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MEETING REVIEW

Extremophiles 2002

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"Extremophiles 2002," held in Naples, Italy, 22 to 26 September 2002, was the fourth international conference on microorganisms growing in very diverse and severe environments, such as high or low temperature, acidic and alkaline conditions, high salt concentrations, and high pressure. About 550 researchers from 30 countries discussed subjects including the ecology, phylogenetics and evolution, molecular and cell biology, genomics, proteomics, enzymology, and structural biochemistry of these fascinating microbes.

EXTREMOPHILES AND THE ORIGIN OF LIFE

In the opening symposium, leading scientists in the field gave their perspectives on the controversial topics of origin and evolution of life on earth and the possibility that life exists outside our planet. Though global phylogenetic models predict that early organisms were hyperthermophiles, this hypothesis is not universally accepted (12). As shown by E. Nisbet (University of London), the preponderance of current geological and geochemical evidence indicates that earth remained hot for several hundred million years, due to frequent meteorite impacts that were capable of heating oceans and the atmosphere up to 100°C. Hyperthermophiles could have been either the first living organisms or the only survivors following such sterilizing events. For these reasons, "hot" versus "cold" origin-of-life theories are hotly debated.

A. Lazcano (Cd. Universitaria, Mexico City, Mexico) pointed out the fact that most molecules with essential biological functions, such as nucleotides, some amino acids, and nucleic acids, are thermolabile. Miller's "primitive soup" hypothesis, implying that the first living organisms used RNA as the information molecule, has been challenged by the "metabolic life " theory. This hypothesis suggests that primordial "life" was nothing other than a series of self-catalyzed reactions based on simple compounds such as CO_2 and CO. Since many reactions are thermodynamically more favorable at high temperatures, it has been suggested that hydrothermal environments were the cradle of this "metabolic life." But Lazcano argued that conversion of this kind of "life" to living organisms required the emergence of a genetic system, raising the problem of nucleic acid stability. Dealing with the latter issue, T. Oshima (Tokyo University of Pharmacy and Life Science) presented data showing that unusual polyamines typical of hyperthermophiles protect both DNA and RNA from denaturation and chemical damage, raising the possibility that these molecules had an essential role in an RNA world.

Another player suggested to have a crucial role in the adaptation of life to very high temperatures, and possibly in the origin of hyperthermophiles, is reverse gyrase, an enzyme inducing positive supercoiling into DNA. P. Forterre (Université Paris-Sud) searched all available genomes for proteins present in all hyperthermophiles and absent in all other organisms. Surprisingly, the only hyperthermophile-specific protein he retrieved is reverse gyrase (8).

One of the central controversial questions concerns the nature (mesophilic or thermophilic) of the last universal common ancestor. Several methods have been applied to infer the environmental temperature of the last universal common ancestor, reaching opposite conclusions. Further comparative genome studies should help to resolve such critical questions. In any case, the discovery of extremophiles living in almost any environmental niche, including those that seem totally inhospitable, expands the limits of life and increases the likelihood that life could have evolved somewhere else in the universe.

EXTREMOPHILE DIVERSITY

The meeting highlighted many recent and important insights into the evolution of the concept of microbial diversity. First, G. Olsen (University of Illinois) stressed that biodiversity under extreme conditions has to be considered not only in terms of the number of group subspecies but also in terms of the genes present in the species and the ecosystems they form. These three levels (species diversity, genetic diversity, and ecosystem diversity) and their interconnections were examined in great detail for very restricted ecological niches, which represent powerful model systems for biodiversity studies.

Ecosystem diversity encompasses the broad differences between ecosystem types and the diversity of habitats and ecological processes occurring within each ecosystem type. J. Reeve (Ohio State University) believes that it is harder to define ecosystem diversity than species or genetic diversity because the "boundaries" of communities (associations of species) and ecosystems are more fluid. Since the ecosystem concept is dynamic and thus variable, it can be applied at different scales, though for management purposes it is generally used to

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group broadly similar assemblages of communities, such as the numbers of microorganisms immured in glacial ice for periods ranging from months to >500 million years. In this respect, G. di Prisco (CNR Institute of Protein Biochemistry, Naples, Italy) stressed the concept that a key element in the study of ecosystems, especially the glacial ones, is the conservation in the natural state of ecological processes, such as energy flows and water cycles, but also the use of suitable tools to avoid external contamination during sampling.

