Signal transduction pathways in Synechocystis sp. PCC 6803 and biotechnological implications under abiotic stress


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Introduction

Organisms, such as prokaryotes, plants, and fish, can acclimate to natural habitats, being more or less capable of sensing changes in the ambient environment and expressing a large numbers of genes. Moreover, those genes give occasion to synthesize specific proteins and metabolites for protection against adversities, such as low-temperature (LT) (Murata & Los, 2006) and hyper-osmotic stress (HO) (Paithoonrangsarid et al., 2004). Considerable findings have indicated that the pathways of transduction among stress signals are regulated by two-component systems in prokaryotes (Sakamoto & Murata, 2002; Suzuki et al., 2000), and that histidine kinases (Hiks) are important sensors in the perception and transduction of abiotic signals in bacteria, cyanobacteria, fungi, and plants (West & Stock, 2001). Moreover, some Hiks perceive stress signals initially as the physical state of membrane lipids changes in unicellular and multi-cellular organisms under LT and HO (Mikami et al., 2002; Paithoonrangsarid et al., 2004). In addition, many studies strongly suggest that serine/threonine kinase (STK) genes in cyanobacteria play essential roles in regulating cellular activities (Han & Zhang, 2001; Yang et al., 2013).

Cyanobacteria feature both bacteria and plants, which makes it an important objective for study of signal transduction systems. The studies on signal transduction systems in cyanobacteria, including two-component signal transduction system and eukaryotic-type STKs, may provide important clues for understanding the stresses in higher plants. unicellular cyanobacteria *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) has become a model cyanobacteria for studying the stresses in biochemistry and molecular biology. Most importantly, the complete nucleotide sequence of the *Synechocystis* genome was determined in 1996 (Kaneko et al., 1996a), and of its plasmids (including 397 genes) in 2003 (Kato et al., 2003). The latter genes were not commonly included in DNA microarrays. At present, functions of some Hiks and STKs have been characterized for providing important information of the construction of libraries of mutants with mutated genes encoding Hiks and STKs. Furthermore, analyses of the stress-inducible genes whose expression are affected by systematic mutagenesis of various signal transduction components, in combination with DNA microarray analyses on genome-wide expression of genes, could help us identify more details of the mechanisms of the perception and signal transduction under abiotic stresses in *Synechocystis*. It includes stress-specific signal sensors, associating transducers, and response regulators (Rres), as well as the co-regulation and crosstalk in various pathways.
Hiks of two-component systems in Synechocystis

Totally, 44 and 3 putative genes encoding Hiks are predicted in the Synechocystis genome (Hik1-44) (Kaneko et al., 1996b; Mizuno et al., 1996) and plasmids (Kaneko et al., 2003), respectively. There are 42 putative genes encoding Rres on the chromosome and 3 on the plasmids. These 47 Hiks and 45 Rres are systematically activated as sensors and transducers in the perception of abiotic signals.

From the whole list of Synechocystis open reading frames (ORFs), 26 sensor kinases have been found, including a transmitter, 16 hybrid sensor kinases (HRs) containing both a transmitter and a receiver, and 38 Rres containing a receiver (Mizuno et al., 1996) (Table 1).

STKs in Synechocystis

Many prokaryotes have been found to harbor STKs and serine/threonine (Ser/Thr) protein phosphatases as their accessory components (Zhang et al., 2007). In Synechocystis genome, STKs has been confirmed by comparing deduced amino acid sequences encoded by open-reading frames with other known amino acid sequences of eukaryotic protein kinases (Mizuno et al., 1996; Zhang et al., 1998b). Furthermore, STKs in Synechocystis are genuine prokaryotic enzymes since their corresponding genes show similar GC content and codon usage as the averages of all possible (ORFs) deduced from the genome (Han & Zhang, 2001). In most cases, cyanobacterial STKs play secondary roles in the acclimation to abiotic changes in Synechocystis.

Canonical STKs contain 12 conserved sub-domains as per Hanks & Hunter (1995), and they fold up to form a common catalytic core, as depicted by 3-dimensional structures of several protein-serine kinases (Zhang et al., 2007). The 12 conserved sequence motifs, about 280bp in length, are named as sub-domains I-V, VIa, VIb, and VII-XI (Hanks & Hunter, 1995). In addition, they are classified into three structural sub-domains with separate roles: N-terminal nucleotide-binding domains containing sub-domains I-IV, C-terminal phosphotransfer and protein–substrate-binding domains containing sub-domains V-VII, and the intervening linker containing a sub-domain V (Kennely, 2003). Additional to the conserved catalytic domains, some STKs have at least one additional domain enforcing STKs with more complicated functions, such as FHA, WD40, PAS, and GAF (Wang et al., 2002). For instance, the FHA domain can participate in a wide range of processes in bacteria, such as intracellular signal transduction, transcription, protein transport, DNA repair, and protein degradation (Zhang et al., 2007). Furthermore, there is a near-perfect correlation between the presence of FHA-containing proteins and STKs and phosphatases in bacterial genomes (Durocher & Jackson, 2002).

