We need to know
We will know

Inscription on the tombstone of David Hilbert, mathematician

Chapter 3
My name is LUCA

I beg your pardon, but isn't that the beginning of a song about "the girl who lives upstairs?"

I don't mean that Luca. Here LUCA stands for the Last Universal Common Ancestor, the living organism that was the mother of all organisms on Earth.

Who was LUCA?

Before this question can be discussed, we need to know if the definition of a species as we know it for animals and plants can also be applied to bacteria.

It was Mrs. Fanny Angelina Hesse whose recommendation made it possible to solidify growth media for bacteria. She suggested using the gelatinizing substance agar (also called agar agar), which was introduced in Robert Koch's laboratory in 1884. The use of agar allowed bacteria to grow on the surface of growth medium, like on the surface of pudding or a slice of bread. On such a surface, clusters of bacteria originating from one cell are formed. These are called colonies. When a tiny portion of a colony of *Escherichia coli* is transferred to fresh agar, new colonies of cells of *E. coli* are recovered (Figure 4). It could then be concluded that the definition of a species could also apply to bacteria. Therefore, elephants arise from elephants, oaks from oaks; *E. coli* yields *E. coli*, and *Staphylococcus aureus* yields *Staphylococcus aureus* and not a bacterium with completely different properties.

In the past 120 years, thousands of bacterial species have been isolated and their properties have been described. However, for many years their evolutionary (phylogenetic) relatedness remained unknown. Of course, efforts were made in numerous laboratories to learn something about this relationship. It was necessary to identify bacterial species on the basis of their properties and to develop strategies to control those causing disease. The actual breakthrough, however, occurred just a quarter of a century ago.

Discover the World of Microbes: Bacteria, Archaea, and Viruses, First Edition. Gerhard Gottschalk.

Figure 4 Colonies of a bacterium on agar growth medium in a Petri dish. When a minute amount of cells is streaked out (starting at 9 o'clock on the dish), cells are so separated that round colonies can develop from single cells. Such a cell community then represents a clone because it originates from one cell. Depending on its size, a colony may contain between 50 and 500 million cells. (Photograph: Anne Kemmling, Goettingen, Germany.)

I have a question regarding Mrs. Hesse. What is agar agar and how did she get the idea to use it to solidify growth media for bacteria?

Fanny Eilshemius was born in 1850 in Laurel Hill, New Jersey (USA), the daughter of German immigrants. While traveling through Europe, she met a medical doctor, Walter Hesse, whom she married. Walter Hesse had a strong interest in bacteriology. He grew bacteria on gelatin surfaces and was very disappointed that the gelatin melted so easily. Fanny remembered a recipe she had from Dutch friends who had lived on the island of Java. They used agar agar, a polymer extracted from algae to solidify deserts. Agar is ideal because hot solutions containing 2 percent agar solidify at about 50 °C and they only melt again upon boiling. In addition, most bacteria do not degrade agar, so the introduction of agar provoked a revolution in microbiological laboratories. Bacteria could then conveniently be grown on agar surfaces in Petri dishes and the properties of pure cultures, those containing only one species, could be studied.

What about the breakthrough you mentioned?

In the 1960s a procedure for sequencing DNA fragments was worked out by the British molecular biologist Frederick Sanger. He developed an elegant method to shorten DNA fragments base by base and to determine whether the last base in the fragment is T, C, G, or A (see Chapter 31 for details). This method was so

ingenious that Frederick Sanger was awarded the Nobel Prize (together with Walter Gilbert and Paul Berg), his second one – see Chapter 28 for the first one. Progress was enormous, and it is no exaggeration to say that now more than a billion base sequences are determined every day using this and more advanced methods. At the University of Illinois in Urbana, the microbiologist Carl Woese adopted Sanger's method to learn something about the phylogenetic relationship of bacterial species. He chose the 16S-rRNA to be sequenced. This RNA is present in all bacteria because it is essential for the formation of the ribosomal protein synthesis factories. A milestone in this research was reached in 1977: Carl Woese and his coworkers published their research paper on the "molecular approach to prokaryotic systematics." The relatedness between bacterial species was determined on the basis of differences in the sequence of 16S-rRNA. At this point, a sensational experiment appeared on the horizon. Ralph S. Wolfe, another eminent microbiologist, was working next to the laboratory of Carl R. Woese. He did pioneering research on methane-producing microorganisms, especially on the biochemistry of the pathways leading to the production of methane. Of course, Ralph Wolfe and Carl Woese talked about their work. They decided to collaborate, but let's have Ralph Wolfe (Champaign-Urbana, Illinois, USA) tell us about the results of their experiments:

