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From the Common Ancestor of all Living Organisms to Protoeukaryotic Cell

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20.1 Introduction

Figure 20.1 shows the phylogenetic tree constructed from the small subunit rRNA genes of living organisms reported by Woese *et al.* (1990), in which the root was placed on the basis of the results presented by Gogarten *et al.* (1989) and Iwabe *et al.* (1989). The species, which is a group of living organisms with sufficient gene interaction to constitute a single gene-pool, has divided into two species at the point Commonote. The species can be called the last and the latest common ancestor of all the living organisms on the earth. We have suggested that the last common ancestor must have already had a rigid genetic system with circular chromosomal DNA, based on the circularity of chromosomal DNAs of eubacteria and archaebacteria (Kondo *et al.*, 1993; Yamagishi and Oshima, 1995). In the first half of the chapter we will discuss the characteristics of the last common ancestor.

The Archaeal line splits into Archaebacteria and Urkaryotes, which is defined by Woese *et al.* (1977) as occurring at the point P. Urkaryotes represent the genes or genetic components of eukaryotes. Eukaryotic species diverge after the point P′. The process from protoarchaebacteria (P) to protoeukaryotes (P′) will be discussed in the second half of the chapter.

20.2 The last common ancestor commonote

Based on the tree topology of Figure 20.1 and parsimony, it is possible to speculate on the characteristics of the last common ancestor. Organisms on both branches of the tree have the same genetic system: the chromosome is replicated by DNA polymerase, transcribed by RNA polymerase, translated by machinery consisting of ribosome, tRNAs, and other factors, using universal codons. These characteristics are likely to have originated from the common ancestor unless these systems have been developed independently.

Based on the principle of parsimony, the characteristics shared by eubacteria and one of the other groups, archaebacteria or eukaryotes, is likely to be that of the common

