Calcium release from *Synechocystis* cells induced by depolarization of the plasma membrane: MscL as an outward Ca$^{2+}$ channel

Lyudmila V. Nazarenko, Igor M. Andreev, Alexander A. Lyukevich, Tatiana V. Pisareva and Dmitry A. Los

Institute of Plant Physiology, Russian Academy of Science, Botanicheskaya Street 35, 127276 Moscow, Russian Federation

Cells of the cyanobacterium *Synechocystis* sp. PCC 6803 are equipped with a mechanosensitive ion channel MscL that is located in their plasma membrane. However, the exact function of the channel in this freshwater cyanobacterium is unknown. This study shows that cells of *Synechocystis* are capable of releasing Ca$^{2+}$ in response to depolarization of the plasma membrane by the K$^+$ ionophore valinomycin in the presence of K$^+$ or by tetraphenylphosphonium (TPP$^+$). A fluorescent dye, diS-C$_3$-(5), sensitive to membrane potential and the metallochromic Ca$^{2+}$ indicator arsenazo III were used to follow the plasma membrane depolarization and the Ca$^{2+}$ release, respectively. The Ca$^{2+}$ release from wild-type cells was temperature-dependent and it was strongly inhibited by the Ca$^{2+}$ channel blocker verapamil and by the mechanosensitive channel blocker amiloride. In MscL-deficient cells, Ca$^{2+}$ release was about 50% of that from the wild-type cells. The mutant cells had lost temperature sensitivity of Ca$^{2+}$ release completely. However, verapamil and amiloride inhibited Ca$^{2+}$ release from these cells in same manner as in the wild-type cells. This suggests the existence of additional Ca$^{2+}$ transporters in *Synechocystis*, probably of a mechanosensitive nature. Evidence for the putative presence of intracellular Ca$^{2+}$ stores in the cells was obtained by following the increase in fluorescence intensity of the Ca$^{2+}$ indicator chlortetracycline. These results suggest that the MscL of *Synechocystis* might operate as a verapamil/amiloride-sensitive outward Ca$^{2+}$ channel that is involved in the plasma-membrane depolarization-induced Ca$^{2+}$ release from the cells under temperature stress conditions.

**INTRODUCTION**

The mesophilic cyanobacterium *Synechocystis* sp. strain PCC 6803 is a unicellular photosynthetic prokaryote that can acclimatize to a wide range of environmental changes (Glatz *et al.*, 1999; Los & Murata, 1999). The complete nucleotide sequence of its genome is now available (Kaneko *et al.*, 1999; Los & Murata, 1999). The complete nucleotide sequence of its genome is now available (Kaneko *et al.*, 1999), and therefore *Synechocystis* may serve as a useful model of both plant-type and microbial cells for studying the molecular mechanisms of stress responses (Glatz *et al.*, 1999; Los & Murata, 1999, 2000).

It is well known that Ca$^{2+}$ plays a role as a secondary messenger in stimulus–response coupling in eukaryotic organisms, including plants, and it is involved in acquisition of their tolerance to a variety of environmental stress conditions (Knight *et al.*, 1997; Sanders *et al.*, 1999; Trewavas & Malho, 1998). In prokaryotic cells, the role of Ca$^{2+}$ in regulation of stress responses has not been clearly demonstrated, but evidence of its involvement in regulation of temperature stress responses, pathogenesis, chemotaxis, differentiation and the cell cycle is now being accumulated (Norris *et al.*, 1996; Holland *et al.*, 1999; Jones *et al.*, 1999).

