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DNA supercoiling regulates the stress-inducible expression of genes in the cyanobacterium

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Summary

Changes in the supercoiling of genomic DNA play important role in the regulation of gene expression. We compared the genome-wide expression of genes in cells of the cyanobacterium *Synechocystis* sp. PCC 6803 when they were subjected to cold, heat, and salt stress, in the presence of novobiocin, an inhibitor of DNA gyrase, and in its absence. The results by DNA microarray and Northern blotting analyses indicated that stress-induced changes in negative supercoiling of DNA decreased stress-induced expression at transcript levels of many genes and increased that of many other genes. These results suggest that stress-induced changes in superhelicity of genomic DNA might provide an important permissive background for successful acclimation of cyanobacterial cells to stress conditions.

Introduction

Changes in supercoiling of genomic DNA play important roles in the regulation of gene expression in response to changes in environmental stress in Gram-negative and Gram-positive bacteria.[1-4] Temperature-dependent alterations in DNA supercoiling were proposed to be one of the sensory mechanisms that alter the expression of genes involved in cold acclimation.[5,6] Salt stress and hyperosmotic stress also affect the degree of negative supercoiling of DNA and regulate the gene transcription.[7-9] Less is known about the effect of heat stress on DNA structure. A few studies demonstrated that heat stress decreased the negative supercoiling of DNA.[10-12]

In principle, studies of changes in supercoiling of DNA had been limited to alterations in plasmid DNAs in *Escherichia coli*, *Bacillus subtilis*, and *Salmonella typhimurium*. Therefore, changes in gene expression due to the change in supercoiling of genomic DNA were mainly assumed on the basis of changes in the linking number of plasmids.[13-15] Recently, we have shown that cold stress causes an increase in the negative supercoiling of the promoter region of the *desB* gene for fatty acid desaturase and directly controls its induction at low temperatures. [6] Another recent report links changes in DNA supercoiling (although measured in a plasmid) to circadian rhythms of gene expression in *Synechococcus elongatus*.[16]

Recent developments of DNA microarray technique, which allow us to follow the genome-wide alterations in gene expression, have markedly increased the impact of the results due to the broadened genome-wide scale of research. Several reports are now available that demonstrate the importance of DNA supercoiling in the regulation of gene expression under osmotic stress in *E. coli*,[9,17] or global transcriptional response to the addition of the inhibitors of DNA gyrase,[17] the key enzyme that controls the degree of supercoiling.

We used novobiocin, an inhibitor of DNA gyrase,[18,19] to examine the effect of changes in DNA supercoiling on genome-wide expression of genes in *Synechocystis* in response to cold, heat, and salt stress conditions. Novobiocin interacts with the ATP-binding site of the B-

subunit of the DNA gyrase, GyrB. We identified many stress-induced genes whose expression is affected by supercoiling of DNA. We discuss these findings in relation to the two-component systems, *e.g.*, the Hik33-Rre26 system for cold stress [20,21]; Hik33-Rre31, Hik34-Rre1; Hik16-Hik41-Rre16, and Hik10-Rre3 for salt stress [22]; and Hik34-Rre1 for heat stress. [23]

Results

Effect of novobiocin on gene expression under normal growth conditions

Novobiocin is an inhibitor of DNA gyrase and thus decreases the extent of negative supercoiling of DNA. To examine whether novobiocin would affect the genome-wide expression of genes, we performed DNA microarray analysis with cells that had been grown under growth conditions, as described in Materials and methods section. Fig. 1 shows that incubation of cells with 50 μg ml^{-1} novobiocin for 60 min affected the expression of only a limited number of genes. Nevertheless, it enhanced the expression of the *gyrB* gene for the B-subunit of DNA gyrase and the *lexA* gene for a regulatory repressor of SOS function. Since novobiocin binds to the B-subunit of DNA gyrase, the increase in the level of *gyrB* transcript might be related to a compensation mechanism to maintain the level of active gyrase in the cell. Why the level of *lexA* transcript was increased by novobiocin is unknown, although Osburne *et al*.[28] showed that novobiocin might affect the SOS response in bacteria.

Novobiocin also inhibited the expression of several genes (Fig. 1). They were the *nlpD* for a lipoprotein, and the *topA* for topoisomerase I, a functional antipode of the DNA gyrase, which caused relaxation of DNA. Since novobiocin inhibited DNA gyrase, down-regulation of the *topA* gene might reflect the action of a compensatory mechanism that is directed to maintain the extent of DNA supercoiling on some steady-state level. The *isiAB* operon for iron stress-induced proteins, whose expression is regulated by various kinds of stress, also appeared among down-regulated genes. It is, however, known that *isiAB* is silent under non-stress conditions.[29] Therefore this down-regulation might reflect some artifact during the microarray analysis (our unpublished observations).

Effect of novobiocin on the cold-inducible expression of genes

Cold shock results in a transient increase DNA supercoiling as demonstrated by studies on bacterial plasmids[5,30] and on the specific region of the cyanobacterial genomic DNA.[6]

Synechocystis cells, which had been grown at 34°C, were pre-incubated at 34°C for 30 min in the presence of 50 μg ml^{-1} novobiocin or in its absence (control). Then, they were further incubated at 24°C for 30 min. Table 1 shows the results of the influence of novobiocin on the cold-induced expression of genes. More than 50 genes were induced by cold with the induction factor higher than 3.0. The effect of novobiocin on cold-induced expression of genes might be mainly divided to two groups. Group 1 included genes whose inducibility by cold was significantly repressed by the presence of novobiocin (Table 1). They were well-known cold-inducible genes[21], such as *ndhD2, crhR, hliB, hliC, fus, rbpA1, rplA, desB*, and some others genes of known and unknown function. Group 2 included genes whose inducibility by cold was significantly enhanced by the presence of novobiocin, whereas their inducibility by cold in control cells was null or only marginal. This group included *feoB, sigD, hik31, hik3, dapA, petC2, pilA1, tatD, ziaA*, and some genes of unknown function.

To confirm the results of microarray assays, we performed Northern-blot analysis of the *hliB* gene for high light-inducible protein in group 1 and *sll1696* in group 2 (Fig. 2). The result revealed that the presence of novobiocin repressed the cold-induced expression of the *hliB* gene and that it significantly enhanced the expression of the *sll1696*.

Novobiocin is an inhibitor of DNA gyrase, which provokes the DNA relaxation. Therefore, the above observations imply that the cold-inducible expression of these genes requires high level of negative supercoiling of DNA. They also indicate that the extent of DNA supercoiling before the addition of novobiocin was too high for the cold-inducible expression of genes in group 2 and that, when the extent of negative supercoiling was decreased to certain levels by the presence of novobiocin, the cold-induced increase in the extent of negative supercoiling was suitable for the expression of these genes.

Effect of novobiocin on the heat-induced expression of genes

Heat stress decreases transiently the extent of negative supercoiling of DNA.[12,31] It was suggested that the dependence on temperature of the activity of DNA gyrase and DNA topoisomerase I might be the key factors for the control of DNA topology under stress conditions. In turn, this might be relevant for the expression of stress-induced genes.

