An alternative methionine aminopeptidase, MAP-A, is required for nitrogen starvation and high-light acclimation in the cyanobacterium *Synechocystis* sp. PCC 6803

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Methionine aminopeptidases (MetAPs or MAPs, encoded by *map* genes) are ubiquitous and pivotal enzymes for protein maturation in all living organisms. Whereas most bacteria harbour only one *map* gene, many cyanobacterial genomes contain two *map* paralogues, the genome of *Synechocystis* sp. PCC 6803 even three. The physiological function of multiple *map* paralogues remains elusive so far. This communication reports for the first time differential MetAP function in a cyanobacterium. In *Synechocystis* sp. PCC 6803, the universally conserved *mapC* gene (*slr0555*) is predominantly expressed in exponentially growing cells and appears to be a housekeeping gene. By contrast, expression of *mapA* (*slr0918*) and *mapB* (*slr0786*) genes increases during stress conditions. The *mapB* paralogue is only transiently expressed, whereas the widely distributed *mapA* gene appears to be the major MetAP during stress conditions. A *mapA*-deficient *Synechocystis* mutant shows a subtle impairment of photosystem II properties even under non-stressed conditions. In particular, the binding site for the quinone Q_B is affected, indicating specific N-terminal methionine processing requirements of photosystem II components. MAP-A-specific processing becomes essential under certain stress conditions, since the *mapA*-deficient mutant is severely impaired in surviving conditions of prolonged nitrogen starvation and high light exposure.

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INTRODUCTION

The starting residue in protein synthesis in bacteria, mitochondria and chloroplasts is always formylmethionine (fMet). Following incorporation into protein, the formyl group is removed by peptide-deformylase activity and the N-terminal methionine residue may then be excised by methionine aminopeptidase (MetAP) activity. This N-terminal methionine excision (NME) pathway affects up to 80 % of cell proteins and is essential for cell viability (Giglione *et al.*, 2003). Although it is known that the N-terminus of proteins is important for protein stability and turnover, cellular targeting or protein function, the broad physiological significance of NME still

remains poorly understood (Frottin *et al.*, 2006). The NME process is highly conserved and the MetAPs of bacteria are similar to MetAPs from mitochondria and chloroplasts, together comprising the MetAP1 family. Eukaryotes contain in addition a second cytoplasmatic MetAP (MetAP2), which is similar to MetAP from archaea (Lowther *et al.*, 1999). MetAPs have become increasingly important in biotechnology due to their essential role in N-terminal processing of recombinant proteins and as effective targets for drug development against cancer, atherosclerosis, angiogenesis or parasites in humans (reviewed by Vaughan *et al.*, 2002). The cleavage probability of N-terminal methionine residues is highly dependent on the following amino acid residue (reviewed by Lowther & Matthews, 2000). Generally, MetAPs display highest activity towards substrates that have small and uncharged second amino acids like glycine, threonine, valine or cysteine (Ben-Bassat *et al.*, 1987). Most bacteria contain only one MetAP-encoding *map* gene, and in these cases, the *map* gene is essential, as in *Escherichia coli* (Chang *et al.*, 1989). In some Gram-positive bacteria, two

Abbreviations: Chla, chlorophyll *a*; DCBQ, 2,6-dichloro-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; MetAP, methionine aminopeptidase; MmapA, *mapA* mutant; NME, N-terminal methionine excision; PAM, pulse amplitude modulated; PQQ, phenyl-*p*-quinone; PSII, photosystem II; RACE, rapid amplification of 5' cDNA ends.

Two supplementary figures are available with the online version of this paper.

or more *map* paralogues have been described, and the *map* paralogues from *Bacillus subtilis* were shown to be differentially expressed (You *et al.*, 2005); however, the functional significance of this redundancy remained unexplained.

In the genome of the unicellular freshwater cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter termed *Synechocystis*) three ORFs encoding potential MetAP1 paralogues were identified, *slr0786*, *slr0918* and *sll0555* (Kaneko *et al.*, 1996; see <http://bacteria.kazusa.or.jp/cyanobase/Synechocystis/index.html>). Unfortunately, the nomenclature of these *map* paralogues was inconsistent in different databases. Today, the UniProtKB/Swiss-Prot database terms the *slr0918* product MAP-A, the *slr0786* product MAP-B and the *sll0555* product MAP-C. The *in vitro* activities of the three *Synechocystis* MetAPs were examined previously (Atanassova *et al.*, 2003). It should be noted that in the abstract and results part of that publication, the terminology for the *slr0786* and *slr0918* product had been inverted. In the results, the *slr0786* product corresponds to c2MetAP-Ia and that of *slr0918* to c1MetAP-Ia. All three MetAPs required Zn^{2+} , Co^{2+} or Mg^{2+} as co-factors and were able to cleave the N-terminal methionine of tested tripeptides. However, the three *map* products exhibited quite different specificities. The c3MetAP-Ia protein (*sll0555* product) displayed the highest activity and was also an effective dipeptidase. By contrast, c2MetAP-Ia (*slr0786* product) had almost no activity *in vitro* with most tested substrates and c1MetAP-Ia (*slr0918* product) showed moderate activity. In spite of this knowledge, the rationale for having three *map* genes in *Synechocystis* remains elusive. In order to simplify and adapt the nomenclature to that now used by UniProtKB/Swiss-Prot, in the following we term the product of *slr0918* MAP-A, that of *slr0786* MAP-B and that of *sll0555* MAP-C, and accordingly the names *mapA*, *mapB* and *mapC* are used for *slr0918*, *slr0786* and *sll0555*.

