Mini-Review

Correspondence

Jörg Soppa

soppa@em.uni-frankfurt.de

From genomes to function: haloarchaea as model organisms

Jörg Soppa

Goethe-University, Biocentre, Institute for Microbiology, D-60439 Frankfurt, Germany

Haloarchaea are adapted to high-salt environments and accumulate equally high salt concentrations in the cytoplasm. The genomes of representatives of six haloarchaeal genera have been fully or partially sequenced, allowing the analysis of haloarchaeal properties *in silico*. Transcriptome and proteome analyses have been established for *Halobacterium salinarum* and *Haloferax volcanii*. Genetic systems are available including methods that allow the fast in-frame deletion or modification of chromosomal genes. The high-efficiency transformation system of *Hf. volcanii* allows the isolation of genes essential for a biological process by complementation of loss-of-function mutants. For the analysis of haloarchaeal biology many molecular genetic, biochemical, structural and cell biological methods have been adapted to application at high salt concentrations. Recently it has become clear that several different mechanisms allow the adaptation of proteins to the high salt concentration of the cytoplasm. Taken together, the wealth of techniques available make haloarchaea excellent archaeal model species.

Introduction

The proposal for a third domain of life, the archaea, was put forward in 1978, but the investigation of halophilic organisms that were subsequently recognized to be archaea preceded this event by far. *Halobacterium halobium*, which was later renamed *Halobacterium salinarum*, was isolated from salted fish about 80 years ago. *Haloferax volcanii*, another commonly studied species, was isolated under the name *Halobacterium volcanii* from the Dead Sea about 30 years ago. Much interest focused on *Hb. salinarum* after the discovery in 1971 of bacteriorhodopsin, a light-driven proton pump that together with the subsequently identified additional haloarchaeal retinal proteins has been intensely studied ever since. Haloarchaea are adapted to high salt environments, i.e. they are not only osmotolerant but typically require molar salt concentrations for cellular integrity and growth. This is explained by the mechanism of osmoadaptation that involves at least equimolar salt concentration in the cytoplasm compared to their environment. The question how the cellular machinery, e.g. proteins and nucleic acids and their specific interactions with one another, has been adapted to conditions that would preclude the function of homologues from mesohalic organisms (non-halophiles) has raised much interest and is still far from being understood.

The continuing interest in these and other questions has driven the development of biochemical, genetic, genomic and cell biological tools, and in many cases haloarchaea have been the forerunners in the archaeal world. Some haloarchaeal species are therefore excellent model systems to solve fundamental biological questions. This review will survey

the state of the art of technologies including genomics, functional genomics and genetics, and discuss recent results including mechanisms for haloarchaeal osmoadaptation.

Genomes and *in silico* genomics

The genome of *Halobacterium* sp. NRC1 was published about five years ago (Ng *et al.*, 2000; <http://zdna2.umbi.umd.edu/>), and shortly thereafter the genome of the closely related type strain *Halobacterium salinarum* was finished (<http://www.halolex.mpg.de>). Both strains contain several replicons; the main chromosome is virtually identical, while surprisingly the smaller replicons were reported to be totally different. In both strains essential genes are located on the smaller replicons and thus *Halobacterium* can be regarded as harbouring more than one chromosome. Comparison to other haloarchaeal genomes became possible recently because the genome sequence of *Haloarcula marismortui* was published (Baliga *et al.*, 2004b, <http://halo.systemsbiology.net>) and the genome sequence of *Haloferax volcanii* is far advanced and the data are publicly available (<http://www.tigr.org/tdb/mdb/mdbinprogress.html>). In addition, sequencing of 1000 random genomic clones of *Ha. marismortui* and three additional species was reported. The sequences cover about 16 % of the respective genomes and allow a first glimpse into the genomes of representatives of three additional haloarchaeal genera, i.e. *Halobaculum gomorrense*, *Natrialba asiatica* and the psychrophilic *Halorubrum lacusprofundi* (Goo *et al.*, 2004). The following features are shared by most or all of these species and thus seem to be typical for haloarchaea in general. They have a major chromosome with a G+C content well above 60 %. The three species with a fully sequenced genome and *Hf. volcanii* have several additional

