JOURNAL OF BACTERIOLOGY, May 1995, p. 2901–2907 Vol. 177, No. 10
0021-9193/95/$04.00+0

Synthesis of the *Caulobacter* Ferredoxin Protein, FdxA, Is Cell Cycle Controlled

SHUI PING WANG,[1] PIL JUNG KANG,[2] YUNG PING CHEN,[1] AND BERT ELY[1]

Department of Biological Sciences, University of South Carolina, Columbia, South Carolina 29208,[1] *and Department of Developmental Biology, Stanford University School of Medicine, Stanford, California 94305-5427*[2]

Received 24 October 1994/Accepted 25 February 1995

The *fdxA* gene was identified upstream of and in the opposite direction from the *Caulobacter crescentus cysC* gene. Analyses of the nucleotide sequence and the deduced amino acid sequence of the *fdxA* gene demonstrated that it encodes a ferredoxin with a molecular mass of 12,080 Da. This ferredoxin has common structural features with ferredoxins that contain a [3Fe-4S] and a [4Fe-4S] cluster, including seven conserved cysteines responsible for the binding of the two clusters. A mutation in the *fdxA* gene was obtained, and the resulting strain did not produce one of the two ferredoxins (FdI) found in *C. crescentus*. Further experiments demonstrated that the *fdxA* gene is temporally expressed in *C. crescentus* and that FdI is required for completion of the cell cycle at 37°C.

Ferredoxins are small, acidic iron-sulfur proteins that are widely distributed among animals, plants, and bacteria. They function as electron carriers in diverse metabolic processes, including photosynthesis, nitrogen fixation, biodegradation of aromatic compounds, and various reactions involved in fermentative metabolism (for a review, see reference 4). Ferredoxins are classified based on the nature of their iron-sulfur cluster(s) and the similarity of their peptide sequences. Ferredoxins in the same subgroup have iron-sulfur clusters with common structural properties and similar spacing of the cysteines in their amino acid sequences (4). Bacterial ferredoxins consist of single polypeptides ranging in mass from 6,000 to 13,000 Da. A number of bacteria possess two or more ferredoxins which function in different biochemical pathways. Some bacteria, e.g., *Chlorobium limicola* (37), have two ferredoxins belonging to one subgroup, possibly generated by a relatively recent gene duplication, while others possess multiple ferredoxins belonging to distinct subgroups (28, 34, 40). In this report, we demonstrate that *Caulobacter crescentus* contains two ferredoxins, designated ferredoxin I (FdI) and ferredoxin II (FdII). FdII is a [2Fe-2Fe]-type ferredoxin encoded by the *fdxP* gene (39). Here we report the identification and characterization of a second ferredoxin gene, *fdxA*, which encodes FdI in *C. crescentus*. In addition, we demonstrate that expression of *fdxA* is regulated by the *C. crescentus* cell cycle.

MATERIALS AND METHODS

Bacteria and growth conditions. Wild-type *C. crescentus* CB15 and mutant strains derived from it were grown in PYE or defined M2 medium at 30°C as described previously (16). *Escherichia coli* strains were grown in LB medium at 37°C (22). The following antibiotic concentrations were used for the selection of recombinant plasmids: kanamycin, 50 mg/liter for solid and 25 mg/liter for liquid media; ampicillin, 100 mg/liter; and tetracycline, 15 mg/liter in LB or 1 mg/liter in PYE.

Molecular techniques. *C. crescentus* chromosomal DNA isolation procedures have been described previously (3, 9). Plasmid isolation, restriction digestion, ligation, transformation, Southern hybridization, and random priming were performed essentially as described by Sambrook et al. (29). DNA fragments were purified by the Geneclean II kit of Bio 101 (La Jolla, Calif.). All restriction enzymes, T4 DNA ligase, and the Klenow fragment of *E. coli* DNA polymerase I were purchased from New England Biolabs (Beverly, Mass.) and used in accord with the manufacturer's recommendations.

Plasmid DNA was introduced by $CaCl_2$-mediated transformation into *E. coli* and by electroporation or mating into *C. crescentus* (8). DNA sequencing reactions were performed based on the dideoxy chain termination method (30) with Sequenase (Amersham/US Biochemical, Cleveland, Ohio). For single-strand sequencing, DNA fragments were cloned into the phagemid pBluescript in both orientations, and single-stranded DNA was rescued by the VCS M13 phage by the procedure recommended by the manufacturer (Stratagene, La Jolla, Calif.). Deaza-dGTP was used in place of dGTP for chain elongation. Nucleotide sequences were analyzed with the Genetics Computer Group programs (6). A *C. crescentus* codon usage table (33) was used in the codon preference analysis.

Isolation and purification of ferredoxins and biochemical assays. *C. crescentus* strains were grown in batch culture in M2 medium at 33°C. Cells were harvested by centrifugation at late growth phase, yielding approximately 2 g of cells per liter of culture. For purification of ferredoxins, 20 g of cells was resuspended in 40 ml of 50 mM phosphate buffer (pH 7.8). Extracts were prepared by sonication as described previously (5). Following centrifugation for 1 h at 39,000 × *g*, the supernatant was passed through a DEAE-cellulose (DE-52) collection column (1.2 by 5 cm, at a flow rate of 10 ml/h) which had been equilibrated with 50 mM phosphate buffer (pH 7.8). The column was washed with 100 ml of phosphate buffer containing 0.1 M NaCl. The column was then inverted, and protein bound to the DEAE-cellulose (brown in color) was blown out of the column from the top. The protein was resuspended in 10 ml of the phosphate buffer and loaded onto a DE-52 concentration column (1 by 10 cm, at a flow rate of 5 ml/h). The ferredoxins were eluted in 10-ml fractions with 0.4 M NaCl in 50 mM phosphate buffer. The peak fraction of ferredoxins was diluted 1:10 with 50 mM phosphate buffer and applied to a DEAE-Sephadex A-50 column (1.2 by 18 cm, at a flow rate of 10 ml/h) equilibrated with 50 mM phosphate buffer (pH 7.8). Protein was eluted with a linear gradient of NaCl over the range from 0 to 400 mM in the same buffer. The peak fractions were pooled and concentrated to 1 ml (final volume) with Centricon-10 microconcentrators (Amicon, Danvers, Mass.). This solution was applied to a Sephadex S-100 column (1.2 by 25 cm, at a flow rate of 4 ml/h) which had been preequilibrated with the phosphate buffer. The fractions eluted from this column resulted in two peaks of brown color. Ferredoxin activity was measured by the ability to substitute for chloroplast ferredoxin in the photoreduction of NADP in an assay employing a ferredoxin-free plant ferredoxin-NAD^+ reductase (5).

