall Glucan Remodelling. Daan M.F van Aalten. *Division of Molecular Microbiology, College of Life Sciences, University of Dundee, Dundee, UK.*
E-mail: d.m.f.vanaalten@dundee.ac.uk

Yeast cell wall remodeling is controlled by the equilibrium between glycoside hydrolases, glycosyltransferases, and transglycosylases. Family 72 glycoside hydrolases (GH72) are ubiquitous in fungal organisms and are known to possess significant transglycosylase activity, producing elongated β(1–3) glucan chains. However, the molecular mechanisms that control the balance between hydrolysis and transglycosylation in these enzymes are not understood. Here we present the first crystal structure of a glucan transglycosylase, *Saccharomyces cerevisiae* Gas2 (*Sc*Gas2), revealing a multidomain fold, with a $(\beta\alpha)_8$ catalytic core and a separate glucan binding domain with an elongated, conserved glucan binding groove. Structures of *Sc*Gas2 complexes with different β-glucan substrate/product oligosaccharides provide "snapshots" of substrate binding and hydrolysis/transglycosylation giving the first insights into the mechanisms these enzymes employ to drive β(1–3) glucan elongation. Together with mutagenesis and analysis of reaction products, the structures suggest a "base occlusion" mechanism through which these enzymes protect the covalent protein-enzyme intermediate from a water nucleophile, thus controlling the balance between hydrolysis and transglycosylation and driving the elongation of β(1–3) glucan chains in the yeast cell wall.

Keywords: glycosidase; mechanism; inhibition

FA1-MS03-O2

Light-Driven Repair of DNA Damage by *Drosophila* (6-4)-Photolyase. Thomas Barends[a], Tatiana Domratcheva[a], Melanie Maul[b], Max Cryle[a], Andreas Glas[b], Sabine Schneider[b], Thomas Carell[b], Ilme Schlichting[a]. [a]*Dept. of Biomolecular Mechanisms, Max-Planck Institute for Medical Research, Jahnstrasse 29, D-69120 Heidelberg, Germany.* [b]*Centre for Integrative Protein Science, Dept. of Chemistry and Biochemistry, Ludwig-Maximilians University Munich, Butenandtstrasse 5-13, D-81377 Munich, Germany.*
E-mail: Thomas.Barends@mpimf-heidelberg.mpg.de

UV-induced intramolecular reactions in DNA result in highly mutagenic lesions such as CPD- and (6-4) photolesions. To counteract this, organisms have evolved light-powered energy and electron transfer-based DNA repair systems, the CPD- and (6-4) photolyases. The mechanism of (6-4) lesion recognition and repair is not well understood. Previously, it was proposed that upon binding of the lesion to the photolyase an oxetane intermediate is formed assisted by the protonation of the lesion's N3 atom by one of two conserved histidines. This oxetane was then proposed to be cleaved after insertion of an electron by the light-excited FAD cofactor [1] [2]. Here, we report that the high-resolution crystal structure of *Drosophila* (6-4) photolyase with bound lesion does not show an oxetane bound to the active site as would have been expected. Moreover, neither of the conserved histidines nor any other potential proton donor is available to protonate the N3 atom to assist oxetane formation. However, integrating data from crystal structures of the photolyase bound to the lesion and repaired DNA and of two mutants with biochemical data and quantum mechanical calculations allows for the proposal of a revised mechanism.

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Keywords: DNA repair; flavoenzymes; photochemistry

FA1-MS03-O3

X-Ray Analyses of Two Evolutionarily Different Threonyl-Trna Synthetases Which Perform a Function by Supplementing Their Defects to Each Other in Crenarchaea. Satoru Shimizu[c],Yoshiteru Sato[c], Ella Czarina Magat Juan[c], Yu-ichiro Miyashita[c], Tsubasa Sagara[a], Kaoru Suzuki[a], Masaru Tsunoda[b], Takeshi Sekiguchi[a], Anne-Catherine Dock-Bregeon[d], Dino Moras[d], Akio Takenaka[b]. [a]*College of Science and Engineering, Iwaki-Meisei University, Iwaki 970-8551, Japan.* [b]*Faculty of Pharmacy, Iwaki-Meisei University, Iwaki 970-8551, Japan.* [c] *Graduate School of Bioscience and Tokyo TokBiotechnology, yo Institute of Technology, Yokohama 226-8501, Japan.* [d]*IGBMC, 67404 Illkirch, France.*
E-mail: atakenak@iwakimu.ac.jp

To maintain the highest fidelity of protein synthesis, twenty kinds of aminoacyl-tRNA synthetases (ARSs) exist in general for twenty kinds of amino acids, each ARS being highly specialized to recognize only the cognate amino acid (A) and the cognate tRNAA. ARS is generally composed of a set of the two domains for catalyzing and for anticodon-binding. For some As difficult to be distinguished, however, ARS contains an additional editing domain in which the mis-acylated non-cognate A is released. It was found that some crenarchaeal organisms have two genes for ThrRS. A multiple-sequence alignment of these gene products (ThrRS-1 and ThrRS-2) against the canonical ThrRSs [1,2] has shown that ThrRS-1 is missing the editing domain and ThrRS-2 is missing the catalytic domain. This suggests that ThrRS-1 and ThrRS-2 perform a function by supplementing the defects to each other in these crenarchaeal organisms. Furthermore, ThrRS-1 is similar to the bacteria Ec-ThrRS [1] and ThrRS-2 is similar to archaea Pa-ThrRS [2]. In addition, ThrRS-2 contains an additional region between the editing domain and the anticodon binding domain. These structural features stimulated us to reveal their tertiary structures. ThrRS-1 (APE0809) and ThrRS-2 (APE0117) from Aeropyrum pernix (Ap), and those