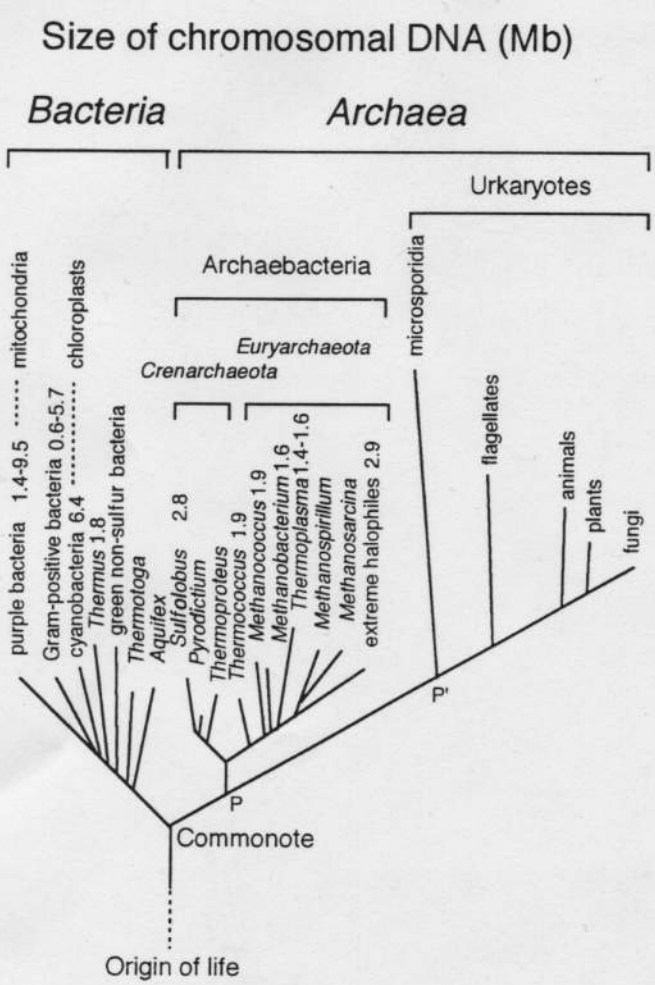

Figure 20.1 Size of chromosomal DNA of microorganisms. The general phylogenetic tree was constructed based on Woese *et al.* (1990) and classified according to Yamagishi and Oshima (1995). P and P′ represent protoarchaebacteria and protoeukaryotes. Data were collected from a review by Krawiec and Riley (1995) and other references (Charlebois *et al.*, 1991; Sitzmann and Klein, 1991; Stetter and Leisinger, 1992; Cohen *et al.*, 1992; Borges and Bergquist, 1993; Kondo *et al.*, 1993; Lopez-Garcia *et al.*, 1992; Tabata *et al.*, 1993).

ancestor. Accordingly, the common ancestor seems to have been an organism of size about 1 μm surrounded by a single lipid membrane. The last common ancestor seems to have had eukaryotic α-type DNA polymerase, because these are found also in archaebacterial and eubacterial branches (Ito and Braithwaite, 1991; Iwasaki *et al.*, 1991; Pisani *et al.*, 1992; Forterre, 1992; Uemori *et al.*, 1993).

Figure 20.1 also summarises the size of chromosomal DNAs of microorganisms. The size varies from several hundreds of kilobases (kb) to several megabases (Mb). The range is rather similar between eubacteria and archaebacteria. It is noted that organisms that branched at points close to the root tend to have genome size of 1.5–2.0 Mb. This size may be that of the chromosome of the common ancestor. These characteristics suggest

