JOURNAL OF BACTERIOLOGY, Nov. 2000, p. 6523–6524 Vol. 182, No. 22
0021-9193/00/$04.00+0

A Gene of *Synechocystis* sp. Strain PCC 6803 Encoding a Novel Iron Transporter

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A mutant of *Synechocystis* sp. strain PCC 6803 disrupted for *sll1878* exhibited greatly reduced Fe^{3+} transport activity. The K_m value of *sll1878*-dependent Fe^{3+} transport in cells grown in iron-replete medium was 0.5 μM. Both the maximal rate and K_m value were increased in iron-starved cells.

While the iron concentration in terrestrial environments is high, the biological availability of this element can be very low, since under aqueous, oxygenic conditions, iron is present as Fe^{3+} (ferric iron), which forms insoluble hydroxides. To promote the acquisition of this element, many bacteria produce extracellular, iron-specific chelators known as siderophores (2, 3, 5, 8). Iron chelation and uptake by cyanobacteria have been reviewed by Boyer et al. (2). The *Synechocystis* sp. strain PCC 6803 genome contains 32 genes that potentially code for nucleotide-binding components of ATP-binding cassette transporters that have no other strong similarity to functionally identified transport polypeptides (6). To determine which of these genes is involved in iron transport, we have analyzed the growth and iron uptake of *Synechocystis* strains in which these putative transport genes have been disrupted. The results suggest that the protein encoded by *sll1878* is a novel iron transporter.

Cells were grown in BG-11 medium (7) buffered by 20 mM *N*-Tris(hydroxymethyl) methyl-2-aminoethanesulfonic acid (TES)–KOH at pH 8.0 under 3% CO_2 in air (vol/vol). To make iron-free BG-11, $MgSO_4$ was replaced by K_2SO_4 and the citric acid, ferric ammonium dicitrate, $CaCl_2$, and trace elements were not initially added to the medium. The medium was treated with Chelex 100 resin (Bio-Rad, Hercules, Calif.) and then supplemented with trace elements and ultrapure $MgCl_2$ and $CaCl_2$ (Ultrapure Chemicals Co., Saitama, Japan). To starve *Synechocystis* for iron, cells were grown in normal BG-11 medium, washed by 20 mM TES-KOH (pH 8.0), and then grown in fresh iron-free BG-11 overnight under continuous illumination with fluorescent lamps at 60 $\mu E\ m^{-2}s^{-1}$.

The mutant lacking *sll1878* (designated M-1) constructed in this study has been deposited in the web site "CyanoMutants" (http://www.kazusa.or.jp/ cyano/mutants/), where the site of insertion of the kanamycin resistance cassette is shown. The wild-type and mutant cells before and after iron starvation were washed with 20 mM TES–KOH buffer and resuspended in fresh iron-free BG-11 at 2×10^9 cells/ml. $^{59}FeCl_3$ solution was added to iron-free BG-11 medium supplemented with various concentrations of cold $FeCl_3$. An aliquot (250 μl) of this solution was mixed with an equal volume of cell suspension in the presence of 1 mM ferrozine (Sigma Chemical Co., St. Louis, Mo.) and incubated at 30°C, either in the dark or light (at 700 $\mu E\ m^{-2}\ s^{-1}$). Uptake was terminated by centrifugation, and the pellet was washed twice with 20 mM Tes–KOH containing 10 mM EDTA before being analyzed for the incorporation of $^{59}FeCl_3$.

Out of 32 *Synechocystis* genes encoding nucleotide binding components of ATP-binding cassette transporters that have not been ascribed any function, we were able to construct 24 separate mutants by inactivating the transporter genes but were unable to attain complete disruption of the remaining 8 genes (*sll0759*, *sll0912*, *sll1276*, *sll1623*, *slr0075*, *slr0251*, *slr0354*, and *slr1735*). All of the mutants except for the one lacking *sll1878* (M-1) grew as well as the wild type on solid, iron-free BG-11 medium, probably utilizing iron that contaminates the iron-free medium or that is carried over from the cell cultures used for the initial inoculum. Wild-type cells grew at a maximal rate at 1 μM Fe^{3+}, while the M-1 mutant grew more slowly at this Fe^{3+} concentration.

The slow growth of the M-1 mutant in iron-free medium was ascribed to a defect in iron acquisition. We assayed the wild-type and mutant strains for the rate of Fe^{3+} transport using $^{59}FeCl_3$ in the presence of ferrozine (inhibits Fe^{2+} transport) (4). Figure 1A shows time courses of $^{59}Fe^{3+}$ accumulation by iron-deprived wild-type and M-1 mutant cells incubated with 10 μM $^{59}FeCl_3$ in the light or dark. The Fe^{3+} uptake proceeded in the dark; light did not have a stimulatory effect on the accumulation of iron over at least a 30-min period. Hence, respiration and other dark metabolic reactions must generate a sufficient supply of ATP to energize Fe^{3+} transport. This is in contrast to the transport of other ions such as Mn^{2+} that is light dependent (1). Since the amount of Mn^{2+} taken up by *Synechocystis* strain PCC 6803 cells is not more than that of Fe^{3+}, it might be expected that ATP produced in the dark would be sufficient to drive Mn^{2+} uptake. However, Mn^{2+} uptake may be linked to immediate incorporation of the cation into protein(s), a process that might be light dependent.