Site-directed mutagenesis. Plasmid pUC4KIXX (Pharmacia, Piscataway, N.J.) was digested with *Xho*I, and the 1.6-kb fragment containing the kanamycin resistance cassette was isolated from an agarose gel. Plasmid pSCW106, containing the 1.45-kb *Sst*I-*Sst*II fragment shown in Fig. 1, was digested with *Sal*I, ligated with the isolated kanamycin cassette, and transformed into *E. coli* XL1-blue (Stratagene). Colonies with both kanamycin and ampicillin resistance were purified, and the identity of the recombinant plasmid was confirmed by restriction analysis. The resulting plasmid, pSCW106Km, containing a kanamycin cassette in the *fdxA* gene, was transferred to *C. crescentus* LS107 (*bla-6 syn-1000*) (1) by electroporation. Kanamycin-resistant colonies were transferred to ampicillin and kanamycin plates in replicate, and colonies that were kanamycin resistant but ampicillin sensitive were identified. This phenotype would be obtained if the wild-type *fdxA* gene were replaced by the mutated version containing the kanamycin cassette. One of the mutants was designated SC3866. To confirm the

* Corresponding author. Phone: (803) 777-2768. Fax: (803) 777-4002. Electronic mail address: Ely@Biol.scarolina.edu.

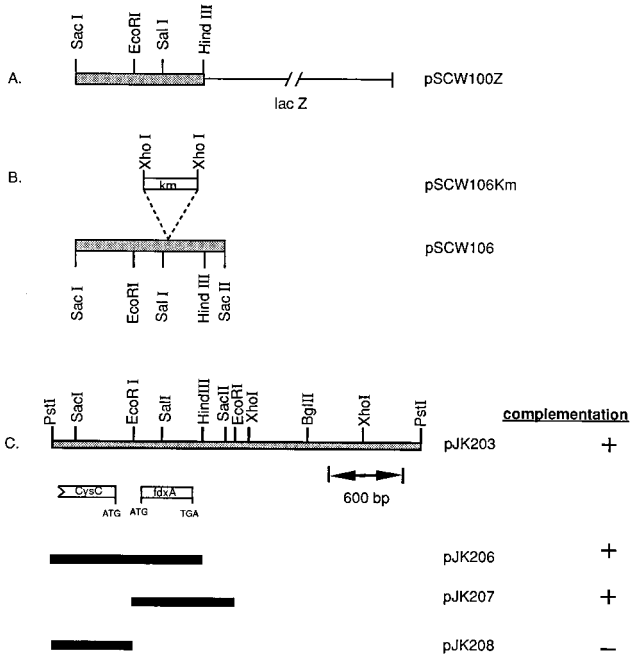

FIG. 1. Restriction map of a *C. crescentus* DNA fragment containing the *fdxA* gene and diagram of recombinant plasmids. (A) pSCW100Z, an *fdxA-lacZ* fusion in the transcriptional fusion vector plac290 (13). (B) pSCW106, a subclone of the *fdxA* fragment on pBluescript KS$^+$ (Stratagene). A kanamycin cartridge disrupting *fdxA* was inserted in pSCW106, creating pSCW106Km. (C) Complementation of LS35 with different subclones. The DNA fragment contained in each subclone is depicted by a solid bar.

mutant genotype, genomic DNA was isolated from SC3866, digested with *Eco*RI and *Hin*dIII, and hybridized with the 0.5-kb *Eco*RI-*Hin*dIII fragment containing the *fdxA* gene. As expected, the 0.5-kb *Eco*RI-*Hin*dIII genomic DNA fragment was shifted to 2.1 kb by the insertion of the 1.6-kb kanamycin cassette (data not shown).

Cell cycle immunoblot and immunoprecipitation. Immunoblot assays were carried out with an antibody raised against the *Azotobacter vinelandii* ferrodoxin I (a gift from Barbara K. Burgess, University of California, Irvine). *C. crescentus* LS107 was synchronized as described previously (11). For immunoprecipitation experiments, strains derived from LS107 and harboring the proper plasmids were cultured and synchronized in minimal M2 glucose medium. Synchronous swarmer cells were obtained by repeated centrifugation in ice-cold M2 medium (25). Swarmer cells (95% pure) were labeled for 10 min with 10 μCi of [^{35}S]methionine per ml of culture. Labeled cells were lysed and treated with *Staphylococcus aureus* A cells, and the cell extracts were immunoprecipitated with antibodies as described previously (14). Samples were analyzed by sodium dodecyl sulfate–12% polyacrylamide gel electrophoresis (SDS–12% PAGE), and the proteins were visualized by autoradiography.

Nucleotide sequence accession number. The nucleotide sequence reported in Fig. 2 has been deposited in the GenBank database under accession number U10035.