Plants and cyanobacteria perform oxygenic photosynthesis, employing a photosystem II (PSII) multiprotein complex to catalyse light-driven electron transport from water to the plastoquinone pool, resulting in oxygen formation. Most components are highly conserved in plants and cyanobacteria. The core of the PSII complex consists of the large subunits D1, D2, cytochrome b_{559} , antenna proteins CP47 and CP43, and a number of low-molecular-mass proteins (for a review see Barber, 2006). The biogenesis of PSII takes place in the plasma membrane, where the PSII core proteins assemble to a complex containing P680-chlorophyll and a functional acceptor site (Q_A and Q_B binding site) (Keren *et al.*, 2005; Zak *et al.*, 2001). The D1 protein, which is directly involved in electron donor and acceptor site formation, is the most sensitive component of PSII since it is highly susceptible to light-induced damage (Aro *et al.*, 1993). A number of proteins, such as the FtsH2 protease, the products of *slr2013* and *slr0286*, as well as PrtA, a periplasmic tetratricopeptide repeat protein, are involved in turnover of PSII in oxygenic phototrophic

organisms (Klinkert *et al.*, 2004; Komenda *et al.*, 2006; Kufryk & Vermaas, 2003; Silva *et al.*, 2003). The mechanism regulating the biogenesis and assembly of the PSII apparatus is still poorly understood and a specific function of N-terminal processing has so far not been described. However, inhibition of N-terminal fMet processing in the eukaryotic alga *Chlamydomonas reinhardtii* and in chloroplasts of *Arabidopsis thaliana* revealed that NME strongly affected the stability of PSII subunits (Giglione *et al.*, 2003).

The physiological function of N-terminal processing in cyanobacteria has been poorly investigated to date. This study aimed to characterize the role of the MetAP paralogues in *Synechocystis* sp. PCC 6803. Here, we describe that the *mapA* product is required for acclimation to high light and nitrogen starvation stress conditions.

METHODS

Growth of cyanobacteria. The transformable wild-type strain of *Synechocystis* strain PCC 6803 (Grigorieva & Shestakov, 1982) and derived *map* mutants (see below) were used in this study. All cells were grown photoautotrophically in liquid BG-11 medium, containing 17.6 mM NaNO_3 (Rippka, 1988) supplemented with 5 mM NaHCO_3 at 28 °C. The mutants were maintained with kanamycin ($50 \mu\text{g ml}^{-1}$). Under standard conditions, cells were incubated under continuous light of $80 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ from white fluorescent tubes (LUMILUX de Luxe Daylight, Osram) and constantly shaken at 130 r.p.m. Growth of the cultures was monitored by measuring the OD_{750} . For growth on plates, BG-11 medium supplemented with 1 mM $\text{Na}_2\text{S}_2\text{O}_3$ and 5 mM NaHCO_3 was solidified with 0.8% agar (Difco). Plates were incubated at 28 °C with continuous light of $50 \mu\text{mol photons m}^{-2} \text{s}^{-1}$.

For starvation experiments, cells of mid-exponential-phase cultures (corresponding to an OD_{750} of 0.4–0.5) were harvested by filtration ($0.45 \mu\text{m}$ Durapore membrane filter HV, Millipore), washed on the membrane and resuspended in combined-nitrogen-free BG-11 medium as described previously (Görl *et al.*, 1998), and incubated as described above. Phycobiliproteins were quantified from whole-cell spectra according to Myers *et al.* (1980). Chlorophyll *a* (Chl*a*) was extracted and quantified as described by Tandeau de Marsac & Houmard (1988). High light exposure was performed with halogen rays (white light, $400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). The cultures were constantly shaken at 130 r.p.m. and incubated at a constant temperature of 28 °C.

Cell viability assays. The ability to resume growth was evaluated on standard BG-11 nitrate agar plates, containing 17.6 mM NaNO_3 . Samples ($100 \mu\text{l}$ of the starved or $10 \mu\text{l}$ of high-light-treated cells) were plated after denoted time points and incubated as described above. After 24 days of incubation, photographs were taken and the plates were further incubated to further observe eventual delayed growth. Additionally cells were stained with the LIVE/DEAD *BacLight* assay (MöBiTec) bacterial viability kit according to the manufacturer's instructions. The dye solution was prepared by diluting $0.3 \mu\text{l}$ of each reagent (A and B) in $0.6 \mu\text{l}$ H_2O , and $0.3 \mu\text{l}$ dye solution was added to $100 \mu\text{l}$ of cells. After 10 min incubation in the dark at room temperature, $20 \mu\text{l}$ cell suspensions were placed onto an agarose-coated object slide. For visualization of live and dead cells, microscopy was performed with a Leica DM5500 fluorescence microscope equipped with a Leica DFC420 camera using blue light excitation (475 nm) and dual-band elimination filters (527/645 nm).

Furthermore, cell recovery was evaluated in liquid medium by adding sodium nitrate (17.6 mM) to the chlorotic cultures, measuring PSII activity by PAM fluorometry as described below and observing pigmentation (see above).

Determination of photosynthetic activity

PSII-dependent oxygen evolving activity. Photosynthetic oxygen evolution was measured *in vivo* using a Clark-type oxygen electrode (Hansatech DW1). Light was provided from a high-intensity white light source (Hansatech L2). Oxygen evolution was measured at room temperature using 2 ml of cell culture (with equal Chl *a* content) with 1000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$.

Pulse amplitude modulated (PAM) fluorometry. *In vivo* PSII fluorescence was analysed *in vivo* in a WATER-PAM chlorophyll fluorometer (Walz). The chlorophyll fluorescence (F_0) of dark-adapted cells was determined and the effective quantum yield (Y) of PSII photochemistry with actinic light of 655 nm ($67\text{--}1127 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) was determined by the saturation pulse method (Genty *et al.*, 1989; Schreiber *et al.*, 1995). Cultures were diluted 1:10 in BG-11 medium before the measurements in a final volume of 2 ml.

The various quinones (125 μM 1,4-benzoquinone, 375 μM 2,6-dichloro-*p*-benzoquinone, 125 μM phenyl-*p*-quinone together with 125 μM potassium ferricyanide) or 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) were added to the cells directly before measuring oxygen evolution or PAM fluorescence.

Mutagenesis. For inactivation of *mapB*, a 810 bp fragment containing the major part of the coding sequence of *mapB* (*slr0786*) was PCR amplified using primers WL139 (5'-CAATAAGGGCCCGGCTCGTATCATGCTAG-3', *ApaI* site inserted – underlined) and WL140 (5'-CCAACCATCTCTAGAAATGTAAGCTC-3', *XbaI* site inserted), digested with the appropriate restriction enzymes and ligated into cloning vector pBluescript SK+. For inactivation of *mapA*, an 883 bp fragment containing the major part of the coding sequence of *mapA* (*slr0981*) was amplified using primers WL129 (5'-GCCCTTGACGGTACCTAGTTGTCAG-3', *KpnI* site inserted) and WL130 (5'-CTTCAGTGACGGCAATGGCATGCTC-3', *SphI* site inserted), digested with the appropriate restriction enzymes and ligated into cloning vector pUC19. The kanamycin-resistance cassette from pUC4K was digested with *HincII* and inserted into *ClaI* sites (blunted) located in the middle of the *mapB* gene and into a unique *HincII* site located in the middle of the *mapA* gene, respectively. The resulting plasmids, pPepM (*mapB*) and pMap (*mapA*) were used to transform *Synechocystis*.