Synechocystis cells, which had been grown at 34°C, were pre-incubated at 34°C for 30 min in the presence of 50 μg ml^{-1} novobiocin or in its absence (control), as above. Then, they were further incubated at 44°C for 30 min. The heat treatment induced the expression of about 30 genes with the induction index higher than 4.0. The effect of novobiocin on heat-induced expression of genes could be divided to three groups. Group 1 included genes whose inducibility by heat was significantly repressed by the presence of novobiocin. These were *htpG, groESL, groEL2, dnaK2, sodB, clpB2* and some other genes of unknown function.[23]

Group 2 represents the genes that were induced in the presence of novobiocin, whereas their inducibility by heat in control cells was insignificant. They were *dnaJ, hypA1, sigB, ziaA, htrA*, and many others, including several representatives of the two-component sensory systems and genes of unknown function (Table 2). It has been suggested that a degree of DNA supercoiling increases under cold stress and decreases under heat stress.[30-33] If heat causes relaxation of DNA, and novobiocin prevents negative supercoiling, it might be that the genes of the second group require rather high level of DNA relaxation for their induction, which normally does not occur during incubation at 44°C for 30 min (in the absence of novobiocin).

Group 3 of unaffected genes is rather small, and it consists of *hspA, clpB1, ctpA*, and one gene of unknown function (lower part of Table 2).

The results of Northern blotting support the data obtained with microarrays. As shown in Fig. 3, novobiocin repressed heat inducibility of *groEL2* gene (group 1), enhanced heat inducibility of *sigB* (group 2), and did not affect the expression of *hspA* (group 3). Thus, we suppose that the heat-induced expression of genes correlates with changes in DNA supercoiling.

Effect of novobiocin on the salt-inducible expression of genes

Salt stress due to NaCl leads to an increase in the extent of DNA supercoiling in bacteria.[9,30] It was demonstrated that salt- and osmo-induced increase in supercoiling of bacterial DNA leads to the activation of the promoter of the *rpoU* operons that encode transport systems for a compatible solute glycine betaine in *E. coli* and *S. typhimurium*.[1,34] It was suggested that the salt-induced change in supercoiling of genomic DNA might play important roles in response and acclimation of cells to new environments.

Synechocystis cells, which had been grown at 34^{o}C, were pre-incubated at 34^{o}C for 30 min in the presence of 50 μg ml^{-1} novobiocin or in its absence (control), as above. Then, they were further incubated at 34^{o}C for 30 min with NaCl at final concentration of 0.5 M. The increase in NaCl concentration induced the expression of more than 100 genes with the induction index higher than 4.0.

Group 1 included the genes whose induction by salt was prevented by the presence of novobiocin. These are *hspA, hik34, clpB1, sigD, sodB, dnaK2*, *ggpS*, and many other salt inducible genes[22] (Table 3). This group included the genes for heat-shock response, as well as *ggpS*, for glucosylglycerol-phosphate synthase, a key enzyme involved in the synthesis of glucosylglycerol, the major compatible solute in *Synechocystis*, and *ggtB* for the glucosylglycerol transport system.

Group 2 included a relatively small number of genes whose inducibility by NaCl was enhanced by novobiocin, whereas their inducibility by NaCl in control cells was insignificant: they were *ziaA, feoB, rre40, dapA* and some genes of unknown function.

Group 3 also included a relatively small number of genes. As discussed above, it consists of the representatives, whose expression was barely affected by the presence of novobiocin: They were *htrA, dnaJ, menG,* and some other genes of unknown function. Among

these genes, there was an *sll1862-1863* operon, which was induced by salt and osmotic stress.[22]

The results of microarray assays were confirmed by Northern-blot analysis of the *hspA* gene for group 1, *feoB for* group 2, and *slr1687* for group 3 (Fig. 4). The result revealed that novobiocin repressed the NaCl-induced expression of the *hspA* gene, enhanced the NaCl-induced expression of the *feoB*, and that it had no effect on the NaCl-induced expression of the *slr1687*.

Discussion

Changes in the extent of DNA supercoiling are associated with a diverse spectrum of environmental stress including temperature stress[6,15,31,35] and hyperosmotic stress.[7,9] These events have been extensively studied in *E. coli* and have been described also in some other bacterial species, such as S. *typhimurium*[1] and *B. subtilis*.[2]

The availability of DNA microarray approach allowed us to investigate the role of novobiocin-affected DNA supercoiling in global expression of genes in *Synechocystis* in response to cold, heat, and salt stress. The analysis revealed that the expression of a large number of stress-inducible genes depends on the extent of genomic DNA supercoiling.

Cold stress

We have previously suggested that the expression of more than half of cold-inducible genes in *Synechocystis* cells are under control of a mechanism that includes the Hik33-Rre26 two-component system.[21] The expression of the other cold-inducible genes is controlled by mechanisms that have not been clarified. Cluster analysis of the results of the present work and of the data obtained previously on the *Synechocystis* mutants defective in *hik33* demonstrated that cold-induced enhancement in DNA supercoiling affected the cold-dependent expression of the majority of genes whose cold-inducibility was not under control

of the Hik33 system (Fig.5). These genes are *crhR*, *rlpA*, *rpbA1* and some others. The mode of activation of these genes under cold stress is still unknown. It would be possible that an increase in negative supercoiling of DNA due to cold stress changes the promoter spacing or availability of regulatory sequences that are recognized by RNA polymerase or cold-induced transcription factors. The importance of an extent of DNA supercoiling for such recognition has been demonstrated in plasmids that carry promoters with various length of spacer or enhancer sequences.[13,36]

It also becomes evident that the majority of genes, which are regulated by cold through Hik33, depend on cold-induced changes in DNA supercoiling. There is only limited information for the mechanism by which the response regulator Rre26, that transfers the cold signal from Hik33, binds to the regulatory regions of the downstream genes.[37] Nevertheless, it's time to suggest the existence of cross talk or interrelations between the two-component signaling switched on by rigidification of cell membranes[38] and changes in the topology of the chromosome.

Heat stress

The only known, recently discovered, histidine kinase that participates in regulation of heat shock response in *Synechocystis*, is Hik34.[23] This sensory kinase also controls many genes induced by hyperosmotic stress[27] and salt stress.[22] Inhibition of DNA gyrase by novobiocin under heat-shock conditions should have decreased the negative supercoiling of DNA. This prevented the induction of major heat-shock genes, such as *groESL*, *cpn60*, *dnaK2*, and *htpG* (Table 2, Fig. 2). By contrast, it enhanced the heat-induced expression of genes of the *dnaJ* family and many genes for proteins of unknown function, as well as the *hik34* gene for heat sensory histidine kinase. Hik34 acts as a repressor of heat-shock genes at physiological temperatures.[23] Thus, the enhanced expression of *hik34* under heat stress by the presence of

novobiocin might have repressed the expression of certain heat-shock genes as observed in Table 2.