RESULTS

Sequence analyses of *fdxA* gene. A clone containing the *C. crescentus cysC* gene was identified by Schoenlein et al. (32). During DNA sequence analysis of this clone, an open reading frame (ORF) was identified within the *Eco*RI-*Hin*dIII fragment, upstream of the *cysC* gene (Fig. 1). Analysis of the DNA sequence indicated that this ORF is 342 bp in length (Fig. 2). A potential ribosome-binding site, GAG, is present 6 bp upstream of the ATG start codon. The ORF could encode a protein containing 113 amino acid residues, with a predicted molecular mass of 12,080 Da. When the deduced amino acid sequence was used to search the GenBank database, we found that the corresponding protein is a homolog of a group of ferredoxins encoded by the *fdxA* genes of various bacteria (Fig.

```
1   GTTTTCACGGAGGTAACCGATTGTCGCGGAGGCGGCATGGCGCCGCCGGGCCACCGCCCC   60
                                        EcoRI
61  AGAAATGCGCGTGCTCTGGCGCGGCGATCAGAATTCTGAGCCAATACGAGAGTAAGCCGC  120

121 ATTGACAAGCGGCCCCCAGACTTCGAAAACGCGCCCGCCGAACTCCGCCCGATCTCAACC  180
          fdxA
181 GAGTTTTCGATGACCTACATCGTCACCGACGCCTGCGTCCGCTGCAAGTTCATGGACTGC  240
          M  T  Y  I  V  T  D  A  C  V  R  C  K  F  M  D  C

241 GTGGAGGTGTGTCCGGTCGATTGCTTCTACGAAGGCGAGAACTTCCTCGTCATCAATCCG  300
    V  E  V  C  P  V  D  C  F  Y  E  G  E  N  F  L  V  I  N  P
                                                 SalI
301 GACGAATGCATCGACTGCGGCGTCTGCGAACCCGAGTGCCCGGTCGACGCGATCAAGCCC  360
    D  E  C  I  D  C  G  V  C  E  P  E  C  P  V  D  A  I  K  P

361 GACACCGAGGACGAGGCCGACGGCAAGTGGCTGAAGATCAACGCCGACTACGCCAAGGTC  420
    D  T  E  D  E  A  D  G  K  W  L  K  I  N  A  D  Y  A  K  V

421 TGGCCGAACATCACGGTCAAGGGCGAGCCGCCCGCCGACCGCGAGGATTTCGAGCGCGAG  480
    W  P  N  I  T  V  K  G  E  P  P  A  D  R  E  D  F  E  R  E

481 ACCGGCAAGTTCGAGAAGTACTTCAGCGAAAAGCCCGGCAAGGGCTCCTGATCCCCGCGA  540
    T  G  K  F  E  K  Y  F  S  E  K  P  G  K  G  S  *
                                     HindIII
541 CGATTTCTCAAGCCCAGAGCGTGACCGCCTCGCGAAAGCTT                    581
```

FIG. 2. Nucleotide sequence and deduced amino acid sequence of the *C. crescentus fdxA* gene. A potential ribosome-binding site (10) upstream of the ATG start codon is underlined.

3). The *C. crescentus* ferredoxin protein has 65.2, 57.3, and 38.7% identity over the entire predicted polypeptide with the related proteins from *Rhodobacter capsulatus* (28), *A. vinelandii* (23), and *Saccharopolyspora erythraea* (7), respectively. The number and the spacing of the eight cysteines are strikingly conserved, i.e., a first Cys-rich domain of CXXCXXXXCXX XCP and a second Cys-rich domain of CXXCXXCXXXCP. Seven of these cysteines have been shown to be involved in the formation of the [3Fe-4S] and [4Fe-4S] clusters in *A. vinelandii* FdxA (15, 35). On the basis of this information, we designated the ORF *fdxA*.

***C. crescentus* has two ferredoxins.** To study the function of the *fdxA* gene product, we partially purified the *C. crescentus* ferredoxin(s). Two ferredoxins were identified and designated FdI and FdII, according to their sequence of elution from the separation column (Fig. 4). Both ferredoxins can substitute for

```
Cc FdxA  MTYIVTDACV RCKFMDCVEV CPVDCFYEGE NFLVINPDEC IDCGVCEPEC  50
Rc FdxA  MTYVVTDNCI ACKYTDCVEV CPVDCFYEGE NTLVIHPDEC IDCGVCEPEC  50
Av FdxA  MAFVVTDNCI KCKYTDCVEV CPVDCFYEGP NFLVIHPDEC IDCALCEPEC  50
Se FdxA  VTYVIAEPCV DVLDKACIEE CPVDCIYEGG RMLYIHPDEC VDCGACEPVC  50

Cc FdxA  PVDAIKPDTE .DEADGKWLK INADYAKVWP NITVKGEPPA DREDFERETG  99
Rc FdxA  PADAIRPDTE .PGMED.WVE FNRTYASQWP VITIKKDPMP DHKKYDGETG  98
Av FdxA  PAQAIFSEDE VPEDMQEFIQ LNAELAEVWP NITEKKDPLP DAEDWDGVKG 100
Se FdxA  PVEAIYYEDD VPDEWAAYTK ANVDFFDELG SPGGAAKVGK VDRDVEPVSS 100

Cc FdxA  KFEKYFSEKP GKGS* 113
Rc FdxA  KREKYFSPNP GTGD* 112
Av FdxA  KLQHLER*         107
Se FdxA  LPPQGE*          106
```

FIG. 3. Comparison of amino acid sequences of the *fdxA* products of *C. crescentus* (Cc), *R. capsulatus* (Rc) (28), *A. vinelandii* (Av) (23), and *S. erythraea* (Se) (7). Vertical bars and colons represent identical and similar amino acid residues, respectively. *C. crescentus* FdxA is 65.2, 57.3, and 38.7% identical to FdxA from *R. capsulatus*, *A. vinelandii*, and *S. erythraea*, respectively. The conserved seven cysteines thought to be the binding sites for the [3Fe-4S] and the [4Fe-4S] clusters are shaded.

