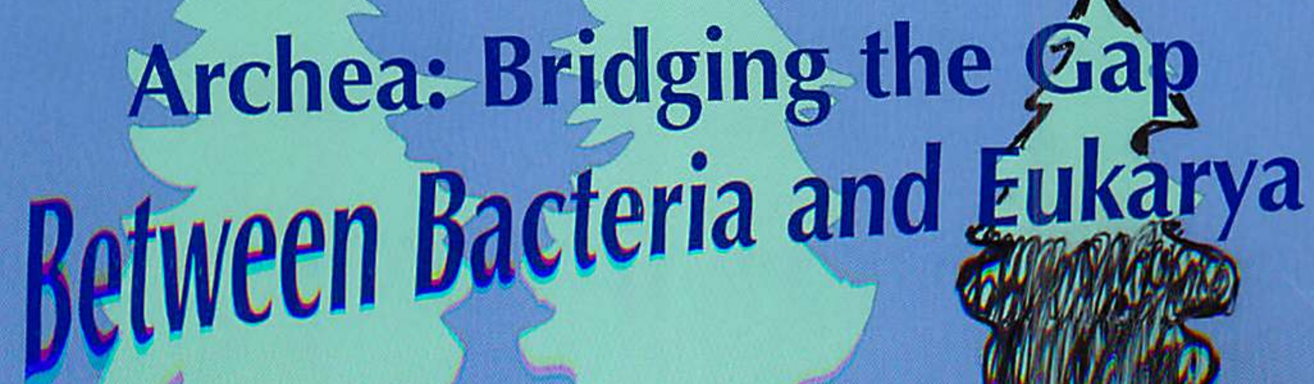


Archea: Bridging the Gap Between Bacteria and Eukarya

Organizers

Dieter Söll

William Whitman

Carl Woese

Car es

Taos, New Mexico

January 9-14, 1999

KEYSTONE SYMPOSIA

on Molecular & Cellular Biology

J Eisen

Shafer
Fernald
Lichtarge

rates of thermophiles vs mesophiles

molecular uses of evolution

good vs. bad evolutionary reconstructions

write paper on how gene trees cannot be representative

can you ID regions of protein prone to convergence

what genes have same phylogeny as RecA, rRNA

tx Deira w/ agent that only causes base damage

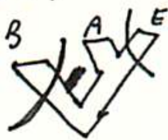
trees of proteosomes

AAO's in thermostable enzymes

correlate RecA's w/ growth T°

curated repair database

what does +/- genes and highest hit mean
- draw onto rRNA tree diff example



- A + TM can be similar if B+E lose genes
- A + TM can be similar if lat transfer (?)
- A + TM can be sim if slow rates

12
used for CH_3 fixation

RecBCDEF?J
RuvABC

2 pathways in Deino..
for Asn tRNA?
why?

Short Papers
- UvrA - ATP + duplication
- UvrB, Mfd, RecG
- Dead boxes
- MutY-Nth
- MutL
- RecQ
- SSB
- LON
- ADA - OGT

A Keystone Symposium

Archaea: Bridging the Gap Between Bacteria and Eukarya

Organizers: Dieter Söll, William Whitman and Carl Woese

Taos, New Mexico • January 9 - 14, 1999

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From the Chairman of the Board

Date: November 24, 1998

To: Friends, Supporters, and Attendees of the Keystone Symposia on Molecular and Cellular Biology

From: Edward A. Dennis, Chair, Board of Directors

The Keystone Symposia on Molecular and Cellular Biology is a free-standing, non-profit 501(c)3 organization dedicated to serving the international scientific community of life scientists. The Keystone Symposia has its origins in Symposia known as the California Membrane Conferences held at Squaw Valley in 1972 and 1973 under the sponsorship of the ICN Corporation. In subsequent years, an expanded number of meetings on a variety of biological topics were organized by Professor C. Fred Fox of the University of California, Los Angeles at various Western ski resorts. These conferences became known as the UCLA Symposia on Molecular Biology.

In 1990, the Symposia organization was transferred to the Keystone Center in Keystone, Colorado, a non-profit 501(c)3 organization which works in the area of Science and Public Policy and Education. The Keystone Center is the leading international mediation organization for resolving disputes involving private, governmental and/or non-profit groups in the environmental sciences. It also administers an innovative school aimed at training students and elementary school teachers in the environmental sciences. Until 1995, the Keystone Symposia, under the Chairmanship of first Dr. Pedro Cuatracasas, then President of Parke Davis Pharmaceutical Research, and then Professor Ralph Bradshaw of the University of California, Irvine, operated as one of the three divisions of the Keystone Center.

In October 1995, under the leadership of Professor Dennis Cunningham of the University of California, Irvine, the Keystone Symposia established itself as a non-profit organization with a phased transition to a complete separation from the Keystone Center. On September 5, 1997, under the Chairmanship of Professor Edward A. Dennis of the University of California, San Diego, this separation was completed and the Keystone Symposia on Molecular and Cellular Biology began a new era as a completely independent non-profit 501(c)3 organization devoted solely to providing outstanding scientific conferences in all areas of the biological and biomedical sciences.

The Keystone Symposia is administered by a Board of Directors with advice from a Scientific Advisory Board composed of outstanding scientists from the university, governmental and industrial sectors. Its excellent staff of 18 employees organizes some 30-40 conferences each year in a variety of Western ski resorts. Last year some 9,000 scientists participated in Keystone Symposia.

The mission of the Keystone Symposia is to develop and administer international symposia of the highest possible quality on topics of interest to researchers in biological, medical and agricultural sciences with the goal of facilitating interdisciplinary information exchange. Please feel free to contact myself or our Executive Director, Jim Bennett, for further information.

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A Keystone Symposium

Archaea: Bridging the Gap Between Bacteria and Eukarya

Organizers: Dieter Söll, William B. Whitman and Carl R. Woese

The Sagebrush Inn, Taos, New Mexico • January 9 - 14, 1999

Saturday, January 9

Time	Event	Location
2 - 7pm	Registration	Chamisa Lobby
6:30 - 7:30pm	Welcome	Chamisa Lobby
7:30 - 8pm	Orientation	Chamisa Ballroom 2
8 - 10pm	**KEYNOTE ADDRESSES**	

***Dieter Söll**, Yale University

Karl O. Stetter, University of Regensburg (025)
The Diversity of Archaea

Claire M. Fraser, The Institute for Genomic Research (012)
Microbial Genome Sequencing: A New Paradigm for Assigning Function from Sequence

---

Sunday, January 10

Time	Event	Location
7 - 8am	Breakfast	Los Vaqueros
8 - 11am	**GENOMIC ANALYSIS**	Chamisa Ballroom 2

***Claire M. Fraser**, The Institute for Genomic Research

Ronald W. Davis, Stanford University
Eukaryal Genomics

Gary Olsen, University of Illinois
Euryarchaeal Genomics

Coffee Break — Chamisa Lobby

Christoph W. Sensen, National Research Council of Canada (021)
Analyzing the Sulfolobus solfataricus P2 Genome

Jeffrey H. Miller, University of California-Los Angeles (015)
Analyzing the Pyrobaculum aerophilum Genome

Time	Event	Location
11am - 1pm	Poster Setup	Chamisa Ballroom 1
4:30 - 6:30pm	**POSTER SESSION 1: Integrating Genomics and Archaeal Biology**	Chamisa Ballroom 1
5:30 - 6:30pm	Social Hour	Chamisa Ballroom 1
8 - 10pm	**INTEGRATING GENOMICS AND BIOCHEMISTRY**	Chamisa Ballroom 2

(Coffee Available ~ Chamisa Lobby)

***John N. Reeve**, Ohio State University

Richard J. Roberts, New England Biolabs, Inc. (020)
Analysis of Restriction Modification Systems from Archaeal Genome Sequences

Patrick Forterre, Université de Paris-Sud (011)
DNA Topology and DNA Topoisomerases in Archaea: A Gold Mine for Topologists and a Puzzle for Evolutionists

August Böck, Universität München (003)
Evolution of Selenocysteine Biosynthesis and Incorporation

Dieter Söll, Yale University (023)
Novel Translational Components in Archaea

Current as of 11/18/98
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Archaea alive and kicking

Monday, January 11

7 - 8am	Breakfast	Los Vaqueros
8 - 11am	**PROCESSING AND MODIFICATION** ***Richard J. Roberts**, New England Biolabs, Inc. **Francine Beth Perler**, New England Biolabs, Inc. (017) *Inteins* **Charles J. Daniels**, Ohio State University (005) *Transfer RNA Maturation*	Chamisa Ballroom 2
	Coffee Break **James A. McCloskey**, University of Utah (014) *RNA Modification* **Nancy Maizels** or **Alan M. Weiner**, Yale University (029) *CCA Addition by tRNA Nucleotidyltransferase: Polymerization without Translocation?*	Chamisa Lobby
11am - 1pm	Poster Setup	Chamisa Ballroom 1
4:30 - 6:30pm	**POSTER SESSION 2: Molecular Processes of Archaea**	Chamisa Ballroom 1
5:30 - 6:30pm	Social Hour	Chamisa Ballroom 1
8 - 10pm	**TRANSCRIPTION AND TRANSLATION** (Coffee Available ~ Chamisa Lobby) ***Charles J. Daniels**, Ohio State University **John N. Reeve**, Ohio State University (019) *Archaeal Histones and Chromatin* **Michael Thomm**, Universität Kiel (028) *Mechanism and Regulation of Transcription in Archaea* **Albert E. Dahlberg**, Brown University (004) *Ribosome Structure and Function* **Patrick P. Dennis**, University of British Columbia (006) *Ribosomal RNA Processing in Sulfolobus acidocaldarius*	Chamisa Ballroom 2

Tuesday, January 12

7 - 8am	Breakfast	Los Vaqueros
8 - 11am	**LESSONS FROM ARCHAEAL STRUCTURAL BIOLOGY** ***Michael W. W. Adams**, University of Georgia **Wolfgang P. Baumeister**, Max Planck Institut für Biochemie (002) *Thermosomes and Proteasomes: From Archetypes to Complex Molecular Machines* **Paul B. Sigler**, Yale University (022) *Molecular Basis for Unidirectional Transcription in Archaea*	Chamisa Ballroom 2
	Coffee Break **Dino Moras**, Université de Strasbourg (016) *Which is the Best Structure of AspRS?* **Douglas C. Rees**, California Institute of Technology (018) *Structural Manifestations of Hyperthermostability in Proteins*	Chamisa Lobby
11am - 1pm	Poster Setup	Chamisa Ballroom 1
4:30 - 6:30pm	**POSTER SESSION 3: Archaeal Cellular Processes, Enzymes, and Evolution**	Chamisa Ballroom 1
5:30 - 6:30pm	Social Hour	Chamisa Ballroom 1

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Tuesday, January 12, continued

8 - 10pm	**ENZYME DIVERSITY** (Coffee Available ~ Chamisa Lobby) ***William B. Whitman**, University of Georgia **Willem M. de Vos**, Wageningen Agricultural University (007) *Glycolytic Enzymes and Their Control in Hyperthermophiles* **Rudolf K. Thauer**, Max Planck Institut für Terrestrische Mikrobiologie (027) *Structure and Catalytic Mechanism of Methyl-CoM-Reductase* **James G. Ferry**, Pennsylvania State University (010) *Contrasting the Physiology and Enzymology of Carbonic Anhydrases Across the Eucarya and Archaea Domains* **F. Robert Tabita**, Ohio State University (026) *Novel Ribulose Bisphosphate Carboxylase/Oxygenase Enzymes of Anoxic Archaea: How do They Work and What are They Doing?*	Chamisa Ballroom 2

Cakes

Jonnycakes

Wednesday, January 13

7 - 8am	Breakfast	Los Vaqueros
8 - 11am	**CELLULAR PROCESSES** ***Willem M. de Vos**, Wageningen Agricultural University **Michael W. W. Adams**, University of Georgia (001) *Hyperthermophilic Adaptations* **Yosuke Koga**, University of Occupational & Environmental Health, Japan (013) *Structure and Biosynthesis of Polar Lipids of Methanogenic and Other Archaea*	Chamisa Ballroom 2
	Coffee Break **William B. Whitman**, University of Georgia (030) *Examination of Biosynthetic Pathways Predicted by Genomic Sequencing in Methanococci* **John L. Spudich**, University of Texas Medical School (024) *Diversification of Function in the Archaeal Rhodopsin Family*	Chamisa Lobby
3 - 5pm	**EVOLUTION AND THE ORIGIN OF LIFE** (Coffee Available ~ Chamisa Lobby) ***Patrick Forterre**, Université de Paris-Sud **Russell F. Doolittle**, University of California-San Diego (008) *Exploring Archaeal Relationships with Protein Clocks* **W. Ford Doolittle**, Dalhousie University, Halifax (009) *Rethinking the Origin of Eukaryotes* **Günter Wächtershäuser**, München, Germany *The Chemoautotrophic Origin of Life*	Chamisa Ballroom 2
7-8pm	Social Hour	Chamisa Ballroom 1
8 - 10pm	Banquet	Chamisa Ballroom 2
9pm - 12am	Entertainment	Chamisa Ballroom 2

Thursday, January 14

Departure

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001 - Adams

Hyperthermophilic Adaptations

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Pyrococcus furiosus (Pf) is being used as a model system to investigate how the primary metabolic pathways of hyperthermophilic archaea are "adapted" to life at extreme temperatures. Pf grows optimally near 100°C by fermenting both peptides and various carbohydrates. It metabolizes carbohydrates by an unusual Embden-Meyerhof pathway in which glyceraldehyde-3-phosphate oxidation is catalyzed by a ferredoxin (Fd)-linked glyceraldehyde-3-phosphate oxidoreductase (GAPOR) rather than by the expected NAD-linked dehydrogenase. GAPOR is unusual in that it contains tungsten, a metal rarely used in biological systems. The pathways of amino acid fermentation in Pf involve transaminases to generate the corresponding 2-keto acid, and four distinct CoA- and Fd-dependent oxidoreductases which produce the CoA derivatives as well as the corresponding aldehyde. It is thought that these aldehydes are oxidized to the corresponding acid by another tungsten-containing enzyme which is termed aldehyde Fd oxidoreductase (AOR). In addition, a third tungstoenzyme, formaldehyde Fd oxidoreductase (FOR), has been purified from Pf, and this enzyme appears to be involved in the catabolism of basic amino acids. Moreover, genomic analyses suggest that Pf contains two additional tungstoenzymes, the functions of which are unknown. This presentation will address the key questions, what is the function of tungsten in the Pf enzymes, do other hyperthermophilic archaea contain tungsten-dependent enzymes, and to what extent is the utilization of this element an adaptation to life near 100°C?

002 - Baumeister

Thermosomes and Proteasomes: From Archetypes to Complex Molecular Machines

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Thermosomes and proteasomes are large multisubunit complexes which play key roles in cellular protein folding and in protein degradation respectively.

20S proteasomes, which are found in all three domains of life are self-compartmentalizing proteases, i.e. the subunits assemble such that the potentially hazardous proteolytic action is confined to an inner compartment. Access to this proteolytic compartment is restricted to unfolded proteins; therefore the proteasome must interact with regulatory complexes which assist in the unfolding and translocation of substrates. While the basic architecture of proteasomes is conserved from archaea to higher eukaryotes there is an increase in complexity resulting from multiple gene duplications and the acquisition of whole modules of subunits. The arrangment of subunits in the eukaryotic 26S proteasome bears testimony of its evolutionary history.

The thermosome, the chaperonin of the archaea, is closely related to the chaperonins found in the cytosol of eukaryotes know as TRiC or CCT; together they are classified as the group II chaperonins. They are more distantly related to the group I chaperonins comprising the bacterial chaperonins (GroEL) and their endosymbiotic descendants. They form toroidal, mostly hexadecameric structures, which in archaea are built from one or two types of subunits, and in eukarya from eigth distinct though related subunits. The evolution of exactly eight gene families seems to have been determined by the fact that eight subunits can be accommodated in each of the two rings in which each subunit occupies a distinct position.

003 - Böck

Evolution of Selenocysteine Biosynthesis and Incorporation

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In bacteria, the amino acid selenocysteine is synthesised from a serine residue charged to a specialised tRNA, $tRNA^{Sec}$, by seryl-tRNA-synthetase. The synthesis involves two enzymes, selenocysteine synthase which exchanges the hydroxyl of serine by a selenol moiety and selenophosphate synthetase, which provides the activated selenium donor, selenophosphate. The incorporation into protein requires a specialised UGA codon and an mRNA structure, the SECIS element, which in bacteria is located immediately 3' of this codon. A specific translation factor, SelB, binds to the SECIS element and carries selenocysteyl-$tRNA^{Sec}$ to the ribosomal A-site.

The basic principle of this tRNA-bound synthesis is conserved in *Archaea* and *Eucarya* although a homolog of the bacterial selenocysteine synthase could not be identified yet. Selenophosphate synthetase, on the other hand, is well conserved in sequence and - with some deviations concerning the composition of the active site amino acids - also in function. A most intriguing difference, however, is that the SECIS element of *Eucarya* and *Archaea* is located in the 3' nontranslated region of the mRNA compared to the location within the reading frame in Bacteria. To resolve the mechanism of the out-of-frame SECIS we have purified $tRNA^{Sec}$ from *Methanococcus jannaschii*, charged it with serine and converted the serine to selenocysteine using the *E. coli* purified selenocysteine biosynthesis enzymes. With the aid of the selenocysteyl-tRNA the SelB homolog of *M. jannaschii* could be identified. Its guanine nucleotide binding characteristics are similar to those of *E. coli* SelB. Whether this SelB homolog also binds the SECIS element is presently under study.

004 - Dahlberg

Ribosome Structure and Function

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A variety of techniques are now being used to examine the structural and functional roles of ribosomal RNA in protein synthesis. In our laboratory we have taken a genetic approach, utilizing a plasmid-encoded rDNA operon. Site directed mutants are introduced at different sites in E. coli 16S and 23S rRNA in an attempt to define specific structural and functional features. An equally useful approach has been random mutagenesis followed by phenotypic selection of suppressor mutants. Using different strains and plasmid constructs it is possible to regulate expression of either the plasmid-encoded or host-encoded rRNA.

Several current projects will be discussed including a putative base-pairing interaction between an enhancer sequence in mRNA and a nucleotide sequence near the 3' end of 16S rRNA, a conformational shift in 16S rRNA during translation, and the utilization of a new approach for isolating antibiotic resistant mutants in rRNA to characterize the tRNA P site in the 30S ribosomal subunit. These projects represent examples by which one can examine the general and specific features of rRNA structure and function in the Bacteria, Eukarya and Archaea. This research was supported by NIH grant GM19756.

005 - Daniels

Transfer RNA Maturation

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Maturation of tRNA primary transcripts follows a complex series of reactions that involves removal of 5' and 3' flanking sequences, addition of the 3' terminal CCA residues, base and sugar modifications, and in some instances the removal of introns. Many of these enzymes are ubiquitous, whereas some appear to be restricted to members of one or two of three Domains: Archaea, Bacteria and Eucarya. This mosaic of characteristics is illustrated in the enzyme systems for intron removal in the Archaea and in the role played by some tRNA introns in tRNA maturation. Early studies on archaeal and eucaryal tRNA intron endonucleases led to the hypothesis that these two groups of introns and their processing systems were evolutionarily distinct. Recent sequence and biochemical studies have established that these enzymes are distantly related and that they are likely to share a common catalytic core. In related studies, we have observed that the archaeal tRNATrp introns possess an unusually high degree of sequence conservation, suggesting that these introns might have a function beyond their participation in intron removal. Close examination of these introns revealed that these conserved sequence elements resembled eucaryal C/D box small nucleolar RNAs (snoRNAs), which are involved in 2'-O-methylation of rRNA in nucleolus. In vitro assays with the Haloferax volcanii intron-containing tRNATrp RNA indicated that this intron directed 2'-O-methylation of residues in both exons, confirming its participation in maturation of its host tRNA. Comparative studies with other members of the Archaea indicated that protein components of the eucaryal snoRNA mediated modification system (fibrillarin and Nop5/56 proteins) are also present in these organisms and provide evidence for the occurrence of this processing system in the shared ancestor to the archaeal and eucaryal Domains.

006 - Dennis

Ribosomal RNA processing in *Sulfolobus acidocaldarius*

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The hyperthermophilic archaeon *Sulfolobus acidocaldarius* contains a single copy 16S-23S rRNA operon in its genome. *In vivo* the primary rRNA transcript is cleaved endonucleolytically 5' to positions -98 and -31 in the 144 nt long 5' ETS and at position +1 at the 5' ETS-16S junction. These cleavages can be reproduced using cell free extract and a synthetic RNA substrate containing the entire 5' ETS and the first 68 nt of the 16S rRNA sequence. Using this assay, a complex and unusual endonuclease activity has been purified to near homogeneity and the features of the substrate that are necessary for accurate and efficient cleavage have been characterized. In crude cell lysates, the activity is sensitive to micrococcal nuclease digestion. On a low salt glycerol gradient, the activity from a crude cell lysate sediments as a broad heterogenous peak at about 55S. After this initial sedimentation, the activity is resistant to nuclease digestion and is already well separated from the bulk of the *Sulfolobus* NOP1 (fibrillarin) and NOP56-58 equivalent proteins (as judged by western blotting). Resedimentation in high salt results in a reduction in the size of the complex to about 20S. Two subsequent chromatographic steps result in activity that is more than 2000-fold pure, contains three major polypeptides, is free of detectable RNA and is highly active in cleavage at positions -98 and -31 and somewhat less active in cleavage at the 5' ETS-16S junction. The recovery of activity appears to correlate with the recovery of one of these polypeptides; an N-terminal sequence has been obtained and efforts to clone the gene have been initiated. The 5' ETS RNA substrate contains three regions of stable secondary structure; the three cleavage sites at positions -98, -31 and +1 are located in unstructured regions following these duplex structures. The consensus at the site of cleavage is (G/A)|(C/A)UU with cleavage occurring 3' to the terminal purine. Deletion analysis indicates that only the consensus sequence at a particular site (and not the associate regions of secondary structure) is required for accurate and efficient cleavage. Moreover, the presence of a purine nucleotide 5' to the site of cleavage is essential.

007 - de Vos

Glycolytic Enzymes and Their Control in Hyperthermophiles.

Willem M. de Vos Laboratory of Microbiology, Department of Biomolecular Sciences, Wageningen Agricultural University, NL

Sugar metabolism in hyperthermophilic Archaea differs from the canonical glycolytic pathways, involves novel enzymes, and shows unique control. In recent years, we have focused our attention on three novel glycolytic enzymes, *viz.* ADP-dependent glucokinase, ADP-dependent phosphofructokinase, and glyceraldehyde-3-phosphate oxidoreductase. These enzymes were first discovered in *Pyrococcus furiosus* but now have found to be distributed among a variety of phylogenetically unrelated hyperthermophilic Archaea. The biochemical properties of these enzymes purified either from their original host or overproducing *E.coli* strains, their genetic organization and expression, and the bioenergetic consequences of their activities will be discussed in relation to their presence in the various Archaea.

008 - Doolittle, R.

Exploring Archaeal Relationships With Protein Clocks

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That the Archaea form a distinctive domain of life was made abundantly clear more than twenty years ago on the basis of (a) ribosomal RNA sequences and (b) a number of biochemical attributes, including the exclusive use of ether linkages in their membranes. Many other characters, however, are shared only with eubacteria, on the one hand, or with eukaryotes, on the other. The situation with regard to protein sequences reflects this unusual trichotomy, making it very difficult to use protein clocks to gauge divergence times. We have attempted to get around the problem by grouping proteins according to their basic phylogeny. Thus, there is a group in which the archaeal sequences are clearly more similar to those of eukaryotes than they are to eubacterial ones. Another group has archaeal sequences being more similar to eubacterial ones, and still a third has the Archaea as the outliers. Many classes of enzyme are found in all three groups. Two kinds of explanation have been explored: (a) rampant horizontal gene transfer, and (b) differential gene loss along different lineages after widespread duplication in early systems.

009 - Doolittle, W.

Rethinking the Origin of Eukaryotes

W. Ford Doolittle. Program in Evolutionary Biology, Canadian Institute for Advanced Research, Department of Biochemistry, Dalhousie University, Halifax, Nova Scotia, B3H 4H7, Canada.

There is at the moment general confidence in the distinctness and coherence of the Archaea and Bacteria, in the notion that eukaryotic cells arose by internal complexification from an archaea-like ancestor, in the (alpha-proteobacterial) endosymbiotic origin of mitochondria and in the primitivity of certain deeply diverging lineages of protists. However, as more genome sequence information becomes available, archaeal and eukaryotic genomes start to look chimeric. It is not clear that there are any genes whose products are so tightly integrated in cellular function that they cannot be replaced. I will review some examples of transfer of genes for integrated functions, and then discuss what kinds of modifications to current theories for eukaryotic origins such data might require. I will ask whether the concept of genomic lineage remains appropriate for deep phylogeny.

010 - Ferry

Contrasting the Physiology and Enzymology of Carbonic Anhydrases across the Eucarya and Archaea Domains

James G. Ferry, Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802, USA.

All carbonic anhydrases are zinc enzymes catalyzing the interconversion of CO_2 and HCO_3^-. The enzyme has been found in virtually every mammalian tissue and cell type where it primarily functions in carbon dioxide and ion transport. Carbonic anhydrase is also abundant in plants and green unicellular algae where it is essential for photosynthetic CO_2 fixation. In contrast, few carbonic anhydrases have been documented in procaryotes. Three distinct classes (α,β, and γ) are proposed based on sequence comparisons. All sequenced carbonic anhydrases from mammals belong to the α class. Plant chloroplast carbonic anhydrases belong to the β class. The enzymes from *Neisseria gonorrheae* and *Escherichia coli* belong to the α and β classes, respectfully. The enzyme purified from the methanoarchaeon *Methanosarcina thermophila* is the only documented γ carbonic anhydrase. No other carbonic anhydrases from the Archaea domain have been identified except for a recently discovered β enzyme in *Methanobacterium thermoautotrophicum*. Crystal structures are available for four mammalian α carbonic anhydrases and the *M. thermophila* γ enzyme. Beyond the ligation of zinc with three histidines, these structures bear no resemblance suggesting convergent evolution. A structure has not been reported for any β carbonic anhydrase. Relatively little is known concerning physiological functions for procaryotic carbonic anhydrases. Carbon dioxide is pervasive in procaryotic metabolism where a variety of functions can be envisioned for carbonic anhydrase; however, the extent to which the three classes occur in the Bacteria and Archaea domains, their molecular structures, and their role in metabolism is vaguely understood. A survey of phylogenetically and physiologically diverse microbes from the Bacteria and Archaea domains suggest that the β and γ carbonic anhydrases are wide spread in both domains and have roles in a variety of metabolic processes. The implications for the occurrence of carbonicanhydrases in procaryotes and the biochemical properties of the archaeal β and γ enzymes will be discussed.

011 - Forterre

DNA topology and DNA topoisomerases in Archaea : a goldmine for topologist and a puzzle for evolutionists.

Patrick Forterre, Cyril Buhler, Danièle Gadelle, Jim C. Wang and Agnes Bergerat.*Institut de Génétique et Microbiologie, Bat 409, Université Paris-Sud, 91405 Orsay Cedex, France, and *Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts 02138, USA*

All cellular organisms contain enzymes that can change the number of topological links in DNA molecules, making transient single-stranded or double-stranded breaks into the phosphodiester backbone (type I and type II DNA topoisomerases, respectively) and forcing the crossing of DNA molecules through each others. During the last years, novel and completely unexpected DNA topoisomerases have been discovered in Archaea, namely reverse gyrase, DNA topoisomerase V and DNA topoisomerase VI.

Reverse gyrase, which combines a helicase and a type I DNA topoisomerase modules into a single polypeptide and produces positive supercoiling, has now been also detected in hyperthermophilic bacteria. In contrast, DNA topoisomerase VI (Topo VI), which is the prototype of a novel family of type II DNA topoisomerase, is only present in Archaea. However, the characterization of one of its two subunits helped in the identification of the protein which initiates meiotic recombination in eucaryotes (SPO11).