In this respect, "hot" environments are more suitable for specific investigation because of restrictive conditions unfavorable for most of the surrounding life forms; in particular, representatives of the *Archaea* have been indicated as good models for diversity-evolution studies. Very interestingly, H. Huber (University of Regensburg) reported the isolation of a nano-sized symbiont, which represents a novel phylum of *Archaea*, the "*Nanoarchaeota*," in a submarine hydrothermal vent. "*Nanoarchaeum equitans*" shows unique features such as small cell size (only 400 nm in diameter), very small genome size (about 500 kb), and growth on the surface of another, "normal-sized" archaeon, a new *Ignococcus* species. The importance of this discovery is clear: this "nano-organism" can provide an extraordinary model to study evolution of thermophily, of minimal cellular and genetic requirement, and of interspecies communications.

An even more delimited field for progress in the biodiversity research is represented by viruses and their hosts in thermal hot springs. In particular, D. Prangishvili (University of Regensburg) and, in the poster session, K. Stedman (Portland State University) asserted that viruses of the *Sulfolobus* genus, especially the *Fuselloviridae*, have provided extensive insights into the comparative genetics of different geographically distributed populations. The increasing efforts in their collection and genome sequencing have been important for the development of molecular genetics in this model hyperthermophile and have also allowed correlations between the geographical distance separating the isolates and their genetic divergence, with implications for virus and, more interestingly, host dispersal and common evolution.

Moreover, many poster presentations dealt with recent advances in the molecular analysis of uncultivated samples from different geographical areas in their specific environments. B. R. Wheeler (University of Delaware), for example, reported in the biodiversity poster session an interesting integrated analytical system which combines molecular genetics and geochemistry to study the variation of microbial communities in hot mud pools in New Zealand in relation to natural fluctuations of chemical composition and temperature.

In general, all biodiversity presentations led to the concept that improvement of cultivation methods together with automated analysis of gene products provided additional insights into complex microbial communities and their "dynamic communications" at the molecular level.

PHYSIOLOGY AND METABOLISM

The need to predict the functions and structures of new proteins discovered via genomic sequencing has led to the rapidly growing field of extremophile proteomics. A flood of new information has been obtained by the codevelopment of appropriate bioinformatic methods for storage and analysis of acquired information in comparative genomics studies. Exhaustive examples of clusters of orthologous genes were provided by K. S. Makarova (National Institutes of Health, Bethesda, Md.).

The explosively increasing number of complete genome sequences, especially for hyperthermophiles, enabled comparative analysis of the general metabolic capacity, and W. de Vos (Wageningen Agricultural University) pointed out that several novel enzymatic conversions are catalyzed for which no genes are already known, whereas some expected genes for various anticipated metabolic pathways are missing. Moreover, if the recent boost of the "postgenomic era" has provided further knowledge of the deep differentiation in the prokaryotic kingdoms of life, it also helped in the identification of common and universal metabolic routes as a consequence of the continuous flow of genetic information exchange among distant and apparently unrelated microbes, as clearly shown by phenomena such as DNA transfer in hot environments, described by B. Averhoff (Ludwig-Maximilians Universitat, Munich, Germany) in her study on the model bacterial system *Thermus thermophilus*. Recently developed proteomics techniques for determining gene function along with differential expression detection and analysis were successfully applied as high-throughput technologies for function discovery and for reconstructing functional networks in extremophiles. The meeting emphasized the modeling of unique biological pathways that attract special interest for both basic and applied research, such as carbohydrate metabolism, and for the understanding of the effect of defective genotype on phenotype as well as the involvement of environmental factors in gene function. An example of these concepts was provided by R. Cannio (CNR Institute of Protein Biochemistry), who demonstrated the involvement of a metabolically uncharacterized gene in lactose metabolism by functional complementation of a natural β-glycosidase-defective mutant of *Sulfolobus solfataricus* (2)

Another important topic, concerning the structure of membrane proteins, was examined by A. J. M. Driessen (University of Groningen) with respect to sugar transporters and motility proteins, such as flagellins. The overall analysis at the biochemical and genome sequence level of *S. solfataricus* P2 revealed common amino acid signal sequences responsible for membrane embedding and, very likely, for cell sorting. In addition, in the poster presented by Z. Szabo, the same group reported the identification of a novel signal peptidase responsible for processing of secreted proteins (1).