Among the 12 putative genes encoding STKs in Synechocystis, seven can encode proteins that belong to the PKN2 subfamily of STKs, namely, spkA (slr1574-75), spkB (slr1697), spkC (slr0599), spkD (slr0776), spkE (slr1443), spkF (slr1225), and spkG (slr0152) (CyanoBase; http://www.kazusa.or.jp/cyano), while five encode proteins (spkH, etc.) of the ABC1 subfamily of STKs (Shi et al., 1998) (Table 2).

Moreover, five ORFs (slr1365, slr0114, slr1860 (icfG), slr1983, and slr2031) encode proteins similar to PPM-family Ser/Thr phosphatases. They all possess a conserved catalytic domain at C-terminal end, with signatures critical to the activity of PPM-type protein phosphatases (Barford, 1996). In addition, all SpkC, SpkD, SpkE, and SpkG have one putative transmembrane segment, while SpkF has four at their C-terminal regions, which makes these STKs localized at membranes as internal signal sequences. Other seven STKs with several putative transmembrane segments could act as membrane receptors in signal transduction (Zhang et al., 1998b).

SpkA, SpkB, SpkC, SpkD, and SpkF were demonstrated as autophosphorylation and phosphorylation of general substrate proteins (Kamei et al., 2001, 2002, 2003). However, SpkE did not show any protein kinase activities, because of a finding consistent with its lack of several key amino acid residues in its kinase motif (Kamei et al., 2002). Studies on STK in Synechocystis are limited. Only SpkA and SpkB were confirmed vital to cellular motility via phosphorylation of membrane proteins (Kamei et al., 2001, 2003). Meanwhile, SpkA activity is essential to the formation of thick pili (Panitchkina et al., 2006). SpkE may be the first one found to play an important role in the post-translational modification of pilin for pili assembly (Kim et al., 2004), and is probably involved in the regulation of nitrogen metabolism (Galkin et al., 2003). SpkC is neither expressive nor inactive under normal conditions, but the ΔspkC mutant strain is sensitive to high-temperature stress (HT) (Personal communication with Zhang XW). However, SpkD is essential for survival of a species and cannot be removed completely (Kamei et al., 2002). Nevertheless, spkD could be strongly affected by NdhR under inorganic carbon deficiency (CiD) (Wang et al., 2004). In our first study, spkG plays as the sensor of high-salt stress (HS) signals directly (Liang et al., 2011), though spkG was found highly down-regulated in iron deficiency under peroxide stress (Singh et al., 2004). However, no expression of spkF could be detected under any condition. In addition, spkH (slr0005) was found responsible to HO, and regulated by a Hik34-Rre1 two-component system (Paithoonrasarid et al., 2004). SpkC, SpkF, and SpkK work one after another to finally phosphorylate the GroES (Zorina et al., 2011).

Summaries of signal transduction under various abiotic stresses

At present, more pathways of stress-induced signal transduction under abiotic stresses are being studied. Hereafter, we summarize signal transduction under abiotic conditions, including LT, HS, HO, hydrogen peroxide stress (HP), high-light stress (HL), HT, and other stresses (phosphate deficiency, PD; CiD; excess nickel ions, ENi; Mn2⁺ deficiency, MnD; K⁺ deficiency, KD; and circadian rhythm, CY etc.).

Signal transduction under LT

LT signals were transferred from Hik33 to Rre26, which regulated the expression of 21 genes including ndhD2, hliA, hliB, hliC, feoB, crp, and genes for proteins with unknown functions (Murata & Los, 2006). Therefore, Hik33 is an important participant in LT-signal transduction, depending on.
the membrane rigidity. Moreover, there were at least two other LT sensors as homologues of DesK and DesR in *Bacillus subtilis*, one depending on membrane rigidity, and another one functions independently in the saturation of membrane lipids (Inaba et al., 2003; Los & Murata, 2004; Mansilla & de Mendoza, 2005).

Moreover, *hik13* (*sll1003*; designations of ORFs) and *hik15* (*sll1353*) genes might be essential for growth under...
Table 2. STKs identified in the genome.

