ELSEVIER

Biochimica et Biophysica Acta 1375 (1998) 140–143

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Short sequence-paper

Sequence and mutational analysis of the *devBCA* gene cluster encoding a putative ABC transporter in the cyanobacterium *Anabaena variabilis* ATCC 29413

Gabriele Fiedler, Matthias Arnold, Iris Maldener *

Universität Regensburg, Lehrstuhl für Zellbiologie und Pflanzenphysiologie, 93040 Regensburg, Germany

Received 19 May 1998; revised 10 July 1998; accepted 4 August 1998

Abstract

The *devBCA* gene cluster (*dev* for development), shown to be essential for envelope formation in heterocysts of *Anabaena* sp. strain PCC 7120, was identified in the gene bank of a second heterocyst-forming strain, *Anabaena variabilis* ATCC 29413. Sequence and structural organization of the three genes, encoding subunits of a presumptive ABC transporter, were nearly identical in both strains. Mutants of *A. variabilis* defective in the *devA* gene were constructed. As *devA* mutants of *Anabaena* 7120, *A. variabilis* mutants were unable to grow on N_2 as sole nitrogen source due to incomplete differentiation of heterocysts.

Keywords: Heterocyst differentiation; Cyanobacterium; ABC transporter

Recently, it was shown that the *devBCA* gene cluster is essential for envelope formation in heterocysts of *Anabaena* 7120 [1,2]. The deduced amino acid sequences of the three *dev* genes show similarity to subunits of ABC transporters [3,4]: *devA* encodes the ATP-binding component [1], *devB* the membrane fusion protein and *devC* the membrane spanning subunit of an ABC exporter [2].

With the first 260 bp of the *devB* gene of *Anabaena* 7120 as a heterologous probe, the *devBCA* cluster from *A. variabilis* could be identified in a gene bank and sequenced. Sequence analysis was performed with the UWGCG package of the University of Wisconsin genetics Computer Group, version 7.3 [5]. The sequence is available in the EMBL Nucleotide Sequence Database under accession number AJ003195. The DNA sequence of the complete *dev* gene cluster of *A. variabilis* (Fig. 1) showed 94% identity to the corresponding sequence of *Anabaena* 7120. Even the region 60 bp 5′ of *devB* revealed 98% identity and the non-coding stretch between *devB* and *devC* was 92% identical, whereas a stretch of 55 bp was missing between *devC* and *devA* in *A. variabilis*. The deduced amino acid sequences of DevB, DevC and DevA show 98%, 97% and 98% identity to the protein sequences from *Anabaena* 7120, respectively. Because the deduced amino acid sequences of DevB, C and A of *A. variabilis* are nearly identical to the corresponding sequences of *Anabaena* 7120 they also show similarities to subunits of ABC transporters as described in Ref. [2]. Accordingly, DevA revealed similarity to the nucleotide-binding subunit, DevB to the membrane fusion protein and DevC to the membrane component of

* Corresponding author. Fax: +49-941-943-3352; E-mail: iris.maldener@biologie.uni-regensburg.de

0005-2736/98/$ – see front matter
PII: S0005-2736(98)00147-3

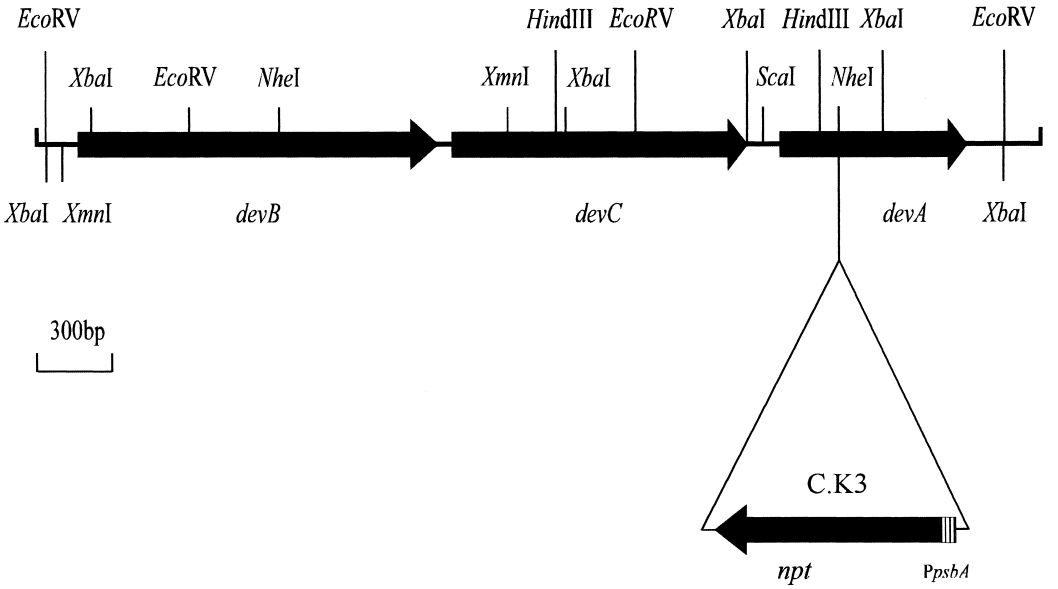
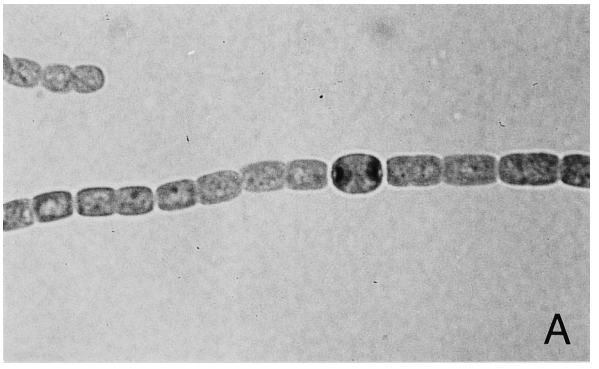


