J. Microbiol. Biotechnol. (2012), **22**(2), 166–169
http://dx.doi.org/10.4014/jmb.1106.06050
First published online October 25, 2011

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Characterization of Stress Responses of Heavy Metal and Metalloid Inducible Promoters in *Synechocystis* PCC6803

Blasi, Barbara[1,2], Loredana Peca[1†], Imre Vass[1], and Peter B. Kós[1*]

[1]*Institute of Plant Biology, Biological Research Centre of the Hungarian Academy of Sciences, Temesvári krt. 62, H-6726 Szeged, Hungary*
[2]*Department of Ecological and Biological Sciences, University of Tuscia, Largo San Camillo De Lellis, 01100, Viterbo, Italy*

Received: June 28, 2011 / Revised: October 13, 2011 / Accepted: October 18, 2011

In several biotechnological applications of living bacterial cells with inducible gene expression systems, the extent of overexpression and the specificity to the inducer are key elements. In the present study, we established the concentration ranges of Zn^{2+}, Ni^{2+}, Co^{2+}, AsO_2^-, and Cd^{2+} ions that caused significant activation of the respective promoters of *Synechocystis* sp. without concomitant unspecific stress responses. The low expression levels can be increased up to 10–100-fold upon treatments with Cd^{2+}, AsO_2^-, Zn^{2+}, and Co^{2+} ions and up to 800-fold upon Ni^{2+} treatment. These results facilitate the development of conditional gene expression systems in cyanobacteria.

Keywords: Cyanobacterial gene expression, heavy metal toxicity, specificity of promoters, *Synechocystis* PCC6803, inducible promoters

In the present study, we aimed to investigate the specificity of changes in gene expression in the unicellular *Synechocystis* PCC6803 species caused by inorganic ions that are generally not present in high concentrations in their normal environmental habitat. We have previously shown [10] that even in low concentrations these toxic ions induce genes that are involved in the specific defense against the respective ions. The corresponding promoters have been used in some applications [8, 9] where side effects of the inducers were irrelevant. Nevertheless, in several applications of inducible gene expression, like triggering enzymes for producing valuable products, or in investigation of functions of unknown genes, side effects may cause basic problems. The aim of the present work was to select inducible promoters and establish the ideal conditions where these promoters can be used in further studies and in biotechnological applications without any disturbances in the metabolism of *Synechocystis* PCC6803.

The set of investigated elements comprised chromium, cobalt, nickel, copper, zink, and arsenic. Each of the elements was investigated in its oxidative state that occur most abundantly in the environment. The studied genes, which show more or less specific response to the selected ions, are listed in Table 1. Although in the *Synechocystis* genome there was no specifically copper-inducible gene found, Cu^{2+} was included among the stress inducers owing to its importance in general stress responses.

High concentrations of metal ions are known to promote a variety of damages at the molecular level, including mostly oxidative stress, or misfolding polypeptide chains (see *e.g.*, [2, 3]). The defense against these types of stresses involves the expression of certain more general stress-responsive genes. These include genes coding for components of redox pathways, which detoxify reactive oxygen species, as well as proteins that are members of chaperon/degradation pathways, which are involved in repairing or degrading misfolded proteins. We selected a set of stress "marker genes" on the basis of literature data available on oxidative stress responses of *Synechocystis* to assess the general metal and metalloid induced gene expression responses (Table 2).

Whether the specific gene expressions and general stress responses occur concomitantly, or there are concentration ranges representative to each response types and lead to different patterns of gene expressions, has not been fully elucidated. The stressors that are usually classified as "heavy metals" (even the metalloid arsenic is frequently included in this popular term) are rather different from one another with respect to their chemical behavior, hence we

*Corresponding author
Phone: +3662 599711; Fax: +3662 433434;
E-mail: kp@brc.hu

†Current address: National Institute of Research and Development for Biological Sciences, Institute of Biological Research Cluj-Napoca, 400015 Romania

Table 1. List of heavy metal and metalloid inducible genes in *Synechocystis* that were used in this study.

ORF ID	Gene name	Activator ion	Function
slr0798	*zia*A	Zn^{2+}; Cd^{2+}	Zinc-transporting P-type ATPase (zinc efflux pump) involved in zinc tolerance
slr0793	*nrs*B	Ni^{2+}; Co^{2+}	Cation efflux pump involved in nickel and cobalt tolerance
slr0944	*ars*B	AsO_2^-	Putative arsenite and antimonite carrier
slr0797	*coa*T	Co^{2+}; Zn^{2+}	Cobalt transport P-type ATPase (cobalt efflux pump) involved in cobalt tolerance

investigated their effects individually in order to see similarities and differences in their gene expression patterns.

