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*Plant Gene Register*

# Nucleotide Sequence of an Operon in *Nostoc* sp. Strain ATCC 29133 Encoding Four Genes of the Oxidative Pentose Phosphate Cycle[1]

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Cyanobacteria are characterized as oxygenic photoautotrophs and use the reductive pentose phosphate (Calvin-Benson) cycle in assimilation of $CO_2$. Based on enzyme assays and isotopic labeling experiments, all cyanobacteria are assumed to use reactions of the oxidative pentose phosphate pathway to generate reductant for maintenance energy in the dark period of natural diel cycles (Smith, 1983). In heterocyst-forming cyanobacteria, the two initial dehydrogenases of the oxidative pentose phosphate pathway, G6PD and 6-phosphogluconate dehydrogenase are about 70-fold more active in heterocysts than in vegetative cells and are thought to provide reductant for nitrogenase activity (Winkenbach and Wolk, 1973).

*zwf*, the gene encoding G6PD, was isolated from a cosmid library of diazotrophic *Nostoc* sp. strain ATCC 29133 genomic DNA maintained in *Escherichia coli* (J. G. Wallis and J. C. Meeks, unpublished data). The cosmid was identified using as a heterologous probe a 500-bp internal fragment of *zwf* from the unicellular cyanobacterium *Synechococcus* sp. strain PCC 7942 (Table I). The 500-bp fragment was the product of PCR amplification of *Synechococcus* 7942 genomic DNA using degenerate primers based on conserved sequences in the *zwf* gene from *E. coli* and *Zymomonas mobilis*. Southern hybridization with the 500-bp fragment yielded restriction fragments of the cosmid that were identical to those of genomic DNA of *Nostoc* 29133. The derived amino acid sequence of *Nostoc* 29133 G6PD has 81% and 61% similarity to the homologous proteins from *Synechococcus* 7942 (Scanlan et al., 1992) and *E. coli* (Rowley and Wolf, 1991), respectively. The provisional identity of *zwf* from sequence homology was supported by G6PD activity assays of plasmid subclones maintained in *Nostoc* 29133, where specific activity increased in parallel with plasmid copy number, and by lack of catalytic activity in a

**Table I.** *Characteristics of a 6672-bp region from Nostoc sp. strain American Type Culture Collection 29133 containing four genes encoding pentose phosphate cycle enzymes*

Organism:
*Nostoc* sp. strain ATCC 29133 (Pasteur Culture Collection 73102, type strain of the species *Nostoc punctiforme*; Rippka and Herdman, 1992).

Techniques:
Genomic library of *Nostoc* 29133 was screened with a 500-bp fragment of *zwf* obtained by PCR from *Synechococcus* 7942 genomic DNA using the following degenerate primers: upstream, 5′-TCCTAAGCTTGAYCAYTAYYTNGGNAA-3′, encoding the conserved peptide DHYLGK; downstream, 5′-CTTCGAATTCKSCCANCKCCARTTRTC-3′, encoding the conserved peptide DNWRW(A/Q). Product cloned as a *Hin*dIII and *Eco*RI fragment into M13mp18. Sequence of both strands of deletion subclones in pBluescript by the dideoxy chain termination method. Computer analysis and comparisons used the Genetics Computer Group group of programs (Devereux et al., 1984) and the NCBI BLAST Network Service.

Methods of Identification:
Comparisons of deduced amino acid sequences with those sequences deposited in major data bases. *zwf* identity was additionally supported by in vitro catalytic assays in overproducing and insertionally inactivated strains of *Nostoc* 29133.

Structural and Functional Features of the Putative Proteins:
Sixteen transcripts, with 10 different 5′ ends, have been detected, which could code for one, two, three, or all four genes. *fbp* is an ORF of 349 amino acids with a predicted molecular mass of 38,593 D; Fbp (EC 3.13.11) hydrolyzes Fru-1,6-bisP to Fru-6-P and orthophosphate. *tal* is an ORF of 381 amino acids with a predicted molecular mass of 41,793 D; Tal (EC 2.2.1.2) reversibly transfers dihydroxyacetone from sedoheptulose-7-P to glyceraldehyde-3-P forming Fru-6-P and erythrose-4-P. *zwf* is an ORF of 509 amino acids with a predicted molecular mass of 58,231; G6PD (EC 1.1.1.49) oxidizes Glc-6-P to 6-phosphoglucono-δ-lactone and $NADPH_2$. The unidentified ORF is 465 amino acids with a predicted molecular mass of 50,595 D. All start codons are ATG and are preceded by putative ribosome-binding sites.

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Abbreviations: *fbp* (Fbp), fructose bisphosphatase; ORF, open reading frame; *tal* (Tal), transaldolase; *zwf* (G6PD), Glc-6-P dehydrogenase.

*Nostoc* 29133 mutant generated by insertational inactivation via homologous recombination.

DNA:RNA blot analysis using an internal 1.4-kb fragment of the *Nostoc* 29133 *zwf* gene revealed mRNA transcripts ranging from 3.3 to 6.0 kb. Continued sequencing of cosmid subclones yielded three additional ORFs contiguous to *zwf*, two of which were identified by sequence comparison as most likely to represent *fbp* (65% amino acid similarity to the homologous protein from *E. coli*; Hamilton et al., 1988) and *tal* (49% similarity to the homologous protein from *E. coli*; Yura et al., 1992); the remaining ORF has no significant similarity to nucleotide or derived amino acid sequences in data bases searched as of April 24, 1994. Involvement of the unidentified ORF in reactions of carbon catabolism is supported by: (a) increased catalytic activity of G6PD when *zwf* was present in *Nostoc* 29133 on multicopy plasmids only when the construct also contained the unidentified ORF and (b) *zwf* is always co-transcribed with this ORF. The operon is organized as *fbp-tal-zwf*-ORF, with the 6-kb transcript initiated at the 5′ end of *fbp*.

G6PD and Tal are specific reactions of the oxidative pentose phosphate pathway. Fbp is most often considered in the context of the regenerative phase of the reductive pentose phosphate pathway and in gluconeogenesis in organisms growing on pentoses or organic acids. In the complete oxidation of Glc to $CO_2$, however, the oxidative pentose phosphate pathway may function as a cycle, with Fbp operating to shift equilibrium toward resynthesis of Glc-6-P from the triose intermediates. Nevertheless, the observations that reduced thioredoxin regulates catalytic activity of the two branch-point enzymes in diametrically opposite ways, activating Fbp (Crawford et al., 1984) and inactivating G6PD (Cossar et al., 1984), may imply that physical and transcriptional linkage of the genes is of little physiological significance.

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