Some reports indicate that the resting level of cytosolic free Ca$^{2+}$ in bacterial (Jones *et al.*, 1999) and cyanobacterial cells (Torrecilla *et al.*, 2000) is in the submicromolar range, i.e. similar to that found in eukaryotic cells. In addition, direct evidence that Ca$^{2+}$ signalling exists in cyanobacteria has become available recently (Torrecilla *et al.*, 2000). However, little is known about the Ca$^{2+}$ transporters, including Ca$^{2+}$ channels, that are responsible for the maintenance of Ca$^{2+}$ homeostasis in cyanobacteria and other prokaryotes (Torrecilla *et al.*, 2000). Most likely, the Ca$^{2+}$ status of cyanobacterial cells is primarily maintained by the activity of Ca$^{2+}$ transport proteins in their plasma membrane (PM). Thus, the difference in the electrical potential across the PM might be essential in controlling the cellular level of Ca$^{2+}$.

Some mechanosensitive ion channels were shown to be responsible for the permeability of cell membranes to...
Ca^{2+} (Ding & Pickard, 1993a, b), and thus they might be involved in Ca^{2+} signalling under certain stress conditions, among which hypo-osmotic stress is well characterized. Cells of *Synechocystis* are equipped with the mechanosensitive ion channel MscL, which is located in their PM. However, the exact function of the channel in this freshwater cyanobacterium, which is normally not exposed to hypo-osmotic stress, is unknown.

One of the approaches to reveal the activity of Ca^{2+} channels in the PM is to follow Ca^{2+} translocation across this membrane in response to perturbation of cellular Ca^{2+} homeostasis with agents that can depolarize the membrane. In the present study, we applied such an approach, with the use of the Ca^{2+}-sensitive dye arsenazo III (Thomas, 1982), to test the functioning of Ca^{2+} transporters in the PM of wild-type *Synechocystis* and of a mutant deficient in the mechanosensitive ion channel MscL. We demonstrate that cells of *Synechocystis* are capable of releasing Ca^{2+} into the assay medium in response to depolarization of the PM caused by treatment of cells with valinomycin in the presence of K^{+} ions. Ca^{2+} release from the wild-type cells was very rapid, temperature-dependent, and inhibited by verapamil (Ca^{2+} channel blocker) and amiloride (mechanosensitive channel blocker). Ca^{2+} release strongly decreased in the ΔmscL mutant cells, and became practically temperature-independent. Our results suggest that MscL is involved in regulation of Ca^{2+} homeostasis in *Synechocystis* cells under temperature-stress conditions.

**METHODS**

**Cyanobacterial strains.** The wild-type strain of *Synechocystis* sp. PCC 6803 was originally obtained from Dr J. G. K. Williams (DuPont de Nemours and Company, Wilmington, DE, USA). Cells were grown photoautotrophically at 32°C under continuous illumination by incandescent lamps at 70 μmol photons m⁻² s⁻¹ and aeration with air containing 2% CO₂, in BG11 medium (Stanier, 1971) buffered with 20 mM HEPES/NaOH pH 7.5. For temperature treatments, cells growing exponentially were harvested by centrifugation at 5000 g for 10 min at 30°C and resuspended in BG11 medium pre-cooled at designated temperatures.

**The MscL-deficient mutant of *Synechocystis*.** A single gene that encodes a homologue of the mechanosensitive channel MscL (srl0875) in *Synechocystis* was identified by homology search in Cyanobase (http://www.kazusa.or.jp/cyano/Synechocystis/index.html) using the amino acid sequence of the MscL of *Escherichia coli* (Sukharev et al., 1994) as a query sequence. A DNA fragment that contained the mscL gene and its flanking regions was amplified from the genomic DNA of *Synechocystis* by PCR with the following primers: mecF (5’-GACACAAGCAGCGGTTAAGTGACG-3’) and mecR (5’-ACCATTACGAGATGTAATTGGTG-3’). The MscL-null mutant (ΔmscL) of *Synechocystis* was produced by inserting a kanamycin-resistance gene, derived from plasmid pUC4KIXX (Pharmacia) by digestion with BamHI, into the unique BglII site of the srl0875 (mscL) gene of *Synechocystis* (Fig. 1A). Transformation of *Synechocystis* cells was done as described by Williams (1988). Complete segregation of the recombinant chromosomes in the mutant strain was confirmed by PCR with the above-listed primers (Fig. 1B).