We will briefly summarize the different topoisomerase activities which have been identified in Archaea and their distribution in the archaeal domain. We will also present our recent advances in the characterization of the *Sulfolobus shibatae* Topo VI. The somewhat erratic distibution of topoisomerases in-between and inside domains will be discussed in relation with present (contradictories) hypotheses about the universal tree of life, but also in relation with the possibility that some present-day cellular proteins involved in DNA metabolism might have a viral (or plasmidic) origin.

012 - Fraser

Microbial Genome Sequencing: A New Paradigm for Assigning Function from Sequence

Claire M. Fraser. The Institute for Genomic Research, 9712 Medical Center Drive, Rockville, MD 20850, USA.

The sequences of more than 20 microbial genomes have been completed during the past three years and work in progress suggests that the number of completed genomes will increase to almost 100 by the end of the decade. With a gene density in microbes on the order of one gene / kbp of DNA sequence and an average genome size of 2-3 Mbp, completed microbial genome sequences will soon represent a collection of more than 200,000 predicted coding sequences. While tremendous biological insights on any given organism derive from analysis of a single genome sequence, we believe that comparative analysis of multiple genomes provides substantially more information on the physiology and evolution of microbial species as well as the ability to better assign putative function to predicted coding sequences. Several examples of how comparative genomics can be exploited to better understand biology will be discussed.

013 - Koga

Structure and Biosynthesis of Polar Lipids of Methanogenic and Other *Archaea*

Yosuke Koga, Masateru Nishihara, and Hiroyuki Morii. Department of Chemistry, University of Occupational and Environmental Health, Kitakyushu 807-8555, JAPAN.

Complete structures of predominant polar lipids from seven species of methanogenic *Archaea* have been elucidated. Four varieties of diether type core lipids and two varieties of tetraether type core lipids were recognized throughout methanogen lipids. Seven kinds of phosphodiester-linked polar groups and three kinds of monosaccharides were found. The distribution of these lipid component parts was analyzed for almost 40 species. It was concluded from the results that lipid structure and composition are determined by the phylogenetic relationship of *Archaea*. The most fundamental distinction of archaeal lipids is the stereochemistry of the glycerophosphate backbone of phospholipids (*sn*-G-1-P). Biosynthesis and maintenance mechanisms of the G-1-P backbone structure are primarily depending on the stereospecificity of G-1-P dehydrogenase, and secondly are depending on G-1-P prenyltransferase. Recently we found third enzyme of etherphospholipid biosynthesis (CDP-unsaturated archaeol synthase). The enzyme studied using various synthetic substrates showed to be specific to geranylgeranyl chains but not stereospecific. The amino acid sequence of G-1-P dehydrogenase does not share any sequence similarity with that of biosynthetic G-3-P dehydrogenase of *E. coli*. Because it would be almost impossible to interchange stereospecificity of the enzymes, it appears likely that the stereostructure of membrane phospholipids of a cell must be maintained from the time of birth of the first cell, when *Archaea* and *Bacteria* would be differentiated.

014 - McCloskey

RNA Modification

James McCloskey, Department of Medicinal Chemistry, University of Utah, Salt Lake City, UT 84112, USA.

While posttranscriptional modification of RNA in general serves to modulate regional structural features, in the thermophilic Archaea (primarily Crenarchaeota) it also appears to play a significant role in thermal stabilization. Ninety-five naturally occurring modified nucleosides are currently known in all forms of RNA (see http://www-medlib.med.utah.edu/RNAmods/RNAmods.html). Greater structural diversity of nucleoside modifications are found in archaeal tRNA (39 known nucleosides), although ribosomal RNA is relatively understudied both with regard to modification structures (16 nucleosides) and their functions. Modification motifs in tRNA are generally more eukaryotic than bacterial; nine nucleosides are unique to the archaea. *Sulfolobus solfataricus* P2 16S rRNA is being studied as a model hyperthermophile for sequence placement and characterization of modified residues. A mean of approximately 38 modified sites are present (70-75°C culture) with relatively heavy ribose methylation, the levels of which appear to increase with culture temperature.

015 - Miller

Analyzing the *Pyrobaculum aerophilum* genome

Sorel Fitz-Gibbon, Department of Microbiology and Molecular Biology Institute, University of California, Los Angeles, Ung-Jin Kim, Biology, California Institute of Technology, Heidi Ladner, Department of Microbiology and Molecular Biology Institute, University of California, Los Angeles, Elizabeth Conzevoy, Department of Microbiology and Molecular Biology Institute, University of California, Los Angeles, Barbara Perry, Biology, California Institute of Technology, Gigi Park, Biology, California Institute of Technology, Karl Stetter, University of Regensburg, Germany, Melvin I. Simon, Biology, California Institute of Technology, Jeffrey H. Miller, Department of Microbiology and Molecular Biology Institute, University of California, Los Angeles.

Pyrobaculum aerophilum is a hyperthermophilic archaeon isolated from a boiling marine water hole that is capable of growth at 104'C. This microorganism can grow aerobically, unlike most of it's thermophilic relatives, making it amenable to a variety of experimental manipulations and a candidate as a model organism for studying archaeal and thermophilic microbiology. We have sequenced the entire genome using a random shotgun approach (3.5X genomic coverage) followed by oligonucleotide primer directed sequencing, guided by our fosmid map. We will report the analysis of the annotated genome.

016 - Moras

Which is the best structure of AspRS ?

D. Moras, Laboratoire de Biologie Structurale, IGBMC, BP163, 67400 Illkirch-Strasbourg, France.

The crystal structure of aspartyl-tRNA synthetase (AspRS) from *Pyrococcus kodakaraensis* was solved at 1.9 Å resolution. The sequence and three-dimensional structure of the catalytic domain are highly homologous to those of eukaryotic AspRSs notably the absence of a large insertion domain present in eubacterial enzymes. In contrast, the N terminal domain, whose function is to bind the tRNA anticodon, is more similar to that of eubacterial proteins. Its structure explains the unique property of archaeal AspRSs to accommodate both $tRNA^{Asp}$ and $tRNA^{Asn}$. A comparison with the known 3D structures of the enzyme from *yeast*, *E. coli* and *Th. thermophlus* clarifies the role of residues present in both archaeal and eukaryotic AspRSs, but absent from the eubacterial enzymes, will be discussed.

017 - Perler

Inteins

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Since the discovery of protein splicing in 1990, more than 70 putative protein splicing elements, termed INTEINS, have been identified in archaea, eubacteria and eucarya (see the INTEIN REGISTRY at InBase: http://www.neb.com/neb/inteins.html). These in-frame insertions (134-548 amino acids) are precisely excised post-translationally from a precursor protein and the flanking sequences are ligated to form a native peptide bond. The protein splicing mechanism has been determined, including roles for many conserved residues. The process is autocatalytic and the intein plus the first downstream residue contain sufficient information to mediate protein splicing. Many inteins are bifunctional proteins with homing endonuclease and protein splicing activities encoded by separate structural domains. The presence of endonuclease activity allows the efficient transfer of intein genes into inteinless alleles. Inteins can thus be inherited both vertically and horizontally. Understanding the mechanism of splicing has allowed researchers at NEB to modify inteins for use in protein purification, protein semisynthesis, peptide ligation, control of enzyme activity and specific modification of selected regions of a single protein.

018 - Rees

Structural Manifestations of Hyperthermostability in Proteins

Douglas C. Rees, Howard Hughes Medical Institute, Division of Chemistry 147-75CH, California Institute of Technology, Pasadena, CA 91125, USA

One of the fascinating questions raised by the existence of hyperthermophilic organisms concerns the stabilization mechanisms for both small and large molecules at elevated temperatures. For the case of hyperthermostable proteins, recent structural and thermodynamic analyses have provided a foundation for addressing these issues. The most general conclusions from these studies are that hyperthermostability is not associated with dramatic increases in the maximal stability of a protein, and that it can be achieved without the requirement for any new types of interactions to stabilize the folded conformation. Rather than being the consequence of any one dominant type of interaction, it appears that the increased thermostability of these proteins reflects a number of subtle interactions involving electrostatic interactions (hydrogen bonds and salt bridges), increased secondary -structure stabilization, surface energies and packing effects.

019 - Reeve

Archaeal Histones and Chromatin

John N. Reeve, Kathryn A. Bailey, Wen-Tyng Li, Suzette L. Pereira, Divia J. Soares and Kathleen Sandman. Department of Microbiology, The Ohio State University, Columbus, OH 43210

All euryarchaeotal genomes sequenced to date contain archaeal histone encoding genes and over 20 very similar but nevertheless different archaeal histone sequences are now available. Residues predicted to be responsible for histone dimer and tetramer formation, for DNA binding and for histone fold stabilization have been changed and structure-function-stability assays of the resulting variants undertaken. Archaeal histones assemble as tetramers into structures, designated archaeal nucleosomes, that circularize linear DNA, protect ~60 bp from micrococcal nuclease digestion, and appear very similar to the structure formed by the histone $(H3+H4)_2$ tetramer core at the center of the eukaryal nucleosome. Archaeal nucleosome positioning sequences have been identified, consistent with a direct role in gene expression, and the possibility that the archaeal nucleosome was the antecedent of the contemporary eukaryal nucleosome will be discussed. Histone fold domains have also recently been identified in several eukaryal transcription factors, and MJ1647 from *Methanococcus jannaschii* encodes an archaeal histone fold with a C-terminal extension. rMJ1647 synthesized in *E. coli* has DNA binding properties consistent with an archaeal histone and removal of the C-terminal extension does not effect the histone fold, dimer formation or DNA wrapping.

020 - Roberts

Analysis of Restriction Modification Systems from Archaeal Genome Sequences.

Richard Roberts, Janos Posfai, Jay Patti, Devon Byrd, Tanya Osterfield and Richard Morgan. New England Biolabs, 32 Tozer Road, Beverly, MA 01915, USA.

Until recently all of the 3000 restriction enzymes known to man had been found by obtaining bacteria and archaea from culture collections or environmental samples and assaying them biochemically and genetically. During the last 15 years, many of these systems have been cloned and sequenced and it is now possible to use quite sophisticated search algorithms to screen new DNA sequences for the presence of methylase genes. Experience among known systems has shown that restriction enzyme genes are found close to their cognate methylase genes.

Analysis of several archaeal genome sequences has been conducted and in *Methanococcus jannaschii*, there are many more methylase genes than would have been expected on the basis of previous biochemical screening. In some cases they could be predicted to form part of restriction modification systems because the adjacent open reading frames were similar to known restriction enzyme genes. Very often, though, the adjacent open reading frames had no homologs in GenBank and were thus candidates either for new specificities or for previously uncloned genes. We have been developing methods to allow these candidate genes to be tested biochemically. Results for *M. jannaschii* and other genomes have been very promising and include the finding of one new specificity that had never been seen before. It seems clear that screening DNA sequence databases will be a very productive method to find restriction enzymes with new specificities.

021 - Sensen

Analyzing the *Sulfolobus solfataricus* P2 genome

Christoph W. Sensen and the Sulfolobus team, National Research Council of Canada, Institute for Marine Biosciences, 1411 Oxford Street, Halifax, NS, Canada B3H 3Z1. E-mail: sensencw@niji.imb.nrc.ca.

The 3 Mbp *Sulfolobus solfataricus* P2 genome is sequenced in an international collaborative effort, including laboratories in Canada, Denmark, France and The Netherlands. More than 80% if the sequence is completed (Sept. 13, 1998). The *Sulfolobus* genome is one of only two crenarchaeote genomes sequenced to date. The genomic data offer an interesting insight into crenarchaeote genome organization.

022 - Sigler

MOLECULAR BASIS FOR UNIDIRECTIONAL TRANSCRIPTION IN ARCHAEA

Otis Littlefield[†&], *Peter Kosa*[†&], *Steven Bell*[*], *Steven Jackson*[*], *Yakov Korkhin*[†&], *and Paul B. Sigler*[†&]; [†]*Departments of Molecular Biophysics and Biochemistry and the* [&]*Howard Hughes Medical Institute, Yale University, New Haven, CT 06511 and* [*]*The Wellcome/CRC Institute, Cambridge, UK.*

The archaeal promoter appears to be a slowly evolving, "stripped down", version of TATA-dependent eukaryal promoters. This has been confirmed progressively with the sequencing of archaeal genomes. Archaeal promoters contain TATA-boxes 20 to 30 base pairs upstream of the transcriptional start site. In archaeal systems, promoter specific, efficient, unidirectional *in vitro* transcription requires only the two basal factors, TBP and TFB (orthologue of TFIIB), both of which have been cloned and overexpressed, purified and crystallized; and the polymerase, which has been purified in multi-milligram amounts. Moreover, there are no archaeal homologues of TFIIA, IIF, IIG, IIH, or SRB's in the archaeal genome. As sequence homology and functional studies would suggest, our crystal structures of archaeal TBP and TBP/TFB/TATA complex showed them to be essentially identical to their eukaryotic pol II counterparts. All but the smallest three subunits of archaeal RNA polymerase subunits share homology with those of eukaryal pol II leading us to believe the arrangement and function of the archaeal components in the preinitiation complex will emulate their counterparts in eukaryotes. In vitro transcription experiments have established that unidirectional transcription is dependent upon an interaction between TFB and a six base pair promoter segment immediately upstream of the TATA-box. This is consistent with results in higher eukarya where TFIIB contacts a similar region (but different consensus sequence) in the pol II promoter. Diffraction quality crystals are under study which contain TBP and TFB in complex with a promoter fragment containing a contiguous directional signal and the TATA box. Thus, the archaeal systems may serve as simplified and robust models of the eukaryotic basal preinitiation assembly.

023 - Söll

Novel Translational Components in Archaea

D. Söll

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The accurate synthesis of aminoacyl-tRNAs is essential for faithful translation of the genetic code and is assumed to be one of the most orthologous processes in biology. This dogmatic view was called into question by the sequencing of a number of archaeal and bacterial genomes which did not contain genes encoding a significant number of aminoacyl-tRNA synthetases. For example, the genomic sequences of the euryarchaeotes *Methanococcus jannaschii* and *Methanobacterium thermoautotrophicum* do not contain open reading frames encoding homologs of the canonical asparaginyl-(AsnRS), cysteinyl-(CysRS), glutaminyl-(GlnRS) and lysyl-tRNA synthetases (LysRS). The use of two-step (indirect) aminoacylation pathways for the formation of Asn-$tRNA^{Asn}$ and Gln-$tRNA^{Gln}$ in some organisms circumvents the need for the enzymes which catalyze one step formation of these molecules, AsnRS and GlnRS. It has now been found that several archaea and bacteria contain a functional class I LysRS with no resemblance to canonical class II LysRSs, with the bacterial examples apparently arising by horizontal gene transfer from archaea. Thus, non-orthologous replacement of essential enzymes such as AsnRS, GlnRS and LysRS is widespread in both Archaea and Bacteria, indicating that while the process of translation is orthologous its constituents are not. This surprising conclusion is supported by the finding that the closely related bacteria *Deinococcus radiodurans* and *Thermus thermophilus*, rather than lacking certain aminoacyl-tRNA synthetases, are able to synthesize Asn-$tRNA^{Asn}$ and Gln-$tRNA^{Gln}$ using both the aminoacylation and transamidation pathways.

024 - Spudich

Diversification of Function in the Archaeal Rhodopsin Family

John Spudich, Department of Microbiology and Molecular Genetics, University Of Texas Medical School, Houston, Texas 77030, USA.

The archaeal rhodopsins are a family of photoactive proteins found in the membranes of *Halobacterium salinarum, Natronobacterium pharaonis, Haloarcula vallismortis* and other halophilic archaeons. Each consists of a single polypeptide that folds into 7 a-helical membrane-spanning segments forming an internal pocket lined by 22 highly conserved residues where the chromophore retinal is bound. The physiological functions of the archaeal pigments divide them into two classes: transport rhodopsins (bacteriorhodopsin, BR, and halorhodopsin, HR) and sensory rhodopsins (SRI and SRII). The former use photoisomerization of retinal to drive electrogenic translocation of ions across the membrane, while the latter use similar photochemical reactions to generate non-electrogenic phototaxis signals via a protein-protein interaction cascade controlling the cells' flagellar motors. The talk will describe recent experiments that shed light on how the common molecular design of archaeal rhodopsins has been modified by nature to provide these distinctly different functions (1).

While sharing a 7-transmembrane-helix structure and retinal binding capacity with the archaeal rhodopsins, visual pigments do not exhibit readily recognizable primary sequence homology. Given the large evolutionary distances between the Archaea and higher animals, the lack of sequence similarity may be a consequence of divergence. The recently observed detailed similarities in the molecular mechanisms of photoactivation of archaeal and visual rhodopsins (2,3) argue for a common origin, or provide a striking example of convergent evolution in protein chemistry, if independent origins are assumed. In keeping with the theme of the meeting, the extensive and compelling evidence for the existence of archaeal rhodopsin-like photosensory proteins in eukaryotic microorganisms will be discussed.

1. Spudich, J.L. (1998) Variations on a molecular switch: transport and sensory signaling by archaeal rhodopsins. Molecular Microbiology 28:1051-1058.
2. Spudich, E.N., Zhang, W., Alam, M., and Spudich, J.L. (1997) Constitutive signaling of the phototaxis receptor sensory rhodopsin II from disruption of its protonated Schiff base-Asp73 salt bridge. Proc. Natl. Acad. Sci. USA. 94:4960-4965.
3. Hoff, W.D., Jung, K.-H. and Spudich, J.L. (1997) Molecular Mechanism of Photosignaling by Archaeal Sensory Rhodopsins. Annu. Rev. Biophys. Biomolec. Struct. 26:223-258.

025 - Stetter

The Diversity of Archaea

Karl O. Stetter, Lehrstuhl für Mikrobiologie, University of Regensburg, D-93053 Regensburg, Germany.

Within the 16S rRNA-based phylogenetic tree of life, archaea represent the third domain of life in addition to the bacteria and eukaryotes. After the discovery by Carl Woese, members of archaea have been isolated so far exclusively from environments "extreme" by anaerobiosis, salinity, heat and thermoacidity which are hostile to ambient forms of life. These cultivated archaea exhibit a great phylogenetic and metabolic diversity. During the last years, investigations of environmental DNA samples reveal the existance of mostly unknown archaeal 16S rRNA genes "everywhere", even in ambient soils and aquatic environments. Therefore, diversity of archaea may be even much higher than known from the cultivated groups.

In my talk, I want to concentrate mainly on hyperthermophilic archaea which represent all the shortest and deep lineages whithin the phylogenetic tree. Hyperthermophiles grow optimally at temperatures between 80 and 113° C. Most of them are able to grow chemolithoautotrophically, using inorganic redox reactions as energy- and CO_2 as carbon sources. In their hot environments hydrogen gas, reduced sulfur and iron compounds may serve as electron donors, while CO_2 and oxidized sulfur, nitrogen and iron compounds may function as electron acceptors, which are present in their hot environments. Alternatively, most hyperthermophilic archaea are able to grow heterotrophically. Based on their requirement of simple substrates and their independence of sunlight, they could thrive in any water-containing hot environment, even on other planets.

026 - Tabita

Novel ribulose bisphosphate carboxylase/oxygenase enzymes of anoxic archaea: how do they work and what are they doing?

F. Robert Tabita, Gregory M.F. Watson, Jae-Pil Yu, and Michael W. Finn, Dept. Microbiology, Ohio State Univ., Columbus, OH 43210 USA

Genomic sequencing of two diverse archaea, *Methanococcus jannaschii* and *Archaeoglobus fulgidus*, indicated potential sequences that encode ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO). Deduced amino acid sequences of the putative large subunits are substantially different from the thousands of form I and form II large subunits currently in the data base. There is also no known role for this enzyme in the metabolism of archaea, although RubisCO is known to catalyze the synthesis of the bulk of biogenically produced organic matter on earth. The ability of RubisCO to catalyze reactions employing either CO_2 or O_2 as gaseous substrates defines the efficiency of this enzyme in metabolism and its ability to sequester atmospheric CO_2. Despite substantial structure-function advances over the years, a defined molecular rationale governing RubisCO specifity has not been elucidated. Since anoxic archaea presumably evolved in the complete absence of oxygen, studies were undertaken to examine the putative RubisCO sequences of *M. jannaschii* and *A. fulgidus*. It was found that known active site residues were conserved and the deduced tertiary structures of each of these potential enzymes were basically similar to known RubisCO structural models. Recombinant enzymes were prepared, and after considerable optimization of enzyme recovery, the *M. jannaschii* enzyme, in particular, was shown to exhibit bonafide RubisCO catalytic activity, with stoichiometric conversion of substrates to products. The enzyme exhibited considerable thermal stability and possessed an unusual salt requirement for maximal activity. Several other catalytic properties were defined; the reversible inhibition of the enzyme by molecular oxygen was especially interesting and unexpected. Despite this, an extremely weak oxygenase activity could be measured. These results suggest that these unusual archaeal enzymes might serve as model systems to probe the basis and evolution of RubisCO CO_2/O_2 specificity.

027 - Thauer

Structure and Catalytic Mechanism of Methyl-CoM-Reductase

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Methyl-coenzyme M reductase is the key enzyme of methane formation in methanogenic archaea. It catalyzes the reduction of methyl-coenzyme M with coenzyme B to methane and the heterodisulfide of coenzyme M and coenzyme B. The crystal structure of the enzyme substrate complex and of the enzyme product complex was determined at 1.45 Å resolution. In both structures the electron density map revealed five modified amino acids located near the active site region: 1-*N*-methyl-His$^{\alpha257}$, 5-methyl-Arg$^{\alpha271}$, 2-methyl-Gln$^{\alpha400}$, S-methyl-Cys$^{\alpha452}$, and thio-Gly$^{\alpha445}$. The mechanism of modification by methylation was resolved. From the two enzyme structures and the properties of its prosthetic group coenzyme F_{430}, a catalytic mechanism is proposed that involves radical intermediates and a nickel organic compound.

Goubeaud, M., Schreiner, G. & Thauer, R.K. (1997) *Eur. J. Biochem. 243*, 110-114.
Shima, S., Goubeaud, M., Vinzenz, D., Thauer, R.K. & Ermler, U. (1997) *J. Biochem. 121*, 829-830.
Ermler, U., Grabarse, W., Shima, S., Goubeaud, M. & Thauer, R.K. (1997) *Science 278*, 1457-1462.

028 - Thomm

Mechanism and regulation of transcription in Archaea

Michael Thomm, Isabell Dahlke, Winfried Hausner, Jens Thomsen and Bernd Goede, Institut für Allgemeine Mikrobiologie, Universität Kiel, D-24118 Kiel, Germany

Initiation of transcription by archaeal RNA polymerases is mediated by two transcription factors, that are homologous to eukaryotic transcription factors TBP and TFIIB. As at RNA polymerase II promoters, TBP bound at the TATA-box is the heart of the preinitiation complex. Entry of the second archaeal factor, TFB, to the TATA-box-TBP binary complex stabilises binding of TBP to the promoter and extends the footprint of TBP to the DNA region upstream of the TATA box.

Although the nature of the archaeal transcriptional machinery is clearly eucaryotic-like, a gene encoding a homolog of the bacterial general transcriptional regulator "Leucine responsive regulatory protein" (Lrp) has been detected in the genome of some Archaea. Pyrococcus-Lrp acts as a transcriptional regulator in vitro. It inhibits specifically transcription form its own gene. This autoregulation is not affected by Leucine. We describe experiments contributing to a molecular understanding of Lrp action.

Furthermore, two differentially expressed archaeal gene loci are presented as models to investigate regulation of transcription in Archaea. The expression of Pyrococcus celB-gene, encoding a beta-glucosidase, is highly stimulated in vivo in the presence of cellobiose as carbon source, the transcription of the dnaK-locus of Methanosarcina, encoding some archaeal molecular chaperones, is dramatically increased by stress. Preliminary evidence suggests that regulation of transcription of the Pyrococcus celB gene is mediated by a bacterial type of activator. The mechanism controlling heat shock gene expression in Archaea seems to differ from both the bacterial and eucaryotic strategies.

029 - Maizels

CCA addition by tRNA nucleotidyltransferase: polymerization without translocation?

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The CCA-adding enzyme builds and repairs the 3′ terminal CCA sequence of all tRNAs by adding one nucleotide at a time, using CTP and ATP as substrates. Unlike all other sequence-specific RNA and DNA polymerases, however, the CCA-adding enzyme does not use a nucleic acid to template nucleotide addition. Thus the protein itself must somehow serve as template, or the enzyme must use a novel mechanism. We have recently shown that the CCA-adding enzyme of *Sulfolobus shibatae* has only a single active site, that the enzyme binds primarily to the acceptor stem (or "top half") of tRNA, and that the tRNA remains immobile on the enzyme surface during addition of CCA. To explain how three nucleotides can be added to tRNA without movement of either the tRNA or the active site, we propose that the growing 3′ terminus of the tRNA progressively refolds to allow the solitary active site to reuse a single nucleotide binding site. The binding site for each successive nucleotide would be created collaboratively by the refolded 3′ terminus and the enzyme, and nucleotide addition would cease when the nucleotide binding pocket was full. The template for CCA addition would be a dynamic ribonucleoprotein structure, in a process we call *collaborative templating*.

030 - Whitman

Examination of biosynthetic pathways predicted by genomic sequencing in methanococci.

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Correct annotation of genomic sequences is still a major problem in the Archaea due to the lack of direct experimental observations and the low similarity of archaeal sequences to bacterial and eukaryal homologs. Two approaches are being utilized to address this problem. In the first approach, genetic tools to experimentally examine the function of ORFs in *Methanococcus maripaludis* are being developed. *M. maripaludis* is a rapidly growing, mesophilic methanogen moderately related to the hyperthermophile *Methanococcus jannaschii*. A facultative autotroph, it can grow in mineral medium on H_2+CO_2 or formate as well as assimilate acetate and amino acids. Genetic tools available in *M. maripaludis* include an efficient transformation system, an expression shuttle vector, and insertional vectors. The expression shuttle vector will be useful for studying structure-function relationships of enzymes with the unusual prosthetic groups that are common in methanogens. By constructing a random library in a suicide vector, insertional mutagenesis of the genome is also possible. Thus, upon isolation of mutants with interesting phenotypes, the physical basis for the mutations can be readily identified. In the second approach, additional computational analyses of putative ORFs are being performed to identify potentially orthologous ORFs in the genomic sequences of the euryarchaeotes. Because biosynthetic pathways should be conserved in these organisms, these analyses will test the annotations for many of the ORFs assigned to these functions. In addition, these analyses should help identify ORFs whose functions can be readily tested genetically.

101 **Analyses of DNA Polymerase Activities in *Aeropyrum pernix*, an Obligate Aerobic Hyperthermophilic Crenarchaeote: Implications on Archaeal DNA Replication**

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The domain Archaea comprises the sub-domains euryarchaeota, crenarchaeota and korachaeota. DNA replication has not been elucidated in any of the subdomains. Our research and those of others have provided evidence that the euryarchaeotes possess, at least, two DNA polymerases, one belonging to family B (PolI) while the other is a hitherto undescribed heterodimeric DNA polymerase (PolII). While no DNA polymerase has been isolated from the korachaeotes, in the crenarchaeotes, *P. occultum* and *S. solfataricus* are reported to contain multiple DNA polymerases. We investigated DNA polymerase activities in *Aeropyrum pernix*, the only obligate aerobic crenarcheote isolated till date, which grows up to 100°C. DNA polymerase activity was determined by fractionation of cell extracts through anion exchange chromatography followed by analysis of each fraction for incorporation of (^{3}H)TTP into activated calf thymus DNA. Two different DNA polymerase activities, one aphidicolin-resistant (PolI) and the other sensitive (PolII), were detected. Both activities were stable above 80°C, but PolII was more thermostable than PolI. These properties are similar to those of PolI and PolII of *P. occultum*, which we characterized previously. Degenerate primers were used to amplify segments of the genes encoding the DNA polymerases. The obtained PCR fragments were cloned and the inserts in recombinant plasmids were sequenced. Two fragments showing extensive similarities to PolI and PolII of *P. occultum* were identified. Using a genome-walking PCR approach, the two genes were completely sequenced and cloned. *A. pernix* PolI showed high amino acid sequence identity to *P. occultum* PolI and family B DNA polymerases found only in the crenarchaeotes, while *A. pernix* PolII showed considerable similarity to the homolog found in both crenarchaeota and euryarchaeota. We hypothesize that both the euryarchaeota and the crenarchaeota contain multiple DNA polymerases. One homolog is aphidicolin-sensitive and common to both subdomains, while the second DNA polymerase in each subdomain is aphidicolin-resistant. Whereas the latter is characterized by a heterodimer in the euryarchaeota, in the crenarchaeota it is a single polypeptide.

