of the crystal structure reveals that the two hemes are closely perpendicular to each other, a geometry that is known to be unfavourable toward facile inter-heme electron transfer. On the basis of this structural information, as well as the relative redox potentials between heme b_H and b_L (150 mV)[3], the authors are hence inclined to favour different electron transfer paths for the two reducing equivalents of the menaquinol substrate during the catalytic turnover of *D. gigas* QFR, as shown in **Fig. 4**.

According to these observations, the authors delineate the paths for electron and proton transfer in *D. gigas* QFR and understand how the anaerobic bacteria utilize the chain of electron transport to harvest the energy. (Reported by Hong-Hsiang Guan)

This report features the work of Hong-Hsiang Guan, Chun-Jung Chen, and their co-workers published in Sci. Rep. ***8****, 14935 (2018).*

TPS 05A Protein Microcrystallog-raphy
TLS 15A1 Biopharmaceutical Protein Crystallography

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

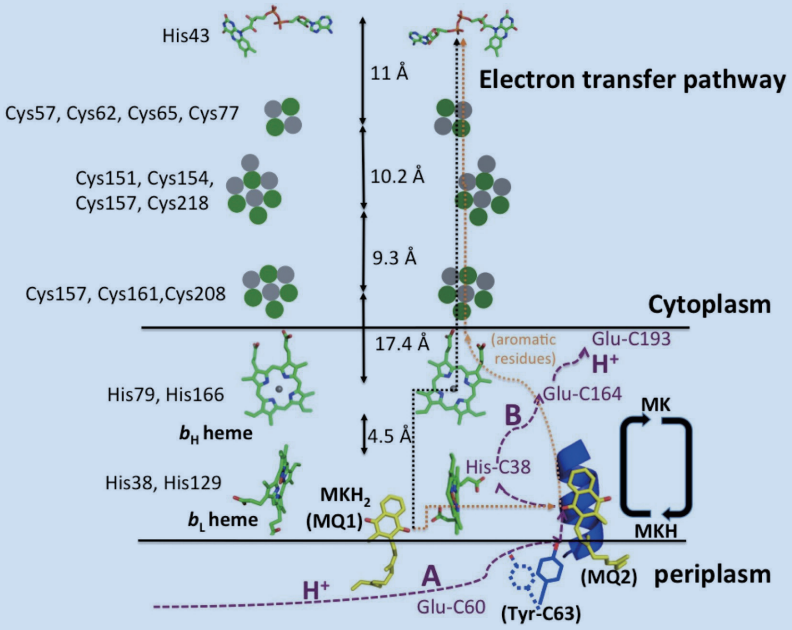

Fig. 4: Delineated electron/proton-transfer paths in *D. gigas* QFR. The fully reduced manaquinol (MKH_2 at the MQ1 site) and the oxidized menaquinone (at the MQ2 site) are shown as yellow sticks. The fully oxidized and semi-oxidized menaquinones are as MK and MKH, respectively. The proposed bifurcation of electron paths (black and orange dotted lines) and the potential flow paths of protons (purple dotted lines) generated in the oxidation of MKH_2, classified as proton paths A and B, are shown. The redox cofactors, FAD (upper sticks), hemes (bottom sticks) and iron-sulfur clusters (grey and green balls), are shown on the electron transfer path. The residues ligating the redox cofactors are black; the proposed proton acceptors are purple. The movement of Tyr-C63 is shown also after binding with MKH with a hydrogen bond. [Reproduced from Ref. 1]

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Asgard Profilin/Rabbit Actin Complex Provides New Clues to Understand Archaea-to-Eukaryote Transition

The origin of the eukaryotic cell is still elusive in modern biology. Combination of the complexstructural and biochemical data shows that Asgard archaea possess a functional eukaryotic-like actin system and indicates that Asgard archaea and eukaryotes share a common ancestor.

The origin of the eukaryotic cell is a major evolutionary conundrum in biology. Metagenomic approaches recently identified genes from Asgard archaea, including Heimdall, Loki, Thor and Odin, which are the closest related prokaryotic relatives of eukaryotes.[1] These proteins produced by archaeal genes are hence supposed to

have eukaryotic features; namely, these Asgard proteins should be involved in membrane maintenance and function, such as vesicular trafficking, *N*-glycosylation, the ubiquitination system, cytoskeletal processes (including actin and profilin homologues) and so on.[2] So far, no functional Asgard protein is available; apart from this situation, an issue about the possibility of eukaryotic contamination of the metagenomes needs to be resolved.[2]