> "Early in his career, Woese had studied the ribosome and was convinced that this organelle was of very ancient origin, that it had the same function in all cells, and that variations in the nucleotide sequence in an RNA of the ribosome could reveal evidence of very ancient events in evolution. He chose the 16S-rRNA and developed a similarity coefficient that could be used to compare the relatedness of two different organisms. By 1976 he had documented the 16S-rRNAs of 60 bacteria.
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> The research program of Ralph Wolfe concerned the biochemistry of methane formation by methanogenic bacteria, an area poorly studied because of the difficulties of cultivating the organisms. Techniques were developed for culture of cells in a pressurized atmosphere of hydrogen and carbon dioxide. By 1976 the structure of two unusual coenzymes, coenzyme M and Factor 420 (a unique deazaflavin), and their enzymology had been elucidated.
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> The conjunction occurred with an experiment designed to examine the 16S-rRNA of methanogens. The pressurized atmosphere technique proved ideal for containing the high level of injected radioactive phosphorus to label the rRNA of growing cells. The two-dimensional chromatograms of the labelled 16S-rRNA oligonucleotides from the first experiment were so different from anything previously seen, that Woese could only conclude that somehow the wrong RNA had been isolated. The experiment was carefully repeated, and this time with the same results: Woese declared, "Wolfe, these organisms are not bacteria!" "Of course they are, Carl; they look like bacteria." "They are not related to anything I've seen." This experiment marked the birth of the archaea!"

This is an important contemporary testimonial. It led to the conclusion that a third form of life exists on our planet, the archaea, in addition to the eukaryotes (animals, plants, fungi) and the bacteria. Ralph Wolfe describes why his colleague Carl Woese chose 16S-rRNA, then he describes his own research during which the techniques for growing large amounts of methane-producing microorganisms were developed. These microorganisms were used for biochemical investigations that led to the discovery of novel "methano-vitamins" such as coenzyme M or factor 420.

The studies in Woese's lab involved growing the methanogenic organisms in the presence of radioactive phosphate. The 16S-rRNA then contained radioactivity because of the phosphate bridges present in the molecule. Fragments were isolated, sequenced, and compared with the 16S-rRNAs of *E. coli* and other organisms. When they compared the sequences obtained, differences were encountered that could not be explained. Both Carl Woese and Ralph Wolfe were speechless. The sequences of all the bacterial species studied before were written in essentially the same language and contained a number of deviations, which gave insight into the distance between two bacterial species in the phylogenetic tree. But what the researchers now discovered was that parts of the text were deleted and parts were written in another language, say in Hebrew. This was something unique, and this also proved to be the case when the 16S-rRNA of other methanogenic organisms was sequenced.

Could you please explain a bit more about these differences in the 16S-rRNAs?

Let us imagine a large mosaic, for instance, the triumphal march of Dionysos in one of the Roman houses in Paphos (Cyprus) discovered in 1962. Like 16S-rRNA, mosaics also consist of thousands of building blocks. If we change something in the border surrounding the scene, this will have little effect on the general impression of the mosaic. It is the same with the sequence of the 16S-rRNA of bacteria. Trends as well as a few variations in the sequences can be recognized, making it possible to determine the relatedness of the organisms from which the 16S-rRNAs were isolated. However, if Dionysos were to be replaced in the mosaic by a mythological priest, then the number and color of the mosaic tiles would be quite different. It is impossible to derive one figure from the other, so a common ancestor must be postulated that gave rise to Dionysos, on the one hand, and to the priest, on the other – an ancestor such as LUCA.