102 **How Much Can We Learn about Archaeal Metabolism Through Genome Comparison?**

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With the determination of the complete genome sequences of four euryarchaeons, it has become possible to compare the proteins encoded in their genomes to their bacterial and eukaryotic counterparts. In an attempt to understand archaeal metabolism, we combined sequence analysis of the proteins encoded in archaeal genomes using iterative BLAST (PSI-BLAST) with the Clusters of Orthologous Groups (http://www.ncbi.nlm.nih.gov/COG) approach, which is based upon all-against-all comparisons and is largely unaffected by the differences in their relative evolution rates. This strategy allowed us to identify candidate proteins for most of the core metabolic pathways in each of the completely sequenced archaeal genomes.

It appears that *Methanococcus jannaschii*, *Methanobacterium thermoautotrophicus*, and *Archaeoglobus fulgidus* have functional bacterial-type pathways for synthesis of all the amino acids, except proline, cysteine, and lysine. In contrast, only methionine, threonine and arginine biosynthetic pathways were found in *Pyrococcus horikoshii*. Biosynthesis of purines and pyrimidines is also similar in bacteria and archaea, with the exception of AICAR transformylase (PurH), which is present only in *A. fulgidus* and is apparently displaced by a non-orthologous enzyme in other archaea. Such coenzymes as NAD and biotin are synthesized by all four organisms; all of them, except *P. horikoshii*, also have genes for riboflavin and cobalamin biosynthesis.

On the other hand, carbohydrate metabolism remains enigmatic. Only the genes for the lower (tri-carbon) part of glycolysis are found in all four archaeal genomes, while the genes for the upper part of glycolysis, pentose phosphate shunt, and the Entner-Doudoroff pathway are all missing. As a result, the mechanisms of formation of ribose, deoxyribose, and hexoses remain unidentified. Proline, cysteine, and lysine biosynthesis pathways are also obscure at this time. Besides, non-orthologous gene displacement is detectable even in the established pathways; only in a few cases both enzyme variants are readily recognized. Clearly, a major experimental effort will be needed to get a comprehensive picture of the metabolic processes in each of the organisms with completely sequenced genomes.

103 **Development of integrative and self-replicating expression vectors for *Methanococcus maripaludis***

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Methanococcus maripaludis is a strictly anaerobic archaeon that produces methane from carbon dioxide and hydrogen or formate and is among the few archaea with a genetic system. Integrative vectors, multiple antibiotic resistance markers, and a shuttle vector are currently available for *M. maripaludis*. In this research, expression vectors were designed and tested.

pDLT44, the first shuttle vector developed for *M. maripaludis*, was constructed by ligation of pMEB.2 into pURB500 (Tumbula *et al.*, 1997). pMEB.2 contains an origin of replication for *E. coli*, β-lactamase for selection in *E. coli*, and the puromycin resistance cassette for selection in methanococci. pURB500 is a cryptic plasmid from *M. maripaludis* strain C5 that contains a methanococcal origin of replication. Many archaea contain oxygen-sensitive metalloenzymes that require unusual cofactors to form a functional enzyme. pWLG30, an expression shuttle vector, was developed to overexpress these archaeal metalloenzyme genes in an anaerobic environment. It was constructed by adding a *NsiI* site downstream of the promoter for the histone-like protein (P_{hmv}) from *Methanococcus voltae*. Downstream of the *NsiI* site are three additional unique sites. Upstream of the P_{hmv} is a unique *ClaI* site that allows removal or replacement of the promoter. The vector allows expression of heterologous genes in *M. maripaludis*. Another utility of the vector is the *in vivo* monitoring of methanococcal promoters. Expression levels in *M. maripaludis* and *Escherichia coli* were tested with the β-galactosidase gene *lacZ*.

Construction of *Methanococcus* integrative vectors with increased versatility began with pMEB.2 (Gernhardt *et al.*, 1990). Removal of the *lacZ* alpha-complementation fragment and the addition of a multiple cloning site (MCS) yielded pWLG11. pWLG11 was developed for insertionally inactivating genes via homologous recombination in *M. maripaludis*. pWLG11 was further modified by the addition of P_{hmvA} upstream of the MCS to form pWLG13. pWLG13 is an integrative expression vector for *M. maripaludis*. Thus, following homologous recombination into the chromosome, the P_{hmv} would drive expression of the genomic copy of the inserted gene. pWLG13 is currently being tested in *M. maripaludis* with the acetohydroxacid synthase (AHAS) gene *ilvB*.

104 **A NOVEL DNA POLYMERASE IN THE EURYARCHAEOTA**

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The propagation of every free-living organism depends on the accurate transfer of genetic information from parent to progeny. Pivotal to this process are the DNA polymerases which undertake various replicative and repair functions. In both Bacteria and Eukarya, multiple DNA polymerases are known to perform the task of replicating the genome. In Archaea, however, the process of DNA replication remains cryptic. To understand replication in these organisms, our experiment was initiated to identify all proteins with DNA polymerizing activity from *P. furiosus*, a member of the subdomain Euryarchaeota. A protein having DNA polymerizing activity with different biochemical properties from that of known DNA polymerases was found. This novel DNA polymerase (PolII) is composed of a heterodimer. We report here that, PolII is highly conserved in the Euryarchaeota. Extensive conservation of amino acid sequence between euryarchaeal DP1s (the small subunit of PolII) and the small subunit of eukaryal DNA polymerase δ was found after careful comparisons. The overall amino acid sequence of DP2 (the large subunit of Pol II) shows no meaningful conservation to any known protein. However, we predicted the amino acid residues in the sequence of Euryarchaeal DP2s for active site located in the distinct motifs which are found in the palm subdomain of all proteins possessing nucleotidyl transfer activity. Hence, the DP2 of euryarchaeal heterodimeric DNA polymerase is proposed to be the catalytic subunit. Immunological experiments show that the proliferating cell nuclear antigen (PCNA) of *P. furiosus* interacts with PolII. It is of interest to note that PCNA also exhibited an interaction with the family B-type DNA polymerase of *P. furiosus* (PolI). Our current studies are to determine if interaction of PCNA with PolII enhances processive DNA synthesis, as well as to investigate the interaction of Pol II to other candidate proteins related to DNA replication. These finding will further substantiate the archaeal/eukaryal relationship.

105 **Isolation of acetate auxotrophs by random insertional mutagenesis of *Methanococcus maripaludis***

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To randomly mutagenize the methane-producing archaeon *Methanococcus maripaludis*, a genomic DNA library was constructed in pMEB.2, a plasmid which carries the puromycin resistance cassette but lacks a methanococcal origin of replication. Following PEG transformation, 33,000 puromycin-resistant transformants were obtained from this library. Seven auxotrophs were then isolated following enrichment with base analogs. For six of these auxotrophs, the putative mutagenic plasmids were isolated by electroporation of plasmid preparations from the methanococci into *E. coli*.

Two of the auxotrophs were of special interest because they required acetate for normal growth. JJ104 was a leaky acetate auxotroph. The plasmid from JJ104, pWDK104, contained partial ORFs homologous to two components of an ABC transporter identified in the *Methanococcus jannaschii* genomic sequence. Transformation of this plasmid back into the wild-type strain yielded transformants with the same phenotype as JJ104, confirming that integration into the genes encoding an ABC transporter produced the observed phenotype. Growth of one of the transformants of pWDK104 was stimulated by the addition of combinations of selenate, molybdate, and tungstate. Presumably, the acetate auxotrophy resulted from the failure to produce the prosthetic group of a key enzyme upon disruption of the transporter and limitation for one of these oxyanions.

The other auxotroph, JJ117, required acetate or cobalamin for growth. Amino acids, other vitamins, cobinamide, and benzylamidazoles had no affect. The plasmid from JJ117, pWDK117, contained most of a large ORF homologous to two decarboxylase homologs in the *M. jannaschii* genome. Of five transformants of the wild type strain by pWDK117, only one was auxotrophic for acetate and cobalamin. By Southern blotting, JJ117 and the auxotrophic transformant appeared to contain tandem repeats of pWDK117 inserted in the genome. In a prototrophic transformant, only a single insertion of pWDK117 had occurred. These results suggest that the auxotrophy was not produced by simple disruption of the decarboxylase gene but may have been caused by more complex interactions.

106 **Comparative genomics of the archaea: universal and unique protein families and emerging evolutionary patterns**

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Comparative analysis of the protein sequences encoded in the four archaeal species with completely sequenced genomes (*Methanococcus jannaschii*, *Methanobacterium thermoautotrophicum*, *Archaeoglobus fulgidus*, and *Pyrococcus horikoshii*) revealed 1326 orthologous sets, 542 of which are conserved in all four species. The proteins that belong to these universal archaeal families comprise 30-35% of the gene complement and should be considered the evolutionary stable core of the archaeal genomes. The core gene set includes the great majority of genes coding for proteins involved in genome replication and expression but only a relatively small subset of metabolic functions. In terms of their phylogenetic affinities, the archaeal protein families are split into "bacterial" and "eukaryotic" ones. Most of the proteins that show the highest similarity to their eukaryotic counterparts or have only eukaryotic orthologs belong to the core set, whereas the mobile component consists primarily of "bacterial" proteins.

The archaeal transcription system is a particularly interesting combination of eukaryotic and bacterial features, with a eukaryotic-type RNA polymerase and several transcription factors but a number of bacterial-type regulators containing the helix-turn-helix domain, some of which form families conserved in all archaea. Detailed analysis of both the core and the mobile components of the archaeal gene complements shows ample evidence of evolutionary events other than simple vertical inheritance, such as differential gene loss and horizontal gene transfer, primarily from bacteria, which results in both non-orthologous and orthologous displacement of a significant number of genes.

Among the core archaeal families, 71 have no orthologs outside the archaea and should be considered unique archaeal characters (synapomorphies). The identification of these synapomorphies underscores the unique evolutionary history of the archaea. Overall, the comparative analysis of archaeal protein families is compatible with an evolutionary scenario whereby the "eukaryotic" families are remnants of an ancestral gene set, while the bacterial families have entered the archaeal lineage by horizontal gene transfer at different stages of evolution and have partially replaced the original genes.

107 **The Process of Primitive Mitosis in *Tritrichomonas foetus***

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The process of mitosis has undergone evolution. This change most probably was accompanied by an increase in genome complexity and the compartmentalization event. Contrary to what happens in higher eukaryotes, where the nuclear breakdown occurs in the beginning of cell division, in the closed type of mitosis the nuclear envelope is maintained totally integral. The nuclear division may be "closed" or "semi-opened" or "opened" depending in the degree of nuclear envelope persistency. In the closed system, the nuclear envelope stands in between the extranuclear spindle and the chromosomes. How then microtubules segregate chromosomes? Several authors sugest kinetochores anchored on the nuclear envelope do this link. In an attempt to explore the primitive closed mitosis process we used as cell model *Tritrichomonas foetus* which is considered by molecular phylogeny one of the most early diverging eukaryotes to be studied to date. *T. foetus* cell division serves as an interesting model to study the mitotic evolutionary path, since it presents a membrane-dependent genomic segregation mode as prokaryotes and also a microtubular spindle apparatus as all eukaryotes. Our results are in disagreement with previous reports in relation to the kinetochore structure/function and also in the description of the behavior of the cytoskeleton during morphogenesis. In an attempt to explore the primitive cell division of *T. foetus* we used several approaches. The first step was to obtain a high index of mitotic cells, therefore, we established a synchronization procedure using hydroxyurea. Living synchronized cells were followed using video enhanced interference contrast microscopy. Scanning electron microscopy, electron microscopy-serial thin section, 3D reconstruction, Panotico staining and confocal laser microscopy of imunofluorescence with anti-tubulin antibody or of DNA fluorescent stain were used to characterize cell morphogenesis. In addition we also produced a hypothetical computer animation of the whole morphogenesis using a representation of major skeletal structures and of the nucleus to picture their overall interaction. Our analyses, based on these combined data, characterized morphogenesis in four phases. In addition we correlated the importance of a bilateral and mirror symmetry of the mitotic apparatus with the events of genomic partition and daughter cell disjunction. On the other hand we also describe the importance of the mastigont system. Analyses of the ultrastructure of the dividing cell rendered information about the details of membrane-microtubule interaction and revealed a complex transformation of the intact nuclear envelope. We observed finger-like expansions on the pole regions of the nuclear envelope and the formation of "bubbles" in between its inner and outer membranes. Microtubules in bundles were observed anchoring onto the nucleus or passing through its interior by a cytoplasmic channel. In the present study we have shown that *T. foetus* mitosis is held not only by the spindle microtubules as in higher eukaryotes but also with the aid of the axostyle and of the flagellar propulsion. Further, we demonstrated alterations of the nuclear envelope which might contribute to realize a still obscure mode of membrane-based chromosome segregation pattern. We propose with this study a better understanding on the evolution of mitosis and we put forward new hypothesis on the segregation mechanism.

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108 **Transformation in Extreme Thermophiles: A High Copy Number, Integrating Shuttle Vector for *Sulfolobus solfataricus*.**

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A number of genomes of thermophilic, extremely thermophilic and hyper-thermophilic archaea have been completely sequenced and analyzed at a sequence level. However there is currently no way to directly correlate any of these genomes with their activity due to the absence of a system for stable transformation and specific gene disruption. The extremely thermophilic archaeon *Sulfolobus solfataricus* is one of the organisms whose genomic sequence is being determined and it has a number of characteristics which make it an attractive system for genetic analysis; heterotrophy, aerobic growth, high plating efficiency and the availability of a large number of extra-chromosomal elements. One of these elements, the virus SSV1, was chosen as the basis for a transformation system for *S.solfataricus*. As well as being the best studied of any archaeal virus, it has a number of features which make it attractive as a transformation system. Viral replication is UV inducible and the genome contains a UV inducible promoter, it integrates specifically into a *S.solfataricus* tRNA gene and does not lyse its host. In a series of experiments we showed that a number of ORFs in the virus genome are required for virus function and that at least one is not required. This allowed us to construct a shuttle vector which is stable both in *E.coli* and *S.solfataricus*. The copy number of the vector is regulated by UV induction. It integrates specifically into the host genome and forms lysogens under certain conditions. It forms infectious virus particles which appear to be larger than the wild type virus. After 3 days of liquid culture the vector spreads to infect all of the cells in that culture. This shuttle vector appears to be an excellent candidate for genetic analysis of the extremely thermophilic archaeon *S.solfataricus*. Complementation and expression studies will be discussed.

109 Transamidation routes to aminoacyl-tRNA formation in *Deinococcus radiodurans*: parallels to the archaea?

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In the absence of the respective tRNA synthetases, some archaea form Gln-tRNA^{Gln} or Asn-tRNA^{Asn} by amidation of the mischarged Glu-tRNA^{Gln} or Asp-tRNA^{Asn} species (1). The genomic sequence of the bacterium *Deinococcus radiodurans* (2) suggested the presence of both glutamyl-tRNA^{Gln} amidotransferase (GluAdT) and aspartyl-tRNA^{Asn} amidotransferase (AspAdT), given the presence of GluAdT homologs (*gatCAB*) and an archaeal-like, non-discriminating aspartyl-tRNA synthetase. Cloned and expressed *gatCAB* from *D. radiodurans* demonstrated both GluAdT and AspAdT activity. However, the lack of a non-discriminating glutamyl-tRNA synthetase activity in *D. radiodurans* restricts the role of the GluAdT to Asn-tRNA formation. As *D. radiodurans* has homologs and the activities of glutaminyl-tRNA synthetase but not of tRNA-independent asparagine synthetase, the role of the GluAdT in this organism may be confined to asparagine biosynthesis. These results are consistent with the previous suggestion (3) that the GatB subunit specifies tRNA-recognition, as *D. radiodurans* has only one *gatB* homolog, in contrast to archaea which possess two *gatB* homologs while lacking glutaminyl- and asparaginyl-tRNA synthetases. As *D. radiodurans* also has asparaginyl-tRNA synthetase, this system is the first example where an aminoacyl-tRNA is formed within the same cellular compartment by both the respective tRNA synthetase and an amidotransferase.

1. Ibba, M., Curnow, A.W. & Söll, D. (1997) *Trends Biochem. Sci.* 22, 39-42.
2. ftp://ftp.tigr.org/pub/data/d_radiodurans/.
3. Curnow, A.W., Hong, K.-W., Yuan, R., Kim, S.-I., Martins, O., Winkler, W., Henkin, T.M. & Söll, D. (1997) *Proc. Natl. Acad. Sci. USA* 94, 11819-11826.

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110 Coenzyme Biosynthesis in the Methanogenic Archaea

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The individual steps in the biosynthetic pathways for the generation of the tetracarboxylic acid moiety of methanofuran (HTCA), coenzyme B (HS-HTP) and methanopterin will be presented. The common link between the biosynthesis of HTCA, coenzyme B and biotin through the common intermediate *trans*-homoaconitate, formed by the protein product of the *aksA* gene, will be described. Identification of the genes involved in biosynthesis (7,8-dihydropteroate synthase) and function (thymidylate synthase) of methanopterin will be given.

111 Non-discrimination of tRNA substrates by aspartyl tRNA synthetases

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In archaea and in some eubacteria, Asn-tRNA^{Asn} is formed by a two step pathway. The first step is mischarging of tRNA^{Asn} by a non-discriminating AspRS. The Asp-tRNA^{Asn} is then converted to Asn-tRNA^{Asn} by a tRNA-dependent amidotransferase.

Productive recognition of tRNA^{Asn} and tRNA^{Asp} by a non-discriminating synthetase requires loss of recognition of the third position of the anticodon of these tRNAs. However, the synthetase must retain its ability to discriminate sufficiently against all other tRNA substrates. In order to determine the structural and functional basis for this non-discrimination, we have cloned and expressed the genes for AspRS1 (discriminating) and AspRS2 (non-discriminating) synthetases from *Deinococcus radiodurans*.

An understanding of the basis for mischarging may shed some light on the evolution of discrimination in the AspRS family, as well as providing clues to the origin of the AsnRS family.

201 **Evolutionary History and Biochemical Characterization of Archaeal Transcription Factors**

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While gene expression in Eucarya utilizes three types of RNA polymerases as well as three distinct protein complexes for polymerase-specific promoter recognition, the homologous archaeal mechanism utilizes one RNA polymerase and at least two basal factors. The archaeal factors involved in transcription initiation are the TATA-binding protein (TBP) and transcription factor B (TFB), which are homologous to eucaryal TBP and TFIIB-TFIIIB proteins respectively. We have cloned and sequenced homologs of eucaryal transcription factors from diverse thermophilic and mesophilic representatives. Phylogenetic analyses indicate that the archaeal sequences form a confident phylogenetic group apart from their eukaryotic homologs. This is consistent with a single member of the TBP and TFB gene families being present in the eucaryal / archaeal most recent common ancestor. These basal transcription factors have been purified and are being assayed for promoter DNA binding activity by gel mobility shift assays and fluorescence anisotropy. Initial results show binding of *Methanoplanus limicola* TBP to a tRNA promoter, while *Methanococcus maripaludis* and *Methanococcus jannaschii* TBP do not exhibit detectable binding under the same gel mobility shift assay conditions. The TFB from the thermophile *Thermococcus litoralis* stabilizes the binding of all TATA-binding proteins assayed. These basal transcription factors bind optimally at a higher ionic strength than that reported for eukaryotic homologs. At optimized salt conditions these proteins show lower promoter binding affinity than their eukaryotic counterparts. A comparative analysis of binding affinities towards different types of promoters will provide insights into basal gene expression in Archaea.

202 **Eucaryal and archaeal features of the *Methanosarcina mazei* S-6 TATA-binding protein (TBP)**

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The TBP gene from the methanoarchaeon *M. mazei* S-6 was cloned and sequenced, and the deduced amino-acid (aa) sequence was compared to archaeal and eucaryal homologs.

The S-6 TBP has 183 aa with two direct 42-aa repeats separated by 51 aa. Homologs of these repeats were found in the eight archaeal and three eucaryal TBPs studied, in which the inter repeat region ranged between 49-51 aa. The percent identity between the repeats was 50-62 and 43 -45 in the archaeal and eucaryal homologs, respectively.

Archaeal TBPs ranged between 181 and 198 aa and aligned with the C-terminal half of eucaryal TBPs (229-344 aa). S-6 TBP does not have an acidic tail but the C-terminal repeat is acidic while the N-terminal repeat is not, in contrast with the archaeal homologs. Identity of all TBPs examined was 30.4-70.2 % and they all showed highly conserved aa at 34 positions. Structure-function correlations, evolutionary comparative analyses and experimental testing of S-6 TBP, are underway.

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203 **Repair of Ultraviolet Light Induced DNA Damage in the Extremely Halophilic Archaea *Haloferax volcanii*.**

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Efficient and accurate DNA repair is critical for minimizing the mutagenic and toxic effects of spontaneous and environmental DNA damage. Early reports on halophilic Archaea suggested that they, in contrast to bacteria and eukaryotes, did not possess the ability to repair UV induced DNA damage in the absence of photoreactiviating light (so called dark repair). Such results were surprising given the extreme resistance of these species to UV and the well documented importance of dark repair in other species. We present evidence for the dark repair of UV induced damage in *Haloferax volcanii*, an extremely halophilic Archaea. We have characterized the dynamics of this process at various intensities of UV and in different phases of the growth cycle. In addition, we are currently working to test whether these Archaea are capable of performing transcription-coupled repair, a process that has been extensively characterized in bacteria, yeast, and mammalian cells. By using strand-specific RNA probes to detect either the transcribed or nontranscribed strand of the *trpABC* operon, we have attempted to determine whether the major UV-induced lesion, the cyclobutane pyrimidine dimer, is removed preferentially from the transcribed strand when this operon is active. We believe that *H. volcanii* can become a model for studies of repair in halophiles and in Archaea.

204 **Comparative and evolutionary studies of DNA repair proteins and pathways by phylogenomic analysis**

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The ability to recognize and repair abnormal DNA structures is common to all forms of life. Comparative studies of DNA repair have been limited by a lack of experimental studies in a wide ecological and evolutionary diversity of species. In this study, we present a global comparative analysis of DNA repair proteins and pathways focusing on what can be learned by analysis of complete genome sequences. For our study, we use a new type of analysis that combines genome analysis and evolutionary reconstructions into one composite phylogenomic analysis. This combined approach is useful because evolutionary reconstructions can improve genome analysis and genome information can improve evolutionary reconstructions. Based on the phylogenomic analysis, we propose a scenario for the evolution of repair proteins and pathways, identifying (1) likely ancient repair pathways (2) pathways that have evolved more recently (3) loss of genes or whole pathways in some lineages (4) gene duplication and lateral transfer events and (5) covergent evolution of some pathways. We discuss how an evolutionary perspective helps understand and even predict the functions of many repair proteins and pathways. In addition, we discuss what our analysis suggests about repair processes in the species for which the complete genomes were analyzed, in particular focusing on the Archaeal species. Finally, we discuss some of the limitations of the comparative genomic approach and present evidence that experimental studies of repair are still needed in many groups of organisms, in particular the Archaea.

205 **Gamma irradiation effect on the hyperthermophilic archaeon *Pyrococcus abyssi***

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It have been shown recently that some hyperthermophilic archaea are highly radioresistant (Kopylov et al., 1993; DiRuggiero et al., 1997).This radioresistance could be related to the efficient DNA repair mechanisms which should correct for DNA damages induced at high temperature in hyperthermophiles. We have initiated the study of radioresistance in *Pyrococcus abyssi* whose genome has been completely sequenced by the Genoscope and which contains a small plasmid that could be used to easily probe for DNA damages. We have tested the effect of gamma irradiation under different physiological conditions on *P. abyssi*. Our data indicate that this archaeon is about 25 times more radioresistant than *E. coli*. The radioresistance of *P.abyssi* is more important at the beginning of the log phase than in stationary phase and is higher in anaerobic condition. We have looked for proteins inducible by gamma irradiation, using one and two dimensional electrophoresis on crude extracts of proteins and on extracts of proteins with affinity for DNA. We found six proteins induced after gamma irradiation that might be involved in DNA repair. Finally, we have studied the plasmid pGT5 of *P. abyssi* after irradiation and observed that DNA strand breaks appear at non lethal doses, indicating that DNA repair systems are indeed necessary for the radioresistance and that pGT5 can be used to detect intracellular DNA damages.
Futhermore, the extreme radioresistance of hyperthermopile archaea was confirmed by the irradiation at 20 and 30 kGy of enrichment cultures obtained at 80°C from deep-sea hydrothermal vents from the Guyamas basin and at the midle-Atlantic Ridge. This allows to isolated new strains of archaea belonging to the genus *Thermococcus*, with resistance levels similar to those of *Deinococcus radiodurans*, which is the most radioresistant organism known until now.

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206 **The DNA-binding protein Tfx from *Methanobacterium thermoautotrophicum*: Structure, DNA-binding properties and transcriptional regulation.**

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In *Methanobacterium thermoautotrophicum* the *fmdECB* operon encoding the molybdenum formylmethanofuran dehydrogenase is directly preceded by an open reading frame *tfx* predicted to encode a DNA-binding protein. The 16.1 kDa protein has an N-terminal basic domain with a helix-turn-helix motif for DNA-binding and a C-terminal acidic domain possibly for transcriptional activation. We report here on the DNA-binding properties of the Tfx protein heterologously overproduced in *Escherichia coli*. Tfx was found to specifically bind to a DNA sequence downstream of the promoter of the *fmdECB* operon as evidenced by electrophoretic mobility shift assays and DNase I footprint analysis. Northern blot hybridizations revealed that transcription of *tfx* is repressed during growth of *M. thermoautotrophicum* in the presence of tungstate. Based on its structure and properties the DNA-binding protein Tfx is proposed to be a transcriptional regulator composed of a basic DNA-binding domain and an acidic activation domain.

207 **Systematic Identification of Novel DNA / RNA Binding Proteins from *Pyrococcus furiosus* by Expression Cloning**

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The complete nucleotide sequence of archaea genomes registered in the database provided 1,700 to 2,000 predicted protein-coding genes per an organism, however, half of these genes did not show any significant similarity to the reported sequences whose functions are known. In order to classify and identify novel factor(s) involved in the regulation of nucleic acid metabolisms such as DNA replication, DNA repair and RNA transcription, we performed a systematic identification by expression cloning of genes for DNA/RNA binding proteins from a hyper-thermophilic archaea, *Pyrococcus furiosus*.

First, we made an expression genomic DNA library using a pRSET plasmid vector (*Invitrogen*) and then, every pooled plasmids (30 clones) was expressed in *E. coli*. The expressed proteins were extracted and heat-treated at 85 ℃ for 15 min to kill endogenous proteins from *E. coli*. The resulting extract was screened by gel retardation assay probed with 20 mers of dAdT, dGdC, rArU and rGrC. So far, we detected about 30 of DNA/RNA binding activities and found that the most of these activities was either dAdT or rArU binding activity. A plasmid clone corresponding to the strongest rArU binding activity was isolated. The responsible gene for rArU binding activity was determined by a series of deletion experiment and DNA sequencing of the insert. The gene encoded a novel protein that was only homologous to a hypothetical protein found in the genome of *P. horikoshii*. The recombinant protein was specifically bound to the rArU probe at high temperature (75 ℃) but not to the rGrC probe.

208 **Characterization of an ATP-dependent Protein Kinase from *Sulfolobus solfataricus***

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The extreme acidothermophilic archaeon, *Sulfolobus solfataricus,* harbors a membrane-associated protein kinase activity. Solubilization required detergents such as octyl glucoside or Triton X-100, suggesting that this activity resides within an integral membrane protein. This protein kinase utilizes ATP as phosphoryl donor and Mn^{2+} as divalent metal ion cofactor. The enzyme exhibits an M_r of ≈ 67 kDa on SDS-PAGE. The protein kinase undergoes autophosphorylation. Two acidic proteins, casein and RCM lysozyme proved to be the most effective exogenous substrates from among those surveyed. A relatively basic peptide modelled after the phosphorylation site on myosin light chains also was phosphorylated by the enzyme. Phosphorylation of the peptide was dramatically stimulated by polyanions such as heparin and DNA. Each of these exogenous substrates were phosphorylated on a threonine residue(s). Alteration of the threonine within the peptide to serine greatly decreased its efficacy as a substrate. Supported by NIH grant R01 GM55067 to PJK and an NSF fellowship to KMB.