Figure 1B shows uptake of Fe^{3+} by wild-type and M-1 mutant cells grown in nutrient-replete medium (upper panel) or by iron-deprived cells (lower panel). The cells were incubated for 5 min in the dark with various concentrations of $FeCl_3$ in the presence of 1 mM ferrozine. Fe^{3+} uptake by the M-1 strain was about one-fifth that of wild-type cells. Fe^{3+} transport activity increased more than fivefold in wild-type cells and two to three times in the M-1 mutant following iron deprivation. The low-level Fe^{3+} transport activity retained in the M-1 mutant suggests the presence of additional Fe^{3+} transporter(s). The difference between the two curves a-b and c-d approximates the activity of the *sll1878*-dependent Fe^{3+} transport. The K_m and V_{max} values for *sll1878*-dependent Fe^{3+} transport, deter-

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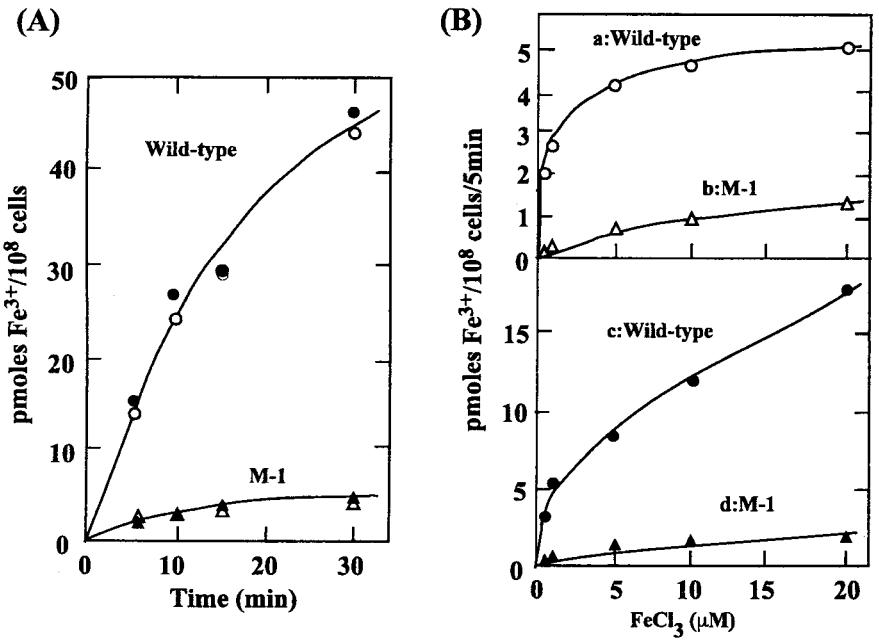

FIG. 1. (A) Time course of $^{59}Fe^{3+}$ uptake by iron-deprived wild-type and M-1 cells, either in the dark (filled symbols) or in the light (open symbols). The concentration of $^{59}FeCl_3$ was 10 μM. (B) Concentration-dependent uptake of $^{59}Fe^{3+}$ by wild-type and M-1 cells grown in complete medium (curves a and b in upper panel) or by iron-deprived cells (curves c and d in lower panel) during a 5-min incubation in the dark.

mined by plotting the reciprocals of curve a-b and curve c-d against the reciprocals of the substrate concentration were 0.5 μM and 3.9 pmol/10^8 cells/5 min, respectively, in the cells grown in nutrient-replete medium and 2.5 μM and 25 pmol/10^8 cells/5 min, respectively, in the iron-deprived cells. Thus, the activity of the *sll1878*-dependent Fe^{3+} transport increased about sixfold after iron deprivation treatment. The affinity of the transporter for the substrate decreased fivefold in the iron-deprived cells.

The product of *sll1878* appears to be a peripheral membrane protein. No citrate is required for *sll1878*-dependent Fe^{3+} uptake, demonstrating that the citrate-iron chelate is not the substrate for this transporter. However, the substrate may be a complex between ferric iron and siderophores produced by *Synechocystis* in response to iron deprivation.

This work was supported by a grant, JPSP-RFTF96L00105, from the Japan Society for the Promotion of Science and a grant from the Human Frontier Science Program to T.O. and by National Science Foundation grant MCB 9727836 to A.R.G.

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