Analysis of the 5' end of *mapA* transcript using a 5'/3'RACE kit (rapid amplification of 5' cDNA ends). For determination of the 5' end of the *mapA* transcript, a 5'/3'RACE kit, 2nd generation (Roche Applied Science) was used according to the manufacturer's instruction. *Synechocystis* wild-type cells in the mid-exponential phase were shifted to nitrogen-free medium and after 3 days of starvation 250 ml of culture (OD_{750} 0.8) was harvested on ice by centrifugation (6000 r.p.m., 10 min, 4 °C) and used for RNA extraction. RNA was isolated by using the RiboPure-Bacteria kit (Ambion) according to the manufacturer's instructions. For the cDNA synthesis of the first strand, 0.5 μg RNA and an antisense gene-specific primer, *map2SP1* (5'-TTCCCAGGTGCCTTCATTGA-3'), located 617 bp downstream of ATG, were used, and for the nested PCR the PCR anchor primer (Roche) and an antisense gene-specific primer, *map2SP2* (5'-TCTTCGGTGACCTTGACCAG-3'), located 372 bp downstream of ATG, were used. Amplification products were checked by agarose gel electrophoresis. The final amplification products were purified with a Gel Purification kit (Qiagen) and subcloned into the pCR II-TOPO vector using the TOPO TA cloning

kit (Invitrogen), and recombinant plasmids were sequenced. The sequence alignment was done with BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>) against the *mapA* (*slr0918*) gene sequence available in Cyanobase (<http://bacteria.kazusa.or.jp/cyano/>).

RNA isolation and semiquantitative RT-PCR. Cells growing under different conditions were harvested by centrifugation (6000 r.p.m., 10 min, 4 °C) and RNA was isolated by using the RiboPure-Bacteria kit (Ambion) according to the manufacturer's instructions. The RNA was eluted in 50 μl water, and to remove the genomic DNA it was treated once with the DNA-free kit (Ambion). The DNase treatment was checked by PCR.

Semiquantitative RT-PCR was performed with the OneStep RT-PCR kit (Qiagen) according to the manufacturer's instructions. The following primers were used. For *mapB*: forward, 5'-GCCTCGG-CATATGCTTGAAT-3'; reverse, 5'-GGAATACCGAACCCAAAGCA-3'. For *mapA*: forward, 5'-TCACCTAGCGCAATGGT-3'; reverse, 5'-TGCTGTGCAATACCCGTTT-3'. For *mapC*: forward, 5'-AGCGG-ACGTTAAACACATGG-3'; reverse, 5'-ATTGCGGTGGGTAAAGG-TTTC-3'. As an RNA-loading control in the PCR the constitutively expressed gene *rnpB* (RNase P subunit B) was used with the following primers: forward, 5'-GTGAGGACAGTGCCACAGAA-3'; reverse, 5'-GGCAGGAAAAAGACCAACCT-3'. First, the optimal cycles required for quantitative amplification of *map1*, *map2*, *map3* and *rnpB* were determined. The following RT-PCR programme was finally used: reverse transcription reaction for 30 min at 50 °C, initial PCR activation step for 15 min at 95 °C and a three-step cycling PCR with denaturing (1 min, 94 °C), annealing step (1 min, 51–57 °C), extension step (1 min, 72 °C) for 26, 24, 22 and 15 cycles for *mapB*, *mapA*, *mapC* and *rnpB*, respectively, and a final extension (10 min, 72 °C). Specificity of amplification was further verified with PCR controls omitting the initial reverse transcription. The PCR products were separated by agarose gel electrophoresis and stained with ethidium bromide. The results were analysed and quantified with the Bio-Rad Quantity One software.

MIC determination. The minimal inhibitory concentration (MIC) of the herbicide DCMU was determined by the serial dilution method in 48-well culture plates. DCMU was diluted in BG-11 medium in a range from 1 μM to 0.01 μM . The final well volume was 1 ml after inoculation. Test organisms were prepared from photoautotrophically nitrate-grown *Synechocystis* cells in the exponential growth phase, which were inoculated in the wells to an initial OD_{750} of 0.1. After incubation (48 h, 28 °C, 30 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) the plates were photographed. The MIC represents the minimal concentration of the herbicide that still inhibits the growth of *Synechocystis* cells. The MIC determinations were done in duplicate.

77 K pigment fluorescence measurements. Fluorescence emission spectra were measured at 77 K in a Perkin-Elmer luminescence spectrometer LS 50-B. *Synechocystis* cells were adjusted to 10 $\mu\text{g Chl a ml}^{-1}$ by dilution with standard BG-11 medium, transferred to cryo tubes and frozen in liquid nitrogen. Fluorescence was excited at 435 nm (± 10 nm) and fluorescence emission was recorded in the range 600–750 nm (± 5 nm, 515 nm cut-off filter) at a speed of 200 nm min^{-1} . The spectra were analysed with FL WinLap.

