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Global transcriptome analysis of salt acclimated *Prochlorococcus* AS9601

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ABSTRACT

Molecular processes leading to salt stress acclimation in the model cyanobacterium *Prochlorococcus* are not known. To address this, we used RNA sequencing (RNAseq) to compare the global transcriptome of two exponential-phase populations of *Prochlorococcus* AS9601 cells – acclimated to high salt (5%, w/v) and normal seawater salt (3.8%, w/v). Experiments showed that salt acclimated cells exhibit slower growth rates with a doubling time almost twice as controls. Approximately 1/3 of the genome was found to be differentially expressed (p-value <0.05), but a considerably large number of these genes are "hypothetical proteins" with unknown function. Transcript abundance were higher for genes involved in respiratory electron flow, carbon fixation, osmolyte/compatible solute biosynthesis and inorganic ion transport. Many of the highly expressed genes are 'high light inducible proteins' believed to be part of the general *Prochlorococcus* stress response. Transcript abundance were lower for genes involved in photosynthetic electron transport and cell division. The relative reduction in transcript abundance for genes encoding proteins containing heme groups and iron transporters suggests cellular iron requirements in salt acclimated cells maybe lower. The results presented here provide the first glimpse into global gene expression changes in *Prochlorococcus* cells due to salt stress.

Introduction

The bacterial phylum cyanobacteria, whose members are metabolically classified as oxygenic photoautotrophs, are widely distributed in nature and play a significant role in primary production and the global carbon cycle (Arrigo, 2005). It is estimated that one half of the world's net primary production (~50 gigatons C year^{-1}) occurs in the oceans (Longhurst et al., 1995). Two genera of cyanobacteria, *Synechococcus* and *Prochlorococcus*, are globally important primary producers that dominate in tropical and subtropical oceans (Partensky et al., 1999). These two cyanobacterial genera are phylogenetically closely related but each has an ecological range in which they are more dominant (Rocap et al., 2003; Johnson et al., 2006; Mella-Flores et al., 2012). Members of *Synechococcus* are mostly present in surface waters that are permanently or seasonally enriched with nutrients from upwelling or coastal inputs. In contrast, *Prochlorococcus* occupies surface waters down to depths of 200 meters of oligotrophic oceans from 40° S to 40° N latitudinal bands. They are genetically and physiologically diverse and are subdivided into ecologically and taxonomically distinct ecotypes and genetic lineages according to their adaptation range to incident light levels/dominance in the ocean vertically with depth (Coleman and Chisholm, 2007). The high-light adapted (HL) ecotypes dominate upper regions of the euphatic zone whereas low-light (LL) adapted ecotypes dominate lower regions of the euphatic zone. The ubiquity of *Prochlorococcus* across horizontal and vertical gradients of the ocean make it the most abundant primary producer on Earth – comprising up to 57% of total phytoplanktonic primary production in the world's oceans (Partensky et al., 1999; Ting et al., 2002).

Given the importance of *Prochlorococcus* in global productivity, uncovering the molecular pathways and mechanisms by which this organism responds to environmental stress is an important area of study. For example, gene expression has been extensively studied in various strains of the organism in response to nutrient limitations (Tolonen et al., 2006; Gómez-Baena et al., 2009; Thompson et al., 2011; Reistetter et al., 2013) light, UV and oxidative stress (Mary, 2004; Kolowrat et al., 2010; Mella-Flores et al., 2012). The importance of these types of studies is compounded by the fact that climate change is causing changes to ocean stratification,

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chemistry and salinity (Boyd, 2013). Reports indicate that ocean salinity is changing at global and regional scales with the surface of subtropical waters generally becoming saltier and high-latitude waters freshening (Durack and Wijffels, 2010; Durack et al., 2012). The impact of these changes is unknown on *Prochlorococcus* populations.

Salt stress challenges cells with reduced water availability and high concentrations of inorganic ions. It is known to inhibit photosynthesis (Allakhverdiev and Murata, 2008) and impact other biological processes such as membrane composition (Huang et al., 2006) and cell division in cyanobacteria (Hagemann, 2011). Long term exposure to salt stress however results in acclimation, a process by which a cell reconfigures its metabolic pathways to adjust to suboptimal growth conditions. In cyanobacteria, salt stress acclimation involves active extrusion of inorganic ions, the accumulation of compatible solutes and the differential expression of many genes (reviewed in Hagemann 2010). The majority of these studies were conducted using marine *Synechococcus* (Ludwig and Bryant, 2012) and fresh water *Synechocystis* (Kanesaki et al., 2002; Marin et al., 2003; Paithoonrangsarid et al., 2004; Shoumskaya et al., 2005; Klähn et al., 2010a). Interestingly, studies are not available on the acclimation of *Prochlorococcus* to salt stress even though it is considered a model organism for cyanobacteria (Partensky et al., 1999). The relatively small genome size (1.7–2.4 mbp) accompanied with the availability of sequenced genomes of isolates from different regions of the world's oceans (http://proportal.mit.edu/) (Kelly et al., 2012) provides an opportunity for global analysis of molecular changes in cells to environmental stress. In the case of salt stress, these studies are also relevant for bioengineering of novel biochemical pathways that are active at high salinities. For these reasons, we undertook this study which aims to elucidate transcriptome changes in *Prochlorococcus* after acclimation to salt (NaCl) stress. The study was carried out using *Prochlorococcus* AS9601, an isolate from the Arabian Sea (Shalapyonok et al., 1998) belonging to HLII clade (Coleman and Chisholm, 2007). Studies exploring the physiology and transcriptome of *Prochlorococcus* AS9601 are not available. For this reason, we chose to conduct our experiments using *Prochlorococcus AS9601* as part of a first attempt to provide details on the transcriptome of this poorly studied strain.

