PERSPECTIVES
INTERNATIONAL MICROBIOLOGY (2004) 7:71–76
INTERNATIONAL MICROBIOLOGY

Vicente Rubio*

Institute of Biomedicine of
Valencia, CSIC, Valencia, Spain

Happy microbes in hostile niches. A symposium on extremophiles

Address for correspondence:
Instituto de Biomedicina de Valencia (CSIC)
Jaume Roig, 11
46010 Valencia, Spain
Tel. +34-963391772. Fax +34-963690800
E-mail: rubio@ibv.csic.es

*President-Elect, Spanish Society for
Biochemistry and Molecular Biology (SEBBM)

Belgium can boast the privilege of having had a microbiologist Nobel laureate (Physiology or Medicine, 1919), Jules-Jean-Baptiste-Vincent Bordet. Today, the country's outstanding microbiological tradition remains alive and well at the Research Institute of the CERIA/COOVI (1 Av. Emile Gryzon, Brussels), the center where Nicolas Glansdorff has worked for most of his scientific career and of which he was director (Fig. 1). The French-speaking-community moiety of this institute is now named Institut de Recherche Jean Marie Wiame, to honor its founding father, who was also the scientific mentor of Nicolas Glansdorff. Werner Maas (New York University) and Luigi Gorini (Harvard University) were Glansdorff's mentors in the United States.

Happy Microbes, convened to honor the officially retiring Nicolas Glansdorff, paid tribute to his paramount interests in both extremophiles and the origin of life. The meeting was held on September 27, 2003, under the auspices of the Belgian Society for Microbiology, in the Etterbeek Campus

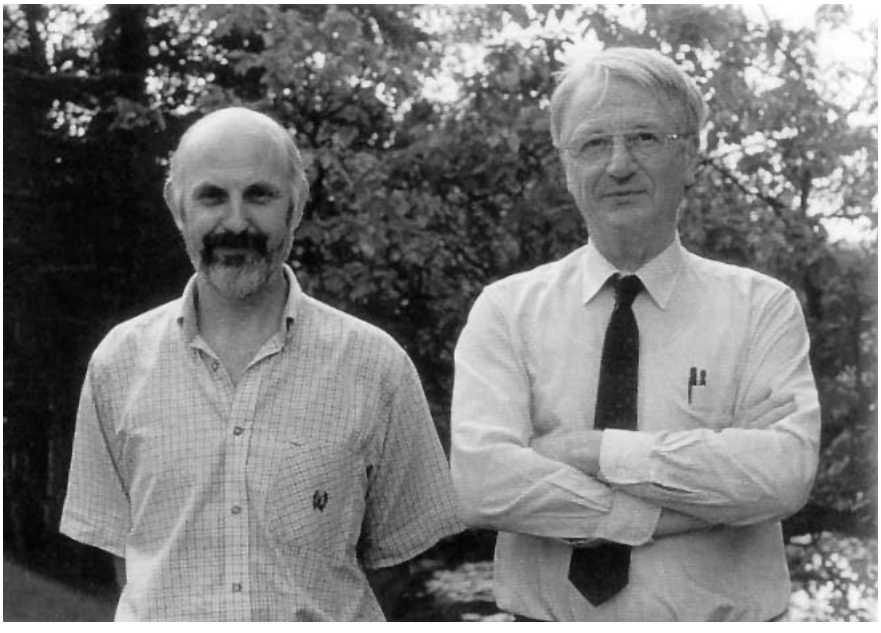

Fig. 1. Nicolas Glansdorff (right) with his colleague Daniel Charlier. [Courtesy of Elisabeth A. Carrey, University College, London.]

of the Vrije Universiteit of Brussels (VUB, the Flemish Free University), the University in which Glansdorff was Professor of Microbiology. The Symposium was organized by Pierre Cornelis (Microbiology, VUB, Brussels), Paul Janssen, Max Mergeay (both at the Belgian Nuclear Research Center, Mol), and Adrianne Toussaint (Université Libre de Bruxelles; the French-speaking counterpart to VUB). An enthusiastic audience of approximately one hundred met to embark on a fascinating experience through space-time and the world of microbiology.

