Short communication

Organization of the Phycocyanin Gene Clusters in *Anacystis nidulans**

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Cyanobacteria (blue green algae) are prokaryotic organisms capable of photosynthesis,[1] with a light-harvesting complex composed mainly of *chl*a and phycobiliproteins. *Chl*a resides in the thylakoid membranes and the phycobiliproteins in the phycobilisomes, which are water soluble complexes attached to the thylakoids.[2–4] The cyanobacterium used in this study, *Anacystis nidulans*, has phycobilisomes containing two core complexes and three rods attached to the core.[5] The phycobilisome is composed of the chromophore-containing polypeptides phycocyanin, allophycocyanin and allophycocyanin B.[3,4,6] The chromophoric proteins α- and β-phycocyanin are located in the phycobilisome rod and their amount is regulated according to the light environment, thereby maximizing the light-harvesting capabilities of the phycobilisome.[7] Hybrid DNA techniques have recently been used to isolate genes from *A. nidulans* and to study their gene organization. The gene coding for the phycobiliprotein β-phycocyanin was recently cloned from *A. nidulans*.[8] The genes coding for phycocyanin have also been cloned from the cyanobacterium *Agmenellum quadruplicatum* and the eukaryotic alga *Cyanophora paradoxa*.[9–11] In the latter organism, the genes coding for α- and β-phycocyanin were found to be clustered such that the gene for β-phycocyanin is located upstream of the α-phycocyanin gene. In this communication we report on the genetic organization of the α- and β-phycocyanin genes in the cyanobacterium *A. nidulans*.

Experimental procedures

The cyanobacterium used in this study was *A. nidulans* 625 (*Synechococcus* 6301).[12] Phages M13 mp8 and mp9, plasmid pUC8 and *Escherichia coli* strains JM83 and JM103 were used for cloning and DNA sequencing.[13] Phage λ-23:30 carrying *A. nidulans* phycocyanin genes has been described elsewhere.[8] DNA isolation and DNA cloning were performed using standard techniques. M13 cloning and dideoxy-DNA sequencing were performed according to directions provided by Amersham International, U.K. *In vitro* DNA labelling and DNA-DNA hybridization were performed as described by Southern.[14] DNA sequences were analysed with the GENEUS computer system.[15] Synthetic oligonucleotides were supplied by SYN-TEK AB, Umeå, Sweden.

Results and discussion

The gene coding for β-phycocyanin of the cyanobacterium *A. nidulans* has previously been cloned from a λ-library.[8] In the course of this study we obtained an M13 phage library contain-

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β-phycocyanin

 1
MetThrPheAspAlaPheThrLysValValAlaGlnAlaAspAlaArgGlyGluPheLeu
ATGACTTTTCATGCTTTCACCAAGGTGGTGGCACAAGCCGATGCCCGTGGCGAATTTTTG
1

21                   SerLeu
SerAspAlaGlnLeuAspAlaLeuSerArgLeuValAlaGluGlyAsnLysArgIleAsp
AGCGACGCCCAACTGGACGCGCTGAGCCGCTTGGTTGCAGAAGGCAACAAACGGATTGAT
61

41
ThrValAsnArgIleThrGlyAsnAlaSerSerIleValAlaAsnAlaAlaArgAlaLeu
ACGGTCAACCGCATCACCGGTAATGCTTCGTCGATCGTCGCTAACGCAGCGCGTGCATTG
121

61                                                  Arg
PheAlaGluGlnProSerLeuIleAlaProGlyGlyAsnAlaTyrThrAsnArgArgMet
TTTGCAGAGCAACCTTCTCTGATTGCTCCTGGCGGCAACGCATACACGAACCGTCGGATG
181

81    +
AlaAlaCysLeuArgAspMetGluIleIleLeuArgTyrValThrTyrAlaValPheThr
GCGGCTTGTCTGCGCGACATGGAAATCATTCTCCGCTACGTGACCTACGCGGTCTTCACC
241

101            Ser              Asp
GlyAspAlaSerIleLeuAspAspArgCysLeuAsnGlyLeuArgGluThrTyrLeuAla
GGCGATGCTTCCATTCTCGACGACCGCTGTTTGAACGGTCTGCGTGAGACCTACTTGGCT
301

121
Ser               Leu
LeuGlyValProGlyAlaSerValAlaGluGlyValArgLysMetLysAspAlaAlaVal
CTGGGCGTGCCCGGTGCATCGGTGGCAGAAGGCGTTCGCAAGATGAAAGACGCAGCTGTG
361

141                                     +        Ile
AlaIleValSerAspArgAsnGlyIleThrGlnGlyAspCysSerAlaIleIleSerGlu
GCGATTGTGAGCGACCGCAACGGCATCACCCAAGGTGACTGTTCAGCGATCATTTCCGAG
421

161
LeuGlySerTyrPheAspLysAlaAlaAlaAlaValAla
CTGGGCAGCTACTTCGACAAAGCTGCTGCTGCAGTTGCCTAG
481


α-phycocyanin

 1
MetSerLysThrProLeuThrGluAlaValAlaAlaAlaAspSerGlnGly
AGTTCCAAGACTCCTCTGACCGAAGCTGTCGCTGCTGCTGATTCGCAAGGA
 1
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Fig. 1. DNA and amino sequences for the α- and β-phycocyanin genes in *A. nidulans.* Positions for amino acids and nucleotides are indicated. Differences between the translated DNA sequence and the amino acid sequence obtained for the sequenced protein are indicated.

