THE TWELFTH EDMOND DE ROTHSCHILD SCHOOL
IN MOLECULAR BIOPHYSICS

MODERN ASPECTS OF HALOPHILISM

PROGRAM
ABSTRACTS

MARCH 26
to APRIL 5, 1989
ISRAEL

SPONSORED BY THE INSTITUT DE BIOLOGIE PHYSICO-CHIMIQUE
(Fondation Edmond de Rothschild, Paris)
THE MAURICE AND GABRIELA GOLDSCHLEGER CONFERENCE FOUNDATION
at the
THE WEIZMANN INSTITUTE OF SCIENCE, REHOVOT
and the
OFFICE OF NAVAL RESEARCH BRANCH OFFICE, LONDON

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GENERAL INFORMATION
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SCHOOL SECRETARIAT AND INFORMATION DESK. The desk will be open as follows:

Sunday March 26 - from 17:00 to 20:30 hrs.
Monday March 27 - from 08:00 to 14:00 hrs.
Tuesday March 28 - from 13:00 to 14:00 hrs.

A representative of ORTRA, the conference travel agent, will be at the Information Desk to assist in reconfirming return flights, to arrange post-meeting tours and accommodation, car-rental, etc. ORTRA Travel agent telephone number 03-664825 (Contact Katrin for further assistance 09:00 - 15:00 hrs daily).

MEALS: Breakfast will be served from 07:00
Lunch and Dinner, as in program

BAR: A cash bar (and snack-bar) in the Hotel Lobby will be open at most hours during the day. Following the evening sessions the bar will be open until midnight, or later if requried.

MIXER: On Sunday March 26 a buffet dinner with complementary drinks will be available for the participants.

IDENTIFICATION BADGES: Name tags should be worn at all times.

COMPLEMENTARY TOUR : A tour of the Old City of Jerusalem is planned for Tuesday afternoon, including the Via Dolorosa, the Cardo and a panoramic view of Jerusalem. Leaving Neve Ilan at 14:15 on Tuesday March 28, followed by reception at the Israel Academy of Sciences and Humanity in Jerusalem.

FOREIGN CURRENCY AND POSTAL SERVICES: Foreign currency can be exchanged at the reception desk of the Guesthouse. Postal services are also available at the reception desk.

Facilities available at the Guesthouse include tennis courts and a heated covered swimming pool. Details at the Reception.

PUBLIC TRANSPORT :
(a) Jerusalem to Neve Ilan:
Bus no. 185 or 186, Leaving platform 8 (Central bus station), Travel time ca. 30 minutes.

Departure times - 06:05 and 06:30 - 21:30 (hourly on the 1/2 hour) and a final bus at 23:00.

(b) Neve Ilan to Jerusalem.

Departures times: 06:05, 06:55, 08:10, 09:10, then 10:20 - 21:20 hourly at 20 minutes past the hour.

(c) Neve Ilan to Tel-Aviv

One bus daily, leaving 08:45. Travel time ca. 1 1/2 hours.

Neve Ilan Guesthouse

Telephone : 02-341241 Telex :26265 NVILN
Address : Neve Ilan Hilltop Resort
D.N. Harei Yehuda 90850, Israel.

WEIZMANN INSTITUTE

LECTURES. All the lectures will be held in the Gerhard Schmidt Lecture Hall, situated in front of the Perlman Building.

MEALS will be served in the large dining-room of the San Martin Faculty Clubhouse.

Breakfast 07:30
Lunch and dinner as per program

IDENTIFICATION BADGES should be worn at all times during the symposium. Participants should also carry their Weizmann Institute Guest Pass while on campus.

INFORMATION. The symposium secretariat and information desk will be in the foyer of the lecture hall, and will be open Sunday April 2 to Tuesday April 4 during morning session coffee breaks. An ORTRA representative will be in attendance on Monday April 3, from 10:00 to 11:00.

BANKS are located within walking distance of the Weizmann Institute. There are no facilities on the campus to exchange foreign currency.

Banking hours:
Sunday through Friday : 08:30 -12:30
Sun., Tues., Thurs. : 16:00 - 18:00

TELEPHONE. Weizmann Institute of Science 08-482111 08-483111
San Martin Faculty Clubhouse 08-483385
On Campus Dial 0 for information
Telex: 381300, Facsimile: 08-466966

Mr. YITZCHAK BERMAN is in charge of all local arrangements.

RECREATION CENTER featuring gymnasium, sauna, tennis courts can be used upon presentation of Weizmann Institute guest pass. The open air swimming pool may not yet be open at this date (depending on weather conditions).

PROGRAM

SUNDAY, MARCH 26

	Arrivals of overseas participants at Neve Ilan Guest House
17:00	Early Registration
19:00	Dinner
21:00	Mixer

MONDAY, MARCH 27 Neve Ilan Lecture Hall

08:00 Registration

08:30 HENRYK EISENBERG
Greetings

MOLECULAR EVOLUTION

08:40 W. FORD DOOLITTLE
Introduction: The halobacteria as archaebacteria

10:00 Coffee

10:30 WOLFRAM ZILLIG
Phylogeny of archaebacterial, eukaryotic and eubacterial RNA polymerases

GENE EXPRESSION

12:00 PATRICK P. DENNIS
Superoxide dismutase from Halobacterium: The gene and its regulation.

13:30 Lunch

15:00 MOSHE MEVARECH
Analysis of the genetic transfer system of Halobacterium volcanii

16:30 FELICITAS PFEIFFER
Genetic instability of Halobacterium halobium: Effects on genome organization and gene expression.

18:00 Close of session

19:00 Dinner

MONDAY, MARCH 27 (Continuation)

CONTRIBUTIONS

20:30	FELIX GROPP *Expression and regulation of Halobacterium halobium phage ϕH genes*
21:00	MARY C. HORNE *Expression and regulation of two gas vacuole protein genes in halobacteria*
21:30	ULRIKE BLASEIO *Spontaneously occuring deletions in plasmids of Halobacterium halobium*
22:00	EVELYN ARNDT *Ribosomal proteins and their gene organisation of Halobacterium marismortui*
22:30	Close of session

TUESDAY, MARCH 28

MOLECULAR EVOLUTION

08:30	W. FORD DOOLITTLE ***Development of physical and genetic means for mapping in halobacteria***
10:00	Coffee
10:30	WOLFRAM ZILLIG ***Archaebacterial virus-host systems***
12:00	AHARON OREN ***Taxonomy of the Halobacterium group and other halophilic procaryotes: Molecular versus classical approaches***
13:30	Lunch
14:15	Departure for tour of Jerusalem
19:30	Reception at Israel Academy of Sciences and Humanities

WEDNESDAY, MARCH 29

GENE EXPRESSION

08:30 PATRICK P. DENNIS
Ribosome component genes in Halobacterium cutirubrium

10:00 Coffee

10:30 MANFRED SUMPER
Halobacterial glycoproteins: Genes, biosynthesis and function

12:00 KNUD H. NIERHAUS
Principles of the ribosomal elongation cycle: A comparison of the translation apparatus in Escherichia coli and Halobacterium halobium

13:30 Lunch

CONTRIBUTIONS

15:00 JOCELYN L. MILNER
Effects of environmental osmolarity on the cytoplasmic solute composition of Escherichia coli: Consequences for protein-DNA interactions

16:00 MARGOT KOGUT
Phenotypic adaptation of eubacterial halophiles to salt and other solutes

16:30 YORAM AVI-DOR and HAGGAI GILBOA
The interrelation between synthesis and uptake of compatible solutes in a halophilic eubacterium

17:00 RACHEL GABBAY-AZARIA
Mechanisms of salt tolerance in the marine cyanobacterium Spirulina subsalsa

WEDNESDAY, MARCH 29 (Continuation)

17:30	P. SELWIN THOMAS *Salt adaptation in Anabaena sp287 and the identification of the sodium sensing DNA fragments of its genome*
18:00	Close of session
18:30	Dinner
20:00	*SPECIAL LECTURE* Dr. ARIEH NISSENBAUM Academic Secretary, The Weizmann Institute of Science *The shipping lanes of the Dead Sea*
21:00	Refreshments

THURSDAY, MARCH 30

PROTEIN STRUCTURE AND FUNCTION

08:30	H.G. WITTMANN *Structure and function of ribosomes as determined by non-crystallographic methods*
10:00	Coffee
10:30	ADA YONATH *Crystallography of ribosomal particles from halo- and eubacteria*
12:00	PAOLA LONDEI *In vitro total reconstitution of active 50S subunits of the halophilic archaebacterium Haloferax mediterranei*
13:00	Lunch
14:30	MOSHE MEVARECH *The halophilic enzyme dihydrofolate reductase of the extremely halophilic archaebacterium Halobacterium volcanii as a model for the structural adaptation of enzymes to extreme salinities*
15:30	RAINER JAENICKE *Structure function relationship of halophilic proteins: Surface-layers and enzymes from halophilic archaebacteria*

THURSDAY, MARCH 30 (Continuation)

17:00	HENRYK EISENBERG *Combined use of ultracentrifugation, light, X-ray and neutron scattering, for the determination of water and salt binding in halophilic proteins*
18:00	Close of session
19:00	Dinner
20:30	GIUSEPPE ZACCAI *Solvent interaction, structure and stabilization of halophilic proteins*
22:00	Close of session

FRIDAY , MARCH 31

08:00	Departure of attendants from abroad for Massada, Dead Sea Spa, Beth Shean, dinner and overnight in Tiberias, Sea of Galilee.

SATURDAY, APRIL 1

08:00	Capernaum, Safed, Acco, Haifa, Carmel
	Return to Weizmann Institute, Rehovot
19:00	Dinner at San Martin Guest House
21:00	Get together at Eisenberg home.

SUNDAY, APRIL 2 Gerhard Schmidt Auditorium, Weizmann Institute

PROTEIN STRUCTURE AND FUNCTION (Continuation)

08:30 JOEL L. SUSSMAN
Protein adaptation to extreme salinity: The crystal structure of 2Fe-2S ferredoxin from Halobacterium marismortui

10:00 Coffee

10:30 MARTIN KESSEL
The cell surface of halobacteria as revealed by two-dimensional and three-dimensional image reconstruction from electron micrographs

ENERGETICS

12:00 DIETER OESTERHELT
Signal transduction in photo- and chemo-taxis of halobacteria

13:30 Lunch

15:00 WALTHER STOECKENIUS
Salt and pH effects on the function of bacteriorhodopsin

16:30 S. ROY CAPLAN
Kinetics and thermodynamics of protein pumping by bacteriorhodopsin

18:00 Close of session

18:30 Dinner

20:00 Jazz Concert
Big band from Rimon School

Refreshments

MONDAY, APRIL 3

08:30	DIETER OESTERHELT *Two pumps, one principle: Light-driven ion transport in halobacteria*
10:00	Coffee
10:30	JANOS LANYI *Mechanism of chloride transport by halorhodopsin*
12:00	YASUO MUKOHATA *Energetics in halobacteria, with special emphasis on their ATP synthase and light -driven pumps*
13:30	Lunch
	Afternoon and evening free

TUESDAY, APRIL 4

08:30	JANOS LANYI *Mechanism of chloride transport by halorhodopsin (continuation)*
10:00	Coffee
10:30	MORDHAY AVRON *The strategy of adaptation to low water potential by the halotolerant alga Dunaliella*
12:00	HARTMUT GIMMLER *The permeability of the plasma membrane of Dunaliella parva and Dunaliella acidophila : Stress and ecophysiological significance*
13:30	Lunch

CONTRIBUTIONS

15:00	GIUSEPPE ZACCAI *Models from neutron diffraction of bacteriorhodopsin structure*
15:30	EBERHARD NEUMANN *Cooperative structural changes induced by electric fields in halophilic purple membranes*
16:00	JAROSLAV KYPR *Unusual DNA double helix promoted by alternating purine-pyrimidine sequences of adenine-thymine pairs*
16:30	CHRISTINE EBEL *Study of the stability and activity of the elongation factor Tu from Halobacterium marismortui at different salt conditions*
17:00	GIUSEPPE BALDACCI *The gene for the EF-Tu from Halobacterium marismortui*
17:30	Close of School
20:00	Closing dinner hosted by Professor Haim Harari, President of The Weizmann Institute of Science

ABSTRACTS OF LECTURES

Introduction: the halobacteria as archaebacteria

W. Ford Doolittle, Department of Biochemistry, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada

I will address myself to the significance of Carl Woese's "discovery" of the archaebacteria to molecular halobacteriology. This discovery emerged from an attempt to place all major bacterial groups within a consistent phylogenetic scheme based on the sequences of ribosomal RNAs. In that scheme, archaebacteria are one of three coequal groups, which may have begun to diverge from each other at the dawn of cellular life. This scenario is radically different from earlier views associated with the "prokaryote: eukaryote dichotomy" of Stanier and Van Niel, which seemed to suggest that eukaryotes arose from within the prokaryotes, only after these had been diversifiying for some two billion years. It motivates investigations into the structure and function of archaebacterial genes. I will attempt an overview of basic features of archaebacterial molecular biology, as we now know them, giving detail only in areas not covered by other speakers.

Relevant literature:

Doolittle, W. F. (1980) Revolutionary concepts in evolutionary cell biology. Trends Biochem. Sci. **5**: 146-149.

Woese, C.R. (1987) Bacterial evolution. Microbiol. Rev. **51**: 221-271.

Charlebois, R.L. and Doolittle, W. F. (1989) Transposable elements and genome structure in halobacteria. *In* M. Howe and D. Berg (eds.), Mobile DNA. ASM Press, in press.