Materials and methods

Culture and growth conditions

Axenic *Prochlorococcus* AS9601 cells were provided by Professor Chisholm (MIT, Cambridge, MA, USA) and maintained in PRO99 natural sea water medium (Moore et al., 2007) prepared from Sargasso Sea water with or without added ultra-pure NaCl, at a temperature of 22 °C with continuous illumination of 45.86 ± 1.41 (SD) μmol photons m^{-2} s^{-2}. A hand-held refractometer was used to verify water salinity measurements.

Experimental set-up and growth measurements

Cultures were acclimated to control and experimental salinities by consecutive transfers from mid-exponential growth into fresh media. Cells were grown in 300 mL batch cultures, and 19 mL were drawn at 48 h intervals for fluorescence measurements and RNA extraction (kept at 4 °C in RNAlater stabilization reagent (Qiagen) at a ratio of 1:3). Fluorescence was measured using a 10 Au fluorometer (Turner Designs, Sunnyvale, CA, USA). Growth rates (μ) were determined by fitting the exponential model ($y = e^{\mu x}$) to points during exponential growth using Microsoft Excel. Doubling times (t_d) were calculated by dividing the natural logarithm of 2 by the growth rate (μ).

RNA extraction

Cells stored in RNAlater were filtered through polycarbonate 0.2 μM filters, and RNA was extracted using the mirVana kit (Ambion) according to the manufacturer's protocol. Genomic DNA was removed using the Tubo DNA-free kit (Ambion), and RNA was concentrated using RNA clean & Concentrator-5 kit (Zymo Research) according to the manufacturer protocols. The concentration of RNA was determined using a Nanodrop spectrophotometer (Thermo Scientific) prior to preparation of cDNA libraries.

RNAseq analysis

51 bp cDNA libraries were prepared from RNA using the TruSeq RNAseq kit (Illumina) omitting the polyA isolation step, multiplexed and sequenced on a single Illumina Hiseq2000 at the BioMicroCenter (MIT, Cambridge, MA, USA). The resulting sequences were aligned against the *Prochlorococcus* AS9601 reference genome, available on NCBI (http://www.ncbi.nlm.nih.gov/), using BWA tool (Li and Durbin 2009) and the HTSeq package (http://www-huber.embl.de/users/anders/HTSeq/) was used to convert mapped sequencing reads to count tables (number of reads mapped to every gene) for differential analysis using the Bioconductor *DESeq* package version 2.13 (Gentleman et al., 2004; Anders and Huber, 2010). DESeq uses a statistical model based on negative binomial distribution using variance and mean to determine *p* values. The model controls for type-I error (the probability of false discoveries) and takes into consideration the count number and expression strength of a gene (Anders and Huber, 2010). Transcript count tables of control samples (cells grown in normal 3.8% salt sea water) were used as a reference for differential analysis. All normalization and quality assessment steps were performed following the DESeq pipeline using default settings prior to calling differential expression at 5% using the *p*-value as criterion of significant change in expression of a gene.

Results and discussion

Salt acclimated cells exhibit slower growth rates

Cells of *Prochlorococcus* AS9601 could not survive past single generation transfers when salt concentrations in sea water exceeded 5% (data not shown), and we suspect 5% to be the salt tolerance threshold of this organism. This places *Prochlorococcus* AS9601 among moderately salt tolerant strains of cyanobacteria (Hagemann 2010). A study involving *Prochlorococcus* SS120, an isolate of the Sargasso sea, revealed that it cannot survive in salt concentrations exceeding 4.1% (Klähn et al., 2010b). The lower salt tolerance threshold of *Prochlorococcus* SS120 could reflect the characteristics of its native environment, namely that the average salt concentration is between 3.5 and 3.8%. In contrast, *Prochlorococcus* AS9601 is an isolate from the Arabian Sea (Shalapyonok et al., 1998), and the average salt concentration in the water is reported to exceed 4.0% (Wang et al., 2013). Therefore, we suspect that the AS9601 strain is adapted to survive in seawater containing higher salt concentrations when compared to *Prochlorococcus* isolates from other parts of the world's oceans.