<table>
<thead>
<tr>
<th>ORF</th>
<th>Locus</th>
<th>Family</th>
<th>Structure feature</th>
<th>Physiological role</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>sll1574-75</td>
<td>SpkA</td>
<td>cbSTKI-other</td>
<td>–</td>
<td>Formation of thick pili, cell motility</td>
<td>(Kamei et al., 2001; Panichkin et al., 2006)</td>
</tr>
<tr>
<td>slr1697</td>
<td>SpkB</td>
<td>cbSTKII</td>
<td>Cys motif, pentapeptide repeats</td>
<td>Cell motility</td>
<td>(Kamei et al., 2003)</td>
</tr>
<tr>
<td>slr0599</td>
<td>SpkC</td>
<td>cbSTKI-other</td>
<td>Pro rich HT, phosphorylation of GroES</td>
<td></td>
<td>(Zorina et al., 2011)</td>
</tr>
<tr>
<td>sll0776</td>
<td>SpkD</td>
<td>cbSTKI-other</td>
<td>–</td>
<td>Post-translational modification of pilin, nitrogen metabolism</td>
<td>(Galkin et al., 2003; Kim et al., 2004)</td>
</tr>
<tr>
<td>slr1225</td>
<td>SpkE</td>
<td>cbSTKI-TM</td>
<td>Cys motif, TM motif</td>
<td>Phosphorylation of GroES</td>
<td>(Zorina et al., 2011)</td>
</tr>
<tr>
<td>slr0152</td>
<td>SpkG</td>
<td>cbSTKI-other</td>
<td>HS</td>
<td>Cell growth under CiD in the presence of glucose</td>
<td>(Liang et al., 2011)</td>
</tr>
<tr>
<td>sll0005</td>
<td>ABC1-like</td>
<td>HO</td>
<td></td>
<td></td>
<td>(Paithoonrangsarid et al., 2004)</td>
</tr>
<tr>
<td>sll1365</td>
<td>PPM3</td>
<td>PPM family</td>
<td>Sensor domain linked to a protein phosphatase domain</td>
<td>Membrane receptors (sensors)</td>
<td>(Zhang et al., 1998b)</td>
</tr>
<tr>
<td>sll1033</td>
<td>PPM family</td>
<td>PPM family</td>
<td>Dephosphorylation of phosphotyrosine-/phosphoserine-containing proteins</td>
<td></td>
<td>(Li et al., 2005)</td>
</tr>
<tr>
<td>sll1387</td>
<td>PPM family</td>
<td>PPP family</td>
<td>Dephosphorylation of phosphoserine-containing proteins</td>
<td></td>
<td>(Li et al., 2005)</td>
</tr>
<tr>
<td>slr0114</td>
<td>ICFG</td>
<td>PPM family</td>
<td>A sensor domain linked to a protein phosphatase domain</td>
<td>Sense light and redox</td>
<td>(Zhang et al., 1998b)</td>
</tr>
<tr>
<td>slr1860</td>
<td>ICFG</td>
<td>PPM family</td>
<td>A GAF domain at N-terminal end</td>
<td></td>
<td>(Zhang et al., 1998b)</td>
</tr>
</tbody>
</table>

LT (Kaneko et al., 1996a; Suzuki et al., 2000a). Hik2 and Rre1 might be the second system to respond to LT signals (Puthiyaveetil, 2009), while other 15 LT-inducible genes were not regulated by Hik33, showing that there were some other unknown sensors and Rres involved in signal transduction under LT. In addition, Hik2 and Hik33 were proposed to be involved in the tolerance of photosystem II to abiotic stress in *Synechocystis* (Puthiyaveetil, 2009).

**Signal transduction under HO and HS**

Currently, five Hik-Rre systems have been identified: Hik33-Rre31, Hik10-Rre3, Hik16-Hik41-Rre17, Hik34-Rre1, and Hik2-Rre1; they appeared to be involved in perception/transduction of HO/HS signals, respectively (Paithoonrangsarid et al., 2004) (Figure 1b and c).

The Hik33-Rre31 system regulates the expression of *fabG*, *gloA*, and *ssl3446* as HO-specific genes, and fully (23%) or partially (58%) contributes to the expression of all osmotsress-inducible genes (Mikami et al., 2002) (Figure 1b). Separately under HS and HO, Hik10 and Rre3 constitute a two-component system that regulates the expression of only *hirA*, a gene for Ser protease. Hik16 and Hik41 act probably as a sensor and an intermediate signal transducer, and then Rre17 is responsible for the transcriptional induction of two stress-inducible genes *sll0938* and *str0967* with unknown functions, and that of *sll0938* as a HS-specific gene. The Hik34-Rre1 system regulates the expression of 19 genes, including some genes of heat shock proteins, and *ssl1107* as a HS-specific gene and *hpsG* as a HO-specific gene (Los et al., 2010) (Figure 1c). The Hik2-Rre1 system is responsible for the induction of *sigB* (coding for an alternative σ subunit of RNA polymerase), and for three other genes with unknown functions under both stresses (Novikova et al., 2007).

Sixty genes have been found to be expressed differently in wild strains and *SpkG* mutants for transport, energy metabolism, protein processing, envelope biogenesis and signal transduction, by analyzing global gene expression in both the *SpkG* mutants and wild strains under normal and HS conditions (Liang et al., 2011). Moreover, it was found that the regulation expression of particular sets of genes by HS is controlled by *spkG* (Liang et al., 2011). *SpkG* was essential for the regulation of the expression of 15 genes (*str0095* etc.), and involved partially in the regulation of 12 genes, including *sll0846* and *str0967* (Liang et al., 2011). However, as shown in Figure 1(c), *str0095* and *sll0846* are regulated by a Hik34-Rre1 system and *str0967* by Hik16-Hik41-Rre17 system. Therefore, *SpkG* co-regulates genes *str0095* and *sll0846* with the Hik34-Rre1 system, and *str0967* with the Hik16-Hik41-Rre17 system under the same stress of HS.

