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ORIGINAL PAPER

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Differential expression of genes influenced by changing salinity using RNA arbitrarily primed PCR in the archaeal halophile *Haloferax volcanii*

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Abstract Extreme halophiles belonging to the domain Archaea require a minimum of ~10% NaCl for growth. Many of these obligate halophiles will continue to grow even as NaCl concentrations approach saturation. The haloarchaeon *Haloferax volcanii* is a model organism in which to study the effects of changes in medium salinity on gene expression, as this organism grows over a wide range of NaCl concentrations, between 12% and 23%, with little effect on growth rate. An RNA arbitrarily primed PCR (RAP-PCR) approach has been applied to identify those genes that are differentially expressed in response to changing salinity. Differences in gene expression can be detected using this methodology, as each sample generates its own unique RNA fingerprint for each growth condition examined. RNA was prepared from *H. volcanii* cultures grown with two different NaCl concentrations in the medium, RAP-PCR was performed, and seven differentially expressed transcripts were identified. These fragments were cloned, sequenced, and subjected to transcript analysis to confirm their regulation. One of the sequences identified in this study displays homology to the eukaryotic Ser/Thr protein kinase Ire1p, a sensor of protein unfolding in yeast and mammalian cells. Evidence for serine phosphorylation in *H. volcanii* is also presented.

Keywords Gene expression · *Haloferax volcanii* · Halophile · RAP-PCR · Sensor kinase

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Introduction

Haloferax volcanii is an extreme halophile belonging to the domain Archaea. *H. volcanii* can be used as a model microorganism for examining the adaptations necessary for dealing with changes in salinity due to its ability to grow over a wide range of NaCl concentrations, from ~12% to 23%, while maintaining a relatively constant growth rate (Mullakhanbhai and Larsen 1975). The study of haloarchaeal adaptations to hypersaline environments has mainly been limited to examination of protein modifications and function [reviewed in Dennis and Shimmin (1997) and Madern et al. (2000)] and the physiological response of the cell to its high-salt environment (Christian and Waltho 1962; Lanyi 1974, 1984; Stoeckenius and Bogomolni 1982; Meury and Kohiyama 1989). There is little known about the molecular and genetic adaptations used by the extreme halophiles for existence in hypersaline environments. Prior studies have identified differentially expressed proteins (Daniels et al. 1984; Mojica et al. 1997) and transcriptionally active regions within the genome (Ferrer et al. 1996) that are responsive to changes in medium salinity in *H. volcanii*. In these studies *H. volcanii* was exposed to several different salinities, ranging from the lower to upper limits for the organism, and examined for a global response to osmotic shifts. It was found that different sets of proteins are expressed dependent on medium salinity (Daniels et al. 1984; Mojica et al. 1997), and that differential expression of transcripts can be identified in situations of both high- and low-salt induction (Ferrer et al. 1996). While little molecular information was revealed in these studies (i.e., no DNA sequence information was obtained from the activated transcripts), they clearly indicated that there are specific genes, thus far unidentified, involved in hypersaline adaptation in the extreme halophiles, which are awaiting detailed investigation.

In this report, a putative sensor kinase and six other genes regulated in response to a shift in salinity have

been identified using the technique of RNA, arbitrarily primed PCR (RAP-PCR). This powerful method is a useful tool for examining differential gene expression in prokaryotes, since accurate identification of differentially expressed transcripts is accomplished using random 10-mer oligonucleotides to create first and second strand cDNA from differing RNA populations. A unique fingerprint is created for each sample and primer used, thereby allowing easy comparison between the strains or conditions examined. Recently, this technique has been used in the deep-sea bacterium *Photobacterium profundum* SS9 to identify genes under the control of the global regulator ToxR (Bidle and Bartlett 2001). This method has also been successfully applied to several other bacterial systems to examine stress response (Wong and McClelland 1994; Benson et al. 2000), in situ growth conditions (Fleming et al. 1998), and response to host infection (Chakrabortty et al. 2000). This is the first report of the use of RAP-PCR in an archaeon.

