Extremophiles (2005) 9: 343–344
DOI 10.1007/s00792-005-0481-0

IN MEMORIAM

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Wolfram Zillig (1925–2005)

Published online: 23 September 2005

Wolfram Zillig, one of the founders of molecular genetics and a strong supporter of the concept that Archaea constituted a third domain of life, died April 23, 2005, 1 week before his 80th birthday.

Wolfram Zillig was a brilliant scientist, providing numerous methods and model systems to the scientific community. His scientific career started in 1952 in Adolf Butenandt's laboratory in Tübingen, Germany, with work on steroid hormones of insects. Already at that time he had become interested in virus particles and the analysis of DNA. He developed the "phenol method" to extract and purify nucleic acids from tobacco mosaic virus nucleoprotein complexes, a general method that is still in use to purify DNA. After postdoctoral work at the University of Wisconsin (Madison, WI, USA), he returned to the Max-Planck Institute in Munich to start his own research group focusing on the gene expression in *Escherichia coli*. He successfully established for the first time an in vitro protein biosynthesis system and synthesized proteins in vitro. The DNA-dependent RNA polymerase (RNAP) always was of special interest to him, and he was the first to recognize that the host RNAP was modified by phosphorylation during infection with T4 or T7 phages.

In 1977 a publication reported that the RNAP of the extremely halophilic *Halobacterium halobium* consisted of only two subunits. Zillig was in doubt about this claim and determined, together with Karl Otto Stetter, the subunit composition of the halobacterial RNAP, which turned out to be very different from the bacterial enzyme. Similar results were determined for the RNAP of the hyperthermophilic *Sulfolobus*, suggesting that the RNAP of these diverse microorganisms was unique. The novel concept of Carl Woese that Bacteria, Archaea and Eukarya constitute the three distinct domains of life was strongly supported by these results. Further phylogenetic analyses of the RNAP sequences from *Sulfolobus acidocaldarius, Thermoplasma acidophilum*, *Halococcus morrhuae*, and *Thermococcus celer* underlined the uniqueness of the archaea and also suggested a common origin of these microorganisms that live in very diverse and extreme environments. Almost even more astounding was the result that the RNAP of the Archaea is more closely related to their eukaryotic counterpart (RNAP II) than to the bacterial RNAP.

Most archaea used for these studies were isolates collected from hot springs all over the world. Zillig sampled many novel archaea in the field and investigated their molecular traits. His strain collection consists of more than 700 isolates; he described the archaeal species *Thermoproteus tenax, Thermococcus celer, Thermofilum pendens, Desulfurococcus, Acidianus ambivalens, Hyperthermus butylicus, Methanothermus fervidus, Sulfolobus shibatae*, *S. solfotaricus, S. islandicus*, and *Picrophilus oshimae*. The last species is the most acidic thermophile known to date and lives at pH 0.5. *Sulfolobus* was developed as a model system to study plasmid variations

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and virus particles; this strain was also used to establish a transformation system for hyperthermophilic archaea.

The viruses of the Archaea were another special research area. Zillig soon recognized that the viruses of hyperthermophilic archaea were unique and exhibited a large variety. He described four different virus families of hyperthermophilic archaea, the *Fuselloviridae*, *Rudiviridae, Lipothrixviridae*, and *Guttaviridae*. The most important virus characterized in his lab was SSV1 of *S. shibatae*, a spindle-shaped particle that is permanently extruded from cells, especially after UV-induction. The genome of SSV1 has been used not only to define the archaeal promoter sequence and to establish an in vitro transcription system, but also to determine DNA uptake after electroporation of the host cell.

The scientific work of Wolfram Zillig started more than 50 years ago with the research on gene expression and *E. coli* phages and culminated in the milestone investigations on archaeal RNAPs, plasmids, and their viruses. Zillig's enormous interest in hyperthermophilic Archaea and his remarkable way of working at the bench up to the age of 77 led to many unexpected discoveries. His charismatic and generous nature, and even more his absolute personal integrity and honesty, were highly impressive to students and co-workers. We will miss his sharp intellect and the inspiration he gave to the development of the field.

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