**Signal transduction under HP**

It is shown in Figure 1(d) that the H$_2$O$_2$-inducible expression of genes *nblA1*, *nblA2* and *ndhD2* are under the control of both Hik33 and PerR, whereas that of *ftsH* is only controlled by Hik33 (Kanesaki et al., 2007). The expression of the other six genes, including *ahpC* gene (an alkyl hydroperoxide reductase) and *perR* gene, was regulated solely by PerR (Houot et al., 2007; Kobayashi et al., 2004). Moreover, previous studies suggested that PerR regulated some
H₂O₂-inducible genes (*ahpC*, *perR*, and *slr0589*) in a negative manner (Kobayashi et al., 2004; Li et al., 2004), and enhanced the expression of other genes (*sll1620*, *sll1550*, and *slr0587*) in a positive manner (Kobayashi et al., 2004; Li et al., 2004), and enhanced the expression of other genes (*sll1620*, *sll1550*, and *slr0587*) in a positive manner (Kobayashi et al., 2004; Li et al., 2004), and enhanced the expression of other genes (*sll1620*, *sll1550*, and *slr0587*) in a positive manner (Kobayashi et al., 2004; Li et al., 2004), and enhanced the expression of other genes (*sll1620*, *sll1550*, and *slr0587*) in a positive manner (Kobayashi et al., 2004; Li et al., 2004), and enhanced the expression of other genes (*sll1620*, *sll1550*, and *slr0587*) in a positive manner (Kobayashi et al., 2004; Li et al., 2004), and enhanced the expression of other genes (*sll1620*, *sll1550*, and *slr0587*) in a positive manner (Kobayashi et al., 2004; Li et al., 2004), and enhanced the expression of other genes (*sll1620*, *sll1550*, and *slr0587*) in a positive manner (Kobayashi et al., 2004; Li et al., 2004), and enhanced the expression of other genes (*sll1620*, *sll1550*, and *slr0587*) in a positive manner (Kobayashi et al., 2004; Li et al., 2004). Despite that many H₂O₂-inducible genes in *Synechocystis* are regulated by *PerR*, the two-component systems play an important role in response to HP positively. Especially, Hik33 was found to be responsible for 22 of the 26 H₂O₂-inducible genes under the control of Hiks (Kanesaki et al., 2007). Hik34 regulates transiently the expression of the *hpg* gene in a negative manner. Induction of genes *sll0939* and *sll0967* for hypothetical proteins, was regulated by Hik16 and Hik41 under H₂O₂ control (Kanesaki et al., 2007). Mechanisms of regulating these H₂O₂-inducible genes by Hik34 and Hik16 plus Hik41 remain uncharacterized to date. Probably, an unknown H₂O₂ sensor, neither a known Hik nor PerR, may exist for regulating the expression of other 45 genes (Kanesaki et al., 2007).

**Signal transduction under HL and HT**

Insertional inactivation of *hik35* (*cph1*) in *Synechocystis* harms the growth of mutant cells under continuous red/far-red light (Fiedler et al., 2004). The mutation of *hik35*, which seems to transfer signals to *rre27*, altered the expression of approximately 20 light-regulated genes (Hubschmann et al., 2005). Hik3 may be involved in blue light-dependent growth (Wild et al., 2002), and Hik44 (*Etr1*) binds ethylene (Los et al., 2010). Hik1, Hik24, and Hik32 were likely to participate in the perception of light-stress signals, which merits further investigation (Los et al., 2010). Hik22 was found to be responsible for 22 of the 26 H₂O₂-inducible genes under the control of Hiks (Kanesaki et al., 2007). Hik34 regulates transiently the expression of the *hpg* gene in a negative manner. Induction of genes *sll0939* and *sll0967* for hypothetical proteins, was regulated by Hik16 and Hik41 under H₂O₂ control (Kanesaki et al., 2007). Mechanisms of regulating these H₂O₂-inducible genes by Hik34 and Hik16 plus Hik41 remain uncharacterized to date. Probably, an unknown H₂O₂ sensor, neither a known Hik nor PerR, may exist for regulating the expression of other 45 genes (Kanesaki et al., 2007).
also in other references (Hsiao et al., 2004; Kappel & van Waasbergen, 2007; Tu et al., 2004).

Hik34 was identified as an important contributor to regulate transiently the expression of heat-shock genes during acclimation to HT and acquisition of thermo-tolerance in Synechocystis (Suzuki et al., 2005). Inactivation of the hik34 gene enhanced the levels of transcripts of several heat-shock genes, in particular, htpG and groESL1. Furthermore, overexpression of the hik34 gene could repress the expression of these heat-shock genes. Moreover, cells with a mutant gene for Hik34 (AHik34 cells) could survive incubation at 48°C for 3 h, while wild-type cells and cells with mutations of other Hiks failed to do so (Suzuki et al., 2005). However, inactivation of hik34 gene shows only an insignificant effect on the global expression of genes upon incubation of mutant cells at 44°C for 20 min. Therefore, it seems that Hik34 is involved in the negative expression regulation of certain heat-shock genes, which might be related to thermo-tolerance in Synechocystis (Suzuki et al., 2005).