replicons with a slightly lower G+C content, and partial genome sequences indicate that this might be true also for the three other species. Some of the smaller replicons of *Halobacterium*, *Haloarcula* and *Haloferax* contain essential genes and thus the occurrence of additional chromosomes might be widespread in haloarchaea. The proteins of all species have a very low isoelectric point as a result of the adaptation to the high salt concentration of the cytoplasm (see below). The genomes of all species harbour insertion elements of different families, although the total number seems to vary and to be considerably smaller for example in *Natrialba* than in *Halobacterium*. In addition, the transposition activity appears to be higher in some haloarchaea, e.g. *Halobacterium*, than in others, e.g. *Haloferax*. It seems to be a general haloarchaeal feature that some genes are present in multiple copies that are single-copy genes in many other archaea. Notable examples are the basal transcription factors TBP and TFB, the replication protein Cdc6, and FtsZ, which is involved in septum formation. In all genomes many genes for transducers have been found, indicating that halophiles can sense and react to a variety of environmental signals, which is in accordance with their high metabolic versatility. In addition, many putative regulatory proteins have been predicted.

As in other microbial species, one problem in making optimal use of the genome sequence information is that a considerable fraction of open reading frames cannot be related to a functional annotation due to the lack of primary sequence similarity with known genes. One possibility to test whether ORFs encoding 'hypothetical proteins' are real genes is to analyse whether they are transcribed. A transcription analysis of 39 ORFs from *Halobacterium* sp. NRC-1 revealed that 30 of them are transcribed at mid-exponential growth phase in complex medium. Thus at least these are real genes, and the remaining nine might well be expressed under different conditions (Shmueli *et al.*, 2004).

An interesting approach tries to raise the fraction of annotated proteins by making systematic use of predictions about possible interactions of putative proteins with proteins of known function. The predictions are based on the location of their genes in operons together with known genes, on synteny, on domain fusions with domains of known function in other species, or on experimentally proven interactions of orthologues in other organisms. In addition, *de novo* structural predictions of small proteins and protein domains and search for tertiary structure similarity to a protein of known function is applied (Bonneau *et al.*, 2004). While this approach is likely to lead to considerable over-prediction, due for example to interactions that occur only in some species, false positives in the yeast two-hybrid system or tertiary structure similarities of analogous proteins, it creates new functional predictions that can be tested experimentally. For experimental verification genetic and functional genomic approaches are of specific importance. Fortunately, many genetic and functional genomic techniques have recently been established for haloarchaea (see below).

A striking example of the importance of experimental verification of bioinformatic predictions is the number of replication origins in the chromosome of *Hb. salinarum*. Bioinformatic genome analyses using different algorithms including the 'Z-curve method' indicated that the main chromosome has two origins of replication (Zhang & Zhang, 2005, 2003). Until very recently it was thought that all prokaryotic circular chromosomes have only one replication origin. However, it was recently shown experimentally that the archaeon *Sulfolobus acidocaldarius* has three origins of replication (Lundgren *et al.*, 2004). This result was in accordance with a prediction of the Z-curve method, and therefore the prediction of two origins for *Hb. salinarum* seemed plausible. Furthermore, both predicted origins were bordered by an allele of the *cdc6* gene, and the proximity of *cdc6* and the replication origin had previously been found in several archaeal species. However, as the two regions were cloned into an origin-lacking vector and tested for autonomous replication *in vivo*, only one of them turned out to be an active replication origin (Berquist & DasSarma, 2003), underscoring the necessity of functional tests for bioinformatic predictions, *in vitro* or at best *in vivo*.

Genetics and functional genomics

Haloarchaea were the first and for many years the only archaea that could be transformed, allowing the development of many molecular genetic tools, e.g. vectors, selection systems or reporter genes. With slight modifications, the transformation procedure that was established about 15 years ago is still widely in use and to my knowledge is successful with every species tested. Transformation efficiency depends on the restriction-modification system present and ranges from 10^2 in *Ha. marismortui* to more than 10^6 in *Hf. volcanii*. Developments in recent years have focused on methods to change the chromosome at will, i.e. to make in-frame deletions of genes or to exchange chromosomal genes with *in vitro*-mutated variants. Early methods were labour intensive and time consuming, but now a so-called pop-in-pop-out method has been established for both *Hf. volcanii* and *Hb. salinarum* (Bitan-Banin *et al.*, 2003; Peck *et al.*, 2000; Wang *et al.*, 2004). For *Hf. volcanii* the system has been further developed and now four different selection principles in synthetic and complex media are available (Allers *et al.*, 2004). The method makes use of designed host strains and plasmids and includes positive as well as negative selection for the introduction of a recombinant DNA fragment into the chromosome and the deletion of the native copy. Mutants can now readily be constructed in only a few weeks, a major breakthrough especially for the experimental verification of proposed functions. A very interesting additional application of designed mutants with an inactivated essential function has been reported recently. They can be used to identify novel non-homologous proteins from other species that are able to complement the missing reaction, and that were hitherto annotated as hypothetical proteins (Levin *et al.*, 2004).