Materials and methods

Bacterial strains and culture conditions

H. volcanii strain WFD11 (DSM 5716) was generously provided by Dr. H.J. Schreier. Cultures were grown aerobically with vigorous shaking at 42°C in a medium containing 12% (optimal) or 20% (high) NaCl (2.1 or 3.5 M), 45 g $MgCl_2{\cdot}6H_2O$, 10 g $MgSO_4{\cdot}7H_2O$, 10 g KCl, 1.34 ml 10% $CaCl_2{\cdot}2\ H_2O$, 3 g yeast extract, and 5 g tryptone. Inclusive of magnesium, calcium, and potassium, which remained constant regardless of NaCl concentrations used, these culture conditions represent a total medium salinity of either 18.5% or 26.5%. However, for the purpose of these studies, only the effects of changing NaCl concentrations were examined. *Escherichia coli* strains used for plasmid maintenance were grown in liquid or solid LB medium at 37°C. When necessary, antibiotics and chromogenic substrates were used in the following concentrations: kanamycin (50 μg/ml); X-gal (40 μg/ml).

RNA preparations

Mid-exponential phase cultures of *H. volcanii* were harvested and RNA was extracted using RNAzol B (Tel-Test; Friendswood, TX, USA), a guanidinium thiocyanate/phenol-based reagent, according to the manufacturer's instructions. RNA was quantified using a UV spectrophotometer and its quality was assessed on an agarose gel prior to the experiments.

RNA arbitrarily primed PCR (RAP-PCR)

RAP-PCR was performed essentially as in Fleming et al. (1998) and Bidle and Bartlett (2001). Arbitrary 10-mer primer kits with G + C contents of 70% (GEN4–70) and 80% (GEN4–80) were obtained from Genosys Biotechnologies (The Woodlands, TX, USA). First strand cDNA synthesis was performed using 200 ng RNA heat denatured for 10 min at 65°C in 20 μl reactions containing the following: 200 μM each dNTP, 5 mM dithiothreitol, 50 U MMLV-RT (Ambion), 1× RT reaction buffer, and 0.4 μM of an arbitrary 10-mer primer. The first strand reaction was performed in an MJ Research mini-cycler (PTC-150) as follows: touchdown from 50° to 30°C in 45-s increments, 37°C for 1 h. Second strand synthesis was performed in 30 μl reactions containing the following: 3 μl of first strand reaction, 0.3 U Taq polymerase (BRL), 20 μM each dNTP, 6% dimethyl sulfoxide, 2 μM each primer (primer 1 is from first strand synthesis and primer 2 is a second arbitrary primer), 2.5 μCi ^{32}P α-dCTP, 1× PCR buffer, and 1.5 mM $MgCl_2$. Cycles for RAP-PCR were: 94°C for 30 s, 40°C for 2 min, and 72°C for 1 min for 40 cycles, with a 10 min 72°C extension on the final cycle. All products were stored at –20°C for up to 1 day prior to electrophoresis. RAP-PCR samples were heated at 92°C for 2 min after the addition of loading dye (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol FF). Samples were then loaded onto a 5% denaturing acrylamide gel (20×42 cm) and electrophoresed at 1,700 V for ~2 h until the xylene cyanol band reached the bottom of the gel. The gels were subsequently dried, marked asymmetrically with a phosphorescent pen for orientation, and exposed to film (Kodak) for 1–3 days at –80°C. Following identification of differentially expressed fragments, bands of interest were isolated from the gel as described in Bidle and Bartlett (2001). Eluted bands were subjected to secondary PCR using the original primers used in RAP-PCR amplification to facilitate cloning into pCR2.1 (Invitrogen) according to the manufacturer's instructions. Clones verified for the correct sized insert were then sequenced to determine putative identity.

DNA sequencing

DNA sequences were determined by thermal cycle dideoxy sequencing with an ABI 373A automated sequencer using fluorescently labeled terminators (Perkin-Elmer) using T7, -M13 reverse, or custom primers. Some sequencing reactions contained 10% DMSO to prevent secondary structure formation within the high-G + C-rich templates. Initial global similarity searches were performed with the BLAST network service (Altschul et al. 1990). Multiple alignments of amino acids were performed using Clustal W (Thompson et al. 1994) in conjunction with the BoxShade program available at http://www.ch.embnet.org/software/BOX_form.html.