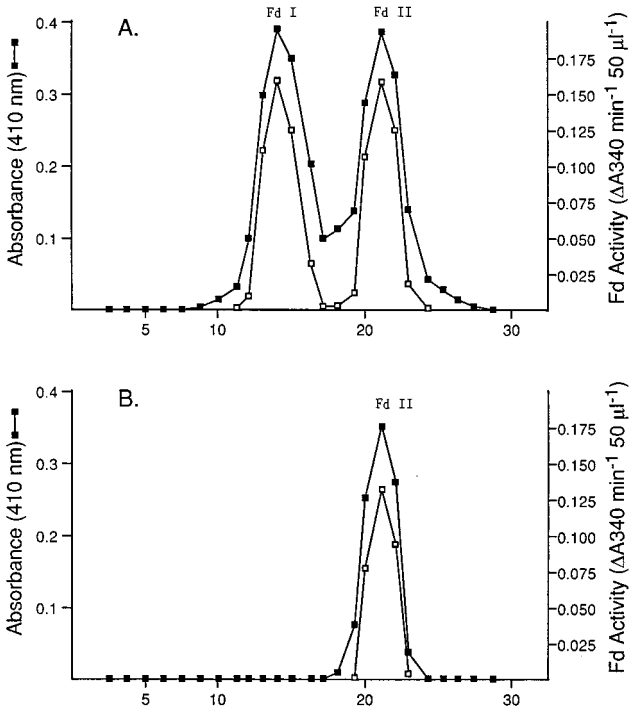

FIG. 4. Purification of ferredoxins from *C. crescentus*. The ferredoxins eluted from a Sephadex S-100 column were identified by the A_{410} (solid squares). Ferredoxin activity was measured by a reconstitution assay with chloroplast photoreduction (open squares). (A) Ferredoxin from LS107; (B) ferredoxin from SC3866.

the spinach chloroplast ferredoxin in the photoreduction assay. However, the two ferredoxins have different visible absorption spectral patterns (data not shown). Thus, it is likely that the two ferredoxins are encoded by two different genes. To determine which ferredoxin is encoded by the *fdxA* gene, the *fdxA* gene was interrupted with a kanamycin resistance cassette as described in Materials and Methods. One of the resulting mutants, SC3866, was chosen for further experiments. When ferredoxins were isolated from SC3866, we found that the ferredoxin peak corresponding to FdI was missing (Fig. 4). Thus, FdI is encoded by the *fdxA* gene.

Cell cycle arrest phenotype of an *fdxA* mutant. Although several *fdxA* genes have been identified, the function of their gene products has not yet been established for any organism. To study the possible function of the *fdxA* gene in *C. crescentus*, the *fdxA* mutant SC3866 was analyzed. This mutant should be identical, at the DNA level, to the parent strain LS107 except for disruption of the *fdxA* gene by the insertion of a kanamycin cartridge. We tested SC3866 in several different growth conditions at 30°C and found no differences in generation time or the developmental cell cycle compared with LS107. However, we found that, in contrast to LS107, SC3866 would not grow at 37°C, suggesting that expression of *fdxA* was required for growth at the elevated temperature.

As part of a separate set of experiments to isolate cell cycle mutants of *C. crescentus* (17), six independent temperature-sensitive mutants which appeared to be defective at different stages of the cell cycle were identified. One of the six mutants, designated LS35, appeared to be arrested at the predivisional cell stage when grown at 37°C. To identify the gene(s) responsible for this mutant phenotype, a cosmid library containing *C. crescentus* genomic DNA was moved into the mutant to complement the temperature-sensitive growth phenotype of LS35. A positive clone was identified and designated pJK203 (Fig. 1C). To locate the gene within pJK203, various DNA restriction fragments were subcloned into broad-host-range plasmid pRK290-20R, a pRK290 derivative (12). The resulting subclones were used to complement the LS35 mutant. The results indicated that the region between the *Eco*RI and *Hin*dIII sites of pJK203 (Fig. 1C) was responsible for the complementation of the LS35 mutant. When the DNA sequence of the *Eco*RI-*Hin*dIII fragment was determined, we found that it was identical to the *Eco*RI-*Hin*dIII fragment of pSCW106. Therefore, the temperature-sensitive mutation in LS35 was located in the *fdxA* gene.

To further characterize the cell cycle arrest phenotype of LS35, the strain was cultured in PYE at 30°C to the early logarithmic phase and then shifted to 37°C. Three hours after the temperature upshift, cells were examined by electron microscopy. As shown in Fig. 5, LS107 grew both at 30°C and at 37°C, and both cultures proceeded through the normal cell cycle, producing stalked cell and swarmer cell progeny. However, at 37°C, the LS107 cells had shorter stalks. The LS35 strain resembled the parent strain at 30°C, but the cells arrested at the predivisional stage at 37°C. With continued incubation at this temperature, the cells elongated but no cell division occurred. Similar results were obtained with SC3866. These results suggest that *C. crescentus* requires the metabolic function of FdI for cell division at the elevated temperature.