From abyssal zones to extraterrestrial space

The title of the first talk, by Daniel Prieur (Centre National de la Recherche Scientifique/Université de Bretagne Occidentale/Ifremer, Brest, France), "From abyssal zones to extraterrestrial space: the quest for microbes", gives a hint of the spirit of the meeting. Prieur reflected on the physicochemical limits to life (temperature, pressure, pH, salinity, exposure to radiation) and illustrated them with specific organisms that challenge these limits. Hyperthermophiles, thermophiles, psychrophiles (low-temperature loving), piezophilic, baroresistant, and radioresistant life forms were paraded across on the screen in a rich panoply demonstrating Nature's experiments to test the limits of biological machinery by colonizing the most hostile niches. I cannot resist mentioning PAV1, the first virus found in hyperthermophilic archaea [2], or the archaea *Pyrolobus fumarii*, which grows at 106ºC and can withstand autoclaving at 120ºC for 1 h, not to mention an organism recently described as growing at 121ºC and resisting exposure to 130ºC [4]. Prieur described the mechanisms of formation of hydrothermal active sites at the ocean bottom and the development of thriving communities of organisms living there. He outlined the strategies used by invertebrates to survive, which are based on endosymbiosis with microorganisms that obtain their energy from oxidation of reduced compounds such as hydrogen sulfide or methane. He considered that the exploration of planet Earth for microbial life may still be incomplete (he mentioned, for example, deep sediments and deep oil reservoirs), and speculated about the possibility of life in other places in the solar system, referring particularly to life-compatible Martian temperatures (although liquid water is non-existent in Mars), and to the Jovian moon Europa, which under a crust of ice hides a liquid ocean where hydrothermal vents may exist at its bottom. Will this be an important element in solving the puzzle of life? The answer may not be found during our generation.

If life at the depths of oceans was a central element of Prieur's presentation, space exploration was a major component of the presentation of Max Mergeay (Belgian Nuclear Research Centre, Mol), "Extremophily in anthropogenic biotopes: *Ralstonia metallidurans* functions expressed in the presence of heavy metals and under space conditions". *Ralstonia metallidurans* is a metal-resistant organism with metabolic functions relevant to the bioremediation of metal-contaminated soils and waters. Mergeay indicated that, although the genome of *R. metallidurans* has not yet been sequenced completely, current genetic evidence indicates that this microorganism is highly specialized in its response to metals and to specific biotopes. Most metal-related functions of *R. metallidurans* appear to be related to the transport and removal of heavy metals, with the participation of plasmid-borne and chromosome-borne genes, whose total number, e.g., for copper, is not less than 14. In addition, a large number of paralogs for typical efflux enzymes are found among the genes of *R. metallidurans*, and experiments using proteomics to define the expression of chromosomal and plasmid genes in response to specific heavy metals were reported. Mergeay also highlighted the fact that genome plasticity is another characteristic that helps this microorganism to adapt to hostile environments; for example, a chromosomal island of *R. metallidurans* with strong resemblance to a 55-kb transposable element enables the bacterium to degrade the pollutants biphenyl and 4-chlorobiphenyl. The outer-space aspect was the description of experiments carried out under the auspices of the European Space Agency, including the growth of solid *R. metallidurans* cultures for one week in the International Space Station, and experiments aboard the Spanish Space Taxi mission (October 2003). Preliminary results have revealed small morphological differences compared to *R. metallidurans* cultures grown under Earth conditions (smaller and rounder cells with higher membrane potentials and decreased permeabilities), and the lack of confluent growth with 10^5 cells per inoculum. Proteomics is being used to explore microgravity effects, and in *R. metallidurans* alterations of acetone metabolism have been found.