Using the 5% apparent salt threshold of *Prochlorococcus*AS9601, cells were acclimated to salt by consecutive transfer into fresh media for three generations prior to transcriptome analysis. The growth curves after third transfer are shown in Fig. 1. Cells growing in 5% salt seawater treatment exhibited a slower growth rate and a doubling time almost twice as long as cells grown in control 3.8% salt sea water. From this experiment, cDNA libraries were prepared using RNA extracted from three independent biological replicates

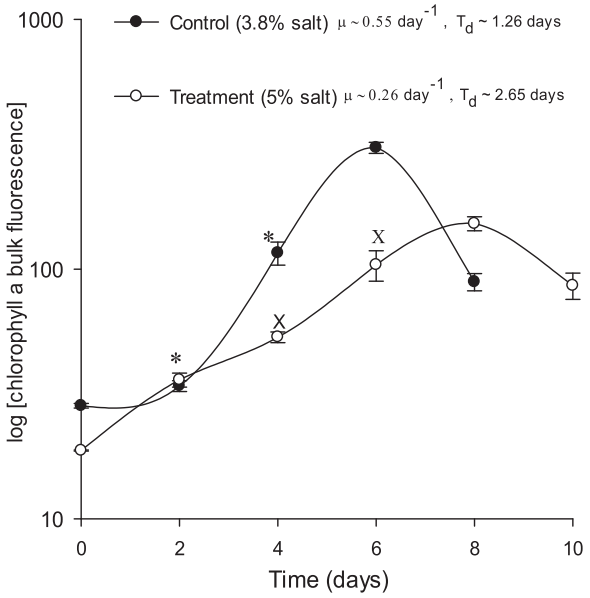

Fig. 1. *Prochlorococcus* AS9601 growth curves for control (normal sea water, 3.8% salt) and treatment (NaCl added to a final concentration 5% salt). The '*' and 'X' indicate the sampling days used for RNAseq analysis for control and treatment respectively.

sampled on 2 consecutive days of exponential growth (see Fig. 1 for sampling days). Approximately 17 million reads form each sample mapped to 1961 Open Reading Frames (ORF) of the *Prochlorococcus* AS9601 genome – of which 98% mapped to 23S and 16S rDNA ORFs and ~300 thousand reads mapped to 1959 ORFs (Supplementary Table 1).

Over 600 genes are differentially expressed in salt acclimated cells

Analysis of sample to sample variation from 2 consecutive days of the growth curve within a treatment group (control or salt treatment) were conducted by plotting the log2fold change in gene expression against the average expression count (Fig. 2). This was a quality assurance step to ensure RNA libraries originated from cells during exponential growth so that changes in gene expression can be attributed to salt stress – this is particularly important because of differences in growth rates of control and salt treated cells (Fig. 1). The DESeq package used in our study applies a negative binomial model using gene counts and only identifies a gene as differentially expressed based on differences between replicates (Anders and Huber, 2010). That is to say, a gene's expression is said to be impacted (i.e. differentially regulated) by treatment (salt) if the change between treated and untreated (control) samples is larger than the change between biological replicates. In this study, significant log2fold changes are determined via the *p*-value (i.e. $p < 0.05$) and depicted with red dots in the figures of normalized counts (Fig. 2). The analysis reveal that there are no significant differences in terms of *p*-value in gene expression between biological replicates from 2 consecutive sampled days within a treatment group (as evidenced by the absence of red dots in the log2fold change plots) (Fig. 2a and b). In contrast, differential expression ($p < 0.05$) can be detected for many genes when the transcriptome of salt treated cells are compared to control cells (Fig. 2c). Furthermore, two main hierarchical tree clusters which align with the experimental factor (control and salt treatment) are observed when differentially expressed genes are plotted using a heatmap (Fig. 2d). From these analysis, two conclusions are drawn (1) cells are in exponential phase in both control and salt treatment because there are no differences in gene expression between biological replicates from 2 consecutive sampled days within a treatment group, and (2) the transcriptome of salt acclimated cells of *Prochlorococcus* AS9601 is different from the transcriptome profile of cells growing in normal sea water salt.