Salt stress

Salt stress due to NaCl leads to an increase in DNA supercoiling in bacteria.[30] In *Synechocystis*, novobiocin prevents the induction of many salt-inducible genes, including those for so called heat-shock proteins, *ggpS* for glucosylglycerol synthase, which is necessary for acclimation of these cells to salt stress. We previously demonstrated that the five two-component systems control different sets of salt-inducible genes.[22] We further mentioned a group of genes whose induction is not regulated by the two-component systems. Here we found that the salt-induced expression of some of such genes (*ggpS*, *ndhR*, *glpD*, *sll1652*, etc.) depended on changes in DNA supercoiling due to NaCl stress.

In addition, relaxation of DNA caused by novobiocin affected the induction of genes that are depressed by Hik33 (*hliABC*-family, *slr1544*, *sll1483*) and Hik34 (mainly the genes for heat-shock proteins). As mentioned above, Hik34 functions as a repressor of *hsp* genes at normal growth temperature.[23] Under NaCl stress, however, Hik34 regulates the expression of a set of genes, probably, in a positive manner. At least the knockout of the *hik34* prevents the expression of a set of NaCl-induced genes.[22] The NaCl-induced activation of the *hik34* gene itself is prevented by novobiocin (Table 3). This may cause repression of *hspA*, *dnaK2*, *sodB*, *sigB*, *clpB1* and many other genes for proteins of unknown function.

The effect of novobiocin-induced changes in DNA supercoiling on gene expression

Since both cold and salt stress should increase the negative supercoiling of DNA, one could expect similar effect of novobiocin on the expression of, at least, some set of genes, which are affected by cold and salt treatments. Indeed, cold and salt stresses induce such genes as *crhR, hliB, hliC, slr1544, sll1483, ssr2016, slr0236* (the latter is not shown in Table 3

of salt-inducible genes with the induction factor of 3.2). Novobiocin prevented cold- and salt-inducibility of these genes. On the other hand, novobiocin severely enhanced the expression of *sll1862-1863*, *feoB, ziaA, sll0462, slr1927, slr1851, tatD* (the latter is not shown in the table of salt-inducible genes with the induction factor of 4.2 in the presence of novobiocin and with induction factor of 1,4 in its absence).

Previously we demonstrated that the histidine kinase Hik33 perceives osmotic stress and cold stress in *Synechocystis* and regulates similar patterns of stress-induced genes.[39] Later it was shown that Hik33 also participates in perception of salt stress.[22] It was hypothesized that membrane-integrated Hik33 might sense stress-induced changes in the fluidity of the cytoplasmic membranes.[39] The present results clearly show similarity in the patterns of cold- and salt-inducible gene expression, and suggest the dependence of gene regulation on global stress-induced changes in DNA supercoiling associated with the activity of DNA gyrase.

It seems that most of cold-induced genes require excess of negative supercoiling of DNA for their induction, whereas most of heat-induced genes require high-temperature-induced relaxation of genomic DNA followed by action of the DNA gyrase, which should maintain the degree of supercoiling of DNA on a certain level, appropriate for efficient transcription (Fig. 6). Inhibition of DNA gyrase activity by novobiocin (Table 2, upper part) silences transcription of a number of heat-inducible genes, and enhances transcription of many genes due to excess of DNA relaxation (Figure 6).

It has been demonstrated in *E. coli* that cold shock causes induction and accumulation of the DNA gyrase subunits.[40] Enzymatic or physical twist in DNA destabilizes its duplex structure and low temperatures and, thus, facilitates formation of the open complex by DNA polymerase, which otherwise could have been thermodynamically impossible. Similar situation appears under salt stress conditions. Under heat shock, however, high levels of negative supercoiling may result in a dangerous destabilisation of the double stranded structure of DNA, and DNA relaxation is considered as a general aspect of thermotolerance.[41]

Cold and heat stresses cause antipodal effects on DNA supercoiling. Patterns of cold-inducible and heat-inducible genes never overlap (Fig 6, A). However, inhibition of DNA gyrase activity by novobiocin leads to enhancement of inducibility of some genes, which normally did not respond sharply to temperature. Genes *ziaA, dapA, tatD, asd, sll1862-1863, sll0462, slr0550, slr1927, slr1851* were induced both under cold and heat stress in the presence of novobiocin. It should be noted that very similar pattern of genes was induced under NaCl stress in the presence of novobiocin (Fig. 6, B). Thus, inability of the inhibited DNA gyrase to maintain the proper extent of supercoiling of DNA results in activation of genes (mostly of yet unknown function) by stress, irrespective of its nature.

Conclusion

DNA microarray-based analysis of gene expression demonstrated that novobiocin, which inhibits stress-induced changes in DNA supercoiling, regulated the transcription of many genes that are involved in stress responses in the cyanobacterium. Changes in supercoiling of the genomic DNA might have provided a permissive background for regulatory proteins, which switch on or off the expression of the downstream genes and ensure successful acclimation of cyanobacterial cells to stress conditions.

Materials and methods

Strain and growth conditions

A glucose-tolerant (GT) strain of *Synechocystis* sp. PCC 6803 was kindly provided by Dr. J.G.K. Williams (Du Pont de Nemours, Wilmington, DE, USA). Cells were cultured at 34^{o}C in BG11 medium[24] under continuous illumination with light from incandescent lamps at 70 μmol photons m^{-2} s^{-1} and were aerated with air that contained 1% CO_2. Upon treatment of *Synechocystis* cells with novobiocin to decrease the extent of negative supercoiling of DNA,

cells were incubated for 30 min under growth conditions in the presence of novobiocin at 50 μg ml^{-1} before application of cold, heat or salt stress.

Cold stress was given by placing culture tubes of cells that had been grown at 34°C to a water bath at 24°C. Hyperosmotic stress was achieved by the addition of 5 M sorbitol in BG-11 medium to the final concentration of 0.5 M to cell cultures. Salt stress was provided by the addition of 5 M NaCl in BG-11 medium to the final concentration of 0.5 M. Equal amounts of BG11 medium were added to control cultures to avoid the effect of dilution of the suspension on the gene expression.

RNA isolation and Northern blot hybridization

Total RNA was isolated using the hot-phenol method.[25] RNA was loaded onto 1.2% agarose gels with formaldehyde at 10 μg per lane and resolved by electrophoresis at 50 V for 2 h. Then RNA was blotted onto nylon membranes (GeneScreen; NEN Research Products, Boston, MA, USA) and hybridized with the radioactively labeled DNA fragments obtained by PCR from the genomic DNA with synthetic gene-specific oligonucleotides.