Not only designed mutants, but also mutants generated by random mutagenesis can be used for the identification of essential elements of a biological process. For most archaeal species this approach is not possible, but the transformation frequency of *Hf. volcanii* is high enough to allow the use of genomic libraries for the complementation of loss-of-function mutants. Complementation of nitrate-respiration-deficient mutants of *Hf. volcanii* has led to the identification of a variety of essential genes for nitrate-respirative growth, including transporters, metabolic enzymes and regulatory proteins (Wanner & Soppa, 1999, 2002 and unpublished data).

The use of two different haloarchaea allows a biological process to be transferred from one species to another, thereby guaranteeing that all essential elements have been identified. As an example, the whole gas vesicle biosynthesis pathway, and subsequently specific steps, were transferred from *H. salinarum* to *Hf. volcanii*, which is naturally devoid of gas vacuole genes, and recent reports include the characterization of the *in vivo* role of two regulatory proteins (Zimmermann & Pfeifer, 2003) and characterization of translational initiation at *gvp* transcripts (Sartorius-Neef & Pfeifer, 2004).

Classical genetic approaches, however advanced they may be, have the intrinsic disadvantage that all genes have to be studied serially, while transcriptome analyses allow parallel investigation of all genes. DNA microarrays have been developed for *Hf. volcanii* as well as for *Hb. salinarum* and have been applied to characterize the regulation of carbon source dependent metabolism (Zaigler *et al.*, 2003), anaerobic respiration (Müller & DasSarma, 2005) and the stress response to UV irradiation (Baliga *et al.*, 2004a; McCready *et al.*, 2005), and to study the role of a transcriptional regulator, Bat, by comparison of wild-type and mutants (Baliga *et al.*, 2002); a variety of further studies are under way. In my opinion it seems to be underestimated that the parameters with the highest influence on data variance are the physiological state of the cells and the experimental setup (provided that the method is well established). For example, the two studies that aimed at a global characterization of the repair of UV damage applied very different experimental strategies. While one study used early exponential phase cells that were kept in the same medium and at the same temperature (McCready *et al.*, 2005), the other study used cells in the transition to stationary phase, and temperature, oxygen availability, medium composition and visible light intensity were changed in the course of the experiment in addition to UV irradiation (Baliga *et al.*, 2002). Not surprisingly, the results were very different. Even if occasionally a study might be questionable, it should be stressed that whenever the physiological state of the culture and the experimental setup are tightly controlled and (in the ideal case) only one parameter is varied, transcriptome studies are extremely informative, allowing for example the analysis of pathway regulation and the elucidation of regulatory hierarchies; and last but not least, they regularly produce unanticipated results that lead to new testable hypotheses.

However, some processes can only be investigated at the protein level, e.g. post-translational modification or processing, intracellular localization of proteins in subproteomes, or persistence of proteins after message degradation. These can be addressed by proteome analysis, and several examples for *Hf. volcanii* and *Hb. salinarum* have been reported (e.g. Karadzic & Maupin-Furlow, 2005; Klein *et al.*, 2005; Tebbe *et al.*, 2005). It was shown that proteome analysis can also be used to improve genome annotation in halophiles. Confident translational start site prediction from the genome sequence alone had been hampered by the very low occurrence of stop codons, resulting in ORFs that are longer than the actual genes. Mass spectroscopic analysis of the cytoplasmic proteome considerably improved gene annotation (Tebbe *et al.*, 2005). Of specific interest is the investigation of the membrane proteome of *Hb. salinarum*. It was determined why membrane proteins cannot be resolved by 2D electrophoresis, the classical proteome analysis method, and a new technique was developed. This allowed the identification of 114 integral membrane proteins, a breakthrough for membrane protein research not confined to archaeal biology (Klein *et al.*, 2005).