Statistical analysis of transcript abundance counts (Supplementary Tables 2 and 3) using a *p*-value <0.05 revealed 627 genes are differentially expressed in salt acclimated cells. Functional categorization of these genes (Table 1 and Supplementary Table 4) according to Cyanobase definitions (Fujisawa et al., 2014) revealed that genes involved in processes related to replication/repair, post-translational modification, defense mechanisms, carbohydrate metabolism, secondary metabolites biosynthesis and RNA are generally induced when compared to controls. In contrast, a proportionally larger number of genes involved in cell cycle control, cell wall/membrane biogenesis and lipid metabolism are repressed. Functional categorization to a cellular process was not possible for 44% of the differentially expressed genes because they have Cyanobase classifications of "general function prediction", "function unknown" or "not in COGs".

Energy and metabolism

Cyanobacteria have both a photosynthetic oxygen-evolving electron transport chain and an oxygen-consuming respiratory electron transport chain. In this study, genes involved in photosynthetic electron transport chain appear to be down-regulated in salt acclimated cells. This includes components of Photosystem II (e.g. *psbB*, *psbT*, *psbJ*), and Photosystem I (e.g. *psaB*, *psaK*, *psaL*), as well as chlorophyll/bacteriochlorophylls encoding genes (e.g. *chlG*, *chlB*, *chlb*, *psaA and psaB*) – range for log2fold change is between −0.2 and −0.85 compared to controls (Table 2). With the exception of three PSII genes – those encoding D1, D2 and CP43 proteins of the reaction center (*psbA* and *psbD*, *PsbC*) increased by log2fold 0.25, 1, and 0.25 respectively (Table 2). Induction of the D1 gene was also reported for salt stressed *Synechocystis* PCC6803 (Kanesaki et al., 2002). However, the effect of salt stress on photosystem gene expression appears to be dependent on the organism under study because in salt acclimated *Synechococcus* PCC 7002 a decrease in PSI transcripts, but no change in PSII transcripts were observed (Ludwig and Bryant, 2012). Salt stress is known to enhance photoinhibition and photosystem damage (Allakhverdiev and Murata, 2008), and result in high turnover of the D1 protein (Aro et al., 1993). The AS9601 strain harbors one copy of the D1 coding gene, and an increase in transcript levels for D1 in salt acclimated *Prochlorococcus* AS9601 could be caused by a relatively high turnover of the D1 protein when compared to other components of the PSII complex. Other genes encoding photosynthetic electron intermediates were also found to be repressed. These includes plastocyanin (petE, −0.53 log2fold), cytochrome b6/b6f (*petA*, *petB*, *petM*, *petD*, *petN*) and cytochrome b559 (*PsbE* and *PsbF*) (Table 2).

The genome of *Prochlrorococcus* AS9601 contains 9 *ndh* genes that encode components of the NADH dehydrogenase complex – transcripts for four of these genes (*ndhF*, *ndhK*, *ndhI* and *ndhB*) were between 0.3 and 0.7 log2fold higher in salt acclimated cells (Table 2). Transcript levels for the remaining *ndh* genes did not change. An increase in transcripts of most genes encoding NADH dehydrogenase complex was also observed in salt acclimated *Synechococcus* PCC 7002 (Ludwig and Bryant, 2012). Several different NADH dehydrogenase complexes coexist in cyanobacteria and are responsible for different functions including respiration, cyclic electron flow and CO_2 uptake (Battchikova et al., 2011). For example, *ndhF* and *ndhD* genes encodes subunit F and D that are involved in cyclical electron flow around PSI, whereas the *ndhB* gene encodes subunit B involved in both respiration and inorganic carbon transport (Battchikova et al., 2011). An increase in transcript abundance for ndh complex genes in *Prochlorococcus* suggests respiration rates