Expression of the *fdxA* gene during the *C. crescentus* cell cycle. To determine if the *fdxA* gene is expressed differentially during the *C. crescentus* cell cycle, two assays were employed. In the first experiment, we used an antibody raised against the *A. vinelandii* FdxA protein for a Western immunoblot. It was found that FdI was present throughout the *C. crescentus* cell cycle (Fig. 6). However, the steady-state level of FdI increased about threefold during the predivisional cell stage. In the second experiment, we used a transcription fusion of the *fdxA* gene to a *lacZ* reporter gene (Fig. 1). When *fdxA* promoter activity was measured by immunoprecipitating pulse-labeled protein with antibody to β-galactosidase, we found that the *fdxA* promoter has two peaks of expression during the cell cycle. One peak corresponds to a 5-fold increase in expression at the time of stalk elongation, and the second corresponds to a more than 20-fold increase in expression in the predivisional cell stage. These peaks of expression are reflected by the cell cycle pattern of accumulation of ferredoxin protein measured by the Western blot analysis.

DISCUSSION

A *C. crescentus* ferredoxin gene, *fdxA*, was identified, and analysis of an insertion mutant demonstrated that *fdxA* encodes FdI. Comparison of the deduced polypeptide sequence of the FdxA protein revealed that it is homologous to the products of the *fdxA* genes from other bacteria, such as *R. capsulatus*, *A. vinelandii*, and *S. erythraea* (Fig. 3). These ferredoxins are believed to be of the [3Fe-4S][4Fe-4S] type (7, 23, 28). The distribution of the cysteines is highly conserved in all the *fdxA* gene products. Seven of the nine cysteines have been shown to be the ligands of the [3Fe-4S] and [4Fe-4S] clusters in *A. vinelandii* ferredoxin I (15, 35). Thus, the *C. crescentus fdxA* gene probably encodes a ferredoxin with a [3Fe-4S] and a [4Fe-4S] cluster.

Expression of the *C. crescentus fdxA* gene is subject to cell cycle control, with two peaks of expression, one during the swarmer to stalked cell transition, and a second stronger peak of expression in predivisional cells (Fig. 6). Both of these times

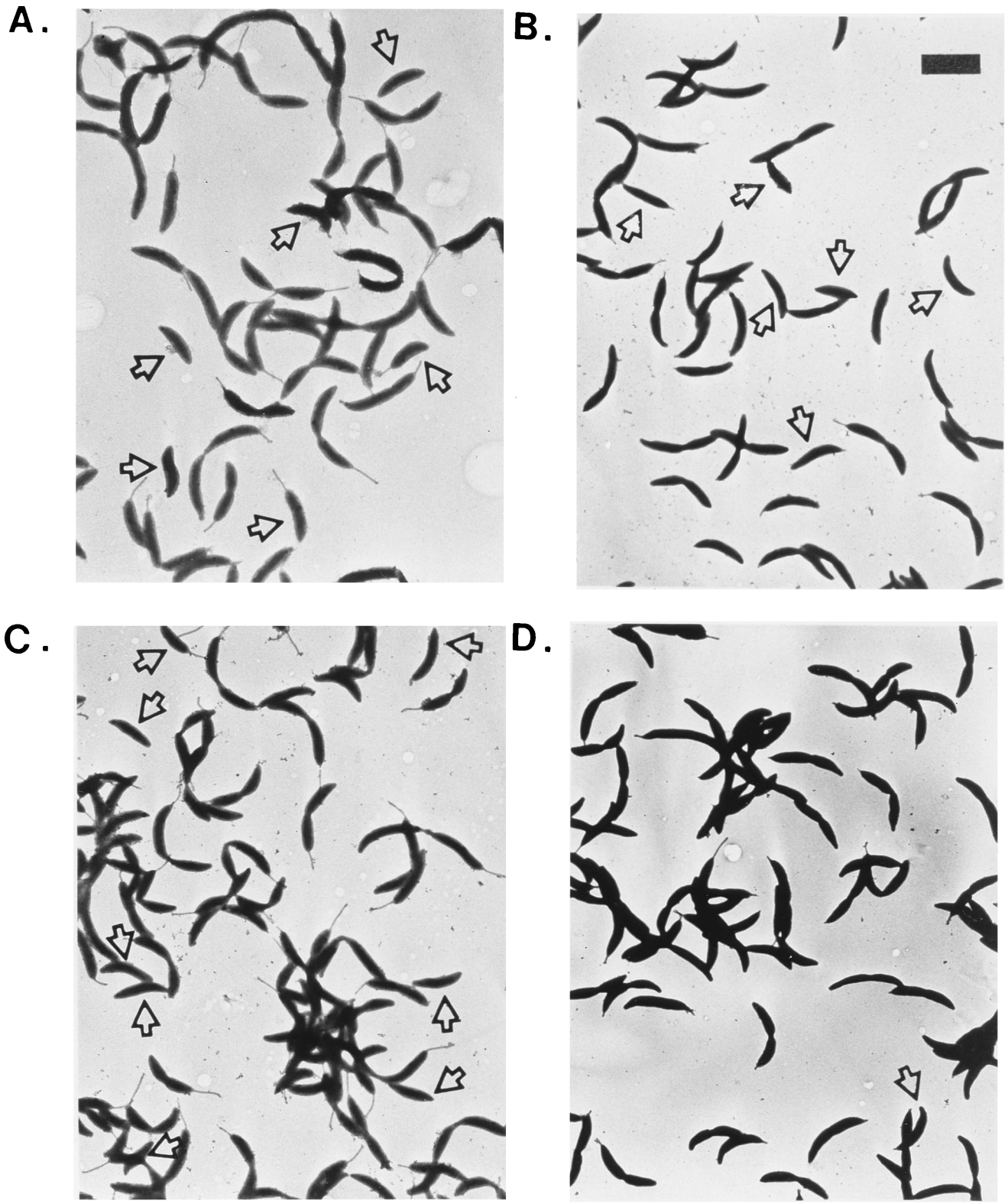

FIG. 5. Electron micrographs of parent strain LS107 (A and B) and the *fdxA* mutant LS35 (C and D). The bacteria were grown in PYE medium at 30°C to the logarithmic growth stage and either maintained at 30°C (A and C) or shifted to 37°C (B and D) for 3 h before examination. The open arrows represent progeny cells (either stalked or swarmer cells).