209 Interaction of Ribosomal Proteins L1 and S8 from Mesophilic and Thermophilic *Methanococcus* Species with Their Specific Binding Sites on rRNA and mRNA

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In Archaea -as in Bacteria- ribosomal protein L1 has a dual function: first as primary rRNA binding protein and second as translational repressor which binds to its own mRNA. The binding site on the mRNA exhibits high similarity in both primary sequence and secondary structure to the binding site on the rRNA. A membrane filter binding assay has been used to examine the interaction of ribosomal proteins L1 and S8 from three different *Methanococcus* species with their specific RNA binding site. The apparent dissociation constant K_d of the homologous *Methanococcus vannielii* L1-rRNA complex is $5 \cdot 10^{-9}$ M, that of the L1-mRNA complex is $1{,}5 \cdot 10^{-7}$ M. L1 proteins from the thermophilic *Methanoccocus thermolithothrophicus* (opt. growth temp. 65°C) and from the hyperthermophilic *Methanococcus jannaschii* (opt. growth temp. 85°C) exhibit an affinity to both rRNA and mRNA which is about one order of magnitude higher than that of their mesophilic counterparts.
The same difference in the binding affinity to their specific 16S rRNA site was observed for mesophilic / thermophilic *Methanococcus* S8 proteins (K_d for the MvaS8/16S rRNA complex: $3{,}1 \cdot 10^{-6}$ M, for the MjaS8/16S rRNA complex: $2{,}7 \cdot 10^{-7}$ M). The strong protein-RNA interaction might make a substantial contribution to the thermal tolerance of ribosomes in (hyper)thermophilic Archaea. The affinity of L1 proteins from all *Methanococcus* species to their mRNA binding site is more than one order of magnitude lower than to their 23S rRNA site. These results fit the requirement of classical regulation of ribosomal protein synthesis in procaryotes.
This work was supported by the Austrian Science Foundation (FWF, grant P12070-MOB to W.P.)

210 Nitrogen Regulation in *Methanococcus maripaludis*

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Little is known about transcriptional regulation in the Archaea. Nitrogen fixation and nitrogen assimilation are regulated systems that can be used as models. We are studying the regulation of *nif* gene expression and *glnA* expression in *Methanococcus maripaludis* using genetic methods.

Six *nif* genes and two *glnB*-like genes composed a single operon that was required for diazotrophic growth. mRNA was fragmented apparently due to mRNA processing. A single transcription start site and a consensus promoter were identified. Expression was repressed by ammonia. Repression depended on the first of two inverted repeat sequences situated between the transcription start and the ribosome binding site. A component of cell extract, from ammonia-grown cells, bound specifically to the first inverted repeat. Both halves of the repeat were required for full repression and binding, suggesting that a dimeric repressor is involved as in the bacterial paradigm. The *glnB*-like genes, assumed to function in nitrogen regulation like their counterparts in Bacteria, were not required for transcriptional regulation of the *nif* operon or for diazotrophic growth.

glnA, encoding the ammonia assimilating enzyme glutamine synthetase, was transcribed from three sites. A weak site appeared constitutive and corresponded to a promoter with limited similarity to consensus. Two stronger sites corresponded to overlapping consensus promoters and were repressed by ammonia. An inverted repeat similar to that involved in *nif* regulation was required for repression. The sequence similarity between the inverted repeats required for repression suggests a common mechanism for transcriptional regulation of nitrogen fixation and ammonia assimilation. Similar inverted repeat sequences were found in the promoter regions of a variety of potential nitrogen-regulated genes in *Methanococcus* and *Methanobacterium* species.

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211 Regulation of the Ribosomal MvaL1 Operon of *Methanococcus vannielii*: A Novel Mechanism of Autogenous Translational Control

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The MvaL1 operon (encoding ribosomal proteins MvaL1, MvaL10 and MvaL12) of the methanogenic Archaeon *Methanococcus vannielii* is autogenously regulated at the level of translation. MvaL1, which is homologous to the regulatory protein EcoL1 of the L11 operon in *Escherichia coli*, was identified as the autoregulator of the MvaL1 operon. The translation of the distal MvaL10 and MvaL12 cistrons is coupled to that of the MvaL1 cistron. MvaL10, the homologue of the regulatory protein EcoL10 of the ß-operon of *E.coli*, does not have a regulatory function. In contrast to all other translational regulatory systems studied so far, the MvaL1 binding site, which shows similarity in both primary sequence and secondary structure of the specific binding site on the 23S rRNA, is located within the structural gene, about 30 bases downstream of the AUG start codon. Specific interaction of MvaL1 with both 23S rRNA and its own mRNA was confirmed by filter binding studies (see abstr. Kraft et al.).
Toeprint experiments revealed that MvaL1 does not inhibit the formation of a functional ternary initiation complex (30S-mRNA-$tRNA_i^{Met}$) and preliminary experiments suggest that even the formation of the 70S complex is not affected by MvaL1. On the other hand we could demonstrate that the autoregulation of MvaL1 occurs at or before the formation of the first peptide bond. Our data suggest a novel mechanism of translational inhibition which is different from the displacement model and the entrapment model described for the regulation of ribosomal protein operons in *E. coli*.
This work was supported by the Austrian Science Foundation (FWF; grant P12070-MOB to W.P.)

212 The 60kd chaperonin of the thermophilic archaeon Sulfolobus solfataricus is an RNA-binding protein that participates in ribosomal RNA processing

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The 60 kD molecular chaperones (chaperonins) are high-molecular weight protein complexes having a characteristic double-ring toroidal shape; they are thought to aid the folding of denatured or newly synthesized polypeptides. These proteins exist as two functionally similar, but distantly related families, one comprising the bacterial and organellar chaperonins and another (the so-called CCT-TRiC family) including the chaperonins of the archaea and the eukaryotes.
We shall report that the chaperonin of the thermophilic archaeon *Sulfolobus solfataricus* is an RNA binding protein that interacts specifically in vivo with the 16 S ribosomal RNA and participates in the early steps of ribosomal RNA maturation, cleaving a precursor rRNA at several specific sites. The features of the cleavage sites and the factors influencing cleavage efficiency will be described. The possible involvement of small RNAs in the processing reactions will also be discussed.
Moreover, we have found that the in vitro reconstitution of *Sulfolobus* 30S ribosomal subunits is enhanced in the presence of the chaperonin, thereby suggesting that the protein complex also facilitates ribosome assembly.
Our results agree with previous work implicating the bacterial chaperonins in RNA protection and/or processing, thus supporting the notion that the chaperonins of all known families have specific and evolutionarily ancient functions in RNA metabolism.

213 **Recombination in the archaea: a Holliday junction resolving enzyme from *Sulfolobus* species**

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The Holliday junction (four-way DNA junction) is a central intermediate of both homologous and some site-specific recombination. The final steps in the pathway of homologous recombination, which serves both to rearrange and repair DNA, occur when a junction specific endonuclease resolves the Holliday junction, giving rise to two recombinant DNA duplexes. Holliday junction endonucleases have been detected in many organisms, ranging from eubacteria and bacteriophage to eukaryotes and pox viruses. To date, the best characterised enzyme of this class is RuvC from *E. coli*, but RuvC is not present in the eucarya or archaea, and the equivalent enzymes involved in genomic DNA recombination in these domains thus remain unidentified.

Using a novel biochemical screen, we have identified and purified a junction-specific endonuclease from *Sulfolobus solfataricus* and the related species *Sulfolobus shibatae*. The enzyme is a metal-dependent endonuclease, highly specific for the structure of the four-way DNA junction. It appears to cleave DNA junctions in a sequence-non-specific manner, in common with the bacteriophage junction resolving enzymes. Protein sequencing is underway to facilitate the identification of the relevant gene, allowing heterologous expression and structural analyses. These studies will shed light on the process of homologous recombination in the archaea, and its relationship with the eubacterial and eucaryal pathways.

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214 **Substrate recognition by class I-type lysyl-tRNA synthetases**

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Lysyl-tRNA synthetases are unique amongst the aminoacyl-tRNA synthetases in being composed of two unrelated families. In most bacteria and all eukarya, the known lysyl-tRNA synthetases are subclass IIc-type aminoacyl-tRNA synthetases whereas some archaea and bacteria have been shown to contain an unrelated class I-type lysyl-tRNA synthetase. We have now examined substrate recognition by a bacterial (from *Borrelia burgdorferi)* and an archaeal (from *Methanococcus maripaludis)* class I lysyl-tRNA synthetase. The genes encoding both enzymes were able to rescue an *Escherichia coli* strain deficient in lysyl-tRNA synthetase, indicating their ability to functionally substitute for class II lysyl-tRNA synthetases *in vivo. In vitro* characterization revealed lysine activation and recognition to be tRNA-dependent, a phenomenon previously reported for other class I aminoacyl-tRNA synthetases. More detailed examination of tRNA recognition has shown that class I lysyl-tRNA synthetases recognize the same elements in $tRNA^{Lys}$ as their class II counterparts; specifically, the discriminator base (N73) and the anticodon serve as recognition elements. The implications of these results for the evolution of Lys-tRNA synthesis and their possible indications of a more ancient origin for tRNA than aminoacyl-tRNA synthetases will be discussed.

215 **Divergence of *Thermus thermophilus* cytochrome *c* oxidase genes: possible transfer from an archaebacterium?**

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The eubacterium, *T. thermophilus* (*Tt*) expresses two heme-copper oxidases: cytochromes caa_3 and ba_3. caa_3 is composed of two subunits each of which is made up of two domains. The larger subunit is a fusion of subunits I and III (using standard terminology), while the smaller subunit is a fusion of subunit II and a cytochrome *c*. ba_3 is a also a two subunit enzyme. The larger subunit corresponds to a somewhat elongated subunit I while the smaller subunit corresponds to a truncated form of subunit II. Physical-chemical characterizations indicate both enzymes function as typical, proton pumping cytochrome *c* oxidases. Comparison of gene sequences indicate that caa_3 is a homolog of eubacterial heme-copper oxidases whereas ba_3 shares little sequence identity (~19 %) with all other heme-copper oxidase, including quinol oxidases from archaea such as *Sulfolobus* (EMBO J. 13, 2516, 1994). This led us to suggest that either caa_3 or ba_3 was obtained from another organism by lateral gene transfer (J. Biol. Chem. 270, 20345, 1995). More recently, Engelhard and co-workers (Eur. J. Biochem. 250, 332, 1997) have identified a cytochrome ba_3 in the archaebacterium, *Natronobacterium pharaonis* (*Np*). The subunit I genes of *Tt* and *Np* cytochromes ba_3 share ~37 % identity and are clearly homologs. This raises the question of whether *Tt* obtained the ba_3 operon from an archaebacterium. (Supported by NIH grant GM35342.)

301 Evolution of archaeal chaperonins by multiple independent gene duplications

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Chaperonins are a family of molecular chaperones that form multisubunit double-ring structures. In Archaea, most chaperonin complexes are 8-fold radially symmetric and consist of two different subunits encoded by paralogous genes. Chaperonin complexes in *Sulfolobus* are an exception, showing 9-fold symmetry. Our phylogenetic analyses reveal that archaeal chaperonin evolution is marked by multiple independent gene duplications. Notably, we demonstrate a strong phylogenetic association between the euryarchaeal chaperonin sequences and a single crenarchaeal paralog. This indicates a gene duplication occurred early in archaeal evolution, prior to the divergence of the euryarchaeotes and crenarchaeotes; thus, one of these paralogs was lost in euryarchaeotes. More recent 'lineage-specific' gene duplications have also occurred in many euryarchaeal species, as well as gene loss: our analyses, corroborated by complete genome sequence, indicate that *Pyrococcus* species have recently lost a chaperonin subunit gene. We performed a PCR-based survey of the Crenarchaeota, cloning and sequencing multiple chaperonin-encoding genes from several *Sulfolobus* species, as well as a single gene from *Desulfurococcus mobilis*; a third previously unknown chaperonin paralog from *Sulfolobus solfataricus* and *S. shibatae* has been discovered. The 9-fold symmetric chaperonin complexes in *Sulfolobus* contrast those present in the crenarchaeote *Pyrodictium*, which contain two chaperonin subunits and are 8-fold symmetric. We hypothesize that *Sulfolobus* chaperonin complexes were ancestrally 8-fold symmetric, and that gene duplication and divergence of the third gene was responsible for the change in chaperonin complex symmetry. Taken as a whole, our analyses describe a pattern of recurrent paralogy within the chaperonin genes of the Archaea which, we argue, could be related to observed differences in chaperonin complex symmetries.

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302 Orientation Of Transcription In Archaea Is Defined By The TFIIB Homologue And Sequences Upstream Of The TATA-Box

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The basal transcription machinery of Archaea is strikingly similar to the core components of the eucaryal RNA polymerase (RNAP) II apparatus. Archaeal promoters contain a TATA-box found 25 base pairs upstream of the transcription start site that forms a ternary complex with the archaeal TATA-Box Binding Protein (aTBP) and TFB (the archaeal homologue of TFIIB). The formation of the TBP/ TFB/ TATA-box ternary complex is required for recruitment of the RNA polymerase to the transcription initiation site, downstream of the TATA-box. Clearly, it is of key importance that transcription occurs in a unidirectional manner at most promoters and, thus, a mechanism must exist to ensure that RNAP is recruited in a directional manner. The simplest model to explain the directional recruitment of RNAP would be to define the polarity of the preinitiation complex at the first step, the binding of TBP to the TATA-box. However, despite extensive analysis in both Eucarya and Archaea, how TBP binds the TATA element in a preferred orientation and how transcriptional polarity is established are poorly understood. Using a combination of *in vitro* transcription and DNA binding studies we test this hypothesis. Remarkably, we find that the principal determinant for the orientation of transcription is not the recognition of the TATA-box by the TATA-box binding protein. Instead, transcriptional polarity is governed by the interaction of the archaeal TFIIB homologue with a conserved motif immediately upstream of the TATA-box. These findings provide important insights into the mechanism and evolution of transcription in Archaea and Eucarya.

303 Conditional-Lethal Mutants of the Thermophilic Archaeon *Sulfolobus acidocaldarius*

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Numerous cellular processes of archaea remain undefined due to the unrelatedness of archaea to well-studied microorganisms and a scarcity of experimental tools for their analysis.

We have isolated and characterized mutants of the thermophilic archaeon *Sulfolobus acidocaldarius* that are able to grow at 70°C but not 81°C, whereas the wild-type grows optimally near the latter temperature. The conditional defects of these thermo-sensitive (Ts) mutants were studied by measuring growth, viability, and distributions of cell mass and DNA content as a function of time after exponential phase liquid cultures were shifted to non-permissive temperature.

The 34 Ts mutants examined were grouped into different phenotypic classes. Several of the mutants arrested in the post-replication stage of the cell cycle after temperature upshift, indicating that they were affected in cellular processes directly or indirectly related to the *Sulfolobus* cell division cycle. In one of the mutants belonging to this group, multiple rounds of chromosome replication occurred in the absence of cell division, showing that the normal interdependence between replication and division was impaired at non-permissive temperature. Implications of other mutant phenotypes are discussed.

The mutant collection may form a starting point for isolation and characterization of novel genes and gene products involved in essential cellular processes in archaea.

304 A membrane bound hydrogenase from methanogenic Archaea highly related to energy conserving NADH:ubiquinone oxidoreductase (complex I)

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Methanogenic Archaea are known to contain two types of [NiFe] hydrogenases designated F_{420}-reducing hydrogenase and F_{420}-non reducing hydrogenase. We have recently purified and characterized a [NiFe] hydrogenase from *Methanosarcina barkeri* only distantly related to these hydrogenases. This novel hydrogenase, designated Ech, is an integral membrane protein and is composed of six different subunits (Ech ABCDEF). Sequence analysis indicates that this enzyme is highly related to three other [NiFe] hydrogenases: *Escherichia coli* hydrogenase-3 (Hyc), *Escherichia coli* hydrogenase-4 (Hyf), and CO-induced hydrogenase from *Rodospirillum rubrum*. These hydrogenases are only distantly related to other [Ni-Fe] hydrogenases. Putative gene clusters encoding related enzymes have also been identified in the genomes of other methanogenic and non-methanogenic Archaea. Within these hydrogenases five subunits are highly conserved. These conserved subunits have homologs in complex I of the respiratory chain of bacteria and mitochondria. In complex I these subunits are assumed to form the essential part of the proton pump. Physiological data indicate that these membrane bound hydrogenases are also proton pumps. The close relationship of these enzymes to complex I indicates that these hydrogenases and complex I have a common ancestor and probably have a similar mechanism for proton translocation. Since these membrane bound hydrogenases catalyze very ancient reactions they may be regarded as the oldest proton pumps in life.

305 **Dilution of Stationary Phase *Sulfolobus* Cultures: Cell Division before Initiation of Chromosome Replication.**

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The cell cycle of organisms from the archaeal genus *Sulfolobus* is characterised by a short pre-replicative (B; G_1) and a long post-replicative (D; G_2, M and cytokinesis) stage. Cells in stationary phase rest in the post-replicative phase with two fully replicated genomes. Since no cells with >2 chromosome equivalents are present in exponentially growing cultures, the prediction would be that stationary phase cells that are re-inoculated into fresh medium should go through cell division before they initiate chromosome replication.

Experiments with stationary phase cells which were diluted at different optical densities all gave the same pattern. The cells first went through a phase corresponding to about a generation time or slightly less during which the optical density increased significantly but no cell division occurred. The cells then went through cell division, such that cells with a single chromosome equivalent appeared in the flow cytometry distributions. Shortly after division, initiation of chromosome replication occurred, as predicted.

The diluted cultures were studied by phase-fluorescence microscopy to investigate how far in advance of division that nucleoid segregation occurred. About 7% of the cells showed segregation of nucleoids in a sample collected 20 min before cell division was observed by flow cytometry.

Interestingly, cells diluted from exponential growth phase stopped dividing and ended up with 2 chromosomes (similar to stationary cells). They then divided back to the normal exponential situation with cells containing one or two chromosomes.

306 **Determination of active site residues of acetate kinase from *Methanosarcina thermophila*.**

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Acetate kinase catalyzes the first step in the activation of acetate for acetotrophic methanogenesis in *Methanosarcina thermophila*. Although the *Escherichia coli* enzyme has been studied extensively, the mechanism remains unclear. A double displacement mechanism with two phosphoenzyme intermediates and a pentacoordinate transition state has been proposed. Chemical modification and site-directed mutagenesis studies have been used to identify amino acid residues important for substrate binding and catalysis in the *M. thermophila* enzyme. We have previously identified the conserved glutamate E384 as a potential phosphorylation site. Chemical modification studies with phenylglyoxal and diethylpyrocarbonate have implicated both arginine and histidine residues at or near the active site. Site-directed mutagenesis has shown that several conserved Arg residues (R91, R175, and R241) are critical for enzymatic activity and are thought to be involved in stabilization of a pentacoordinate transition state. Site-directed mutageneis has shown that none of the conserved His residues are essential for activity. Replacement of H180 with Ala resulted in nearly complete loss of catalysis with little change in K_m; however, other replacements at H180 restored significant levels of activity. Replacements at other conserved His residues have been shown to affect k_{cat} to a lesser extent while replacement at H90 only affected the K_m for acetate.

307 **Intrinsic Resistance of *Natronococcus occultus* to Potassium Tellurite**

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Natronococcus occultus, a haloalkaliphilic archaeon, was examined for its resistance to potassium tellurite. Cells grown on a defined medium, containing 1 mM K_2TeO_3, transported the heavy metal and reduced it to metallic tellurium. This resulted in the deposition of intracellular tellurium crystals and the formation of black colonies on solid medium. Cell-free extracts of tellurite-grown *N. occultus* catalyzed the reduction of potassium tellurite. The inducible enzyme activity was dependent on NADH oxidation and occurred only under reducing conditions. This is the first report of an archaeon which is resistant to this normally toxic oxyanion.

308 **Structural and functional studies on the chaperonin TF55 from the archaeon Sulfolobus.**

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TF55 from archaea and its homologue from the eukaryotic cytosol, know as TriC or CCT, form a distinct subfamily of chaperonins that do not depend on a co-chaperonin for protein folding activity.

In archaea chaperonins form either eight or nine rings of one or two different subunits. TF55 from *S. solfataricus* is a nine fold symmetrical chaperonin which consists of two different subunits in a 2:1 ratio.

Oligomers of TF55 have been successfully crystallized in two dimension via their interaction with phospholipid monolayers at the air-liquid interface. Large coherent 2D-crystalline areas with p312 symmetry (a=b=162 Å, γ=60°) have been obtained and two-dimensional projection structures of the end-on arrays of the chaperonin were produced by electron microscopy. The chaperonin forms nine fold symmetrical rings with a three fold symmetry indicating a $((\alpha_2\beta)_3)_2$ double ring structure. A three dimensional model of the chaperonin at 18 Å resolution was reconstructed.

Under the crystallization condition used the chaperonin forms a double ring which was 162 Å in diameter and 175 Å height. Due to the absence of ATP the chaperonin is in an open "barrel" conformation with a large central cavity of 100Å in diameter in the middle of the ring complex and 70Å towards the top and the bottom.

The two different subunits of TF55 have been successfully cloned and expressed in *E. coli*. The two subunits alone form as well an oligomeric ring structures which show nine-fold symmetry for the beta subunit but eight fold symmetry for the alpha subunit. The recombinant alpha subunit oligomers were successfully crystallized in three dimensions by the vapor diffusion method. Three dimensional crystals of this protein obtained diffracted to a maximal resolution of 2.8 Å. A complete data set has been obtained and the determination of the protein structure is currently in progress.

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309 **Isolation and Characterization of an Acid Phosphatase from Thermoacidophilic Archaeon *Sulfolobus acidocaldarius***

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An acid phosphatase (APase) from thermoacidophilic archaeon *Sulfolobus acidocaldarius* was isolated, partially purified, and characterized. The enzyme was purified to 26-fold by ethanol fractionation, ion exchange chromatography, and gel filtration chromatography with overall recovery of 10 %. We have not found any other APase activity in the preparations. This partially purified APase was used in kinetic studies and analysis of optimal pH and temperature.

The optimum pH and temperature of the APase from *S. acidocaldarius* for *p*-nitrophenylphosphate as a substrate were approximately 5.0 and 70 °C, respectively. The apparent *Km* value was 1.9 mM. The APase activity was inhibited by some cations Fe^{3+}, Zn^{2+}, and Mn^{2+}, and also by EDTA. A native molecular mass of the enzyme was estimated about 20 kDa on a gel filtration chromatography. In order to confirm whether the APase is active in the monomeric form, we attempted to elute the enzyme from SDS-polyacrylamide gels using disk gel electrophoresis apparatus and renature the enzyme. The APase activity was recovered from SDS-gels in the 20 to 30 kDa range. This result, together with gel filtration analysis, suggests that the APase from *S. acidocaldarius* is active in the monomeric form.

310 **Methanopterin: Functional Differences from Folate**

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Tetrahydromethanopterin (H_4MPT) is a cofactor which plays a functional role in methanogens and some other Archaea that is partly analogous to that played by tetrahydrofolate (H_4F) in other organisms. This presentation will review the functional similarities and differences between H_4F and H_4MPT. Both cofactors carry C1 fragments between formyl and methyl oxidation levels. Nitrogen atoms N^5 and/or N^{10} bind the C1 fragment. The chemical environment of N^5 is similar in H_4F and H_4MPT but the environment of N^{10} differs: in H_4F it is linked by conjugation to the electron-withdrawing carbonyl group of para-aminobenzoyl glutamate; H_4MPT lacks the carbonyl group. Some consequences are as follows. On H_4F the C1 redox pathway is reversible between formyl and methylene, but irreversible to methyl. Formyl is attached to the reactive N^{10}, where it enters purine biosynthesis. The only "way out" from methyl H_4F is via methionine biosynthesis (the methyl H_4F trap of pernicious anaemia). On H_4MPT the C1 redox path is fully reversible between formyl and methyl. Oxidation from methyl is used in some anaerobic archaeal energy pathways; methylotrophic methanogens and some sulphate reducers. Formyl is attached to N^5 on H_4MPT where it cannot be used for purine biosynthesis. Serine hydroxymethyltransferase (reversible) and thymidylate synthase (irreversible) operate with methylene H_4MPT, but with enzymes that are distinct from H_4F-utilizing enzymes. The methylene fragment is bridged between N^5 and N^{10} on both cofactors but the rate-limiting chemistry for the above reactions is on N^5. H_4MPT (and related pterins lacking the carbonyl group) can be viewed as an archaeal evolutionary invention, tuning C1 metabolism for novel energy pathways.

311 **Phylogenetic analysis of proteasome alpha genes detected in the Crenarchaea and lower Eukarya using degenerate PCR**

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A pair of degenerate primers was designed to the most conserved regions of alpha proteasome subunits. The forward primer is a 38-mer that recognizes Prosomal Consensus Box I: [FW]-S-P-[DES]-G-[RKH]-[LIV]-[FYG]-Q-[VI]-[ED]-YA. The reverse primer is a 35-mer that recognizes Prosomal Consensus Box III: R-P-[FY]-G-[VT]-[SA]-[LFT]-[LI]-[IFY]-[AG]-G-X-D. The universal utility of these primers has been demonstrated by using them to amplify known alpha proteasome genes from the yeasts *S. cerevisiae* and *S. pombe*. These primers were then used to amplify alpha proteasome genes from the crenarchae *Sulfolobus solfataricus*, *Thermofilum librum*, and *Pyrobaculum islandicum*, as well as from the deeply branching euryarchaeon *Methanopyrus kandleri* and the lower eukaryote *Trichomonas vaginalis*. PCR products were gel purified, cloned, and sequenced. A single alpha gene was amplified from each of the crenarchae, but interestingly, two distinct alpha genes were detected in *Methanopyrus*. A single alpha gene without an interprimer intron has so far been isolated from *Trichomonas*. Translated sequences were trimmed of amino acids encoded by the primers and were aligned with euryarchael, eubacterial, and eukaryotic alpha proteasome sequences from the databases. The aligned amino acid sequences represent just less than half of the full-length proteins, corresponding to amino acids 28–130 of the *Thermoplasma acidophilum* protein. As expected, the *S.cerevisiae* sequences fall into seven different groups using actinomycetes as an outgroup. The *Trichomonas* protein groups with PsmA5 subunits. The *Thermofilum* sequence and one of the *Methanopyrus* sequences group with thermophilic and methanogenic euryarchae, respectively, while the other of the *Methanopyrus* sequences forms a grouping with the two other crenarchae. This degenerate PCR strategy is being employed to investigate the distribution of alpha proteasome genes in order to deduce the evolutionary history of this gene family.

312 **Creation of Genetic Information by DNA Polymerase of the Hyperthermophilic Archaeon *Thermococcus litoralis***

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DNA polymerase is an enzyme needed for replication of cellar DNA containing genetic information. The reaction requires a single-stranded template DNA, a short complementary primer DNA or RNA, and four deoxyribonucleoside triphosphates (dNTPs). Evidently no genetic information is created in this reaction. We have discovered that DNA polymerase of the hyperthermophilic marine archaeon *Thermococcus litoralis* (*Tli*) can synthesize DNA in the complete absence of added primer and template DNAs, implying that genetic information is "created."