Immunoblot analysis of MAP-A. For generation of MAP-A specific antibodies, the ORF *slr0918* (*mapA*) was amplified via PCR (primers: forward, 5'-GGAATTCATATGGGCGACACTATTACCCT-3'; reverse, 5'-CCGCTCGAGTTACTCCCTAGGGTCAAATTTCC-3'; *NdeI* and *XhoI* sites, respectively, underlined), purified and cloned into pET-15b (Novagen), generating an N-terminal His₆-tag fusion. The resulting plasmid was transformed into *E. coli*-BL21(DE3), and the cells were grown in LB medium with 100 μg ampicillin ml^{-1} at 37 °C, to an OD_{600} of 0.8. Protein overproduction was induced with

1 mM IPTG and incubation at 20 °C for 24 h. The cells were harvested (6000 r.p.m., 10 min, 4 °C) and broken in lysis buffer (50 mM NaH₂PO₄, 400 mM NaCl, 10 mM imidazole, 1 mM PMSF, pH 8.0) by sonification on ice. The MAP-A protein was purified with an Ni-NTA column (His-Select cartridge, Sigma) according to the manufacturer's description, using wash buffer (20 mM Tris/HCl, 400 mM NaCl, 20 mM imidazole, pH 8.0), and the protein was eluted with 250 mM imidazole. The elution buffer was exchanged by dialysing the protein with 100 mM K₂HPO₄, 100 mM KH₂PO₄, 100 mM KCl, 100 mM NaCl, 50 % (v/v) glycerine. The purity of the protein was determined by SDS-PAGE and used for generation of antibodies in rabbits (Pineda Antikörper-Service, Berlin). For immunoblot analysis, cyanobacterial cell extracts were prepared by breaking the cells with glass beads (110 µm diameter) using a FastPrep (MP Biomedicals) followed by centrifugation (3500 g, 10 min, 4 °C). Cell extract was separated by ultracentrifugation (100 000 g, 60 min, 4 °C). Protein compositions were assessed by electrophoresis in a denaturing 12.5 % SDS-PAGE and 7 µg samples of total protein from the soluble and the particulate fraction were loaded per lane. Immunodetection was performed with polyclonal antibodies raised against MAP-A and visualized by chemoluminescence reaction (LumiLight, Roche).

RESULTS

Survey of cyanobacterial *map* sequences and inactivation of *Synechocystis map* genes

In the genome of *Synechocystis*, three different ORFs code for MetAPs: *slr0918*, *slr0786*, and *sll0555* (Kaneko *et al.*, 1996). A survey of the available cyanobacterial genome data (see Cyanobase, <http://bacteria.kazusa.or.jp/cyano/> and GenBank, <http://www.ncbi.nlm.nih.gov/Genbank>) revealed the presence of close homologues of the *Synechocystis mapC* (*sll0555*) gene in all cyanobacteria whose genome has been sequenced to date. By contrast, *mapA* (*slr0918*) homologues are restricted to about half of the cyanobacterial strains. A comparison with a recent phylogenetic tree of cyanobacteria (Voß *et al.*, 2007) revealed that the cyanobacteria which only contain the *mapC* homologue belong to one phylogenetic cluster, containing unicellular freshwater and marine *Synechococcus* and *Prochlorococcus* strains. Both *mapA* and *mapC* homologues are present in all other strains, including the nearest relatives of *Synechocystis*, namely *Crocospheera watsonii* and *Cyanosphaera* PCC 7424, as well as in all filamentous strains, and the most deeply branched species *Gloeobacter violaceus* and thermophilic strains *Synechococcus* JA-2-3Ba and *Synechococcus* JA-3-3Ab (see Supplementary Fig. S1, available with the online version of this paper). A third *map* gene has so far only been identified in *Synechocystis*. Inactivation of the three *map* genes in *Synechocystis* was attempted by insertion of a kanamycin-resistance gene in the respective coding regions. Homozygous mutants were obtained for *mapA* (*slr0918*) and *mapB* (*slr0786*), which were used for further analysis. The inability to fully segregate the *mapC* mutation indicates that MAP-C may be essential for *Synechocystis*. Initial screening experiments under various growth conditions indicated that the *mapA* mutant (MmapA) was severely impaired in surviving

periods of prolonged nitrogen starvation (chlorosis), whereas the *mapB* mutant was unaffected under such conditions. Therefore, the phenotype of the MmapA strain was investigated in more detail.

Phenotype of MmapA during nitrogen chlorosis

Unicellular non-diazotrophic cyanobacteria including *Synechocystis* undergo chlorosis under conditions of nitrogen starvation (reviewed by Schwarz & Forchhammer, 2005). As documented in detail for *Synechococcus elongatus*, the cells rapidly bleach due to the degradation of phycobiliproteins (Collier & Grossman, 1992). Prolonged starvation leads to a strong reduction of photosynthetic activity to a basal level of approximately 0.1 % compared to vegetative cells, and maintenance of this residual activity is essential for keeping the cells alive (Sauer *et al.*, 2001). Comparing nitrogen chlorosis in the MmapA mutant with wild-type cells showed that the initial chlorosis responses, the degradation of phycobiliproteins and Chl_a, as well as PSII decay, occurred in the same manner in the wild-type and the *mapA* mutant (Fig. 1a).

To investigate the ability to reinitiate growth after prolonged periods of chlorosis, the viability of the cells was analysed with different assays. Recovering cells on nitrogen-replete agar showed that 7 days after nitrogen step-down, the MmapA cells still recovered like the wild-type, after 13 days of nitrogen starvation, the MmapA cells displayed only a very low plating efficiency and after 35 days chlorosis, no more colonies could be recovered on nitrate-replete agar, regardless of how long the plates were incubated (Fig. 1b).

In addition, the recovery of chlorotic cells was assayed by supplementing liquid cultures with nitrate and observing the repigmentation and measuring PSII activity in recovering cultures. PAM fluorometry proved to be a very sensitive method for detecting emerging PSII activity from chlorotic cells (Sauer *et al.*, 2001). After 7 days of chlorosis, the MmapA cells were still able to recover PSII activity as detected by the emergence of PSII quantum yield, but could not do so after 35 days of chlorosis (Fig. 1c), confirming the plating efficiency under the same conditions (Fig. 1b). Furthermore, 35-day-old chlorotic cultures of the MmapA strain could no longer regenerate pigmentation and growth. This is in sharp contrast to the wild-type, whose ability to recover from chlorosis was not affected by prolonged starvation (Fig. 1). The loss of viability of the MmapA cells after 35 days chlorosis was further confirmed by LIVE/DEAD BacLight staining. Red-fluorescent spots, indicating a loss of cell viability (Görl *et al.*, 1998), were visible in MmapA cells but not in wild-type cells (Fig. 1c, right panel). After 63 days of chlorosis, the wild-type cells still exhibited residual variable chlorophyll fluorescence as measured by PAM fluorometry, indicative of remaining PSII activity. By contrast, no variable fluorescence was observed in the MmapA cultures starved for the same length of time, indicating a total loss of PSII activity (not shown). Taken