Confirming the regulation of RAP-PCR fragments by changing salinity

To verify differential expression of the fragments identified in RAP-PCR, a quantitative PCR approach was used (Benson et al. 2000; Bidle and Bartlett 2001). This method relies first on the creation of random cDNA molecules from a RNA population, followed by gene-specific PCR. Briefly, first strand cDNA was created using RNA from *H. volcanii* strains following the same RT conditions stated above with the exception that 2 μM of random hexamers were used instead of a single arbitrary primer. Quantitative PCR was then performed using custom made 18-mer primer pairs specific for each sequence being tested and 2.5 μCi ^{32}P α-dCTP. Cycles of PCR were as follows: 92°C, 1 min; 50°C, 1 min; 72°C, 1 min for 25 cycles. Synthesis of a PCR product of the correct size and expression pattern was considered verified for differential expression of the original RAP-PCR result. All quantitative RAP-PCR experiments were performed in duplicate to ensure reproducibility.

Isolation of the locus-bearing genes encoded by clones B2 and B5

Genomic DNA from *H. volcanii* was isolated as described in Ng et al. (1995) and digested with either *Bam*HI, *Kpn*I, *Not*I, *Sph*I, or *Xho*I restriction enzymes (Gibco-BRL). Restriction digests were electrophoresed through a 0.8% agarose gel in duplicate and subsequently transferred to a nylon membrane (Osmonics) for Southern analysis. Each membrane was probed with the PCR product from either clone B2 or B5 which was randomly primed using [α-^{32}P] dCTP and the RadPrime kit (Gibco-BRL). Hybridizations were performed at 62°C in QuikHyb hybridization solution (Stratagene) overnight. Each membrane was washed under stringent conditions (Ausubel et al. 1993) and exposed to autoradiographic film Kodak) overnight at –80°C with an intensifying screen.

Western blot analysis

Haloferax volcanii cultures were grown to mid-exponential phase in either optimal or high-salt medium. Cells were harvested in a microcentrifuge and cell pellets were first resuspended in a lysis buffer containing the serine phosphatase inhibitor NaF (50 mM HEPES, pH 7.5, 20 mM $MgCl_2$, 20 mM KCl, 100 mM NaCl, 1 mM EDTA, pH 8, 1 mM DTT, 50 mM NaF). Lysed cells were then directly added to gel loading buffer and equal amounts of protein were separated by SDS-PAGE and then transferred to a PVDF membrane for 2 h at 60 V. The membrane was first blocked for 1 h in TBST (150 mM NaCl, 10 mM Tris-Cl, pH 8, 0.05% Tween 20, 5% bovine serum albumin). Primary rabbit anti-phosphoserine antibody (Research Diagnostics) was added to the blocking solution at a concentration of 0.5 μg/ml and allowed to incubate for 2 h. After washing in TBST, the membrane was incubated for 1 h with goat anti-rabbit IgG antibody–horseradish peroxidase (HRP)–conjugated secondary antibody (Research Diagnostics). After washing, visualization of immunoreactive proteins was detected by chemiluminescent HRP substrate using the Supersignal Substrate system (Pierce) and autoradiographic film (Kodak) exposure.

Nucleotide sequence accession numbers

The sequences of the partial gene fragments identified in this work are deposited in GenBank under accession nos. AF315626–29, AF315631, and AF454091–93.

Results and discussion

Identification of differentially expressed genes in response to changing salinity

Differential gene expression between *H. volcanii* cultures grown in optimal (12%) or high (20%) NaCl conditions was examined using 36 different random primer combinations in this RAP-PCR analysis. The reproducibility of the technique was first tested by performing RAP-PCR in duplicate using the same primer set with RNA that had been isolated on separate occasions from cultures of *H. volcanii* grown in optimal or high-NaCl conditions. The results of this experiment yielded nearly identical results (data not shown), thus all subsequent RAP-PCR experiments were performed only once. A total of 17 unique bands were obtained from an approximate total of 700 bands examined that clearly displayed differential regulation (data not shown). Fragment sizes ranged from ~750 bp to 100 bp, although bands smaller than 180 bp were not further examined. Differentially expressed RAP-PCR fragments were purified from each gel and re-amplified using the appropriate primers to verify that the correct sized fragment was isolated and to facilitate cloning. Cloned PCR products were sequenced to determine putative gene function.