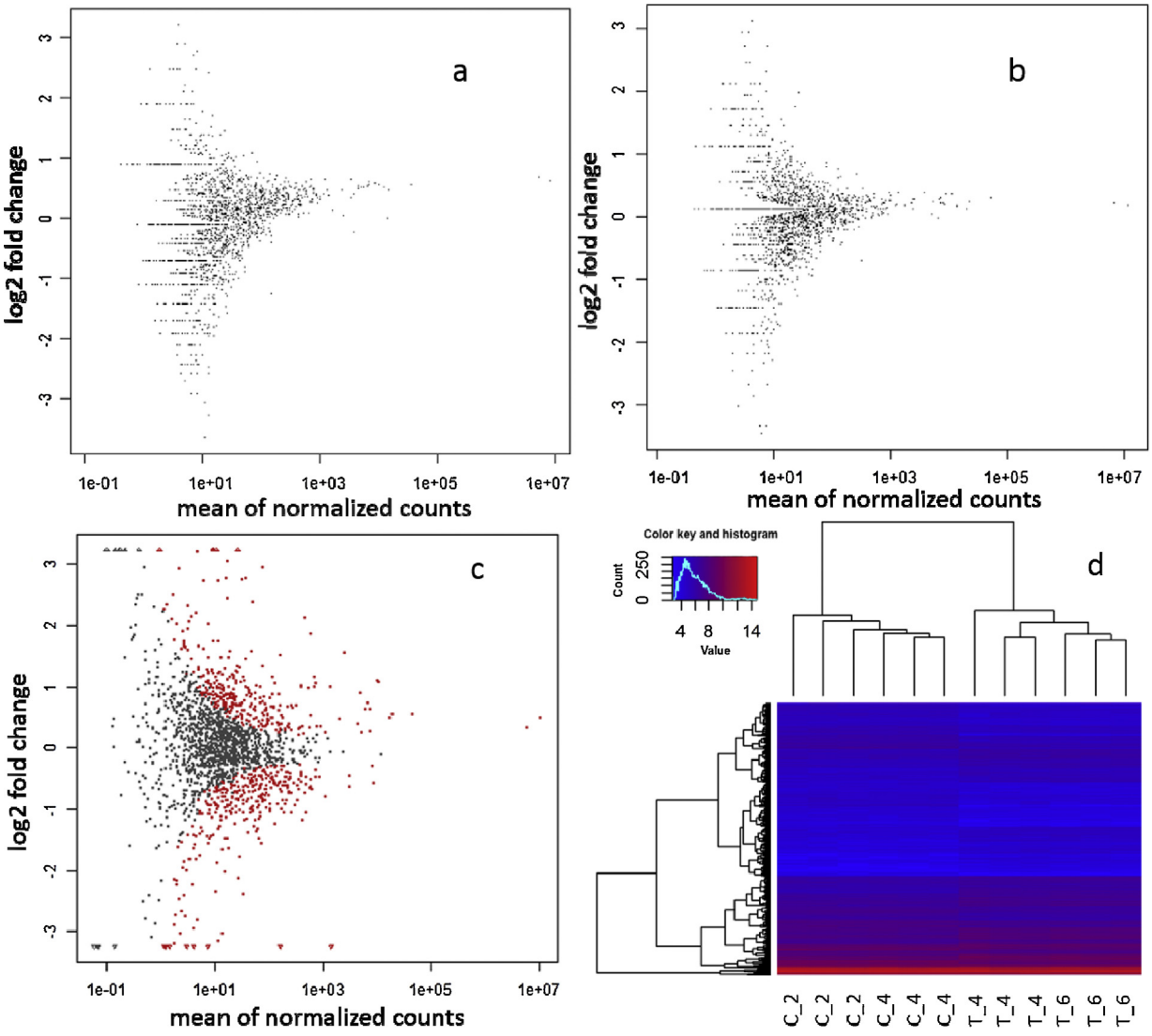

Fig. 2. (a) Plot of the log2fold change against the mean of normalized counts for "control versus control"; (b) plot of the log2fold change against the mean of normalized counts for "salt treatment versus salt treatment"; (c) plot of the log2fold change against the mean of normalized counts for "control versus salt treatment" coloring in red genes that are differentially regulated ($p < 0.05$); (d) heatmap showing the expression data of differentially expressed genes ($p < 0.05$) in salt acclimated cells using control as a reference. C_2 and C_4 refer to the control samples (three biological replicates each) from the second and fourth days of the growth curve, while T_4 and T_6 refer to the salt treatment samples (three biological replicates each) from the fourth and sixth days of the growth curve. A color scale from blue to red is used to illustrate the level of expression, low being blue and high being red respectively. The dendrogram above the heatmap clearly indicates a strong separation between control and treatment samples. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

could be higher in salt acclimated cells when compared to controls. This is further supported by an increase in transcripts for genes involved in the degradation of glycogen (*glgx*; log2fold change 0.83) and carbohydrates (e.g. *gap2*; log2fold change 0.87 and *pdhB*; log2fold change 0.38) (Table 2). However, transcript abundance for genes encoding subunits of the main terminal oxidase, *ctaE*, *ctaC* and *cyoB* (cytochrome c oxidase, COX) of the respiratory electron transport chain are the same in salt acclimated and control cells. Transcripts for enzymes and proteins that are central to carbon fixation and concentration – RubisCO (*rbcL* and *rbcS*) and carboxysome shell protein (*csoS1*, *csoS2 and csoS3*) are up-regulated in salt acclimated AS9601 cells (Table 2). An induction of carbon fixation genes was also observed in salt acclimated strains of *Synechococcus* and *Synechocystis* (Kanesaki et al., 2002; Marin et al., 2004; Pandhal et al., 2009; Ludwig and Bryant, 2012). A relationship exists between induced carbon fixation and respiration mediated by NADH dehydrogenase, because NADH dehydrogenase mutants of *Synechocystis* PCC6803 required higher concentrations of CO_2 for growth (Ogawa, 1991). The co-upregulation of ndh and carbon fixation genes in this study suggests a similar relationship exists in *Prochlorococcus* AS9601.