The DNA synthesis was observed in a reaction mixture containing 20 mM Tris-HCl (pH 8.8), 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 6 mM $MgSO_4$, 200 μM dNTPs and 1 μg/ml highly purified *Tli* DNA polymerase, but not containing added template and primer DNAs. The DNAs synthesized were 0.2 to 50 kbp in length after 4 h, and were linear and double-stranded as demonstrated by electron microscopy. They had mostly repetitive sequences of (TATCTAGA)*n*, (TAGATATCTATC)*n*, or the like. When the temperature or the ionic strength of the reaction condition was changed, the sequence of the product DNA changed, *e.g.*, (TAAT)*n* at 69 °C, (TATCCGGA)*n* at 84 °C or (TATCGCGATAGCGATCGC)*n* at 89 °C in 10 mM KCl, while the sequence was (TATCTAGA)*n* in 0 mM KCl, (TATATACG)*n* in 50 mM KCl or (TATAGTTATAAC)*n* in 100 mM KCl at a fixed temperature (74 °C). When the pH of the reaction condition was changed from 6.8 to 10.8, the size of the product DNA decreased, but its sequence did not. With homology searches of these sequences, similar repetitive sequences were found in natural genomes of various organisms.

These results demonstrate that DNA polymerase of the hyperthermophilic marine archaeon *T. litoralis* isolated from a hydrothermal vent near Naples can synthesize a variety of DNAs *ab initio*, and that the characteristics of the DNA thus created are markedly influenced by reaction conditions. Our findings suggest that genetic information can potentially flow from protein to DNA, that protein is a driving force of evolution, and that such protein-made information is strongly influenced by environmental factors. It may be possible that such genetic information created by protein emerged on the primitive earth at a certain stage of the evolution of life.

313 Antibiotic resistance of Tryptophanyl-tRNA synthetase

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The crystal structure of *B. stearothermophilus* tryptophanyl-tRNA synthetase (TrpRS) complexed with ATP and indolmycin has been determined at 2.8 A resolution. Indolmycin is an analog of tryptophan and inhibits prokaryotic TrpRS specifically, in a manner of competitive inhibitors.· According to the structure, indolmycin makes two hydrogen bonds with D132 and H43, which are not observed in the structure of TrpRS with other tryptophan analogs. Including the H43 residue, several mutants were screened to raise indolmycin resistance.

Genetic analysis and steady state kinetics of these mutants revealed the following facts. First, H43 residue is essential for the efficient inhibition by indolmycin. H43N mutation confers the indolmycin resistance without affecting the affinity for tryptophan. This residue is conserved in almost all known bacterial TrpRSs, while it is not observed in any eukaryotic TrpRS. This explains the species specificity of indolmycin. Also, the reason why H43 residue is so conserved can be accounted for by the fact that the rate constant of H43 was less than 70 % of the wild type enzyme. Furthermore, one of the isolated indolmycin resistant strains carried single mutation K218E, which is a putative tRNA binding site. In fact, the mutant shows a high Km for tRNA. This mutant suggests the significance of the role tRNA plays in the amino acid discrimination of TrpRS.

315 Characterization of Lactate Dehydrogenase from the Sulfate-Reducing Thermophilic Archaean, *Archaeoglobus fulgidus*.

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Support NSF-Idaho EPSCoR-93192.

Prokaryotes from the domain Archaea are the best understood inhabitants of life from extreme environments and are a rich source of heat-stable enzymes for research and biotechnology. We study *Archaeoglobus fulgidus*, a member of the only sulfate-reducing group in this domain. *A. fulgidus* lives in an anaerobic environment at 83oC using lactic acid as a sole carbon and electron source, reducing sulfate to hydrogen sulfide. *A. fulgidus* produces enzymes similar to the sulfate-reducing eubacteria as well as the methanogenic Archaea. The sequence of the entire *A. fulgidus* genome has recently been completed, making it an ideal organism for research to better understand thermophilic enzymes and the physiology of sulfate-reduction.

We have found that *A. fulgidus* grows on both L and D forms of lactic acid. This organisms produces two forms of the enzyme lactate dehydrogenase that can discriminate between these enantiomers of lactic acid. This research is aimed at understanding the little known NAD-independent lactate dehydrogenases (LDH) that recognizes D-lactic acid. In order to purify the NAD-independent D (-) lactate dehydrogenase (d-LDH), *A. fulgidus* cells were grown under strict anaerobic conditions at 83oC. Cells were lysed by passage through a French pressure cell and d-LDH was purified by ultracentrifugation, ammonium sulfate precipitation and column chromatography.

To produce larger quantities of the d-LDH and study its thermophilic properties, the gene (*ldh*) encoding the d-LDH was cloned into an *E. coli* expression system. The *ldh* gene was cloned behind the *malE* gene, which encodes the maltose binding protein (MBP), in the pMAL expression vector. The resulting MBP-d-LDH fusion protein was expressed at low levels in *E. coli* under aerobic conditions at 37oC. As is the case with many Archaea, the ldh gene is almost exclusively encoded by the rare *E. coli* arginine codons (AGG/AGA) which led to low levels of recombinant expression. The tRNA that recognizes this codon, which is encoded by the *dnaY* gene on the pUBS520 vector, was cloned into the *E. coli* strain carrying the *malE-ldh* expression vector. This resulted in high levels of MBP-d-LDH fusion protein expression. D-LDH activity was assayed in native polyacrylamide gels at 60oC using D-lactate as electron donor and PMS and MTT as the artificial acceptors.

The activity of the recombinant fusion protein was indistinguishable from the native protein from *A. fulgidus*. Furthermore, the recombinant enzyme maintained activity after exposure to oxygen and at temperatures of 80oC. These results indicate that the stability of the d-LDH recombinant protein is similar to the native protein and that activity does not require a unique cofactor. Continuation of this work will help us to better understand the factors that confer thermostability to the enzymes such as d-LDH and how this particular LDH carries out electron transfer reactions in the absence of NAD.

314 Separation of Glycerolipid Biosynthesis and Glycerol Catabolism by Glycerophosphate Enantiomers in *Archaea*

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Glycerophosphate (GP) backbone of phospholipids in Archaea is *sn*-glyceol-1-phosphate (G1P), which is an enantiomer of bacterial and eucaryal counterpart, *sn*-glycerol-3-phosphate. (G3P). This is one of the most fundamental features characteristic of members of each domain, and so far, no exception has been found. GP-forming activities were surveyed in various archaeal species including *Methanobacterium thermoautotrophicum*, *Methanosarcina barkeri*, *Halobacterium salinarum*, two strains of *Pyrococcus* sp., and *Thermoplasma* sp. All of the cell-free homogenates revealed the ability to form G1P from dihydroxyacetonephosphate (DHAP). G3P-formation from glycerol or DHAP were also detected in the cell-free homogenates of four heterotrophic archaea. These results showed that G1P, the *Archaea*-specific phospholipid backbone, is produced from DHAP by G1P dehydrogenase not only in methanogens but also in the other archaea while exogeneous glycerol is catabolized via G3P. This means that the anabolism and the catabolism of GP are separated by the GP enantiomers in archaeal cells.

316 Evolutionary persistence of histones and nucleosomes

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All cells have architectural proteins to compact and organize their chromosomes. Prokaryotes have evolved a variety of unrelated small, basic DNA-binding proteins that includes histones in the *Archaea* and the HU family in *Bacteria*, while virtually all *Eukarya* employ histones. Histones may have prevailed during the evolution of the *Eukarya* because of their extended interactions with DNA. Contemporary archaeal histones and nucleosomes are clearly related to the evolutionary ancestors of their eukaryal counterparts, as archaeal and eukaryal nucleosomes exhibit a conserved protein structural motif, the histone fold, and similary recognize DNA structural features.

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317 Heterologous expression and purification of functionally active archaeabacterial rhodopsins in E. coli

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Three archaeabacterial retinal proteins bacteriorhodopsin (BR) from *Halobacterium salinarium*, halorhodopsin (pHR) and the photoreceptor sensory rhodopsin II (pSR-II) from *Natronobacterium pharaonis* were successfully expressed in *E. coli* using the method of Shimono et al., (1997). Flash photolysis experiments with isolated membrane preparations show that all pigments are directly incorporated into the plasma membrane of the bacteria.
For the isolation of the rhodopsins the *E. coli* membranes were solubilized with a detergent and Ni-NTA affinity chromatography was applied to purify the C-terminal His-tagged proteins.
The UV/VIS absorption spectra and ESI-mass spectra of the products demonstrate that this procedure provides highly purified retinal pigments in a yield of about 1 mg/L culture medium for pSRII. From a detailed comparison of the photochemical properties almost no differences to the homologous expressed proteins are observed in the case of pSR-II and pHR. However, the photocycle kinetics of BR expressed in E. coli was significantly distinct from that of the wild type.

Shimono, K., M. Iwamoto, M. Sumi, and N. Kamo *FEBS Letters*. 420:54-56 (1997).

318 Identification and Characterization of a Novel Ferric Reductase from the Hyperthermophilic Archaeon *Archaeoglobus fulgidus*

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Little is known about the acquisition and metabolism of iron by the Archaea. The purpose of this study was to investigate ferric iron reduction in the Archaeon *Archaeoglobus fulgidus*. *A. fulgidus* is a sulfate-reducing hyperthermophilic Archaeon first isolated from marine thermal vents in Southern Italy.
Archaeoglobus fulgidus contains high Fe(III)EDTA reductase activity in its soluble protein fraction. The corresponding enzyme, which constitutes about 0.75% of the soluble protein, was purified 175-fold to homogeneity. Based on SDS PAGE, the ferric reductase consists of a single subunit with a M_r of 18,000. Using native PAGE, the enzyme also ran at a M_r of 18,000 suggesting that the native ferric reductase is a monomer.
Both NADH and NADPH serve as electron donors for complexed ferric iron reduction. FMN is required as catalytic intermediate for Fe(III) reduction. Electron acceptors include Fe(III)EDTA and other Fe(III) complexes. Ferric reductase also utilizes FMN as electron acceptor in place of Fe(III) complexes which classifies the enzyme as a NAD(P)H:flavin oxidoreductase.
N-terminal sequence analysis of the purified ferric reductase resulted in the identification of the hypothetical gene, AF0830, of the *A. fulgidus* genomic sequence of The Institute for Genomic Research database. The AF0830 gene locus is hereby assigned to encode a ferric reductase or, alternatively, a NAD(P)H:flavin oxidoreductase. No significant homology exists to any other protein with known function suggesting that the *A. fulgidus* ferric reductase belongs to a new family of enzymes.
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319 Morphology Revisited: The phylogeny of shape determining genes

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Two separate strategies to combat osmotic pressure exist in extant life. One in which an outer, stress-bearing fabric lends rigidity and shape to the organism (prokaryotic) and the second, an internal framework of membranes and cytoskeletal components (eukaryotic). Integral to these strategies is the ability to divide. As peptidoglycan is the exclusive cell wall constituent in the Bacterial Domain, it has been proposed that its ubiquitous presence *dictated* certain aspects of bacterial morphology at the point of the bacteria's last common ancestor (Siefert and Fox, Microbiology, 144:2803-2808, 1998). Therefore, tracing genes involved in peptidoglycan synthesis and cell division of the Bacterial domain as well as genes identified as related to these proteins by previous investigations, will provide clues to cellular evolution.
Using genomic information from 13 complete genomes publicly available as well as partial genomes, we have investigated the evolution of shape determining/cell division genes across the three domains. Six genes, *mre*, *ftsA*, *dnaK*, *ftsZ*, *rodA*, and *ftzW* were tracked and their presence or absence in each organism noted. Parsimony and distance algorithms to deduce phylogenetic relationships were applied to the aligned data sets of each gene. The results can be explained by a hypothesis that posits a series of gene duplications. The ATPase/peptide binding family (HSP70/DnaK), which exhibits homology to murein synthesizing genes (*mre*), is most likely the result of a gene duplication of an ancestral *dnaK*-like gene, occurring after the Bacterial/Archaeal split, hence its ubiquity yet unique presence in the Bacteria. Likewise, we argue a second gene duplication of this *mreB* gene, early in Bacterial evolution but subsequent to the Archaeal/Bacterial split, resulting in the cell division protein *FtsA*. This explains both its absence in the Archaea, which has been bothersome (Doolittle, Nature, 392:339-342, 1998), and its intimate association with a morphological system based on peptidoglycan in place in the Bacteria at this time. The second family of related proteins involves the *cell division* protein ftsZ ubiquitous in Bacteria, Archaea, and chloroplasts. Our results substantiate that this is an ancient protein, and most likely its primitive form was central to a last common ancestor's ability to divide. We again propose that the weak homology between ftsZ and tubulins is a result of a variant of the ancestral ftsZ cell division protein that ultimately became associated exclusively with the eukaryotic cell and provided an alternate strategy for cell organization now represented by the eukaryotes. In a likewise manner, the low sequence homology of actin to the HSP70/*DnaK* family may well represent a gene duplication in a last common ancestor with similar segregation. A statistical approach that would allow divergence times based on the gene families identified and the series of gene duplications hypothesized will serve to strengthen the hypothesis, a step closer in defining the nature of the last common ancestor.
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320 Cloning, Expression and Characterization of Novel Cytochromes P450 from the Archaea: The Ancestral P450 Structure?

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The cytochrome P450 monoxygeases catalyze the exceedingly difficult chemistry of unactivated carbon oxidation with exquisite regio- and stereo- selectivity. P450 genes number over 800 and are found in all life forms in the biosynthesis of cellular regulatory compounds, such as the steroid hormones, as well as in catabolic detoxification of xenobiotics. With the proliferation of genetic information, it is natural to focus on proteomic questions. Is there an ancestral P450 gene? We have focused on questions related to the mechanisms of the P450 monoxygenases and envision multiple functions for this enzyme super family in not only atmospheric dioxygen metabolism but also via reductive chemistries operating in totally anaerobic environmental niches. In order to address these issues we have cloned, expressed, purified and characterized two interesting P450 systems. A P450 from *Sulfolobus solfataricus*, and a shortened protein from *Methanococcus jannaschii*, which we believe to represent the core domain structure of this ubiquitous class of enzymes The *Sulfolobus* gene is expressed to high yield in *E. coli*, and has been fully characterized by optical, Raman, and paramagnetic resonance spectroscopy. The enzyme is found to be highly thermo- and baro- stable, offering an exciting platform for the engineering of commercially important biotransformations. Structural information rationalizes experimentally measured of ultra-fast ligand dynamics and the control of redox flow via protein - protein recognition. This detailed characterization of the first P450 systems from the *Archaea* offers insight into evolutionary connectivity and the design of redox active sites. Our work is supported by grants from the National Institutes of Health.

321 **Plant-type β carbonic anhydrases extend into the Archaea domain**

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Carbonic anhydrases are zinc enzymes catalyzing the interconversion of carbon dioxide and bicarbonate [CO_2 + $H_2O \leftrightarrow HCO_3^-$ + H^+]. Three distinct classes (α, β, and γ) of carbonic anhydrase are recognized and appear to have arisen independently with no sequence similarities among each of the classes. The β class of carbonic anhydrase is composed primarily of plant chloroplast enzymes with the only documented prokaryotic enzyme being the *Escherichia coli* CynT. An open reading frame was identified in the genome of the thermophilic methanoarchaeon *Methanobacterium thermoautotrophicum* ΔH with a deduced sequence that is 34.3% identical to that of CynT. The gene product, designated as Cab (carbonic anhydrase beta), was produced in *E. coli*, purified to electrophoretic homogeneity, and found to have carbonic anhydrase activity. Native gel filtration chromatography and metals analysis suggest Cab is a homotetramer and contains one zinc per subunit. EXAFS studies indicate the catalytic zinc is coordinated by two cysteines, one histidine, and one or two water molecules. No loss of activity was observed after incubation of Cab for 15 minutes at 75°C. These results show that β carbonic anhydrases extend not only into the Archaea domain but also into the thermophilic prokaryotes.

322 **Phylogenetic Diversity of Archaea in Deep-sea Hydrothermal Vent Environments**

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Recent molecular phylogenetic surveys of naturally occurring microbial community in various hot water environments have revealed that the phylogenetic diversity of thermophilic microorganisms is far greater than previously proposed. Deep-sea hydrothermal vents are likely major hot water environments harboring a great diversity of thermophilic microorganisms but are behindhand with the molecular phylogenetic exploration. Here we report the phylogenetic characterization of diverse archaeal communities in deep-sea hydrothermal vent environments by the PCR-mediated small subunit rRNA gene (SSU rDNA) sequencing.

The mixed population DNAs are directly extracted from the chimneys and sediment of hydrothermal vent systems in Japan. Based on the sequence analysis of partial rDNA amplified with an Archaea-specific primer, the archaeal population in the deep-sea hydrothermal vent environments consisted of phylogenetically diverse groups. Most of archaeal rDNA sequences are the uncultivated types of sequences and reveal distant relationship not only to the cultivated species but also to the unidentified species found in other hot water environments. The phylogenetic analysis of these archaeal rDNA sequences revealed the existence of uncultivated, potentially ancient groups of archaea diverged deeply from the root of both Crenarchaeota and Euryarchaeota. These findings extend our view to archaeal diversity in hot water environments and phylogenetic organization of these organisms.

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Effect of light, anaerobiosis and nitrogen concentration on Glycerol and Lipid synthesis in *Dunaliella salina*

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Dunaliella salina is a halophilic green alga which photosynthetically or with a number of organic compounds synthesizes glycerol. Synthesis of glycerol and lipids is regulated by a number of environmental factors. Majority of lipid synthesized is polar lipid in presence of light and adequate supply of nitrogen. Nitrogen deficient cells showed a decrease in dry weight, chlorophyll a and protein content but there was an increase in the amounts of glycerol, lipid and carotenoid. Nitrogen deficiency inhibits cell division and therefore the quantity of storage product per cell is increased. Dark grown nitrogen deficient cells of *Dunaliella salina* accumulates glycerol and lipids under heterotrophic and phototrophic conditions.

Cellular lipid shows an increment , chlorophyll content decreased in nitrogen deficient cells at high light intensity. Anaerobiosis triggered lipid synthesis and prompted the breakdown of reserved glycerol in dark and light.

Anaerobiosis under nitrogen depleted medium showed a higher lipid content than under nitrogen sufficient medium.

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324 **Site Directed Mutagenesis Study of the Mechanism of Gamma Class Carbonic Anhydrase from M. Thermophila**

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Carbonic anhydrase gamma (CA–γ) from *M. thermophila* catalyzes the interconversion of carbon dioxide to bicarbonate during growth on acetate. We have explored the structure-function relationship of two regions in this novel enzyme by site-directed mutagenesis and chemical rescue. One region containing two acidic glutamate residues (Glu62, Glu84) appears to function in a novel proton transport pathway for carbonic anhydrase. An external solvent-exposed Glu84 residue was mutated to Ala, Gln, Asp and His. The Asp and His mutants had native like activity, while the Ala and Gln mutants exhibited very low activity, unless 50 mM imidazole was added to the buffer. The second region of interest contains the basic Arg59 residue, which may transiently bind bicarbonate anions prior to export or import from the active site. *Mutagenesis* of this residue to Lys, His, Glu, Gln, Met, Cys and Ala indicated that only Lys was catalytically active in MOPS buffer, while Ala and Cys mutants could have activity partially restored upon addition of 50 mM guanidinium hydrochloride. These results suggest a mechanistic role for the Arg59 residue, as well as a structural role in a salt bridge between subunits of the enzyme. This work was supported by a Research Training Grant fellowship from the National Science Foundation.

325 **Crossing bacterial/archaeal boundaries**

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Recently we discovered genes, enzymes and coenzymes in a methylotrophic α-proteobacterium, *Methylobacterium extorquens* AM1, which were thought to be unique to methanogenic archaea (Science 281, 99-102). We purified and characterized two tetrahydromethanopterin-dependent enzymes, a novel $NADP^+$-specific methylene tetrahydromethanopterin-dependent dehydrogenase and methenyl tetrahydromethanopterin cyclohydrolase which are proposed to be involved in formaldehyde oxidation to CO_2 in *M. extorquens* AM1.

We now discovered that other methylotrophic and methanotrophic proteobacteria of the α, β, and γ-group also contain methenyl tetrahydromethanopterin cyclohydrolase activity and the respective gene. Phylogenetic trees will be presented to show the sequence distances among bacteria and archaea implicating a long evolution of tetrahydromethanopterin-dependent enzymes.

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326 **Synthesis of cysteinyl-$tRNA^{Cys}$ in methanogenic Archaea**

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Aminoacyl-tRNA synthetases, a class of highly conserved enzymes, charge tRNA with the amino acid required for protein biosynthesis. The genomic sequences of two Archaea, *Methanobacterium thermoautotrophicum* (1) and *Methanococcus jannaschii* (2) reveal no gene encoding cysteinyl-tRNA synthetase (CysRS). Nevertheless Cys-$tRNA^{Cys}$ is required for protein biosynthesis and a $tRNA^{Cys}$ gene is present in both genomes. To compensate for a missing CysRS, Archaea could obtain Cys-$tRNA^{Cys}$ by misacylation of $tRNA^{Cys}$ with serine by seryl-tRNA synthetase (SerRS) and subsequent thiolation in a reaction similar to the formation of selenocysteinyl-$tRNA^{Secys}$ (3).

To investigate this possibility SerRS was purified from *Methanobacterium thermoautotrophicum* and cloned and expressed from *Methanococcus maripaludis*. Both enzymes charge their homologous as well as *E. coli* tRNA with serine. Mobility shift experiments of charged tRNA on acid-urea PAGE indicated that Cys-$tRNA^{Cys}$ is not made via Ser-$tRNA^{Cys}$ in these organisms. We are now looking for direct charging of $tRNA^{Cys}$ with cysteine in *Methanobacterium thermoautotrophicum* extracts. Cysteine-dependent ATP-PP_i exchange as well as TCA-unsoluble radioactivity could be measured in protein extracts showing the presence of CysRS in this organism.

(1) Smith, D. R., L. A. Dourette-Stamm, C. Deboughery, H. Lee, J. Dubois, et al. 1997. J. Bacteriol. 179, 7135-7153
(2) Bult, C. J. et al. 1996. Science, 273, 1058-1073
(3) Baron, C. and A. Böck.. 1995. In: Söll, D. and RajBhandary, U. (ed.), tRNA: Strucure, biosynthesis and function. ASM Press, Washington, D.C., 529-544

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A1 Molecular Physiology and Pathology of Membrane Traffic	Sep 15, 1998	Nov 9, 1998	Thomas C. Südhof and Richard H. Scheller	Santa Fe	Jan 9-14
A2 Archaea: Bridging the Gap Between Bacteria and Eukarya	Sep 15, 1998	Nov 9, 1998	Dieter Söll, William Whitman and Carl Woese	Taos	Jan 9-14
A3 Frontiers of NMR in Molecular Biology VI	Sep 15, 1998	Nov 9, 1998	Stanley J. Opella, Angela Gronenborn and Gerhard Wagner	Breckenridge	Jan 9-15
J3 Type 2 Cytokines in Allergy and Helminth Infections J4 Asthma	Sep 15, 1998	Nov 9, 1998	Fred D. Finkelman, Stephen J. Galli, Joseph F. Urban and Robert L. Coffman Dean D. Metcalfe, Robert Lemanske, Lanny Rosenwasser and Jeffrey Drazen	Lake Tahoe	Jan 9-15
A4 Molecular and Cellular Biology of Gene Therapy	Sep 15, 1998	Nov 13, 1998	Bonnie W. Ramsey, Mark Kay, Malcolm Brenner and Alan Smith	Salt Lake City	Jan 14-20
B1 Experimental Models of Immune Dysregulation and Mucosal Inflammation: Impact on the Understanding and Treatment of the Inflammatory Bowel Diseases	Sep 21, 1998	Nov 16, 1998	Warren Strober, Donna Rennick and Caroline Whitacre	Santa Fe	Jan 16-21
B2 Immunogenetics of Human Disease - MHC/TCR and Peptide	Sep 21, 1998	Nov 16, 1998	Chella S. David, Gunther Hammerling and C. Garrison Fathman	Taos	Jan 16-22
B3 Chemokines and Chemokine Receptors	Sep 21, 1998	Nov 18, 1998	Stephen Hunt III, Steven Kunkel, John Westwick and Gregory LaRosa	Keystone	Jan 18-23
B4 Macrophage Biology	Sep 21, 1998	Nov 23, 1998	Samuel C. Silverstein, Siamon Gordon and Ralph Steinman	Keystone	Jan 22-28
B5 Endocrine Disruptors	Sep 30, 1998	Nov 30, 1998	Kenneth S. Korach and George M. Stancel	Granlibakken	Jan 31 - Feb 5
B6 Aging: Genetic & Environmental Influences on Life Span	Oct 2, 1998	Dec 2, 1998	Judith Campisi and Jan Vijg	Tamarron	Feb 2-7
B7 Ocular Cell and Molecular Biology	Oct 5, 1998	Dec 4, 1998	Steven E. Wilson and Joe Hollyfield	Keystone	Feb 5-10
B8 Interactions and Intersections in Plant Signaling Pathways	Oct 8, 1998	Dec 8, 1998	Nam-Hai Chua and Venkatesan Sundaresan	Coeur D'Alene	Feb 8-14
C1 B Lymphocyte Biology and Disease	Oct 8, 1998	Dec 8, 1998	Edward A. Clark, Thomas J. Kipps and Michel C. Nussenzweig	Taos	Feb 8-14
C2 Molecular Mechanisms in DNA Replication and Recombination	Oct 16, 1998	Dec 16, 1998	Jerard Hurwitz and Stephen Kowalczykowski	Taos	Feb 16-22
C3 At the Dawn of the New Millennium: The Future of Drug Discovery	Oct 21, 1998	Dec 21, 1998	David W. Robertson, Catherine D. Strader, Marvin L. Bayne and Leslie J. Browne	Granlibakken	Feb 21-26
C4 Inflammatory Paradigms and the Vasculature	Oct 23, 1998	Dec 23, 1998	David Stern, Judith Berliner, David Hajjar, Steven Kunkel and John Harlan	Santa Fe	Feb 23-28
C5 Metalloproteases: Chemistry, Biology and Medicine	Oct 26, 1998	Dec 23, 1998	Hideaki Nagase, J. Frederick Woessner and Judith S. Bond	Tamarron	Feb 25- Mar 3
C6 Molecular Mechanisms in Alzheimer's Disease	Nov 3, 1998	Jan 4, 1999	Bruce A. Yankner and Dennis J. Selkoe	Taos	Mar 3-8
C7 Immunological and Biological Aspects of Therapeutic Protein Delivery to the Lungs	Nov 3, 1998	Jan 4, 1999	John Patton, Aleksander Blum, Ron Wolffe, Michael Matthay, Joe Brain and David Bice	Tamarron	Mar 3-8
C8 The Functions of Small GTPases	Nov 6, 1998	Jan 6, 1999	Susan Ferro-Novick and Larry Feig	Santa Fe	Mar 6-11
J5 Infections of the Nervous System: Host-Pathogen Interactions J6 Effectors of Inflammation in the CNS	Nov 9, 1998	Jan 8, 1999	W. Ian Lipkin, Opendra Narayan, William Hickey and Maggie So Scott R. Barnum, Mark Emmerling and Robert Ames	Taos	Mar 9-14
D1 Molecular Pathogenesis of Bone Disease	Nov 16, 1998	Jan 15, 1999	Steven L. Teitelbaum, Louis V. Avioli and F. Patrick Ross	Granlibakken	Mar 15-20
D2 The Molecular Basis of Cancer	Nov 16, 1998	Jan 15, 1999	Carol Prives, George Vande Woude and Arnold Levine	Taos	Mar 15-21
D3 Molecular and Cellular Biology of Transplantation	Nov 20, 1998	Jan 21, 1999	Jeffrey L. Platt and Kathryn Wood	Lake Tahoe	Mar 21-26
D4 Tolerance and Autoimmunity	Nov 25, 1998	Jan 26, 1999	Tak W. Mak, Hans Wigzell, Jean-François Bach and Diane Mathis	Keystone	Mar 26 - Apr 1
J7 Molecular Mechanisms for Gastrointestinal Cancer J8 Lipid Mediators: Recent Advances in the Understanding of Molecular Biology, Biochemistry and Pharmacology	Dec 1, 1998	Feb 1, 1999	Raymond N. DuBois, C. Richard Boland and Anil K. Rustgi Floyd H. Chilton and K. Frank Austen	Keystone	Apr 1-7
E1 Apoptosis and Programmed Cell Death	Dec 7, 1998	Feb 5, 1999	John A. Cidlowski and J. John Cohen	Breckenridge	Apr 6-11
X1 Specificity in Signal Transduction X2 Oncogene Networks in Signal Transduction	Dec 9, 1998	Feb 9, 1999	Norbert Perrimon and Tony Pawson Jacalyn H. Pierce, George Vande Woude and Silvio Gutkind	Keystone	Apr 9-14
X3 Protein Folding, Degradation and Molecular Chaperones X4 Protein Folding, Modification and Transport in the Early Secretory Pathway	Dec 10, 1998	Feb 10, 1999	Franz-Ulrich Hartl and Sue Wickner Linda M. Hendershot and Randall J. Kaufman	Copper Mountain	Apr 10-16
X5 DNA Vaccines: Immune Responses, Mechanisms, and Manipulating Antigen Processing X6 Molecular Approaches to Human Viral Vaccines	Dec 11, 1998	Feb 12, 1999	Margaret A. Liu and Jay Berzofsky Ann Arvin & Harry Greenberg, Mary Lou Clements-Mann, Douglas Richman and Bernard Roizman	Snowbird	Apr 12-17
E2 The PPARS: Transcriptional Links to Obesity, Diabetes and Cardiovascular Disease	Dec. 21, 1998	Feb. 26, 1999	Bruce Spiegelman and Ronald M. Evans	Keystone	Apr 28 - May 2