Of the 17 clones originally isolated, only seven clones were chosen for further analysis. This was due to the fact that the remaining ten clones identified in these studies either failed to re-amplify the excised band (four clones) or were not confirmed for differential expression (two clones). Furthermore, four clones also were shown to encode 16S or 23S rDNA, an artifact often associated with the use of this technique (Nage et al. 2001).

To verify the accuracy of the pattern of expression found in the original RAP-PCR analysis, transcript analysis was performed using a quantitative PCR method (Benson et al. 2000; Bidle and Bartlett 2001). The regulation of all seven genes examined was confirmed using gene-specific PCR primers and cDNA prepared from RNA isolated from *H. volcanii* cultured with either 12% or 20% NaCl in the growth medium. When all of the RAP-PCR clones were examined using this method, the pattern of gene expression matched that seen in the original RAP-PCR analyses (Fig 1). These experiments confirmed that three of the seven genes found are maximally expressed in the presence of high NaCl in *H. volcanii* (clones B2, B3, and B8) while the remaining four genes' expression appears to be completely repressed at 20% NaCl (clones B5, B11, B12, and B15).

Characterization of salinity-regulated genes

A summary of genes present in the National Center for Biotechnology Information (NCBI) nucleic acid sequence databases available through the BLAST network service (Altschul et al. 1990) which showed greatest similarity to the partial gene sequences found in this study is shown in Table 1. The probability (*P*) values (Karlin and Altschul 1990) obtained from BLASTX ranged from 6×10^{-59} to 0.009.

Of the seven partial open reading frames found, two appear to have some involvement with environmental stress signaling. One of the differentially regulated gene sequences identified in this study encodes a protein displaying a serine/threonine kinase domain that is 57%

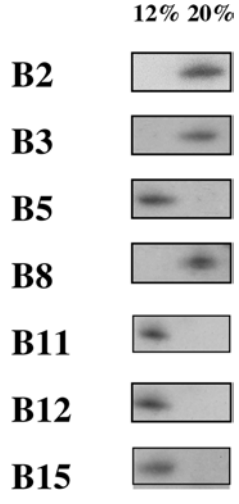

Fig. 1 Confirmation of differential gene expression of RAP-PCR fragments in *Haloferax volcanii* grown with either 12% or 20% NaCl. Quantitative PCR was performed using gene-specific primers as described by Benson et al. (2000) and Bidle and Bartlett (2001). Clone designations are given *on left*

Table 1 Summary of partial gene sequences regulated by changing salinity identified using RAP-PCR. The percentage Identity:Similarity and *P* values were obtained from BLASTX. Gene sequences were also compared directly against the NRC-1 genome available through NCBI

Clone/ size (bp)	Closest relative (accession number)	*P* value	% Identity: Similarity	Homology in NRC-1 genome	Gene expression at 12% or 20% NaCl
B2 (609)	*Saccharomyces cerevisiae* Ire1p protein kinase (U10556)	$6e^{-48}$	57:70	NO	20%
B3 (433)	*Vibrio cholerae* serine deaminase (AE004415)	$6e^{-59}$	84:75	YES (0.02, 31:52)[a]	20%
B5 (254)	*Salmonella typhimurium* putative methyl-Accepting chemotaxis protein (AE008755)	$2e^{-06}$	33:49	NO	12%
B8 (668)	*Deinococcus radiodurans* aldo/keto reductase (AE002058)	$8e^{-37}$	46:67	YES ($1e^{-13}$, 35:58)	20%
B11 (550)	*Halobacterium* sp. NRC-1 hypothetical proteins:			YES	12%
	Vng6397 h (AE005167)	$4e^{-5}$	85:88		
	Vng0650c (AE005012)	$9e^{-24}$	44:65		
B12 (608)	*Saccharomyces cerevisiae* YFW1 gene (U11583)	0.009	27:41	NO	12%
B15 (560)	*Pseudomonas syringae* transposase (AF170066)	0.002	38:60	YES (0.54, 24:41)	12%