An interesting observation made in this study is the repression of a gene encoding a periplasmic ABC-type Fe^{3+} transporter (*afuA*) by a −0.61 log2fold change (Table 2). A relationship between iron homeostasis and the osmotic stress response has been reported for one bacterium – *Chromohalobacter salexigens* (Argandoña et al., 2010). The study showed that *C. salexigens* required less iron at high salt concentrations because cells produced less protein – the assumption being that many proteins contain Fe^{3+} at their active site. A similar phenomenon could be occurring in *Prochlorococcus* because genes encoding heme containing proteins (e.g. cytochrome b6-f) and Reiske-iron sulfur protein (*petC*) are down-regulated in salt acclimated cells compared to control cells (Table 2). Interestingly as well, salt acclimated cells have lower transcript abundance for the iron free electron transfer gene, flavodoxin (*isiB*) (Table 2). This gene is reportedly induced in iron stressed cells (Thompson

Table 1
Functional categorization of differentially expressed (p-value <0.05) genes in salt acclimated cells using Cyanobase definitions.

Functional categories	Total no. Cyanobase	Total no. differential expressed	No. induced	No. repressed	Prevalent expression profile
Translation	131	44	22	22	Equal
Transcription	33	11	6	5	Equal
Replication, recombination and repair	66	24	15	9	Induced
Cell cycle control, mitosis and meiosis	17	4	0	4	Repressed
Defense mechanisms	19	8	8	0	Induced
Signal transduction mechanisms	22	7	3	4	Equal
Cell wall/membrane biogenesis	102	26	11	15	Repressed
Intracellular trafficking and secretion	15	4	2	2	Equal
Posttranslational modification, protein turnover, chaperones	86	33	21	12	Induced
Energy production and conversion	76	39	23	16	Induced
Carbohydrate transport and metabolism	54	18	11	7	Induced
Amino acid transport and metabolism	125	32	17	15	Equal
Nucleotide transport and metabolism	50	11	6	5	Equal
Coenzyme transport and metabolism	94	27	13	14	Equal
Lipid transport and metabolism	44	15	5	10	Repressed
Inorganic ion transport and metabolism	51	19	9	10	Equal
Secondary metabolites biosynthesis, transport and catabolism	27	9	7	2	Induced
RNA	43	18	17	1	Induced
General function prediction only	138	44	27	17	U[a]
Function unknown	84	21	9	12	U
Not in COGs	687	211	78	133	U
Other (pseudogenes not in Cyanobase)		2	2	0	U
Total	1964	627	312	315	

[a] U = Unknown because assignment of function to these genes is ambiguous.

et al., 2011) and is often used as a biochemical marker for iron limitation in phytoplankton (Roche et al., 1996) because it replaces the iron requiring electron transfer protein ferredoxin. In this study, there were no differences in ferredoxin expression levels (encoded by *petE*) between control and salt acclimated cells indicating that cells are not iron limited. Further studies are required to investigate the link between iron requirement and salt acclimation in *Prochlorococcus*.

Transporters and compatible solutes

Cells acclimate to salt/ionic stress by activation of ion transporter systems and the accumulation of compatible solutes (Hagemann, 2011). Compatible solutes are low-molecular weight organic compounds, typically non-charged, that accumulate to high concentrations in osmotic stressed cells without negatively impacting metabolism. The most common compatible solutes utilized by moderately salt tolerant cyanobacteria are sucrose, glucosylglycerol (GG) and glucosylglycerate (GGA), whereas in halophiles glycine betaine (GB) is most common (Klähn and Hagemann, 2011). In *Prochlorococcus*, sucrose is reported to be the major compatible solute to accumulate in response to salt stress (Hagemann, 2011), but some isolates, for example SS120, Natl2a and MIT9312, are also reported to accumulate GGA in small amounts in response to osmotic stress (Klähn et al., 2010b). Genes encoding enzymes involved in GB biosynthesis are normally absent in *Prochlorococcus*, with the exception of two strains, MIT9313 and MIT9303 (Scanlan et al., 2009). In *Prochlorococcus* AS9601, genes for GB biosynthesis and the *ggpS* gene involved in GG biosynthesis are absent indicating GB and GG are not produced by the organism. Instead, the AS9601 genome contains orthologs for the two genes involved in GGA biosynthesis – *gpgS*, encoding glucosyl-phosphoglycerate synthase and *gpgP*, encoding glucosyl-phosphoglycerate gpgS (*A9601_08961* and *A9601_08981* respectively) (Hagemann, 2013). In this study, the gpgS ortholog (*A9601_08961*) is up-regulated by a log2fold value of 0.66 in salt acclimated cells (Supplementary Table 4) but there are no differences in transcript abundances for the *gpgP* ortholog (*A9601_08981*) when compared to control cells. Transcripts for a gene (*A9601_08971*) encoding glycoside hydrolase family 13 that is involved in carbohydrate and sucrose metabolism is present at higher levels in salt acclimated cells compared to controls (log2fold 0.43, Table 2). Also, there is a 0.52 log2fold increase in transcript abundance of genes encoding sodium transporters (*citT* and *trkG*) in salt acclimated cells when compared to control cells. Collectively, the data suggests that active extrusion of sodium ions and accumulation of osmolytes, GGA and sucrose, are involved in *Prochlorococcus* AS9601 acclimation to salt stress.