Major attention in this session was devoted to stress responses, since, as highlighted by A. Macario (University of Albany), extremophiles are exposed more than other organisms to a variety of stressors in their natural habitats: physical (heat, cold, and radiation), mechanical (compression, decompression, and shearing), and chemical-biochemical (sudden shift from anaerobiosis to aerobiosis or vice versa; changes in pH or osmolarity). Thus, antistress mechanisms in microbes that thrive at the extremes of life can serve as models for the study of the general defense strategies evolved by all organisms. Reports have focused mainly on antistress processes aiming to protect cellular macromolecules, with considerable interest for molecular chaperones and, more generally, for cold and heat shock factors. Intriguingly, G. Juez (University

Miguel Hernandez, Alicante, Spain) remarked that many of the stress response factors in haloarchaea are multistressor inducible; that is, different stress agents can be counterbalanced by the same protein-mediated cell response. It has been shown that there is a frequent overlap between osmotic stress and high-temperature responses in both halophilic and thermophilic prokaryotes. Very interestingly, (hyper)thermophilic *Archaea* and *Bacteria* accumulate high intracellular concentrations of unusual small molecules, namely, of the so-called compatible solutes. H. Santos (New University of Lisbon) demonstrated that, among other "hypersolutes," mannosylglycerate is preferentially distributed among marine (hyper)thermophilic organisms and is highly efficient in the protection of enzymes against thermal inactivation. This finding suggests that these molecules could play a role in the thermoprotection of cell components in vivo.

GENE EXPRESSION

Most of the reports of this session concerned recent advances in transcription regulation in (hyper)thermophilic *Archaea*, a topic that has lagged behind, mainly due to the lack of tools for studies in vivo. Indeed, although the molecular biology of these organisms has received much attention in recent years (3, 11), first-generation host-vector systems became available only recently, and their applicability is still limited.

Given the availability of several sequenced genomes of extremophiles, several reports concerned gene expression studies from a genome-wide perspective. As an example, M. W. W. Adams (University of Georgia) reported the use of *Pyrococcus furiosus* microarrays to study global variation of gene expression in response to different growth conditions (cold shock, oxidative shock) and to investigate primary metabolism (10). He showed that cold shock results in massive down-regulation of more than 200 open reading frames (ORFs), whereas about 20 genes were upregulated. Several of these ORFs were also upregulated in oxidative shock, whereas no homologs of bacterial oxidative shock were retrieved. The effects of S^0 and of the primary carbon source on gene expression were analyzed with this approach.

Microarrays of *P. furiosus* and *Thermotoga maritima* were also used by W. de Vos (Wageningen Agricultural University) to study the major metabolic pathways, including glycolytic pathways and degradation of sugar polymers, and by R. Kelly (North Carolina State University) to study stress response. He reported that in *P. furiosus*, heat shock induces several genes involved in proteolysis, molecular chaperones such as the thermosome TF55, a small heat-shock protein (Hsp20), two CDC48-like proteins, and several glycosyl hydrolases.

C. Schleper (Darmstadt University of Technology) reported the identification, by differential display analysis, of genes induced by heat shock in *S. solfataricus*. These included molecular chaperones (the thermosome and a subunit of the prefoldin) and several ORFs of unknown functions. Heat shock promoters were able to drive heat-induced expression of β-galactosidase (encoded by the *Sulfolobus lacS* gene) in vivo in one of the first experiments using a reporter gene in hyperthermophilic *Archaea*. However, the transformation vector was not stably maintained and could be used only for transient-transfection experiments. Pioneering in vivo studies were also reported by G. Fiorentino (University of Naples) for studying the promoter of the *S. solfataricus* alcohol dehydrogenase gene, which is regulated by the enzyme substrate benzaldehyde. The identification of the protein factors responsible of this regulation is in progress (7).