[a](*P* value, % identity: similarity) for NRC-1 homolog using BLASTN

Fig. 2 Partial amino acid alignment of the putative *Haloferax volcanii* sensor kinase hvIre1p with its homolog in yeast, Ire1p. Identical residues are in *black* and similar residues are in *gray*. The invariant Lys (K) residue within the Ire1p kinase subdomain II (Tirasophon et al. 1998) is indicated by an *asterisk*

identical and 70% similar to the eukaryotic sensor kinase Ire1p (Fig 2). Ire1p is a sensor of the unfolded protein response pathway in yeast and mammalian cells (Cox et al. 1993; Mori et al. 1993; Tirasophon et al. 1998). The *H. volcanii* homolog, which has been named hvIre1p, is transcriptionally upregulated in response to an increase in medium NaCl concentration and might function to respond to protein unfolding in the cell. Despite the fact that haloarchaea contain proteins that have evolved to withstand the detrimental effects of high salinity (Dennis and Shimmin 1997), even so-called halophilic proteins will shift towards unfolded forms as salinity increases above optimal levels. The discovery of a homolog to a sensor kinase that is activated in response to protein unfolding is thus consistent with known salt effects on proteins. Since the optimal salinity for *H. volcanii* growth is approximately 12%, it can be envisioned that despite the organism's ability to grow at higher salinities, an 8% increase in the salinity to 20% will begin to cause some protein unfolding or 'salting-in.' Such an event could then catalyze the activation of hvIre1p transcription and in turn initiate an adaptive response to protein unfolding via phosphorylation of one or more target proteins involved in the response. This hypothesis is currently being investigated.

Another gene that appears to be somehow involved in environmental stress signaling was found in clone B5. This clone encodes a protein containing the amino acid motif GGDEF. This motif is found in several known bacterial response regulators as well as in a number of putative open reading frames from recent genome studies, including *Salmonella typhimurium* and *E. coli*. Among the known bacterial response regulators with a similar motif are the response regulator CelR2 from *Rhizobium leguminosarum* bv. *trifolii* [(Ausmees et al. 1999); accession number AF121341] and PleD from *Caulobacter crescentus* [(Sommer and Newton 1989); accession number L42554]. While these proteins exert different functions, both are hypothesized to be involved in two-component signal transduction. CelR2 is involved with the regulation of cellulose synthesis, while PleD is an important component of the regulation of cell division processes (Sommer and Newton 1989; Ausmees et al. 1999). Interestingly, another protein that was among the clone B5 BLAST output sequences which contain the GGDEF motif is the YkoW protein from *Bacillus cereus* [(Okstad et al. 1999); accession number AJ243712]. This as yet uncharacterized protein also contains PAS domain sequences which are sensing modules found in all three domains of life and which

function in prokaryotes in the global regulation of metabolism, behavior, and development [reviewed in Taylor and Zhulin (1999)]. While the complete sequence of clone B5 will need to be determined to ascertain whether it also contains PAS domains, the possibility is nevertheless intriguing that this gene may also possess these types of regulatory sequences.

The remainder of the genes isolated in this study encode products that display varying degrees of identity and similarity to a diverse range of proteins (Table 1). However, many of these clones, notably clones B12 and B15, show at best weak identity to known proteins in the database. Obtaining additional DNA sequence information from these clones will undoubtedly aid in the positive identification of these genes. These studies are currently in progress.

Linkage of genes encoded by clones B2 and B5

To assess whether the sensor kinase hvIre1p encoded by clone B2 is linked to the response regulator encoded by clone B5, Southern analysis was performed. The pattern of hybridization seen was identical in both experiments regardless of the probe used, indicating that the sensor kinase and response regulator are linked (Fig 3). Using different combinations of primers specific for clone B2 or B5 in PCR analysis, it was also determined that the putative response regulator (clone B5) lies upstream of the sensor kinase (clone B2; data not shown). Interestingly, these genes display inverse regulation to one another (see Fig. 1) with the sensor kinase being upregulated in the presence of high salt and the response regulator being downregulated under the same conditions. The implication of this inverse regulation is presently unknown, although the gene encoded by clone B5 could potentially act as a negative regulator of hvIre1p function.