Highly induced/suppressed genes

Only 69 genes are identified as differentially expressed when we changed the filter criterion of significance from only meeting the p-value requirement ($p < 0.05$) to having to meet both (1) false discovery rate (p-adjusted) <0.05 and (2) magnitude of log2fold change with values greater than 2 (highly induced) or less than −2 (highly suppressed) (Supplementary Table 5). Of those 69 genes, 41 encoded hypothetical proteins, 8 encoded possible high light inducible proteins and the remaining genes either had known or putative functions in the *Prochlorococcus* A9601 genome. Of those that are annotated; genes involved in secondary metabolite biosynthesis (*A9601_03711* and *A9601_03031*); cell wall membrane biogenesis (*murI*), amino acid transport/metabolism (*A9601_04581*), energy production/conversion (*rbcL*, *rbcS*, *cso2*), RNA (tRNAs for glycine and valine), and ABC transporter (A9601_10521) appear to be highly-induced in salt acclimated cells (Table 2). Conversely, genes that appear to be repressed in salt acclimated cells are those involved in cell cycle control (*minE*), lipid transport and metabolism (*A9601_04791*) and cell wall/membrane biogenesis (*men*C) (Table 2).

To gain further insight into the potential role of unclassified or unknown genes, we searched for orthologs using best hit pairs from BLAST (using 97% identity cut-off) and subsequently conducted a brief literature search to determine if similar genes and/or proteins were reported in salt stress studies involving other

Table 2
List of differentially expressed genes ($p < 0.05$) in salt acclimated *Prochlorococcus* AS9601 cells that were discussed in text ("Results and Discussion" section). Transcriptome of control cells (growing in normal sea water salt) was used as reference.

Gene ID	Definition	*p*-Value	Adjusted *p*-value	Log2fold
Energy				
A9601_0071	Photosystem II protein X PsbX	0.01	0	−0.44
A9601_1851	Possible Photosystem II reaction center Z protein	0	0	−0.77
psbA	Photosystem II PsbA protein (D1)	0	0.04	1.07
psbB	Photosystem II PsbB protein (CP47)	0.02	0.05	−0.45
psbC	Photosystem II PsbC protein (CP43)	0.03	0.1	0.27
psbD	Photosystem II PsbD protein (D2)	0.01	0.04	0.25
psbE	Cytochrome b559 subunit alpha	0	0.06	−0.66
psbF	Cytochrome b559 subunit beta	0	0.09	−0.73
psbH	Photosystem II reaction center protein PsbH	0	0	−1.04
psbI	Photosystem II reaction center I protein PsbI	0	0.02	−0.88
psbJ	Photosystem II reaction center protein PsbJ	0	0	−0.85
psbK	Photosystem II reaction center protein PsbK precursor	0	0	−1.01
psbL	Photosystem II reaction center L	0	0	−0.86
psbM	Possible Photosystem II reaction center M protein	0	0	−1.17
psbN	Photosystem II reaction center protein N	0.04	0	−0.6
psbO	Photosystem II manganese-stabilizing protein	0	0	−0.64
psbT	Photosystem II reaction center protein T	0.01	0.1	−0.32
psaA	Photosystem I P700 chlorophyll a apoprotein A1	0	0	−0.59
psaB	Photosystem I P700 chlorophyll a apoprotein A2	0.04	0.06	−0.29
psaC	Photosystem I subunit VII	0	0.01	−0.58
psaD	Photosystem I protein PsaD	0	0	−1.07
psaE	Photosystem I reaction center subunit IV	0	0.07	−0.68
psaF	Photosystem I PsaF protein (subunit III)	0	0	−0.8
psaI	Photosystem I subunit VIII (PsaI)	0	0	−0.59
psaK	Photosystem I PsaK protein (subunit X)	0	0.01	−0.46
psaK	Photosystem I PsaK protein (subunit X)	0	0.01	−0.46
psaK	Photosystem I PsaK protein (subunit X)	0	0.01	−0.46
psaL	Photosystem I reaction center protein subunit XI	0	0.01	−0.48
psaM	Photosystem I reaction center subunit XII	0	0	−1.49
chlB	Light-independent protochlorophyllide reductase	0	0	−0.62
chlG	Bacteriochlorophyll/chlorophyll a synthase	0	0	−1.01
Electron transport and intermediates				
isiB	Flavodoxin FldA	0	0.05	−0.73
ndhB	NAD(P)H-quinone oxidoreductase subunit B	0	0.06	0.56
ndhF	NAD(P)H-quinone oxidoreductase subunit F	0	0.03	0.54
ndhI	NADH dehydrogenase subunit I	0	0	0.72
ndhK	NADH dehydrogenase subunit B	0.01	0.07	0.35
petA	Cytochrome b6f complex subunit PetA	0	0.06	−0.63
petB	Cytochrome b6	0.01	0	−0.48
petC	Rieske iron–sulfur protein	0	0	−0.92
petD	Subunit IV of the cytochrome b6f complex	0	0.04	−0.43
petE	Plastocyanin	0	0	−0.53
petM	Cytochrome b6f complex subunit PetM	0	0	−1.24
petN	Cytochrome b6-f complex subunit VIII	0	0	−0.8
Carbon metabolism, concentration and fixation				
glgX	Putative isoamylase	0	0	0.83
gap2	Glyceraldehyde 3-phosphate dehydrogenase(NADP+)	0	0	0.87
ccmK	Carboxysome shell protein CsoS1	0	0	1.02
csoS2	Carboxysome shell protein CsoS2	0	0	2.12
csoS3	Carboxysome shell protein CsoS3	0.01	0	1.23
pdhB	Pyruvate dehydrogenase E1 beta subunit	0.01	0.03	0.37
rbcL	Ribulose bisophosphate carboxylase	0	0	1.57
rbcS	Ribulose bisphosphate carboxylase, small chain	0	0	1.86
Other genes				
afuA	ABC-type Fe3+ transport system periplasmic	0	0	−0.61
A9601_03711	Bacterial-type phytoene dehydrogenase	0	0	2.39
A9601_04581	Possible cystathionine gamma-synthase	0	0	2.01
A9601_08971	Glycoside hydrolase family 13	0.02	0.01	0.43
A9601_19201	Sucrose phosphate synthase	0	0.02	−0.52
A9601_tRNAGly	tRNA-Gly	0	0.01	2.15
A9601_tRNAVal	tRNA-Val	0	0.01	2.06
citT	Putative sodium/sulfate transporter, DASS family	0.03	0	0.52
menC	Putative O-succinylbenzoate synthase	0	0.02	−2.74
minE	Possible septum site-determining protein MinE	0	0	−1.65
murI	Putative aspartate and glutamate racemase	0	0.01	2.21
trkG	Possible sodium transporter, Trk family	0.04	0.14	0.52