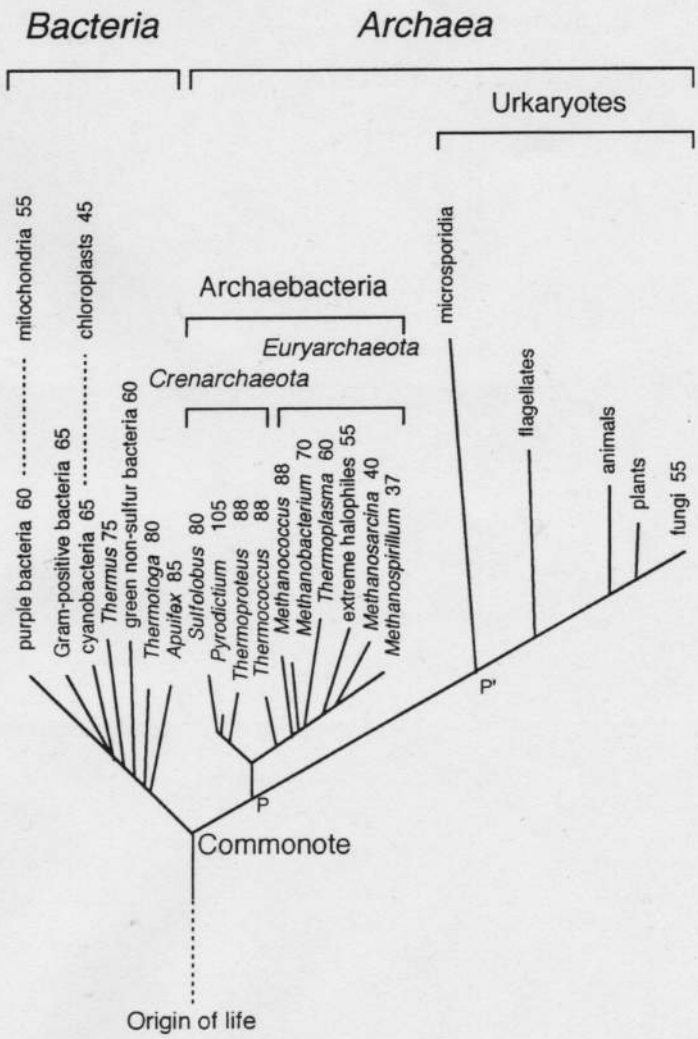

Figure 20.2 Optimum growth temperatures of microorganisms. The optimum growth temperature of each species or the optimum temperature of the most thermophilic species in each group is shown. The data are collected from the work of Brock (1978), *Bergey's Manual of Systematic Bacteriology* (1989) and other references (Huber *et al.*, 1986; Huber *et al.*, 1992). Only data obtained from laboratory culture experiments are included. The general phylogenetic tree was constructed based on Woese *et al.* (1990) and classified according to Yamagishi and Oshima (1995). P and P′ represent protoarchaebacteria and protoeukaryotes, respectively.

that the last common ancestor commonote was a organism very similar to the contemporary eubacteria and archaebacteria.

Figure 20.2 summarises the optimum growth temperatures of microorganisms. The antiquity of thermophilic microorganisms has been proposed by several authors (Pace *et al.*, 1986; Achenbach-Richter *et al.*, 1987; Pace, 1991; Burggraf *et al.*, 1992). The proposal was based on the findings that the many eubacterial groups contain thermophilic microorganisms, and the ultrathermophilic microorganisms can be found at a position close to the root in the general phylogenetic tree.

However, Gogarten-Boekels *et al.* (1995) have discussed the possible effects of heavy meteorite bombardment on the early evolution of life on the earth. Gogarten-Boekels *et al.* (1995) and Forterre (1996) discussed the possibility that the selection of thermophilic phenotype can explain the distribution of thermophilic organisms in the general philogenetic tree. Thus the common ancestor need not necessarily have been an ultrathermophile. Nevertheless, these authors also agree that the ancestors of life on the earth were thermophilic at some stage in the early history of the evolution of life. Figure 20.2 gives further support to the antiquity of thermophilic microorganisms on the earth. An organism close to the root of the tree tends to have higher growth temperature. An organism that branched far from the root tends to have lower optimum growth temperature. This result can be interpreted as meaning that the organism with lower optimum growth temperature branched when the ambient temperature had decreased to that temperature. The temperature at which the protoeukaryotic organism separated from the protoarchaebacteria may be 50–60 °C: eukaryotes can grow at the highest temperature. It is also likely that both the ancestors of eubacteria and of archaebacteria were ultrathermophilic microorganisms.

Figures 20.1 and 20.2 also show the principal classification of the living organisms. We have proposed including archaebacteria and eukaryotes (Urkaryotes) in the same taxon Archaea (Yamagishi and Oshima, 1995). The taxon Archaea thus defined is monophyletic whatever the branching within the group. This proposition is based on the fact that the most important branching point of the living organisms on the earth is point C in Figures 20.1 and 20.2. Accordingly, we think it appropriate to make a principal division at this point. We also think it appropriate to retain the common name archaebacteria for the group consisting of Crenarchaeota and Euryarchaeota. We have previously proposed to handle the eukaryotes as the composition or chimera of prokaryotes (Yamagishi and Oshima, 1995).

20.3 The process from the protoarchaebacteria to protoeukaryotic cells

Figure 20.1 suggest that the ancestor of the eukaryotes separated from the ancestor of archaebacteria somewhere near the point P. If there is a distinct line between the point P and the branching point of Euryarchaeota and Crenarchaeota, then the protoeukaryotes evolved from the protoarchaebacteria. Accordingly, there is no special archaebacterial species which is especially closely related to the protoeukaryotes as a whole. Nevertheless, it is still possible that some of the contemporary archaebacteria retain some of the characteristics of the protoarchaebacteria.

There are large differences between eukaryotic cells and archaebacterial cells. The largest difference is the presence of nucleus and other organelles such as mitochondria and chloroplasts in eukaryotic cells. The endosymbiotic origin of mitochondria and chloroplasts is well established. The mitochondrion originated from a microorganism related to proteobacteria (Yang *et al.*, 1985). The chloroplast originated from cyanobacteria (Palenik and Heselkon, 1992).