Although the basic mechanism of transcription in *Archaea* is fairly well understood, less is known about the mechanisms of gene regulation. The basal transcription apparatus of *Archaea* is a simplified version of the eukaryal one: a preinitiation complex containing TBP (TATA-binding protein) and TFB (transcription factor B) is assembled at the corresponding TATA box-BRE (TFB-responsive element) sequences in promoters and recruits RNA polymerase (5). Despite the eukaryotic character of the basal transcription apparatus, many regulators like those in the *Bacteria* can be found in *Archaea*. In particular, members of the Lrp (leucine-responsive regulatory protein) family are present in almost all *Bacteria* and *Archaea*. In the *Bacteria*, these proteins are involved in regulation of amino acid metabolism, whereas their physiological role in *Archaea* is unknown. A few archaeal Lrp-like proteins have been characterized recently; all of them acted as repressors of their own genes by preventing binding of RNA polymerase or of the TBP/TFB (transcription factor IIB orthologue) complex. J. van der Oost (Wageningen Agricultural University) described a novel member of this family, the LysM protein from *S. solfataricus*, which regulates the *lys* cluster for lysine biosynthesis. Indirect evidence suggests that LysM is a transcriptional activator, because its affinity for DNA is higher in the absence of lysine, when transcription of the *lys* genes is maximal.

M. Thomm (Institut fur Allgemeine Mikrobiologie, Kiel, Germany) investigated the mechanism of regulation of heat shock response in *P. furiosus*. He showed that the protein Phr acts in vitro as a transcriptional repressor of its own and of two heat-induced genes (coding for the small heat shock protein, Hsp20, and an AAA+ ATPase). Phr binds to a DNA sequence overlapping the transcription start sites and prevents RNA polymerase recruitment to the promoter. Three sequences conserved in the binding sites of Phr were proposed as consensus regulatory sequences of heat shock promoters.

L. Tutino (University of Naples) described a strategy for developing genetic tools for expression of heterologous proteins in cold-adapted bacteria. This system might help the exploitation of industrial processes requiring low temperatures and ameliorate the recovery of proteins forming inclusion bodies in mesophilic hosts, since hydrophobic interactions inducing protein aggregation are reduced at low temperatures.

Data on the mechanism of translation initiation in *Archaea* are scarce. Genomic sequences have shown that many archaeal genes are organized in operons and have Shine-Dalgarno (SD) sequences upstream of their start codons as in the *Bacteria*, whereas single-gene transcriptional units lack SD sequences, as in the *Eukarya*. P. Londei (University of Rome) described the use of cell-free translation assays to show that in the absence of SD sequences, leaderless initiation is used as the default mechanism and does not require a specific mRNA-rRNA interaction. A number of translation factors have been expressed and purified, and their functions are being studied.

GENOMICS

R. Garrett (Copenhagen University) reported on a comparative analysis of the genomes of three *Sulfolobus* species (*S. solfataricus*, *S. acidocaldarius*, and *S. tokodaii*). They are characterized by the presence of several mobile elements, which are classified into two main groups: the autonomous insertion sequence (IS) elements and the nonautonomous miniature inverted repeat element (MITE)-like elements. The number and diversity of IS elements vary considerably among *Archaea*. The genus *Sulfolobus* (in particular *S. solfataricus*) is very rich in these elements, whereas other archaeal species, such as "*Pyrococcus abyssi*" and *Methanococcus jannaschii*, contain single copies of putative IS elements and *Methanobacterium thermoautotrophicum* contains none. Whereas eukaryal IS elements contain multiple ORFs, the archaeal ones range from 0.5 to 2 kb and usually encode only transposases and sometimes additional enzymes, like DNA resolvases. The MITEs are the result of a deletion within an IS element (type I MITEs) or are derived by accruing terminal inverted repeats (type II MITEs). They are common in the eukaryal genomes and are also abundant in the *S. solfataricus* and *S. tokodaii* genomes. The distribution of the above mobile elements in *S. solfataricus* was found not to be casual, suggesting that the DNA replication origin (*oriC*) and terminus (*terC*) regions act as barriers against their mobility.