J1/J2, J3/J4, J5/J6, J7/J8, X1/X2, X3/X4, and X5/X6 are joint sessions.
Registration for one conference allows attendance at either meeting (pending space availability)

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2000 KEYSTONE SYMPOSIA

	Conferences	Organizers	Site	Date
A1	Gene Therapy: The Next Millennium	Inder M. Verma and Elizabeth G. Nabel	Keystone	Jan 6-12
A2	Cancer, Cell Cycle and Therapeutics	James Roberts, Stephen H. Friend and Richard D. Klausner	Steamboat Spgs	Jan 8-13
A3	Molecular Biology of the Cardiovascular System	Jeffrey M. Leiden and Mark C. Fishman	Snowbird	Jan 12-17
A4	Mitochondrial Dysfunction in Pathogenesis	John J. Lemasters and Anna-Liisa Nieminen	Santa Fe	Jan 15-20
A5	Stem Cells, Asymmetric Cell Division and Cell Fate	Margaret T. Fuller, Chris Doe and Irving L. Weissman	Keystone	Jan 17-22
A6	Innate and Acquired Immunity at Mucosal Surfaces	Martin F. Kagnoff and Lloyd F. Mayer	Sagebrush	Jan 18-23
A7	Cellular Immunity & Immunotherapy of Cancer	Olivera J. Finn and Michael T. Lotze	Santa Fe	Jan 21-27
B1	Signaling 2000	Tony Hunter and Tony Pawson	Keystone	Jan 22-28
B2	Genetics of Alcohol and Substance Abuse	Jeanne M. Wehner, John C. Crabbe and Wade H. Berrettini	Granlibakken	Jan 23-28
J1	Genetics, Pathogenesis and Ecology of Emerging Viral Diseases	Michael J. Buchmeier and Clarence J. Peters	Taos Civic Ctr	Jan 24-30
J2	Pathogen Discovery: From Molecular Biology to Diseases	Georg Hess and Helen Lee	Taos Civic Ctr	Jan 24-30
B3	Transposition & Other Genome Rearrangements	Nigel D.F. Grindley, Jef D. Boeke, Tania A. Baker and Ronald H. Plasterk	Santa Fe	Jan 27-Feb 2
B4	T Lymphocyte Activation, Differentiation and Death	Laurie H. Glimcher, William E. Paul, Gerald R. Crabtree and Harvey I. Cantor	Keystone	Jan 28-Feb 3
J3	The Dynamics of the Cytoskeleton	Elaine V. Fuchs and Ronald D. Vale	Keystone	Feb 3-9
J4	Intercellular Junctions: Short-Range Interactions Fundamental to the Development, Differentiation and Homeostasis of Cellular Assemblies	Bruce R. Stevenson, Daniel A. Goodenough and Richard Fehon	Keystone	Feb 3-9
C1	Molecular Epidemiology: A New Tool in Cancer Prevention	Frederica Perera and Curtis C. Harris	Sagebrush	Feb 10-15
C2	Chromatin Structure and Function	Gary Felsenfeld, Gary Karpen and Michael Grunstein	Tamarron	Feb 12-18
J5	Diabetes Mellitus: Molecular Mechanisms, Genetics and Prospects for New Therapy	C. Ronald Kahn and Alan R. Saltiel	Taos Civic Ctr.	Feb 16-22
J6	Molecular Control of Adipogenesis and Obesity	Bruce M. Spiegelman and Jeffrey M. Friedman	Taos Civic Ctr.	Feb 16-22
C3	Signals and Signal Perception in Biotic Interactions in Plants	Richard A. Dixon and Maria J. Harrison	Sagebrush Inn	Feb 22-27
C4	T Cell/APC Determinants of Immune Defense Against Microbial Infections	Robert L. Modlin, Foo Y. Liew and Margaret A. Liu	Santa Fe	Feb 24- 29
C5	Macromolecular Assemblies at Work: Application of Physics, Chemistry and Mathematics to Biology	Stephen K. Burley and John Kuriyan	Tamarron	Feb 24-Mar 1
D1	Cell Biology of Virus Entry, Replication and Pathogenesis	Michael B.A. Oldstone, Ari Helenius and Richard W. Compans	Taos Civic Ctr	Feb 29-Mar 5
D2	Experimental and Clinical Regulation of Angiogenesis	Robert S. Kerbel and Jeffrey M. Isner	Doubletree Hotel	Mar 2-7
D3	Assembly of Signaling Networks	Susan S. Taylor and Jack E. Dixon	Taos Civic Ctr	Mar 6-12
D4	Potassium Channels: Structure, Function and Therapeutic Utilities	Valentin K. Gribkoff and Leonard K. Kaczmarek	Granlibakken	Mar 11-16
D5	Genetic Bases of Brain Development and Dysfunction	Mary E. Hatten and Huda Y. Zoghbi	Sagebrush Inn	Mar 18-23
E1	Advances in Human Breast and Prostate Cancer	Dennis J. Slamon and Donald S. Coffey	Lake Tahoe	Mar 19-24
E2	Joint Regulation of Signaling Pathways by Integrins and Growth Factors	Martin A. Schwartz and Lewis C. Cantley	Breckenridge	Mar 25-31
E3	Nuclear Receptors 2000	Ronald M. Evans, Kathryn B. Horwitz and Bert W. O'Malley	Steamboat Springs	Mar 25-31
E4	Keystone Millennium	David Baltimore	Keystone	*Mar 31-Apr 4*
E5	Mechanisms of Immunologic Tolerance and its Breakdown	David W. Scott, Anne Cooke and Marc K. Jenkins	Steamboat Springs	Mar 31-Apr 6
E6	Novel Biological Approaches to HIV-1 Infection Based on New Insights into HIV-Biology	Robert C. Gallo, Didier Trono and Joseph G. Sodroski	Keystone	Apr 4-10
E7	Cytokines and Disease	Alan Sher, David J. Cosman, Robert L. Coffman and Warren J. Leonard	Snowbird	Apr 8-14

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Alan Sher, David J. Cosman, Robert L. Coffman and Warren J. Leonard

Cell Activation and Signal Transduction: Lipid Second Messengers IV
George M. Carman, John H. Exton and Sarah Spiegel

FAX
505 758 1949
messages 505 758 2254

Dieter Soll
. criticized late $$
-should study Archaea for some interesting feature they have not b/c they are Archaea

ARCHAEA
REACHAA
~~AREA~~

UR-Metabolism

has to be very careful from drawing conclusions from 16s rRNA for metabolism

a deep branching ~~methanogen~~ methanopyrus has a new lipid (~~2-3-di-ortho-~~) pre Archaeol ... which was thought to be a primitive lipid

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ARCHAEA
REACHAA
~~ARCH~~

UR-Metabolism

Karl Stetter

- 1st Archaea recognized (by Woese) were extremophiles
  - Methanogens - anaerobic ones

  Extreme Halophiles

  Extreme thermophiles
  Sulfolobus

- Where else are they
  - Pace, Delong et al w/ 16s rRNA show high diversity
    - in cold environments
    - in hot environments

- Hyperthermophiles (>80°C optimal T°)

- Deep branching species in rRNA tree are thermophiles
∴ Was original organism hyperthermophile?

maybe b/c earth was hotter then

Where are they today
- volcanoes
- vents
- deep hot rocks (eg in oil reserves)
  - found same species as in vents (Arch. fulgidus
  - suggests this means they could be on other planets

Extreme thermophiles

- special equipment needed to grow these

- modes of nutrition highly variable

Ferm. heterotrophs { Pyrococcus furiosus - motile, fermentative heterotroph
{ AEP2 - can grow on almost anything (including DNA)

Facultative chemoorganotroph

suggests that 16S is NOT a good predictor of metabolism - close "relatives" in 16S tree v. different

A fulgidus
- genome said 50 fatty acid metabolic genes
- grows on fatty acids
  - crude oil +
  - crude oil water extracted +
  - " " non-water -

has to be very careful from drawing conclusions from 16S rRNA for metabolism

a deep branching methanogen (methanopyrus) has a new lipid (~~2-3-di-ortho-~~) pre Archaeol ... which was thought to be a primitive lipid

Pyrodictium abyssi -- grows up to 110°
- forms network of tubules
- cells embedded w/in tubules
- penetrate thin surface layer -- connecting periplasmic space of cells w/ each other

glycoprotein
· 5 subunits
· 3 subunits ID'd
· no homologs to these
· resist 140°C

suggests a primitive multicellular example

Sensitive to shearing

Pyrolobus fumarii highest T°
- can use v. low O_2 to gain energy
- 90°C too cold
- optimum 100+

Strategy for IDENTIFICATION/ISOLATION based on rRNA

① novel 16s
② specific oligo probes
③ cell ID, whole cell hyb w/ probe → dead
④ isolation by "optical tweezers" via morphology (if pronounced morphology)
⑤ cultivate single cell

Did this w/ vent sample from E. Pacific rise

were able to grow up Koryarchaeota
Pyroplasma - best at 100°C, pH 7.

Ron Davis

Yeast
- still don't know # of genes
- v. dense,
- extra space - not sure what for

Emphasizes the need for Genetics to discover f(x)

Chip Technology
- Oligo arrays
- Microarrays

Says don't use clones b/c can't keep track

So -- use PCR's to amplify orfs

Says smaller arrays is better
Biggest problem is glass slides ... ∴ should buy commercial stuff

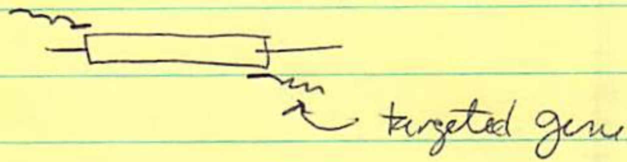
Affy whole genome yeast array
Suggests cost will go down as supply increases b/c
demand from pharmac. is so high.

How deal with data

Most of genes that they know f(x) are highly expressed
Some correlated expression profiles are clustered in genome

Says that there is a value in good databases for others to access data

Deletion analysis
Consortium to do set of deletions



only need about 45bp of homology

Delete all ORFs in 4 different strains
MATa
MATα
..

Since he doesn't think they can keep track of strains - so use genetic bar codes

So have bar code + other stuff they need to add for primers ... lots of $$$

Essential Genes
- 17% of ORF'S cannot be deleted in haploids
- 1/3 of these are uncharacterized
- 2x as likely to be w/in 5Kb of another essential gene

- only 2.5% of essential genes have a homolog w/in genome

but >10% of non-essential genes have homologs

- Can use in competitive environments (non pure culture)

Gene dosage effects

SCHMUCK

Euryarchaeal Genomics - Gary Olsen

Salvador Dali quote

There is/was a history
How well do hypothesis fit the data?

Why care about molecular phylogeny
- Relationships among organisms
- Natural populations

Histories of genes
- origin of metabolism
- gene families origins + structure
- lat. transfer

Patterns of D.

16s tree
- may or may not represent species tree

FRAMEWORK FOR THINKING ABOUT ORGANISMS GENES + GENOMES

Rooting the tree

Phylogenetic Methods
- need a model of evolution to test how well the data fit hypothesis

Just b/c they aren't perfect doesn't mean you can't test hypothesis

16s rRNA tree

- if take it as hypothesis of organismal evolution can use it to test hypothesis

e.g. origin of symbioses between methanogens + ciliates

e.g. origin of hyperthermophiles

- some mesophiles come out from w/in thermos
- can traces assuming ancestor was thermophiles
- ~~analogy~~.

SUGGEST THERMOPHILES HAVE EVOLVED SLOWLY

- many cases of closely related extreme thermos + mesophiles

- suggests there are recurring Δ's related to thermophiles

Biosynthesis Status of M. jann. aa synthesis
Pathway complete: arg asn asp glu gln gly thr
Steps missing: His Ile Leu. Lys Phe Ser Trp Tyr Val
Unknown: ??

Published in Science

How much energy required for synthesis in the environment?
Good correlation -- the "free" aa are missing steps in pathway

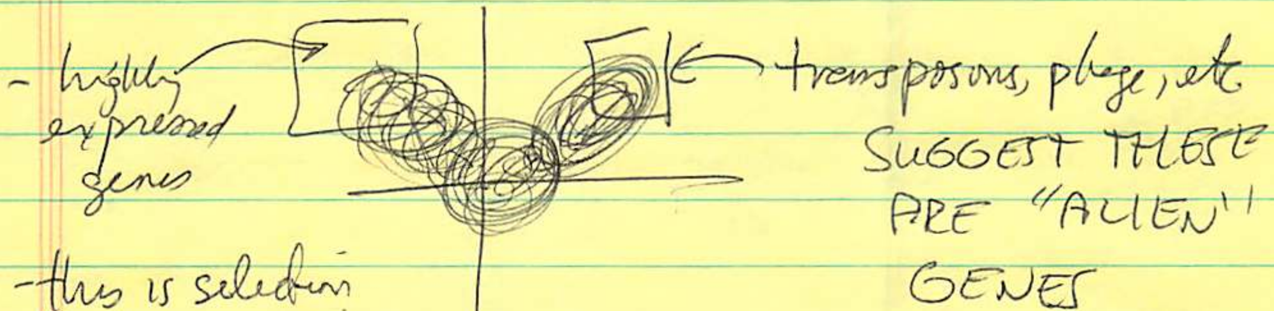
cannot assign NEW F(x) from genome analysis

LATERAL TRANSFER

How frequent is transfer

1991 paper on codon usage in Ecol genome Danchin

Codon usage in all Ecoli genes - factor analysis



- this is selection for high expression so it will be maintained

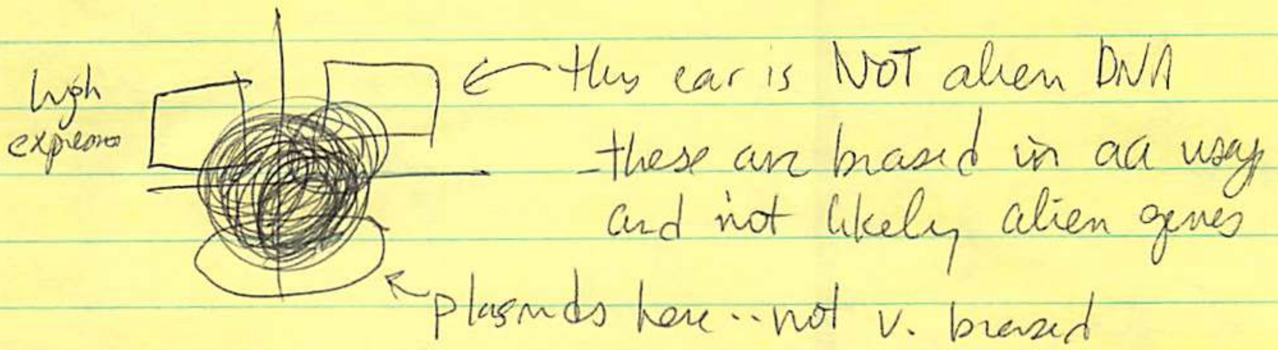
- this should "absorb" over time

- should take ~100 million to "equilibrate"

- leads to ~1 genome every billion yrs.

Lawrence + Ochman --- similar conclusion

Archaea

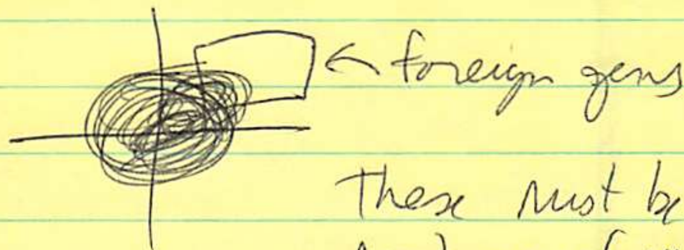


Can correct for aa usage

Source must be different than receptor

A. fulgidus - as Stetter sugg it is v. diff from close relatives

Genome sugg AF has "normal methogens" genes as well as extras



These must be recent genes.
Most are of UNID'd f(x)
Most core genes have normal usage.

more reducing environments in past means synthesis in past may have been different

Solfolobus

- 60% done
- will release soon
- 6 large contigs

use MAGPIE program

Cruz
Griffey

Gonzalez

Rodriguez from Sweeney

Sea
Oak

1 Min ⑤
2 Det - Gonzalez ⑤
3 Bal - 4
4 Bos
5 NY -
6 Cle
7 TB - Winn, Boggs
8 Cal
9 Oak
10 Sea Griffey
11 Tex
12 Chi -
13 KC - Sweeney
14. Tor - Cruz

Solfolder

8 tRNAs w/ introns

. many repeats

-18 Kb repeat -- completely identical

-6-7 diff. insertion elements

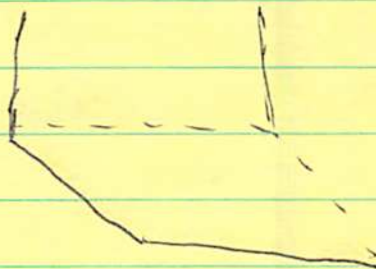
(gattaatccc aaaagg aatt ...) repeated over + over

Classes of gene

- use this for annotation

-

can plot 3D



JEFF MILLER

Euryarch { M. jann therm
Pyrococcus horikoshii
A. Fulgidus
H

Pyrobaculum aerophilium
- optim growth 101°C
- Nitrate reducer
- facultative microaerobe
- facultative heterotroph
- 100% plating efficiency

Decided to do reverse genetics

- 2.2 Mb 52% GC
- expected 1.7 mb so did only 4x coverage for 1.7
- so this wasn't enough

Sorel Fitz-Gibbon

- ~1 month until data release
-

58% best hit to Archa

45 ABC tporters
no catalase
- many leaderless transcripts
1 rRNA
no histone
no Z component
2 family B pols

has rev. gyrase
- no glutaminyl tRNA synt.
- no G/C skew

DEAMINATION , U during replication

Crystallize - trying to do all proteins
- beginning of consortium - 20 labs
-

Repair

Koulis et al - Ung Activity

- NOVEL UN Damage activity not homologous to anything

- ORF2 = Nth activity
ORF1 = like MutY - but only in sequence
= has UNG activity

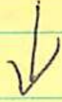
- GT mismatch glycosylase - human + Coli
- no homology to UNGs so good examples

- GU MM glycosylase
- does not cleave U in ssDNA

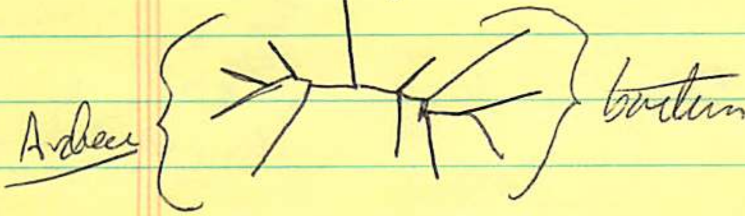
D. radiodurans
- chromosome degrade but comes back
- reassembled correctly

Pyrobaculum does not appear to be deamination resistant

use character analysis



1000 "characters"



So get similar
true to all

− +/−

− − − −

Richard Morgan - Restriction Systems in Archaea

Modifications
+
endonuclease

I Restriction → specificity → modification
II modifier protects DNA + endonuclease that cleaves
III Restriction → → Modification

Type IIs → many types
- some w/ control genes
- some have two modification systems (e.g. those w/ asymmetric sites)
* some work like NER cleaving on both sides
- VSR

≈1/3 of organisms examined have a recognizable type II activity

- how ID restriction enzymes
  - search for methylases
  - nearby ORFs are usually REs (which are not highly conserved)

- modifications
  - 5me-C - highly conserved
  - aminos - 3 classes, less conserved

- Genome analysis
  - most genomes have many likely genes
  - many probably due to lateral transfer
    - e.g. in H. pylori there is another strain w/ an RE inserted in one region

H. influenzae

- GTYRAC - sites are underrepresented relative to expected
- GTRYAC - more close to expected

could be due to codon usage

Restriction systems

- Bacteria : many
- Archaea : many
- Euk : none

A. fulgidus

- type I good hits
- type III - good hit for methylase
- type II - two good hits

M. thermo

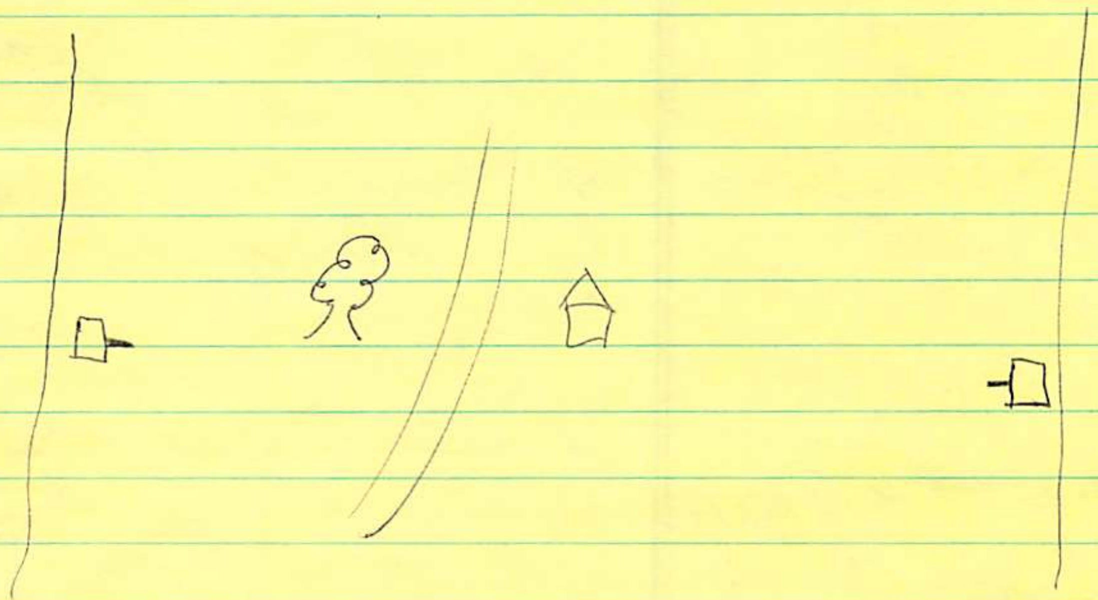
- type I - good hit
- type II - good hit

Is there a correlation betw II

P. horikoshii
- 4 type II like
- one v. similar to 3 GATC endos
- 18 aa insertion in recognition domain } so seq. may not work
- no detectable GATC methylation

M. jannaschii
3 type I
8 good type II
4 more potential type II's

does f(x) as GATC endo { - one like GATC endo
- with nearby GATC methylase } w/ ORF in between



Patrick Forterre - Gyrase

- 1st grant was rejected

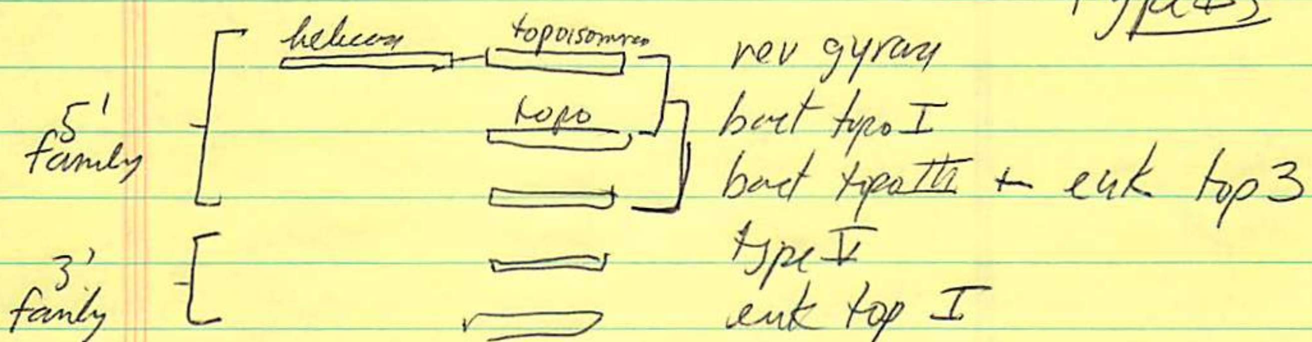
8 families of topoisomerases

	ω-protein	type I
	swivelase	
	gyrase	
in Archs + thermo	reverse gyrase	
	topo III	
	topo IV	
only in Arch	topo V	
only in Arch	topo VI	

monomers: type I = ss breaks
multimers: type II = ds breaks

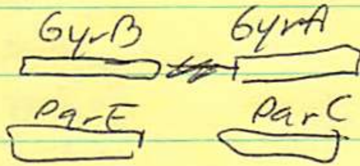
Supercoiling -
negative in all species
Positive only in Archaea

Reverse Gyrase

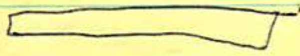


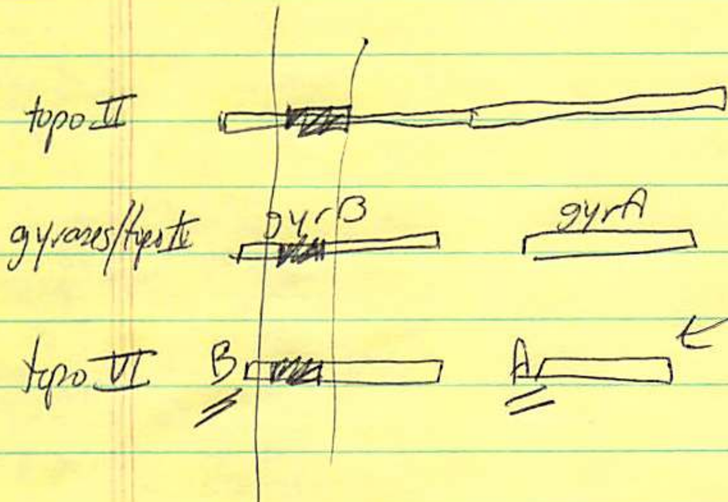
X FILES

<u>type II</u>



euk top II





- similar in seq to spo11
- allowed to predict activity ... only 1 Y conserved in spo11's

- similar ATP domain to HSP90 + MUTL

X-tal of HSP90 + MUTL show similar domains

In vitro activity of type VI A + B

Suggests may have similar f(x) to spo11 (initiation of recombination)

DNA does not denature w/ high T° in hyperthermophiles b/c topology constrains DNA

So this is why GC in DNA does not need to be high in thermos but RNA GC has to be high