cyanobacteria (Supplementary Table 6). Unknown genes that appear to be repressed in this study showed similarity to genes present in other strains of *Prochlorococcus* or in *Synechococcus* strains – the majority of which seem to encode proteins that are involved in cell cycle regulation and control. In contrast, the majority of induced genes showed similarity to high light inducible proteins – known to be part of the general stress response in *Prochlorococcus* (e.g. Mella-Flores et al., 2012) as well as ATP

Binding Cassettes (ABC) – type transporters. Involvement of ABC-type transporters in osmoregulation and translocation of compatible solutes has been reported in cyanobacteria and other bacteria. For example, an ABC transporter in *Bacillus subtilis* is involved in Na^+ export (Cheng et al., 1997), an ABC transporter found in *Escherichia coli* is involved in translocation of a wide range of compatible solutes (Gul and Poolman, 2013) and in *Synechocystis* sp. strain PCC 6803 – more than half of the induced proteins were periplasmic ABC-type transporters or hypothetical proteins (Marin et al., 2004; Huang et al., 2006).

Conclusion

Understanding the process of salt acclimation in cyanobacteria has implications in biotechnology and could lead to bioengineering of novel pathways that are active in saline and/or brackish water. It can also provide insight on the impact of rising ocean salinity due to climate change on primary production in the world's oceans. In this study, we used RNAseq to identify the major biochemical pathways that are impacted in salt acclimated cells of *Prochlorococcus* AS9601. Global transcriptome analysis suggests that salt acclimated cells increase respiratory electron flow, carbon fixation, osmolyte/compatible solute biosynthesis and inorganic ion transport. It also suggests that salt acclimated cells are reducing photosynthetic electron transport reactions and slowing down cell division. Specific observations made in this study that warrant further investigation are; (1) reduction in transcript abundance for genes encoding proteins containing heme groups or iron, as well as iron transporters suggests cellular requirement in salt acclimated cells maybe lower, and (2) increase in transcript abundance for genes encoding high light inducible suggests a link between light and salt stress. This study provides the first glimpse of the global transcription changes in *Prochlorocccus* cells due to salt stress, but the lack of functional assignment for a large number of genes remains a challenge for molecular research involving this model organism.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.micres.2015.04.006

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