Sequencing of the first psychrophile genomes was among the highlights of the congress. H.-P. Klenk (Epidauros Biotechnologie AG, Bernried, Germany) talked about the sequencing of the *Desulfotalea psychrophila* genome. This microorganism is a sulfate-reducing gram-negative bacterium that was chosen because of its low optimal growth temperature (10°C) and its frequent occurrence in permanently cold Arctic marine sediments, where it represents 4% of all *Bacteria*. The 3.66-Mbp genome of this species contains 91 tRNA- and rRNA-encoding genes and about 3,330 ORFs. Half of the latter ORFs show a clear sequence similarity to genes encoding proteins of known function, whereas the remaining half correspond to conserved hypothetical proteins from other sequenced genomes or have no significant similarity to sequences for any known protein. At least 50 genes coding for ABC transporters were identified. In addition, two operons encoding proteins essential for the cobalamin biosynthesis were described, as well as about 70 genes belonging to vitamin and cofactor synthetic pathways.

R. Cavicchioli (University of New South Wales, Sydney, Australia) described the genome sequencing of two methanogens: the psychrophile *Methanogenium frigidum* (optimal growth temperature, 15°C) and the psychrotolerant *Methanococcoides burtonii* (optimal growth temperature, 23°C). Since the complete genome sequences of five methanogens, with optimal growth temperatures ranging from 110°C (*Methanopyrus kandleri*) to 37°C (*Methanosarcina mazei*), are already available, the completion of the *M. frigidum* and *M. burtonii* chromosomal sequences will be of great value in order to understand the molecular mechanisms responsible for cold adaptation in the archaeal domain.

A. Slesarev (Fidelity Systems, Inc., Gaithersburg, Md.) described the peculiar features of *Methanopyrus kandleri*. This organism, isolated from a deep thermal vent in the Gulf of California, is the only known hyperthermophilic methanogen that grows at temperatures up to 110°C. Another interesting peculiarity of this species is the presence of high concentrations of 2,3-disphosphoglycerate (1.1 M) and potassium ions (3.3 M) inside the cells. The *M. kandleri* genome (about 1.6 Mbp) was completely sequenced and was found to contain 1,662 ORFs, among which 74 contributed to new clusters of orthologous groups. Interestingly, proteomic studies revealed the methanogenic nature of *M. kandleri* together with similarities to the halophiles.

DNA REPLICATION, REPAIR, AND RECOMBINATION

The investigation of DNA replication, repair, and recombination mechanisms in hyperthermophilic archaeal species is a very active field because *Archaea* are useful model organisms for the study of these important DNA transactions in the more complex eukaryal systems (4).

This session was opened by M. F. White (University of St. Andrews), who described recent progress on the structural analysis of the *S. solfataricus* single-stranded DNA binding protein. The second part of the talk concerned the *S. solfataricus* homolog of the eukaryal nucleotide excision repair (NER) endonuclease XPF. This protein, like all other components of the NER machinery, is highly conserved in all eukaryotes and is involved in repair of UV-induced DNA damage and recombination pathways. Corresponding ORFs are present in *Archaea*, although none of them has been functionally characterized. The endonuclease activity of *S. solfataricus* XPF was shown to be greatly stimulated by the *Sulfolobus* proliferating cell nuclear antigen (PCNA)-like factor. M. Ciaramella (CNR Institute of Protein Biochemistry) analyzed the effects of UV irradiation on transcription of NER genes of *S. solfataricus*. A putative *cis*-acting sequence that is located upstream of the UV-induced genes was identified.

Since DNA undergoes different forms of damage at high temperatures, hyperthermophiles are believed to have evolved very efficient systems to protect and repair their genomes. The biochemical characterization of the Y-family DNA polymerase from *S. solfataricus* (DNA Pol Y1) was the subject of a talk by P. Grùz (National Institute of Health Sciences, Tokyo, Japan). The family Y of DNA polymerases represents a novel widespread group of enzymes that are able to incorporate deoxynucleotides in DNA templates which contain various kinds of lesions, such as uracil and inosine residues, abasic sites, thymine dimers, and so on. Moreover, the replicative B family DNA polymerase from *S. solfataricus* (DNA Pol B1) was shown to recognize uracil on the DNA template and to stall synthesis 4 to 6 bases upstream of it. This special "read-ahead" function was previously identified in the B family DNA polymerase from *P. furiosus*.

The crystal structure of *S. solfataricus* DNA Pol Y1 in ternary complexes with DNA and an incoming nucleotide, either paired or mispaired, was described by W. Yang (Laboratory of Molecular Biology, National Institutes of Health, Bethesda, Md.). This protein possesses the hand-like overall structure, with palm, fingers, and thumb domains, typically found in all known polymerases. However, *S. solfataricus* DNA Pol Y1 makes a few contacts with the template base and the incoming nucleotide. Thus, base selection is more relaxed at the active

site, and this explains the low fidelity of this enzyme, as well as its ability to bypass damaged sites on the template.