‘Halophilic adaptation’ of methodologies

As haloarchaea not only grow in high-salt conditions but also have a high intracellular salt concentration, many standard protocols cannot be used as such, but they must be adapted to these conditions. However, the problems are often smaller than anticipated, and many solutions are already established. At first sight protein isolation and biochemistry seem to be difficult, but the problem is mostly imaginary. While the favourite child of ‘mesohalic’ biochemists, ion-exchange chromatography, in many cases cannot be used, all other principles are readily available, and numerous proteins have been isolated and characterized. Crystallization and X-ray diffraction have been used to generate high-resolution structures of soluble proteins, membrane proteins in several functional states, membrane protein complexes and the large subunit of the ribosome (selected recent examples: Zeth *et al.*, 2004; Schobert *et al.*, 2003; Gordeliy *et al.*, 2002; Tu *et al.*, 2005).

In other cases, the addition of salt and optimization of conditions is not enough, but alternative solutions have to be found. One possibility is to find halophilic proteins that can replace the usual mesohalic counterparts. This has been done for resistance genes, reporter genes, and genes allowing conditional selection. Recent examples are the usage of the BgaH reporter enzyme for the *in vivo* analysis of constitutive and regulated promoters, and the development of methods for gene inactivation (Gregor & Pfeifer, 2005; Allers *et al.*, 2004). A second possibility is to find a mesohalic protein that is salt-tolerant, and a recent example is the application of a bacterial cellulose-binding domain for affinity purification of fusion proteins and protein complexes from haloarchaea (Irihimovitch *et al.*, 2003). A third possibility is to modify a mesohalic protein until it can withstand high salt conditions; a recent example is the application of a modified

GFP for the *in vivo* analysis of proteasome function (Reuter & Maupin-Furlow, 2004). A fourth possibility is to use an alternative method, if techniques are intrinsically salt sensitive. Examples are the use of co-affinity isolation instead of co-immunoprecipitation (Zimmermann & Pfeifer, 2003) or a pull-down approach instead of an electrophoretic mobility shift assay to analyse DNA protein interactions (Soppa & Link, 1997).

For some applications, regular mesohalic assays can be used after dilution to reduce the salt concentration, e.g. for the quantification of metabolites (Zaigler *et al.*, 2003). It should be noted that very fast cell lysis can be achieved by simple dilution and thus haloarchaea are ideally suited for the determination of biologically unstable metabolites like ATP or the isolation of intact RNA.

Halophilic adaptation of organisms

As already mentioned, all halophilic archaea studied balance the high osmolarity of their environment by having an at least equimolar intracellular salt concentration, KCl instead of NaCl in well-energized cells. It was recognized long ago that typical haloarchaeal proteins differ from mesohalic proteins by having a high fraction of acidic residues and a reduced fraction of basic residues. The genome sequences have corroborated that result and shown that a theoretical 2D gel of a haloarchaeon differs considerably from that of other organisms (Tebbe *et al.*, 2005). As had been proposed before, structure determination of some soluble haloarchaeal proteins showed that they have a high concentration of negative charges on the surface of the folded protein. Earlier it had been proposed that this leads to the binding of a network of hydrated cations, but a few recent reports have modified that picture and, in addition, have shown that the mode of haloadaptation can be different for individual proteins. The malate dehydrogenase of *Ha. marismortui* was found to have strong binding sites for some cations as well as anions, and loosely bound many more cations than mesohalic enzymes in the natural solvent (Ebel *et al.*, 2002). This might turn out to be true for typical haloarchaeal proteins. Consistent with the absence of the usual high excess of acidic residues, the dihydrofolate reductase of *Hf. volcanii* was proposed to be mainly adapted to high salt concentrations

by replacing large hydrophobic with small less hydrophobic residues, thus requiring a higher salt concentration for folding than the *Escherichia coli* enzyme (Wright *et al.*, 2002). The halophilic nature of a ferredoxin from *Hb. salinarum* was found to rely on an extra domain, not present in mesohalic orthologues, that comprises 1/3 of acidic residues (Marg *et al.*, 2005). This mechanism might allow lateral integration of genes from mesohalic species. While the haloadaptation of proteins has been characterized in detail in several cases, similar studies have not yet been performed for the adaptation of interactions of biomolecules, protein–protein or protein–nucleic acid, to high salt concentrations.