In eukaryotes, membrane remodelling is regulated cooperatively by various proteins, such as the ARP2/ARP3 complex, vasodilator-stimulated protein and profilin/actin complex. Protein sequence analysis shows that Asgard archaea have eukaryotic-like actins that share a sequence identity (58–60%) with human cytoplasmic β-actin and other eukaryotic actins (52–62%). Expectedly, the results of phylogenetic analysis and structural modelling indicate that Asgard actins are structurally conserved and might possess eukaryotic-like properties, but Asgard profilin-like proteins exhibit a lower sequence identity (11–17%) to human profilin-1 and other eukaryotic profilins (7–24%). To investigate the properties of Asgard profilin-like proteins, a research team led by Robert C. Robinson (Institute of Molecular and Cell Biology, A*STAR, Singapore) solved the structure of Loki profilin-1 alone and complex structures of Loki profilin-1/rActin (rActin stands for rabbit α-actin), Loki profilin-2/rActin and Odin profilin/rActin. All diffraction data sets were collected at **TPS 05A**.[3]

Figures 1(a) and 1(b) show basically that Loki profilin-1 and human profilin have a similar overall topology; merely helices and loops of varied lengths, particularly the "Loki loop", can be observed. Structural

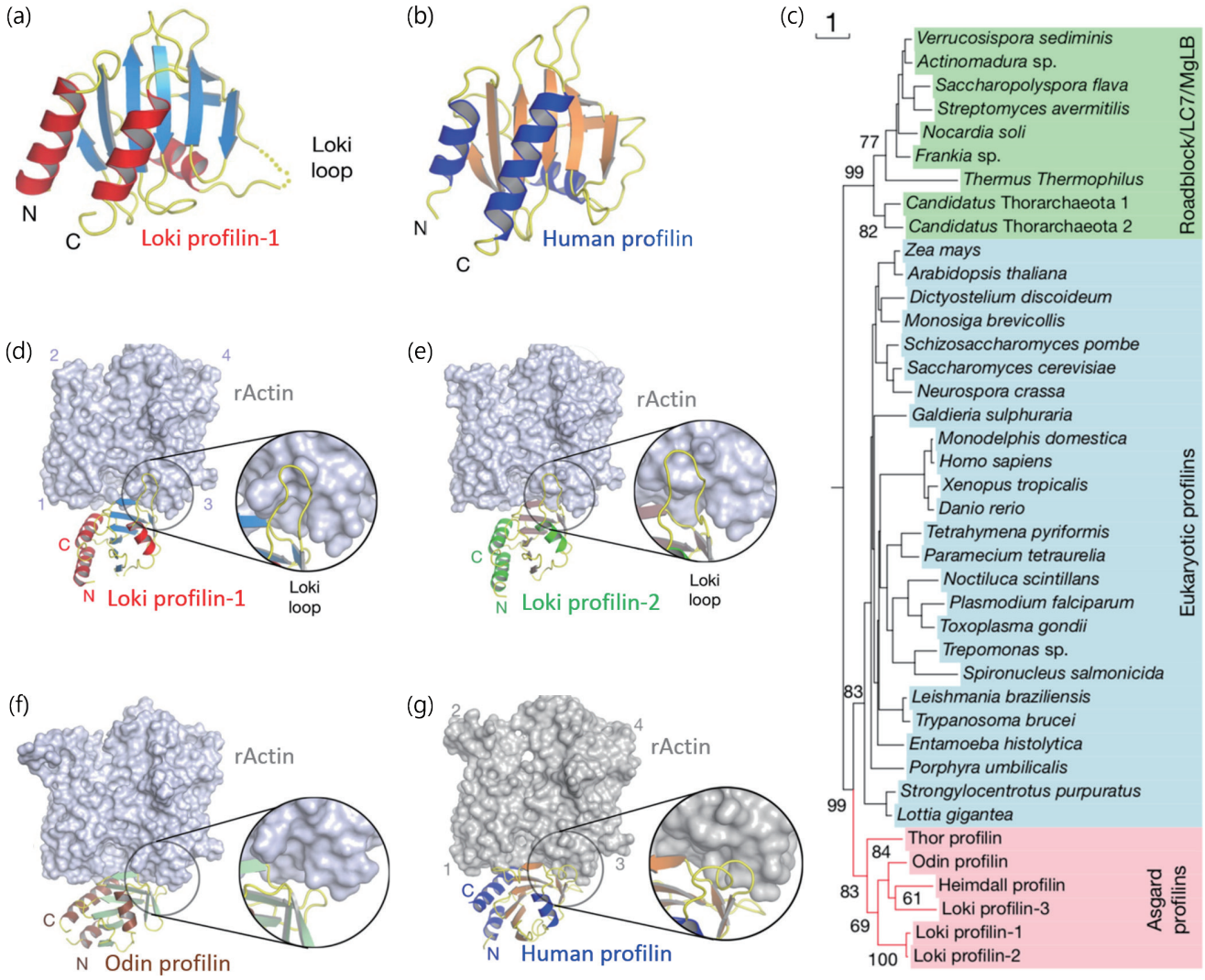

Fig. 1: (a) Overall structure of Loki profilin-1. The partially disordered Loki loop is shown as a dotted line. N and C stand for N-terminal and C-terminal, respectively. (b) Overall structure of human profilin for comparison (PDB code: 1FIL). (c) Phylogenetic tree of Asgard and eukaryotic profilins. (d) Complex structure of Loki profilin-1 with rActin. rActin is presented as a surface. (e) Complex structure of Loki profilin-2 with rActin (f) Complex structure of Odin profilin with rActin. (g) Complex structure of human profilin with rActin (PDB code: 2PAV) for comparison. [Reproduced from Ref. 3]

comparison of Loki profilin-1 with all published eukaryotic profilins indicates that the root-mean-square deviation between them is from 2.3 to 2.7 Å. A subsequent profilin phylogenetic analysis clearly revealed that Asgard and eukaryotic profilins are classified into two distinct clades (**Fig. 1(c)**), even though they are related to each other.