The structural and functional interactions between the DNA polymerase processivity factors homologous to the eukaryal PCNA and replication factor C (RFC) from the euryarchaeon *P. furiosus* were discussed by Y. Ishino (Kiushiu University, Fukuoka, Japan). The conformational changes of *P. furiosus* PCNA upon its interaction with *P. furiosus* RFC were presented, as well as the PCNA ring-opening mechanism. These studies are based on the three-dimensional (3D) structure of the *P. furiosus* PCNA complexed with a synthetic peptide which corresponds to the 50-amino-acid C-terminal tail of the *P. furiosus* RFC large subunit. Biochemical features of RFC were also studied by J.-P. Raffin (Ifremer, Plouzane, France), who demonstrated that ATP hydrolysis is not required for DNA polymerase stimulation or PCNA loading by RFC from "*P. abyssi*."

S. D. Bell (MRC, Cambridge, United Kingdom) presented a model for coupling lagging-strand synthesis and Okazaki fragment processing in the crenarchaeon *S. solfataricus*. This is based on the finding that in vitro the three *Sulfolobus* PCNA-like factors assemble into heterotrimers in an ordered way, as indicated by surface plasmon resonance analyses. The *S. solfataricus* heterotrimeric PCNA was shown to simultaneously interact with DNA Pol B1, flap endonuclease 1, and DNA ligase I. A structural and functional characterization of flap endonuclease 1 from *Pyrococcus horikoshii* was reported by I. Matsui (National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan).

F. Matsunaga (Université de Paris-Sud) presented in vivo and in vitro studies on the mechanisms of DNA replication initiation in hyperthermophilic *Archaea*. He described the identification in "*P. abyssi*" (as well as in *S. acidocaldarius*) of short RNA-primed replication intermediates, whose structure is similar to that of Okazaki fragments from the eukaryal organisms. F. M. Pisani (CNR Institute of Protein Biochemistry) described the biochemical properties of a minichromosome maintenance protein-like DNA helicase from *S. solfataricus*. The strand displacement activity of this enzyme is stimulated by the *S. solfataricus* single-stranded-DNA-binding protein. This finding was further substantiated by demonstrating, with immunological techniques, that a physical interaction takes place between these two replication factors.

G. Lipps (University of Bayreuth) reported on the biochemical characterization of a novel protein encoded by the "*Sulfolobus islandicus*" plasmid pRN1. This 904-amino-acid polypeptide turned out to be a multifunctional replication factor which has ATP-dependent 3′-5′ DNA helicase activity, DNA polymerase activity, and DNA primase activity.

Among the posters on the mechanisms of DNA replication, recombination, and repair, the work carried out by A. Seybert from D. B. Wigley's laboratory (Clare Hall Laboratories, Herts, United Kingdom) on the characterization of RFC from *Archaeoglobus fulgidus* is worth mentioning. By analytical ultracentrifugation analyses it was demonstrated that the *A. fulgidus* RFC is a complex of one large and four small subunits. The contribution of the individual subunits to the overall ATPase activity of *A. fulgidus* RFC was investigated by mutagenesis, and strong evidence for allosteric regulation of nucleotide binding and hydrolysis during the PCNA loading cycle was reported.

A poster by E. Jolivet (IUEM-CNRS, Plouzane, France) and colleagues presented the analysis of cell response to ionizing radiation in hyperthermophilic *Archaea* isolated from deep-sea hydrothermal vents. "*P. abyssi*" and three *Thermococcus* strains were found to be highly radioresistant; however, none of these strains exhibited a specific DNA protection system. The mechanism of radioprotection is under investigation (9).