We assumed that significant specific gene inductions occur at moderate toxic effects; that is, in the order of a magnitude of the half growth inhibitory concentrations (IC_{50}), estimated using our previous growth inhibition data [10].

When applying individual ion treatments, the following results were observed. The half growth-inhibitory concentration of Cd^{2+} is about 6.5 μM. The expression of the cadmium- and zinc-specific *zia*A gene is highly induced at as low as 2 μM Cd^{2+}, and its expression is high throughout the investigated concentration range (Fig. 1A). Above 8 μM Cd^{2+}, however, other unspecific stress genes are induced. This result shows that a cadmium-specific response is present up to 4 μM, and above this concentration a general stress occurs.

The half growth-inhibitory concentration of $NiCl_2$ is about 27 μM. The nickel-specific *nrs*B is the only overexpressed gene at 5 μM, and at this concentration it already reaches its highest expression, showing that the metal-specific response is already fully triggered (Fig. 1B). The general stress-related genes start to be induced at 10 μM Ni^{2+} and their transcript level remains roughly the same at higher concentrations.

The IC_{50} for arsenite is around 2 mM. The *ars*B gene is already highly induced at 80 μM (Fig. 1C). The general stress-related genes are not induced up to 0.7 mM AsO_2^- concentration, and the limit between the specific and unspecific response is in the range between 0.7 and 2 mM AsO_2^-.

The IC_{50} of copper is around 2 μM. At 0.5 μM Cu^{2+}, none of the stress genes were significantly overexpressed (Fig. 1D), whereas most of these had higher than 2-fold induction over 1.25 μM, so the stress response had started at 1.25 μM Cu^{2+} and it increased with increasing concentrations.

The IC_{50} of Zn^{2+} is between 8 and 16 μM. Incubation with Zn^{2+} results in full overexpression of *zia*A already at 4 μM, whereas *coa*T shows increased level of expression throughout the tested range (Fig. 1E). At the highest tested concentration (32 μM Zn^{2+}), four stress genes show significant overexpression.

The IC_{50} value is around 8 μM for Co^{2+}. At 1 μM concentration, *coa*T is already highly induced, whereas the other cobalt-inducible gene *nrs*B has significant overexpression only over 2 μM (Fig. 1F). In the tested concentration range, only *hsp*A gets overexpressed among the general stress genes starting at 2 μM.

Our data strongly suggest that these ions exert their toxicity at higher concentration *via* protein denaturation, oxidative stress, and in part, damaging components of the photosynthetic apparatus. Hence, it is important to keep the concentrations low enough for avoiding those detrimental effects when using the corresponding promoters in delicate biotechnological applications.

We found that in the standard culture medium, the base expression levels of *zia*A, *coa*T, and *nrs*B genes are very low. Taking both base expression levels and rates of induction into account, the investigated promoters exhibit different behaviors (Table 3) that can be beneficial in biotechnological applications of cyanobacteria. The promoter–

Table 2. The *Synechocystis* genes that were used as markers of general and oxidative stress.

ORF ID	Gene symbol	Function	Rate of overexpression
slr1738	*per*R	Transcription factor	12^a, 7^b, 2.6^c, 4.3^d
sll1621	*ahp*-C	Peroxiredoxin gene	13^a, 13.1^b, 2.6^c, 26^d
sll1666	*dna*J	DNA J-like protein	5.1^b
sll0247	*isi*A	Iron stress chlorophyll-binding protein	8^a, 11^b
sll1514	*hsp*A	Heat shock protein A	76^a, 6^b
slr1544	*lil*A	Light harvesting-like protein	131^a, 9^b
ssl0452	*nbl*A1	Phycobilisome-degrading protein	18^a, 4^b
sll2012	*sig*D	Group 2 RNase polymerase Σ factor	4.7^a, 8^b

The applied stress conditions were as follows: 0.25 mM H_2O_2 for 20 min (a) [5]; 1.5 mM H_2O_2 for 30 min (b) [7]; 10 μM methyl viologen for 15 min under conditions of normal light (c: 50 μE m^{-2} s^{-1}) or high light (d: 200 μE m^{-2} s^{-1}) [6]. For detailed descriptions of the experiments see the respective publications.