Haloarchaea do not live at constant salt concentrations, but in many natural settings are exposed to changing salinities due to evaporation or rain, and thus also the intracellular conditions change considerably. While extreme halophiles like *Hb. salinarum* require at least about 2 M salt, moderate halophiles like *Hf. volcanii* have a growth optimum slightly higher than 2 M but can grow from about 1 M to saturation. Thus haloarchaea are excellent models to study osmoadaptation over an extreme range of salt concentrations. Several recent studies have identified genes that are differentially expressed at different salt concentrations (Bidle, 2003; Choi *et al.*, 2005; Jäger *et al.*, 2002) or detected *de novo* synthesis of dimeric lipids upon an osmotic downshift (Lopalco *et al.*, 2004), but the opportunities are clearly underexploited.

Hot news from haloarchaea

It is not surprising that the developments described above have enabled tremendous progress in various areas of haloarchaeal biology, including chromosome maintenance, transcriptional regulation, protein export and degradation, gas vesicle synthesis, or motility and sensing. Some results have been of great significance for other species, e.g. the discovery that the twin-arginine translocation pathway not only transports a few redox proteins containing prosthetic groups, but is a widespread general transport mechanism for folded proteins. Additional breakthroughs have been high-resolution structures of the large subunit of the ribosome or of membrane proteins, including a receptor transducer complex that will have a great impact on the understanding of bacterial chemotaxis. Further news is the

Table 1. Commonly used haloarchaeal species and selected features

Species	Genome sequence	Transcriptome/ proteome analysis	Genetic system/ pop-in-pop-out	Selected research areas
<i>Haloarcula marismortui</i>	Published	–/–	+/–	Ribosome structure, enzymes
<i>Halobacterium salinarum</i> and NRC-1	Published	+/+ membrane proteome	+/+	Retinal proteins, motility, sensing, gas vesicles, cell cycle, DNA repair, anaerobic metabolism
<i>Haloferax volcanii</i>	Complete	+/+	+/+	Transcriptional and metabolic regulation, protein transport and degradation
<i>Natronomonas pharaonis</i>	Published	–/–	–/–	Sensory rhodopsin II + transducer

isolation of hitherto uncultivated species including the fascinating stamp-like square archaeon and the discovery that haloarchaea are much more widespread than anticipated. These results have been summarized in detail elsewhere (Soppa, 2005).

Coda

During recent years the genomes of several haloarchaeal species have been fully or partially sequenced. Functional genomic analyses as well as sophisticated methods to manipulate the chromosome have been established for *Hb. salinarum* and *Hf. volcanii*. In addition, many methods have been adapted for application under high salt conditions. Therefore, today several haloarchaeal species are excellent model organisms that are used for the investigation of many biological questions. Commonly used species and selected features are summarized in Table 1.

