【Grant-in-Aid for Scientific Research (S)】

Understanding the origin of eukaryotes through marine benthic archaea

Principal Investigator	Japan Agency for Marine-Earth Science and Technology, Institute for Extra-cutting-edge Science and Technology Avant-garde Research (Super-cutting-edge Grand and Advanced Research Program), Principal Researcher IMACHI Hiroyuki Researcher Number:20361933
Project Information	Project Number : 22H04985 Project Period (FY) : 2022-2026 Keywords : archaea, the origin of eukaryotes, anaerobes, syntrophy

Purpose and Background of the Research

●Outline of the Research

Eukaryotes (complex organisms including animals, plants, fungi, and amoeba) are thought to have evolved from *Prokaryotes* (structurally simple and unicellular organisms), yet, “how” this took place remains a major mystery. Prokaryotes can be divided into *Archaea* and *Bacteria*. Current data suggest that the first eukaryotic cell emerged through an “archaeon” engulfing a bacterium and fusing into a single biological entity. The engulfed bacterium is today’s mitochondrion. On the other hand, although the host archaeon is thought to have inhabited the seafloor, the appearance, way of life, and evolutionary path from archaea to eukaryotes remain unclear. Under these circumstances, we have been trying to culture archaeal species from seafloor environments for many years to elucidate them (KAKENHI grants 18687006, 21687006 & 24687011). Through 12 years of strategic experimentation, we finally captured the first archaeon closely related to the eukaryote ancestor, named strain MK-D1 (Imachi et al., 2020. Nature; KAKENHI grants 15H02419 & 19H01005).
We found that MK-D1 has unique features that are not found in any other prokaryotes, including that its genome encodes a repertoire of proteins that are only found in eukaryotes, and that it changes its cell morphology while proliferating slowly like eukaryotes. Based on these features, we hypothesize that the host archaeon (i.e., ancestor of us eukaryotes) acquired these features to survive in the extreme environment of the oligotrophic seafloor, and the features unintentionally formed the basis for its evolution into eukaryotes. To answer this hypothesis, we will conduct a polyphasic study combining culture experiments, microscopic observations, omics analysis, and chemical analysis to describe our ancestral archaeon and to understand why our complex eukaryotes came into being. In particular, this study aims to elucidate the evolutionary process surrounding "the genes, cell morphology, and way of life of our ancestral archaeon”, "how it took in other bacterium”, and "the path to cellular complexity”.

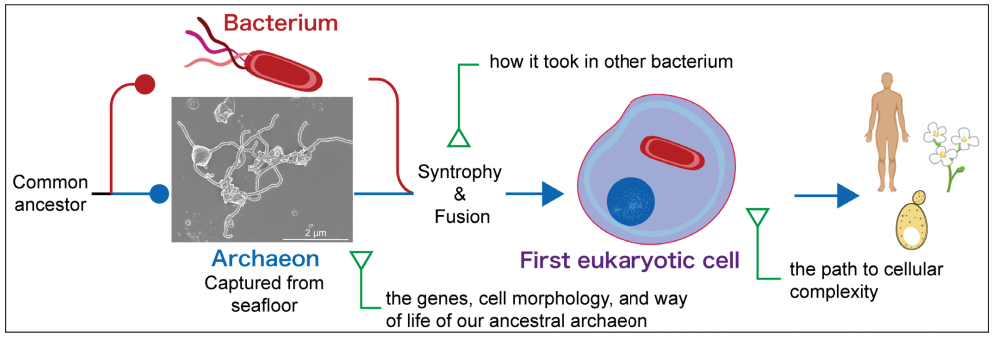

Figure 1. Overall image of this research project

●Hypothesis led by the features of MK-D1

MK-D1 grows slowly like eukaryotes, extends long protrusions outside the cells, and releases many membrane vesicles (Fig. 2). Normally, prokaryotes devote most of their energy to proliferation and successive generations and do not build complex structures on the outside of the cell. On the other hand, eukaryotes use their energy more for maintenance and building complex external organs than for proliferation. In other words, MK-D1 is like a eukaryote in how it slowly proliferates and turns much of its energy into maintaining the cell and building external structures (i.e., protrusions and membrane vesicles) by processing intracellular components. The archaeal ancestor of eukaryotes might have similar features to MK-D1.

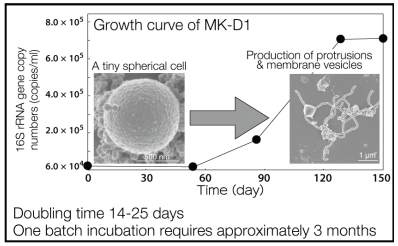

Figure 2. Growth curve and cell morphological change of MK-D1

Expected Research Achievements

This study consists of the following four research topics. Finally, based on the results obtained from the topics, we will describe our ancestral archaeon and propose a new model for the origin of eukaryotes.

●Elucidation of the mechanisms of cell maintenance and processing by MK-D1

We aim to elucidate the mechanisms of when and how MK-D1 acquires and consumes nutrients, and how it maintains cells and processes cellular components (i.e., protrusion formation and membrane vesicles production). For this purpose, MK-D1 will be cultured under various conditions, and morphological and metabolic changes will be traced by combining analysis of nutrients in the culture medium, microscopic observation, and omics analysis. In parallel with these culture experiments, we will also elucidate the components and functions of protrusions and membrane vesicles. Based on morphological similarities and proteomic analysis, we speculate that the protrusions are formed by actin filaments. Therefore, we will perform immunostaining targeting actin filaments to clarify their localization in MK-D1 cells. For membrane vesicles, we will clarify their contents and the amount released.

●Elucidation of the effects of oxygen

The accumulation of oxygen triggered the evolution from archaea to eukaryotes. During this evolution, the archaeal ancestor had to abandon its oxygen-intolerant function. To infer the metabolisms discarded by the archaeal ancestor, we will perform comparative genomics on archaea. In addition, we will estimate the timing of the intracellular symbiosis switch by conducting culture experiments to determine the oxygen tolerance capacity of MK-D1.

●Analysis of genomic information on diverse archaea

The genomes of diverse archaea related to the origin of eukaryotes are acquired from various seafloor samples by metagenomic analysis. The acquired genome information will be subjected to comparative genome analysis and gene evolution analysis to reconstruct the archaeal ancestor's genome to infer the genes and way of life.

●Cultivation of diverse archaea related to the origin of eukaryotes

We aim to obtain cultures of new archaea related to the origin of eukaryotes and elucidate their properties. Cultured archaea will be studied for the above-mentioned cell maintenance mechanisms and the effects of oxygen.

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