Evidence of serine-threonine phosphorylation in *Haloferax volcanii*

Given the fact that a putative serine–threonine kinase is present in the genome of *H. volcanii*, it was of interest to determine if proteins phosphorylated on serine residues could be detected in *H. volcanii* at both optimal and high salinity. Evidence for tyrosine phosphorylation in *H. volcanii* has already been demonstrated (Smith et al. 1997). Crude cell extracts from *H. volcanii* grown under optimal and high salt conditions were prepared and treated with a serine phosphatase inhibitor prior to Western analysis using phosphoserine antisera. As seen in Fig 4, at least ten different proteins in the cell lysate from high salt conditions displayed immuno-reactivity with the antibody, while no reactivity was detected under optimal salt conditions. When cell lysates were not treated with phosphatase inhibitors, no visible bands could be detected in either condition (data not shown). These results clearly demonstrate that (1) phosphoserine proteins can be detected in *H. volcanii*; and (2) high salinity appears to favor the phosphorylation of several proteins as compared with optimal salinity.

Do other archaeal halophiles use genes similar to those found in *H. volcanii* for hypersaline adaptation?

Recently, the genome sequence of the extreme halophile *Halobacterium* species NRC-1 was completed (Ng et al. 2000). This organism grows optimally with ~25% NaCl in the growth medium, well above the optimal requirement for *H. volcanii* (~12%). In an attempt to identify potential homologs of *H. volcanii* RAP-PCR fragments in NRC-1, its genome sequence was examined using the "Search Microbial Genomes" function available through NCBI. Two of the seven clones were found to have significant relatedness to genes found in NRC-1, two clones were found to have weak relatedness, and three clones, including the putative protein kinase and response regulator, were not found in the genome. Results of this survey can be seen in Table 1. Given the diverse environments from which these two organisms were isolated and the significantly different NaCl requirements for growth, these results are not entirely surprising. However, it will be of interest in future examinations of other haloarchaeal genomes to assess

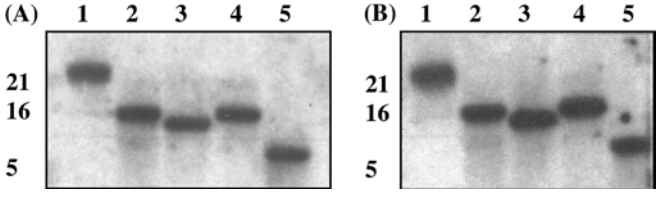

Fig. 3A, B Southern analysis demonstrating that the sensor kinase and response regulator encoded by clones B2 and B5, respectively, are linked. **A** *Haloferax volcanii* genomic DNA digests probed with clone B2. Lane 1 *Bam*HI, lane 2 *Kpn*I, lane 3 *Not*I, lane 4 *Sph*I, lane 5 *Xho*I. **B** *Haloferax volcanii* genomic DNA digests probed with clone B5. *Lanes* are the same as in **A**. Molecular weight size standards are given *at left* in kilobases (kb)

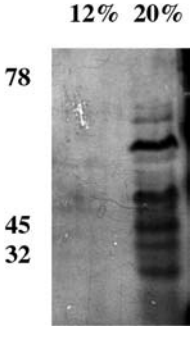

Fig. 4 Western analysis of phosphoserine proteins in *Haloferax volcanii*. Whole cell lysates were probed with anti-phosphoserine antibodies and detected by chemiluminescence. Molecular weight markers (kDa) given *at left*. *12%*, 12% NaCl in growth medium; *20%*, 20% NaCl in growth medium

whether closer relatives of *H. volcanii* possess similar genes.