Unfortunately, enough pure Asgard actins for biochemical experiments failed to be produced using a heterologous host expression system. As mentioned above, however, the authors postulated that the mammalian actin might bind to Asgard profilins, including Loki profilin-1 and -2 and -3, Heimdall profilin, Odin profilin and Thor profilin, because of the greater sequence identity between Asgard and eukaryotic actins. Once rActin interacts with Asgard profilins, Asgard profilin/rActin should become an active form to initiate or to sustain the actin polymerization that was detected with a pyrene-actin assay. According to the results of these pyrene-actin assays, the actin polymerization is observable in the presence of Asgard profilins, except Thor profilin.

Figures 1(d)–1(g) show the interactions of Asgard profilins with rActin in a slightly different way. Comparison of **Figs. 1(a) and 1(d)** reveals that the Loki loops become ordered upon rActin binding; the binding site is near the surface of rActin subdomain 3. Compared with **Fig. 1(g)**, the C-terminal helices of Loki profilin-1 and -2 are slightly moved from the binding region on actin subdomain 1. **Figures 1(f) and 1(g)** indicate that Odin profilin and human profilin bind to rActin in a similar orientation. Among four profilins, Odin profilin has the most compact structure, which makes sense to explain why Odin profilin exists in a geothermal environment (Yellowstone National Park). The common binding modes elucidate that Asgard archaea possess functional eukaryotic-like profilin- actin systems.

In eukaryotes, actin filaments can be nucleated and elongated via polyproline-profilin binding; the binding mode involves π-π interactions between prolines and aromatic residues located in the N- and C-terminal helices of profilin (**Fig. 2(a)**). **Figures 2(b)–2(d)** indicate that no aromatic residues can be found, and insufficient space exists between N- and C-terminal helices for polyproline binding. In addition, the isothermal titration calorimetry (ITC) data ($\Delta H = 0$) support the structural information that Asgard profilins do not bind to polyproline motifs. These important