PHYLOGENY OF ARCHAEBACTERIAL, EUKARYOTIC AND EUBACTERIAL RNA POLYMERASES

W. Zillig, G. Pühler, H. Leffers*, F. Gropp, P. Palm, H.-P. Klenk and F. Lottspeich

Max-Planck-Institut für Biochemie, 8033 Martinsried, FRG

*Biostructural Chemistry, Aarhus University, Aarhus, Denmark

The genes for the large components, B resp. B" and B', A and C of the DNA-dependent RNA polymerases of the archaebacteria Sulfolobus acidocaldarius and Halobacterium halobium are arranged, in this order, in reading units corresponding to the rpoBC operon of E. coli. Yet the open reading frame in front of the operons, which is highly homologous between the two archaebacteria and the open reading frames downstream are different from those found in corresponding positions in eubacteria. The protein of the corresponding genes of Methanobacterium thermoautotrophicum (Berghöfer et al. 1988) is the same as in H. halobium.

Whereas the organization of these RNA polymerase component genes in archaebacteria thus resembles that in eubacteria, the sequence of the archaebacterial components resembles much more that of the corresponding eukaryotic components, especially those of eukaryotic nuclear RNA polymerases II and III, than that of the eubacterial ß and ß' subunits. The sequences of the archaebacterial genes resp. RNA polymerase components were aligned with all known sequences for B and A components of eukaryotic B and A and eubacterial ß and ß' components. The AC genes split, in which the archaebacterial A gene corresponds roughly to the first two thirds and the archaebacterial C gene to the last third of the eukaryotic resp. eubacterial ß' gene, appears characteristic for the archaebacteria. The B"B' split found in H. halobium and M. thermoautotrophicum seems characteristic for the methanogens and extreme halophiles.

Phylogenetic dendrograms were constructed from the sequence similarity data of the alignment by the distance matrix method of Fitch and Margoliash (1967) by DNA parsimony analysis employing a program of Felsenstein (1984) by the maximal likelihood method of Felsenstein (1973) and by the supposedly rate invariant evolutionary parsimony algorithm of Lake (1987). In all cases, the dendrograms for the B components are less complete but resemble those obtained for the A components. The eukaryotic pol II

and/or pol III A component lineages are close neighbors of the archaebacterial A+C component lineage. The significance of the data does not allow a decision whether they share a short stem or have two separate roots close to each other in the tree. The eukaryotic pol I lineage, however, has its root in the tree significantly distant from those of pol *II and pol III. It bifurcates with the eubacteria.*

These data support the coherence of the archaebacterial kingdom derived from rRNA sequence data (Woese and Olsen, 1986). In addition, they suggest that the eukaryotic cytoplasm (eucyte), i.e. the real eukaryotic compartment of the eukaryotic cell, arose either by a fusion of different ancestors or by an acquisition event similar to but different from those which gave rise to the organelles, or involved horizontal gene transfer. The formally also possible alternative assumption that the eubacteria and/or the archaebacteria arose from within the eukaryotes by reduction event(s) appears much less probable.

Introduction of more primitive (deeper branching and short) representatives of all kingdoms should increase the significance of the suggested phylogeny.

References

Berghöfer, B., Kröckel, L., Körtner, C., Truss, M., Schallenberg, J. and Klein, A. (1988) Relatedness of archaebacterial RNA polymerase core subunits to their eubacterial and eukaryotic equivalents. Nucl. Acids Res. 16:8113-8128

Felsenstein, J. (1973) Maximum likelihood and minimum-steps method for estimating evolutionary trees from data on discrete characters. Syst. Zool. 22:240-249

Felsenstein, J. (1984) Distance methods for inferring phylogenies: A justification. Evolution 38:16-24

Fitch, W.M. and Margoliash, E. (1967) Construction of phylogenetic trees. Science 15:279-284

Lake, J.A. (1987) A rate-independent technique for analylis of nucleic acid sequences: evolutionary parsimony. Mol. Biol. Evol. 4:167-191

Woese, C.R. and Olsen, G.J. (1986) Archaebacterial phylogeny: perspectives on the urkingdoms. Syst. Appl. Microbiol. 7:161-177

Superoxide Dismutase from Halobacterium: The Gene and Its Regulation

PATRICK P. DENNIS AND BRUCE P. MAY

Halobacterium cutirubrum contains a single superoxide dismutase activity. The activity functions in the high ionic strength intracellular environment to provide protection against the toxic effects of the superoxide anion. The enzyme has been purified and partially characterized. It is most likely tetrameric with a subunit M_r of 25000, contains Mn as a metal ion cofactor and has optimal activity in 2M KCl. The amino terminal sequence was determined and used to prepare an oligonucleotides complementary to the DNA encoding the protein. A 1.1 Kbp fragment of genomic DNA was isolated and sequenced. The fragment encodes a polypeptide of 200 amino acids that is 40% identical with eubacterial Mn-containing SODs and unrelated to the Cu-Zn enzymes found in eucaryotes. The transcript of the SOD gene is initiated one nucleotide in front of the ATG translation initiation codon and truncated at a major termination site 30 nucleotides beyond the coding region. The 5' flanking region lacks the consensus sequences associated with most other archaebacterial promoters. The acitivity of the SOD gene is regulated; paraquat, an intracellular generator of the superoxide anion, causes an increase in sod mRNA and SOD protein and anerobic growth conditions cause a reduction in sod mRNA and SOD protein. The synthetic oligonucleotide used to isolate the sod gene hybridizes weakly to a second genomic DNA fragment. This fragment was cloned and sequenced; it contains an open reading frame encoding a SOD-like protein. The gene is transcribed but the amount of mRNA is not altered by paraquat. At the protein level, there is 82% amino acid sequence identity between the authentic and the SOD-like proteins. The four amino acid residues involved in metal ion binding are conserved. The function and enzymatic activity of this SOD-like protein has yet to be established.

References

May, B. and P. Dennis (1987) J. Bacteriol. 169, 1417-1422.
May, B., P. Tam and P. Dennis (1989) Canadian J. Microbiol., in press.
May, B. and P. Dennis (1989) submitted for publication.

Analysis of the genetic transfer system of *Halobacterium volcanii*.

Moshe Mevarech, Ilan Rosenshine and Ronen Tchelet
Department of Microbiology, George S. Wise Faculty of Life Sciences
Tel Aviv University, Israel

The extremely halophilic archaebacterium *Halobacterium volcaii* is an excellent subject for studies on the genom organization of archaebacteria. This organism is prototrophic, requiring for growth only simple carbon and nitrogen sources. Unlike many other halobacteria, the genom of *H. volcanii* is stable and spontaneous mutations occur only at very low frequencies. Two recent discoveries substantiate *H. volcanii* as a model organism for genetic studies in archaebacteria. Few years ago (1) we demonstrated that when two auxotrophic mutants of *H. volcanii* are grown together on solid surface, genetic exchange takes place. More recently, a transformation protocol was developed by which DNA can be introducd into the the cells of *H. volcanii* (2). The purpose of this paper is to analyze the natural genetic exchange system of *H. volcanii*.

Already from the preliminary genetic analysis of the transfer system, it was evident that there is no donor or recipient (in terms of eubacterial conjugation). It was found that each cell can be a donor or/and a recipient of genetic information. In order to explain this observation two models were proposed: a) the genetic exchange takes place by fusion between the cells (like in eukariotic sexual mating); b) the genetic exchange is by conjugation, but every cell is able to donate and receive genetic information. In order to distinguish between these two models, mutants were labelled by cytoplasmatic markers prior to the mating. If the genetic exchange requires cellular fusion, each recombinant will contain the cytoplasmatic markers. If, however, the genetic exchange is similar to conjugation, half of the recombinants will contain the markers and half will not conain the markers. As cytoplasmatic markers we have used natural plasmids of *H. volcanii* (2,3). We found that half of the recombinant cells contained the plasmids and half did not, indicating that the mechanism of transfer resembles "classical" conjugation.

The process of conjugation was studied in details. First the kinetic of the process was established. Then the genetic status of the exconjugants was shown to be a stable diploidic or merodiploidic. Electron micrographs of cells grown on solid surfaces show that the cells produce "bridges" composed of the surface glycoproteins and cytoplasmatic continuity. In order to show that these bridges are made *de novo* and are not the result of incomplete devisions, two population of cells were mixed and grown together on solid surface. Cells of one of the populations was marked on the surface with biotin. Using avidin-gold complexes, it was possible to see labelled cells conneced to unlabelled ceels by bridges.

Another evidence for the existence of cytoplasmatic continuity between cells was obtained by crossing, again, cells that contain plasmids with cels that do not contain plasmids. This time, however, before selecting for recombinants, the surface glycoprotein was removed by reducing the Mg^{++} concentration. All the recombinants obtained by this way contained the plasmids, indicating that following the removal of the surface glycoprotein the two spheroplasts connected by bridges fused together.

In conclusion, it seems that the unusual conjugation system of *H. volcanii* comprises a special genetic exchange system which has some features of both- the conjugation system of prokaryotes and the more complicate sexual genetic exchange system of eukaryotes.

References

1. Mevarech, M. and Werczberger, R. (1985) J. Bacteriol. 162 461-462.
2. Charlebois, R.L., Lam, W.L., Cline, S.W. and Doolittle, W.F. (1987) Proc. Natl. Acad. Sci. U.S.A. 84 8530-8534.
3. Rosenshine, I. and Mevarech, M. (1989) Can. J. Microbiol. in press.

GENETIC INSTABILITY OF HALOBACTERIUM HALOBIUM: EFFECTS ON GENOME ORGANIZATION AND GENE EXPRESSION.

Felicitas Pfeifer
Max-Planck-Institut für Biochemie, D-8033 Martinsried, Fed. Rep. of Germany

The high frequency of spontaneous mutations affecting phenotypic markers of *Halobacterium halobium* is due to the action of various insertion elements (ISH-elements). Nine of these elements (ISH1, ISH2, ISH23, ISH24, ISH26, ISH27, ISH28, ISH1.8, and ISHS1) have been identified in *H. halobium* - mainly through the analysis of mutants in bacteriorhodopsin synthesis (1-4). The copy number of these elements varies from two to more than ten (5). Most of these are found in regions of the genome consisting of a lower G+C content (FII-DNA, 58% G+C) than the main part of the genome (FI-DNA, 68% G+C). Within the FII-DNA many of these elements are located on pHH1, the 150 kb major plasmid of *H. halobium*. As a result of the activity of these insertion elements the plasmid population appears to be rather dynamic - rearrangements, insertions and deletions occur frequently during the growth of the halobacterial cell (6).

The effect of the genetic instability on gene expression has been studied with respect to two genes: (a) the *bop* gene encoding bacterio-opsin, the protein moiety of bacteriorhodopsin in the purple membrane, and (b) on the two gas vacuole protein genes (*p-vac* and *c-vac*) of *H. halobium*. The *bop* gene located in FI-DNA is affected with a frequency of 10^{-4}. About 50 different Bop mutants have been studied; more than 95% are caused by the integration of an insertion element within or up to 3800 bp upstream of the *bop* gene coding region (1,7,8). One mutant (M86) contained a 1883 bp deletion which occurred 1953 bp upstream of the *bop* gene and involved the recombination of an eight base pair repeated DNA sequence (8). The distribution of the mutations affecting *bop* gene expression led to the identification of two other genes (*brp* and *bat*) located upstream of the *bop* gene (8,9).

H. halobium contains two different, though highly homologous genes encoding the hydrophobic protein of the gas vacuole. One of these genes (*p-vac*) is located on pHH1, whereas the second gene (*c-vac*) is found in the chromosomal FI-DNA (10). The two proteins derived from these genes differ by the exchange of two amino acids (position 8 and 29) and in a deletion encompassing 3 amino acids near the carboxy terminus of the *p-vac* protein. The *p-vac* gene is expressed constitutively, whereas gas vacuole synthesis due to the *c-vac* gene expression develops during stationary growth in *p-vac* deletion mutants. The dynamics of pHH1 affect the *p-vac* expression with a frequency of 10^{-2} - 10^{-5} while *c-vac* mutants are obtained with a frequency of 10^{-5} (11). Insertion elements (mainly ISH2 but also others) are found to be integrated up- or downstream of the *p-vac* coding

sequence in the majority of the *p-vac* mutants (10,11). About 30% of the *p-vac* mutants are, however, due to deletions encompassing the entire *p-vac* gene region (6). These deletions are usually caused by insertion elements as well; i.e. ISH2 or ISH27 are found to be involved in recombinations with subsequent deletions encompassing DNA adjacent to the element (13).

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EXPRESSION AND REGULATION OF *HALOBACTERIUM HALOBIUM* PHAGE ΦH GENES

Felix Gropp, Peter Palm and Wolfram Zillig

Max-Planck-Institut für Biochemie, D-8033 Martinsried, F. R. G.

Among archaebacterial viruses the temperate bacteriophage ΦH of *Halobacterium halobium* strain R_1 is the most extensively studied on the molecular level.
We describe 5 distinct modes of ΦH gene expression:
1. Transcription of phage ΦH during lytic growth on the sensitive host bacterium (*Halobacterium halobium* strain R_1) .
2. Transcription of the circularized prophage ΦH1 in strain $R_1$24.
3. Transcription of the L-region of ΦH present as 12 kb plasmid in the immune strain R_1L.
4. Transcription during the lytic growth of phage mutants containing an ISH23/50 in the immune strain R_1L
5. Transcription during lytic growth of ISH23/50-insertion mutants in the sensitive host bacterium R_1 showing enhancement of early transcripts.

The sequential expression of the phage genome is described along with a detailed analysis of the transcription of early lytic, constitutive, and immunity genes that map in the L-region.

The putative promoter sequences determined for several phage genes are compared with the upstream sequences of the *H. halobium* DNA-dependent RNA polymerase large subunit genes and with the gene for the ribosomal protein S12-homolog of *H. halobium*. The similarity of these putative promoter elements revealed conserved motifs that are discussed in relation to the TATA-box motif recognized by the eukaryotic DNA-dependent RNA polymerase II.