Detection of Ca^{2+} release from the cells induced by depolarization of the PM. Ca^{2+} release from the cells was monitored by changes in absorbance of the metallochromic Ca^{2+} indicator arsenazo III in the assay medium (2 ml) (20 mM HEPES/BTP, pH 7.2, 3 mM MgSO₄, 27 μM arsenazo III) (Thomas, 1982). The cells were resuspended in 2 ml of the assay medium (100 μg total cellular protein per measurement). Ca^{2+}-dependent changes in absorbance of arsenazo III were recorded with a Hitachi 537 spectrophotometer operating in double-beam or double-wavelength (650–720 nm) mode. Measurements were carried out in unsterred cuvettes (light path 1 cm) that were kept at designated temperatures. The assay medium merely contained Ca^{2+} at the levels of contamination that could originate from the chemicals and the cell samples. All other details of experimental procedures are given in figure legends.

**Detection of putative Ca^{2+} stores within *Synechocystis* cells.** The presence of putative intracellular Ca^{2+} stores in the cyanobacterial cells was assayed by recording the increase in chlorotetra-cycline (CTC) fluorescence during the uptake of this Ca^{2+} indicator by the cells (Dixon et al., 1984). The assay was performed in the same medium (1 ml) as used for detection of Ca^{2+} release from the cells but lacking magnesium sulfate and supplemented with CTC at 25 μM. The fluorescence measurements were carried out at room temperature in an unstirred 1 cm light-path cuvette of a Hitachi 850 fluorescence spectrophotometer set at 380 nm (excitation) and 530 nm (emission).

**Detection of membrane potential on the PM of *Synechocystis* cells and its dissipation in the presence of valinomycin/K⁺ or tetraphenylphosphonium (TPP⁺).** The presence of membrane potential on the PM of *Synechocystis* cells was assayed by recording fluorescence changes of the potential-sensitive cyanine dye diS-C₃-(5) ( Waggoner, 1974). The assay medium (1 ml)
had the same composition as that used for detection of Ca\(^{2+}\) release from the cells, but it was supplemented with 1 \(\mu\)M diS-C\(_{3}\)-(5). Fluorescence of diS-C\(_{3}\)-(5) was measured at desired temperature in an unstirred 1 cm light-path cuvette of a Hitachi 850 fluorescence spectrophotometer set at 620 nm (excitation) and 670 nm (emission).

**RESULTS**

**Ca\(^{2+}\)** release from cells probed with arsenazo III and the presence of membrane potential on their PM

Cyanobacterial cells were washed in a Ca\(^{2+}\)-free medium and incubated in the assay medium with the Ca\(^{2+}\) indicator arsenazo III. Under these conditions, addition of two different compounds to the assay medium, the potassium-specific ionophore valinomycin in the presence of K\(^{+}\) ions or Ca\(^{2+}\) ionophore A23187, induced rapid efflux of Ca\(^{2+}\) from the cells (Fig. 2). It is important to note that a qualitatively similar effect was observed after addition of the lipophilic cation TPP\(^{+}\) to the cells.

As shown in Fig. 2(A), the release of Ca\(^{2+}\) from the cells was expressed as a change in absorbance of arsenazo III resulting from the formation of a complex of this dye with Ca\(^{2+}\) ions (Thomas, 1982). Fig. 2(B) shows a time-course of the valinomycin/K\(^{+}\)- and TPP\(^{+}\)-induced Ca\(^{2+}\) release and demonstrates that these two processes appeared to be too rapid for their kinetics to be resolved.

