学位論文の要旨

Abstract of Thesis

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学位論文題目 Title of Thesis（学位論文題目が英語の場合は和訳を付記）

Exploring the archaeal origin of eukaryotic protein machineries
（真核生物タンパク質装置のアーキア起源の探索）

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Life on earth can be classified into two groups of organisms based on cellular structure. Prokaryotes, which comprise bacteria and archaea, are simple, unicellular cells that lack a nucleus. Eukaryotes, including plants and animals, have more complex cell organizations and contain a membrane bound nucleus. Investigating the potential origins of the eukaryotic cell and the emergence of its cellular functions are important considerations for predicting the characteristics of organisms that participated in eukaryogenesis. Recently, metagenomic sequencing has identified Asgard archaea, whose genomes contain genes which have potential homologs to eukaryotic signature protein (ESP) genes. Many of these ESPs encode protein machineries hypothesized to be involved in membrane maintenance and function, by analogy to the eukaryotic proteins. Specifically, ubiquitin, glycosylation, endosomal sorting complexes, and dynamic cytoskeleton systems required for vesicle trafficking and membrane remodelling, are predicted in the Asgard genomes. The Robinson laboratory has previously characterized some components of protein machines that are encoded in Asgard archaea genomes and have compared them to their eukaryotic counterparts. This dissertation is focused on studying the putative cytoskeleton and membrane trafficking proteins from Asgard archaea to gain insight into which interactions have been maintained or evolved during eukaryogenesis.

α/β-tubulins polymerize to form eukaryotic microtubules, and understanding their emergence is a critical question in eukaryogenesis. In this work, we have elucidated the structure and dynamics of Asgard tubulin polymerization from hydrothermal-living Odinarchaeota (OdinTubulin) in order to understand the evolution of eukaryotic microtubules. Determination of the X-ray structures of OdinTubulin in the apo form and bound to GTP or GDP, together with sequence analysis, confirmed that OdinTubulin has diverged significantly from prokaryotic homologs FtsZ and CetZ, and branches in the same clade as eukaryotic tubulins in phylogenetic trees. GTP-bound OdinTubulin, refined at 1.62 Å, is most similar to α- and β-tubulins within a microtubule protofilament. OdinTubulin has a nucleotide sensor motif, which lies in the intermediate domain, connecting the nucleotide from the lower subunit (-) to the nucleotide in the upper subunit (+) through a bonding network of cations and water molecules. The GDP-bound structure revealed that the phosphate ion is released following hydrolysis, and is replaced by three water molecules. Time course crystallography of GTP-soaked crystals, combined with electron microscopy of the OdinTubulin filaments, indicated conformation changes throughout a protofilament resulting from GTP hydrolysis to GDP. OdinTubulin polymerized optimally in the presence of GTP and Mg^{2+} and formed tubules at high temperature, consistent with the temperature of the Yellowstone Lower Culex Basin hot spring where its metagenome was sampled. The OdinTubulin tubules are constructed from 2-5 layers of short discontinuous

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curved protofilaments that spiral around the wall of tubule, more similar to FtsZ, rather than running parallel to its length as in microtubules. Thus, this study proposes that tubulin originated in archaea and the switch to microtubules filament morphology was an important adaption during eukaryogenesis.

A primitive regulated actin cytoskeleton has been demonstrated to exist at the protein level in Asgard archaea, including homologs of actin, and its regulators, profilin and cofilin/gelsolin, which control the assembly and disassembly of actin filaments, respectively. However, functional gelsolins have only shown for one phylum, Thorarchaeota (Thor). Thus, in the second part of this thesis we investigated the evolution of regulated actin dynamics by characterization of two domain gelsolins (2DGels) from Heimdallarchaeota (Heim) and Lokiarchaeota (Loki). Sequence analysis revealed that Heim2DGels and Loki2DGels have C-terminal extensions which are distinct between Loki and Heim phyla and show significant variability within each phylum. In the structures, Loki2DGel sequesters two subunits of G-actin and Heim2DGel a single G-actin. Domain 1 of Heiml2DGel binds in the classic site between actin subdomains 1 and 3, similar to Loki2DGel and human gelsolin. Comparison of the calcium-binding sites between Loki- and Heim2DGels reveals the additional calcium-binding sites, beyond the Type I and II sites, are different in the two structures. Loki2DGel, but not Heim or Thor, has an unusual WH2-like motif (LVDV) between the gelsolin domains, in which the aspartic acid coordinates a calcium ion at the interface with actin. *In vitro* activities of Asgard 2DGels on actin dynamics show that Loki and Heim 2DGels can sever, cap actin filaments, and sequester actin monomers under calcium control. Furthermore, expression of Asgard 2DGels in the U2OS human cell line disrupted actin filaments in response to calcium release. In summary, these data indicate that the last Asgard archaea common ancestor likely possessed a calcium-regulated 2DGel actin-filament depolymerization system, and that the emergence of calcium regulation of actin dynamics predates eukaryogenesis. However, calcium regulation of actin dynamics has continued to evolve since the Asgard archaea and eukaryotes last common ancestor.

Characterization of the potential membrane-trafficking components encoded by Asgard archaea genes is critical to understand whether Asgard archaea organisms are capable of generating eukaryotic membrane fusion. In particular, the Rab-GTPase superfamily plays an important role in intramembrane traffic in eukaryotes, which includes vesicle formation and fusion. The dimerized roadblock and longin domain proteins are involved in the regulation of Rab-GTPases, in the transport protein particle (TRAPP) complex and the regulator complex in eukaryotes. The TRAPP complex also includes subunits unique to this complex, such as TRAPPC3. In this section, we determined the X-ray structures of Rab GTPase, roadblock and TRAPPC3 proteins from Thor. Superimposition of these structures onto the eukaryotic TRAPP and regulator complexes structures indicate that Thor has many of the basic building blocks that are found in these complex eukaryotic membrane-regulating machines. In particular, we show that the Thor Rab is a close structural homolog of eukaryotic Rab1, the Asgard roadblock proteins adopt long and short versions as seen in the human regulator complex, and that the Thor TRAPPC3-like protein is and intermediate between a 4-vinyl reductase (V4R) domain and the TRAPPC3 protein found in the TRAPP complex. We conclude that Asgard archaea have many of the components of Rab-directed membrane targeting complexes.

Taken together, this dissertation has produced experimental data to address the potential origins of the eukaryotic cell. We find evidence that the emergence of the calcium-controlled actin regulation predated eukaryogenesis. We predict that microtubules are younger than the actin cytoskeleton, emerging during eukaryogenesis. Finally, we find structural evidence that Asgard archaea, and the eukaryotic common ancestor, will have complex membrane regulating systems. Thus, we provide further support to the idea that Asgard archaea and eukaryotes share a common ancestor, and that the cytoskeleton and Rab-directed membrane regulation were likely important drivers in eukaryogenesis.