Expression and Regulation of Two Gas Vacuole Protein Genes in Halobacteria

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Halobacteria, like many aquatic microorganisms, produce proteinaceous gas-filled structures called gas vacuoles that regulate the cells bouyancy in a water column. The production of gas vacuoles by a halobacterial species is evident from the marked opacity of liquid cultures and single colonies in contrast to the translucent cultures of non-producers, as well as the relative ability of the individual cells to float to the surface in liquid culture. The halobacterial species *Halobacterium halobium* in particular, exhibits a high degree of variability in gas vacuole production, the translucent mutant colonies arising at a frequency of 10^{-2} and the subsequent spontaneous "revertants" occuring at an even higher frequency (1,2). The genetic basis for this phenotypic variation has only recently been studied at the molecular level (3,4,5). We have demonstrated that *H. halobium* contains two highly homologous (86%), but differentially regulated gas vacuole protein genes (*vac*); one is located on the major plasmid pHH1 and encodes a protein of 76 amino acids (*p-vac*), while the other is a chromosomal gene (*c-vac*) that encodes a slightly larger protein of 79 amino acids (3). In addition, we have determined that several other independently isolated halobacterial species (GN101, SB3, YC819-9) also contain a chromosomal gas vacuole protein encoding gene which is homologous (95-100%) to *c-vac*, but that these strains all lack a plasmid encoded *vac* gene. The complete nucleotide coding sequences for all of the respective genes has been determined and the comparison alignments made (3,6). Northern analysis determined the approximate transcript lengths for the respective *vac* gene(s) of each halobacterial species, and S1 nuclease mapping experiments indicated that the 5' end for *p-vac* and *c-vac* transcripts mapped at the same relative nucleotide while the chromosomal gene transcripts for *vac* from GN101, SB3, and YC819-9 started at exactly the same nucleotide.

Phase contrast microscopic observations during a time course of growth of cultures from various halobacterial species and strains, and subsequent Northern analysis of the isolated RNA samples from the respective time points demonstrated that the *p-vac* gene is expressed constitutively while the *c-vac* gene more tightly regulated (6). In those halobacterial species containing only the chromosomal *vac* gene (both the natural isolates and *H. halobium* pHH1 deletion variant *p-vac* mutant strains), the chromosomal gene is not expressed until stationary phase. In contrast *H. halobium* wild type expresses *p-vac* at a high level throughout the growth cycle, while the *c-vac* gene transcript could not be detected on the same time course Northern blot. Quantitative S1 nuclease experiments performed on the RNA time point samples representing the maximum level of transcription for either the *p-vac* or *c-vac* gene demonstrated that the plasmid gene from *H. halobium* wild type is transcribed at 10-15 fold higher levels than that for the *c-vac* gene in *p-vac* deletion mutants.

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Spontaneously occuring deletions in plasmids of *Halobacterium halobium*

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The 150 kbp major plasmid pHH1 carries a gene for the gas vacuole protein as well as various insertion elements (ISH). Vac$^-$ mutants can be observed with a frequency of 10^{-2}. This high rate of change in the gas vacuole phenotype is due to genomic rearrangements of the plasmid; i. e. the integration of an additional insertion element or a deletion of the region encompassing the gas vacuole protein gene (1).
Four deletion derivatives of the 34 kbp *vac*$^-$ mutant plasmid pHH4 were chosen for further analysis (2). Following restriction endonuclease mapping the fusion regions of four pHH4 derivatives were characterized by DNA sequence determination. We could show that insertion elements are involved in the deletion event. Recombination at one terminus of ISH2 led to the formation of the 15 kbp and 17 kpb plasmids pHH7 and pHH6, while ISH27 was involved in the formation of the two variants pHH8 (6 kbp) and pHH9 (5 kbp) (3). The 1386 bp element ISH27 was characterized on the DNA and transcriptional level. Analysis of various copies of ISH27 indicated that they constitute an insertion element family. At least three different types could be identified (4).
The four kbp of unique DNA present in all deletion plasmids should be important for plasmid maintenance and replication. This *H. halobium* replicon might be useful for the construction of a H. halobium transformation vector.

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manuscript in preparation

Ribosomal proteins and their gene organization of Halobacterium marismortui

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Since ribosomes are present in all pro- and eukaryotic organisms, they are excellent objects for studying the molecular evolution between distantly related organisms. We have so far determined the complete amino acid sequences of 23 ribosomal proteins from the archaebacterium Halobacterium marismortui, and compared their sequences with those from eubacteria and eukaryotes in order to elucidate the relationship of these organisms. The results show that several of the halobacterial ribosomal proteins are related to their eukaryotic counterparts whereas others are related to the eubacterial ones. In addition, some halobacterial proteins are homologous to both their eubacterial and their eukaryotic counterparts.

In addition to the protein-chemical studies, we have recently succeeded in cloning a chromosomal DNA-fragment of H. marismortui which contains several genes for ribosomal proteins. This was done by using an oligonucleotide probe based on the amino acid sequence of H. marismortui protein L25 (HL25). The sequence analysis of the cloned DNA-fragment revealed that it contains the genes for the proteins HL1, HL6, HL25, HL4, HS18, HL23, HS3 and HL33 and corresponds to the S10 operon of E. coli. Furthermore, the following results were obtained: 1) In the analysed operon the gene for protein HL1 which corresponds to that for EL3 in E. coli is located next to the promoter. This finding is different from the S10 operon of E.coli in which the gene for ES10 is the first one. 2) The gene for the EL16-equivalent protein is totally missing in this operon of H. marismortui. 3) Based on the locations in the operons, the gene for protein HL6 should be equivalent to that for protein EL4 in E. coli. On the other hand the homology between them is very low. 4) In contrast to the genes in eukaryotes, no introns in the ribosomal protein genes of H. marismortui were found.

Development of physical and genetic means for mapping in halobacteria.

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My laboratory is developing approaches, along several fronts, to map the genome of *Halobacterium volcanii*. (i) *Physical mapping*. Both "top-down" methods, using pulsed-field gel electrophoresis, and "bottom up" technology, with cosmid clones, are being attempted. To date, we have cosmids representing perhaps 95% of the genome, ordered into about twenty contigs. Cloned genes are being mapped to these cosmids by hybridization. (ii) *Transformation methods*. We have detected and now optimized DNA uptake systems of several kinds -- transfection with phage phiH, transformation with marked and unmarked versions of the *H. volcanii* plasmid pHv2, and transformation to prototrophy with total cellular DNA. These methods can be used in classical genetic mapping and complementation analyses. (iii) *Development of shuttle vectors*. Resistance to mevinolin, an inhibitor of HMGCoA reductase, arises spontaneously at very low frequency. A DNA fragment conferring resistance has been cloned into pHv2, and a shuttle vector selectable and maintainable in both *H. volcanii* and *Escherichia coli* has been developed.

The combined use of these methods to begin a study of the biochemical genetics of tryptophan biosynthesis in *H. volcanii* will be discussed.

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ARCHAEBACTERIAL VIRUS-HOST SYSTEMS

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In order to compare gene expression mechanisms, especially signal structures and controls in archaebacterial genomes with those operating in eubacterial and eukaryotic genomes, we have isolated several archaebacterial viruses including bacteriophage ØH of Halobacterium halobium, the virus-like particle SSV1 of Sulfolobus sp. B12 and the Thermoproteus tenax viruses TTV1,1,3 and 4.

We have sequenced the whole genome of SSV1 (P. Palm and B. Grampp, unpublished) and the major part of the genome of TTV1 (H. Neumann and V. Schwass, unpublished) and the early region of the genome of H. halobium phage ØH (F. Gropp and P. Palm, unpublished). We have localized a number of genes for structural proteins (envelope and DNA-binding) and a large number of open reading frames in these genomes. S1 and reverse transcriptase primer-extension mapping of transcript starts, in several cases secured by in vitro-capping experiments uncovered a unique consensus of the putative archaebacterial promoter consisting of a significantly conserved Box B around the transcript start and an AT-rich box A containing the sequence XTTA some 25 nucleotides upstream of box B. This situation resembles others mapped in archaebacteria by other groups and is similar to the consensus of the eukaryotic pol II promoter (Bucher and Trifonov, 1986). The transcripts often terminate after oligo T runs. But this situation appears less general than the promoter consensus.

These data are in accord with the structural resemblance of archaebacterial and eukaryotic RNA polymerase.

Several control mechanisms, e.g. the immunity conferred by the early region of phage ØH and its breakthrough by certain insertion mutants of the phage, the UV-induction of the T_{ind} gene of the Sulfolobus-like particle SSV1, its influence on DNA replication and virus production and the control of the carrier state of TTV1 are investigated. Translation signals and translation controls, the organization of genes and the interaction of the viral and the host genomes are under study and shall be discussed.

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Taxonomy of the Halobacterium group and other halophilic procaryotes: molecular versus classical approaches

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The extremely halophilic archaebacteria (Halobacterium and related organisms) form a heterogeneous group, whose evolutionary status is becoming increasingly clear, but whose taxonomy is at present in a state of great confusion. Reasons for this are:

1. The discrepancies between results obtained by the classical morphological - physiological approach (the approach culminating in the species descriptions in the latest editions of Bergey's Manual) and the more recent studies involving molecular characterizations, especially of DNA and RNA.
2. Many of the strains isolated, notably those that are the most popular objects of study, have not been effectively and validly described conform the requirements of the Bacteriological Code. Two of the most used species names (Halobacterium halobium and Halobacterium marismortui) are invalid: no type strains exist, and they are not included in the approved list of bacterial names.

Because of the heterogeneity within the Halobacterium group, as apparent from studies on their physiology, DNA-DNA and DNA-RNA hybridization studies [6], and studies on the lipid components, proposals have been made to split the former genus Halobacterium into three genera: Halobacterium (e.g. H.salinarium), Haloferax (e.g. H.volcanii) and Haloarcula (e.g. H.vallismortis; also "H.marismortui" belongs to this group) [5].

The novel approach of ribosomal RNA sequence determinations and comparison supports the above classification to a large extent. Sequences of 5S and 16S rRNA molecules from H.vallismortis and "H.marismortui" showed an extremely great similarity [1,3]; however, on the basis of morphological and physiological properties the two strains proved definitely distinct [3].

Analysis of 16S ribosomal RNA nucleotide sequences has also been extremely helpful in the elucidation of the taxonomic status of obligately anaerobic halophilic bacteria. During the past six years a number of anaerobic, fermentative, moderately halophilic bacteria have been isolated from hypersaline environments such as sediments of the Dead Sea and Great Salt Lake [2,4]. At least part of these isolates are able to produce endospores. New genera were created (Haloanaerobium, Halobacteroides, Sporohalobacter) to include these isolates. Only the technique

insight that all these strains are closely related to each other - and that a strain from the Dead Sea that was formerly described as Clostridium lortetii belongs to this new group, and is unrelated to the genus Clostridium proper. The eubacterial anaerobic halophilic bacteria do not show a clear relationship with any of the recognized subgroups within the eubacterial kingdom, with the possible exception of the spirochetes, and a new family, the Haloanaerobiaceae, was created to include these novel organisms.

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Ribosome component genes in Halobacterium cutirubrum

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The ribosome of Halobacterium cutirubrum consists of 16s, 23s and 5s rRNAs and approximately 60 separate proteins. The three rRNA species are produced from a single copy transcription unit. Transcription initiation occurs at five tandomly arranged promoters located between 200 and 800 nucleotide upstream from the beginning of the 16s rRNA gene. The 16s and 23s rRNA genes are surrounded by inverted repeat sequences. These complementary sequences presumably are capable of forming helical stems that contain the recognition sites for excision of precursor 16s and 23s rRNAs from the primary transcript. These processing sites resemble the tRNA intron excision site and may indeed by cleaved by the intron excision enzyme system. The rRNA transcription unit also contains an alanine tRNA in the 16s - 23s intergenic space and a cysteine tRNA distal to the 5s rRNA gene.

The large ribosome subunit contains a distinct stalk structure that is composed of a 4:1 complex between protein L12e and L10e; the complex associates with the 23s rRNA binding protein, L11e. The stalk structure and its resident proteins are universally conserved in all ribosome; it functions in factor binding and associated GTPase activities during the protein synthesis cycle. A fourth protein L1e also binds 23s rRNA and is located on the 50s ridge; it functions directly to stabilize peptidyl tRNA binding to the P site and indirectly with the factor binding center to enhance GTPase activity. A 5.2 Kbp fragment of H. cutirubrum DNA containing the L11e, L1e, L10e and L12e and two other genes (ORF and NAB) has been cloned and sequnced. The ribosomal protein genes are clustered in the same order as that in E. coli although the transcription pattern is quite different. Transcripts from the region include (i) abundant monocistrimic L11e and tricistronic L1e, L10e and L12e, (ii) less abundant bicistronic NAB-L11e and monocistronic NAB transcripts and (iii) a very rare monocistronic ORF transcript. The 74 nucleotide long untranslated leader of the L1e-L10e-L12e transcript contains a region that has a sequence and structure almost identical to a region within the binding domain for the L1e protein in 23s rRNA and highly similar to the E. coli L11-L1 mRNA leader sequence that has been implicated in autogeneous translational regulations. Other transcripts are initiated at or adjacent to the ATG translation initiation codons.

The consensus sequence for the promotors of rRNA and ribosomal protein gene is TTCGA ... 4 to 10 nucleotide ... TTAA ... 25 - 26 nucleotides ... initiation site; termination generally occurs on polyT tracks following GC rich regions.