Ca\(^{2+}\) release induced by valinomycin appeared only in the presence of K\(^{+}\) ions in the assay medium, and no release was observed in the absence of K\(^{+}\) (Fig. 2A). This finding suggests that valinomycin catalyses the influx of K\(^{+}\) ions from the assay medium into the cells, thus leading to depolarization of the PM, i.e. a collapse of the membrane potential which is maintained by the cells under optimal growth conditions. To validate this suggestion, the putative sensitivity of membrane potential on the PM of the *Synechocystis* cells to the combined action of valinomycin and K\(^{+}\) ions in the assay medium was directly tested by using the potential-sensitive probe diS-C\(_{3}\)-(5). Fig. 3(A) shows the kinetics of distribution of this probe between the assay medium and the cells. It can be seen that valinomycin added to the cells after achieving a steady state of diS-C\(_{3}\)-(5) fluorescence quenching had practically no effect on this value. However, subsequent addition of K\(^{+}\) ions to the cells initiated a pronounced increase in fluorescence of the probe, clearly indicating depolarization of the PM. Moreover, a similar, and even more marked, effect was also observed in the presence of TPP\(^{+}\), which is known as a simulator of the action of valinomycin/K\(^{+}\), suggesting that depolarization of the PM indeed occurs (Fig. 3A).

The valinomycin/K\(^{+}\)-induced Ca\(^{2+}\) release took place in both the wild-type and the ΔMscL mutant cells and displayed similar kinetic properties in the two strains (Fig. 2B). However, the intensity of the valinomycin/K\(^{+}\)-induced Ca\(^{2+}\) release was three times lower in the mutant cells than in the wild-type cells. This could be explained by the fact that the ΔMscL mutant cells in their resting state are more depolarized than the wild-type ones. However, in the mutant cells, the fluorescence response of diS-C\(_{3}\)-(5) induced by TPP\(^{+}\) at 32°C (Fig. 3B, upper part) appeared to be even higher than that observed in the wild-type cells, pointing to a higher extent of PM polarization in the ΔMscL mutant cells.

**Detection of putative Ca\(^{2+}\)** stores inside the cyanobacterial cells

The above findings strongly suggest that valinomycin/K\(^{+}\)-induced release of intracellular Ca\(^{2+}\) treated with of the cells induced mobilization of Ca\(^{2+}\) from intracellular Ca\(^{2+}\) stores. Although the resting level of intracellular free Ca\(^{2+}\) in *Synechocystis* cells is expected to be low enough and similar to the intracellular Ca\(^{2+}\) levels reported for a variety of eukaryotic cells (Knight et al., 1997; Trewavas & Malho, 1998), the presence of Ca\(^{2+}\) stores

![Fig. 2. Valinomycin/K\(^{+}\)- and TPP\(^{+}\)-induced Ca\(^{2+}\) release from wild-type and ΔMscL cells of *Synechocystis* into the assay medium.](image-url)
cannot be excluded. In order to test this suggestion, we attempted to detect the presence of such stores in the cells by following fluorescence of the Ca$^{2+}$ indicator CTC, which is capable of monitoring sequestered Ca$^{2+}$ at concentrations of 0.1–30 mM (Dixon et al., 1984). Fig. 4 shows a gradual increase in CTC fluorescence that results from its passive equilibration with the entrapped Ca$^{2+}$ within both the wild-type and mutant cells. The kinetics of this process for both types of cells was about the same, and the observed fluorescence signal after achieving a steady-state level is rapidly reversed by the subsequent addition of the Ca$^{2+}$ chelator EGTA to the assay medium. Thus, the observed fluorescence emanated primarily from CTC–Ca$^{2+}$ complexes bound to some unknown structures within the cells.