APPLICATIONS

Extremophiles are best known in all biomedical fields as the source of one of the most novel enzymes of last century: the thermostable DNA polymerase. The significant impact of *Taq* DNA polymerase from *Thermus aquaticus* on life science, in diagnostic techniques and in forensic applications, is undeniable; PCR is used worldwide for reverse genetics and genome sequencing and analysis. For these reasons, thermostable DNA polymerases are still the object of study, and efforts are being made to isolate enzymes which synthesize DNA with increased fidelity, higher speed, and longer extension. Therefore, it is not surprising that one-third of the reports presented in this session dealt with DNA polymerases from thermophiles. T. Imanaka (Kyoto University) described the "*Thermococcus kodakaraensis*" DNA polymerase, which is one of the most interesting enzymes to be applied in PCR, with an increased fidelity and extension rate. In a similar vein, A. Gardner (New England Biolabs, Beverly, Mass.) showed that archaeal DNA polymerases, and in particular the Vent DNA polymerase mutant Ala488Leu, have a remarkable preference for the incorporation of acyclo- and dideoxynucleotide triphosphates compared to the *Taq* polymerase. This peculiarity is exploited in DNA sequencing and single-nucleotide-polymorphism detection by incorporating dye-labeled terminators, and these polymerases are proposed to outcompete mesophilic and thermophilic analogs. Another example of how the study of thermophilic DNA polymerases can have a direct impact on the development of novel technologies for human health was the use of these enzymes for methylation-specific PCR (R. L. Momparler, Université de Montréal). This technique can be used to identify methylated cancer-related genes for early cancer diagnosis; thermostable DNA polymerases, which specifically amplify methylated targets, would widen the applicability of this technique.

Although DNA polymerases are certainly the best example of technologically important enzymes, the capability of extremozymes to best perform and to survive extreme operational condition is appealing for industrial applications. In this regard, enzymes involved in carbohydrate modification and binding are of particular interest. Cyclodextrin glycosyltransferases, amylomaltase, and glucan branching enzymes from moderate thermophiles can be used to exploit starch as a source of cyclodextrins and to reduce the viscosity of this polymer by maintaining its high molecular weight, and they can be used as thermoreversible gelling agents to replace gelatin, which is at high risk for harboring the agent of bovine spongiform encephalopathy (L. Dijkhuizen, University of Groningen). In addition, N. Aghajari (Centre National de la Recherche Scientifique, Lyon, France) reported on the transglycosylation

activity in the crystalline state of a psychrophilic α-amylase, which can be promising for the synthesis of oligosaccharides in solution, and S. D'Auria (CNR Institute of Protein Biochemistry) showed how a glucose dehydrogenase from a thermophile can be used to prepare a nonconsuming glucose sensor by exploiting the intact binding ability of the apoenzyme.

The applicability of extremozymes is often hampered by their limited availability from natural sources; a report from P. Bergquist (Macquarie University, Sydney, Australia) showed how fungal expression systems can be utilized to produce a variety of thermophilic proteins by using the cellulose-inducible promoter *cbh1*. In this regard, C. Schiraldi (Naples Second University) tackled the problem of the production and the downstream processing of hyperthermophilic glycosyl hydrolases expressed in *Escherichia coli* by an integrated approach involving a novel microfiltration bioreactor, followed by the permeabilization of the host cells and the immobilization of the biocatalysts.

In the more general picture of the novel frontiers in the application of extremophiles, an unexpected finding was that a RNA endonuclease from *Methanococcus jannaschii*, involved in vivo in the splicing of archaeal pre-tRNAs, is able to cleave RNAs in mammalian cells at specific sites and to promote RNA arrangements and *trans*-splicing events in cooperation with an endogenous ligase (G. Tocchini-Valentini, CNR Institute of Cell Biology, Rome, Italy); this novel activity could be used to modify the function of target RNAs.