Fig. 1. Nitrogen-starvation-induced chlorosis and recovery in *Synechocystis* wild-type and MmapA cells. (a) Left: time-course of phycobiliprotein (circles) and Chl a (triangles) decay in wild-type (filled symbols) and MmapA (open symbols) cultures after shift to combined-nitrogen-depleted medium. The pigments were determined from whole-cell absorption spectra as described (see Methods), and normalized to the value immediately after the shift to nitrogen-free medium (100%). Right: PSII quantum yield was determined by PAM fluorometry (see Methods) and normalized to the value immediately after the shift to nitrogen-free medium (100%). The values represent the mean of three independent experiments. The standard deviations were below 5%. (b) Nitrate-grown culture (+N) and nitrogen-starved cultures after 7, 13 and 35 days of chlorosis (-N) were plated on nitrate-replete BG-11 agar plates and photographed after 24 days incubation under standard conditions. (c) Starved cultures were supplemented with 17.6 mM NaNO_3 and incubated as before. At denoted time points the PSII quantum yield was determined by PAM fluorometry. The 100% value corresponds to the value determined before the induction of nitrogen starvation. The recovery of wild-type (filled symbols) was almost identical after 13 days (circles) and 35 days (triangles) of chlorosis. MmapA cells (open symbols) recovered slowly after 13 days chlorosis (circles), but no regeneration of PSII quantum yield was detectable after 35 days chlorosis (triangles). Three independent experiments revealed standard deviations below 5%. The pictures to the right show *Synechocystis* cells from a 35-day-old nitrogen-starved culture stained with LIVE/DEAD *BacLight* viability stain. Uptake of red colour indicates cell death.

together, these data strongly suggested that MAP-A activity is necessary to maintain cell viability and residual PSII activity during long-term nitrogen chlorosis.

Phenotype of MmapA during high light exposure

To study the effect of high light exposure on MmapA cells, cultures were exposed to a photon fluence rate of $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ of white light, while shaking them in flasks with ambient air. In the course of high light exposure, light-saturated rates of oxygen evolution were measured, cell viability was analysed by using LIVE/DEAD *BacLight* viability stain and plating efficiency was tested on standard BG-11 agar plates. In wild-type cells, photosynthetic oxygen evolution under light-saturated conditions first increased in response to high light exposure, presumably as a result of a rapid acclimation to the increased photon flux density. After more than 20 h of high light exposure, a decrease of oxygen evolution was observed, which stabilized at a level corresponding to approximately 50 % PSII activity compared to the initial value (Fig. 2a). In contrast to the wild-type, the *mapA* mutant could not cope with high light exposure. Photosynthetic oxygen evolution started to decrease immediately following high light treatment, reaching after 24 h a level of approx. 58 %, which further declined with prolonged incubation (Fig. 2a). Growth of wild-type cells occurred during the entire high light exposure time, whereas the MmapA cells stopped growing after 28 h, as observed by measuring OD_{750} (not shown). Live-dead staining confirmed a loss of viability of MmapA cells as visualized by a loss of green fluorescence and appearance of red fluorescence. After 57 h of high light treatment, no more viable cells of MmapA were observed by live-dead staining (Supplementary Fig. S2). In agree-

ment with these data, plating efficiency of the MmapA cells decreased strongly during high light treatment (Fig. 2b). Together, the results of this study implied that the function of the *mapA* product is essential for acclimating to high light stress.

To investigate the damage to the photosynthetic apparatus of the MmapA cells during high light exposure in more detail, low-temperature (77 K) fluorescence emission spectra with excitation light absorbed by Chl *a* (435 nm) or phycobiliproteins (600 nm) were acquired. Fig. 3 shows the spectra from wild-type and MmapA cells before (a) and after (b) 18 h high light treatment, a time point where most MmapA cells were still viable. Excitation at 600 nm sensitizes mostly phycocyanin; the 655 nm emission peak, to which the spectrum was normalized, reveals fluorescence from phycocyanin, the 687 nm peak results from the phycobilisome core and PSII Chl *a* with a PSII Chl *a* shoulder at 697 nm, and the 720 nm peak results from emission of PSI Chl *a*. Excitation at 435 nm sensitizes Chl *a* and reveals fluorescence of PSI and PSII (Allen *et al.*, 1989). The spectrum was normalized to PSI emission at 717 nm to reveal differences in PSII. The fluorescence maximum at 685 nm and the shoulder at 695 nm originate mainly from chlorophylls of the PSII core complex. As shown in Fig. 3, the fluorescence spectrum of MmapA showed a subtle difference compared to the wild-type before high light exposure. In the 435 nm excitation spectrum, a slight decrease of chlorophyll emission at 695 nm, corresponding mainly to the antenna protein PsbB (CP47) of PSII, was observed. After 18 h of high light exposure, the MmapA strain now differed markedly from the wild-type: in 600 nm excited samples the phycobilisome core/PSII peak was strongly reduced compared to the wild-type. In the

Fig. 2. Sensitivity of *Synechocystis* wild-type and MmapA cells to high light. (a) *Synechocystis* wild-type (filled circles) and MmapA (open circles) cultures were grown to the mid-exponential phase, and at time 0 the cultures were exposed to high light ($400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$). At denoted time points, photosynthetic activity was determined by measuring oxygen evolution as described in Methods. The 100 % values correspond to the values determined before the illumination process. (b) Plating efficiency of *Synechocystis* wild-type and MmapA cells after 6 and 24 h of high light (HL; $400 \mu\text{mol photons s}^{-1} \text{m}^{-2}$) exposure, or as a control, incubated for 24 h under a fluence density of $40 \mu\text{mol photons s}^{-1} \text{m}^{-2}$ (LL).

Fig. 3. 77 K fluorescence emission spectra of intact *Synechocystis* wild-type and MmapA cells. (a) Spectra from cells exposed to moderate light ($60 \mu\text{mol photons m}^{-2} \text{s}^{-1}$); (b) spectra of cells after illumination with high light ($400 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 18 h. Left, 600 nm excitation spectra, normalized to 655 nm emission; right, 435 nm excitation spectra, normalized to 717 nm emission. The emission spectrum of *Synechocystis* wild-type cells is represented by solid lines, that of MmapA cells by dashed lines. The values represent the means of two independent measurements, which differed by less than 10%. a.u., arbitrary units.