References

- Allers, T., Ngo, H. P., Mevarech, M. & Lloyd, R. G. (2004). Development of additional selectable markers for the halophilic archaeon *Haloferax volcanii* based on the *leuB* and *trpA* genes. *Appl Environ Microbiol* **70**, 943–953.
- Baliga, N. S., Pan, M., Goo, Y. A. & 7 other authors (2002). Coordinate regulation of energy transduction modules in *Halobacterium* sp. analyzed by a global systems approach. *Proc Natl Acad Sci U S A* **99**, 14913–14918.
- Baliga, N. S., Bjork, S. J., Bonneau, R., Pan, M., Iloanusi, C., Kottemann, M. C., Hood, L. & DiRuggiero, J. (2004a). Systems level insights into the stress response to UV radiation in the halophilic archaeon *Halobacterium* NRC-1. *Genome Res* **14**, 1025–1035.
- Baliga, N. S., Bonneau, R., Facciotti, M. T. & 12 other authors (2004b). Genome sequence of *Haloarcula marismortui*: a halophilic archaeon from the Dead Sea. *Genome Res* **14**, 2221–2234.
- Berquist, B. R. & DasSarma, S. (2003). An archaeal chromosomal autonomously replicating sequence element from an extreme halophile, *Halobacterium* sp. strain NRC-1. *J Bacteriol* **185**, 5959–5966.
- Bidle, K. A. (2003). Differential expression of genes influenced by changing salinity using RNA arbitrarily primed PCR in the archaeal halophile *Haloferax volcanii*. *Extremophiles* **7**, 1–7.
- Bitan-Banin, G., Ortenberg, R. & Mevarech, M. (2003). Development of a gene knockout system for the halophilic archaeon *Haloferax volcanii* by use of the *pyrE* gene. *J Bacteriol* **185**, 772–778.
- Bonneau, R., Baliga, N. S., Deutsch, E. W., Shannon, P. & Hood, L. (2004). Comprehensive de novo structure prediction in a systems-biology context for the archaea *Halobacterium* sp. NRC-1. *Genome Biol* **5**, R52.
- Choi, J., Joo, W. A., Park, S. J., Lee, S. H. & Kim, C. W. (2005). An efficient proteomics based strategy for the functional characterization of a novel halophilic enzyme from *Halobacterium salinarum*. *Proteomics* **5**, 907–917.
- Ebel, C., Costenaro, L., Pascu, M., Faou, P., Kernel, B., Proust-De Martin, F. & Zaccari, G. (2002). Solvent interactions of halophilic malate dehydrogenase. *Biochemistry* **41**, 13234–13244.
- Goo, Y. A., Roach, J., Glusman, G. & 7 other authors (2004). Low-pass sequencing for microbial comparative genomics. *BMC Genomics* **5**, 3.
- Gordeliy, V. I., Labahn, J., Moukhametzianov, R. & 8 other authors (2002). Molecular basis of transmembrane signalling by sensory rhodopsin II-transducer complex. *Nature* **419**, 484–487.
- Gregor, D. & Pfeifer, F. (2005). *In vivo* analyses of constitutive and regulated promoters in halophilic archaea. *Microbiology* **151**, 25–33.
- Irihimovitch, V., Ring, G., Elkayam, T., Konrad, Z. & Eichler, J. (2003). Isolation of fusion proteins containing SecY and SecE, components of the protein translocation complex from the halophilic archaeon *Haloferax volcanii*. *Extremophiles* **7**, 71–77.
- Jäger, A., Samorski, R., Pfeifer, F. & Klug, G. (2002). Individual *gvp* transcript segments in *Haloferax mediterranei* exhibit varying half-lives, which are differentially affected by salt concentration and growth phase. *Nucleic Acids Res* **30**, 5436–5443.
- Karadzic, I. M. & Maupin-Furrow, J. A. (2005). Improvement of two-dimensional gel electrophoresis proteome maps of the haloarchaeon *Haloferax volcanii*. *Proteomics* **5**, 354–359.
- Klein, C., Garcia-Rizo, C., Bisle, B., Scheffer, B., Zischka, H., Pfeiffer, F., Siedler, F. & Oesterhelt, D. (2005). The membrane proteome of *Halobacterium salinarum*. *Proteomics* **5**, 180–197.
- Levin, I., Giladi, M., Altman-Price, N., Ortenberg, R. & Mevarech, M. (2004). An alternative pathway for reduced folate biosynthesis in bacteria and halophilic archaea. *Mol Microbiol* **54**, 1307–1318.
- Lopalco, P., Lobasso, S., Babudri, F. & Corcelli, A. (2004). Osmotic shock stimulates *de novo* synthesis of two cardiolipins in an extreme halophilic archaeon. *J Lipid Res* **45**, 194–201.
- Lundgren, M., Andersson, A., Chen, L., Nilsson, P. & Bernander, R. (2004). Three replication origins in *Sulfolobus* species: synchronous initiation of chromosome replication and asynchronous termination. *Proc Natl Acad Sci U S A* **101**, 7046–7051.
- Marg, B. L., Schweimer, K., Sticht, H. & Oesterhelt, D. (2005). A two-alpha-helix extra domain mediates the halophilic character of a plant-type ferredoxin from halophilic archaea. *Biochemistry* **44**, 29–39.
- McCready, S., Müller, J. A., Boubriak, I., Berquist, B. R., Ng, W. L. & DasSarma, S. (2005). UV irradiation induces homologous recombination genes in the model archaeon, *Halobacterium* sp. NRC-1. *Saline Systems* **1**, 3.
- Müller, J. A. & DasSarma, S. (2005). Genomic analysis of anaerobic respiration in the archaeon *Halobacterium* sp. strain NRC-1: dimethyl sulfoxide and trimethylamine N-oxide as terminal electron acceptors. *J Bacteriol* **187**, 1659–1667.
- Ng, W. V., Kennedy, S. P., Mahairas, G. G. & 40 other authors (2000). Genome sequence of *Halobacterium* species NRC-1. *Proc Natl Acad Sci U S A* **97**, 12176–12181.
- Peck, R. F., DasSarma, S. & Krebs, M. P. (2000). Homologous gene knockout in the archaeon *Halobacterium salinarum* with *ura3* as a counterselectable marker. *Mol Microbiol* **35**, 667–676.
- Reuter, C. J. & Maupin-Furrow, J. A. (2004). Analysis of proteasome-dependent proteolysis in *Haloferax volcanii* cells, using short-lived green fluorescent proteins. *Appl Environ Microbiol* **70**, 7530–7538.
- Sartorius-Neef, S. & Pfeifer, F. (2004). *In vivo* studies on putative Shine-Dalgarno sequences of the halophilic archaeon *Halobacterium salinarum*. *Mol Microbiol* **51**, 579–588.
- Schobert, B., Brown, L. S. & Lanyi, J. K. (2003). Crystallographic structures of the M and N intermediates of bacteriorhodopsin: assembly of a hydrogen-bonded chain of water molecules between Asp-96 and the retinal Schiff base. *J Mol Biol* **330**, 553–570.
- Shmueli, H., Dinitz, E., Dahan, I., Eichler, J., Fischer, D. & Shaanan, B. (2004). Poorly conserved ORFs in the genome of the archaea *Halobacterium* sp. NRC-1 correspond to expressed proteins. *Bioinformatics* **20**, 1248–1253.
- Soppa, J. (2005). From replication to cultivation: hot news from haloarchaea. *Curr Opin Microbiol* **8**, 737–744.