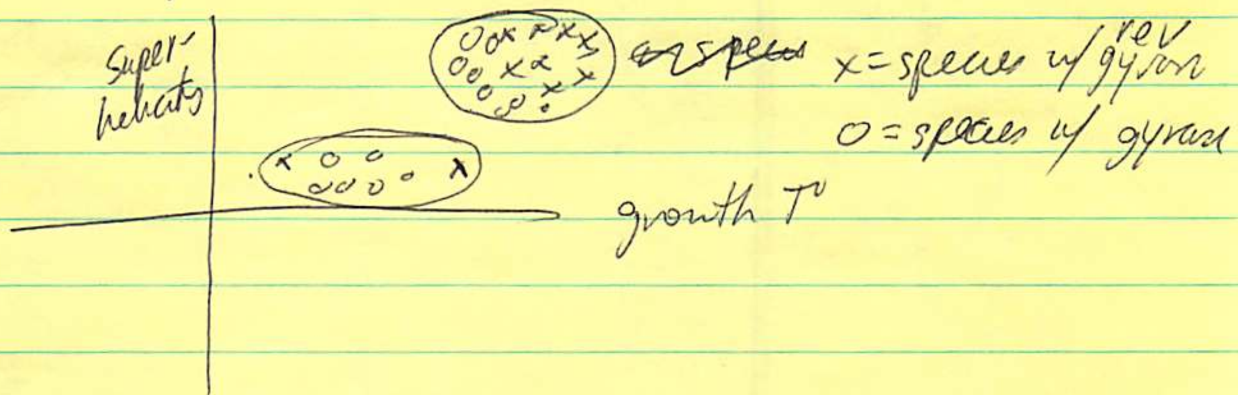
suggest rev. gyrase could be adaptation to high T°

X FILES

TRANSCRIPTION + TOPOLOGY

Sequence periodicity in genomes of bacteria + Archaea suggest ~~that~~

Presence of rev. gyrase does NOT always predict whether genome is relaxed OR not

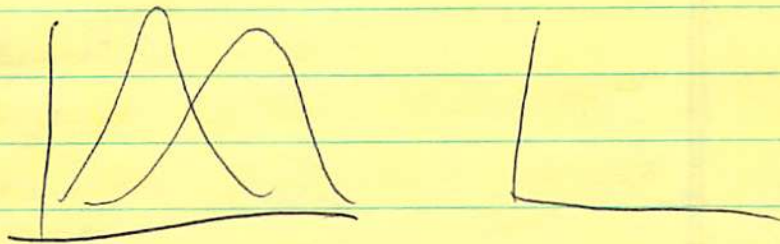


Genomes	G	RG
Aquifex	+??	++
Thema	+	+

Shock + TOPOLOGY

Heat shock → ↑ in :
Cold shock → ↓

Thinks that this is determined by diff T° optimums of diff enzymes



All 3 domains	topo I/III topo II-A
Only in A + E	topo VI-A
" " A + B	gyrase + rev. gyrase
" " E	topo I (swivelase
" " B	topo IV
" " A	topo VI
" " meth. kandleri	topo V

SUGGEST MANY CASES OF NON-ORTHOLOGOUS GENE DISPLACEMENT

Sel C

selA in ~~[illegible]~~ netjn was based on presence of conserved motif in one region

selB - netjn has large deletion relative to other sel B's

selB -- in E coli is like a elongation factor for

D. Soll

Direct aminoacylation

Indirect pathway

Distribution + origin of non-canonical

AA tRNA synthetases

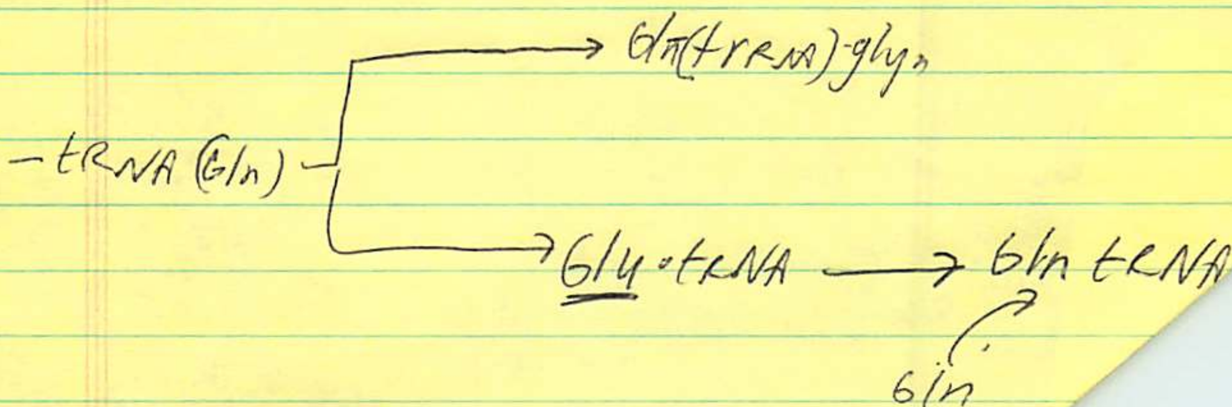
can divide into 2 main classes

I :

II :

only 4 completed genomic sequences contain a complete set based on homology

missing GlnRS in all Arch + most bact
AsnRS in many Arch + some bact

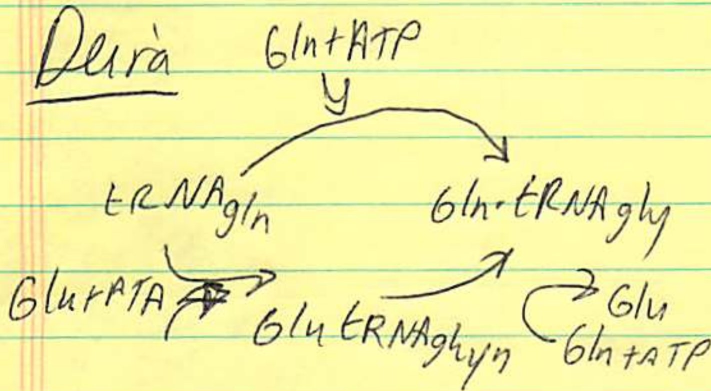


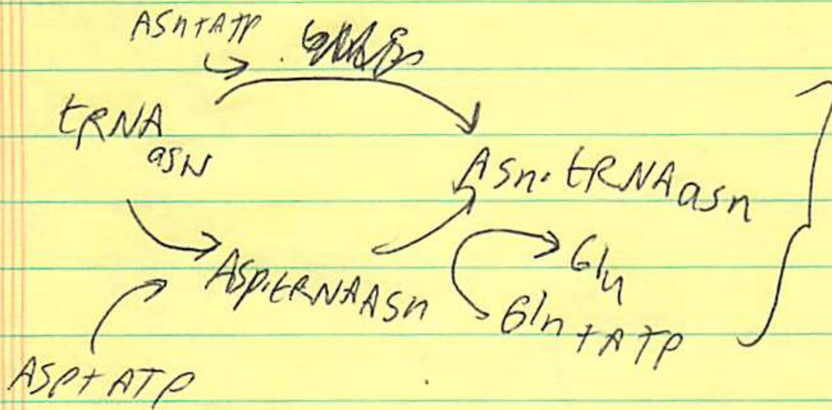
METABOLISM
IS
AMAZING

Glu-tRNA^{Gln} amidotransferase

- no ID w/ known proteins
- essential for growth

Poster -- no acknowledg of TIGR
- D. Soll -- no mention of TIGR in talk





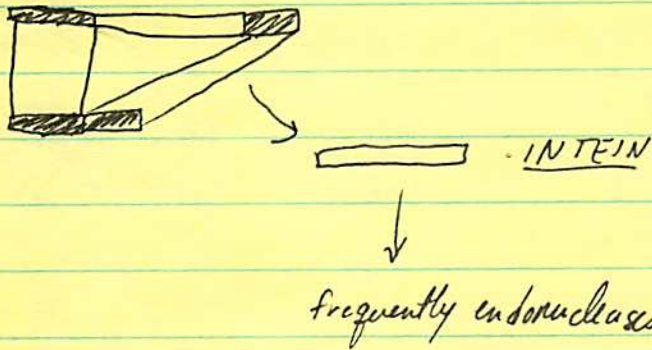
suggest that this pathway may be there to make asparagine

Evolution of Seryl-tRNA syn
see JBC

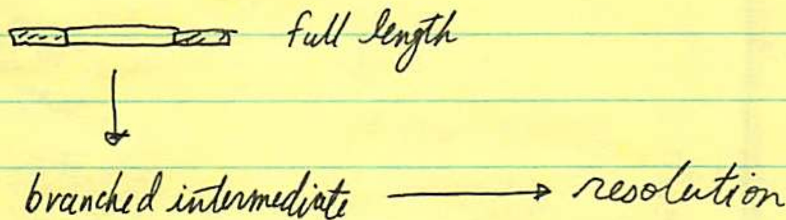
DIETER
R
Y

B

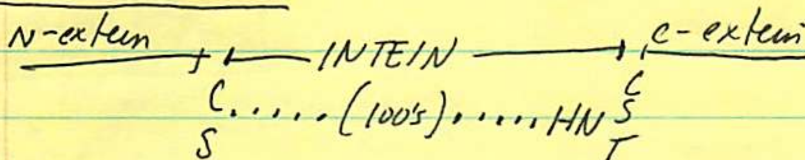
INTEINS



to test ... inserted intein into deep vent polymerase
... followed precursors in-vitro



Conserved motifs



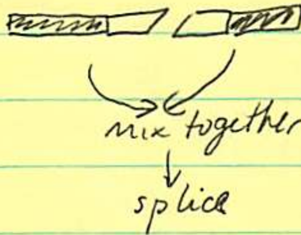
Intein Cleavage Vectors

① protein purification
- modify intein to have epitope tag
- modify intein so that it only cuts one side

② protein synthesis
- can synthesize two components and then link them together w/ C-term thio-ester

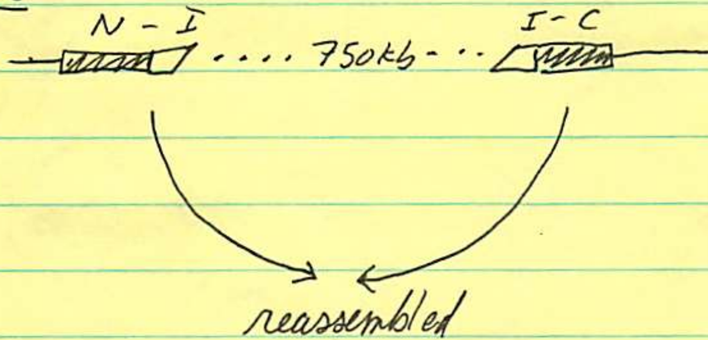
③ controllable splicing?
④ splicing in-trans

INTEINS



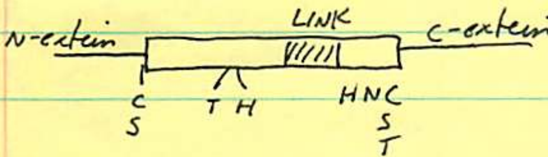
PNAS 95:9226
[illegible] et al

DNA E

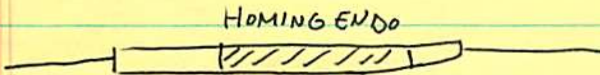


Protein Splicing rescues cell from separation of protein

INTEINS



Homing Endos - Belfort + Roberts 97
- mediate gene conversion event to insert



SPLICING STRUCTURE

- V. similar to hedgehog autocat. domain
    - used to attach to cholesterol in membrane

INTEINS

- .5 genes have multiple inteins
- 3 inteins in M. tub + M. lep are in diff. locations in same gene
- 55 inteins are in DNA repair, replication, and gene expression
    but 1/2 are prob. derived from each other

- thinks that the inteins move around in viruses + phage

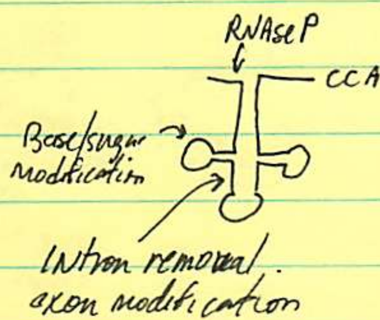
Virus
- Chlo
- Bassu phage

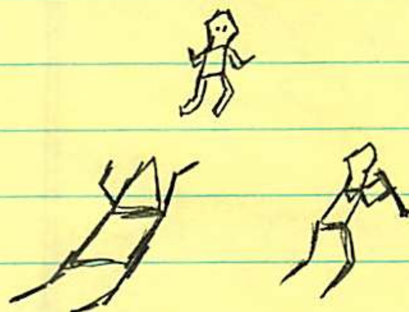
Many archaea - most inteins are in Archaea
many eubacteria

ARE THEY REALLY NEUTRAL?

Charlie Daniels - tRNA Maturation in Archaea

transcription → tRNA → tRNA maturation → translation





RNAse P complexes

	RNA(nt)	RNA catalysis	proteins
E.coli ~~Bacteria~~	373	+	1(14kDa)
Yeast	482	-	10 (20-100 kDa)
M.jannaschii	274	+/-	?

tRNA introns
- widespread NOT ubiquitous
- intron position in yeast conserved (37-38)
- intron position freq. conserved in Archaea (37-38) but NOT all

WAR

tRNA endonucleases
- highly conserved motif in Archaeal also found in two of the yeast nuclear tRNA endonuclease components

tRNA trp introns

-sequence highly conserved among archaea
-except M. jannascii

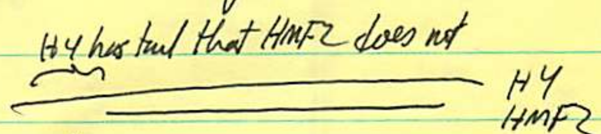
WARP
WARD -does it have a f(x)
WARN -suggestion came from rRNA maturation in euks
WARE
WARM -showed that Archaeal system similar to snoRNA processing
WARS
WART
WARY

John Reeve

started w/ protein purified that bound DNA

How to package DNA?
- eukaryotes only do it one way → nucleosomes
- prokaryotes do it many ways

Isolated HMF-2 - very similar in sequence to histone H4



this is site of acetylation

HU - does NOT have similar structure

Nucleosomes

H1/H2 dimer } Archaeal nucleosomes v. similar
H3/H4 dimer }

M. jannaschii 5 histones ... can make all types of dimers
M. thermo 3
M. 2

Able to circularize DNA using linear piece + HMF

In-vivo
- isolate protein + DNA → X link → digest → get nucleosomes

Are the histones localized in-vivo?

M. jannaschii
works better
w/ genome
b/c have
all problems

M. fervidus →
- diff. genes bound to histones more than others
↓
looked at sequence of these genes
↓
get localized assembly

Questions
- no detectible modification of histones but most work in-vitro
- (b) ... have not found a homolog
- HISTONES in crenarchaeotes
  - not detectible

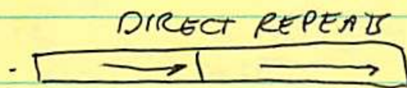
M. Thomm

- mechanism + regulation of txn in Archaea

Separating txn complexes in P. furiosis
- 3 components
- RNA polymeras — like pol II in euks
- aTBP — like TBP
- aTFB — like TFIIB

transcription in-vitro

TBP in P. furiosis



...and then

- binds to TATA box

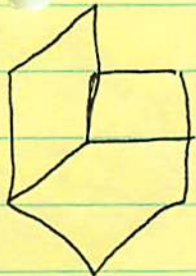
TFIIB
- doesnt bind DNA on own
- enhances TBP footprint

RNA pol

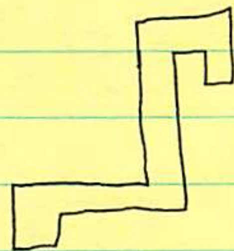
CRP homolog ... homolog of bact txn regulator
- binds to DNA

DNAK of M. mazei

Ribosome Assembly



BIOLOGY
IS FUN.



making rRNA mutations

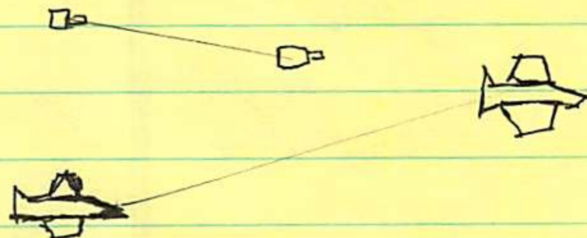
I DON'T CARE.

3 stories

① how does mRNA feed through 30S

BEATCAL ② using new system w/ deletions in all 7 genomic rRNAs to study Ab resistance

BE AT CAL ③ A-site of tRNA



B EAT

rRNA processing in Solfolobolobolos
Sulfolobus acidocaldarius

Aa Bb Cc Dd Ee Ff

Gg Hh Ii Jj g K k

y

ABCDEFGHIJK

yY
LMNOP. y

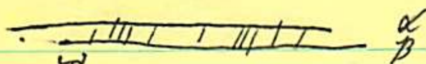
yY
y

Q S Y

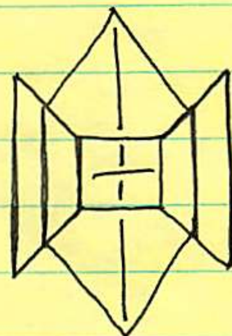
Archaea Proteosome

- less complex than eukaryotic

- Thermoplasma acidophilum
  - two subunits: $\alpha + \beta$
  - related to each other

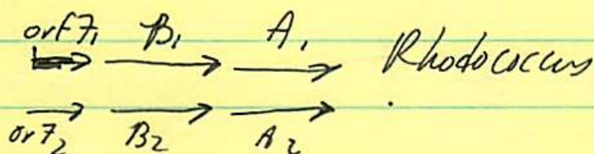


  - pro peptide
  - cleaved off in autocatalytic activity
  - this exposes active site
- crystal structure shows $\alpha + \beta$ v. similar in structure
- N-terminal extension of α
- experiments suggests they are NOT essential



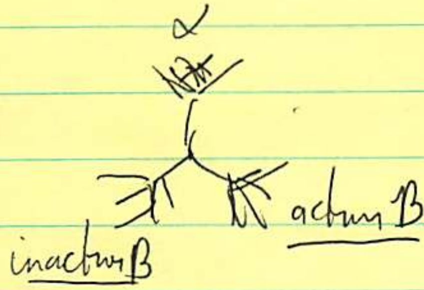
Genome sequences show most Arch have sim. proteosome components
- but also show many other proteases (e.g. lon)
- this may explain why they are not essential

Proteosome in bacteria



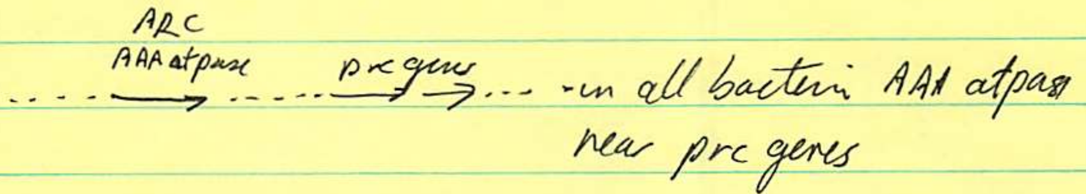
$\beta_1 + \beta_2$ v. closely related
$\alpha_1 + \alpha_2$ v. closely related } suggests lateral transfer b/c of diff GC content

and tree shows ~~$\beta_1 + \beta_2$ v. close to each other~~



In tree Rhodococcus
$\alpha_1 + \alpha_2$ next to each other
$B_1 + B_2$ next to each other

E coli	Thermoplasma	Rhodococcus	Yeast
v. simple	more complex	more complex	most complex
HslV	α, B,	α, B, orf7	7α + B

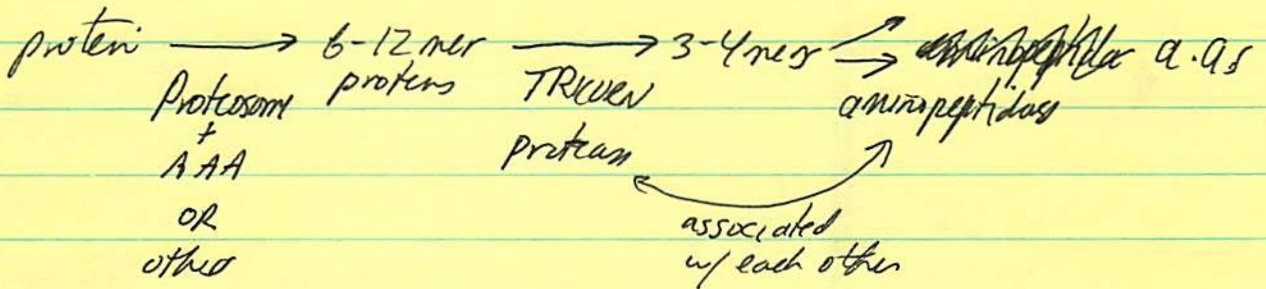


- in all bacteria AAA atpase near prc genes

~~AAA~~

26S proteosome
- regulatory complex in yeast
- v. flexible structure

protein degradation in Thermoplasma



- genomes show few homologs of TRICORN
- so went in + isolated Pyrococcus complex + has similar function but little sequence similarity

pyrococcus
- has some clon sequences in C. elegans

Higher eukaryotes
have a diff system to perform oligo peptidase

Molecular Basis for Unidirectional TXN in Archaea

Newcomer to this field.
- Came to Archaea b/c of reduced complexity

SIMPLER here not always near ancestral

Pre-initiation complex
- eukaryotic complex growing at 30 kD/month
- Archaea much less complex

Archaea
- TBP, TFB, pol - can give unidirectional txn
- extremophile enzymes increases ~~sample~~ range of biochemistry

Any 2nd grader who has taken a course in eukaryotic biology knows...

TBP
- mentioned that there is NOE TFIIA homolog in any Archaeal genomes ... maybe the reason for some of the differences betw euk + Arch TBPs

TF~~IIB~~-
- similar in structure to cyclins

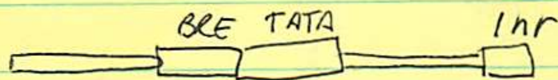
changing the tire metaphor ... applies in BIOLOGY .. want some "looseness" in initial binding and then it can tighten down

So... how does unidirectional txn occur?

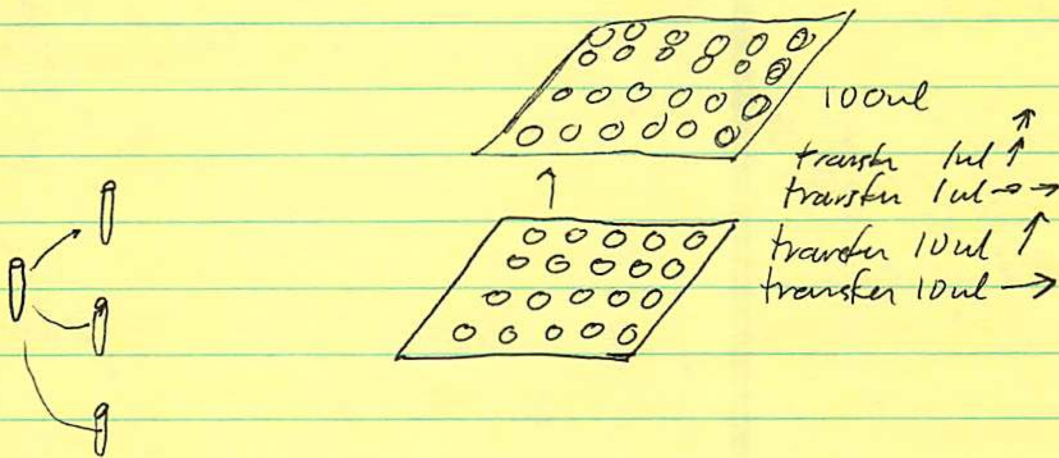
~~TFII~~ TFIIB .. orients TBP on DNA .. makes it asymmetrical

R. Ebright
- pointed out that ~~[illegible]~~ TFIIB have characteristic signature of HTH proteins

What defines polarity of Arch. txn?

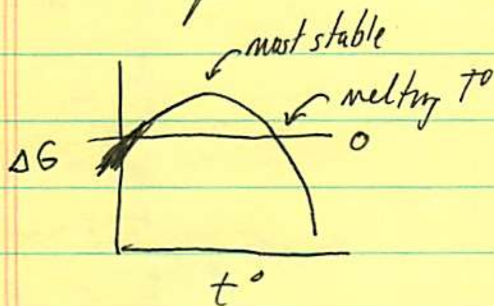


polarity determined by interaction of TFB w/ BRE



Doug Rees

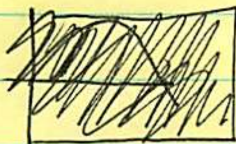
- max. stability of hypertherm. proteins is not much diff than "normal"
- relationship betw structure + stability is subtle
- relationship betw " " + T° dep. of stability is subtle



From Normal Proteins

- there are some things that correlate w/ stability
- but majority of effect is due to # of residues (more # = more stable)
-

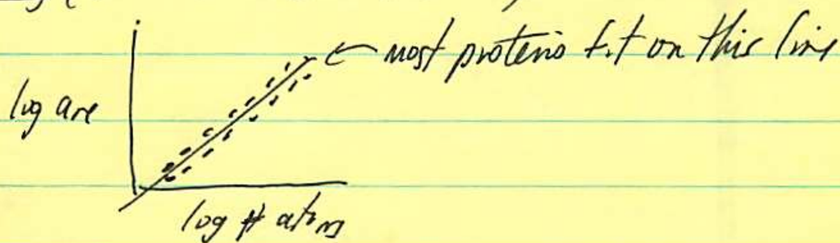
How shift "normal" proteins to thermostable ones



(1) shift curve to the right (and ∴ may not have higher max. stability)

Structures suggest some factors that are important

- salt bridges may help -- both betw. residues + w/ cofactors
- packing (surface area vs. volume)



- coefficient of thermal expansion - perhaps they expand less than "normal" proteins

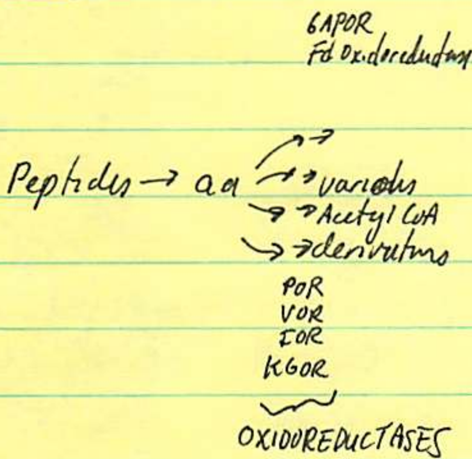
Mike Adams - Adaptation of hyper-thermophiles

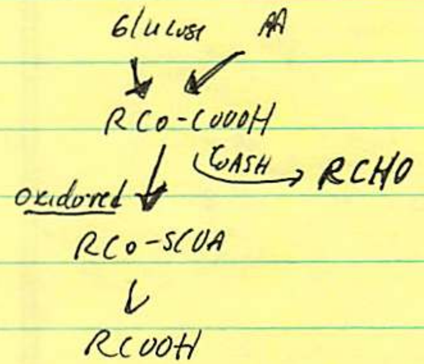
P. furiosis

- obligate anaerobe
- tungsten important to growth (W)
- maybe similar to Molybenum proteins ... many of which have cofactors

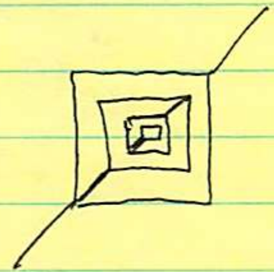
Glucose → → G-3-P → → → Acetate

GAPOR
Fd Oxidoreductase





GAPOR, FOR, AOR - Tungsten containing



EMLTAC
DEAGLC

Agenne

- all are Fd dependent
- all have single W per subunit
- also two additional ones in the genome
- many have 4+ Fe
- 3D structures v. similar
- W site coordinated w/ apterrin
- some use 2 pterrins
- suggests that in some places 3D structures line up w/o much seq. similarity
- can structures help w/ functional predictions?

What about others

	AOR	FOR	GAPOR	Other
PF	+	+	+	++
PH	+	+	+	+++
Pyobaculum	+	+		+
Archf	+			+++
Methjn			+	

WHY Tungsten?