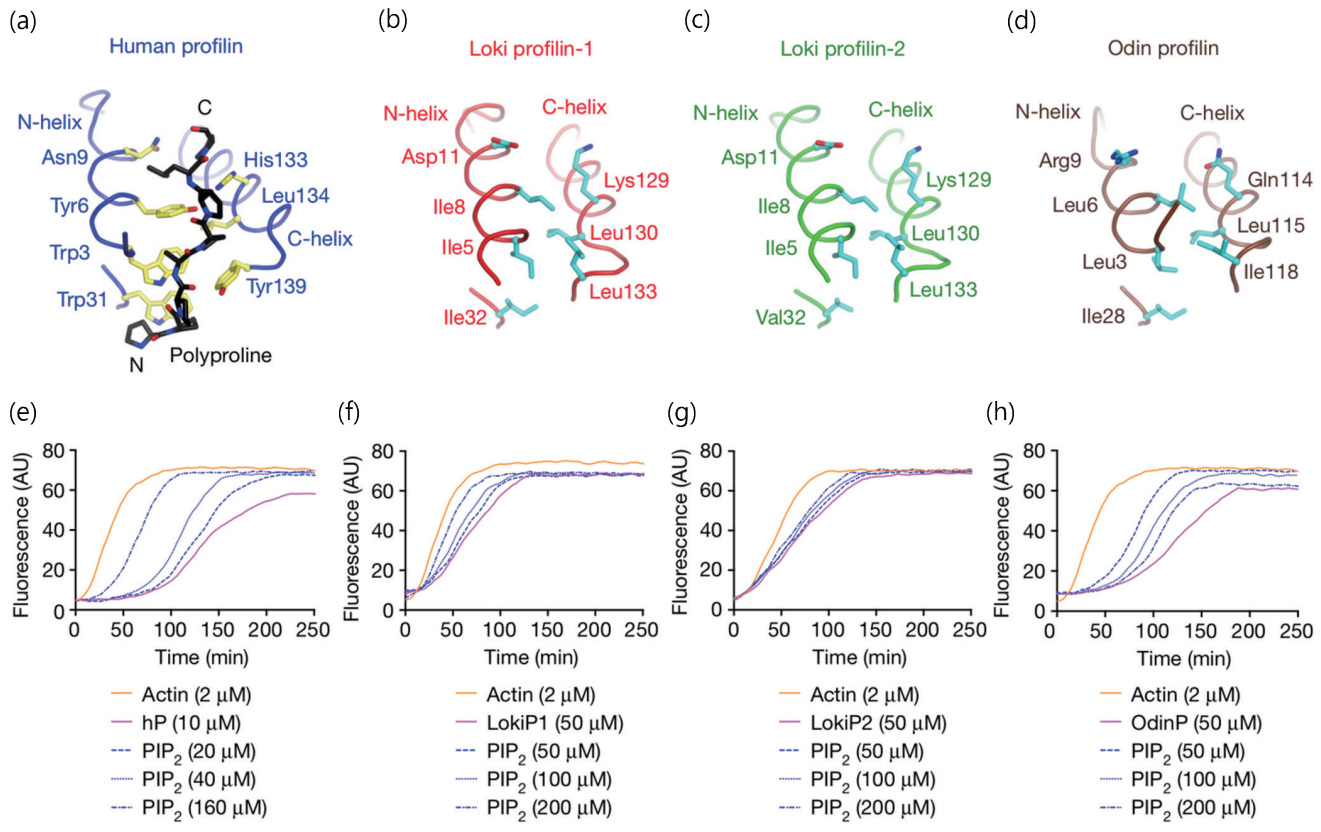

Fig. 2: (a) Interaction network of human profilin with polyproline. The polyproline (PDB code: 2PAV) is shown in black. The binding residues on human profilin are labeled in blue. (b)–(d) Residues corresponding to those presented in (a), from (b) Loki profilin-1, (c) Loki profilin-2 and (d) Odin profilin. (e) Pyrene-actin polymerization profiles of rActin (orange) added to human profilin (pink) and with PIP_2 at increasing concentrations (blue). (f)–(h) Profiles similar to those presented in (e), for (f) Loki profilin-1, (g) Loki profilin-2 and (h) Odin profilin. [Reproduced from Ref. 3]

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.

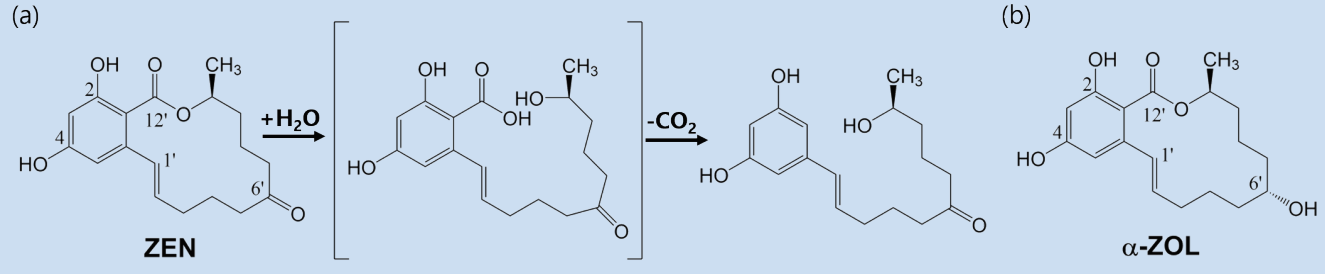

Fig. 1: (a) The hydrolytic process of ZEN by ZHD. Left: The structure of ZEN. Middle: Intermediate. Right: Nontoxic product. (b) The structure of α-ZOL. The carbon numbering 1–6 in the phenyl ring and 1′–12′ in the lactone ring. [Reproduced from Ref. 4]