ing β-phycocyanin-specific DNA fragments and we used the M13 phage library to obtain DNA sequences for parts of the β-phycocyanin gene. The sequences obtained were used to construct different specific synthetic oligonucleotides hybridizing to various regions within the β-phycocyanin gene. Using the different oligonucleotides to prime DNA synthesis from various M13 clones, we obtained a complete DNA sequence for the β-phycocyanin gene from *A. nidulans* 625 (Fig. 1). The DNA sequence was translated into amino acids and the derived amino acid sequence was compared with the published amino acid sequence obtained for the β-phycocyanin polypeptide from *A. nidulans.*[16] The comparison revealed an almost identical amino acid sequence (Fig. 1). However, some differences were found: the translated DNA sequence displays duplicate amino acids at position 78 (Arg-Arg) and at position 157 (Ile-Ile), whereas the results for the sequenced protein show only single amino acids. Furthermore, there are several serine-leucine replacements. From the DNA sequence it is clear that no signal peptide is present in the protein, and that the predicted protein starts with the methionine at position 1 as shown in Fig. 1. This, and the additional amino acids arginine and isoleucine render a protein with a total of 173 amino acids, compared to the 170 amino acids determined by amino acid sequencing of the β-phycocyanin polypeptide.[16] Using one synthetic oligonucleotide hybridizing close to the *C*-terminal end of the β-phycocyanin gene, the end of the gene and the region downstream from its stop codon were sequenced. An analysis of this DNA sequence reveals an open reading frame which starts with an ATG located about 50 nucleotides downstream from the β-phycocyanin stop codon. The *N*-terminal part of the translated protein derived from the DNA sequence and the published *N*-terminal sequence for α-phycocyanin are identical.[17] We therefore consider the existence of an α-phycocyanin gene located downstream from the gene for β-phycocyanin confirmed. It is interesting to note that the untranslated region is only about 50 nucleotides long, and thus shorter than the untranslated region between the large and small subunits of ribulosebisphosphate carboxylase in *A. nidulans.*[18] Data obtained by protein sequencing of phycobiliproteins indicate the possibility that α- and β-phycocyanin polypeptides are evolutionarily related and could have arisen from one common ancestral gene.[19] The data now emerging from cloning and DNA sequencing of phycocyanin genes, such as the one described in this study, strongly support this hypothesis. In all cases so far studied, the two genes for the phycocyanin proteins are closely linked and the gene coding for β-phycocyanin is located upstream from the gene coding for α-phycocyanin.[9–11] Interestingly enough, this genetic organization is also preserved in the eukaryotic alga *C. paradoxa,* making the hypothesis that the chloroplast

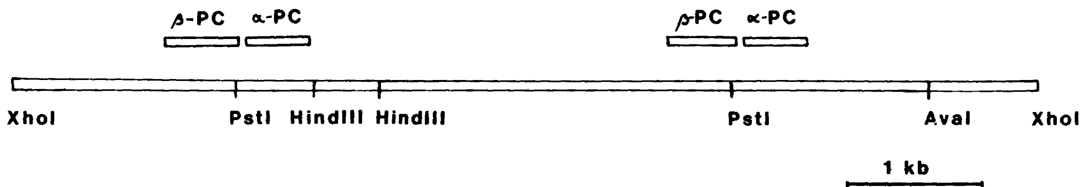

Fig. 2. Gene organization of the *A. nidulans* chromosome containing the α- and β-phycocyanin genes. Sites for restriction endonucleases *Xho*I, *Hind*III, *Ava*I and *Pst*I are shown.

may have evolved from cyanobacteria seem reasonable. In the course of this study, we obtained an M13 phage, M13-9T22, carrying a β-phycocyanin-specific *Taq*I fragment. We used this M13 phage as a β-phycocyanin-specific probe to compare the genetic structure of our λ-clone with that of the *A. nidulans* 625 chromosome. The results showed that the probe hybridized to identical restriction endonuclease fragments in the λ-phage and the *A. nidulans* chromosome, indicating that no DNA sequence alterations had occurred during the construction and preparation of the λ-phage (data not shown). However, the probe hybridized to two *Hind*III fragments, despite the fact that the probe did not contain any *Hind*III site. This result suggested that the phycocyanin gene could be duplicated on the *A. nidulans* genome. To further investigate the possible β-phycocyanin gene duplication we performed specific subclonings from the λ-23:30 phage into plasmid pUC8. Plasmid DNA sequencing confirmed the existence of two β-phycocyanin genes (Fig. 2), and from the restriction endonuclease map constructed we found that the intergenetic region between the two phycocyanin gene clusters is about 2.5 kb (Fig. 2). We believe that the gene duplication of the α- and β-phycocyanin genes present in *A. nidulans* reveals a true evolutionary pathway for certain bacteria, possibly related to the natural growth environment. The DNA sequences so far obtained for the two phycocyanin gene clusters in *A. nidulans* show that the coding regions are similar, if not identical. The gene duplications must therefore have arisen late in evolution and certainly later than the duplication giving rise to α- and β-phycocyanin. The question arises as to why *A. nidulans* has evolved duplicate phycocyanin gene clusters and whether the two gene clusters are functionally active. In the future, it will be interesting to see which genes are located between the two phycocyanin gene clusters. The idea that most polypeptides coding for proteins in the phycobilisomes are evolutionarily related and could have arisen from one common ancestral gene makes it possible that the intergenetic region between the two phycocyanin genes contains genes coding for other polypeptides within the phycobilisomes.[19] A more detailed analysis using gene technology will be able to answer these questions.

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