### Signal transduction under other abiotic stresses

DNA microarray analysis revealed that the expression of all PD-inducible genes was completely eliminated by inactivation of either Hik7 or Rre29, and Rre29 binds to the upstream flanking regions of three genes at repetitive PyTTAAPyPy (T/A)-like sequences (Py represents a pyrimidine) (Suzuki et al., 2007) demonstrated that another component, SphU (slr0741), was important in the negative regulation of PD signals. In addition, expression of a gene for alkaline phosphatase (slr0654) and those of two different operons that encode subunits of the phosphate-transport system (a phosphate-binding protein, Slr0679; PstS, Slr0680; PstC, Slr0681; PstA, Slr0682; and PstB, Slr0683) were induced under PD (Hirani et al., 2001).

CiD induced the regulatory genes slr1214 (rre15), slr1292 (rre11), slr1594 (ndhr), sigD, sigG, and sigH (Wang et al., 2004). In particular, slr1214 (rre15), showed especially the earliest induction, implying a role for the early response to CiD (Wang et al., 2004). The sole and markedly up-regulated Hik was a phytochrome-like protein Hik1 (slr1393, 1.79-fold at 200 min), while the induction of rre15 was triggered before the growth inflection provoked by CO₂ downshift (2.29-fold at 180 min) (Wang et al., 2004). We speculate that the CiD signal may be transferred from Rre15 to Hik1, then to Rre11.

The operon that contains genes for Rre33 (NrsR; slr0797) and Hik30 (NrsS; slr0798) is located 118 bp upstream of the nrsBACD operon, a nickel-resistance operon (slr0793-slr0796) required for the export of Ni²⁺ from the cytoplasm, and hik30-rre33 operon is transcribed in the opposite direction. NrsR binds to the promoter region of the nrsBACD operon (Lopez-Maury et al., 2002). Moreover, Ni²⁺-dependent inducibility of nrsBACD operon disappeared and cells became very sensitive to ENi when hik30 and rre33 genes were inactivated. It appeared that the NrsSR system (Hik30-Rre33) acted positively to regulate the Ni²⁺-dependent expression of the nickel-resistance operon (Lopez-Maury et al., 2002).

Due to mutations of hik27 (slr0640) and rre16 (slr1837), both Hik27 and Rre16 regulated the expression of three genes, namely, mntC, mntA, and mntB (Yamaguchi et al., 2002). The three genes constitute the mntCAB operon that encodes subunits of the ABC-type Mn²⁺ transporter. It showed that Hik27 and Rre16 constituted a two-component system, acting as the sensor and signal transducer of MnD (Ogawa et al., 2002).

The Hik20 (slr1590) in Synechocystis is homologous to KdpD in Escherichia coli, which was identified as a sensor of KD (Jung et al., 2000). Rre19 (Slr11592) is homologous to KdpE, which represents a cognate Rre. Thus, it seems that the Hik20-Rre19 system is involved in transducing signals when the supply of K⁺ ions is deficient. Screening of the Hiks mutant library under non-stress conditions allowed Hik20, as an important contributor, to regulate the expression of the kdpABC operon in Synechocystis. Mutation of hik20 enhanced the levels of transcripts of kdpA and kdpB, which is presumed to encode components of the K⁺ ion transport system, and those of several other genes, such as pilA-pilB-sll1696 operon (see “List of experimental data available” at http://wwwgenome.jp/kegg/expression). However, mutation of Rre19 did not enhance the levels of kdpA and kdpB transcripts, which suggested that the Hik20-Rre19 system is not entirely homologous to the KdpD-KdpE system (Walderhaug et al., 1992). In addition, Hikg (KdpD, slr1731) belonging to the Kdp system, one of three K⁺ transport systems in Synechocystis, was also reported to be involved in acclimating to KD (Matsuda & Uozumi, 2006) (see “Two-component system-Synechocystis sp. PCC 6803” at http://wwwgenome.jp/keggbin/show_pathway?org_name=syn&mapno=02020&mapscale=1.0&show_description=show).

Recently, an observation suggested that Hik8 might be involved in the regulation of CY in Synechocystis (Osanai et al., 2005), while Hik8 (an ortholog of SasA) and Hik24 (an ortholog of CikA) have been identified as signal transducers associated with a circadian clock-related protein KaiC in Synechococcus sp. PCC 7942 (Ito et al., 2009; Mackey et al., 2008). Hik8 transferred a phosphate group to Rre31 (an ortholog of RpaA), in the presence of KaiC, and acted as a major mediator of CY that regulates the oscillation of genes expression (Mackey et al., 2008). In addition, it has been reported that Hik8 (slr0750) regulates positively the expression of sugar catabolic and anabolic genes in Synechocystis (Singh & Sherman, 2005). SigE and Hik8 regulate positively and independently or cooperatively, the cph1-rcp1 and other genes expression for sugar catabolism (including glycolysis, the OPP pathway, and glycogen catabolism) under control of the diurnal rhythm. However, only Hik8 positively regulates the sugar anabolic gene, SigE not (Osanai et al., 2005).