- Soppa, J. & Link, T. A. (1997).** The TATA-box-binding protein (TBP) of *Halobacterium salinarum*. Cloning of the *tbp* gene, heterologous production of TBP and folding of TBP into a native conformation. *Eur J Biochem* **249**, 318–324.
- Tebbe, A., Klein, C., Bisle, B. & 7 other authors (2005).** Analysis of the cytosolic proteome of *Halobacterium salinarum* and its implication for genome annotation. *Proteomics* **5**, 168–179.
- Tu, D., Blaha, G., Moore, P. B. & Steitz, T. A. (2005).** Structures of MLSBK antibiotics bound to mutated large ribosomal subunits provide a structural explanation for resistance. *Cell* **121**, 257–270.
- Wang, G., Kennedy, S. P., Fasiludeen, S., Rensing, C. & DasSarma, S. (2004).** Arsenic resistance in *Halobacterium* sp. strain NRC-1 examined by using an improved gene knockout system. *J Bacteriol* **186**, 3187–3194.
- Wanner, C. & Soppa, J. (1999).** Genetic identification of three ABC transporters as essential elements for nitrate respiration in *Haloferax volcanii*. *Genetics* **152**, 1417–1428.
- Wanner, C. & Soppa, J. (2002).** Functional role for a 2-oxo acid dehydrogenase in the halophilic archaeon *Haloferax volcanii*. *J Bacteriol* **184**, 3114–3121.
- Wright, D. B., Banks, D. D., Lohman, J. R., Hilsenbeck, J. L. & Gloss, L. M. (2002).** The effect of salts on the activity and stability of *Escherichia coli* and *Haloferax volcanii* dihydrofolate reductases. *J Mol Biol* **323**, 327–344.
- Zaigler, A., Schuster, S. C. & Soppa, J. (2003).** Construction and usage of a onefold-coverage shotgun DNA microarray to characterize the metabolism of the archaeon *Haloferax volcanii*. *Mol Microbiol* **48**, 1089–1105.
- Zeth, K., Offermann, S., Essen, L. O. & Oesterhelt, D. (2004).** Iron-oxo clusters biomineralizing on protein surfaces: structural analysis of *Halobacterium salinarum* DpsA in its low- and high-iron states. *Proc Natl Acad Sci U S A* **101**, 13780–13785.
- Zhang, R. & Zhang, C. T. (2003).** Multiple replication origins of the archaeon *Halobacterium* species NRC-1. *Biochem Biophys Res Commun* **302**, 728–734.
- Zhang, R. & Zhang, C. T. (2005).** Identification of replication origins in archaeal genomes based on the Z-curve method. *Archaea* **1**, 335–346.
- Zimmermann, P. & Pfeifer, F. (2003).** Regulation of the expression of gas vesicle genes in *Haloferax mediterranei*: interaction of the two regulatory proteins GvpD and GvpE. *Mol Microbiol* **49**, 783–794.