	W vs	Mo
T° stab	HIGH	low
O_2 sens	sensitivity	stable
red potential	low	variable
Kinetics	slow	fast

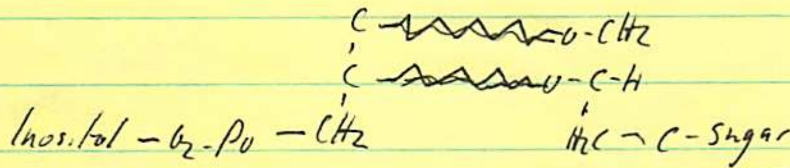
Growth on W
- Pyrobaculum aerophilum reqs W at high T°
- but it can grow at lower T° w/ Mo

Why AOR in peptide fermentation
- aldehydes generated from 2 keto acids only at high T°
- Fd 2e/1e conversion

Why FOR used?
- replace NAD dep enzymes

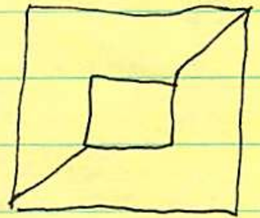
Why GAPOR?
- maybe 1,3 bisphos too unstable
- replace NAD dep enzymes

Structure + Biosynthesis of polar Lipids



Methanococcus maripaludis

mesophile
autotroph
GC = 33%
lyses in distilled H_2O



Tools

- PEG transformation
- selectible markers
- self replicating shuttle vector
- reporter genes
- selection for conditional mutants w/ uracil analogs

Expression Vectors -

Integrative vectors -

Random insertional mutagenesis -

- cut up gDNA
- ligate into non-replicative plasmid w/ pur
- select for pur resistance
- isolate plasmid

(1) permease | ATPase | Isolate gene that when mutated led to ~~growth~~ on acetate requirement

- In ABC transporter family.
- Maybe ion transporter
- Similar to MJ gene ID'd as Sulfur tporter

(2) JJ117 -

- acetate + vitamins stim growth
- cobalamin supports growth but cobinamide does not
- sequence is sim to MJ0010 (39%) MJ0016 (28.2%)
- there are homologs of phosphonopyruvate decarboxylase in Strep. hygroscopicus
- original mutant looked like had multiple integration of vector

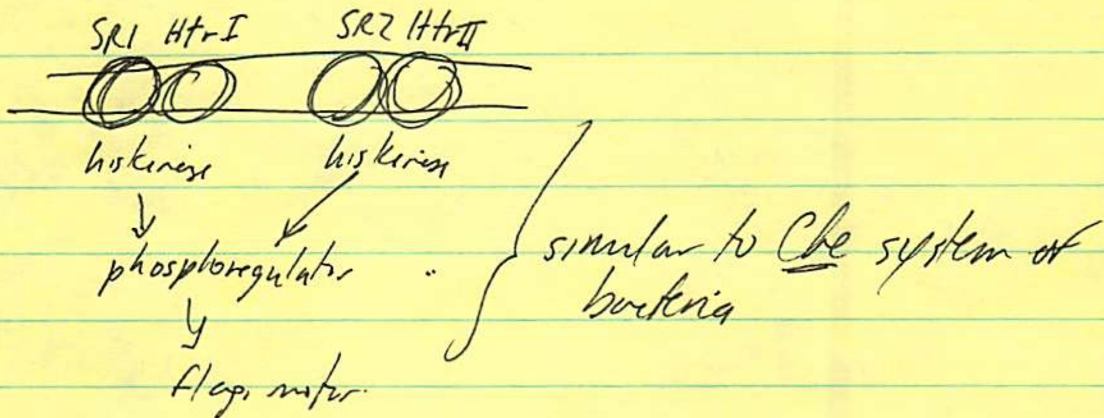
↓ (cell sketch) ↓ mutant

J. Spudich - Rhodopsins

Archaeal rhodopsins
Relationship to euk visual pigments
Archaeal rhodopsins homologs in euks.

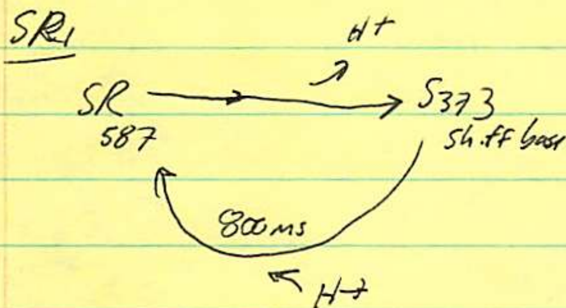
Halobacteria

- phototactic -- attracted to orange light
- two sensory rhodopsins - SR1, SR2
- BR = } light driven ion channels - H^+ out of cell
- HR = } - Cl^- into cell



- SR1 can work as a light driven transporter + must be modified to work as sensor

SR1



- Htr1 affects the reprotonation of $SR_{373} \to SR_{587}$

Deletion Mutants used to map f(x) domains

Similarities + Differences to Euk rhodopsin

	A	Euk
retinal use	+	+
retinal	trans	cis
seven helices	+	+
isomerization w/ light	+	+
steric trigger w/ CH_3	+	+
proton transfer helix 6	+	+

Is this homology or convergence
- high conservation among BR, SRI, SRII in retinal binding pocket
- but poorly conserved elsewhere
- some aa's similar between Archael + Euk rhodopsins in retinal binding region
- but overall little aa similarity in retinal binding region

Similarity
: topology, activation ..

Non-similarities
aa sequence

Arch Rhodopsin's in eukaryotes?
- 1st suggestion was experiments in Chlamy phototaxis which was retinal-like
- break came from EST project for N. crassa which seemed to encode Archael-like rhodopsins
- sequence is v. similar to Archael rhodopsins
- protein absorption is like Arch. rhodopsins

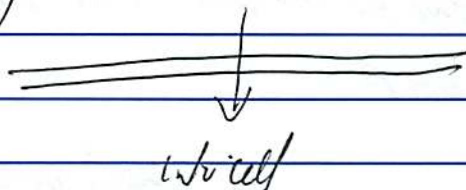
KEYSTONE SYMPOSIA
on Molecular and Cellular Biology

Sugar utilization in hyperthermophiles

CelB/LamA operon
celB = glycosidase xtallized
lamA = glucanase

celB = ~~under~~ txn controlled by B-linked sugars
no induction w/ maltose + some others
rapid induction w/ others

lamA = laminarin →(LamA) laminaribiose
(seaweed)



How does Pyrococcus utilize glucose?
- genome does not have Hexokinase like gene ...

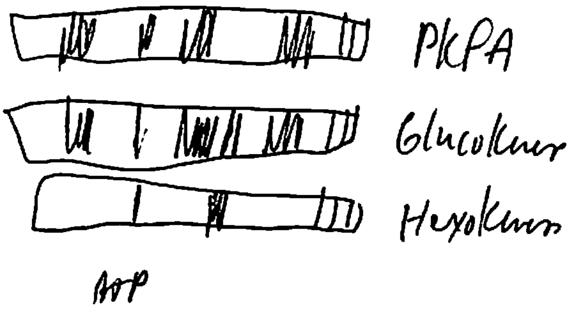
ADP-Dependent Kinases

glucose
↓ ADP dep. glucokinase
glucose 6-P
↓
F-6-P
↓ ADP-dep phosphofructokinase = PfkA
F-1-6-DP

Genes + activity in Pyrococcus, Thermococcus + M. jannaschii

So seq. sim. to GlkA

Why use ADP not AMP?
- ADP is more stable at high T
in-vitro



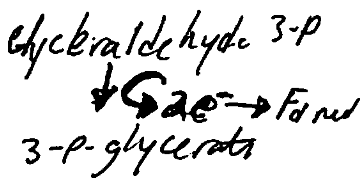
Formation of triosephosphates

Galk
- highest sim to T. maritima
- only uses ATP
- exclusive for D-galactose

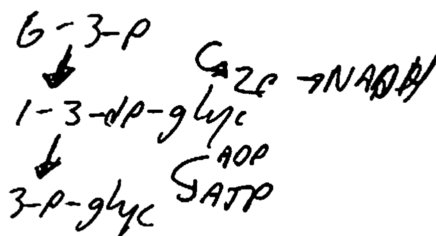
Production and Disposal of Reducing Environments

① Fd-conversion of GAP

~~GAPOR~~ GOR



~~most species~~ GAP



} can be reversed for gluconeogenesis

So...why do it this way and lose the extra ATP?

Suggests that GOR is on for degrading glucose + GAP is used for gluconeogenesis.

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RUBISCO

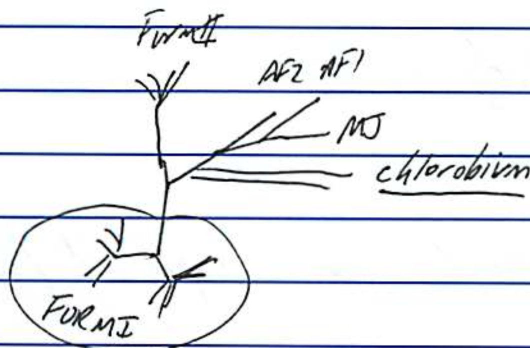
- 1000's of sequences
- form I's can be divided into many groups
- form II form one group

tau ≃ specificity

form IA	25-50
IB	75-80
IC	45-75
ID	~100
II	~15

How learn about RUBISCO
① mechanistic / str(x) / f(x)
② directed evolution
- biological selection
- mutagenesis
- shuffling
③ microbial biodiversity - take advantage of Natures surprises

Why do obligate anaerobic Archaea have RUBISCO?

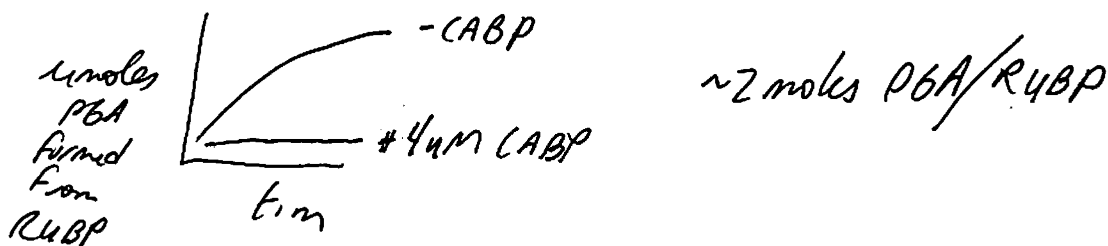


Archaea RUBISCO's

- catalytic sites conserved
- so...maybe "real"
- structure-threading looks good

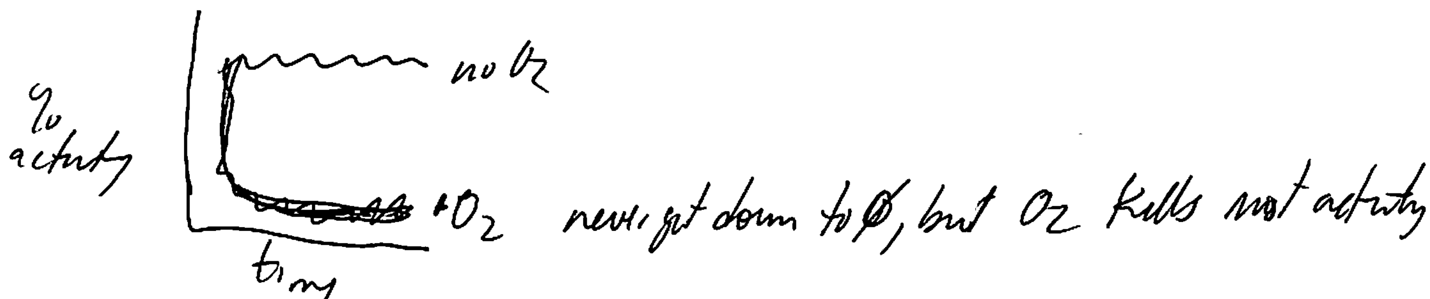
Metjn RUBISCO

- cloned + purified



~2 moles PGA/RUBP

- thermally stable



never got down to Ø, but O_2 kills most activity

- this is reversible (add O_2 scavenger)

- specificity?
  - this enzyme can catalyze an oxygenase activity in presence of O_2
  - little carboxylase activity w/ O_2

- expression
  ARCFU rbcL1 not expressed rbcL2 expressed (under conditions used)

- How make RUBP not known

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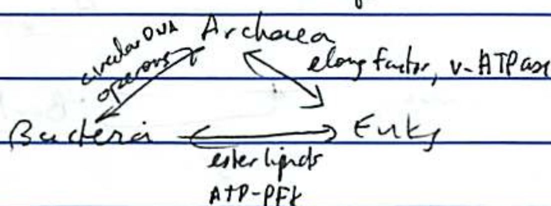
Russ Doolittle

- protein clocks are not like Atomic clocks
- Carl Woese the great non-present organizer of the meeting
- no talk about this can start w/o some kind of homage to Carl Woese

Just what is the universal tree

"Kandler's Triangle"



Assumes History can be reconstructed w/ sequences

Problems w/ reconstruction

① some trees are weird

Clocks

- most proteins change at characteristic rates + these rates never run backwards
- don't want slow Δ'ing genes or fast Δing genes
- most enzymes are in middle so chose all enzymes

% similarity

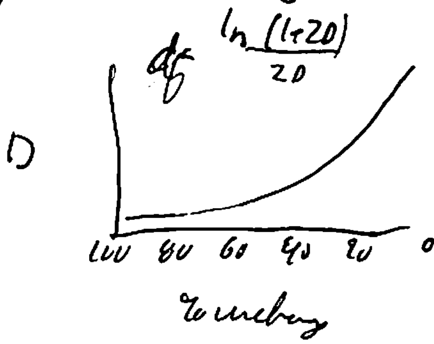
e.g. Animals vs. Fungi avg % sim = 55%

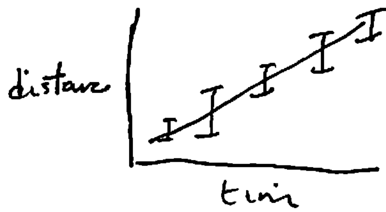
Bacteria vs Euks avg % = 37%

- Least squares tree

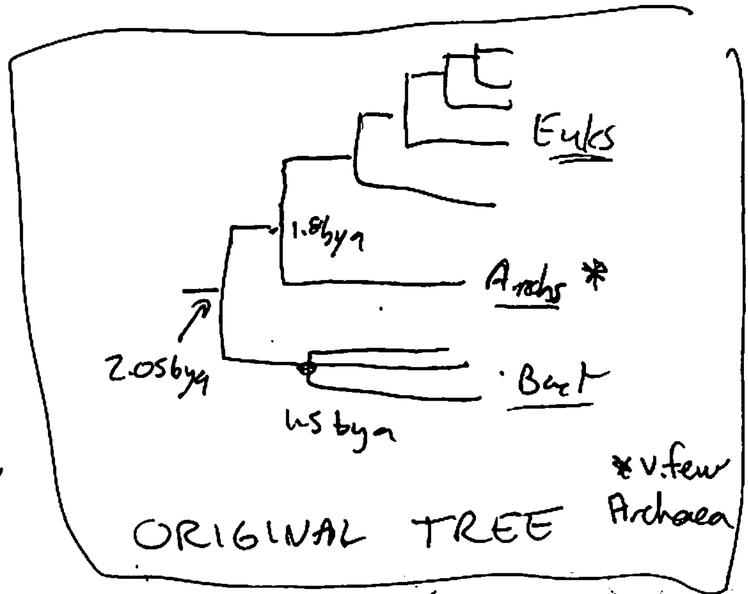
root of tree placed to constrain rates as much as possible

Extrapolate backwards





1 - distance has errors
2 - fossil dates have errors



Lateral transfer is a rejuvenating experience it makes you look younger than you really are.

(1) If tree is average can it be bootstrapped

(2) but many genes showed more similarity B+A, B+E NOT A+E

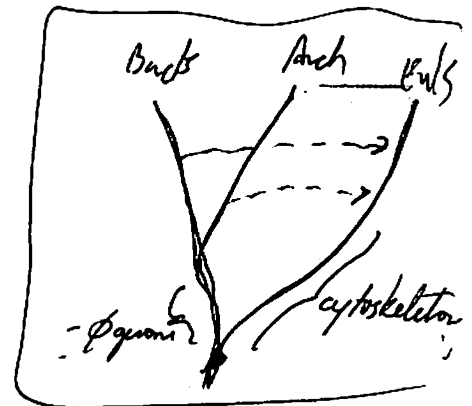
this is not surprising

took just B+A

- excluded genes that looked like lat. transfers

searches for most similar genes between A+B

- 48-67% EO



when did Bacterial like prots show up in euks? 42% = 2 bya

" " archaeal prots share common ancestor w/ euks = about same

suggests B+A share common ancestor

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WFD

What does it mean to say Archaea are a bridge

- 3 domain woese tree has dominated theories of euk origin
  ① mt genomes are degenerate descendants of genomes of symbiotic α-proteos
  ② symbiosis was made possible by evolution of cytoskeleton + endomembranes
  + there were protoeuk. that branched before
  ③ some proto euks still exist
  ④ protoeuks evolved from archaea

① Mitochondrial endosymbiosis
  - proposed by Margulis
  - triumph of molecular evolution
  - still confirmed by trees - rRNA + cpn60 (Viale et al)

② Symbiosis made possible by evolution of cytoskeleton + endomembranes
  - Stanier + vanNiel 1962 ... sugg. diff betw proks + euks
    was mostly cellular architecture
  - 1970 by R Y Stanier
    - sugg. euks don't need to evolve new metabolis b/c they eat bacteria
      or b/c they keep bacteria intracellularly

③ Some such protoeukaryotes survive to this date (as "Archezoa")
  - Cavalier-Smith 1980's began to argue that amitochondrial protists
    that never had mitos would exist
  - many of these species/groups have features that suggest they
    never had mitos
  - supported by rRNA trees which showed microsporidia, metamonads,
    etc were deep branching

- also went along w/ other observations on ~~[illegible]~~ then deep branches

(4) protoeuks evolved from Archaea

(a) why believe this?

(a) strong resemblance betw. txn, replication etc of Archaea + Euks
e.g. Zillig 1980

(b) rooting of universal tree w/ duplicated genes
Iwabe et al

- tRNA syn this still works
- EF's still work

Which to still believe?

(1) mito are desc. from α's

(2)(3) problems w/ Archezoa

(a) all euks prob. had mitos

- e.g. Trichomonas has hsp70 in nucleus that is mito-like
Giardia has cpn10, 60, 70 + valyl tRNA syn.

(b) the existance of 2ary amitochondrial euks sugg. mitos not needed

(c) Martin theory reqs NO cytoskeleton

(3) (d) Archezoa NOT deeply branching

- microsporidia in prot trees are NOT deep branching

(e) "Deepest" euk lineages show many advanced features

- including most gene duplications
- and PRP8

(4) did protoeuks evolve from Archaea?

- many euk. nuclear genes are of bacterial origin NOT mitochondrial
- archaea are themselves contaminated by bacterial genes

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What tree we get depend on what ~~tree~~ gene we get.

Why pick certain genes?
① can't be transfered (BUT C. Squires show rRNA from
- EF can be transfered   prothes works in E.col: D's)

② majority of genes give same tree
BUT this not true

Rooting of ~~Euka~~ tree is getting shaky.
Not only is rooting complicated
Rooting of tree is clouded in mists of uncertainty

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The past is unobservable so we must start with a theory

- Monocellular cells too complex to spontaneously exist

-

- 3 theories

① 1st organisms were vessicles - engaged in photosynthesis (Morowitz)
- then they invented genetic machinery to rule membranes

] THINKS THIS IS TOO COMPLICATED

② RNA world
- then these molecules invented everything else

- THINKS THIS IS TOO COMPLICATED + THAT ASSUMPTION OF BROTH w/ ACTIVATED NUCS TOO FAR FETCHED

③ begin w/ a metabolism
- then metabolism must invent everything else
- genetic material
- does NOT assume need a prebiotic broth
- requires evolution independent of nucleic acids

How get metabolism
① replace reproduction with chain reaction
- starting RXN → propogation RXN → termination RXN
branch RXN's

- suggests that branch RXN's are the "mutations" of evolution
- if branch RXN increases autocatalytic mechanism

claims that all mutations are like this

EVOLUTION IS A LIBERATION PROCESS
↓
1st step of freedom is runaway cons

Evolution by dual feedback

e.g. ribozymes → make proteins that make themselves
coenzymes →
nucleic acids → make proteins that make themselves

Universal Principles of Metabolism

① must have a core metabolism - initiation + prop. cycle
- must have peripheral metab. w/ feedback loops

② evolutionary centralism
- center is more immune from change (that's why oldest metabolism pathways are central)

③ need to convert current enzyme ~~[illegible]~~ processes into possible CHEMICAL processes early on

CURRENT	UR-METABOLISM
catalytic HS-groups	H_2S
metal clusters	metal sulfides
Thioesters	Thio acids
O, N	replace w/ S
Keto groups (CH_2CO)	thio end groups
Reducing power	$HS + FeS \to FeS_2 + 2e^- + H^+$ OR $h\nu$
Enzyme surfaces	sulfide mineral surfaces
Cellular metabolism	cellular metabolism

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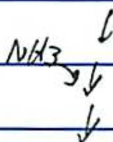
Initiation Pathway
got CO_2 fixation w/ replacement rules

Get chains of RXN's w/o enzymes
- enzymes are "acts of desperation" to allow colonization of one hostile chemical space after another

So... can get activated acetic acid

Branch RXN's
- Glut. synthase ... replace w/ inorganic chemicals
- Peptide formation ... activated
  - get dynamic library of peptides

$CO + H_2S$



NH_3 →

Peptides (can modify RXN's)

Quarrel over Protein vs. nucleic acids
Rather like quarrel betw. Stalin and Trotsky.

Early biochemistry = coordination chemistry
Early biocatalysts = transition metals

Life begins w/ chemical necessity and it has invented genetic chance.

How does genetic machinery arise?
- everything is a catalyst

Eigen -- hypercycle

explanandum and explanans are identical

Chemical energy is ??and specific ... photoenergy is horrible

chemical potential is the prime mover of the origin of life

Archaea: Bridging the Gap Between Bacteria and Eukarya (A2)

Sagebrush Inn, Taos, New Mexico • January 9 - January 14, 1999

Organizers: Dieter Söll, William Whitman and Carl Woese

Abstract Deadline: September 15,1998 • Early Registration: November 9, 1998

Saturday, January 9

Registration (2:00 pm - 7:00 pm)

Welcome (6:30 pm - 7:30 pm)

Orientation (7:30 pm - 8:00 pm)

Keynote Addresses (8:00 pm - 10:00 pm)

- **• Dieter Söll, Yale University**
- **Karl O. Stetter, University of Regensburg**
  The Diversity of Archaea
- **Claire M. Fraser, Institute for Genomic Research**
  Microbial Genome Sequencing: A New Paradigm for Assigning Function from Sequence

Sunday, January 10

Breakfast (7:00 am - 8:00 am)

GENOMIC ANALYSIS (8:00 AM - 11:00 AM)

- **• Claire M. Fraser, Institute for Genomic Research**
- **Ronald W. Davis †, Stanford University**
  Eukaryal Genomics
- **Gary Olsen, University of Illinois**
  Euryarchael Genomics
- **Christoph Sensen, National Research Council of Canada**
  Analyzing the Sulfolobus solfatharicus P2 genome
- **Jeffrey H. Miller, University of California-Los Angeles**
  Analyzing the Pyrobaculum aerophilum genome

Poster Setup (11:00 am - 1:00 pm)

POSTER SESSION 1: Integrating Genomics and Archaeal Biology (4:30 pm - 6:30 pm)

Social Hour (5:30 pm - 6:30 pm)

INTEGRATING GENOMICS AND BIOCHEMISTRY (8:00 PM - 10:00 PM)

- **• John N. Reeve, Ohio State University**
- **Richard J. Roberts, New England Biolabs**
  Analysis of Restriction Modification Systems from Archaeal Genome Sequences
- **Patrick Forterre, Universite de Paris-Sud**
  DNA Topology and DNA Topoisomerases from Archaea: A Goldmine for Topologists and a Puzzle for Evolutionists
- **August Böck, Universitat Munchen**
  Evolution of Selenocysteine Biosynthesis and Incorporation
- **Dieter Söll, Yale University**
  Novel Translational Components in Archaea

Monday, January 11

Breakfast (7:00 am - 8:00 am)

PROCESSING AND MODIFICATION (8:00 AM - 11:00 AM)

- **• Richard J. Roberts, New England Biolabs**
- **Francine Beth Perler, New England Biolabs**
  Inteins
- **Charles J. Daniels, Ohio State University**
  Transfer RNA Maturation
- **James A. McCloskey, University of Utah**
  RNA Modification
- **Alan M. Weiner, Yale University**
  CCA Addition by tRNA Nucleotidyltransferase: Polymerization without Translocation?

Poster Setup (11:00 am - 1:00 pm)

POSTER SESSION 2: Molecular Processes of Archaea (4:30 pm - 6:30 pm)

Social Hour (5:30 pm - 6:30 pm)

TRANSCRIPTION AND TRANSLATION (8:00 PM - 10:00 PM)

- **• Charles J. Daniels, Ohio State University**
- **John N. Reeve, Ohio State University**
  Archaeal Histones and Chromatin
- **Michael Thomm, University of Kiel**
  Mechanism and Regulation of Transcription in Archaea
- **Albert E. Dahlberg, Brown University**
  Ribosome Structure and Function
- **Patrick P. Dennis, University of British Columbia**
  Ribosomal RNA Processing in Sulfolobus Acidocaldarius

Tuesday, January 12

Breakfast (7:00 am - 8:00 am)

LESSONS FROM ARCHAEAL STRUCTURAL BIOLOGY (8:00 AM - 11:00 AM)

- **• Michael W. Adams, University of Georgia**
- **Wolfgang P. Baumeister, Max Planck Institut**
  Thermosomes and Proteasomes: From Archetypes to Complex Molecular Machines
- **Paul B. Sigler, Yale University**
  Molecular Basis for Unidirectional Transcription in Archaea
- **Dino Moras, Universite de Strasbourg**
  Which is the Best Structure of AspRS?
- **Douglas C. Rees, California Institute of Technology**
  Structural Manifestations of Hyperthermostability in Proteins

Poster Setup (11:00 am - 1:00 pm)

POSTER SESSION 3: Archaeal Cellular Processes, Enzymes, and Evolution (4:30 pm - 6:30 pm)

Social Hour (5:30 pm - 6:30 pm)

ENZYME DIVERSITY (8:00 PM - 10:00 PM)

- **• William B. Whitman, University of Georgia**
- **Willem M. De Vos, Wageningen Agricultural University**
  Glycolytic Enzymes and Their Control in Hyperthermophiles
- **Rudolf K. Thauer, Max Planck Institut**
  Structure and Catalytic Mechanism of Methyl-CoM-Reductase
- **James G. Ferry, Pennsylvania State University**
  Contrasting the Physiology and Enzymology of Carbonic Anhydrase Across the Eucarya and Archaea Domains
- **F. Robert Tabita, Ohio State University**
  Novel Ribulose Bisphosphate Carboxylase/Oxygenase Enzymes of Anoxic Archaea: How do They Work and What are They Doing?

Wednesday, January 13

Breakfast (7:00 am - 8:00 am)

CELLULAR PROCESSES (8:00 AM - 11:00 AM)

- **• Willem M. De Vos, Wageningen Agricultural University**
- **Michael W. Adams, University of Georgia**
  Hyperthermophilic Adaptations
- **Yosuke Koga, University of Occupational and Environmental Health**
  Structure and Biosynthesis of Polar Lipids of Methanogenic and Other Archaea
- **William B. Whitman, University of Georgia**
  Examination of Biosynthetic Pathways Predicted by Genomic Sequencing in Methanococci
- **John L. Spudich, University of Texas**
  Diversification of Function in the Archaeal Rhodopsin Family

Wednesday, January 13

EVOLUTION AND THE ORIGIN OF LIFE (3:00 PM - 5:00 PM)

- **• Patrick Forterre, Universite de Paris-Sud**
- **Russell F. Doolittle, University of California-San Diego**
  Exploring Archaeal Relationships with Protein Clocks
- **W. Ford Doolittle, Dalhousie University**
  Rethinking the Origin of Eukaryotes
- **Günter Wächtershäuser, Munich, Germany**
  The Chemoautotrophic Origin of Life

Social Hour (7:00 pm - 8:00 pm)

Banquet (8:00 pm - 10:00 pm)

Entertainment (9:00 pm - 12:00 pm)

Thursday, January 14

Departure (7:00 am - 10:00 am)

(A2) Archaea: Bridging the Gap Between Bacteria and Eukarya

New Participant Addresses as of 1/4/99

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