This study used the technique of RAP-PCR to identify genes that are differentially expressed in response to changing salinity in the archaeal halophile, *H. volcanii*. This is the first application of this technique for use in Archaea. Seven genes whose response was either induced or repressed in the presence of high NaCl concentrations were revealed from these studies. Of these seven genes, two appear to be involved in some aspect of environmental stress signaling.

One of the genes identified in this study appears to encode an eukaryotic-like sensor kinase involved in the unfolded protein response in yeast and mammalian cells (Cox et al. 1993; Mori et al. 1993; Tirasophon et al. 1998). The discovery of this gene is noteworthy, as several homologs of eukaryotic protein kinase sequences have been reported in both Bacteria and Archaea [reviewed in Leonard et al. (1998)], although in Archaea all have been identified only by genome surveys (Smith and King 1995). Evidence of eukaryotic-like protein phosphorylation has been demonstrated among diverse members of Archaea, including Ser/Thr phosphorylation in *Sulfolobus acidocaldarius* (Skorko 1984; Kennelly et al. 1993; Leng et al. 1995) and Tyr phosphorylation in *S. solfataricus*, *Methanosarcina thermophila*, and *H. volcanii* (Smith et al. 1997). This is the first description of the actual isolation and preliminary characterization of a putative archaeal Ser/Thr kinase gene.

The role of many of the salinity-regulated genes uncovered in this study is at present unclear. However, a combination of mutational analyses and recovery of full gene sequence is currently being employed to begin identifying the role of each of these genes in hypersaline adaptation in *H. volcanii*. The information obtained from these studies might allow us to gain a better overall understanding of the molecular and genetic mechanisms used by the extremophilic haloarchaea to thrive in hypersaline environments.

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References

Altschul SF, Gish W, Miller W, Myers WE, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Ausmees N, Jonsson H, Hoglund S, Ljunggren H, Lindberg M (1999) Structural and putative regulatory genes involved in cellulose synthesis in *Rhizobium leguminosarum* bv. *trifolii*. Microbiology 145:1253–1262

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (1993) Current protocols in molecular biology. Wiley, New York

Benson NR, Wong RM-Y, McClelland M (2000) Analysis of the SOS response in *Salmonella enterica* serovar *Typhimurium* using RNA fingerprinting by arbitrarily primed PCR. J Bacteriol 182:3490–3497

Bidle KA, Bartlett DH (2001) RNA arbitrarily primed PCR survey of genes regulated by ToxR in the deep-sea bacterium *Photobacterium profundum* strain SS9. J Bacteriol 183:1688–1693

Chakrabortty A, Das S, Majumdar S, Mukhopadhyay K, Roychoudhury S, Chaudhuri K (2000) Use of RNA arbitrarily primed-PCR fingerprinting to identity *Vibrio cholerae* genes differentially expressed in the host following infection. Infect Immun 68:3878–3887

Christian JHB, Waltho JA (1962) Solute concentration within cells of halophilic and non-halophilic bacteria. Biochim Biophys Acta 65:506–508

Cox JS, Shamu CE, Walter P (1993) Transcriptional induction of genes encoding endoplasmic reticulum resident proteins requires a transmembrane protein kinase. Cell 73:1197–1206

Daniels CJ, McKee AHZ, Doolittle WF (1984) Archaebacterial heat-shock proteins. EMBO J 3:745–749

Dennis PP, Shimmin LC (1997) Evolutionary divergence and salinity-mediated selection in halophilic Archaea. Microbiol Mol Biol Rev 61:90–104

Ferrer C, Mojica FJM, Juez G, Rodriguez-Valera F (1996) Differentially transcribed regions of *Haloferax volcanii* genome depending on the medium salinity. J Bacteriol 178:309–313

Fleming JT, Yao W-H, Sayler GS (1998) Optimization of differential display of prokaryotic mRNA: application to pure culture and soil microcosms. Appl Environ Microbiol 64:3698–3706

Karlin S, Altschul SF (1990) Methods for assessing the statistical significance of molecular sequence features by using general scoring schemes. Proc Natl Acad Sci USA 87:2264–2268

Kennelly PJ, Oxenrider KA, Leng J, Cantwell JS, Zhao N (1993) Identification of a serine/threonine-specific protein phosphatase from the archaebacterium *Sulfolobus solfataricus*. J Biol Chem 268:6505–6510