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The first procaryotic glycoprotein was discovered in Halobacteria [1-3] and identified as the hexagonally arranged protein subunit of the cell surface layer. Halobacteria are able to synthesize five more glycoproteins and these were identified as the subunits of the flagellar filaments [4].

The structure of these glycoproteins was worked out in detail [5]. The cell surface glycoprotein contains O-linked disaccharides and in addition two different types of saccharides in novel N-glycosidic linkages [6,7]. One type represents a repeating unit pentasaccharide, the other one is an oligosaccharide which consists of glucose and sulphated glucuronic acid residues. Only the latter type of saccharide is also found to be present in halobacterial flagellins.

Biosynthetic studies have revealed that dolichol-linked saccharides serve as precursors in halobacterial glycoprotein synthesis [8]. In addition, a transient methylation of dolichyl oligosaccharides appears to be an obligatory step in this process [9]. Assembly of the N-glycosidic linkage takes place at the cell surface.

The genes encoding the cell surface glycoprotein as well as all halobacterial flagellins were cloned and analysed [10,11]. The primary structure of the surface glycoprotein combined with a three-dimensional reconstruction from electron micrographs [12] suggests a model of the halobacterial cell wall.

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"Principles of the ribosomal elongation cycle: A comparison of the translation apparatus in Escherichia coli and Halobacterium halobium"

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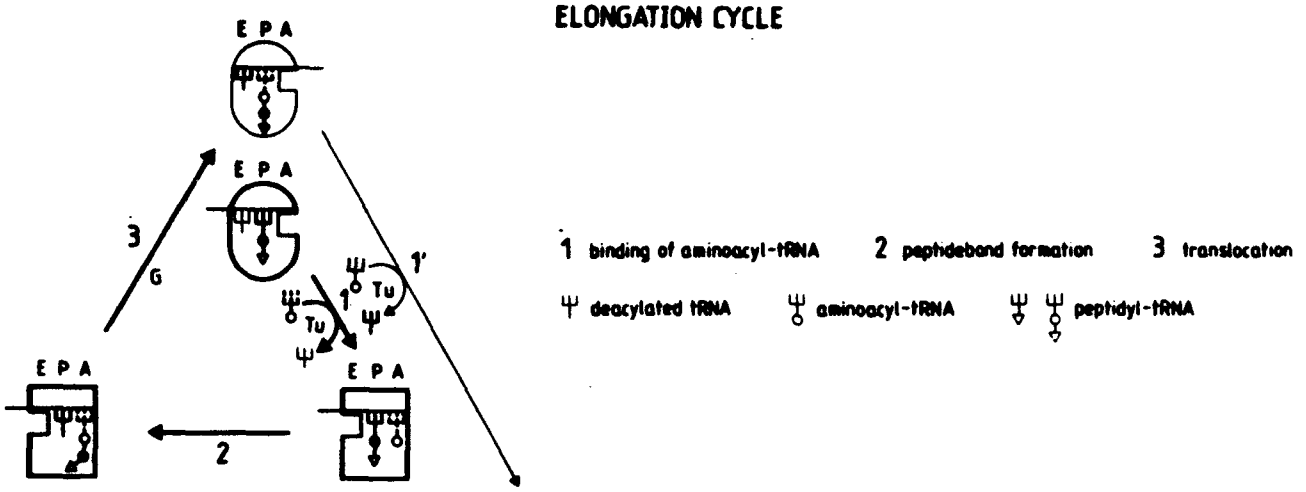
The ribosomal elongation cycle signifies a sequence of reactions which result in the prolongation of a peptidyl-residue by one amino acid. A tRNA binds as aminoacyl-tRNA to the ribosome and is thus responsible for the input of the new amino acid according to the codon present in the A site, the binding site for aminoacyl-tRNA.

In addition to the standard conditions for E. coli ribosomes we used a translational system optimized for the extreme halophile H. halobium (1) which requires 6 M monovalent cations in vitro. Nitrocellulose filtration cannot be used for tRNA binding assays at these high salt concentrations, since even the non-bound tRNA is quantitatively collected on the filter, but rather sucrose-gradient centrifugation had to be applied. Identical results were obtained with ribosomes of both organisms applying different methods (2,3):

The ribosome contains three tRNA binding sites, the A, P and E sites. These sites show a remarkable discriminatory capacity against the various tRNAs (aminoacyl-, peptidyl- and deacylated tRNA) present on the ribosome during elongation: The E site binds exclusively deacylated tRNA, A and P site can bind simultaneously an aminoacyl-tRNA, whereas a ribosome can bind only one peptidyl-tRNA at a time, although this tRNA can be present in either A or P site (exclusion principle for peptidyl-tRNA binding).

Functional analyses revealed again the same pattern (3-6). The first and the third site (A and E) are allosterically linked via negative cooperativity: Occupation of the A site results in a drastic decrease of the E-site affinity for tRNA and vice versa. As a consequence the ribosome assumes two different functional states in the course of the elongation cycle. In the pretranslocational state the high-affinity sites are A and P (E: low affinity), in the posttranslocational state P and E (A: low affinity). Both high affinity sites are occupied simultaneously with tRNA, and the adjacent tRNAs undergo simultaneously codon-anticodon interaction.

It follows that in the course of two elongation cycles the tRNA passes through the ribosome by subsequently occupying three different binding sites: The A, P and E sites.



These findings have led to the allosteric three-site model (Figure) which has important implications for the accuracy and rate of the coding process, for the translocation process, and for our understanding of the inhibition mechanisms of some antibiotics (7).

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Effects of Environmental Osmolarity on the Cytoplasmic Solute Composition of *Escherichia coli*: Consequences for Protein-DNA Interactions.

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When the osmolarity of the intracellular environment of *Escherichia coli* is elevated a series of physiological responses are elicited. The intracellular concentrations of certain solutes increase and osmotic balance and growth are restored. K^+, glutamate and trehalose are accumulated in cells grown under osmotic stress in minimal medium. The intracellular accumulation of glycine betaine, choline, and/or proline, when these solutes are provided exogerously, is concomitant with a reduced intracellular accumulation of K^+, glutamate and trehalose. Thus, the intracellular solute environment is a reproducible function of extracellular solute concentration and composition. These cytoplasmic solutes should not be viewed as inert. *In vitro*, all affect the noncovalent assembly and interactions of biopolymers. In particular, K^+ exerts dramatic effects on specific and nonspecific protein-nucleic acid interactions *in vitro*. However, these same interactions apparently are relatively insensitive to changes in the intracellular K^+ concentration *in vivo*. The cellular solutes other than K^+ exhibit preferential exclusion from the local environment of globular proteins and consequently stabilize the native state and assemblies of reduced solvent-exposed surface. Mechanisms must operate in *E. coli* to compensate for the potentially disruptive effects of variations in the intracellular solute environment on macromolecular processes. The limits of medium osmolarity which permit growth of *E. coli* may reflect the effective limits of such compensatory mechanisms.

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PHENOTYPIC ADAPTATION OF EUBACTERIAL HALOPHILES TO SALT AND OTHER SOLUTES

By

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Most eubacterial halophiles have the ability to adapt phenotypically to a wide range of external salinity from low to high salt concentrations, in marked contrast to the archaebacterial halophiles which are genotypically adapted to habitats of high salinity (1). The central question is how do eubacterial halophiles switch from growth at low salinity (< 1.0 M NaCl) to high salinity (up to 4.0 M NaCl).

We have studied one of these organisms, *Vibrio costicola*, and followed some of the changes manifested when it undergoes a shift-up in salinity (2,3). We have also determined the minimum concentrations of salt and solutes for growth, and the role of osmotic effects in haloadaptation (4,5). One of the most obvious responses to external salinity is an alteration in membrane phospholipid composition (2,3), which parallels the time-course of changes in growth rate (6) and apparently does not involve protein synthesis (3,4).

These studies have highlighted the role of the cell membrane in haloadaptation (7), not only because it must be involved in regulating the cytoplasmic ionic environment (8,9), but also as the most probable site for the sensing mechanism(s) that trigger the cellular response(s) to ionic and osmotic changes in the environment (7).

Recently, we have developed a cell-free system from *V. costicola* with which to investigate salt and solute effects on the enzymes of phospholipid synthesis *in vitro*. In addition, in collaboration with Professors Golboa and Avi-Dor (Technion, Haifa), we have determined for the first time the true intracellular free Na^+ concentration in *V. costicola*, and its dependence on external salinity, by means of ^{23}Na-NMR spectroscopy.

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The Interrelation Between Synthesis and Uptake of Compatible Solutes in a Haliphilic Eubacterium

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The cytoplasm of halophilic eubacteria is not highly ionic and organic compounds of low molecular weight (s.c. compatible solutes) replace some of the intracellular salt (1). In a large number of moderately halophilic bacteria glycine - betaine was found to be the major compatible solute which accumulated under conditions of high salinity (2). To the above class of organisms belongs bacterium Ba_1 which was isolated from the Dead Sea and which is known to accumulate glycine-betaine when the latter or its precoursor, choline is present in the medium (3). The aim of the present work was to find out whether the cells of bacterium Ba_1 relay on glycine-betaine as the principle osmoregulator also when they are grown on a defined medium with glucose or succinate as the single carbon source. Natural abundance ^{13}C-NMR spectroscopy was used to identify the intracellular organic solutes. It was found that when the salt concentration was below 0.6M then α,α-trehalose was the major organic solute in the protein-free cell extract, whereas above this concentration the major solute was identified as 1,4,5,6-tetrahydro-2 methyl-4-

pyrimidinecarboxylic acid (ectoine). Ectoine is a novel cyclic amino acid which was discovered by Galinski et al. (4) in a phototrophic, halophilic eubacterium. The intracellular concentration of ectoine increased nearly linearly with the extracellular salt concentration. There was no evidence for the presence of glycine-betaine in measurable amounts. When the minimal medium was supplemented with glycine-betaine, the latter accumulated in the cells and suppressed the synthesis of ectoine but not that of α, α-trehalose. In contrast, addition of proline to the medium inhibited the formation of α, α-trehalose but not that of ectoine. Hence, glycine-betaine and ectoine on the one hand, and proline and α, α-trehalose on the other, seem to belong to different classes of osmoprotectants. The main point which emerged from this investigation is that the nature of the principle solute which is used by the bacterial cell for osmoregulation is under strict control. It depends both on the external salt concentration and on the nature of the available carbon sources in the medium.

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Mechanisms of Salt Tolerance in the Marine Cyanobacterium *Spirulina subsalsa*

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Glycinebetaine was found to be the major organic substrate accumulating under hypersaline growth conditions in the halotolerant cyanobacterium *Spirulina subsalsa*. In addition to its proposed role as osmolite, glycinebetaine is shown to specifically protect enzymatic activity. Glucose-6-phosphate dehydrogenase from *S. subsalsa* obtained full activity in the presence of NaCl at concentrations as high as 1.5 M, provided that comparable concentrations of glycinebetaine were also present in the reaction mixture. Kinetic analyses indicated that glycinebetaine protected the enzyme against both NaCl-induced decrease in V_{max} and reduction in affinity to glucose 6-phosphate. The alternative osmolites, glycerol and proline, protected the enzyme against the reduction in V_{max} but not against the the reduction in affinity to glucose 6-phosphate.

The respiration activity in protoplasts from *S. subsalsa* exhibited higher rates of O_2 uptake in cells grown in hypersaline medium. NaCl added to the reaction enhanced O_2 uptake, and this effect was specific to sodium ions. Plasma membranes presented high rates of cytochrome-oxidase activity, which were enhanced by NaCl. High rates of cytochrome-c oxidation by NADH were observed, and were found to be more sensitive to NaCl than cytochrome-c oxidation. These findings demonstrate the enrichment of respiratory ETS activity, including cytochrome-oxidase in the plasma-membrane of marine *Spirulina*. The plasma-membranes were found to contain also active H^+-ATPase which was enhanced by NaCl and found sensitive to vanadate and DCCD. The enhancement of cytochrome-oxidase and H^+-ATPase activities by NaCl suggests that these enzymes, functioning as proton-pumps, are involved in the extrusion of sodium ions from salt adapted *S. subsalsa* cells, and are assumed to be coupled to a Na^+/H^+ antiporter located on the plasma-membranes.

Salt adaptation in Anabaena sp 287 and the identification of the sodium sensing DNA fragments of its genome.

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Cyanobacteria are a group of prokaryotic micro-organisms performing higher plant-style photosynthesis. Many of them are capable of fixing atmospheric molecular nitrogen under aerobic conditions and are used to provide in situ fertilization to crop plants. Certain cyanobacteria such as Spirulina sp are used as food supplements for cattle and poultry. Their ability to adapt to all environments also allows them to play a major role in soil reclamation. We examined the response of a freshwater nitrogen fixing cyanobacterium Anabaena sp 287 to salt stress and it showed the final tolerant level 0.15 M NaCl. At 0.2 M NaCl all metabolic activities were inhibited and by the subsequent degradation of phycobiliproteins followed by chlorosis the filaments met death. The tolerance level was increased by the addition of combined nitrogen to the medium for temporary adaptation and by NTG mutagenesis for permanent adaptation. Under nitrogen fixing conditions sucrose acted as primary osmoregulator and alanine, trehalose and glucose as secondary osmoregulators. The cellular nitrogen status was inversely proportional to the cellular sugar levels. Cultural growth of Anabaena sp 287 in combined nitrogen and the salt tolerant mutant Anabaena STM 007 performed photosynthesis, respiration and nitrogen fixation at 0.2 M NaCl. Anabaena STM 007 adapted to strict halophilism and did not accumulate sugars in the presence of salt however it showed changes in the cellular soluble protein profile. Similarly it showed adaptation in all physiological properties. Due to the characteristic presence of a peptidoglycan layer in cyanobacteria they release many pool constituents to the medium during down- and upshock. The salt tolerant mutant Anabaena STM 007 was further improved for proline overproduction in the case of Anabaena STM 304 and histidine release to the medium in the case of Anabaena STM 340 by developing resistance to amino acid analogues. Providing down- and upshock treatments to immobilized cyanobacteria amino acids were recovered after the release from the filaments. Sau3A digested cyanobacterial DNA was spliced with BamH1 digested pBR 322 and transformed to E. coli RM1, an alanine transport negative mutant and the recombinant plasmids bearing sodium sensing fragments of cyanobacterial DNA of various sizes were obtained.

