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.*
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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

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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.*
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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.

[1] Kim, S., Malhotra, K., Smith, C., Taylor, J. and Sancar, A., **1994**, J. Biol.Chem. 269, 8535-8540 [2] Zhao, X., Liu, J., Hsu, S., Zhao, S., Taylor, J. and Sancar, A., **1997**, J. Biol. Chem. 272, 32580-32590

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.*
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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