data are consistent with the Asgard archaea metagenomes; Asgard profilins hence truly derive from the Asgard metagenome and are not the result of eukaryotic contamination.

Phosphatidylinositol-4,5-biphosphate (PIP_2), a membrane phosphoinositide, regulates the activities of many actin-binding proteins, such as Dia2, cofilin, profilin and so on.[4] Although archaea have no ability to synthesize PIP_2, they contain other lipids with inositol phosphate head groups. The authors hence attempted to know whether PIP_2 affects the actin polymerization. **Figures 2(e)–2(h)** show that all actin polymerization profiles have a similar trend; these patterns elucidate the mitigated effects of the Asgard profilins in inhibiting actin polymerization at increasing PIP_2 concentration. PIP_2 is not, however, the natural phospholipid for Asgard profilins; for this reason a large PIP_2 concentration is necessary in this assay.

In summary, five conclusions have been generated according to the structural information and biochemical data. (1) Actin phylogenetic analysis and structural modelling show that Asgard actins are highly conserved and might have eukaryotic-like properties. (2) Pyrene-actin assays reveal that Asgard profilins regulate polymerization of rActin in vitro, even though the two species diverged more than 1.2 billion years ago. (3) The compelx structures, including Loki profilin-1/rActin, Loki profilin-2/rActin and Odin profilin/rActin, indicate that Asgard archaea have functional eukaryotic-like profilin-actin systems. (4) Structural comparison, ITC data and metagenomics analysis elucidate no eukaryotic contamination in Asgard metagenomes. (5) Pyrene-actin assays unveil that Asgard archaea have phospholipid-sensitive actin-regulating profilins. Taken together, Asgard archaea and eukaryotes share a common ancestor. (Reported by Chun-Hsiang Huang)

This report features the work of Robert C. Robinson and his collaborators published in Nature ***562****, 439 (2018).*

TPS 05A Protein Microcrystallography

- Protein Crystallography
- Biological Macromolecules, Protein Structures, Life Science

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ZHD: A ZEN-Hydrolyzing Enzyme for Detoxification

Zearalenone (ZEN), an estrogenic mycotoxin, results in severe health problems in human beings. According to the results of analysis and bioassay of the complicated structures, ZHD is capable of hydrolyzing ZEN and its more toxic derivative a-zearalenol (α-ZOL) to decrease their toxicity.

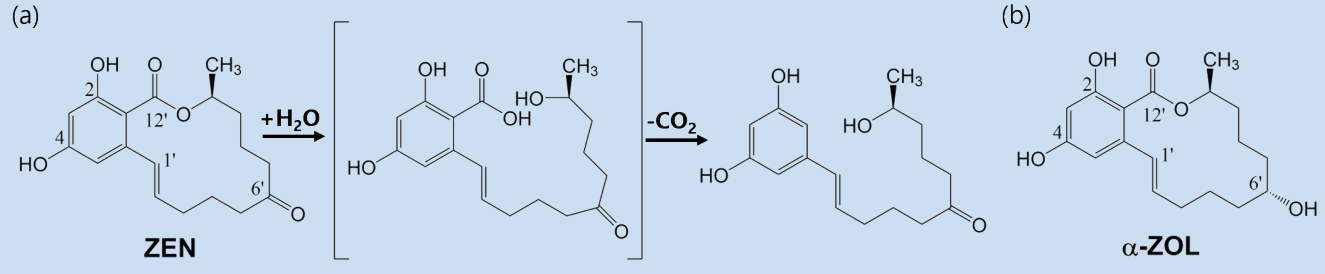

Fig. 1: (a) The hydrolytic process of ZEN by ZHD. Left: The structure of ZEN. Middle: Intermediate. Right: Nontoxic product. (b) The structure of α-ZOL. The carbon numbering 1–6 in the phenyl ring and 1′–12′ in the lactone ring. [Reproduced from Ref. 4]

Zearalenone (ZEN; **Fig. 1(a)**), an estrogenic mycotoxin produced by some *Fusarium* and *Gibberela* species, is widely detected in musty grains, including corn, wheat, barley and so on. ZEN causes not only reproductive disorders in farm animals, such as infertility, abortion and other breeding problems, but also hyperestrogenic syndromes in human beings; it thus causes significant economic loss.[1] To overcome the mycotoxin contamination, using a biocatalyst to remove ZEN in a substrate-specific way should be an approach superior to traditional chemical and physical methods. In 2002, Takahashi-Ando *et al*. identified a new enzyme, ZHD101 from *Clonostachys rosea*, that catalyses cleavage of the ester bond of ZEN into a nontoxic product (**Fig. 1(a)**). This information provides a key clue for a mycotoxin biodegradation strategy.[2]