Lanyi J (1974) Salt-dependent properties of proteins from extremely halophilic bacteria. Bacteriol Rev 38:272–290

Lanyi JK (1984) Bacteriorhodopsin and related light energy convectors. In: Ernster, L (ed) Bioenergetics. Elsevier, Amsterdam, pp 315–348

Leng J, Cameron AJ, Buckel S, Kennelly PJ (1995) Isolation and cloning of a protein-serine/threonine phosphatase from an archaeon. J Bacteriol 177:6510–6517

Leonard CJ, Aravind L, Koonin EV (1998) Novel families of putative protein kinases in Bacteria and Archaea: evolution of the "eukaryotic" protein kinase superfamily. Genome Res 8:1038–1047

Madern D, Ebel C, Zaccai G (2000) Halophilic adaptation of enzymes. Extremophiles 4:91–98

Meury J, Kohiyama M (1989) ATP is required for K^+ active transport in the archaebacterium *Haloferax volcanii*. Arch Microbiol 151:530–536

Mojica FJM, Cisneros E, Ferrer C, Rodriguez-Valera F, Juez G (1997) Osmotically induced response in representatives of halophilic prokaryotes: the bacterium *Halomonas elongata* and the archaeon *Haloferax volcanii*. J Bacteriol 179:5471–5481

Mori K, Ma W, Gething MJ, Sambrook JF (1993) A transmembrane protein with cdc2/CDC28-related kinase activity is required for signaling from the ER to the nucleus. Cell 74:743–756

Mullakhanbhai MF, Larsen H (1975) *Halobacterium volcanii* spec. nov., a Dead Sea halobacterium with a moderate salt requirement. Arch Microbiol 104:207–214

Nage AC, Fleming JT, Sayler GS, Beattie KL (2001) Screening for ribosomal-based false positives following prokaryotic mRNA differential display. Biotechniques 30:988–996

Ng W-L, Yang C-F, Halladay JT, Arora P, DasSarma S (1995) Isolation of genomic and plasmid DNAs from *Halobacterium halobium*. In: Robb FT, Sowers KR, DasSarma S, Place AR, Fleischmann EM, Schreier HJ (eds.) Archaea: a laboratory manual. Cold Spring Harbor Press, Cold Spring Harbor, NY, pp 179–184

Ng WV, et al (2000) Genome sequence of *Halobacterium* species NRC-1. Proc Natl Acad Sci USA 97:12176–12181

Okstad OA, Gominet M, Purnelle B, Rose M, Lereclus D, Kolsto A-B (1999) Sequence analysis of three *Bacillus cereus* loci carrying PlcR-regulated genes encoding degradative enzymes and enterotoxin. Microbiology 145:3129–3138

Skorko R (1984) Protein phosphorylation in the Archaebacterium *Sulfolobus acidocaldarius*. Eur J Biochem 145:617–622

Smith RF, King KY (1995) Identification of a eukaryotic-like protein kinase gene in Archaebacteria. Protein Sci 4:126–129

Smith SC, Kennelly PJ, Potts M (1997) Protein-tyrosine phosphorylation in the *Archaea*. J Bacteriol 179:2418–2420

Sommer JM, Newton A (1989) Turning off flagellum rotation requires the pleiotrophic gene *pleD*: *pleA*, *pleC* and *pleD* define two morphogenic pathways in *Caulobacter crescentus*. J Bacteriol 171:392–401

Stoeckenius W, Bogomolni RA (1982) Bacteriorhodopsin and related pigments in halobacteria. Annu Rev Biochem 51:587–611

Taylor BL, Zhulin IB (1999) PAS domains: internal sensors of oxygen, redox potential, and light. Microbiol Mol Biol Rev 63:479–506

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Tirasophon W, Welihinda AA, Kaufman RJ (1998) A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells. Genes Dev 12:1812–1824

Wong KK, McClelland M (1994) Stress-inducible gene of *Salmonella typhimurium* identified by arbitrarily primed PCR of RNA. Proc Natl Acad Sci USA 91:639–643