### Co-regulations and crosstalk among the perception and subsequent transduction of abiotic signals

Screening of various signal transduction under diverse conditions presents sets of co-regulations and crosstalk...
regulations among signal transduction systems, as well as crosstalk among various stresses. We divide them preliminarily into five classes and summarize them below.

Co-regulations under the abiotic stresses

*Synechocystis* recognizes HS and HO as different stresses, due to the fact that they produce different effects on the cytoplasmic volume, although mechanisms common to the responses to each form of stress might also contribute to the same genes expression in *Synechocystis*. HO and HS. In *Synechocystis* enhances general expression of some genes for an HL-inducible protein (*hliA*), for the synthesis of glucosylglycerol (*ggpS* and *gldP*), for a sigma factor (*sigD*), and for heat-shock proteins (*hspA*, *dnaK2*, *groEL2*, and *clpB1*) (Kanesaki et al., 2002; Murata & Suzuki, 2006) (Figure 2a).

In addition, the expression of four *hli* genes (*hliA*, *ssl2542*, *hliB*, *ssr2595*; *hliC*, *ssl1633*; and *hliD*, *ssr1789*) in *Synechocystis* was also induced by HL (He et al., 2001), LT, and nutrient starvation (NS) (Latifi et al., 2009). Furthermore, association of these proteins with the photosystem II has been demonstrated (Yao et al., 2007). Subunits D2 and D3 (NdhD2, Slr1291; and NdhD3, Sll1733) of NADH dehydrogenase are involved in the cyclic flow of electrons in photosystem I and also the uptake of CO2, respectively. (Okawa et al., 2000b). The expression of these heat-shock genes, such as *hspA*, *dnaJ*, *dnaK2*, and *hpgG*, were also induced transiently by HP (Kanesaki et al., 2007), HT (Inaba et al., 2003; Suzuki et al., 2005), HL (Hihara et al., 2003), UV irradiation (Huang et al., 2002), HO (Paithoonrangsarid et al., 2004), and HS (Shoumskaya et al., 2005). Therefore, these results show that the induction of heat-shock proteins responds timely to various kinds of stresses in general. Although Hik34 also regulated the expression of *slr1634* and *slr1808* (hemA) genes in a negative manner, the above-mentioned abiotic stresses did not enhance the expression of these genes (Kanesaki et al., 2007).

Talks among the signal transduction systems under different abiotic stresses

Since Hiks have been shown, sometimes, to be sensors or transducers of abiotic or intracellular stimuli (Mizuno et al., 1996), some Hiks in *Synechocystis* might also be expected to have similar functions. For example, Hik33 is involved in the responses to various abiotic stresses; however, mechanisms of the responses differ. Hik33 was originally identified as DspA, a chemical sensor of drugs, such as inhibitors of photosynthesis. Soon after, NblS, a putative homologue of Hik33 in *Synechococcus elongatus* PCC 7942, was identified as a sensor of HL and NS (van Waasbergen et al., 2002). Since the degree of considerable homology between NblS and Hik33 amounts up to 58% at the amino acid level, recent studies suggest that Hik33 may also play roles in response to HL (negatively) in *Synechocystis* (Hsiao et al., 2004; Tu et al., 2004). However, the Hik33 does not seem to contribute significantly to the transduction of nutrient-related signals in *Synechocystis* (Zabulon et al., 2007). Moreover, in *Synechocystis*, strong evidence shows that DspA, as a regulator controlling the expression of many genes, was involved in HS responses (Marin et al., 2003). In addition, analyses indicate that Hik33 is a bifunctional sensor to LT (Suzuki et al., 2001) and HO (Kanesaki et al., 2007).

However, we are not sure how a single two-component system perceives and transduces more than one type of abiotic signals in stress-specific manner (Los & Murata, 2002, 2004; Mikami et al., 2002). Generally, Hik33-Rre26 contributes to the regulation of gene expression under LT and HL by regulating different sets of genes in response to each respective stress, while Hik33-Rre31 does so upon exposure

Figure 2. Some of two different stress-inducible genes are the same and some are different (Mikami et al., 2002; Murata & Suzuki, 2006).
of cells to HO and HS. Nevertheless, experiments data prove that Hik33 perceives the LT signals, then also transduces the signals to Rre31, and thus regulates genes expression, for adapting temperature changes (Zhang, 2008). Furthermore, it is worth mentioning that three genes respectively homologous to hik33, rre26, and rre31, are encoded by the plastid genome of some red and golden-brown algae (Duplessis et al., 2007).