In a digestive system, ZEN turns into a more toxic derivative, a-zearalenol (α-ZOL; **Fig. 1(b)**), *via* gut microbes or intracellular enzymes. Compared to ZEN, α-ZOL shows greater estrogenic activity (> 90-fold) and stronger estrogen receptor-binding activities (> 10–20 times). ZHD101 also detoxifies α-ZOL through the same hydrolytic mechanism of ZEN, but the ZHD101 activity against α-ZOL is ~40% less than that against ZEN even though the two compounds have similar chemical structures (**Fig. 1**). Rey-Ting Guo (Tianjin Institute of Industrial Biotechnology) recently solved the structure of ZHD101 in complex with α-ZOL and suggested why ZHD101 has less activity against α-ZOL.[3] To identify further potential ZHD-family members, a research team led by Guo found a novel ZHD from *Rhinocladiella mackenziei* (RmZHD) *via* data-mining in Genbank; its activities against ZEN and α-ZOL are greater than ZHD101 by ~1.66-fold and ~1.2-fold. They also determined the structures of RmZHD alone and in complex with two substrates, ZEN and α-ZOL. All diffraction data sets were collected at **TPS 05A**, **TLS 15A1** and **TLS 13C1**.[4]

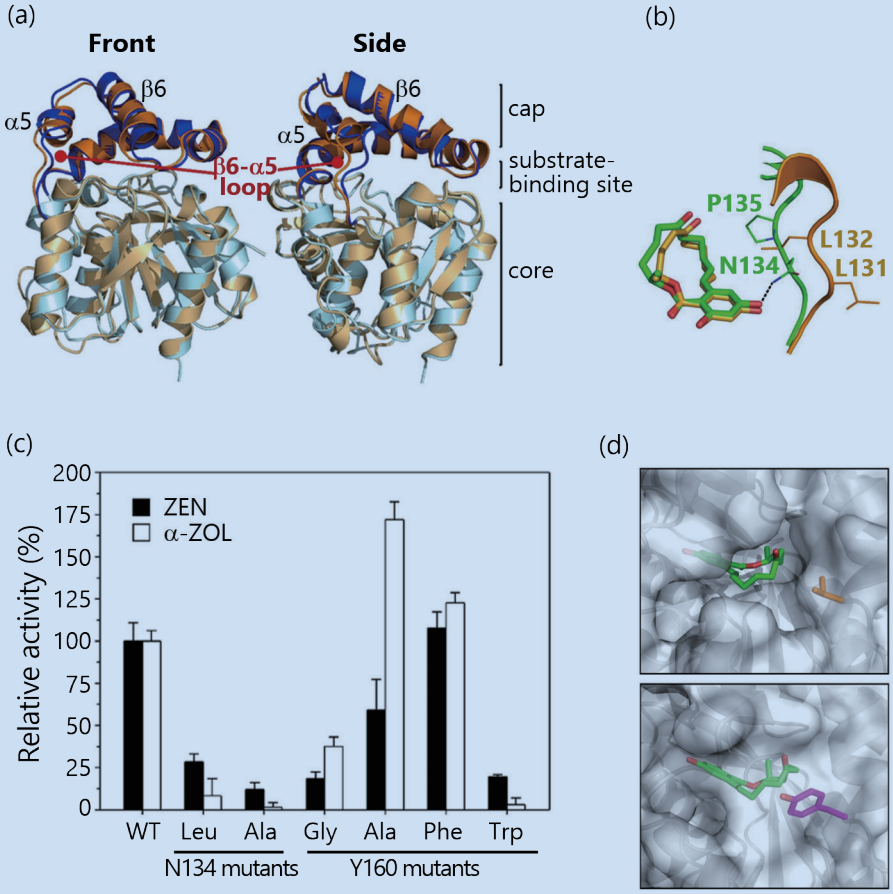

Fig. 2: (a) Overall structural comparison of RmZDH (blue) and ZHD101 (orange). The cap and core domains of RmZHD and ZHD101 appear in deeper and lighter colors, respectively. (b) The β6-α5 loop superimposition of RmZHD/ZEN (green) and ZHD101/ZEN (orange). The dashed line denotes a hydrogen bond. (c) Activity assay. The wild-type activity against ZEN and α-ZOL serves as reference (100%). All measurements were conducted in triplicate; the results are presented as average ± SD. (d) Open conformation (left panel) and closed conformation (right panel). The bound ZEN molecules (green), ZHD101 V158 (orange) and RmZHD Y160 (purple) are displayed as stick models. [Reproduced from Ref. 4]