The search for LUCA and the origin of DNA

Time travel to the ancient past was the subject of two presentations, by Anthony Poole (Arrhenius Laboratories, Stockholm University), and by Patrick Forterre (Institute de Génétique et Microbiologie, Université de Paris Sud at Orsay). These two presentations went as far back as LUCA, the last universal common ancestor, and asked questions such

as "What does the RNA world tell us about the last universal common ancestor?" (Poole's presentation) and the "Origin and evolution of DNA and DNA replication mechanism: the viral hypothesis" (Forterre's presentation). The core of Poole's presentation was that, if we derive from an RNA world, a search into the antiquity and evolution of RNA processing may provide the most detailed insight into the origin and evolution of the three domains of life. An RNA-based high-fidelity RNA polymerase must have been an absolute requirement of the RNA world, even before cells appeared on the evolutionary stage, and the ribosome may have derived from this ancient copying ribozyme. Furthermore, limitations to the size of double-stranded RNA genomes, and fidelity-copying considerations, in addition to the time needed for copying the entire RNA genome, have led Poole to believe that the primeval RNA genome had many origins of replication, and that it was probably composed of multiple individual segments of double-stranded RNA. This definitely rings a bell regarding similitude to present-day eukaryotic chromosomes. Poole challenged the view that, since prokaryotes are simpler, they must have come first. The genome of prokaryotes may, instead, have shrunk as a consequence of stress factors (for example, thermoreduction), which would have eliminated what we now consider "junk" material (e.g. introns; do introns derive from the previous RNA-based catalytic machinery that became superseded by a protein-based machinery?). Therefore, the LUCA may have had many features of the eukaryotic genome and may have been mesophilic!

Not less provocative was the presentation of Forterre. In response to the title "who invented DNA and why was it invented?" (or in French, as set out by Forterre, "qui a inventé l'ADN? et pourquoi?"), he provided a sharp answer: viruses did, and they did it to escape counterattack from their hosts. The conventional view that viruses are chunks of cellular RNA or DNA that escaped from cellular chromosomes long ago, and later on incorporated additional genes from their hosts is, according to Forterre, challenged by the homologies found between viruses having very distantly related hosts and by evidences of gene flow from viruses to cells. Viruses may actually be very ancient and may have played a pivotal role in the transition from the RNA to the DNA world. Relevant to this transition, thymidine appears to have been "invented" at least twice over, and several proteins involved in DNA metabolism originated a number of times independently. Since DNA can be considered a modified form of RNA, and the modifications are introduced by protein enzymes, DNA may have appeared at a late stage of the RNA world, well after the invention of protein enzymes. If DNA had been invented by viruses, DNA-making enzymes would be viral. Indeed, Forterre provided comparative genomic clues suggesting that most proteins involved in DNA metabolism might have a viral origin.

A database of microbial mobile elements

After traveling to the past, a bit of reflection into the present and even into the near future was welcome. This was provided by Ariane Toussaint (Service de Conformation des Macromolecules Biologiques et de Bioinformatique, ULB, Brussels), who discussed "Prokaryotes in the third millennium: a new evaluation of our life-support system". Toussaint's reflection did not focus on life itself, but on our comprehension of life. It was based on bioinformatics, a wonderful tool that allows us to travel across taxon barriers and to search entire living worlds from our personal-computer. One can readily agree with Toussaint that the time is close when, on the basis of pure genomic information, we will be able to reconstruct virtually the makings and workings of an entire microorganism, or at least to explain all of its metabolic activities. But, of course, this depends extremely on an accurate and reliable gene annotation system, which, given the extent of the genomic effort, has to be automatized. Toussaint points out that a significant or large fraction of a given organism's genome (amounting in some cases to 20–30% of the whole genome) comprises what she calls the "horizontal gene pool", made up of prophages and other mobile elements. Many biological activities are dependent on those elements, as is very well known for plasmids conferring virulence, metal resistance, etc. The present annotation of these elements gives little insight into their function. To improve this situation, Toussaint presented a new classification of bacterial and archaeal mobile elements based on a dissection of the functional modules of these elements. The database is called ACLAME (http://aclame.ulb.ac.be) and is publicly accessible. Volunteers wishing to participate in maintaining the database are welcome!