Let's now look at the sequences of the 16S-rRNAs of *Escherichia coli* (1542 bases long) and *Bacillus licheniformis* (1548 bases) as representatives of the bacteria and of *Methanosarcina mazei* (1474 bases), *Archaeoglobus fulgidus* (1492 bases), and *Methanosphaera stadtmanae* (1480 bases), representing the archaea. The alignment of these sequences results in a beautiful picture (Figure 5), which was done with the aid of a computer program that searches for maximum correspondence of the sequences. In order to attain this sequence homology, the computer takes sequences apart and introduces gaps. Several archaeal "gaps" are apparent, including some large ones, but there are also a few bacterial gaps. Identical sequences

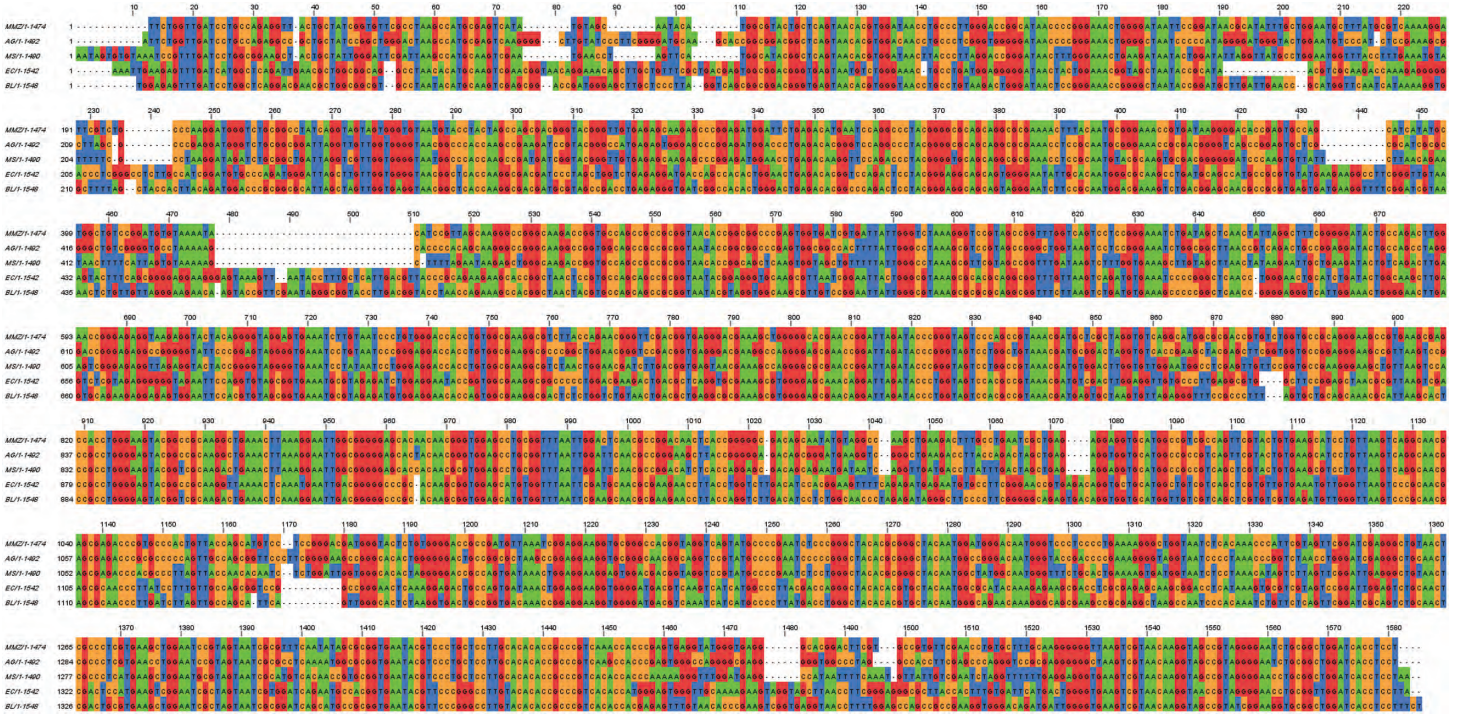

Figure 5 Base sequence of the 16S rRNAs of three archaea (*Methanosarcina mazei, Archaeoglobus fulgidus,* and *Methanosphaera stadtmanae)* and two bacteria (*Escherichia coli* and *Bacillus licheniformis*). The depiction is based on a ClustalW-alignment under standard conditions (W.A. Larkin *et al.*, Bioinformatics 23, 2947, 2007). Visualization was done with Jaliew using the standard color code for nucleotides. (C. Clamp *et al.*, Bioinformatics 20, 416, 2004). Gaps were inserted into the sequences to achieve maximal agreement. (Adaptation: Antje Wollherr, Goettingen, Germany.)