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Expression of transposons Tn1, Tn5, Tn7, Tn9, Tn10 and phage Mu and the unstability of Tn5 generated hydroxyproline overproduction mutations in the salt tolerant nitrogen fixing bacterium, Azotobacter chroococcum MKU1.

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Transposons Tn1, Tn5, Tn7, Tn9 and Tn10 were transferred to Azotobacter chroococcum MKU1 via conjugation using the transposon delivering vectors pGS18, pGS9, pBEE7, pGS27 and pBEE10 respectively. Phage Mu was transferred using the RP4::Mucts61 plasmid-transposon cointegrate and produced 10^5 phages ml^{-1} after heat induction at 42°C; using the tra function of plasmid RP4 and its specific pili formation, the cointegrate made A. chroococcum MKU1 as an efficient donor in transferring chromosomal genes and Mu facilitated in vivo gene cloning by enhancing the frequency of transfer from 10^{-6} - 10^{-7} (during RP4 transfer) to 10^{-3} - 10^{-4}. The other transposons resulted in transposition frequencies of approximately 10^{-6}. Among them Tn5 was extensively used to isolate pigment deficient mutants by metranidazole selection in order to check the overproduction and release of hydroxyproline to the medium. A. chroococcum MKU1, a nitrogen fixing gram negative bacterium produces a dark brown pigmentation during its latelog-stationary phase of cultural growth in liquid as well as solid Jensen's medium when sucrose is used as carbon source and depending on the nutrient supply the colour production varies. Mutants isolated at the frequency of 10^{-5} instead of storing pigment inside the cells excreted into the medium along with hydroxyproline imparting a pinkish brown colour to the cultures. Radioactive labelled (C^{14}) sucrose was added to the growing cultures and the excreted labelled amino acids of the culture filtrate was separated by Dowex column chromatography. Among the amino acids hydroxyproline constitute 70 to 75% of the total and this evidenced the possible conversion of carbon skeletons from sucrose to hydroxyproline. The hydroxyproline overproduction varied among the colonies isolated by metronidazole selection and the mutants were highly unstable during subculturing. This is due to the loss of the mutation during segregation since A. chroococcum has been reported to have 20-25 chromosome equivalents per cell. Cloning of this mutation in E. coli for a stable expression as done for Serratia marcescens amino acid overproduction mutations will be useful for industrial applications. This salt tolerant strain also possess a 3Kb indigenous plasmid which provided a partial role in salt tolerance. Curing of the plasmid or co-integrate formation with RP4::Mu showed reduced levels of salt tolerance and pigment production in the parent.

Structure and Function of Ribosomes as Determined by non-crystallographic Methods

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Current research on the structure of bacterial ribosomes is concentrating on the elucidation of the architecture of the ribosomal particles, i.e. the spatial arrangement of the proteins and the RNA strands in situ. To this end, the following methods are being applied: X-ray structural analysis of three-dimensional crystals of ribosomes and their subunits, three-dimensional image reconstruction from two-dimensional crystalline sheets of ribosomal particles, immuno electron microscopy, neutron scattering studies, assembly maps based on the reconstitution of ribosomal subunits from their individual components, elucidation of the neighborhood between ribosomal components by protein-protein, protein-RNA and RNA-RNA cross-linking. The combination of these approaches has already yielded a considerable insight into the architecture of ribosomes, especially from E. coli and Bacillus stearothermophilus.

Since ribosomes occur in all organisms, they are ideal objects for evolutionary studies. The complete sequences of many ribosomal proteins and RNAs from various classes of organisms have been determined. Comparison of these sequences led to interesting conclusions about the evolution of ribosomes and their components.

My lecture will give an overview about the structure of ribosomes as elucidated by non-crystallographic methods, and Dr. A. Yonath will talk about X-ray structure analysis and image reconstruction of ribosomal particles. Furthermore, I will summarize our current knowledge on the molecular mechanism of protein biosynthesis and on the inhibition of this process by antibiotics.

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CRYSTALLOGRAPHY OF RIBOSOMAL PARTICLES FROM HALO- AND EU-BACTERIA

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Ribosomes are the cell organelles where protein biosynthesis takes place in every living cell. They are complex assemblies composed of several strands of RNA and a large number of different proteins arranged into two subunits which associate upon initiation of protein synthesis. The molecular weights of bacterial ribosomes are 2.3×10^6 for the assembled particles, 1.45×10^6 for the large subunit, and 8.5×10^5 for the small subunit. Aiming at the unraveling of their three-dimensional structure we have crystallized wild-type, mutated and modified ribosomal particles as well as their complexes with other components involved in biosynthesis of proteins. The best crystals are of the large ribosomal subunits from Halobacteria marismortui. These diffract to 4.5Å All crystals ware grown from functionally active particles, and, despite the natural tendency of isolated ribosomes to disintegrate, the crystalline material has retained its integrity and biological activity for long periods.

Monofunctional reagents of heavy-metal-clusters were covalently bound to free sulfhydryl groups on the surface of the ribosomes, or on an isolated ribosomal component which was consequently incorporated into the core particle. Particles depleted from a specific protein were obtained either genetically (e.g. mutation of B. stearothermophilus) or by mild chemical procedures. So far, no mutation which results in a removal of a ribosomal protein from Halobacterium marismortui, could be introduced, probably due to its resistance to antibiotics. However, for detaching selected proteins from these ribosomes, we took advantage of the delicate equilibrium of the di- and mono valent ions present in the natural environment of these ribosomes, as well as of the major role that Mg^{++} plays in their rigidity. Attempts to label the detached proteins with metal-clusters will be discussed.

Three-dimensional reconstruction studies performed on ordered arrays of whole ribosomes and their large subunits led to a model showing a void space at the interface between the two subunits, which is large enough to accommodate the non-ribosomal components involved in protein biosynthesis; a groove in the small subunit, rich in rRNA which may be the site for binding mRNA and tRNA; and a tunnel, of up to 25Å in diameter, and around 100Å in length, which spans the large subunit and may provide the exit path for the nascent polypeptide chain.

Based on preliminary studies we suggest that the walls of the exit tunnel are rich in rRNA and in hydrophobic amino-acid residues, both in eu- and Halobacteria.

This work was carried out in collaboration with Max-Planck-institute for Molecular Genetics, D-1000 Berlin 33.

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Characterization of Crystals of Small Ribosomal Subunits

IN VITRO TOTAL RECONSTITUTION OF ACTIVE 50 S SUBUNITS OF THE HALOPHILIC ARCHAEBACTERIUM *Haloferax mediterranei*

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The ribosomes of extremely halophilic bacteria are unique in that they contain mostly acidic, instead of basic, proteins, and are unstable unless in the presence of nearly-saturating concentrations of salt, typically potassium chloride (1-9).

To get some insight into the mechanisms of RNA-protein interaction and ribosome folding in hypersaline environments, we have worked out a system for *in vitro* total reconstitution, starting from the dissociated RNA and protein components, of the large ribosomal subunits of the halophilic archaebacterium *Haloferax mediterranei*. Successful reassembly of ribosomal particles fully active in poly(U)-directed polyphenylalanine synthesis requires a 2 h incubation at 42-44 °C in the presence of high salt (3.2 M KCl) and high magnesium (60 mM) concentrations. The requirement for KCl is quite stringent, other salts (e.g. ammonium sulfate or ammonium chloride) being scarcely or no effective in promoting subunit reconstitution.

A progressive lowering of the salt concentrations in the reconstitution mixture results in the formation of particles having greatly decreased sedimentation coefficients and reduced or absent synthetic activity. Structural analysis of such defective particles shows that they still contain most of the ribosomal proteins, even when reconstitution is performed at KCl concentrations as low as 0.5 M. This indicates that monovalent cations probably exert their strongest influence at the level of the three dimensional folding of the ribonucleoprotein particle.

For a further discussion of the specificity of RNA/protein interactions in halophiles, data will also be presented regarding the ability of halophilic ribosomal RNA and proteins to recognize non-halophilic counterparts in a variety of experimental conditions.

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The halophilic enzyme dihydrofolate reductase of the extremely halophilic archaebacterium *Halobacterium volcanii* as a model for the structural adaptation of enzymes to extreme salinities.

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The extremely halophilic archaebacteria of the genus *Halobacteriacae* are adapted to survive and grow at extreme salinities. In these bacteria the osmotic balance is maintained by accumulation, in the cell, of multimolar concentration of KCl. The entire biochemical machinary is, thus, adapted to function at very high salt concentrations. The study of the structural properties of the halobacterial enzymes might, therefore, shed light on the question of adaptaition of proteins to extreme salinities. The enzyme dihydrofolate reductase (DHFR) is an excellent subject for comparative studies on the relationships between structure and function. The three dimensional structures of DHFRs of *E. coli* (1), *Lactobacillus caseii* (2) and chicken (3) were determined at high resolution. In spite of the great variations in the amino acid sequences among these proteins, the three dimensional structures show a great deal of resemblance.

The extremely alophilic bacterium *Halobacterium volcanii* is very sensitive to the antifolate inhibitors trimethoprim and methotrexate. Resistant mutants to these drugs arise spontaneously at frequences of 10^{-10} -10^{-9}. In all the cases analyzed so far, it was found that the basis for the resistance is an amplification of the region of the chromosome that codes for the enzyme dihydrofolate reductase (DHFR) (4). This amplification causes an overproduction of the enzyme and by that alleviating the inhibition of the drug.

These trimethoprim resistant mutants enabled us the quick isolation of the gene coding for the halobacterial DHFR (h-DHFR) and ubsequently the determination of the nucleic acid sequence of the coding region. Moreover, since the basis of drug resistance is the overproduction of the enzyme, the drug resistant mutants are a very good source for large amounts of the enzyme.

A comparison of the amino acid sequence of h-DHFR to the sequence of DHFRs from other sources reveals a simmilar degree of homology as exists among the non-halophilic enzymes (25-30%). A distinctive feature of h-DHFR is its high net negative charge. This feature is characteristic to many enzymes isolated from halophilic bacteria (5).

h-DHFR was purified to homogeneity using a series of ammonium sulfate mediated chromatographies (6). The biochemical properties of the purified enzyme were analyzed. As in the case of other halophilic enzymes, the salt concentration affects both, the activity of the enzyme and its stability. A detailed analysis of the effect of salt concentration on the kinetic parameters of the enzymatic reaction indicates that it affects the V_{max} but not the K_m. Interesting relationships were found between the effect of salt concentration and pH on the enzymatic activity. In the pH range of 4-7 the enzymatic activity increases with the increase of proton concentration. At pH 4 the activity is more than twenty folds higher than at pH 7. At pH 7 the enzymatic activity depends on ionic strength, being higher at higher salt concentrations. This dependence becomes less pronounced as the pH decreases. At pH 4 the enzymatic activity does not depend any more on the ionic strength. The interpretation of these observations is that the limiting step in the enzymatic activity is the hydrid transfer reaction between the NADPH and the dihydrofolate (7,8). This reaction is stimulated by the protonation of a single aspartic acid side chain (Asp 29). The pK_a of this residue is probabely influenced by the screening of the surface charge of the protein. At low pH the proton concentration is high enough to protonate the aspartic acid side chain and therefore the salt has no more effect on the reaction rate.

The effect of salt concentration on the stability of the h-DHFR is rather complicated. At concentrations higher than 2M the enzyme is stable for a long time. As the salt concentration is reduced the enzyme becomes unstable. However, whereas in the cases of h-MDH and other halophilic enzymes the inactivation is a first order process (9) in h-DHFR the kinetics of inactivation is more complicated, hinting to the the possibility that the enzyme undergoes a sequence of conformational changes, losing its activity gradually.

It is hoped that using 'site directed mutagenesis' techniques the primary structure of the enzyme will be modified, enabling the detailed study of the effect of the structure of the enzyme on its halophilic properties.

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STRUCTURE-FUNCTION RELATIONSHIP OF HALOPHILIC PROTEINS: SURFACE-LAYERS AND ENZYMES FROM HALOPHILIC ARCHAEBACTERIA

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Biomolecules have evolved their specific functional properties by optimizing the balance of intra- and intermolecular forces in their respective cellular environment. Alterations of the environment required either compensation of the hostile external parameter, or molecular adaptation [1].

In recent years, the three-dimensional structure of a wide variety of proteins, including samples from extremophilic organisms, have been elucidated at the atomic level. As taken from the data base presently available, the astronomically big reservoir of possible primary structures (10^{220} for a domain size of 170 amino-acid residues) yields only a relatively small number of stable topologies. Their free energy of stabilization ΔG_{stab} is minute compared to the total molecular energy. Molecular adaptation to extremes of physical conditions refers to marginal alterations of the intramolecular interactions. E.g. for extreme thermophiles (in comparison with mesophilic homologs), $\Delta\Delta G_{stab}$ amounts to the equivalent of only a few additional hydrogen bonds, hydrophobic interactions or ion pairs. Clear-cut predictions with respect to the correlation of primary structure and protein stability are presently not possible [2].