Life in the cold

Most other contributions remained at the present point of the space-time intersection, dealing with more "mundane" topics, related mainly to microorganisms that live under non-usual thermal conditions. Thus, psychrophilic organisms—one of the favorite topics of Nicolas Glansdorff, who has garnered a reputation as a real first-hand (and foot, given his Arctic trekking) connoisseur of the frozen world—were

the subject of the presentations of Charles Gerday (Université de Liege, Belgium) and Nick Russell (Wye campus of Imperial College, London). Gerday's presentation on "Cold-active enzymes" defined properties generally found among enzymes of cold-adapted organisms. These properties include their high specific activity at low temperatures—although their activities are lower than those of their mesophilic counterpart enzymes at the corresponding environmental temperature—and high thermosensitivity, which reflects increased flexibility. Gerday reported on experiments with an α-amylase from an Antarctic microorganism, an enzyme that is inactivated upon heating at a temperature lower than that needed to unfold the enzyme. The unfolding is reversible (a rare but real treat for microcalorimetrists; most large proteins melt irreversibly) and the enzyme has the lowest recorded stability for any reversibly unfolded protein. Crystallographic studies provide some insight into the mechanism of this low stability, mainly a decrease in weak interactions such as salt bridges and hydrophobic interactions, and decreased interactions between different domains. Structure-guided site-directed mutagenesis experiments in Gerday's laboratory have generally confirmed these proposals. The take-home message is that psychrophilic enzymes trade stability for structural flexibility, and that minimization of weak interactions is the strategy of this trade.

Nick Russell's presentation on "Membrane lipids of cold-adapted Antarctic bacteria in the relation to real-time adaptation and evolutionary aspects", illuminated the fact that our real understanding of this topic is still quite limited. True, it is well-accepted that a number of psychrophilic bacteria sport large amounts of polyunsaturated fatty acids in their membranes, but it is quite an enigma why other bacteria, equally exposed to permanent low temperatures, do not. The theory that polyunsaturated fatty acids are needed to increase membrane fluidity is simplistic and insufficient. In fact, apparently, certain degrees of polyunsaturation lead to shorter effective chain-lengths and may result in a tendency of phospholipids to form deadly micelles rather than to organize as bilayers. Thus, there must be more to this mechanism than simply how fluid the membrane is at a certain temperature. Clearly, more biophysical experiments examining the consequences of lipid composition on bilayer and membrane properties are needed, especially those replicating extreme conditions—not only because of the cold—of both the Antarctic seas and the Antarctic rocky soils. In addition, specific metabolic processes leading to the synthesis of these unsaturated fatty acids, and the control of these metabolic processes, require further studies of cold-living bacterial species. Therefore, we were left with an enthusiastic question mark, meaning that greater efforts have to be focused on this topic, not only for reasons of pure knowledge, but also because the demand for polyunsaturated fatty acids in the form of food supplements that do not taste fishy is now "cool" (to use temperature-related terminology). After all, psychrophiles may offer an opportunity to the biotechnologist, in these days of science-industrial parks and spin-off companies.