As Hik33 not only acts as a sensor of several types of stress but also regulates different sets of genes under diverse conditions, Hik33-Rre26 system regulates the same sets of genes in response to diverse stresses. Taking only one instance, there are three distinct sets of genes, either of whose LT-inducible and HO-inducible expression is regulated by Hik33 (Figure 2b). The first set of genes is entirely or partially regulated by Hik33 under HO solely. It is likely that FabG (Slr0330; 3-ketoacyl-ACP reductase), that is required for the elongation of fatty acids, might be involved in the synthesis of fatty acids or lipopolysaccharides, whereas MurF (Slr1351; UDP-N-acetylmuramoylalanyl-N-glutamyl-2,6-diaminopimelate-d-alanyl-d-alanine ligase) required for the assembly of peptidoglycans, might be involved in the maintenance of cell wall integrity. Moreover, the expression of some genes for phosphate-transport system (phosphate-binding protein, Slr0679; PstS, PstC, PstA and PstB) and for heat-shock proteins (GroES, Slr2075; DnaJ, Slr0093; GroEL, Slr2076; GroEL-2, Slr0416; DnaK, Slr0170; and HtpG, Slr0430) was also regulated by Hik33 (Mikami et al., 2002). Initiation factor IF-3 (InfC, Slr0974), 50S ribosomal proteins (Rpl34, Smr0011), and tryptophanyl-tRNA synthetase (TrpS, Slr1883) were involved in the control of translation (Mikami et al., 2002). Hik33 also regulated the genes for proteins that are involved in signal transduction pathways, such as sensory Hik34 (Slr1285) and adenylate cyclase CyaA (Slr1991) (Mikami et al., 2002).

The second set of genes is regulated by Hik33 after a downward shift in temperature. The Hik33-regulated genes encode proteins that are involved in gene expression (Crh, Slr0083; and Fus, Slr1105) and the regulation of photosynthesis (NdhF, Slr2099; and CytM, Slr1245), and that in response to oxidative stress (XthA, Slr1854; and GsbH, Slr1238) and membrane function (LivF, Slr1881; and DesB, Slr1441) (Mikami et al., 2002). As well, these genes for an elongation factor EF-G (Fus, Slr1105), for GTP cyclohydrolase I (FolE, slr0426), and for an unknown functional protein (Ycf39, slr0399) were regulated by Hik33 (Mikami et al., 2002).

Another set of genes is the same as two different stress-inducible genes regulated by Hik33. Proteins induced by strong light (HilaA, Slr1254; Hlb, Ssr2595; and Hlic, Slr1633) may be involved in the regulation of photosynthesis, whereas members of subunit D2 (NdhD2, Slr1291) of NADH dehydrogenase were involved in the cyclic flow of electrons in photosystem I and in the uptake of CO2 respectively (Mikami et al., 2002). Furthermore, SigD (Slr2012), a 70kDa sigma factor, is a transcription factor. This set of genes also include genes for cell-surface lipoprotein (Slr1483), solanesyl diphosphate synthase (Sds, Slr0615), and several unidentified proteins (Slr1544, Slr1191, Slr1483, Slr1541, Slr0082, and Slr0668). Therefore, Hik33 might be a global “multi-stress” sensor, recognizing different stresses in different mechanisms, which is as yet unknown (Mikami et al., 2002).

Co-regulations and crosstalk regulations of the signal transduction systems under certain abiotic stresses

There is co-regulation in signal transduction systems not only under different conditions, but also under the same stress. For example, under HS, slr0095 and slr0846 are co-regulated by the Hik34-Rre1 system and SpkG, and slr0967 by the Hik16-Hik41-Rre17 system and SpkG (Figure 1c). Moreover, crosstalk regulations exist in two-component systems transferring the same stress signals in Synechocystis as in E. coli (Verhamme et al., 2002). From Figure 1(b) and (c), we can find that the specificity crosstalk between the Hik34-Rre1 system and the Hik2-Rre1 system is also revealed via Rre1.

Crosstalk among the two-component systems and the STK systems

Findings demonstrate that the signals, due to HO, that induce the expression of some genes are probably perceived by unknown mechanisms that differ from typical Hik-Rre two-component ones (Murata & Los, 2006). In both cases for ethylene response in the plant Arabidopsis thaliana (Chang et al., 1993) and for high osmolarity adaptation in the yeast S. cerevisiae (Maeda et al., 1994), the two-component systems act upstream of a cascade of STKs in the same signal transduction pathway. Moreover, it is a very interesting discovery that several genes encoding STKs or phosphatases are in the same cluster as those encoding members of two-component systems, or as the gene slr1983 who encodes a protein containing both a Rre domain and a protein Ser/Thr phosphatase domain. The slr0114 cluster contains slr0114 encoding a protein Ser/Thr phosphatase and slr0115 encoding a DNA-binding Rre. Similarly, the STK gene slr1697 (spkB) is followed immediately by slr0921 (req25), although these two genes are transcribed in opposite directions. The slr0776 cluster is in the same situation, since it contains one STK gene slr0776 (spkB) and one HR gene slr0779 (Zhang et al., 1998b). In addition, an ORF (req21, slr1982) upstream of slr1983 encodes a Hik protein, which may cooperate with slr1983 as a two-component system (Mizuno et al., 1996).