435 nm excitation spectrum, the MmapA cells showed a clear reduction of the 695 nm fluorescence corresponding mainly to CP47, as compared to high-light-treated wild-type cells, indicative of damage of PSII and confirming the results from the photosynthetic oxygen evolution measurements.

Expression pattern of the three *map* genes under different growth conditions

To investigate the expression pattern of the three *map* genes during photoautotrophic growth in nitrogen-replete and nitrogen-starved conditions, as well as during high light exposure, semiquantitative RT-PCR was performed with gene-specific *mapB*, *mapA* and *mapC* primers (see Methods). Under nitrogen-replete growth, all three *map* genes appeared to be expressed (Fig. 4a) and their levels did not significantly change at different phases of growth (not shown). Nitrogen starvation enhanced the expression of *mapB* and *mapA* genes, whereas the expression of *mapC* clearly decreased (Fig. 4a). *mapB* expression reached a maximum after 24 h, and subsequently expression decreased. By contrast, *mapA* expression increased up to 72 h, and the level was maintained at high levels even after 144 h. In accord, an increase of MAP-A protein in the soluble cell-free extract was observed during nitrogen starvation by Western blot analysis (not shown). Similar to nitrogen-stress conditions, high light exposure induced the expression of *mapB* and *mapA* genes, whereas the *mapC* gene was tuned down after 24 h (Fig. 4b).

To gain insights into the control of *mapA* expression, the *mapA* promoter region was analysed. The 5' end of the *mapA* transcript was determined by the 5'RACE method (see Methods). The potential transcriptional start point (tsp), as deduced from this experiment, is located 57 bp upstream of the predicted ATG start codon. Interestingly, 84 bp upstream of the potential tsp, a sequence resembling an NtcA-binding motif was found (Fig. 5). The motif exhibits one base deviation from the canonical GTA-N₈-TAC motif, and therefore resembles the non-canonical NtcA motifs found in certain NtcA-regulated genes that are expressed under severe nitrogen starvation, such as *glnN* (Reyes *et al.*, 1997; Aldehni & Forchhammer, 2006). Attempts to generate NtcA-deficient mutants in *Synechocystis* have failed so far (Garcia-Dominguez *et al.*, 2000), preventing examination of *mapA* expression by mutant analysis. Due to the low affinity of NtcA for non-canonical sites, binding cannot reliably be detected by mobility shift assays (Reyes *et al.*, 1997; Jiang *et al.*, 2000). In fact, using the *mapA* upstream region as a probe, only a faint gel-shift by NtcA could be observed (data not shown); thus the proof for regulation of *mapA* by NtcA awaits further elucidation.

MAP-A-deficient cells display an altered quinone acceptor site in PSII

PSII activity as determined by oxygen evolution did not reveal differences between wild-type and MmapA cells that were grown under standard conditions (not shown).

Fig. 4. Expression pattern of *map* genes during different growth conditions. Total RNA was isolated from wild-type cells during different photoautotrophic growth conditions: (a) nitrogen-starved conditions (-N) and (b) after high light exposure (HL). After 0, 6, 24 and 144 h, semiquantitative RT-PCR was performed with specific *mapB*, *mapA*, and *mapC* primers (for details see Methods). The constitutively expressed *rnpB* gene served as a control for the nitrogen step-down experiment. Three independent experiments, of which one is shown, yielded almost identical results. For the high light experiment, total RNA served as a loading control, since *rnpB* expression was affected by high light conditions. A representative of three independent experiments is shown.

However, PAM fluorescence analysis indicated subtle differences between mutant and wild-type cells under non-stressed conditions. The MmapA cells showed appreciably more variable fluorescence (F_v) under almost saturating light ($1127 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) than the wild-type (Fig. 6a, arrows), indicating a more efficient fluorescence quenching under high actinic light in the

mutant. To reveal whether this difference might be caused by altered electron acceptor availability, PSII activity was tested with the artificial electron acceptor 1,4-benzoquinone in combination with potassium ferricyanide (each at 125 μM). At low fluence rates (67 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) no difference in variable fluorescence between the two strains was apparent (Fig. 6b). However, when high actinic light (1127 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) was turned on, appreciable differences between mutant and wild-type cells appeared. Whereas the wild-type was not affected by benzoquinone/ferricyanide (compare Fig. 6a and b), the mutant displayed – after the initial rapid fluorescence increase – a second (slow) phase of fluorescence increase, which did not occur in the wild-type. Finally, the mutant reached fluorescence levels that greatly exceeded the initial maximal fluorescence (F_m') of dark-adapted cells. Saturation light pulses yielded no more variable fluorescence (F_v), indicating complete closure of acceptor sites in the presence of benzoquinone/ferricyanide (compare MmapA in Fig. 6a and b). This result could indicate that, in contrast to the wild-type, benzoquinone is an unfavourable PSII electron acceptor in MmapA. In accord with this interpretation, oxygen evolution in the presence of 1,4-benzoquinone showed an approximately 40 % decrease in the mutant compared to the wild-type (Table 1).

To investigate whether the inhibitory effect of benzoquinone depends on the chemical structure of the acceptor molecule, light-saturated oxygen-evolving activity was determined in the presence of various quinone derivatives, which differ in size and molecular mass (Table 1). In wild-type cells, 1,4-benzoquinone, 2,6-dichloro-*p*-benzoquinone (DCBQ) and phenyl-*p*-quinone (PPQ) led to equal oxygen evolution rates. However, in MmapA cells, PSII-dependent oxygen evolution depended on the type of quinone. Whereas 1,4-benzoquinone substantially inhibited oxygen evolution, DCBQ led only to a slight reduction of PSII activity, and PPQ did not impair PSII activity (Table 1).