Protein chip technologies of the 21st century

Carrying on the biotechnological tune was the presentation of Vehary Sakanian (FRE-CNRS-ProtNeteomix, Université de Nantes, France) on "Protein chips: high throughput technology for basic and applied science". Sakanyan, the founder and head of ProtNeteomix, a spin-off company of the University of Nantes, has developed protein-chip technologies that include protein production "in vitro" (although "in vivo"-produced proteins, either recombinant or in natural extracts, can also be used), protein fixation to a solid support in microarray form without chemical cross-linking, labeling of a probe (either a protein, an antibody, a peptide, a nucleic acid, or a small ligand) with near-infrared fluorescent dye, and probe binding and fluorescent detection. The technologies are extremely useful for the detection and mapping of interactions, protein profiling, and target screening using candidate labeled drugs. In addition, they may have important diagnostic potential (for example, to search for specific tumor biomarkers). Several examples were illustrated. Of these, I mention here only—because of the basic science and the use of the arginine repressor (ArgR) from both a mesophile and a thermophile—the comparison of DNA-binding sites and strength of the interactions, both in the absence and in the presence of arginine, of the ArgRs from *Escherichia coli* and *Bacillus stearothermophilus* [3]. Protein-chip technology appeared to me to be extremely useful for any microbiology laboratory working in the field of gene regulation, and I had the impression that Sakanyan would welcome interesting and sound scientific collaborations.

"Hot" topics

The remaining presentations dealt—literally—with hot topics since they, either directly or indirectly, addressed questions concerning proteins from thermophiles. Raymond Cunin (VUB, Brussels), an aspartate transcarbamylase expert, talked about "Allosteric regulation of enzymes: from

mesophilic model to hyperthermophiles (from the aspartate transcarbamylase of *Escherichia coli* to that of *Pyrococcus furiosus*)". He summarized the main traits of the well-characterized allosteric regulation of *E. coli* aspartate transcarbamylase, an oligomeric enzyme that is a classic in studies of allostery, given its clear-cut cooperativity for aspartate and the relatively early date at which its structure was determined. Cunin recollected very elegant experiments, including several crucial ones from his own laboratory, that allowed the dissection of the intramolecular signal-transduction process, from the binding site of the allosteric regulators to the Zn domain of the regulatory subunit, and from there to the catalytic subunit that is responsible for carrying out the enzyme reaction. His conclusion: control is in the small details. A few structural elements embedded in the basic protein framework dictate the pattern of regulation in this well-characterized enzyme. Another part of Cunin's presentation dealt with ongoing work in his laboratory on the aspartate transcarbamylase from the hyperthermophilic and barotolerant archaea *Pyrococcus abyssi*. This enzyme resembles the *E. coli* enzyme, but is much more thermostable and, according to Cunin, it has a much greater affinity for the inhibitor CTP. Protein engineering is being used to explore the basis of these differences with the *E. coli* enzyme, and the state of the art was reviewed.

Aspartate transcarbamylase, this time from the thermoacidophilic archaea *Sulfolobus acidocaldarius*, was the subject of another presentation, by University of Gent (Belgium) scientists Dirk de Vos and Jozef van Beeumen ("How does the structure of *Sulfolobus acidocaldarius* aspartate transcarbamylase cope with the extremely high temperatures of its environment?"). To gain insight into previous observations on the stability and regulatory properties of this allosteric enzyme, its crystal structure in the T (tense, or low affinity for the substrate showing cooperativity, aspartate) state was determined. Significant structural differences with the mesophilic *E. coli* enzyme were reviewed in the presentation, and were interpreted in the context of thermal stability and kinetic and allosteric properties differences.

My own presentation "Structural bases of feed-back control of arginine synthesis in *Pseudomonas aeruginosa* and *Thermotoga maritima*", dealt with the mesophilic and thermophilic versions of the controlling arginine biosynthetic allosteric enzyme acetylglutamate kinase. The use of a thermophilic enzyme as an investigative tool (in fact, I highlighted the advantages of using thermophilic enzymes for crystallographic work) and the major role of arginine biosynthesis in Glansdorff's scientific career, justified this contribution to the *Happy Microbes* symposium. In many organisms, acetylglutamate kinase regulates arginine biosynthesis. In those species, such as *E. coli*, in which the control of arginine synthesis is exerted at the step of acetylglutamate synthesis, there are also non-regulated versions of this enzyme. I focused my discussion on three issues: (1) how a short N-terminal amino acid sequence extension characterizes those acetylglutamate kinases inhibited by arginine; (2) how the removal of this extension causes the enzyme to become arginine-insensitive; and (3) the role of this extension, which is shaped as a linked α-helix, in interlacing three adjacent acetylglutamate homodimers (the basic unit of acetylglutamate kinase is a very stable homodimer) into doughnut-like hexamers that undergo conformational changes triggered by the binding of arginine. Thus, by combining enzyme engineering, biochemical techniques, and X-ray crystallography, our results have shown how and where arginine binds, why it causes inhibition (by preventing the approach of the two bound substrates), and how arginine binding moves the nearby N-terminal helix by triggering a conformational change that spans the entire hexameric doughnut.