Protein stability is determined mainly by the free energy of solvation of the polypeptide backbone and the amino-acid side chains. In the case of extreme halophiles (with cytoplasmic salt concentrations close to saturation), this general principle enforces a compromise between groups of the protein prone to hydration, and excess salt present in the solvent medium. Maximum hydration is provided by acidic side chains, especially glutamic acid. On the other hand, salting-out is promoted by decreased polarity of a molecule. From this one would expect

that adaptation to halophilic conditions may be accomplished by increasing the ratio of polar vs unpolar groups [3].
Halophilic dihydrofolate reductase, lactate dehydrogenase and malate dehydrogenase may be considered good examples obeying the given adaptation mechanism. Their reversible unfolding (as a function of salt concentration) will be discussed in order to illustrate the structure-function relationship of halophilic enzymes and their folding-association behavior [4].
In considering enzymes, full catalytic function requires the salt-induced decrease in flexibility to be compensated by some mechanism. Obviously, the decrease in hydrophobicity serves this purpose. Non-halophilic lactate dehydrogenase with and without its hydrophobic N-terminal dekapeptide may be considered as an example [5,6].
Further means of increasing protein hydration is ion binding or conjugation of a protein with a highly sulfated glycomoiety. As shown by Eisenberg and coworkers [3], preferential ion binding is common to all halophilic proteins investigated so far. The surface-layer protein of *Halobacterium halobium* will be discussed as an example. The protein exhibits typical properties of a highly charged polyelectrolyte. Binding of divalent ions alters its association pattern in a characteristic way which may be tentatively correlated with morphological changes of the microorganism in the presence and absence of Mg^{2+} [7].

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Combined use of ultracentrifugation, light, X-ray and neutron scattering, for the determination of water and salt binding in halophilic proteins

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Proteins of extreme halophiles complex unusual amounts of water and salt in their natural environment. Unusually large particles are formed the composition and low resolution structure of which may be determined by solution techniques. A basic experimental technique is sedimentation in the ultracentrifuge of native and denatured proteins, at varying concentration of low molecular weight salts. Values for the individual binding of water and of salt are derived from solution density dependence of density increments $(\partial\rho/\partial c)_\mu$, which determine sedimentation in the ultracentrifuge. In strict analogy to the sedimentation analysis forward scattering of light, X-rays and neutrons is analyzed in similar terms, the mass density increment being replaced by the analogous refractive index, electron density and scattering length density increments. Combination of these equations in pairs is particularly useful in the case of neutron scattering due to the negative scattering length of water. Study of the angular dependence of scattering provides additional information for the determination of protein shape, size, volume and surface.

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Solvent interaction, structure and stabilisation of halophilic proteins.

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Many of the halophilic proteins that have been purified are only stable in highly concentrated solutions of certain salts (1). They tend to unfold below a threshold salt concentration that is still relatively high (e.g. 2.5 M NaCl or KCl) (1). Malate dehydrogenase from *Halobacterium marismortui* (hMDH) was found to have exceptional solvent interactions with water and salt molecules in conditions in which it is stable, leading to an effective salt concentration associated to the protein in significant excess over that in the solvent (1-3). Concomitant with protein unfolding these exceptional solvent interactions are lost at non-physiological salt concentrations (4). Recent work has shown that this protein can also be stabilised by high concentrations of certain "salting-in" ions (that usually favour protein unfolding) (5). These and most other results on hMDH structure and stability cannot be understood in terms of the usual effects of salts on protein structure.

A novel stabilisation model was proposed for halophilic malate dehydrogenase that can account for all observations, so far (5). The model results from the analysis of data on the protein in salt solutions chosen for their different effects on protein structure (potassium phosphate, a strongly "salting-out" agent, $MgCl_2$, which is "salting-in", NaCl and KCl that are mildly "salting-out"). Different protein stabilisation mechanisms are dominant in different salt solutions in which this enzyme is active. Thus, in molar concentrations of strongly "salting-out" phosphate ions, the mechanisms are similar to those in non-halophilic soluble proteins, in which the hydrophobic interaction dominates. In high concentrations of KCl (similar to the physiological environment of the protein), NaCl or $MgCl_2$, on the other hand, a solution particle is formed in which the protein dimer interacts with large numbers of salt and water molecules (the mass of solvent molecules involved is approximately equivalent to the protein mass). It was proposed that, under these conditions, the main stabilisation mechanism is the formation of hydrate bonds between the protein and hydrated salt ions. Model predictions are in agreement with all experimental results, and, as expected, the thermodynamic stability of the solution particle close to room temperature is observed to be mainly enthalpy driven. This is unlike the case of non-halophilic soluble proteins in which entropy terms dominate stabilisation close to room temperature. The stabilisation model is discussed in terms of a structural model

proposed previously from solution small angle scattering data in which the solvent interactions take place on loops extending outwards from the protein core (2).

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Protein Adaptation to Extreme Salinity: The Crystal Structure of 2Fe-2S Ferredoxin from *Halobacterium Marismortui*

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As a model for the adaptation process of a protein to an extremely saline environment, we have investigated the crystal structure of a 2Fe-2S ferredoxin from the halophilic archaebacterium *Halobacterium marismortui* (HmFd). Ferredoxin represents a good choice for studying haloadaptation because it is ubiquitous from prokaryotes to higher plants, and because the 3-D structure of a non-halophilic ferredoxin from *Spirulina platensis* (SplFd) with the same 2Fe-2S prosthetic group is known. HmFd is similar to plant ferredoxins except that it is longer (MW = 14,000 daltons), and richer in negatively charged residues. Thirty four out of its 128 amino acid residues are either ASP or GLU. The sequence alignment of HmFd with the nonhalophilic ferredoxin SplFd reveals extensive homology in the core parts, particularly in the vicinity of the four CYS residues that comprise the ligands for the Fe-S cluster. However, the homology is progressively weaker farther from the active site in the direction of both termini. We have determined the crystal structure of a 2Fe-2S ferredoxin from *Halobacterium marismortui* at 2.5 Å resolution. The most striking feature of the structure is the presence of a spatially distinct domain made up of regions from the amino- and carboxy termini. This hydrophilic domain may provide the necessary amount of water binding residues to allow for the solvation of the protein in an aqueous phase where free water is scarce, in much the same way as a hydrophobic leader sequence causes solvation of a protein in a lipid bilayer.

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The cell surface of Halobacteria as revealed by two dimensional and three-dimensional image reconstruction from electron micrographs

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We will first review the information available on the structure of the halobacterial cell surface before the introduction of digital image processing techniques. The first micrograph of the cell surface of *Halobacterium halobium* (Houwink, 1956) already showed evidence that the surface is covered by an hexagonal array of morphological units with an approximate spacing of 15nm.

In order to more fully understand the results obtained for the halobacterial cell surface using digital image processing we will review in detail the methodologies applied: a) Specimen preparation b) Electron microscopy c) Optical diffraction d) Computer image processing of two dimensional projections culminating in a full three-dimensional reconstruction (Baumeister and Engelhardt, 1987).

We will review the results obtained with the above methodologies over the past several years in the examination of halobacteria from different saline habitats. a) The square bacterium and other species from the Gavish Sabkha in Southern Sinai (Kessel&Cohen, 1982;Kessel et al, 1985) (b) *Halobacterium halobium* originally isolated from rotting fish c) *Halobacterium volcanii* isolated from the Dead Sea (Mullakhanbai and Larsen, 1975).

We will consider *H. volcanii* in detail being the first species of halobacteria for which a 2.0nm resolution three dimensional reconstruction was produced (Kessel et al, 1988a,b). The reconstruction shows a 4.5nm high dome shaped morphological unit with a small orifice at the apex of the dome opening inwards into a funnel shape towards the cell membrane. The mophological units are connected to each other by extensions from the base of the dome meeting at a three fold axis of symmetry. We will consider a model proposed for the cell surface glycoprotein (CSG) of *H. volcanii* and *H. halobium* based on the three dimensional reconstruction and the information derived from the amino acid sequence of the CSG following cloning of the gene from *H. halobium* (Lechner and Sumper, 1987), and based on evidence from an early x-ray diffraction study (Blaurock et al, 1976).

We will also consider of the behaviour of the cell envelope and particularly the CSG under different ionic conditions in order to explain maintenance of cell wall integrity.

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Signal transduction in photo- and chemotaxis of halobacteria

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Halobacteria grow in brines and salt ponds by respiration, fermentation or photosynthesis and are able to sense chemicals and light. Photosynthesis of halobacteria is optimal in green light. In search of these conditions the cells sense the light qualities UV, blue and green and respond to stimuli by a phobic or attractive response. All photobiological reactions are mediated by retinal proteins. Cellular movement is created by a flagellar bundle pushing or pulling the cell by clockwise (cw) or counterclockwise (ccw) rotation. Statistical mode-distribution keeps the spatial coordinates constant unless a stimulus reaches the cell. Then, with a prolonged (green) or shortened (UV, blue) run, the cell moves to or away from the respective light source.

A signal transduction chain connects stimulus reception with the motor response and will be described in more detail for the chain starting at the photoreceptor P_{480}. This retinal protein occurs in halobacterial cells constitutively and mediates a photophobic response. In principle, a single photon absorbed in one of the approximately 300 receptors can cause a stop response of the motor. Amplification occurs through a photocatalytic process. The response time (t_R) is connected with the stimulus level (photon exposure $F = I \cdot \tau$) by the equation

$$t_R = \frac{b}{I\tau} + t_{min}.$$

The constant is related to properties of the photoreceptor, such as quantum yield, molar extinction and concentration, but also to the gain of the signal transduction chain. The molecular components of the transduction chain are not yet identified but involvement of membrane potential changes have been excluded. To demonstrate participation of diffusible

components, a mutant which is unable to switch its flagellar motor and thereby swims straight for long times has been isolated. By manipulation of its permeability and by incubation with wild type extracts, spontaneous and stimulated switching could be introduced. By using this reconstitution as an assay, a factor could be purified which is a small molecule and could be replaced by fumarate.

Chemotaxis in halobacteria is less well characterized, but also in this case receptors are linked with the flagellar motor via a signal transduction chain. At least part of the chain must be identical to that of mediating phototaxis since chemo- and photostimuli can be integrated by the cell. Adaptation to both types of signals involves a methylation and demethylation system which has been characterized recently.

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SALT AND pH EFFECTS ON THE FUNCTION OF BACTERIORHODOPSIN.

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Bacteriorhodopsin (bR) is a retinal pigment, found in halobacteria. It functions as a light energy converter through a cyclic photoreaction, which causes the electrogenic translocation of protons across the cell membrane and thus provides metabolic energy for the organism. In intact cells, bR occurs as crystalline aggregates in the cell membrane, known as the purple membrane (pm). The structure and function of bR are described in most modern biochemistry texts; the details of the translocation mechanism, however, are still not completely understood. The kinetics of the photoreaction in the "photocycle" can be followed spectroscopically. The early reactions have time constants from the femtosecond to microsecond range. The later reactions are usually in the millisecond range; they are, however, strongly salt- and pH-dependent and can be slowed down by one or two orders of magnitude under still physiological conditions [1].

The pm is characterized by an unusually high surface charge, which is mainly due to its high content of acidic lipids. Acidification or deionization of purple membrane (pm) suspensions reversibly shifts the purple color of bacteriorhodopsin (bR) to blue, due to a shift of its absorption maximum from 568 to 605 nm. The apparent pK of the transition depends on the cation concentration, and occurs near pH 3.0 in suspensions of native pm in distilled water [2, 3]. After rigorous removal of cations, however, the membrane remains blue at the highest pH obtainable in deionized water. Further acidification of blue membrane with HCl restores the purple color with an apparent pK of ~0.5. This effect is caused by the Cl^- anion. When most of the acidic native lipids are removed and/or repalced by neutral lipids, deionization does not induce a color change, the pK of the purple-to-blue transition shifts to ~1.5 and becomes independent of salt concentration. In the lipid-depleted membranes, titration with HCl only causes partial conversion of bR to the blue form because the acidic purple form appears before the transition is complete [4]. In pm titrations with H_2SO_4, the acidic purple form is not observed and the lipid-depleted membrane undergoes a full transition. Despite the increasing proton concentration, the color change of bR is only partial in neutral lipid environments. We conclude that these color changes of bR are caused by conformational changes of the protein and that in the purple-to-blue transition in the native membrane, high proton concentration at the membrane surface induces the protein conformational change. The surface pH is determined by the dissociation state of acidic lipids in the membrane, and also by the electrolyte concentration in the medium. Cations have an indirect effect on bR color by changing the ion composition at the membrane surface. Calculations based on this surface model adequately describe the effect of cations under all experimental conditions.

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KINETICS AND THERMODYNAMICS OF PROTON PUMPING BY BACTERIORHODOPSIN

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It is generally assumed that ion pumps are completely coupled and that all uncoupling may be attributed to external leaks. However, there is no thermodynamic or kinetic justification for this assumption. Recently several reports have appeared showing that a certain degree of intrinsic uncoupling is indeed present in ion pumps and transport systems. Analysis of the six-state model of a redox proton pump studied by Pietrobon and Caplan (1985) shows the regulatory influence of the thermodynamic forces on the extent and relative contributions of redox slip and proton slip. Comparison between simulated behavior and experimental results leads to the conclusion that the typical relation between rate of electron transfer and proton potential difference $\Delta\mu_H$ found in mitochondria at static head is a manifestation of intrinsic uncoupling in the redox pumps, which may well have physiological significance for the efficiency of oxidative phosphorylation (Pietrobon et al., 1986).