Since these data strongly indicated an altered Q_B site in the *mapA* mutants, the effect of the PSII Q_B site inhibitor DCMU was investigated. In the presence of a sublethal concentration of DCMU (0.01 μM), actinic light of 67 μmol photons m⁻² s⁻¹ already caused a strong fluorescence increase in the wild-type, due to partial closure of the PSII acceptor sites by DCMU. In contrast, PAM fluorescence in the MmapA cells was not severely affected by DCMU, and the cells exhibited appreciable variable fluorescence under low and high actinic light conditions (Fig. 6c), indicating that the acceptor sites were not as efficiently blocked by DCMU as in the wild-type. To independently confirm this result, the sensitivity to DCMU was tested by determining the MIC and oxygen evolution. The MIC of MmapA cells for DCMU was 0.16 μM, whereas 0.04 μM DCMU was sufficient to inhibit growth of the wild-type (Fig. 7a). Furthermore, oxygen evolution in MmapA cells was clearly less sensitive towards DCMU

Fig. 5. Sequence of the 5' end of the *mapA* transcript (*slr0918*). Total RNA from nitrogen-starved *Synechocystis* wild-type cells was isolated and analysed by using the 5'RACE method, as described in Methods. Sequencing the cDNA revealed the 5' end of the *mapA* transcript (+1), indicating a potential transcription start site (tsp). The potential NtcA-binding site upstream of the tsp is aligned to the NtcA-binding site in the promoter of the *glnA* gene (*slr1765*) (<http://www.ncbi.nlm.nih.gov/>). Below, the consensus canonical NtcA-binding sequence is shown (Luque *et al.*, 1994). The putative NtcA-binding motif is highlighted in bold and the possible tsp is underlined.

Fig. 6. PAM chlorophyll fluorescence analysis of *Synechocystis* wild-type and MmapA cells. Cultures were grown in standard BG-11 medium (OD_{750} 0.5) and samples were taken for measuring chlorophyll fluorescence in the dark (F_0) and under actinic light of 67 or 1127 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (in part c, 865 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$), as indicated below the graph (* stands for $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). Maximal chlorophyll fluorescence (F_m) was induced by 0.2 s saturating red-light pulses (7000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, indicated by 'x'). PAM fluorescence analysis of *Synechocystis* wild-type (right) and MmapA (left) cells under various conditions: (a) without additions; (b) in the presence of 125 μM 1,4-benzoquinone and 125 μM potassium ferricyanide, added directly before measurement; (c) in the presence of 0.01 μM PSII inhibitor DCMU, added directly before measurement. The arrows below the traces designate actinic light on or off; the arrows at the top point towards the saturating light pulses at high actinic light. The analysis was performed three times, generating almost identical results.

Table 1. Photosynthetic oxygen evolution in *Synechocystis* wild-type and MmapA cells in the presence of different quinones and potassium ferricyanide

Oxygen evolution was measured in the presence of 125 μM 1,4-benzoquinone, 375 μM 2,6-dichloro-*p*-benzoquinone (DCBQ) and 125 μM phenyl-*p*-quinone (PPQ) in the presence of 125 μM potassium ferricyanide as described in Methods. Cells of mid-exponential-phase cultures grown in BG-11 medium under standard conditions were used (see Methods). The values were normalized to the oxygen evolution rate of untreated samples [100 %, corresponding to 286 $\mu\text{mol O}_2$ (mg Chl*a*)⁻¹ h⁻¹ for the wild-type and 298 $\mu\text{mol O}_2$ (mg Chl*a*)⁻¹ h⁻¹ for MmapA cells]. The results from three independent experiments with standard deviations are shown.

Strain	Oxygen evolution (%)		
	1,4-Benzoquinone	DCBQ	PPQ
Wild-type	106.2 $\pm$ 8.3	114.6 $\pm$ 11.8	121.1 $\pm$ 8.4
MmapA	65.9 $\pm$ 3.6	88.3 $\pm$ 3.2	118.5 $\pm$ 7.11

than that in wild-type cells (Fig. 7b). Together, these data support the conclusion that the *mapA* mutant has an altered quinone acceptor site.

Fig. 7. Susceptibility of *Synechocystis* wild-type and MmapA to DCMU. (a) MIC determination by serial dilution of DCMU in BG11 medium in 48-well culture plates as indicated and inoculation with *Synechocystis* wild-type or MmapA cells (for details see Methods). (b) Photosynthetic oxygen evolution in the presence of various DCMU concentrations by wild-type (filled circles) and MmapA cultures (open circles). Error bars show the standard deviations from three independent measurements. The 100 % values correspond to the values determined without DCMU [243 $\mu\text{mol O}_2$ (mg Chl*a*)⁻¹ h⁻¹ for wild-type and 301 $\mu\text{mol O}_2$ (mg Chl*a*)⁻¹ h⁻¹ for MmapA].

DISCUSSION

As deduced from its universal distribution in cyanobacteria, MAP-C appears to be the dominant and housekeeping MetAP. Its predominant expression under non-stressed conditions corresponds to its predicted role as major MetAP during healthy photosynthetic growth. By contrast, MAP-A seems to fulfil a specialized role. Its expression is enhanced under conditions of stress and it seems to be required for the synthesis of a stress-resistant photosynthetic apparatus. The distribution of *mapA* homologues among the various cyanobacterial lineages is intriguing. Comparison of its occurrence in the various strains with the corresponding phylogenetic tree of their 16S rRNA (see Supplementary Fig. S1) indicates that *mapA* could be an ancient cyanobacterial gene that was lost in the lineage of marine *Synechococcus* and *Prochlorococcus* strains due to their reductive genome evolution (Voß *et al.*, 2007). The conservation of *mapA* in multicellular cyanobacteria as well as in the physiologically versatile *Synechocystis* group indicates that *mapA* confers a selective advantage for those cyanobacteria, which inhabit complex environments. This conclusion is in accord with the observed differential expression pattern of *map* genes, with *mapC* being mainly expressed during vegetative growth and *mapA* being induced during stress conditions. The *mapA* gene is located immediately upstream of *slr0919*, a gene encoding a hypothetical protein. Since the kanamycin-resistance gene inserted in the MAP-A mutant is transcribed in the same direction as the *mapA* gene, a polar effect on the downstream *slr0919* gene seems highly unlikely. A third MetAP gene, *mapB*, has so far only been identified in *Synechocystis*. It is transiently induced upon stress treatment, but the corresponding mutant did not show any impairment. This corresponds to previous biochemical analysis of the *Synechocystis map* products, which revealed that MAP-B (c2MetAP-Ia) hardly displayed enzymic activity (Atanassova *et al.*, 2003). The physiological role of the *mapB* product remains elusive.