In addition to the symbiotic nature of eukaryotic cells, there are several other differences in characteristics between archaebacteria and eukaryotes. The sizes of the cells are different: eukaryotic cells are about ten times larger in diameter than prokaryotic cells. The genome size of eukaryotes is also much larger than that of prokaryotic cells in general. There are also extremely complex membrane systems in eukaryotic cells: endoplasmic reticulum, lysosome, vacuole, Golgi body, etc.

20.3.1 *The structure of macrocells of* Thermoplasma

We have analysed some characteristics of the cell wall-free archaebacterium *Thermoplasma acidophilum*. *Thermoplasma* is an acidothermophilic archaebacterium with optimum growth temperature of 50–60 °C. The archaebacterium was found by Darland *et al.* (1970) and was analysed extensively by Searcy and his colleagues (Searcy *et al.*, 1981; Hixon and Searcy, 1993). This archaebacterium is proposed to be the candidate for the archaebacterium which derived the nuclear and cytoplasmic moiety of eukaryotic cells (Searcy *et al.*, 1981; Margulis, 1993).

We have isolated several new strains of *Thermoplasma* from hot springs near Tokyo (Yasuda *et al.*, 1995) and have analysed microbiological characteristics of the strains. The characteristics are indistinguishable from those of *Thermoplasma acidophilum* (Yasuda *et al.*, 1995). The cells of the type strain are irregular (Hixon and Searcy, 1993). We have analysed the cellular structure by scanning electron microscopy (SEM). Strain HO-121 showed irregular cell shape similar to that reported for the type strain of *Thermoplasma acidophilum*, although one of the strains, HO-51, showed spherical cells with smooth surface. Another strain, HO-12, had a spherical cell surface with knobs.

The cells of these strains formed cotton-like aggregates which could be seen with the naked eye after the prolonged incubation of the culture, and the structure of this cotton-like aggregate was investigated by SEM. Individual spherical cells were recognisable in the aggregate of the cells of strain HO-51; the structure is shown schematically in Figure 20.3A. The aggregated cells of strain HO-54 showed significantly different structure; the cells are connected each other to form a continuous lamellar structure and individual cells could not be distinguished. The structure is shown schematically in Figure 20.3B. It appeared that strain HO-54 formed multinucleate cells.

The lamellar structure may be the form of the intermediate stage between the proto-archaebacteria and protoeukaryotic cells. Figures 20.3 A and B show the cellular structures we have observed in the *Thermoplasma* strains HO-51 and HO-54, respectively. If we assume that the extralamellar structure is decreased in volume, and the cytoplasmic compartment of the lamellar structure is increased in volume, the expected structure can be represented as shown in Figure 20.3C. The extralamellar structure is connected to the outside and the topology is the same as the inside of the endoplasmic reticulum of eukaryotic cells. Thus the continuous transition from Figure 20.3A through 20.3B to 20.3C can explain the process from protoarchaebacteria to protoeukaryotic cells. The increase in cell volume and the genome size and formation of endoplasmic reticulum can be explained by this process.

The model presented here does not deny the endosymbiotic theory of the origin of organelles surrounded by double membranes, such as mitochondria and chloroplasts in eukaryotic cells. Instead, the larger size of the protoeukaryotic cell rather enables the endosymbiosis of other prokaryotic microorganisms. The model also explains the origin of the single-membrane-bounded structures in eukaryotic cells, i.e. vacuole, lysosome, Golgi body, etc., as well as endoplasmic reticulum. These single-membrane-bounded structures may originate from cytoplasmic membrane of multinucleate cells of protoarchaebacteria.