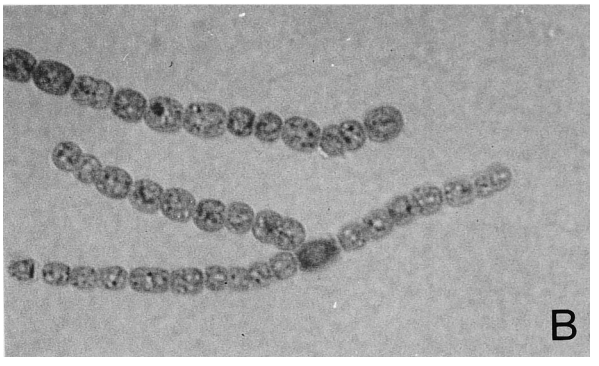
Fig. 1. Restriction map of the *dev* gene cluster of *A. variabilis*. The three open reading frames *devA*, *devB* and *devC* encoding the subunits of the presumptive ABC exporter are shown as filled arrows, indicating the direction of transcription. Restriction sites were mapped by sequencing. The site of directed insertion of C.K3 into *devA* is shown. The *npt* gene encoding neomycin phosphotransferase under control of the *psbA* promoter was used as selection marker in conjugation experiments.

ABC exporters. These data suggest that the DevBCA proteins in *A. variabilis* resemble an ABC exporter.

To investigate the physiological role of the *devBCA* gene cluster in *A. variabilis*, a site-directed mutation of the *devA* gene was done. The fragment containing the *devA* gene was cloned into pRL271 [6] and the C.K3 cassette bearing the *npt* gene [7] was inserted into the unique *Nhe*I site, located in the *devA* gene (Fig. 1). After transfer to *A. variabilis* via conjugation [8], double recombinants (DR87) were selected as described [9,10]. The correct insertion of the interrupted gene through a double recombination event was shown by Southern blot analysis of the DNA from several randomly chosen colonies (data not shown).

The phenotype of the *devA* mutant DR87 was characterized and compared to the phenotype of wild-type *A. variabilis*, and was identical to the phenotype of the *devA* mutant M7 of *Anabaena* 7120 [11,1,2]. Forty-eight hours after deprivation of com-





→

Fig. 2. DAB oxidation in intact filaments of wild-type *A. variabilis* and mutant strain DR87. Filaments were deprived of combined nitrogen for 48 h and then incubated for 30 min in DAB solution in the dark. (A) DAB deposition is seen only in the polar region of wild-type heterocysts. (B) In filaments of the *devA*-mutant DR87, several heterocyst-like cells can be detected which show no oxidation of DAB.