We cannot help doubting whether the crosstalk and co-regulation between a couple of two-component systems, as well as between two-component systems and the STK system in Synechocystis play a significant role in the responses to more and more complicated surroundings. It is similar that the crosstalk regulations among group 1(sigA) and four group 2 (sigB, sigC, sigD, and sigE) sigma factors, and cooperation of group 2 sigma factors could be important for the adaptation of cyanobacteria Synechococcus sp. PCC7942 and Synechocystis to different abiotic and physiological conditions (Imamura & Asayama, 2009; Lemeille et al., 2005; Osnai et al., 2005; Yoshinura et al., 2007). Most important of all, we find that the growth of the ΔspkG mutant strain is almost completely unaffected at a high NaCl concentration (4%) in contrast to the wild strain, but impaired at a higher NaCl concentration (5%) after 2 days (Liang et al., 2011; Zhang, 2008). There is also the same phenomenon in the
two-component systems including Hik33 under HS, namely, the growth of the ΔHik33 mutant strain was not obviously different at a high NaCl concentration (4%) in contrast to the wild strain (Zhang, 2008). Therefore, we speculate that there must be cooperation or crosstalk between STKs and two-component signal transduction systems.

Crosstalk between oxidative stress and other stresses

The subject of increasing studies and a large amount of experimental data, abiotic stresses cause ion imbalance and hyperosmotic stresses and the part played by ROS in biological processes points to a strong crosstalk between ROS homeostasis and other cellular stress-inducible networks (Sarwat et al., 2013). We summarize the most important and the most-studied connections in Synechocystis.

Presently, an increasing number of enzymatic and Regulatory activities (such as non-enzymatic antioxidants, several different carotenoids, polyunsaturated fatty acids, vitamins, anticancer and antiviral drugs) as a natural arsenal to response to oxidative stress are strictly controlled by redox-based reactions in the cell (Habib et al., 2011; Skjanes et al., 2013). In addition, many antioxidants in higher plants are important redox signaling components that interact with biomembrane-related compartments (Shao et al., 2008), which may exist in Cyanobacteria involved in signal transduction under LT. Some data highlight the possibility that PerR may function as a peroxide-sensing repressor in Synechocystis, working with some Hiks, Hik33, Hik34, Hik41, and Hik16. Screening thioredoxins target proteins in Synechocystis showed the catalase-peroxi-dase enzyme KatG, the 1-Cys Prx and the Type II Prx peroxiredoxins, as thioredoxins-regulated proteins (Lindahl & Florencio, 2003; Perez-Perez et al., 2006). The removal of nitrate from the growth medium would induce the expression of a prx gene encoding 2-Cys Prx; meanwhile, transcription of several genes encoding peroxiredoxins was induced in response to HS (Stork et al., 2005). Similarly, the expression of isi (iron-stress inducible) genes isiA and isiB was highly induced by NaCl treatment in Synechocystis (Vinnemeier et al., 1998). Furthermore, it has been shown that high salinity would lead to oxidative stress in cyanobacteria. Since cyanobacterial cells facing HS exhibit a high-demand for ATP synthesis, it may be due to that any physiological condition that decreases the ATP to NADPH balance would result in ROS production during oxidative stress (Latifi et al., 2009). Similarly, Synechocystis mutant deficiency in the synthesis of ferredoxin quinone reductase (FQR) was sensitive to HL, which is in agreement with this hypothesis (Yeremenko et al., 2005). In addition, UV-B irradiance can indirectly cause oxidative stress by the production of ROS, as the production of ROS and lipid peroxidation upon exposure to UV-B irradiation increased to 5 days and then decreased to preirradiation levels (He et al., 2002). UV-A irradiation could cause similar effects (Latifi et al., 2009).

In a word, LT, HL, HS, or certain NL may destabilize the balance between oxidants and antioxidants in the cells, leading to oxidative stress, and then rouse an array of signal transduction pathways in turn cope with these stresses.

Conclusion

It is for the first time to confirm a STK and two-component system together constituting a complex network of signal transduction systems in Synechocystis under HS. By parity of reasoning, the cyanobacterial signal transduction systems are complex networks beyond imagination (Figure 3), acclimating cyanobacteria to complex and diversified abiotic stresses. However, the association of two-component systems with STKs systems remain to be revealed, and how the two signal transduction systems come into being as an integral network presses for a solution.

Besides, as abscisic acid (ABA) plays an important role in improving plant tolerance to cold (Xue-Xuan et al., 2010) and a few reports show that abiotic stresses such as salt stress can induce ABA biosynthesis in cyanobacteria (Manickavelu et al., 2006), the interaction and correlation between ABA and two-component systems or STKs systems requires further research.
Declarations of interest

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