**Action of Ca$^{2+}$ channel blockers on Ca$^{2+}$ release**

In order to elucidate the nature of the putative Ca$^{2+}$ channels that are involved in the valinomycin/K$^+$-induced Ca$^{2+}$ release from the cells, two inhibitors were applied: verapamil, known as a PM Ca$^{2+}$ channel blocker (Hosey & Lazdunski, 1988), and amiloride, a reported inhibitor of mechanosensitive ion channels (Hamill et al., 1992). Even at relatively low concentrations, these inhibitors strongly suppressed Ca$^{2+}$ release from the wild-type cells of *Synechocystis* (Fig. 5). The inhibitory effects of verapamil and amiloride were dependent on their concentrations. The blockers reduced the Ca$^{2+}$ release from the cells by 70–80% when applied at concentrations of 10–100 μM (Fig. 5).

Our attempts to apply inorganic channel blockers, LaCl$_3$ or GdCl$_3$, were unsuccessful, because these compounds strongly interacted with arsenazo III and thus interfered with the monitored response.

---

**Fig. 3.** Detection of membrane potential on the PM of the wild-type and mutant cells of *Synechocystis* PCC 6803 with the potential-sensitive fluorescent dye diS-C$_3$-(5), their depolarization in the presence of valinomycin/K$^+$ or TPP$^+$ (A) and effect of temperature on this process (B). Where indicated, 4.5 μM valinomycin, 120 mM KCl and 10 mM TPPCl were added to the cells. The charts represent the mean of four independent measurements.

**Fig. 4.** Kinetics of passive CTC equilibration with the entrapped Ca$^{2+}$ within wild-type and ΔMscL mutant cells of *Synechocystis* PCC 6803. A reversal of the CTC–Ca$^{2+}$ fluorescence signal was initiated by the addition of 2 mM EGTA to the assay medium. The chart represents the mean of three independent measurements.

**Fig. 5.** Effect of verapamil and amiloride on the valinomycin/K$^+$-induced Ca$^{2+}$ release from wild-type and ΔMscL mutant cells of *Synechocystis*. Cells were grown at 32°C under continuous illumination, and the inhibitors were added to the suspensions to reach the designated concentrations. Ca$^{2+}$ release from cells was monitored as the absorbance of arsenazo III. Light grey bars, wild-type; dark grey bars, ΔMscL mutant. Each bar represents the mean of results from three independent experiments. Standard error bars were too small to show clearly and have been omitted.
Effect of temperature on the Ca\(^{2+}\) release and membrane potential of the cells

Since the activity of Ca\(^{2+}\) channels in plants may be modulated by temperature (Ding & Pickard, 1993b; Plieth, 1999), we addressed the question whether the release of Ca\(^{2+}\) caused by depolarization of the PM is temperature-dependent in Synechocystis cells. Fig. 6 shows the Ca\(^{2+}\) release from the cells at 20 and 30°C. It can be seen that the intensity of the observed response of arsenazo III in wild-type cells appeared to be markedly lower at 20°C than that at 30°C.

The intensity of the valinomycin/K\(^{+}\)-induced Ca\(^{2+}\) release was more than three times lower in the ΔMscL mutant cells than that in the wild-type cells of Synechocystis (Fig. 2). Concentration dependencies of the verapamil/amiloride inhibition of the Ca\(^{2+}\) release exhibited only little difference between the wild-type and mutant cells (Fig. 5). At the same time, the Ca\(^{2+}\) release from the mutant cells appeared to exhibit only slight sensitivity to temperature, and even slightly increased upon a decrease in temperature from 30°C to 20°C, as compared to that in the wild-type cells (Fig. 6).

The observed suppression of the Ca\(^{2+}\) release from the wild-type cells at 20°C could be due to enhanced depolarization of the cell membranes at lower temperatures. We tested this possibility by direct recording of the membrane potential of the cells at different temperatures with the use of diS-C\(_3\)-\((5)\). Fig. 3(B) shows that both the wild-type and mutant cells exhibited pronounced depolarization of the PM in response to a temperature decrease from 32 to 20°C, as judged by a marked reduction of the TPP\(^{+}\)-induced increase in diS-C\(_3\)-\((5)\) fluorescence with a decrease in temperature. This figure also demonstrates another notable fact, already mentioned above, that the resting membrane potential of the mutant cells also declines with a decrease in temperature, and their behaviour in this respect differs only slightly from that of the wild-type cells.