In bacteriorhodopsin the photoreaction proceeds at an essentially fixed rate, and hence a $\Delta\mu_H$-controlled slip regulation would provide a "safety valve" as was suggested some years ago (Caplan, 1982; see also Eisenbach and Caplan, 1979). This has been observed by a number of workers (e.g. Westerhoff and Dancshazy, 1984). A detailed compartmental analysis of light-induced proton movement in bacteriorhodopsin vesicles has been given by Klausner et al. (1982). The fitting of a compartmental model to a large number of experimental data required the introduction of an arbitrary "control function". It is shown by means of the Hill diagram method (Hill, 1977) that this control function is a straight-forward consequence of intrinsic uncoupling. Reaction slip increases as the proton gradient increases. As Westerhoff and Dancshazy have pointed out, this property in pumps which constitute the first step in a free-energy transducing pathway permits those pumps to be at least partially controlled by the processes constituting the later steps.

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Two Pumps, One Principle: Light-driven Ion Transport in Halobacteria.

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Halobacteria synthesize two light-driven ion pumps when respiration by lack of oxygen or fermentation by lack of arginine stop. Light then is the only alternative energy source. Photosynthetic growth is dependent under these conditions on the proton pump bacteriorhodopsin mediating photophosphorylation. The inwardly directed proton pump halorhodopsin, on the other hand, allows to accumulate chloride ions inside the cell necessary for maintenance of isotonic conditions during growth.

Both ion pumps have been characterized in much detail by physiological, biochemical and biophysical methods. In both molecules the retinal moiety occurring as a protonated Schiff's base undergoes a thermally reversible photochemical *trans*-13*cis* isomerization and is considered to be the light-triggered switch for ion translocation in both molecules. Both molecules consist of 7 transmembrane helical parts which form a ring with retinal spanning its diameter. The helices are amphipathic in their amino acid side chain distribution and by all likelihood face with their hydrophobic parts the outside lipid environment, whereas the hydrophilic areas are forming the inner surface of the molecule. It is this part of the two ion pumps which is conserved most. Identical amino acid

residues in both molecules serve two functions: Maintenance of the helical structure of the transmembrane sections including the tilt of some of them. Prolines, threonines, serines and some other residues might be involved in this function. Another group of conserved residues is thought to interact with retinal to provide this moiety with the specific properties of optical absorption, stereochemical photo-isomerization and to catalyze its double-bond rotations. A third group of residues which should not be conserved must deal with the different ion specificities of the two pumps allowing the conductance of the proton and the chloride ion to and from the Schiff's base. Here specifically two aspartic acids (D96 and D85) and two arginines (R108 and R200) should be involved in proton and chloride binding, respectively. This concept of a mechanistic model with retinal as an ion switch transferring ions between two binding sites with subsequent passive diffusion in and out of the molecule through the intrahelical pore space will be discussed. It is described in a recent TIBS article (February 1989 issue) which also cites the relevant literature.

MECHANISM OF CHLORIDE TRANSPORT BY HALORHODOPSIN Janos K. Lanyi, Department of Physiology and Biophysics, University of California, Irvine, CA 92717, U.S.A.

The halobacterial retinal protein, halorhodopsin (HR), which functions as a light-driven electrogenic chloride pump in *H. halobium*, is a small integral membrane protein, which undergoes various photoreactions including rapid cyclic isomerisation of the retinal from all-*trans* to 13-*cis*. Since this photocycle contains several chloride-dependent equilibria, it is probably directly related to the translocation of chloride ions across the protein which spans the membrane. A gated optical multichannel analyser is used for measurements of the HR photocycle. We tested the system with bacteriorhodopsin (the proton pump of *H. halobium*, BR), and from the known scheme of this pigment developed criteria for manipulating the obtained spectra in calculations of the absorption spectra of the photocycle intermediates and the kinetics of their rise and decay. The results for BR agree with data reported by a large number of other authors. The results for HR in the presence of chloride are consistent with earlier data, and reveal an additional intermediate, not previously seen, in the sub-usec time-scale. Although a deprotonated intermediate is not in the HR photocycle, a one-by-one comparison of the rest of the intermediates observed for BR and HR indicates a striking similarity between the photocycles of the two bacterial rhodopsins. This was previously not apparent, perhaps because the experimental approaches to the spectroscopy of the two pigments were different and the data were thus more fragmented. The similarity between the photocycles of BR and HR has important implications for the functional differences between these two pigments.

The primary structure of HR is now available, and arguments based on plausible secondary and tertiary structures for

the protein suggest that only a few arginine residues, and possibly the protonated Schiff base, participate in the intramembrane translocation of chloride. Cloning and sequencing of a second halorhodopsin, from *Natronobacterium pharaonis*, provided the means for comparing two different proteins with the same transport function, since there is only 65% sequence identity between these proteins. The conserved residues are those which play a role in chloride transport, while those which are different between these proteins and BR are likely to be involved in proton transport.

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Energetics in Halobacteria
with special emphasis on their ATP synthase and light-driven pumps

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I. ATP synthase

Halobacteria synthesize ATP through respiration and/or by $\Delta\tilde{\mu}H^+$ formed by light-energized retinal protein pumps (1-3). The machinery which phosphorylates ADP to ATP is also driven by a pH jump (outside acidic) at a pH optimum of 6.8 (4,5). The proton motive force was determined to be at most -90 mV at [ADP]av = 2.1 mM, [Pi]av = 17.9 mM and [ATP]av = 39 μM at 30°C. This suggest that more than 3 protons are required for synthesizing 1 ATP. This figure is larger than that for F_oF_1 ATPase.

The ATP synthase includes a $(86 + 64 \text{ kDa})_2$ = 320 kDa head piece (6,7) which is released from the inner face of plasma membrane as an ATPase. (An ATPase from H. saccharovorum is reported to be 300 kDa with 83 and 60 kDa subunits (8)). The optimal conditions for this ATPase are pH = 5.8 and 1.5 M Na_2SO_4 and Mn^{2+} is more effective than Mg^{2+} (7). The ATPase is inhibited by nitrate.

The polyclonal antibody raised against this (Halo)-ATPase cross reacted not only with the ATPases of other kinds of archaebacteria, Sulfolobus, Methanobacterium and Methanosarcina, but also with the so-called anion-sensitive (V-type (9); nitrate sensitive) H^+-ATPase of tonoplast of yeast and red beet (10). The antibody reacted little to F_1-ATPase (F-type (9); azide-sensitive) from various sources and Ca^{2+}-ATPase (P-type (9); vanadate-sensitive). So far F-type ATPase itself has not been detected in halobacteria (and other archaebacteria either). The cross reaction (positive or negative) can be confirmed by the reverse reactions to (Halo)-ATPase with the antibodies of those other types of ATPases.

These results indicate that the ATP synthase functioning in halobacteria (or archaebacteria; we classify it as "A-type" ATPase is far from an F-type ATPase which has been believed to be the only ATP synthase ubiquitously distributed in respiring organisms, but is more closely related to V-type ATPases in eukaryote. (The above observation was confirmed with Sulfolobus ATPase by the homology of the primary amino acid sequences among ATPases from Sulfolobus (A), plant vacuole (V) and mitochondria (F).

A-type ATPase requires more protons per ATP than F-type ATPase. When two types of ATPases are incorporated in eukaryote F-type works more efficiently (with less H^+ debt) as synthase and A-type as H^+ pump which is now known as V-type.

II. Retinal protein pumps

Halobacterium halobium contains bacteriorhodopsin (H^+ pump; 11) and halorhodopsin (Cl^- pump, 13) as light-energy transducers. Halobacterium sp. aus-1 and aus-2, collected in Western Australia, also contain bR-like new light-driven H^+ pumps. The M_r are all around 26 kDa (4). We named them

Archaerhodopsin and Archaerhodopsin-II, respectively.The photochemical and spectroscopic properties of aR and aR-2 are quite similar to those of bR, although aR in the "claret" membrane (a purple membrane-like fraction containing aR and bacterioruberin in 1:1) shows salt concentration dependency in the yield of the M intermediate by flash photolysis.

However, the homology in primary amino acid sequence is 59% between bR and aR (14), while it is 83% between aR and aR-2 (presently 60% of the aR-2 sequence are resolved). BR may be evolved in the Gondwanaland and aR's afterwards in Australia/ Antarctica.

The hydropathy profile of aR implies seven trans-membrane helices just like bR. The helices C (roughly from Ala(90/84) to Ala107/Val101 in aR/bR) and G (containing retinal-binding Lys(222/216)) of both aR and bR are very well conserved with identical amino acids upto 92% and 88%, respectively. Two carboxyl residues (Asp(91/85) and Asp(102/96) of aR/bR) on the C helix are conserved in both aR and bR (but missing in hR) (14), suggesting their key involvement in proton pumping as FTIR (15) and the site-directed mutagenesis (16) data suggested.

More pumps with naturally different sequences would be found in other strains of halobacteria. Comparison of the primary structures and functions of such pumps will give us further information, which, in some parts especially in view of evolution, is unique and different from that obtainable from other methods.

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The Strategy of Adaptation to Low Water Potential by the Halotolerant alga *Dunaliella*

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Halotolerant organisms developed two types of strategies to cope with living in media containing high sodium chloride concentrations (Avron, 1986). The problem is how to maintain osmotic equilibrium while avoiding the deleterious effects to the normal operation of most enzymic systems of high intracellular ionic strength, and particularly high sodium concentrations.

In the strategy adopted by the Halobacteria, potassium is accumulated intracellularly at a concentration which is isoosmolar with the external sodium concentration. In the second strategy, adopted by most other halotolerant organisms, "compatible solutes" are accumulated intracellularly (Brown, 1976). These are selected organic molecules which provide osmotic equilibrium with minimal damage to the cells' metabolism. The accumulated organic solute varies with the species and the salt stress, but most fall into two groups: low molecular weight polyols such as glycerol and mannitol or zwitterionic molecules such as proline and betaine (Ben-Amotz and Avron, 1983).

The unicellular alga *Dunaliella* is the only eucaryotic organism which was shown to thrive in media containing various concentrations of salt, from 0.1 M to 5.5 M, and to show excellent adaptation to changes in salt concentration. When growth conditions are optimal, the doubling time is about 6 hours. When the salt concentraiton is augmented to saturation, the doubling time is limited by the salinity to about 72 hours. Algal adaptation to fluctuations in salt concentration is relatively rapid and is a function of the extent of the osmotic shock and the rapidity of the transition. However, rapid and drastic lowering of the salt concentraiton will burst the cells.

The mechanism by which the alga adapts to various salt concentrations is termed the osmoregulatory mechanism. In *Dunaliella*, it was shown to function through the ability of the algae to vary the intracellular concentration of mostly a single substance, glycerol (Ben-Amotz and Avron, 1981). When adapted to grow in media containing widely different salt concentrations, the intracellular glycerol concentration is directly proportional to the extracellular salt concentration, and osmotically sufficient to account for most of the required osmotic pressure. When grown with high salt concentration, the cell contains over 50% glycerol, but, nevertheless, little to no glycerol is found in the growth medium (Ben-Amotz *et al.*, 1982). *Dunaliella* possesses, in addition, a mechanism which prevents equilibration of the extracellular salt with the cell interior. Thus, cells grown in media containing between 1-4 M sodium chloride contain no more than 0.1M sodium chloride intracellularly (Bental et al., 1988b; Karni and Avron, 1988). Like most living cells, *Dunaliella* accumulates potassium intracellularly, but to an osmotically minor level. Cells grown in media containing 1-4 M NaCl and 0.001-0.005 M KCl contain 0.1-0.2 M potassium intracellularly (Avron, 1986)

When exposed to higher or lower salt concentrations in their media, the algae behave like perfect osmometers, rapidly shrinking or swelling. Within minutes after the rapid volume change, synthesis or elimination of glycerol is initiated and continues for a few hours until the cell volume returns to its original size. Glycerol synthesis under hypertonic conditions and glycerol elimination under hypotonic conditions are independent of protein synthesis and occur in the light or in the dark (Sadka et al., 1989). The compatible nature of glycerol permits enzyme function at the very high concentrations which exist inside the cells.

Three novel enzymes have been identified and characterized in *Dunaliella* and are suggested to form the core of a specialized "glycerol cycle". The first, dihydroxyacetone reductase, catalyzes the interconversion of glycerol and dihydroxyacetone via the reduction and oxidation of $NADP^+$ and NADPH, respectively. The second, dihydroxyacetone kinase, mediates the phosphorylation of dihydroxyacetone by ATP and the third, DL-glycerol-1-phosphatase, specifically dephosphorylates DL-glycerol-1--phosphate (Ben-Amotz and Avron, 1981).

Glycerol synthesis depends on the supply of reduced carbon from the photosynthetic carbon reduction cycle or from stored nonosmotically active polysaccharides such as starch (Bental et al., 1988a). Subjecting the algae to a hypertonic stress activates the pathway converting stored polysaccharides such as starch, and carbon dioxide via the photosynthetic carbon cycle, to dihydroxyacetone phosphate. Reduction to glycerol phosphate followed by the action of the specific phosphatase results in the observed accumulation of glycerol. When the cell is subjected to a hypotonic stress, a pathway leading to the oxidation of glycerol to dihydroxyacetone and the phosphorylation of the latter to dihydroxyacetone phosphate is activated. The dihydroxyacetone phosphate accumulated is then converted to stored polysaccharides via the classical starch synthetic pathway.

The signal which triggers the osmoregulatory response in *Dunaliella* has not been identified. Changes in concentration of soluble metabolites participating in the metabolism of glycerol, such as ATP, $NADP^+$, glycerol, dihydroxyacetone, or even protons, may induce the metabolic response to osmotic shock. Alternatively, changes in membrane parameters, such as membranal tension, may serve as the triggering signal. It was recently demonstrated that inhibition of the plasma membrane ATPase of these cells prevents an upshock osmoregulatory response (Oren-Shamir et al., 1989).