DNA microarray analysis

DNA microarrays of *Synechocystis* (CyanoCHIP ver. 1.6) were purchased from TakaraBio Co. Ltd. (Otsu, Japan). The microarray carried 3079 of the 3264 open reading frames of *Synechocystis*. Cy3 dye- and Cy5 dye-labeled cDNAs used for hybridization were synthesized by reverse transcription of 20 μg of the total RNA.[26,27] Hybridization was conducted at 65°C for 16 h. After incubation the microarrays were rinsed with 2x SSC (1x SSC is 150 mM NaCl and 15 mM sodium citrate) at room temperature, with 2x SSC at 60°C for 10 min, with 0.2x SSC, 0.1% SDS at 60°C for 10 min, and finally with distilled water at room temperature for 2 min. Moisture was removed with an air spray prior to analysis in the array scanner (GMS418; Affimetrix, Woburn, MA). Each signal was quantified with the ImaGene ver. 5.5 software

(BioDiscovery, Los Angeles, CA). To calculate the level of the transcript of each gene, its signal on the microarray was normalized by reference to the total intensity of signals from all genes with the exception of genes for rRNAs. Each experiment was repeated 2 or 3 times. Cluster analysis has been performed with KeggArray built-in software at http://www.genome.jp

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Fig. 1.

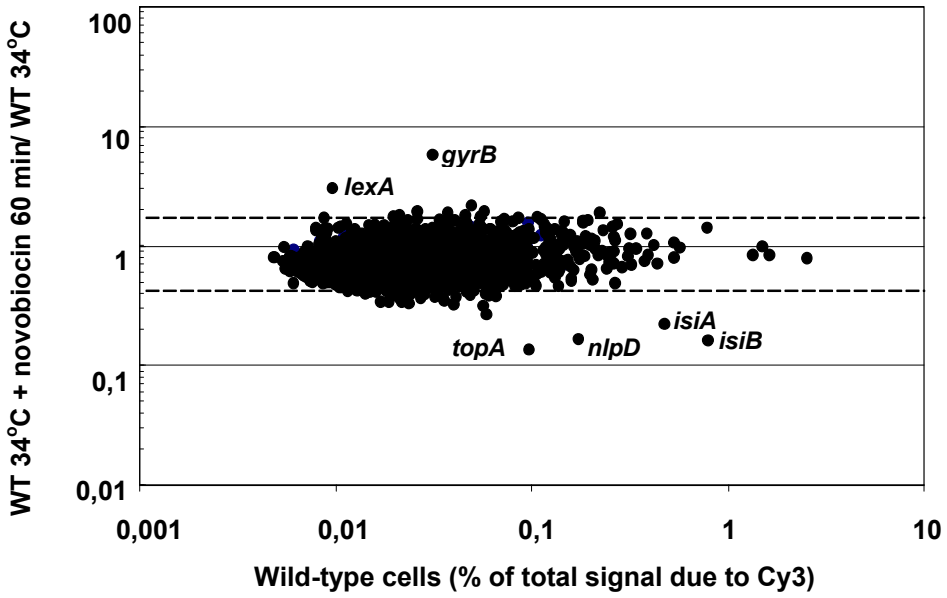

Fig. 1. Microarray analysis of the effect of novobiocin on the expression of genes in *Synechocystis* cells. Cells, which had been grown at 34°C under normal growth conditions (control), were exposed to novobiocin at 50 μg ml^{-1} for 60 min (sample). Genes whose expression was significantly affected are marked.

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Fig. 2.

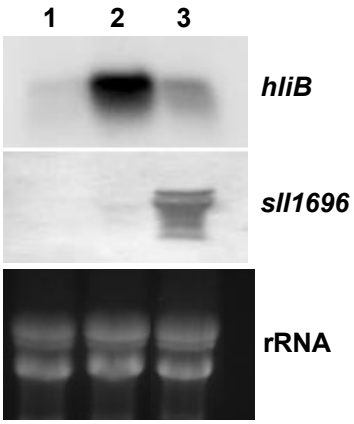

Fig. 2. Cold-induced gene expression examined by northern blotting. Cells were grown at 34^{o}C (1) and incubated for 30 min at 24^{o}C in the absence of novobiocin (2) or in the presence (3) of novobiocin at a final concentration of 50 μg ml^{-1}. Samples were loaded at 30 μg of total RNA per lane.

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Fig. 3.

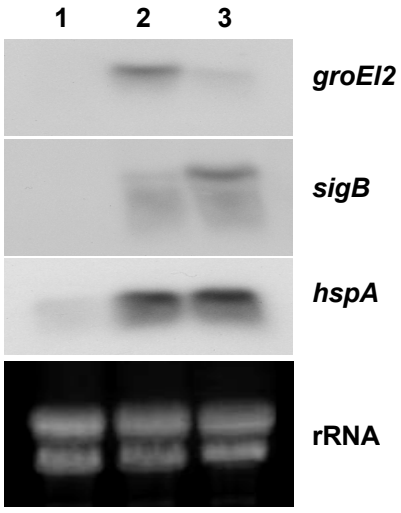

Fig. 3. Heat-induced gene expression examined by northern blotting. Cells were grown at 34^{o}C (1) and incubated for 30 min at 44^{o}C in the absence (2) or in the presence (3) of novobiocin at final concentration of 50 μg ml^{-1}. Samples were loaded at 5 μg of total RNA per lane.

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Fig. 4.

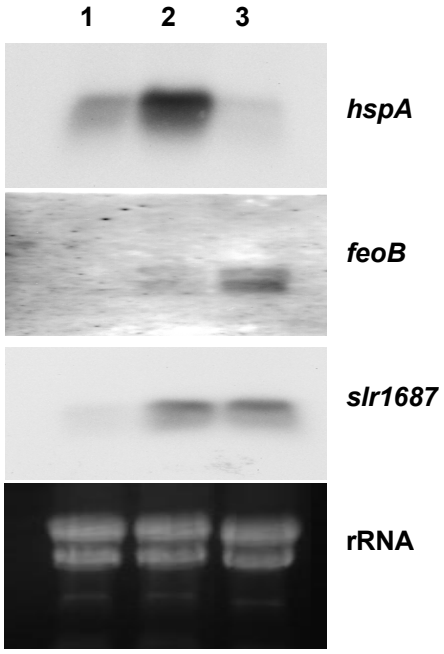

Fig. 4. Salt-induced gene expression examined by northern blotting. Cells were grown at 34°C (1) and incubated for 30 min at 34°C with 0.5 M NaCl in the absence (2) or in the presence (3) of novobiocin at a final concentration of 50 μg ml^{-1}. Samples were loaded at 10 μg of total RNA per lane.

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Fig. 5.