DISCUSSION

Release of Ca\(^{2+}\) from the putative intracellular Ca\(^{2+}\) store(s) of cyanobacterial cells

Our results allow us to conclude that valinomycin/K\(^{+}\)-treated cells of Synechocystis exhibit the mobilization of intracellular Ca\(^{2+}\) rather than its release from the cell wall. There are three lines of evidence for this conclusion. First, the Ca\(^{2+}\) release was observed only in the presence of valinomycin/K\(^{+}\) or TPP\(^{+}\), i.e. under conditions providing depolarization of the PM (Fig. 2). Second, the Ca\(^{2+}\) release was strongly suppressed by the Ca\(^{2+}\) channel blocker verapamil and the mechanosensitive channel blocker amiloride (Fig. 5). Both inhibitors are believed to block the channels that are located in the PM of cells (Hosey & Lazdunski, 1988; Hamill et al., 1992). Third, the valinomycin/K\(^{+}\)-induced Ca\(^{2+}\) release from the cells was simulated by the Ca\(^{2+}\) ionophore A23187 (Fig. 2). Taken together, these findings indicate that the Ca\(^{2+}\) mobilization from the cells is mediated by the interaction of the compounds with the PM.

The release of Ca\(^{2+}\) from Synechocystis cells suggests that the Ca\(^{2+}\) gradient, which is directed towards the extracellular medium, exists on the PM. This gradient might be formed due to the presence of intracellular Ca\(^{2+}\) store(s) in the cells. This proposal is supported by the results showing the increase in fluorescence intensity of CTC resulting from its passive equilibration with the entrapped Ca\(^{2+}\) within the cells (Fig. 4). Although the nature of the detected Ca\(^{2+}\) store(s) remains unclear, the putative Ca\(^{2+}\) pool in the cells is most likely associated with the PM, because their treatment with EGTA resulted in a rapid disappearance of the fluorescence signal from CTC (Fig. 4). A recent report on the cyanobacterium Anabaena PCC 7120 also indicated the presence of intracellular Ca\(^{2+}\) stores in these cells (Torrecilla et al., 2000).

Nature of the putative Ca\(^{2+}\) channels in the PM of the cyanobacterial cells

The results obtained by application of the ion channel blockers verapamil and amiloride (Fig. 5) allow us to suggest that the release of Ca\(^{2+}\) from the intracellular space occurs through a special type of Ca\(^{2+}\) channels, most likely voltage-gated, in the PM. The transient nature of the verapamil/amiloride-sensitive Ca\(^{2+}\) release is in accordance with this conclusion, because there is evidence that during sustained depolarization voltage-gated Ca\(^{2+}\) channels undergo very rapid transition to a nonconducting, inactive state (Cens et al., 1999). This feature of the Ca\(^{2+}\) release and the conditions that are required for its activation suggest that opening of voltage-gated Ca\(^{2+}\) channels in the PM results from its depolarization.
In eukaryotic cells, such channels are responsible, as a rule, for Ca\textsuperscript{2+} influx into the intracellular space under environmental stress conditions (Knight et al., 1991; Hamilton et al., 2000). In cyanobacteria, however, nothing is known about the functioning of Ca\textsuperscript{2+} channels in the PM. It is interesting to note that in the cyanobacterium *Anabaena PCC 7120*, verapamil did not inhibit a transient burst of intracellular free Ca\textsuperscript{2+} induced by an increase in concentration of external Ca\textsuperscript{2+}, but was able to maintain the resting value of free Ca\textsuperscript{2+} at a rather high level (Torrecilla et al., 2000). However, it remains unclear whether this effect was, in fact, due to interaction of verapamil with some outward Ca\textsuperscript{2+} channels in the PM.