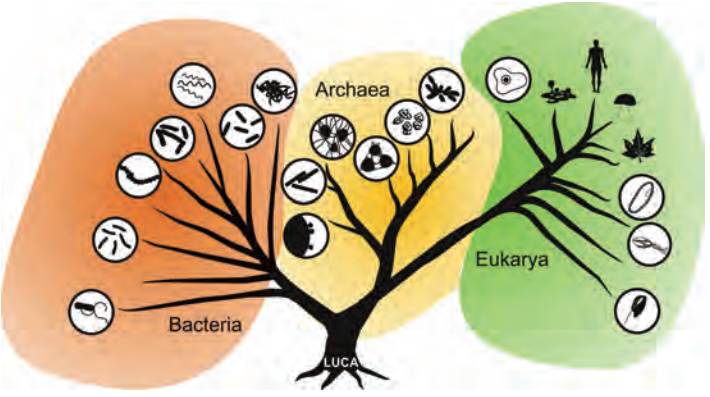

Figure 6 Phylogenetic tree of the three domains of all living organisms, depicted clockwise from left to right: Bacteria: *Aquifex*, *Bacteroides*, cyanobacteria, proteobacteria, Spirochaeta, Bacilli, green filamentous bacteria. Archaea: *Nanoarchaeum equitans* (small dots) *attached to Ignicoccus hospitalis*, *Thermoproteus, Pyrodictium, Methanococcus*, *Methanosarcina*, halophilic archaea. Eukarya: *Entamoeba*, mucilaginous fungi, humans, fungi, plants, ciliates, trichomonads, diplomonads. LUCA (at the bottom) stands for the Last Universal Common Ancestor. (Diagram: Anne Kemmling, Goettingen, Germany.)

can be seen around 810, 1440, and 1540. Looking at Figure 5 as a whole, the impression is that the upper three sequences are related as well as the lower two. These differences made history.

Carl Woese recognized the tremendous importance of his discovery. In the case of the methanogenic organisms, he had sequenced the 16S-rRNA of an organism that phylogenetically does not have much in common with the bacteria. This new domain of organisms was originally called archaebacteria; later the word "bacteria" was omitted, so now we speak of archaea. The terms methanobacteria or methane-forming archaebacteria are no longer used; instead, they are called methanoarchaea.

When these results were published, many microbiologists opposed such views. But there was also a lot of support, for instance by the German microbiologists and molecular biologists Otto Kandler, Wolfram Zillig, and Karl Otto Stetter, who later made important contributions supporting the archaeal concept. In the US, acceptance was slow but became enthusiastic when major textbooks adopted the concept of three domains of life.

Now we will jump from the situation in 1977 to the present and look at an actual phylogenetic tree. It is apparent in Figure 6 that, beginning with LUCA, evolution proceeded to the domains of Archaea and Eukarya, from which the domain of Bacteria branched off early in evolution. Not only methane-forming bacteria belong to the archaea but also the so-called extremophilic microorganisms, which will be discussed in Chapter 6.

Readers not so familiar with this concept of evolution will find it difficult to accept the idea that two of the three domains of life on our planet are devoted to

microorganisms. The third one is reserved for plants, fungi, animals, and us. We may not forget that bacteria and archaea, on the evolutionary time scale, were by themselves during most of the biological evolution on Earth. One might even ask the question why microorganisms allowed the evolution of higher organisms – an interesting but difficult question. But still, what were the properties of LUCA? It can be assumed that it was a bacterial- or archaeal-like organism that lived in the absence of oxygen. LUCA was a fermenting organism or an organism that converted sulfur and molecular hydrogen to hydrogen sulfide. Archaea performing this kind of fermentation are still present on our planet. How LUCA evolved and how our current atmosphere developed with oxygen as the indispensable element will be outlined in the next two chapters.