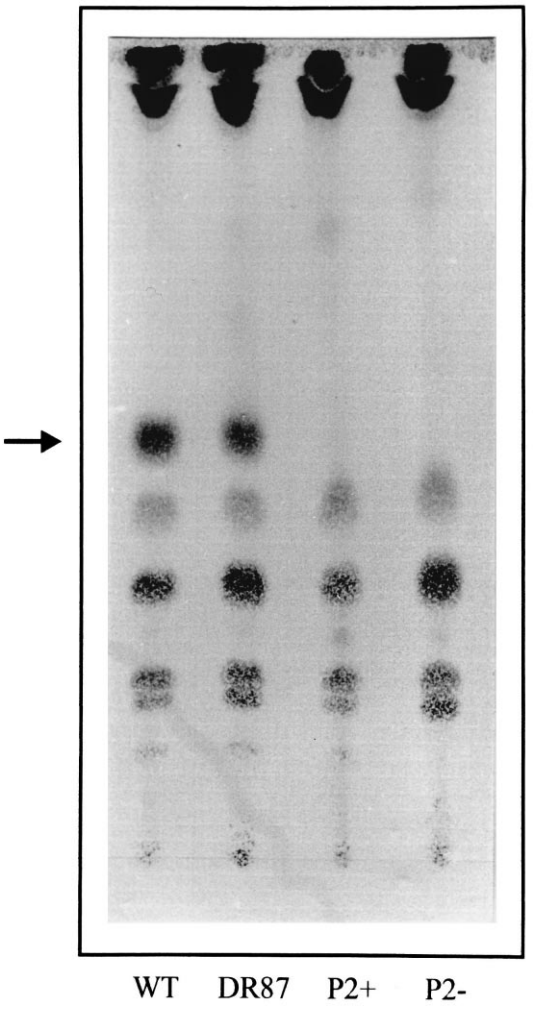


Fig. 3. Thin-layer chromatography of glycolipid extracts from cultures containing 50 μg chlorophyll [16]. The position of the heterocyst-specific glycolipids is indicated by an arrow. Cultures of wild-type *A. variabilis* (wt) and *devA* mutant DR87 (DR87) were deprived of NO_3^- for 48 h. Extracts of NO_3^- grown (P2+) and NO_3^--deprived cultures of *Anabaena* 7120 mutant P2 (P2−) were used as a Hgl^- control, because this mutant is not able to synthesize heterocyst-specific glycolipids [11].

bined nitrogen, DR87 showed spaced differentiated cells (Het^+), and the filaments started to fragment (Fig. 2B). The envelope of the heterocysts looked less refractile in the mutant (Hen^-) than in the wild-type *A. variabilis* (Fig. 2) in the light microscope. Heterocyst-specific glycolipids were identified in thin-layer chromatography [12] of extracts of mutant and wild-type filaments (Hgl^+) as seen in Fig. 3. After staining with diaminobenzidine (DAB), brown precipitates were detected near the poles of wild-type heterocysts showing the heterocyst-specific oxidation of DAB in the dark and indicating high respiratory activity [13] (Fig. 2A). No oxidation of DAB could be detected in heterocysts of the *devA* mutant (Dab^-) (Fig. 2B). *DevA* mutants of both strains are not able to grow aerobically on N_2 as sole nitrogen source (Fox^-). Nomenclature of the mutant phenotypes was adapted from Ref. [11].

Based on 97% identity between the Dev protein sequences from the two *Anabaena* species, we suggest that the putative ABC transporter plays a similar role in heterocyst differentiation (for a review see [14]) of *A. variabilis* and *Anabaena* 7120. We hypothesize that the DevBCA transporter exports glycolipids or an enzyme that is needed for assembly of the laminated layer outside the cell wall of developing heterocysts [2]. Since no *devBCA* homologues were found in the genome of the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 [15], the DevBCA exporter may be important for heterocyst forming cyanobacteria only.

The authors would like to thank Dr. Margret Sauter and Dr. Michael Schütz for critical reading of the manuscript. The work was supported by the Deutsche Forschungsgemeinschaft.

References

[1] I. Maldener, G. Fiedler, A. Ernst, F. Fernández-Piñas, C.P. Wolk, J. Bacteriol. 176 (1994) 7543–7549.
[2] G. Fiedler, M. Arnold, S. Hannus, I. Maldener, Mol. Microbiol. 27 (1998) 1193–1202.
[3] C.F. Higgins, S.C. Hyde, M.M. Mimmack, U. Gileadi, D.R. Gill, M.P. Gallagher, J. Bioenerg. Biomembr. 22 (1990) 571–592.
[4] T. Dinh, I.T. Paulsen, M.H. Saier Jr., J. Bacteriol. 176 (1994) 3825–3831.
[5] J. Devereux, P. Haeberli, O. Smithies, Nucleic Acids Res. 12 (1984) 387–395.
[6] T.A. Black, Y. Cai, C.P. Wolk, Mol. Microbiol. 9 (1993) 77–84.
[7] J. Elhai, C.P. Wolk, Gene 68 (1988) 119–138.
[8] C.P. Wolk, A. Vonshak, P. Kehoe, J. Elhai, Proc. Natl. Acad. Sci. U.S.A. 81 (1984) 1561–1565.
[9] Y. Cai, C.P. Wolk, J. Bacteriol. 172 (1990) 3138–3145.
[10] I. Maldener, W. Lockau, Y. Cai, C.P. Wolk, Mol. Gen. Genet. 225 (1991) 113–120.
[11] A. Ernst, T.A. Black, Y. Cai, J.-M. Panoff, D.N. Tiwari, C.P. Wolk, J. Bacteriol. 174 (1992) 6025–6032.

[12] F. Winkenbach, C.P. Wolk, M. Jost, Planta 107 (1972) 69–80.
[13] M.A. Murry, A.G. Olafsen, J.R. Benemann, Curr. Microbiol. 6 (1981) 201–206.
[14] C.P. Wolk, A. Ernst, J. Elhai, in: D.A. Bryant (Ed.), The Molecular Biology of Cyanobacteria, Kluwer Academic, London, 1994, pp. 769–823.
[15] T. Kaneko, S. Sato, H. Kotani, A. Tanaka, E. Asamizu, Y. Nakamura, N. Miyajima, M. Hirosawa, M. Sugiura, S. Sasamoto, T. Kimura, T. Hosouchi, A. Matsuno, A. Muraki, N. Nakazaki, K. Naruo, S. Okumura, S. Shimpo, C. Takeuchi, T. Wada, A. Watanabe, M. Yamada, M. Yasuda, S. Tabata, DNA Res. 3 (1996) 109–136.
[16] H. Mackinney, J. Biol. Chem. 140 (1941) 315–322.