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THE PERMEABILITY OF THE PLASMA MEMBRANE OF *DUNALIELLA PARVA* AND *DUNALIELLA ACIDOPHILA*: STRESS- AND ECOPHYSIOLOGICAL SIGNIFICANCE

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The algal genus *Dunaliella*, characterized by the lack of a rigid cell wall, includes two species of extreme, but different stress resistance. The halotolerant *D. parva* is able to grow at salinities up to 5 M NaCl, whereas the acid resistant *D. acidophila* exhibits optimal growth at pH 1.0. Both algae use glycerol as osmoticum, are excluders of the main ions of the medium (Na^+,Cl^-, respectively H^+, SO_4^{2+}) and the mode of osmoregulation is a volume regulation. The maintenance of high internal glycerol levels and the exclusion of Na^+, respectively H^+ requires a low permeability of the plasma membrane (PM). The permeability properties of the PM of both algal species will be surveyed and their ecophysiological significance discussed.

Experimental data demonstrate that in general P_S values of the PM of *Dunaliella* are significantly lower than the corresponding values for plasma membranes of higher plant cells and of other algae. The increased resistance for passive permeation of a variety of hydrophilic and lipophilic non-electrolytes must be due to the special chemical composition of the *Dunaliella* PM. However, Collander plots demonstrate an approximately linear relationship between log P_S and log $K_r/MW^{1.5}$ if solutes are divided into two classes (high and low hydrogen bonding capacity), simimarly as it was observed for other plant cell membranes. The fact that the slope of the Collander line for the PM of *Dunaliella* is similar to those measured from Collander lines for membranes of higher plant cells and other algae indicates that the permeation of solutes through the PM of *Dunaliella* is based on similar principles as observed for other membranes and that the mass selectivity is the same. The physiological benefit of a tight PM for *Dunaliella* (e.g. for the retention of glycerol) will be discussed as well as possible drawbacks (e.g. the requirement of massive CO_2 uptake during photosynthesis).

A low permeability of the PM for H^+ is of special importance for the pH homeostasis of *D. acidophila*. This algae maintains a cytoplasmic

pH of 7.2 at an external pH of 1.0. In this case the membrane potential (E_m) and the surface potential contribute additionally to a low permeation of H^+: Normally plant cells (including *D. parva*) possess a negative E_m in the order of -50 to -200 mV. However, *D. acidophila* is unique because of its positive E_m (between + 40 and + 100 mV, depending upon the energization of the cells). A positive E_m suppresses the passive H^+ influx into the cytoplasm. The positive surface potential, which is the result of the charge distribution of phospholipids at a pH_{ext} of 1.0, amplifies this effect. The effect of the positive E_m on ion fluxes and the internal ion content will be described. Finally it will be shown that the low pH of the medium potentially protects *D. acidophila* cells against the action of some lipophilic solutes (e.g. herbizides, ionophores and inhibitors), which easily enter *D. parva* cells (pH_{ext} 7.6). This is due to the fact that many nitrogen containing compounds, which at neutral pH values are uncharged, are positively charged at pH 1.0. The positive E_m and the positive surface potential suppress the passive uptake of positively charged solutes into *D. acidophila*.

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Models from neutron diffraction of bacteriorhodopsin structure.

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Bacteriorhodopsin (BR) is a retinal binding protein of molecular weight 26000 arranged on a very well ordered two dimensional hexagonal lattice in purple patches on the plasma membrane of *Halobacterium halobium*. It functions as a light-driven proton pump with a photo-cycle length of the order of milliseconds. Since the publication of its structure to 7 Å resolution (1), showing it to be made up of seven transmembrane alpha helices, and the determination of its amino acid sequence (2,3), the challenge of fitting sequence to structure has been met by different experimental approaches, including neutron diffraction. The basis of the neutron diffraction experiments is that neutrons are scattered with significantly different amplitudes by different hydrogen isotopes. The preparation of suitably labelled purple membrane samples is facilitated by the fact that the bacterium lends itself well to *in vivo* deuterium labelling, and also by the extraction and reconstitution procedures that were developped in the past few years for membrane proteins.

A set of neutron diffraction experiments on BR with deuterium labelled amino-acids will be reviewed. There is a logical progression in the complexity of sample preparation and data analysis in these experiments leading to the most recent in which a model is proposed for the helices around the end of the BR molecule close to where the retinal is bound.

- First, by using well -defined culture media, all the amino-acids of a certain type were deuterated in the labelled sample. Of these, valine and phenylalanine were the most useful because of their inhomogeneous distribution in the sequence. Diffraction data from these samples set constraints on the rotation of the sequence helices, placing their hydrophobic sides towards the postulated lipid environment on the outside of the molecule (4).

- Second, the BR molecule was reconstituted and recrystallised from two chymotryptic fragments (2 helices and 5 helices, respectively) one of which was fully deuterated in all its amino acids. The diffraction data placed the 2 helix label in the structure (5, 6), but because the deuteration was too extensive (a considerable part of the label was in the putative extra-membrane loops) information was not obtained on the position or rotation of individual helices.

- Third, the two previous approaches were combined. BR was reconstituted from chymotryptic fragments in which only certain amino acids were labelled. These were chosen to be well inside the transmembrane portion of the helices. The analysis of diffraction data from these samples leads to very strong predictions on the location and rotational setting in the structure of two helices, and constraints for a third (7).

- In a partial structural model of BR, the new results are combined with previous neutron diffraction results on the location of the retinal (8,9), and on the hydration of the membrane (10-12).

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COOPERATIVE STRUCTURAL CHANGES INDUCES BY ELECTRIC FIELDS IN HALOPHILIC PURPLE MEMBRANES

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Structure and function of biological membranes are vitally dependent on the electric membrane polarization; prolonged depolarization of the plasma membrane causes cell death. The natural cross-membrane electric fields affecting the protein/lipid dielectrics are on the order of 100 kV/cm. The inhomogeneous fields originating from ionic groups and adsorbed small ions are of the same order of magnitude; these fields are restricted to the interfacial compartments adjacent to the membrane surface. Externally applied electromagnetic fields which are strong enough to compete with the intrinsic membrane fields, cause structural rearrangements in the proteins and in the lipid bilayer parts (1-6).

For example, electro-optic data of aqueous suspensions of purple membranes indicate that bacteriorhodopsin exhibits conformational flexibility in electric field pulses (1-30 kV/cm, 1 100μs). The electric dichroism shows two kinetically different structural transitions within the protein molecule (1). The electrically induced rearrangements comprise a rapid ($\tau \approx 1\ \mu s$), but concerted, change in the orientation of both retinal and tyrosine and /or tryptophan side chains. These angular changes of position are accompanied by changes in the local protein environment of the chromophores. A slower relaxation mode ($\tau \approx 100\ \mu s$) involves alterations in the microenvironment of aromatic amino acid residues and is accompanied by pK-changes of at least two types of proton binding sites, leading to a sequential uptake and release of protons.

Light scattering data are consistent with the maintenance of the random distribution of the membrane discs within the short duration of the applied electric fields.

The kinetics of the electro-optic signals and the steep dependence of the relaxation amplitudes on the electric field strength suggest a saturable induced-dipole mechanism and a rather large reaction dipole moment of

$$\Delta m = 1.1 \times 10^{-25}\ C\ m \qquad (1)$$

(= 3.3×10^4 debye) per cooperative unit at $E = 1.3 \times 10^5$ V/m (1). The large reaction dipole moment

$$\Delta M = N_A \Delta m = 6.62 \times 10^{-2}\ C\ m/mol \qquad (2)$$

is indicative of appreciable cooperativity in the probably unidirectional transversal displacement of ionic groups on the surfaces of, and within, the bacteriorhodopsin proteins of the membrane lattice.

The numerical value of ΔM thus reflects ionic polarization involving ion pairs of the protein, inclusively the intrinsic Ca/Mg-ions and the ionic atmosphere of the entire membrane disc.

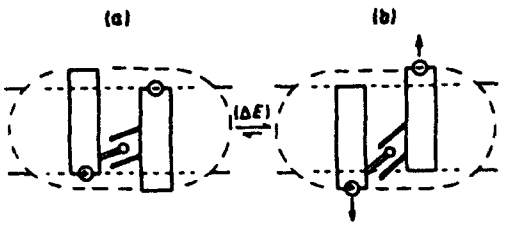

Fig. 1. Principle of the saturable induced-dipole mechanism causing positional changes of side chains in helical membrane proteins. In bacteriorhodosin helical parts with different net charge may move transversal to the membrane plane in opposite directions when the electric membranefield is increased, (a)→ (b). The geometrically limited increase in the distance of the charge centers is equivalent to a saturable induceddipole moment. The transversal displacement of at least one of the two helical parts can thereby cause a concerted rotational shift of the retinal (= 0) and of aromatic amino acid side chains which may sandwich the retinal chromophore.

The data of detergent treated purple membranes suggest that detergent binding interferes with the protein/protein lattice interactions. At low concentrations of Triton X-100 the detergent appears to loosen the crystalline-like bacteriorhodopsin lattice structure. At higher detergent content, however, the protein/protein contacts seem to be strengthened, facilitating long-range interactions. Finally, at very high detergent concentration the protein lattice is dissolved.

Photobleaching disrupts the retinal Schiff's base bond. Thereby the internal fluidity of the protein increases as well as the internal flexibility of the neighbouring unbleached proteins. The bleaching experiments suggest that the protein polarization in external electric fields is independent of whether the retinal is covalently bound or not. Furtheron, the field-induced uptake and the release of protons are not affected by the state of the neighbouring proteins. The proton transfer is directly coupled to the existence of the intact Schiff's base.

Adressing the functional aspect of field-induced structural rearrangements in bacteriorhodopsin there are some similarities between the photocycle and the electro-optic cycle of rotational chromophore displacements and conformational transitions in the protein part. In particular, there is a hyperpolarizing increase in the absolute value of the electric membrane potential difference during light induced proton pumping. This increase in the membrane field is concomitant with a decrease in the proton transport.

The temporal coincidence and the opposite sequence of field-induced pH-changes compared with the light-induced pH-changes suggest a direct functional role of the electric field changes. The increase in the electric membrane field resulting from proton pumping may exert a negative feed-back (reducing proton transport) via an electric field effect directly on the structure of bacteriorhodopsin, switching off pump activity.

A further functional aspect of the bacteriorhodopsin data may be of general fundamental importance. Compared with the rapid induction of the electric field-mediated structural changes (us range), annealing of the changes after the electric impulse is very slow (ms). The field induced conformational transitions therefore exhibit memory properties.

THE TWELFTH EDMOND DE ROTHSCHILD SCHOOL IN MOLECULAR BIOPHYSICS
" MODERN ASPECTS OF HALOPHILISM "

Unusual DNA Double Helix Promoted by Alternating Purine-Pyrimidine Sequences of Adenine;Thymine Pairs

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ABSTRACT

High concentrations of CsF induce extensive and unusual changes in the CD and ^{31}P NMR spectra of poly(dA-dT) and related DNA molecules. The resulting spectral properties are qualitatively distinct from those of the known A-, B- and Z-DNA conformations. These observations prompted us to postulate an existence of the fourth type of double helix which we call X-DNA. This conformer can be induced at physiological conditions in poly(amino^{2}dA-dT). X-DNA appears to display the surface morphology similar to Z-DNA but the arrangement of base pairs like A- or B-DNA. Detailed molecular structure of this new type of double helix is not yet known.

Abstract :

Study of the stability and activity of the elongation factor Tu from *Halobacterium marismortui* at different salt conditions.

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The elongation factor EF-Tu is required for the biosynthetic pathway. When complexed with GTP, it interacts with an aminoacyl-tRNA forming a ternary complexe. This complexe can deliver the aminoacyl t-RNA on the A site of a ribosome, allowing the use of the aminoacid for the elongation of the polypeptide chain. The halophilic elongation factor hEF-Tu from *Halobacterium marismortui* has been purified and characterised(1).

We have studied the stability and the activity of hEF-Tu at different concentrations of salts (ammonium sulphate, sodium or potassium chloride), by the measurement of the exchange with GDP : in its stable state, the protein is associated with GDP. hEF-Tu is only stable at high concentrations of salts, but is generally more active in medium concentration range. The kinetics of denaturation of the protein at various NaCl and KCl concentrations have been measured. A preliminary structural characterisation of the protein in solution has been done by neutron scattering, analytical centrifugation and light scattering.

(1) F. GUINET , R. FRANK, R. LEBERMAN, Eur. J. Biochem., 172, 687, (1988).

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THE GENE FOR THE EF-Tu FROM HALOBACTERIUM MARISMORTUI

We sequenced a portion of the Halobacterium marismortui genome inserted in an EMBL3 recombinant phage bank screened with a labeled mixture of oligonucleotides corresponding to the N-terminal extremity of the EF-Tu.

This DNA region contains an open reading frame of 421 aminoacid residues. The 5'-end of the gene exactly corresponds to the first 23 N-terminal aminoacids of the protein. The apparent relative mass of the purified protein calculated by SDS-page electrophoresis is about 60 000 ; in contrast that of the predicted peptide is about 46 000. Thus, we confirmed the primary structure of the 3'-end of the gene and the presence of the stop codon by directly sequencing this DNA region, i.e. without prior insertion of it in a recombinant phage bank.

A labeled probe corresponding to this gene hybridizes to an RNA band of about 1 700 nucleotides. This size fits well with a 421 long aminoacid peptide. The comparison of the predicted peptide with the 428 aminoacid long EF-Tu from archaebacterium Methanococcus vannielii shows a high degree of homology. Consensus regions characteristic of EF-Tus are also present in the gene from H. marismortui. The percentage of acidic amino-acids is higher than in non halophilic EF-Tus. The comparison of the predicted secondary structure with that of the E. Coli EF-Tu shows an overall conservation of the N-terminal GTP-binding region.

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