The importance of the NME pathway for PSII biogenesis was previously demonstrated by inhibition of N-terminal fMet-excision in chloroplasts of *Chlamydomonas reinhardtii* and *Arabidopsis thaliana* (Giglione *et al.*, 2003). Upon inhibition, the photosynthetic activity decreased and the PSII proteins D1, D2, CP43, CP47 and PsbH accumulated to lower levels due to enhanced degradation. Loss of stability was probably caused by impaired assembly of the complex. Examination of PSII properties of the *mapA*-deficient mutant revealed a subtle aberration already under non-stressed vegetative growth. In particular, the quinone acceptor site of PSII seemed to be affected, as deduced from the studies using chemicals reactive with the Q_B site. In wild-type cells, benzoquinone mediates electron transfer from PSII to the artificial oxidant ferricyanide by accepting the electrons from the Q_B site. However, in the *mapA* mutant, the presence of benzoquinone/ferricyanide clearly impairs PSII-dependent oxygen evolution and leads to a slow and continuous increase in PAM chlorophyll

fluorescence in response to high actinic light (Fig. 6, Table 1). The latter effect could be caused by permanent binding of reduced benzoquinone to the Q_B pocket, thereby causing acceptor site inhibition. In the *mapA* mutant the degree of inhibition by quinone derivatives depended on their particular structure (Table 1). 1,4-Benzoquinone binds directly to the Q_B site, while DCBQ binds to Q_A and Q_B sites, but more tightly to the Q_A site (Mayers *et al.*, 1993). The stronger inhibition by 1,4-benzoquinone thus implies a Q_B site modification in the *mapA* deficient mutant. This conclusion agrees with DCMU being significantly less inhibitory for the *mapA* mutant than for wild-type cells. DCMU binds synergistically and non-covalently to the Q_B binding pocket and displaces the natural quinones due to its higher affinity for the binding site (Oettmeier, 1999). As a consequence, electron transport in PSII breaks down, which inhibits cell growth and finally leads to cell death (Rutherford & Kriger-Liszky, 2001).

The Q_B binding pocket is built from amino acids of the D1 protein together with some low-molecular-mass proteins (Kern *et al.*, 2005). Resistance to DCMU can already be caused by subtle modification of the D1 protein such as single amino acid substitutions (Dalla Chiesa *et al.*, 1997; Oettmeier, 1999). Thus, it is possible that an N-terminal processing defect in the MmapA cells could result in altered D1 conformation that leads to enhanced DCMU resistance. Alternatively, another component of PSII could depend on MAP-A processing and could indirectly affect the Q_B site. Deletion mutants in *psbK* and *psbX* genes, encoding accessory PSII subunits at the Q_B site, resemble the MmapA phenotype in certain aspects. A *psbK*-deletion mutant in *Synechocystis* is less DCMU sensitive than wild-type cells (Ikeuchi *et al.*, 1991). The low-molecular-mass subunit PsbX is essential for binding and turnover of quinone molecules at the Q_B site. A *psbX*-deletion mutant showed normal photoautotrophic growth and oxygen evolution, despite having a decreased amount of PSII (Funk, 2000), but showed lower oxygen evolution in the presence of benzoquinone, as is the case for MmapA. Interestingly, the N-termini of the small accessory proteins PsbK and PsbX show an N-terminal methionine excision probability of 99 % or 90 %, respectively, according to TerminiNator prediction (<http://www.isv.cnrs-gif.fr/terminator2/>). Taking these results together, the phenotype of the *mapA* mutant has similarities to the reported phenotypes of several mutants of PSII components; however, none of them matches the MmapA completely. This almost pleiotropic phenotype suggests that several proteins of the PSII apparatus could be targets for MAP-A processing. The reduced fluorescence from the CP47 antenna chlorophyll, as revealed by fluorescence emission spectroscopy, agrees with a subtle inherent distortion of the PSII apparatus in the *mapA* mutant. It remains to be elucidated which PSII components are directly dependent on MAP-A processing.

Clearly, *Synechocystis* requires the *mapA* product under conditions of severe and prolonged nitrogen starvation as

well as under permanent high light exposure, since cells lacking MAP-A lose viability during these treatments. The loss of viability could be related to the stability of the photosynthetic machinery. PSII activity is essential for surviving high light exposure and recovering after nitrogen starvation. Under high light conditions, PSII-dependent oxygen evolution ceases in the *mapA* mutant (Fig. 2), indicating an increased susceptibility to this stress. In accord with the reduced oxygen evolution, the 77 K fluorescence emission spectra confirmed damage to PSII (Fig. 3). As outlined above, MAP-A seems to be required for correct processing of PSII component(s). Stable assembly of PSII is especially important under conditions of prolonged nitrogen chlorosis. The cells survive this precarious condition by minimizing protein synthesis, albeit conserving a low level of photosynthetic activity for maintenance metabolism (Sauer *et al.*, 2001). Under these conditions, stability of PSII becomes more important than under vegetative growth, but the capacity for PSII biogenesis and repair is limited. The expression pattern of the *mapA* gene matches this increased requirement for MAP-A (Fig. 4). Under vegetative growth conditions, MAP-C activity is presumably sufficient for the biogenesis of an almost intact photosystem. However, under stress, MAP-A function becomes vital. There are two, not mutually exclusive, explanations for the dependence on MAP-A under the conditions of stress. First, the downregulation of *mapC* expression under stress conditions could make the NME pathway dependent on MAP-A. The incorrect N-terminal maturation of newly synthesized proteins might cause a severe impairment of PSII biogenesis and stability, similar to that observed in the above-mentioned experiments of NME inhibition in *Chlamydomonas* and *Arabidopsis* chloroplasts (Giglion *et al.*, 2003). A second explanation could be differential N-terminal processing under stress conditions. Since MAP-A exhibits different substrate specificities compared to MAP-C *in vitro* (Atanassova *et al.*, 2003), N-terminal processing could be qualitatively different under stress or vegetative growth. MAP-A-dependent processing could be involved in the biogenesis of a photosystem which is optimized to sustain stress conditions and which under normal conditions is synthesized only in low amounts. A specificity of certain PSII proteins for MAP-A processing is suggested by the subtle phenotype of the *mapA* mutant under standard growth conditions, where *mapA* is expressed only at low levels (Fig. 4). To gain further insights, proteomic analysis of the *mapA*-deficient mutant grown under stressed and non-stressed conditions is required.

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