Based on **Fig. 2(a)**, two vital structural differences illustrate why RmZHD has a greater activity against ZEN and α-ZOL than ZHD101. First, compared with ZHD101, the β6-α5 loop of RmZDH is nearer the substrate-binding site. The N134 side chain, located in the β6-α5 loop, forms a hydrogen bond to O4 of the phenyl ring of ZEN upon substrate binding, but a similar conformational change cannot induce in ZHD101 (**Fig. 2(b)**). To investigate the catalytic role of N134, two mutants, N134L and N134A, were purified for activity assay. **Figure 2(c)** indicates that N134 plays a crucial role in RmZHD catalysis because both mutants display much small activity against ZEN and α-ZOL than the wild type.

Second, the entry to the substrate-binding pocket is covered with a larger residue, Y160, in RmZHD (a so-called closed conformation), but the open conformation can be observed in ZDH101 because the corresponding residue, V158, is smaller (**Fig. 2(d)**). To elucidate the catalytic role of Y160, some mutants were constructed, including Y160G,

Y160A, Y160F and Y160W, to conduct activity assays. Notably, the result shows distinct effects on ZEN and α-ZOL. In regard to ZEN, compared with the wild type, all mutants except Y160F having equal activity possess less ZEN-hydrolyzing activity, which indicates that Y160 is the most suitable residue for ZEN interaction (**Fig. 2(c)**). As regards α-ZOL, compared to the wild type, only Y160A exhibits an increase, more than 70%, in α-ZOL hydrolyzing activity (**Fig. 2(c)**). The complex structure of Y160A/α-ZOL indicates that the unfavourable close contact (the side chain of Y160) disappears; Y160A thus makes an active-site environment suitable for α-ZOL binding and hydrolysis, which conclusion is supported by data from isothermal titration calorimetry (ITC).

In summary, Guo and his collaborators determined the crystal structures of ZHD101 and RmZHD. Comparison of the two enzyme structures clearly shows that two vital structural differences (the β6-α5 loop and Y160) are involved in the catalytic reaction. These results provide the important molecular mechanism of substrate binding and catalysis for the ZHD family of enzymes; this critical information can be applied for further protein engineering and genetic modification to diminish the mycotoxin. (Reported by Chun-Hsiang Huang)

This report features the work of Rey-Ting Guo and his collaborators published in ACS Catal. ***8****, 4294 (2018).*

TPS 05A Protein Microcrystallography
TLS 13C1 SW60 – Protein Crystallography
TLS 15A1 Biopharmaceuticals Protein Crystallography

- Protein Crystallography
- Biological Macromolecules, Protein Structures, Life Science

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AmbP3: A Proficient Enzyme Involved in the Normal and Reverse Prenylation of Hapalindoles

Compounds related to hapalindole can serve as potential pharmaceutical leads. This study is the first to elucidate how a single enzyme catalyses normal and reverse prenylation of hapalindoles. These findings provide vital clues to the engineered biosynthesis of prenylated natural products.

Hapalindoles, produced from Cyanobacteria, are bioactive secondary metabolites with diverse chemical structures; compounds related to hapalindole are considered to have pharmacological effects, such as antibacterial, antifungal, insecticidal and antimitotic activities.[1,2] Prenylation is a crucial reaction to increase the chemical complexity of these compounds; namely, the prenylated compounds typically improve the bioactivities because the prenyl moiety makes the compound more lipophilic. In general, the prenylation of two types, normal and reverse, is performed by prenyltransferases (PTases). It is hence important to understand the catalytic mechanism of PTases for future engineering approaches to synthesize unnatural bioactive compounds.

Some PTases, including DMATS (dimethylallyl tryptophan synthase), Fnq26 and TleC, conduct normal or reverse prenylation depending on the substrates, which means that these PTases have the potential for plasticity of substrate binding. No structural data were, however, available to elucidate how a single enzyme switches the two distinct prenylation modes upon different substrates binding. For this reason, a research team led by Ikuro Abe (Graduate School of Pharmaceutical Sciences, University of Tokyo) determined the structure of *Fischerella ambigua* AmbP3 alone and complex structures of AmbP3/DMSPP (DMSPP is a DMAPP analogue), AmbP3/DMSPP/hapalindole U (reverse pathway in **Fig. 1(a)**) and AmbP3/DMSPP/hapalindole A (normal pathway in **Fig. 1(b)**). All diffraction data sets were collected at **TLS 15A1**.[3]