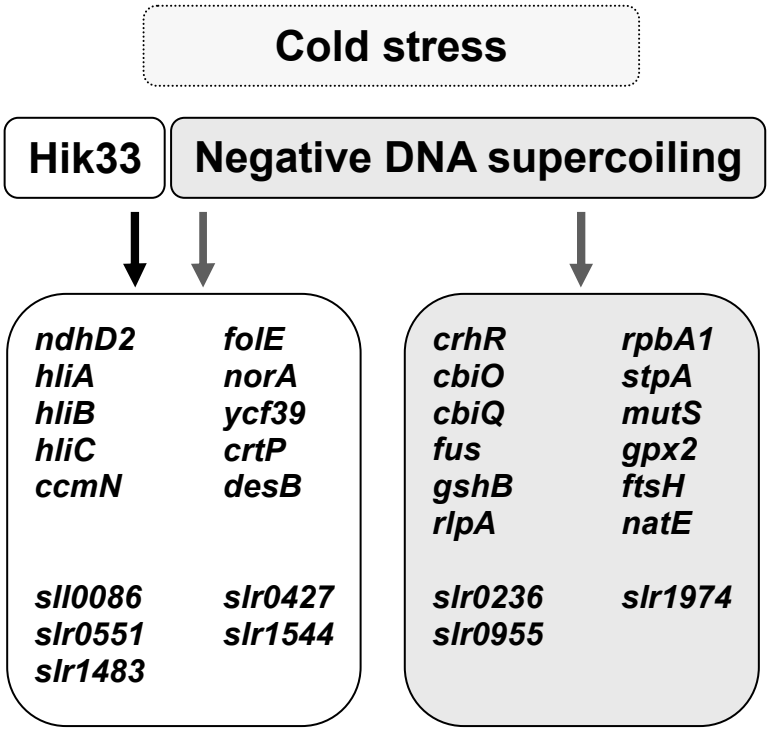

Fig. 5. Cold-induced genes controlled by Hik33 (black arrow) and by changes in DNA supercoiling (gray arrows). Cold-induced changes in DNA supercoiling affect the expression of the Hik33-controlled genes. Genes depicted in gray box are not under control of the sensory histidine kinase Hik33, and they are regulated by DNA supercoiling.

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Fig. 6.

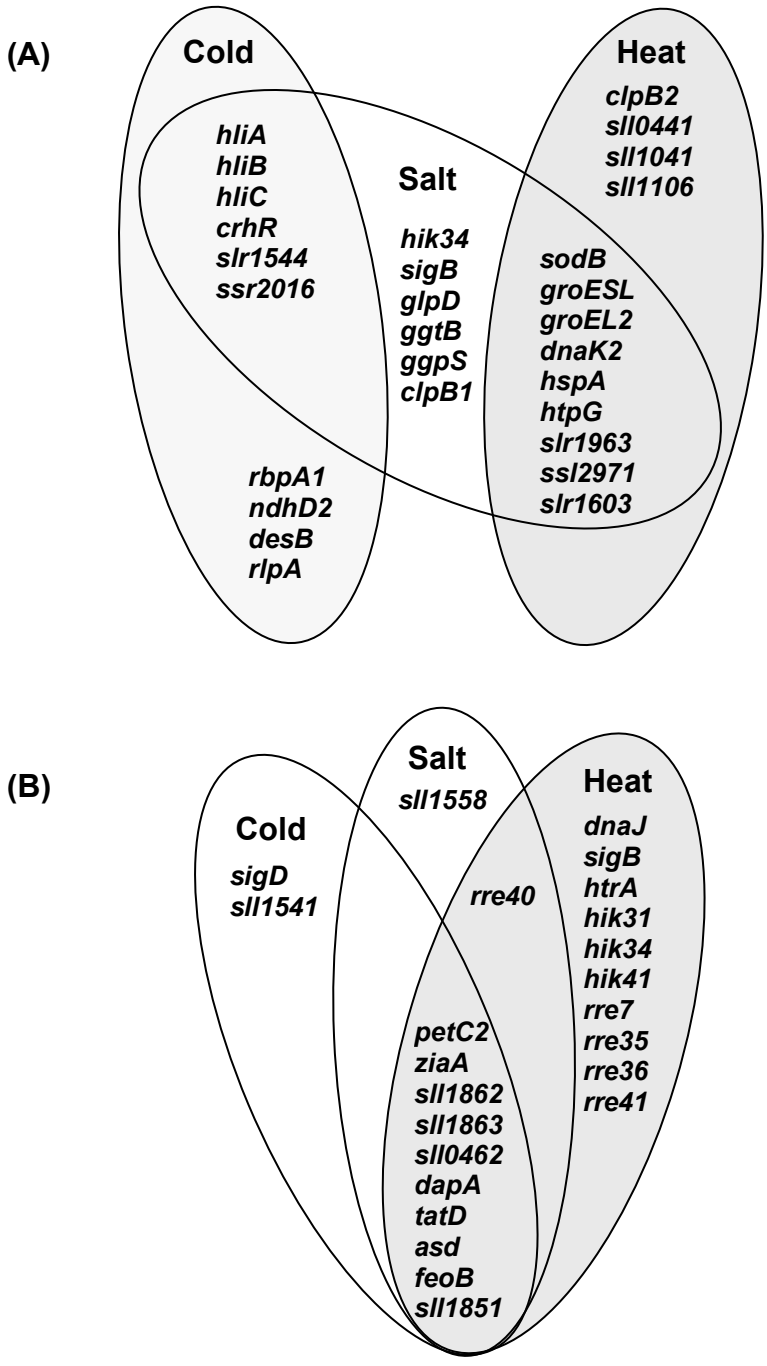

Fig. 6. Genes affected by the increase in negative supercoiling (A) and by relaxation (B) of DNA.

Table 1. The effect of novobiocin on cold-induced gene expression.