Glansdorff's *Naturphilosophie*

The final presentation was by Glansdorff himself and was entitled "Reflections of a microbiologist at the dawn of the twenty-first century". Although Glansdorff paid tribute to microbiology and requested more public recognition of this discipline, most of his talk transcended the limits of pure microbiology and even biology, and asked larger questions about ourselves and human evolution. His perspective was heavily colored by neo-Darwinian theory in its more stringent and mathematical use of natural selection as an algorithm. I would say that the subject of his talk falls in the mainstream of what used to be called natural philosophy. He challenged the concept of evolution producing increasing complexity. Instead, he prefers to think, with Stuart Kaufmann [5], that evolution increases order. Following Kaufmann, natural selection works the best at the boundary between order and randomness, generating self-organizing autonomous systems. Self-replication is now a property of certain molecules but must have arisen as a property of the whole autonomous system, which thus had lots of time to perfect itself by increasing its efficiency until self-replicative molecules arose and were selected. This bears directly on the question of the origin of life. A second question raised by Glansdorff concerned the origin of ethics, justice, altruism, and cooperative social action. Do these traits arise purely as a consequence of natural selection? His answer

was yes. In his thinking, he follows the seventeenth-century philosopher Baruch Spinoza and the present-day neurologist Antonio Damasio [1]. I fear misrepresenting Glansdorff's elaborate thinking, but those interested in reading his complete talk can request it to Dr. Paul Janssen (pjanssen@sckcen.be). On the whole, he considers the development of ethics, the sense of justice and of brotherhood, to have arisen by natural selection, and he believes that those practicing aggressive, competitive capitalism as though it were a natural steady state of human societies are wrong. Furthermore, he believes that conscious ("sentient") beings are those that give meaning to the Universe.

Glansdorff ended his talk by reflecting on the future. Following Robert Bradbury, he sees an exponential increase in intelligence on planet Earth, and he concludes that, if this rate of increase continues according to the same law, the depositaries of such a large amount of intelligence will be very different from us in a not so distant future. Will they be of our flesh and bones or will they be other types of machines, perhaps based on the development of artificial intelligence? Or will our technology—being relatively advanced, but our ethical development as a group being much more primitive—lead us to destruction, leaving only a few as the seeds for further evolution? After all, Newton predicted that everything would stop by the year 2060, and that an assembly of saints, including himself, would take control then. Glansdorff bade goodbye by saying "see you in 2060". As a Newtonian saint, I am sure.

References

1. Damasio A (2003) Looking for Spinoza. Harcourt, New York
2. Geslin C, Le Romancer M, Erauso G, Gaillard M, Perrot G, Prieur D (2003) PAV1, the first virus-like particle isolated from a hyperthermophilic euryarchaeote, *Pyrococcus abyssi*. J Bacteriol 185: 3888–3894
3. Ghochikyan A, Karaivanova IM, Lecocq M, Vusio P, Arnaud MC, Snapyan M, Weigel P, Guevel L, Buckle M, Sakanyan V (2002) Arginine operator binding by heterologous and chimeric ArgR repressors from *Escherichia coli* and *Bacillus stearothermophilus*. J Bacteriol 184:6602–6614
4. Kashefi K, Lovley DR (2003) Extending the upper temperature limit for life. Science 301:934
5. Kaufmann S (1993) The Origins of Order: Self-organization and Selection in Evolution. Oxford University Press, New York