Increase in the size of a cell provides space for larger chromosomal DNA. Multinucleate cells contain many copies of genomic DNA. The increase in genome size is initially just that of the total number of copies. Multiple copying of the genomic DNA is expected to accelerate mutation of genes. Although a gene may lose its original function through mutations, other copies of the gene can support the function if it is necessary. Gene duplications are often found in many organisms and they are considered to play a role in the creation

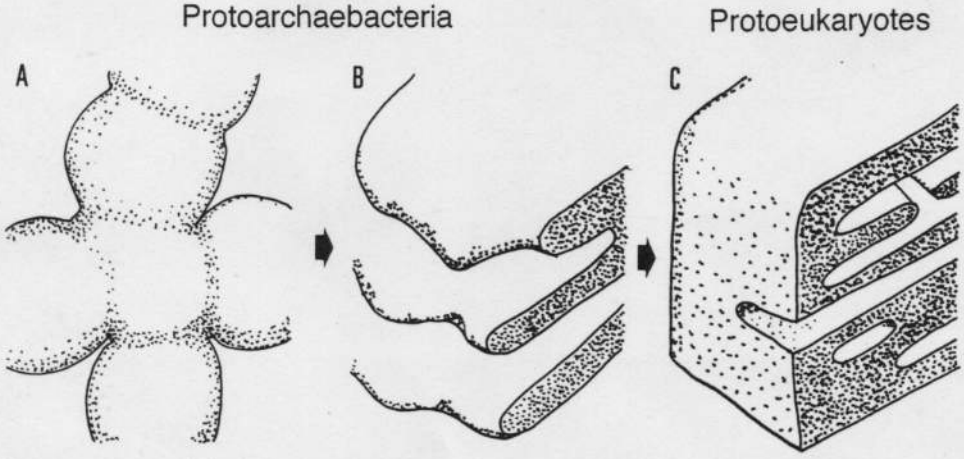

Figure 20.3 Model of the process from the protoarchaebacteria to protoeukaryotes. (A) and (B) represent the structures of cells observed in strains of *Thermoplasma*. Expansion of the cytoplasmic space of the structure from (B) to (C) can explain the formation of protoeukaryotic cells. The process can explain the increase in size of the cells and in genome size, and the origin of endoplasmic reticulum and other single-membrane-surrounded structures in eukaryotic cells.

of new functional genes. Multiple copies of chromosomal DNA must facilitate the creation of new genes.

In general, cells have to incorporate substrates from outside to inside and export waste matter from inside to outside. Such transportation becomes difficult in larger cells. The multinucleate cells found in the strain HO-54 retained the extracellular space as the channel system through the cytoplasmic space. These channel system must support the transport of compounds between internal cytoplasmic space and the external medium.

20.3.2 *Cytoskeleton in* Thermoplasma *cells*

The significant differences in the cellular structures of the cells of different strains of *Thermoplasma* suggest the presence of mechanical structure to maintain the respective shape of the cells. Because there is no cell wall around the cell membrane, there must be some structural component which supports the membrane from the cytoplasmic space. Cells of strain HO-51 were treated with Triton X-100, DNAase and RNAase. The solution was centrifuged and the pellet was investigated by atomic force microscopy (AFM). AFM revealed membrane structure of size about 1 μm (S. Kasas, K. Ito, G. Takhashi, A. Ikai, T. Oshima and A. Yamagishi, unpublished). The membrane structure is overlapped with mesh-like structure. The average hole size of the mesh was 30 nm × 18 nm, and the average height of the mesh was 1.6 nm. It is not clear whether the mesh is flat and attached to the back side of the cytoplasmic membrane in the cell. Alternatively, the mesh-like structure may be formed by flattening of three-dimensional mesh or network during the drying process of preparation of the structure for AFM observation.

Nevertheless, the structure was retained after treatment with neutral detergent Triton X-100 and recovered by centrifugation. The mesh portion resisted the pressure of the tip of an atomic force microscope during observation, suggesting the mechanical strength of the mesh-like structure. The structure seems to represent cytoskeleton in *Thermoplasma*

cells. Hixon and Searcy (1993) reported the formation of mesh-like structure from the cell extract of *Thermoplasma* in the presence of Ca^{2+} and ATP. The relation between the structure reported by Hixon and Searcy (1993) and the mesh-like structure observed in Triton-treated cell in our experiments is not yet clear.