MACROMOLECULAR STRUCTURE-FUNCTION RELATIONSHIPS

The molecular reasons for the adaptation of proteins from extremophiles to adverse environmental conditions is one of the most intriguing topics in this field, as confirmed by the mass of data accumulated over the years on extremozymes. A combination of computational, biochemical, and structural evidence supports the hypothesis that ion pair formation, hydrogen bonds, and hydration, rather than hydrophobic interactions, play relevant roles in the stabilization of enzymes from extremophiles. The reports of several groups showed the importance of ion pair interactions: an iron-sulfur protein from *S. acidocaldarius* (R. Ladenstein, Karolinska Institutet, Huddinge, Sweden), a triosephosphate isomerase from *Thermoproteus tenax* (R. Hensel, Universitat Essen), and a heat shock protein from *P. furiosus* (F. Robb, Center of Marine Biotechnology, Baltimore, Md.) show increased surface charges and electrostatic bonds between subunits. Moreover, the comparison of malate dehydrogenases from extremophiles (halophiles, thermophiles, and psychrophiles) and mesophiles by neutron scattering experiments suggested that salt bridges and ion binding would increase the resilience of the proteins from extremophiles (G. Zaccai, Centre National de la Recherche Scientifique, Grenoble, France). Similarly, Michael Danson (University of Bath) remarked that adaptation to high temperatures of citrate synthase was achieved by extended ionic networks both within and between the subunits. This conclusion was reached by careful inspection of the 3D structure of five enzymes from organisms growing optimally from 10 to 100°C. The importance of subunit interactions not only for thermostability but also for thermoactivity led this speaker, together with Roy Daniel (University of Waikato, Hamilton, New Zealand), to propose that an equilibrium model based on subunit dissociation can describe the variation of enzyme activity with temperature and time. This new concept of "real" temperature optimum (T_{opt}) best fitted with oligomeric enzymes, which show a T_{opt} significantly lower than would be expected from their thermostability (6). This model allows this phenomenon to be distinguished from cases of "apparent optimum," in which protein instability is the major factor ruling the enzyme thermal activity.

The high-resolution (1.85-Å) 3D structure of the alcohol dehydrogenase from *S. solfataricus* was presented (A. Zagari, University of Naples); the enzyme showed significant differences in the orientation of the catalytic domain and in the coordination of the catalytic zinc compared to other known structures. Moreover, comparison with the 3D structure of a mutant of the same enzyme showed that a single mutation (Asn249Tyr) decreases the coenzyme affinity and increases the activity about sixfold, even at low temperatures, with no effect on protein stability.

Interesting examples of engineered enzymes were also reported by G. Perugino (CNR Institute of Protein Biochemistry), who showed that thermophilic β-glycosyl hydrolases from *S. solfataricus*, *P. furiosus*, and *Thermosphaera aggregans* can be modified in their active sites to produce an innovative class of biocatalysts, named glycosynthases, which efficiently synthesize oligosaccharides but do not hydrolyze them.

Despite the large amount of biochemical and structural data available for enzymes from thermophiles, relatively little is known concerning psychrophilic enzymes. In this regard, G. Feller (University of Liège) showed by mutational analysis that α-amylase from the bacterium *Pseudoalteromonas haloplanktis* reaches the required conformational flexibility to perform catalysis at low temperature by increasing both the k_{cat} and the K_m. Mutational analysis and directed evolution experiments on an alkaline phosphatase and a chitinase from Antarctic bacteria led to similar conclusions (V. Bouriotis, University of Crete, Heraklion, Greece). In addition, the characterization of trypsin Y from Atlantic cod expressed in *Pichia pastoris* and *E. coli*, reported by H. Palsdottir (University of Iceland), showed that this enzyme has an unexpected predominant chymotrypsin activity and a subzero T_{opt}.

The section "Macromolecular Structure-Function Relationships" had the highest number of poster presentations, with more than 100, and although many reported interesting results, they are too numerous to be listed here. Poster P175, presented by J. Massant (Department of Microbiology, Vrije University, Brussels, Belgium), was of particular interest, as it dealt with a topic that is still largely unexplored: the in vivo stability of thermolabile substrates utilized by enzymes from hyperthermophiles. The authors provided evidence that the ornithine carbamoyltransferase and the carbamoylphosphate synthase from *P. furiosus* associate, forming a multienzyme complex, to prevent the thermodenaturation of carbamoylphosphate, a compound that has a half-life of less than 2 s under *P. furiosus* growth conditions.

In the conference closing lecture, G. Antranikian (Technical University Hamburg-Harburg) gave an expansive overview of the opportunities offered by modern molecular techniques for the exploitation of the genetic diversity of extremophiles and

unlocking nature's own technology. The genomes of extremophilic *Bacteria* and *Archaea* that have already been sequenced (about 20) and those in progress (more than 30) are expected to disclose a variety of biocatalysts with different physicochemical properties. Examples ranged from thermophilic enzymes for carbohydrate metabolism (amylases, CGTase, and branching and debranching enzymes) to psychrophilic lipases, proteases, cellulases, and proteases possessing catalytic activity below the freezing point of water.

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