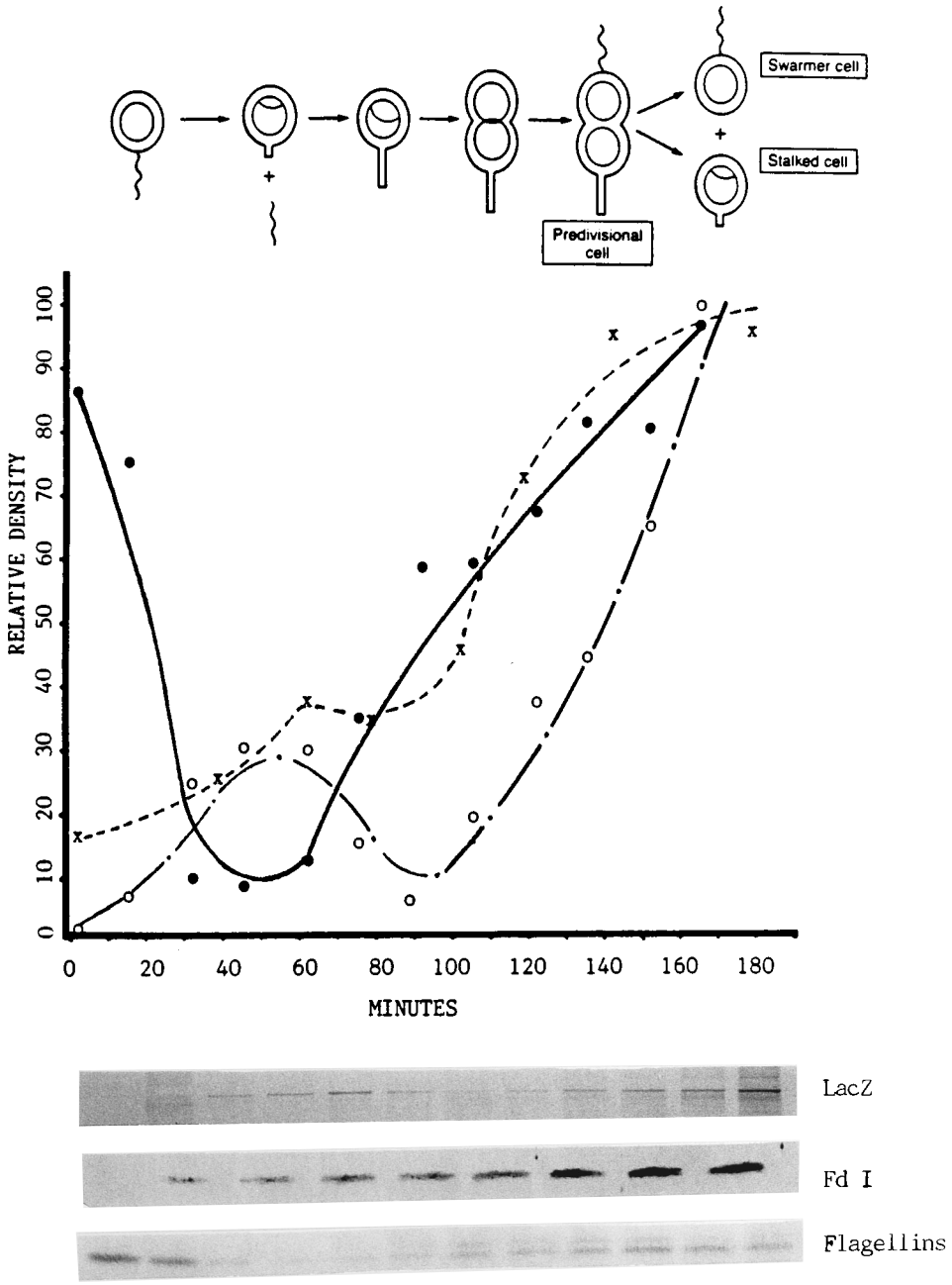

FIG. 6. Expression of an *fdxA-lacZ* fusion and steady-state levels of FdxA in the cell cycle. Synchronous cells of LS107 harboring plasmid pSCW100Z were pulse labeled with [^{35}S]methionine at different time points in the cell cycle, and expression of the *fdxA-lacZ* fusion was assayed by immunoprecipitation with anti-β-galactosidase antibodies (○). Accumulation of FdI protein was assayed by Western analysis of a synchronous culture of LS107 with antibodies against *A. vinelandii* FdxA protein (×). The flagellins were also assayed with antiflagellin antibodies as a control for synchrony (●). The data points represent a summation of the synthesis of the three flagellin proteins (29, 27, and 25 kDa).

coincide with periods of increased rates of membrane synthesis, the first during stalk formation and the second prior to cell division. Thus, FdI may be involved in some aspect of membrane biogenesis (see below). FdI protein accumulates in the cell in a pattern that reflects the changes in the rate of synthesis (Fig. 6). Thus, protein turnover rates appear to be relatively low. This raises an important question. How can FdI protein levels be sixfold lower in swarmer cells than in predivisional cells? One possibility is that FdI is selectively degraded in swarmer cells. However, no drop in overall FdI levels was observed when cell division occurred (Fig. 6). Alternatively, FdI may segregate preferentially to stalked cells during cell division. Preferential protein segregation during cell division is well documented in *C. crescentus* (2, 24), and the Lon and DnaK proteins have been shown to segregate preferentially to stalked cells (27). Further experiments will be needed to determine if a similar phenomenon occurs with FdI.