20.3.3 *Tetraether lipid biosynthesis in* Thermoplasma

The membrane lipids in archaebacteria are different from those in eubacteria and eukaryotes. Cytoplasmic membrane of *Thermoplasma* consists of tetraether lipids with some diether lipids. The ether lipid biosynthetic pathway is essentially the same as that of isoprenoid biosynthesis. Tetraether lipids have a structure which is made by connecting the adjacent diether lipid molecules at the heads of their isopranyl alcohol moiety.

We have analysed the effect of squalene epoxidase inhibitors on tetraether lipid biosynthesis (T. Kon, A. Yamagishi and T. Oshima, unpublished). The squalene epoxidase inhibitor terbinafine inhibited the growth of *Thermoplasma*, while the growth of *Escherichia coli* and *Halobacterium* was not inhibited by the inhibitor.

We have analysed the effect of terbinafine on the biosynthesis of tetraether lipids in *Thermoplasma*. *Thermoplasma* cells were cultured with [^{13}C]mevalonic acid. The cells were harvested and lipids were extracted from the cells. The lipids were analysed by thin-layer chromatography (TLC) after acid methanolysis. Significant incorporation of ^{13}C was observed in the tetraether lipid fraction on TLC. Terbinafine significantly affected the ether lipid biosynthesis. The radioactivity recovered in the tetraether lipid fraction was reduced to about 10% of the control by the addition of 0.1 mg/ml terbinafine. The radioactivity of the diether lipid fraction was increased simultaneously. [^{13}C]Mevalonic acid and the inhibitor were removed by centrifugation and the cell pellet was resuspended in fresh culture medium. After incubation of the cells in the fresh medium, cells were harvested and lipids were analysed. The radioactivity in the diether fraction was decreased and that of tetraether fraction was increased. These results clearly indicate that the tetraether lipids are synthesised from the diether lipids. These results also indicate that the biosynthesis from diether lipids to tetraether lipids is inhibited by terbinafine.

It is interesting to note that terbinafine is a specific inhibitor of squalene epoxidase, which catalyses the reaction in the biosynthesis of steroids in eukaryotes. These results suggest that the structure of the enzyme catalysing the tetraether lipid biosynthesis and that of the squalene epoxidase may be similar at least at the site of the inhibition by terbinafine. It is also important to note that the biosynthesis of squalene in eukaryotic cells is localised in microsomes. The location is compatible with the scheme presented in Figure 20.3: the endoplasmic reticulum of eukaryotic cells originated from the cytoplasmic membrane of protoarchaebacteria.

20.4 Conclusions

Although the question whether the common ancestor commonote was thermophilic or not is still a matter of debate, it is likely that the ancestors both of eubacteria and archaebacteria were ultrathermophilic microorganisms.

The multinucleate lamellar structure of macrocells was found in a strain of the acidothermophilic cell wall-less archaebacterium *Thermoplasma*. We propose that the formation of multinucleate cells of protoarchaebacteria participated in the process of forming

the protoeukaryotic cells before the endosymbiosis of mitochondria and chloroplasts. The multinucleate cells must have contributed to the increase in cell size and genome size, and to the formation of single-membrane-surrounded systems such as endoplasmic reticulum and vacuoles in eukaryotic cells.

20.5 Summary

The early history of the evolution of living organisms on the earth is discussed based on the analysis of thermophilic archaebacteria. We propose that the formation of multinucleate macrocells is the important step in the process from protoarchaebacteria to protoeukaryotic cells. The increase in both cell volume and size of genome and formation of a single membrane surrounding intracellular structures such as endoplasmic reticulum in eukaryotic cells can be explained by the formation of multinucleate cells with lamellar structure.

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