ORF no.	Gene	Product	IF -NB	IF +NB
Genes whose cold inducibility was markedly reduced by novobiocin treatment				
slr1291	*ndhD2*	NADH dehydrogenase I	24.4 ± 1.5	12.5 ± 0.2
slr1544		Hypothetical protein	19.3 ± 1.0	5.8 ± 0.4
slr0083	*crhR*	ATP-dependent RNA helicase	14.0 ± 0.7	5.9 ± 0.5
ssr2595	*hliB*	High light inducible protein	8.0 ± 0.2	2.9 ± 0.3
ssl1633	*hliC*	CAB/ELIP/HLIP superfamily	6.4 ± 0.1	2.3 ± 0.2
sll1483		Periplasmic protein	6.4 ± 0.1	3.9 ± 0.1
slr1105	*fus*	Elongation factor EF-G	6.0 ± 0.2	1.4 ± 0.1
sll0517	*rbpA1*	Putative RNA binding protein	5.2 ± 0.4	1.6 ± 0.1
ssr2016		Hypothetical protein	4.9 ± 0.2	2.3 ± 0.1
slr0955		tRNA/rRNA methyltransferase	4.7 ± 0.1	2.2 ± 0.2
sll0384	*cbiQ*	ABC-type cobalt transport permease	4.6 ± 0.1	1.2 ± 0.1
slr0236		Hypothetical protein	4.6 ± 0.1	1.7 ± 0.1
slr0423	*rlpA*	Rare lipoprotein A	4.5 ± 0.3	0.7 ± 0.2
slr0400		Inorganic polyphosphate/ATP-NAD kinase	4.5 ± 0.2	2.5 ± 0.2
sll1911		Hypothetical protein	4.5 ± 0.3	1.6 ± 0.1
slr1254	*crtP*	Phytoene desaturase	4.4 ± 0.1	1.2 ± 0.1
slr0616		Hypothetical protein	4.4 ± 0.1	1.8 ± 0.1
sll0385	*cbiO*	Cobalt transport ATP-binding protein	4.2 ± 0.4	1.8 ± 0.2
slr1436		Hypothetical protein	4.1 ± 0.2	2.8 ± 0.2
slr1974		Putative GTP-binding protein	3.9 ± 0.1	1.6 ± 0.1
sll1770		ABC1-like	3.9 ± 0.1	1.7 ± 0.2
sll1772	*mutS*	DNA mismatch repair protein MutS	3.8 ± 0.3	1.3 ± 0.1
sll0815		Hypothetical protein	3.8 ± 0.1	1.4 ± 0.2
slr0401		Spermidine/putrescine binding protein	3.7 ± 0.6	2.0 ± 0.2
slr1747		Hypothetical protein	3.6 ± 0.1	1.4 ± 0.1
sll0185		Hypothetical protein	3.5 ± 0.2	0.5 ± 0.1
slr1992	*gpx2*	Glutathione NADPH peroxidase	3.4 ± 0.2	0.9 ± 0.1
slr1238	*gshB*	Glutathione synthetase	3.4 ± 0.1	1.9 ± 0.1
sll1441	*desB*	Acyl-lipid desaturase (omega-3)	3.6 ± 0.1	0.4 ± 0.1
Genes whose cold inducibility was enhanced by novobiocin treatment				
sll1862		Hypothetical protein	3.2 ± 0.1	31.9 ± 2.1
sll1863		Hypothetical protein	3.6 ± 0.2	25.7 ± 1.7
slr1392	*feoB*	Ferrous iron transport protein B	4.3 ± 0.2	21.5 ± 0.4
slr1185	*petC2*	Rieske iron sulfur protein	1.2 ± 0.1	17.0 ± 0.8
sll1541		Lignostilbene-alpha,beta-dioxygenase	6.5 ± 0.1	14.5 ± 1.0
sll1696		Hypothetical protein	1.7 ± 0.1	14.0 ± 0.7
sll0462		Hypothetical protein	2.4 ± 0.2	13.8 ± 0.5

slr1927		Hypothetical protein	5.3 ± 0.3	11.7 ± 2.0
slr0550	*dapA*	Dihydrodipicolinate synthase	2.5 ± 0.2	11.5 ± 0.3
slr1851		Hypothetical protein	0.8 ± 0.1	11.2 ± 0.6
slr0798	*ziaA*	Zinc-transporting P-type ATPase	2.4 ± 0.2	9.2 ± 0.4
sll0360		Hypothetical protein	2.9 ± 0.1	9.0 ± 0.8
sll2012	*sigD*	RNA polymerase sigma factor	4.1 ± 0.1	8.9 ± 0.5
sll0790	*hik31*	Two-component sensor histidine kinase	2.6 ± 0.4	8.8 ± 0.5
sll1786	*tatD*	Putative deoxyribonuclease, TatD	2.0 ± 0.2	8.6 ± 0.4
slr0549	*asd*	Aspartate semialdehyde dehydrogenese	2.3 ± 0.3	8.0 ± 0.4
sll1124	*hik3*	Two-component sensor histidine kinase	2.0 ± 0.1	5.9 ± 0.2
sll1694	*pilA1*	Cyanobacterial pilin	0.8 ± 0.1	4.1 ± 0.4

Cells, which had been grown under normal conditions and then incubated in the presence (50 μg ml^{-1}) or absence of novobiocin at 34°C for 30 min, were incubated at 24°C for 30 min. Each value indicates the ratio of the level of the transcript in cold-stressed cells in the presence of novobiocin to that in cold-stressed cells in the absence of novobiocin. The numbering of open reading frames (ORFs) corresponds to that in the database on the Cyanobase website (http://bacteria.kazusa.or.jp/cyano/Synechocystis/). This table lists the heat stress-inducible genes with induction factors higher than 3.0 in control cells (average of values from 2-3 independent experiments). The entire list can be accessed at http://www.genome.jp/kegg-bin/get_htext?htext=Exp_DB&hier=1. Genes that had been used as probes in Northern blotting are underlined. IF – induction factor; NB – novobiocin. The table represents the results of two independent experiments.

Table 2. The effect of novobiocin on heat-induced gene expression

ORF	gene	Product	IF -NB	IF +NB
Genes whose heat inducibility was markedly reduced by novobiocin treatment				
sll0430	*htpG*	HtpG, heat shock protein 90	43.2 ± 2.1	10.2 ± 1.4
slr2075	*groES*	10 kD chaperonin, GroES protein	30.2 ± 1.1	5.4 ± 0.4
slr2076	*groEL1*	60 kDa chaperonin 1, GroEL1	23.4 ± 3.0	4.4 ± 0.4
sll0416	*groEL2*	60 kDa chaperonin 2, GroEL2	16.7 ± 2.5	3.6 ± 0.2
sll0170	*dnaK2*	DnaK protein 2, heat shock protein 70	15.5 ± 1.5	9.6 ± 0.2
slr1963		Water-soluble carotenoid protein	12.9 ± 1.5	5.7 ± 0.2
slr1516	*sodB*	Superoxide dismutase	10.6 ± 2.0	4.5 ± 0.2
sll0441		Hypothetical protein	9.4 ± 0.7	1.2 ± 0.1
ssl2971		Hypothetical protein	9.2 ± 0.5	4.2 ± 0.3
sll1041		ABC transporter ATP-binding protein	8.3 ± 0.8	0.9 ± 0.1
sll1106		Hypothetical protein	6.6 ± 0.3	1.0 ± 0.1
slr1603		Hypothetical protein	6.1 ± 0.4	3.9 ± 0.4
sll1621		Membrane protein	6.0 ± 0.2	1.6 ± 0.2
slr0156	*clpB2*	ClpB protein	4.8 ± 0.3	0.8 ± 0.1
slr1634		Hypothetical protein	4.3 ± 0.2	0.2 ± 0.1
sll0575	*rfbB*	Export system ATP-binding protein	4.3 ± 0.2	0.8 ± 0.1
Genes whose heat inducibility was enhanced by novobiocin treatment				
slr0093	*dnaJ*	DnaJ protein, molecular chaperone	10.3 ± 0.7	88.4 ± 3.0
slr1674		Hypothetical protein	22.6 ± 2.1	40.9 ± 2.6
slr0095		O-methyltransferase	3.7 ± 0.2	40.6 ± 3.5
sll1652		Hypothetical protein	1.9 ± 0.1	41.0 ± 2.5
sll0939		Hypothetical protein	3.0 ± 0.1	36.2 ± 2.8
sll0843		Hypothetical protein	1.9 ± 0.1	33.3 ± 3.1
slr1675	*hypA1*	Putative hydrogenase	15.9 ± 1.0	32.1 ± 3.0
slr0798	*ziaA*	Zinc-transporting P-type ATPase	0.9 ± 0.2	31.2 ± 2.0
sll0306	*sigB*	RNA polymerase group 2 sigma factor	11.5 ± 1.0	26.8 ± 2.2
sll0938		N-succinyldiaminopimelate aminotransferase	1.3 ± 0.3	26.0 ± 1.4
slr0272		Hypothetical protein	1.2 ± 0.1	25.5 ± 2.3
slr2037		Hypothetical protein	1.0 ± 0.1	20.8 ± 1.4
slr1185	*petC2*	Rieske iron sulfur protein	1.0 ± 0.1	19.2 ± 0.8
slr1398		Hypothetical protein	1.2 ± 0.2	19.2 ± 1.6
slr1851		Hypothetical protein	1.7 ± 0.1	18.9 ± 1.1
sll1549		Salt-enhanced periplasmic protein	1.5 ± 0.2	18.5 ± 1.0
slr1204	*htrA*	Serine protease HtrA	5.6 ± 0.4	18.2 ± 1.2
sll1863		Hypothetical protein	1.2 ± 0.1	18.2 ± 1.2
slr0967		Hypothetical protein	5.1 ± 0.4	16.8 ± 2.1
slr1915		Hypothetical protein	6.8 ± 0.8	15.7 ± 1.4
slr1413		Hypothetical protein	4.4 ± 0.2	18.0 ± 2.4
slr0271		Hypothetical protein	1.6 ± 0.3	17.2 ± 1.3
sll0790	*hik31*	Sensor histidine kinase Hik31	0.7 ± 0.1	16.0 ± 1.1
sll1862		Hypothetical protein	1.4 ± 0.2	15.2 ± 0.5
sll0528		Hypothetical protein	6.3 ± 0.5	14.1 ± 1.1
sll0877		Hypothetical protein	1.1 ± 0.1	13.9 ± 1.0
sll0038	*rre36*	Two-component response regulator	0.9 ± 0.1	13.3 ± 2.1
slr1916		Esterase	1.5 ± 0.1	13.2 ± 0.5
sll1849		Hypothetical protein	1.2 ± 0.3	13.1 ± 1.0
ssl3769		Hypothetical protein	0.8 ± 0.1	12.6 ± 2.0