(ST0966 and ST2187) from Sulfolobus tokodaii (St) were overexpressed in E. coli, purified, and crystallized. The crystal structure of Ap-ThrRS-1 has been successfully determined at 2.3 Å resolution, as the first example. Ap-ThrRS-1 is a dimeric enzyme, the two identical subunits being associated to each other. Each subunit is composed of the two domains for the catalytic reaction and for the anticodon-binding, as expected. Their structures are similar to those of Ec-ThrRS. The amino acid residues essential for the catalysis and for the anticodon recognition are highly conserved at the positions and in the orientations. The essential editing domain of ThrRS is completely missing in Ap-ThrRS-1 as expected, suggesting the necessity of the second enzyme ThrRS-2 for editing. Since the N-terminal sequence of Ap-ThrRS-2 is similar to the sequence of the editing domain of Pa-ThrRS, Ap-ThrRS-2 is expected to catalyze de-aminoacylation of the misacylated serine moiety at the CCA terminus.

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Keywords: aminoacylation; threonyl-tRNA synthetase; protein synthesis

FA1-MS03-O4

Crystallographic Snapshots of Iterative Substrate Translocations During Nicotianamine Synthesis in Archaea. Cyril Dreyfus[a], David Lemaire[b], David Pignol[a], Pascal Arnoux[a]. [a]*Laboratoire de Bioénergétique Cellulaire, CEA, DSV, IBEB, Saint-Paul-lez-Durance, F-13108, France.* [b]*Laboratoire des Interactions Protéine Métal, CEA, DSV, IBEB, Saint-Paul-lez-Durance, F-13108, France.*
E-mail: cyrille.dreyfus@cea.fr

Nicotianamine is a ubiquitous metabolite in plants that is able to bind heavy metals both *in vitro* and *in vivo* [1]. It is the main precursor in phytosiderophore synthesis and also an important metal chelator allowing long distance iron transport and sequestration. The action of NA is not restricted to iron homeostasis but extends to that of other metal ions such as Cu^{2+}, Zn^{2+}, Mn^{2+} and Ni^{2+} [2].
Nicotianamine synthase (NAS) is the enzyme catalysing NA synthesis by the condensation of three aminopropyl moieties of S-adenosylmethionine (SAM) and the cyclization of one of them to form an azetidine ring (Fig. 1). An intriguing feature of NAS, when compared to other aminopropyltranferase enzymes, is that it uses three molecules of SAM without any other aminopropyl acceptor. By comparison, spermidine synthase and spermine synthase, two enzymes belonging to the aminopropyltransferase family, use putrescine or spermidine as their respective acceptors and are limited to only one aminopropyl transfer [3]. The NAS gene family has long been considered to be plant-specific. However, recent sequencing projects have revealed *nas*-like genes in the genome of various organisms including plants, fungi and archaea [4].
Here we report six crystal structures of an archaeal NAS from *Methanothermobacter thermautotrophicus* either free or in complex with its product(s) and substrate(s) [5]. These structures reveal a novel fold arrangement with a C-Terminal Rossman-fold domain topped by a NAS specific alpha helical N-Terminal domain. Combined together our work depict an original reaction mechanism taking place in a buried reaction chamber located between the N and C-Terminal domains. This reaction chamber is open to the solvent through a small inlet and a single active site allows the selective entrance of only one substrate at a time that is then processed and translocated stepwise.

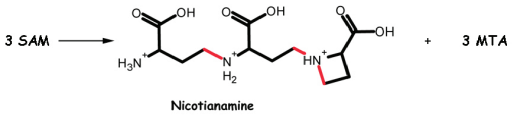

Figure 1: Biosynthesis of nicotianamine (novel bonds created during the reaction are coloured in red).

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Keywords: structure and mechanism; siderophores; metal ligands

FA1-MS03-O5

Substrate Recognition and Catalysis of Polysaccharide Lyases. Sine Larsen[a], Michael McDonough[a], Majbritt Thymark[a], Malene H. Jensen[a], Harm Otten[a], Leila Lo Leggio[a], Torben Borchert[b], Lars H. Christensen[b], Henrik Frisner[b], Carsten Sonksen[b]. [a]*Department of Chemistry, University of Copenhagen, Denmark.* [b]*Novozymes, Bagsværd, Denmark.*
E-mail: sine@kemi.ku.dk

Lyases cleave the backbone of polysaccharides by a β-elimination mechanism, which results in a double bond in the non-reducing end of the cleaved substrate. Six out of the 21 families of polysaccharide lyases contain enzymes that assist in the degradation of the pectic network of the primary cell wall of plants [1]. We report recent results on investigations of enzymes assigned to Family 1 and 4. The pectate lyases in Family 1 act on the smooth region of pectin, homogalacturonan (HGA) a homopolymer of (1,4)-α linked D galaturonic acid (GalUA) residues. Pectin has "hairy" rhamnogalacturonan regions (RG-I) interspersed and the Family 4 lyases act on this part of pectin. The backbone of RG-I is composed of alternating rhamnose (Rha) and galacturonic acid (GalUA) residues with [,2-α-L-Rh-(1,4)-α-D-GalUA-(1,] as the repeating unit, the family 4 lysases cleave the (1,4)-α-glycosidic bond. Among the differences