Ferredoxins have been studied extensively because of the important biological roles that they play in nitrogen fixation (28, 31) and biodegradation of aromatic compounds (18, 26,

36) in microorganisms, in photosynthesis of the green plants, and in various other reactions involved in fermentative metabolism (4). Different ferredoxins may have specific functions, although their principal biochemical role is to serve as an electron carrier. The protein products of the *fdxA* genes are the most intensively studied ferredoxins (15, 23, 28), but their function is the least understood. In *A. vinelandii*, disruption of the *fdxA* gene with a kanamycin cartridge resulted in growth properties virtually identical to those of the wild type (23), although growth was affected if the flavodoxin gene was mutated as well (21). On the other hand, attempts at disruption of the *fdxA* genes in *R. capsulatus* and *S. erythraea* were unsuccessful (7, 28), suggesting that the *fdxA* gene product may be essential for the growth of these organisms. However, no specific biological function for *fdxA* has been determined. We demonstrated that expression of *fdxA* is required for *C. crescentus* to grow at elevated temperatures. Microscopic examination revealed that the *fdxA* mutant was arrested at the predivisional stage. Thus, at the elevated temperature, the cell cycle appeared to be arrested in the *fdxA* mutant. It is significant that the cell cycle arrest occurs at one of the peaks of *fdxA* expression. This concordance suggests that *fdxA* is involved in some process which is critical for cell division at elevated temperatures.

One possible role for FdI is that it is required for the synthesis of some membrane phospholipids in *C. crescentus*. We noticed that the *fdxA* mutant has a phenotype similar to that of a fatty acid auxotroph of *C. crescentus*, AE6001, in that both are arrested at the predivisional stage (14, 19). In plants, it has been shown that a ferredoxin was required for a stearoyl-acyl carrier protein desaturase which catalyzes the principal conversion of saturated fatty acids to unsaturated fatty acids (38). In *C. crescentus*, expression of *fdxA* was increased at the time of stalk elongation and during cell division. Since the stalk contains an extension of the cell membrane, large amounts of membrane phospholipids need to be synthesized during the period of stalk elongation as well as prior to cell division (20). Furthermore, *C. crescentus* growing at different temperatures could have different membrane fluidities, which are dependent on the saturation-desaturation status of the fatty acids of the membrane phospholipids. If FdI were involved in saturation-desaturation enzyme processes in *C. crescentus*, it might be required at the higher temperature to maintain an appropriate level of membrane fluidity. At the lower temperature, the process involving FdI may be less important because of the effect of temperature on membrane fluidity. Thus, the effect of *fdxA* mutations on cell division would be indirect. Alternatively, *C. crescentus* may have a protein which can substitute for FdI at the lower temperature but not at the higher temperature. In either case, this type of functional role for FdI would be consistent with the fact that FdI homologs are widely distributed in the bacterial kingdom.

ACKNOWLEDGMENTS

We thank Betty Branham for help in preparation of the manuscript.

P. J. Kang was a Postdoctoral Fellow in the laboratory of Lucy Shapiro at the Stanford University School of Medicine. This work was supported by grants GM34765 to B. Ely and GM32506-12/5120MZ to L. Shapiro.