slr0518	*abfB*	Arabinofuranosidase	1.6 ± 0.2	12.2 ± 0.9
sll0360		Hypothetical protein	1.1 ± 0.2	10.8 ± 0.5
slr1676		Hypothetical protein	1.5 ± 0.3	11.4 ± 0.7
slr1927		Hypothetical protein	0.5 ± 0.1	10.6 ± 0.5
slr0549	*asd*	Aspartate beta-semialdehyde dehydrogenese	0.7 ± 0.1	10.2 ± 0.8
slr1906		Hypothetical protein	0.9 ± 0.2	10.0 ± 0.5
slr0210	*hik9*	Sensor histidine kinase Hik9	1.1 ± 0.1	9.9 ± 0.8
slr1245		Transcriptional regulator	1.1 ± 0.2	9.6 ± 0.8
slr0550	*dapA*	Dihydrodipicolinate synthase	0.6 ± 0.2	8.5 ± 0.5
sll0462		Hypothetical protein	0.6 ± 0.1	8.2 ± 0.3
slr1042	*rre7*	Two-component response regulator	1.0 ± 0.1	6.8 ± 0.2
slr1285	*hik34*	Sensor histidine kinase Hik34	3.1 ± 0.2	6.5 ± 0.2
sll0039	*rre35*	Two-component response regulator	0.7 ± 0.1	5.7 ± 0.2
sll1786	*tatD*	Putative deoxyribonuclease, TatD	0.3 ± 0.1	4.3 ± 0.1
slr1305	*rre41*	Two-component response regulator	1.1 ± 0.2	3.8 ± 0.3
Genes whose heat inducibility was not affected by novobiocin treatment				
sll1514	*hspA*	16.6 kDa small heat shock protein	66.2 ± 3.2	52.8 ± 2.5
slr1641	*clpB1*	ClpB protein	58.9 ± 3.0	40.2 ± 4.1
sll0846		Hypothetical protein	17.0 ± 2.0	15.9 ± 0.9
slr0008	*ctpA*	Carboxyl-terminal processing protease	4.8 ± 0.4	4.4 ± 0.2

Cells, which had been grown under normal conditions and then incubated in the presence (50 μg ml^{-1}) or absence of novobiocin at 34°C for 30 min, were incubated at 44°C for 30 min. Each value indicates the ratio of the level of the transcript in heat-stressed cells in the presence of novobiocin to that in heat-stressed cells in the absence of novobiocin. The numbering of open reading frames (ORFs) corresponds to that in the database on the Cyanobase website (http://bacteria.kazusa.or.jp/cyano/Synechocystis/). This table lists the heat stress-inducible genes with induction factors higher than 4.0 in control cells (average of values from 2-3 independent experiments). The entire list can be accessed at http://www.genome.jp/kegg-bin/get_htext?htext=Exp_DB&hier=1. Genes that had been used as probes in Northern blotting are underlined. IF – induction factor; NB – novobiocin. The table represents the results of two independent experiments.