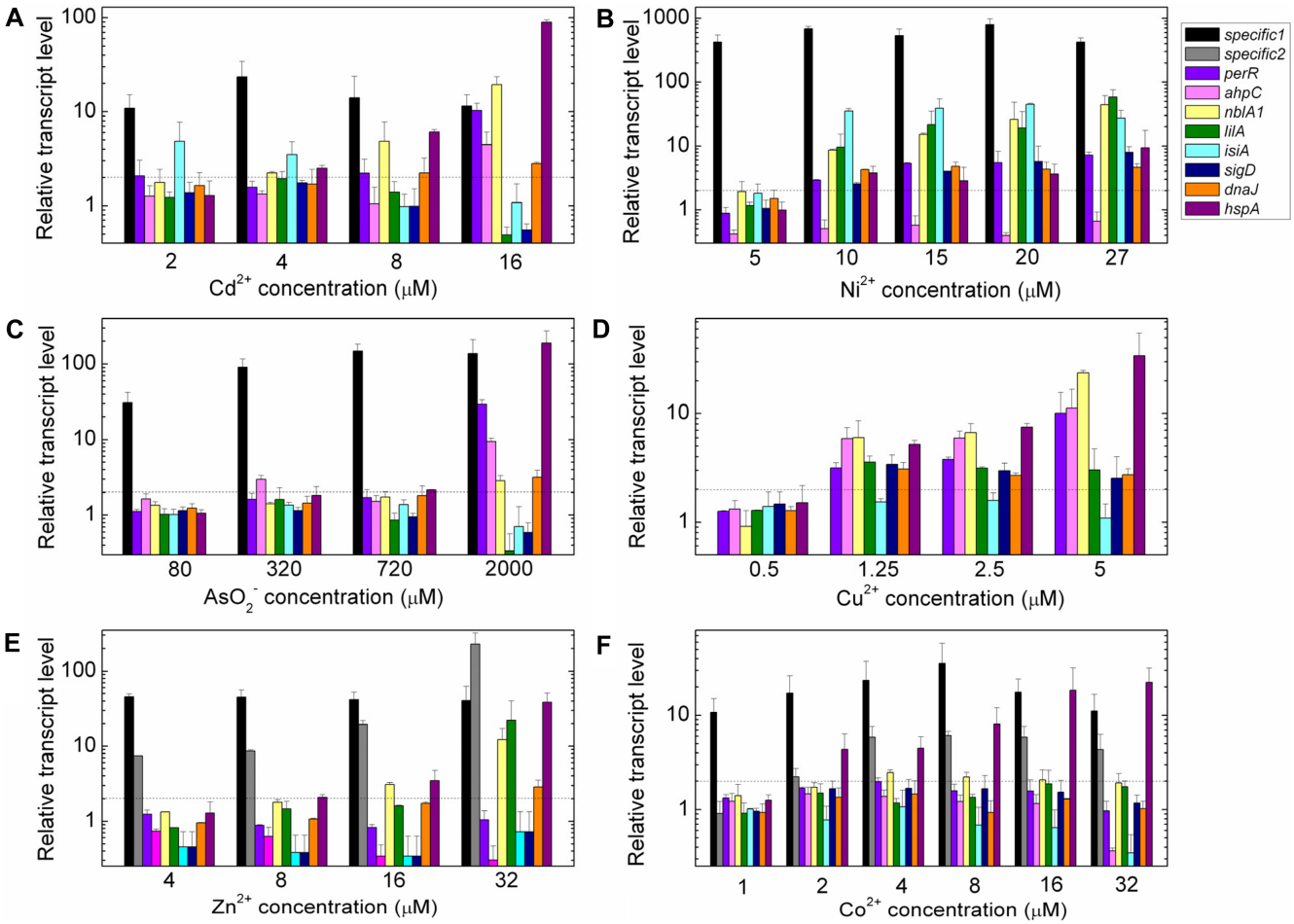

Fig. 1. Extent of gene overexpression by heavy metal treatments.
Synechocystis cultures were treated with the indicated concentrations of inorganic salts as described in the Materials and Methods. The rates of gene expressions are presented relative to the levels in the nonsupplemented BG-11 solution and expressed as fold upregulation. The specific genes are *zia*A (Cd^{2+} treatment); *nrs*B (Ni^{2+}); *ars*B (AsO_2^-); *zia*A (black bar); and *coa*T (grey bar) (Co^{2+} and Zn^{2+}). The mean values and standard errors were calculated from three independent experiments.

operator system of *ars*B allows some moderately low level of base expression of the gene downstream of the promoter, which can then be overexpressed by up to two orders of magnitude upon addition of up to 0.5 mM AsO_2^-. The *zia*A and *coa*A systems have very low base expression levels and can be used for over 10-fold overexpression of the genes downstream. The promoter–operator system of *nrs*B also has a rather low base expression level and allows a remarkable 2.5 order of magnitude overexpression of the regulated gene at 5 μM of Ni^{2+}.