REFERENCES

1. **Alley, M. R. K., and J. Gober (Stanford University).** Personal communication.
2. **Alley, M. R. K., J. Maddock, and L. Shapiro.** 1992. Polar localization of a bacterial chemoreceptor. J. Cell Biol. **6:**825–836.
3. **Barrett, J. T., R. H. Croft, D. M. Ferber, C. J. Gerardot, P. V. Schoenlein, and B. Ely.** 1982. Genetic mapping with Tn*5*-derived auxotrophs of *Caulobacter crescentus*. J. Bacteriol. **149:**888–898.
4. **Bruschi, M., and F. Guerlesquin.** 1988. Structure, function and evolution of bacterial ferredoxins. FEMS Microbiol. Rev. **54:**155–176.
5. **Chen, Y., and D. Yoch.** 1989. Isolation, characterization, and biological activity of ferredoxin-NAD^+ reductase from the methane oxidizer *Methylosinus trichosporium* Ob3b. J. Bacteriol. **171:**5012–5016.
6. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:**387–395.
7. **Donadio, S., and C. R. Hutchinson.** 1991. Cloning and characterization of the *Saccharopolyspora erythraea fdxA* gene encoding ferredoxin. Gene **100:** 231–235.
8. **Ely, B.** 1979. Transfer of drug resistance factors to the dimorphic bacterium *Caulobacter crescentus*. Genetics **91:**371–380.
9. **Ely, B.** 1982. Transposition of Tn*7* occurs at a single site on the *Caulobacter crescentus* chromosome. J. Bacteriol. **151:**1056–1058.
10. **Ely, B.** 1992. DNA sequence of the 3′ end of the *Caulobacter crescentus* 16S rRNA gene. Nucleic Acids Res. **20:**1423.
11. **Evinger, M., and N. Agabian.** 1977. Envelope-associated nucleoid from *Caulobacter crescentus* stalked and swarmer cells. J. Bacteriol. **132:**294–301.
12. **Gober, J. W., and L. Shapiro.** 1992. A developmentally regulated *Caulobacter* flagellar promoter is activated by 3′ enhancer and IHF binding elements. Mol. Biol. Cell. **3:**913–926.
13. **Gomes, S., and L. Shapiro.** 1984. Differential expression and positioning of chemotaxis methylation proteins in *Caulobacter*. J. Mol. Biol. **177:**551–568.
14. **Hodgson, D., P. Shaw, M. O'Connell, S. Henry, and L. Shapiro.** 1984. *Caulobacter crescentus* fatty acid-dependent cell cycle mutant. J. Bacteriol. **158:**156–162.
15. **Howard, J., T. Lorsbach, D. Ghosh, K. Melis, and C. Stout.** 1983. Structure of *Azotobacter vinelandii* 7Fe ferredoxin. J. Biol. Chem. **258:**508–522.
16. **Johnson, R., and B. Ely.** 1977. Isolation of spontaneously derived mutants of *Caulobacter crescentus*. Genetics **86:**25–32.
17. **Kang, P. J., and L. Shapiro.** 1994. Cell cycle arrest of a *Caulobacter crescentus secA* mutant. J. Bacteriol. **176:**4958–4965.
18. **Koga, H., E. Yamaguchi, K. Matsunaga, H. Aramaki, and T. Horiuchi.** 1989. Cloning and nucleotide sequences of NADH-putidaredoxin reductase gene (*camA*) and putidaredoxin gene (*camB*) involved in cytochrome P-450cam hydroxylase of *Pseudomonas putida*. J. Biochem. **106:**831–836.
19. **Letts, V., P. Shaw, L. Shapiro, and S. Henry.** 1982. Synthesis and utilization of fatty acids by wild-type and fatty acid auxotrophs of *Caulobacter crescentus*. J. Bacteriol. **151:**1269–1278.
20. **Mansour, J. D., S. Henry, and L. Shapiro.** 1981. Phospholipid biosynthesis is required for stalk elongation in *Caulobacter crescentus*. J. Bacteriol. **145:** 1404–1409.
21. **Martin, A. E., B. K. Burgess, S. E. Iismaa, C. T. Smartt, M. R. Jacobson, and D. R. Dean.** 1989. Construction and characterization of an *Azotobacter vinelandii* strain with mutations in the genes encoding flavodoxin and ferredoxin I. J. Bacteriol. **171:**3162–3167.
22. **Miller, J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
23. **Morgan, T., D. Lundell, and B. Burgess.** 1988. *Azotobacter vinelandii* ferredoxin I: cloning, sequencing, and mutant analysis. J. Biol. Chem. **263:**1370–1375.
24. **Nathan, P., S. L. Gomes, K. Hahnenberger, A. Newton, and L. Shapiro.** 1986. Differential localization of membrane receptor chemotaxis proteins in the *Caulobacter* predivisional cell. J. Mol. Biol. **191:**433–440.
25. **O'Neill, E. A., and R. A. Bender.** 1987. Periodic synthesis of phospholipids during the *Caulobacter crescentus* cell cycle. J. Bacteriol. **169:**2618–2623.
26. **Peterson, J., M. Lorence, and B. Amarneh.** 1990. Putidaredoxin reductase and putidaredoxin. J. Biol. Chem. **265:**6066–6073.
27. **Reuter, S. H., and L. Shapiro.** 1987. Asymmetric segregation of heat-shock proteins upon cell division in *Caulobacter crescentus*. J. Mol. Biol. **194:**653–662.
28. **Saeki, K., Y. Suetsugu, K. Tokuda, Y. Miyatake, D. Young, B. Marrs, and H. Matsubara.** 1991. Genetic analysis of functional differences among distinct ferredoxins in *Rhodobacter capsulatus*. J. Biol. Chem. **266:**12889–12895.
29. **Sambrook, J., E. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
30. **Sanger, S., S. Nicklen, and A. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
31. **Schatt, E., Y. Jouanneau, and P. Vignais.** 1989. Molecular cloning and sequence analysis of the structural gene of ferredoxin I from the photosynthetic bacterium *Rhodobacter capsulatus*. J. Bacteriol. **171:**6218–6226.
32. **Schoenlein, P., L. Gallman, and B. Ely.** 1988. Use of transmissible plasmids as cloning vectors in *Caulobacter crescentus*. Gene **70:**321–329.
33. **Schoenlein, P., L. Gallman, M. Winkler, and B. Ely.** 1990. Nucleotide sequence analysis of the *Caulobacter crescentus flaF* and *flbT* genes and an analysis of codon usage in organisms with GC-rich genomes. Gene **92:**17–25.
34. **Shanmugam, K. T., B. B. Buchanan, and D. I. Arnon.** 1972. Ferredoxins in light- and dark-grown photosynthetic cells with special reference to *Rhodospirillum rubrum*. Biochim. Biophys. Acta **256:**477–486.

35. **Stout, C. D.** 1989. Refinement of the 7Fe ferredoxin from *Azotobacter vinelandii* at 1.9Å resolution. J. Mol. Biol. **205:**545–555.
36. **Subramanian, V., T. Liu, W. Yeh, C. Serdar, L. Wackett, and D. Gibson.** 1985. Purification and properties of ferredoxin$_{TOL}$. J. Biol. Chem. **260:**2355–2363.
37. **Tanaka, M., M. Haniu, K. T. Yasunobu, M. C. W. Evans, and K. K. Rao.** 1975. The amino acid sequence of ferredoxin II from *Chlorobium limicola*, a photosynthetic green bacterium. Biochemistry **14:**1938–1943.
38. **Thompson, G. A., D. E. Scherer, S. Foxall-Van Aken, J. W. Kenny, H. L. Young, D. K. Shintani, J. C. Kridl, and V. C. Knauf.** 1991. Primary structures of the precursor and mature forms of stearoyl-acyl carrier protein desaturase from safflower embryos and requirement of ferredoxin for enzyme activity. Proc. Natl. Acad. Sci. USA **88:**2578–2582.
39. **Wang, S. P., Y. P. Chen, and B. Ely.** 1995. A ferredoxin, designated FdxP, stimulates *p*-hydroxybenzoate hydroxylase activity in *Caulobacter crescentus*. J. Bacteriol. **177:**2908–2911.
40. **Yoch, D. C., and R. P. Carithers.** 1979. Bacterial iron-sulfur proteins. Microbiol. Rev. **43:**384–421.