Table 3. The effect of novobiocin on NaCl-induced gene expression

ORF no.	Gene	Product	IF - NB	IF + NB
Genes whose NaCl inducibility was markedly reduced by novobiocin treatment				
sll1514	*hspA*	16.6 kDa small heat shock protein	70.2 ± 2.1	15.1 ± 0.6
sll0528		Hypothetical protein	69.5 ± 2.5	4.4 ± 0.6
sll0939		Hypothetical protein	51.9 ± 3.0	9.4 ± 0.7
slr1285	*hik34*	Histidine kinase Hik34	44.0 ± 3.4	1.6 ± 0.4
slr0967		Hypothetical protein	42.0 ± 1.5	5.5 ± 0.3
slr1544		Hypothetical protein	25.7 ± 1.1	2.1 ± 0.1
sll0846		Hypothetical protein	25.7 ± 1.0	5.3 ± 0.1
slr1963		Water-soluble carotenoid protein	22.8 ± 0.7	1.0 ± 0.1
slr1641	*clpB1*	ClpB protein	22.5 ± 0.5	2.4 ± 0.1
sll0306	*sigB*	RNA polymerase sigma factor	21.4 ± 1.5	2.2 ± 0.1
slr1603		Hypothetical protein	20.4 ± 1.5	1.0 ± 0.1
slr0959		Hypothetical protein	18.1 ± 0.9	5.9 ± 0.3
sll1722		Hypothetical protein	16.6 ± 0.4	1.6 ± 0.2
sll1167	*pbp*	Penicillin-binding protein 4	15.9 ± 0.3	1.4 ± 0.3
slr1516	*sodB*	Superoxide dismutase	15.5 ± 0.4	2.2 ± 0.3
sll1594	*ndhR*	Transcriptional regulator	15.0 ± 1.2	0.7 ± 0.1
slr1971		Hypothetical protein	13.7 ± 0.7	2.4 ± 0.2
slr1704		Hypothetical protein	13.3 ± 0.5	3.3 ± 0.2
sll1085	*glpD*	Glycerol-3-P dehydrogenase	12.8 ± 0.5	2.0 ± 0.2
ssl2542	*hliA*	High light inducible protein	12.6 ± 0.3	7.1 ± 0.1
slr1915		Hypothetical protein	12.6 ± 0.3	4.1 ± 0.3
sll1483		Periplasmic protein	11.6 ± 1.2	2.9 ± 0.1
sll0170	*dnaK2*	Heat shock protein 70	11.5 ± 0.7	0.9 ± 0.1
sll1884		Hypothetical protein	11.1 ± 0.4	1.3 ± 0.1
slr1686		Hypothetical protein	11.0 ± 0.4	4.9 ± 0.2
ssr2595	*hliB*	High light inducible protein	10.9 ± 0.4	2.1 ± 0.2
slr1485		Phosphatidylinositol phosphate kinase	10.5 ± 0.3	4.3 ± 0.2
sll0938		N-Succinyldiaminopimelate aminotransferase	10.3 ± 0.5	6.0 ± 0.4
sll1797	*ycf21*	Ycf21 gene product	9.8 ± 0.4	1.3 ± 0.1
sll1330	*rre37*	Two-component response regulator	9.5 ± 0.3	1.5 ± 0.1
slr0529	*ggtB*	Glucosylglycerol transport system	9.0 ± 0.2	1.3 ± 0.1
sll1652		Hypothetical protein	8.8 ± 0.2	3.3 ± 0.1
sll1566	*ggpS*	Glucosylglycerol-phosphate synthase	8.8 ± 1.1	1.1 ± 0.1
ssl2971		Hypothetical protein	8.7 ± 0.6	2.0 ± 0.1
slr0581		Hypothetical protein	8.6 ± 0.3	1.8 ± 0.1
slr1738		Hypothetical protein	8.1 ± 0.3	0.9 ± 0.1
sll1773		Hypothetical protein	7.8 ± 0.4	0.6 ± 0.1
sll1086		Hypothetical protein	7.7 ± 0.5	3.1 ± 0.1
sll1621		Membrane protein	7.3 ± 0.3	1.1 ± 0.1

sll1965		Hypothetical protein	7.3 ± 0.7	1.3 ± 0.3
sll1723		Hypothetical protein	6.8 ± 0.5	1.2 ± 0.2
sll1620		Hypothetical protein	6.7 ± 0.5	0.8 ± 0.2
slr0251	*ycf85*	ABC transporter ATP-binding protein	6.3 ± 0.2	1.0 ± 0.2
sll0416	*groEL2*	60 kDa chaperonin 2, GroEL2	6.2 ± 0.2	0.6 ± 0.1
sll1549		Salt-enhanced periplasmic protein	6.2 ± 0.3	1.6 ± 0.2
slr1192		Alcohol dehydrogenase family	5.9 ± 0.2	1.2 ± 0.1
slr0530	*ggtC*	Glucosylglycerol transport system	5.9 ± 0.4	1.5 ± 0.2
slr2006		Hypothetical protein	5.9 ± 0.2	1.4 ± 0.1
slr0083	*crhR*	ATP-dependent RNA helicase	5.8 ± 0.2	2.9 ± 0.1
sll1724	*icsA*	LPS glycosyltransferase IcsA	5.7 ± 0.2	1.7 ± 0.1
sll0005		Hypothetical protein	5.7 ± 0.6	0.8 ± 0.1
slr0853	*rimI*	Ribosomal alanine acetyltransferase	5.6 ± 0.9	1.5 ± 0.1
slr0974	*infC*	Translation initiation factor IF-3	5.5 ± 0.3	1.2 ± 0.2
ssl1633	*hliC*	CAB/ELIP/HLIP superfamily	5.4 ± 0.3	1.9 ± 0.2
sll0169		Hypothetical protein	5.4 ± 0.4	0.5 ± 0.2
slr0589		Hypothetical protein	5.4 ± 0.2	0.9 ± 0.2
slr1894		Hypothetical protein	5.4 ± 0.2	1.6 ± 0.1
slr0746	*stpA*	Glucosylglycerolphosphate phosphatase	5.1 ± 0.2	1.6 ± 0.1
sll1491		Periplasmic WD-repeat protein	5.0 ± 0.5	0.6 ± 0.1
Genes whose NaCl inducibility was markedly enhanced by novobiocin treatment				
sll1862		Hypothetical protein	172.1 ± 5.5	372.3 ± 5.0
sll1863		Hypothetical protein	166.5 ± 4.0	267.6 ± 4.2
sll0462		Hypothetical protein	1.8 ± 0.2	23.0 ± 1.1
slr1927		Hypothetical protein	2.4 ± 0.2	16.2 ± 0.4
slr0798	*ziaA*	Zinc-transporting P-type ATPase	4.3 ± 0.4	13.2 ± 0.8
slr1851		Hypothetical protein	2.5 ± 0.2	11.8 ± 0.4
sll1558		Mannose-1-phosphate guanyltransferase	4.3 ± 0.4	11.3 ± 0.9
slr1392	*feoB*	Ferrous iron transport protein B	4.6 ± 0.3	11.2 ± 1.1
sll1544	*rre40*	two-component response regulator	1.1 ± 0.1	9.1 ± 0.9
slr0550	*dapA*	Dihydrodipicolinate synthase	1.4 ± 0.2	7.0 ± 1.1
Genes whose NaCl inducibility was not affected by novobiocin treatment				
slr1687		Hypothetical protein	15.7 ± 2.1	13.8 ± 2.0
slr1204	*htrA*	Serine protease HtrA	11.4 ± 1.5	7.5 ± 0.5
slr0093	*dnaJ*	DnaJ protein. heat shock protein	9.9 ± 0.5	12.8 ± 2.6
slr1916		Esterase	7.7 ± 0.4	5.5 ± 0.5
sll1653	*menG*	Phylloquinone methlytransferase	6.5 ± 0.3	4.4 ± 0.6
slr0095		O-Methyltransferase	6.0 ± 0.9	7.8 ± 0.6

Cells, which had been grown under normal conditions and then incubated in the presence (50 μg ml^{-1}) or absence of novobiocin for 30 min, were incubated with 0.5 M NaCl for 30 min.

Each value indicates the ratio of the level of the transcript in salt-stressed cells in the presence of novobiocin to that in salt stressed cells in the absence of novobiocin. The numbering of open reading frames (ORFs) corresponds to that in the database on the Cyanobase website (http://bacteria.kazusa.or.jp/cyano/Synechocystis/). This table lists the salt stress-inducible genes with induction factors higher than 4.0 in control cells (average of values from 2-3 independent experiments). The entire list can be accessed at http://www.genome.jp/kegg-bin/get_htext?htext=Exp_DB&hier=1. Genes that had been used as probes in Northern blotting are underlined. IF – induction factor; NB – novobiocin. The table represents the results of two independent experiments.