**MscL as a Ca\textsuperscript{2+} channel**

The data obtained with the ΔMscL mutant of *Synechocystis* suggest that the MscL mechanosensitive ion channel operates in the PM of these cells as a substantial contributor to the Ca\textsuperscript{2+} release induced by the membrane depolarization. Ca\textsuperscript{2+} release was significantly suppressed in the mutant cells as compared to that observed in the wild-type cells (Figs 2, 5 and 6). The data presented in Figs 3 and 4 show that alternative explanations for this result based on a lower value of the transmembrane Ca\textsuperscript{2+} gradient on the PM of the mutant cells, or a greatly increased initial depolarization of these cells as compared to those of the wild-type cells, can be excluded.

The temperature dependence of the Ca\textsuperscript{2+} release dramatically changed in the mutant cells as compared to that in the wild-type cells (Fig. 6). A possible explanation for this phenomenon is temperature dependence of the activity of the MscL (Ding & Pickard, 1993b; Kikuyama & Tazawa, 2001), which might be the reason for the observed temperature dependence of the Ca\textsuperscript{2+} release in the wild-type cells of *Synechocystis PCC 6803*.

The intensive Ca\textsuperscript{2+} release from the wild-type cells at 32 °C caused by the valinomycin/K\textsuperscript{+}-induced depolarization of the PM and relatively low intensity of such release at 20 °C might be due to the fact that cold stress itself causes depolarization of the PM and thus simulates the valinomycin/K\textsuperscript{+} effect (Fig. 3B). Such an action of cold stress was most likely responsible for the observed attenuation of the valinomycin/K\textsuperscript{+}-induced Ca\textsuperscript{2+} release from the cells. Since the mutation in MscL only strongly inhibited (by about 50%) but did not abolish completely the depolarization-induced Ca\textsuperscript{2+} release, it is reasonable to suggest that additional Ca\textsuperscript{2+} channels other than MscL are present in the PM of the cells of *Synechocystis*. Taking into account the fact that in the ΔMscL mutant cells the Ca\textsuperscript{2+} release was also observed and it was sensitive to verapamil and amiloride, it is possible that other putative Ca\textsuperscript{2+} channels also have a mechanosensitive nature.

In the ΔMscL mutant cells of *Synechocystis*, the Ca\textsuperscript{2+} release was only slightly affected by temperature, suggesting that the MscL might be the main channel that controls the Ca\textsuperscript{2+} release under temperature stress conditions.

At present, the mechanisms that activate the MscL as a result of depolarization of the PM of *Synechocystis* remain unclear. At the same time, despite the generally accepted model of stretch-induced opening of the MscL (Blount & Moe, 1999; Sukharev, 1999; Martinac, 2001; Sukharev et al., 2001), some recent reports demonstrate both the voltage-induced and stretch-independent activation of mechanosensitive ion channels, as well as voltage-induced changes in membrane tension (Gil et al., 1999; Reifarth et al., 1999; Zimmerman & Sentenac, 1999). The latter effect, which is probably caused by changes in electromechanical compression of the lipid bilayer (Needham & Hochmuth, 1989; Menconi et al., 2001), provides a possible explanation for our results as well. Such an interpretation is in accordance with the known cross-sensitivity of cell membrane ion channels to factors of different physical nature, such as temperature and osmotic stresses (Ding & Pickard, 1993a, b; Martinac et al., 1990; Jones et al., 2000; Marchenko & Sage, 2000; Kikuyama & Tazawa, 2001). Based on the data cited above, it could be expected that the MscL of the freshwater *Synechocystis* may be activated, albeit to different extents, by different stress factors.

**ACKNOWLEDGEMENTS**

This work was supported in part by grants from Russian Foundation for Basic Research (no. 00-04-48421a and 01-04-99416) to D. A. L., and no. 01-04-06160 to A. A. L.

**REFERENCES**