It is noteworthy that the promoter of the plastocyanin *pet*E gene can be used in conditionally inducible constructs [4] but the 0.3 μM $CuSO_4$ content of the culture medium causes its full activation making it virtually to not have

Table 3. Suggested promoters for heavy metal and arsenite inducible gene constructs.

Inducer ion	Inducible promoter	Recommended maximal concentration	Uninduced expression level	Fold overexpression
AsO_2^-	*ars*B	720 μM	Moderate	100
Cd^{2+}	*zia*A	2 μM	Low	10
Co^{2+}	*coa*T	1 μM	Low	10
Ni^{2+}	*nrs*B	5 μM	Low	400
Zn^{2+}	*zia*A, (*coa*T)	4 μM	Low	40, (8)

transcriptional regulation [1]. Therefore, the biotechnical application of the corresponding promoter is tedious and inconvenient.

It is also noteworthy that as the *hsp*A gene gets overexpressed the most readily in every treatment, its promoter offers a convenient way of assessing early emergence of the general cellular stress state.

The suggested ion concentrations do not result in nonspecific gene expression, as far as the investigated genes are concerned; hence, these concentrations of the otherwise toxic ions are not harmful to *Synechocystis* and can be safely used for artificial switching of genes cloned downstream of the promoters. Development of plasmid vectors utilizing these promoter–operator systems are under way in our laboratory.

Acknowledgment

This work was supported by the Hungarian Granting Agency OTKA (K68036). Barbara Blasi was supported with a grant from the Faculty of Mathematical, Physical and Natural Sciences of the University of Tuscia.

REFERENCES

1. Briggs, L. M., V. L. Pecoraro, and L. McIntosh. 1990. Copper-induced expression, cloning, and regulatory studies of the plastocyanin gene from the cyanobacterium *Synechocystis* sp. PCC-6803. *Plant Mol. Biol.* **15:** 633–642.
2. Brostrom, C. O. and M. A. Brostrom. 1998. Regulation of translational initiation during cellular responses to stress. *Prog. Nucleic Acid Res. Mol. Biol.* **58:** 79–125.
3. Cuypers, A., J. Vangronsveld, and H. Clijsters. 1999. The chemical behaviour of heavy metals plays a prominent role in the induction of oxidative stress. *Free Radic. Res.* **31(Suppl):** S39–S43.
4. Huang, H. H., D. Camsund, P. Lindblad, and T. Heidorn. 2010. Design and characterization of molecular tools for a synthetic biology approach towards developing cyanobacterial biotechnology. *Nucleic Acids Res.* **38:** 2577–2593.
5. Kanesaki, Y., H. Yamamoto, K. Paithoonrangsarid, M. Shoumskaya, I. Suzuki, H. Hayashi, and N. Murata. 2007. Histidine kinases play important roles in the perception and signal transduction of hydrogen peroxide in the cyanobacterium, *Synechocystis* sp. PCC 6803. *Plant J.* **49:** 313–324.
6. Kobayashi, M., T. Ishizuka, M. Katayama, M. Kanehisa, M. Bhattacharyya-Pakrasi, H. B. Pakrasi, and M. Ikeuchi. 2004. Response to oxidative stress involves a novel peroxiredoxin gene in the unicellular cyanobacterium *Synechocystis* sp. PCC 6803. *Plant Cell Physiol.* **45:** 290–299.
7. Li, H., A. K. Singh, L. M. McIntyre, and L. A. Sherman. 2004. Differential gene expression in response to hydrogen peroxide and the putative PerR regulon of *Synechocystis* sp. strain PCC 6803. *J. Bacteriol.* **186:** 3331–3345.
8. Liu, X. and R. Curtiss III. 2009. Nickel-inducible lysis system in *Synechocystis* sp. PCC 6803. *Proc. Natl. Acad. Sci. USA* **106:** 21550–21554.
9. Peca, L., P. B. Kos, Z. Mate, A. Farsang, and I. Vass. 2008. Construction of bioluminescent cyanobacterial reporter strains for detection of nickel, cobalt and zinc. *FEMS Microbiol. Lett.* **289:** 258–264.
10. Peca, L., P. B. Kos, and I. Vass. 2007. Characterization of the activity of heavy metal-responsive promoters in the cyanobacterium *Synechocystis* PCC 6803. *Acta Biol. Hung.* **58:** 11–22.