Plant Physiol. (1992) 98, 1538–1540
0032-0889/92/98/1538/03/$01.00/0

Received for publication December 16, 1991
Accepted January 10, 1992

Plant Gene Register

Barley (*Hordeum vulgare*) Gene for CP29, a Core Chlorophyll *a/b* Binding Protein of Photosystem II[1]

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The CAB[2] protein CP29 is distinguished from LHCII proteins by its higher Chl *a/b* ratio (3–5 *versus* 1.2) (2, 6) and its relative abundance in the Chl *b*-less barley mutant, chlorina-f2, which is nearly depleted for LHCII (3, 6). In tomato, two CP29 polypeptides have been characterized, and the gene for one of them has been isolated (4). CP29 from barley was seen as a single band (3) or a double band (6) in gel fractionation studies.

A genomic clone encoding CP29 was fortuitously isolated from a barley cv Bomi library by hybridization with a cDNA clone, pKG2252 (1), encoding a LHCII type I CAB protein. Subsequently, five cDNA clones were isolated, they were all derived from the gene, and in combination the cDNA sequences covered the mRNA sequence from bp +3 to three different polyadenylation sites. The derived amino acid sequence is only about 50% homologous to LHCII type I CAB proteins and was found instead to be closely related to tomato CP29 (4). In tomato, tryptic peptide sequences from a type II CP29 have been determined (4). In these short regions, the barley CP29 is more similar to the tomato type I sequence than the two tomato sequences are to each other, suggesting that the barley CP29 gene codes for a type I protein. The N terminal of the mature CP29 proteins is not known, and the transit peptide processing sites in the tomato or the barley precursor sequence cannot be recognized by similarity to other chloroplast proteins. From *in vitro* translation/chloroplast import studies, the tomato transit peptide was estimated to about 50 amino acids (4). The N-terminal 61 amino acids in the barley and tomato CP29 precursors are at most 32% homologous (with several gaps) and the high homology starts at aspartate 62.The noncoding regions are completely diverged in the barley and tomato gene (Fig. 1, Table I). However, the barley and tomato CP29 promoters share a region of 21 of 24 identical nucleotides about 160 bp from the transcription start site that may constitute a *cis*-regulatory element. The promoter proximal region also contains three GATA boxes implicated in the regulation of several plant genes, *e.g. cab-E* from tobacco (5).

[1] This work was supported by The Danish Biotechnology Programme.

[2] Abbreviations: CAB, Chl *a/b* binding protein; LHC, light-harvesting complex; bp, base pair.

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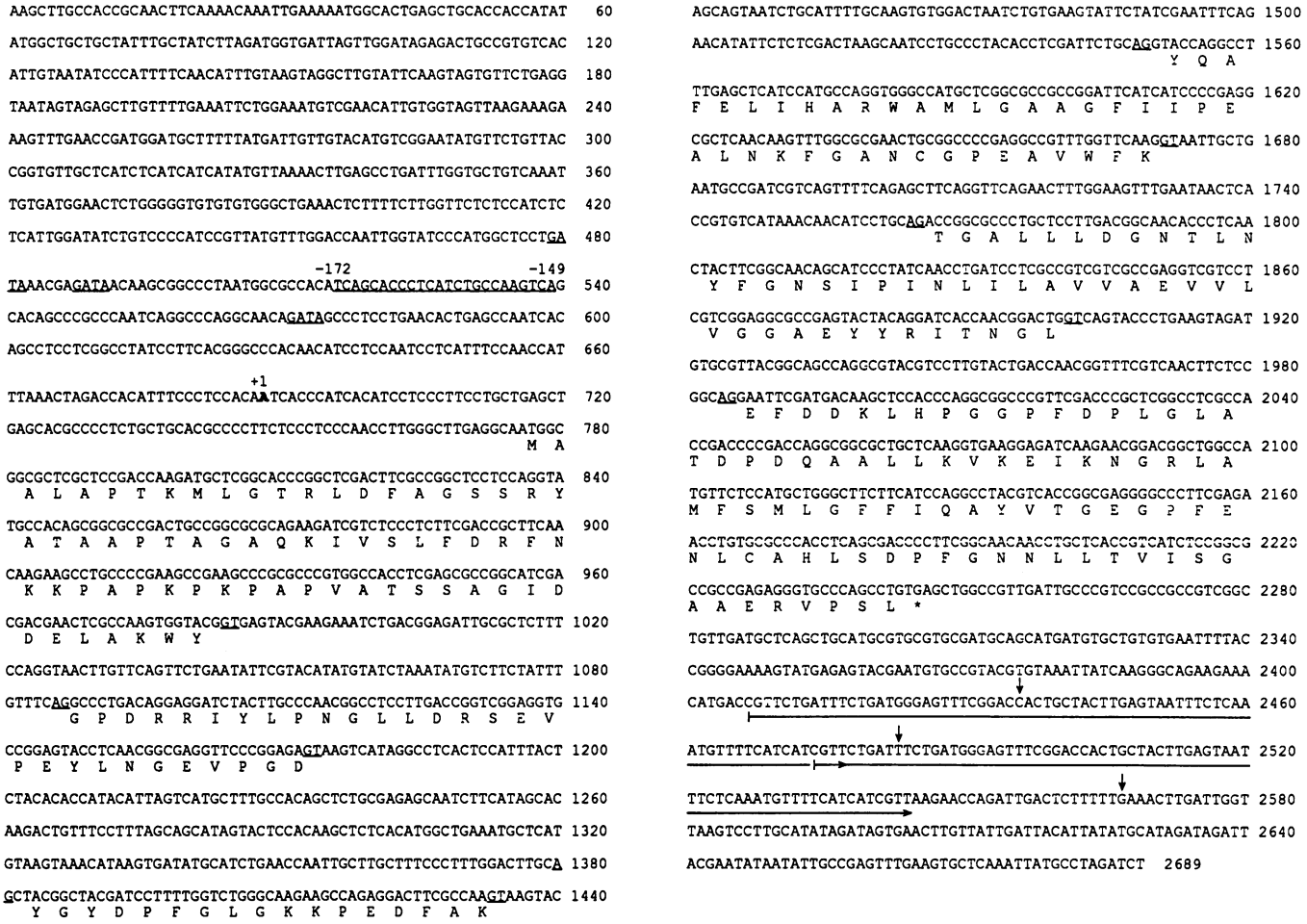

Figure 1. Nucleotide sequence of a barley CP29 gene and the derived amino acid sequence. The transcription start site is marked +1. Putative regulatory elements and intron splice sites are underlined. Sequences of cDNA clones start at +3 and terminate at the polyadenylation sites shown with down arrows. Horizontal arrows below the sequence show a 70-bp direct repeat.

Table I. *Characteristics of a CP29 Gene from Barley*

Organism:
Hordeum vulgare (barley), cv Bomi.
Location:
The gene was found on a 5.5-kilobase pair *Hin*dIII fragment. Genomic location not known.
Gene Function:
Codes for a light-harvesting CAB protein from PSII equivalent to tomato CP29 type I.
Isolation:
The gene was isolated from a partial *Hin*dIII library constructed in λL47.1 using a PSII type I *cab* cDNA as probe.
Techniques:
Subcloning in pBS−; dideoxynucleotide sequencing of plasmid DNA. Transcription start site determined by S1 nuclease mapping.
Method of Identification:
Exons were identified by comparison with barley CP29 cDNA clones. The derived amino acid sequence of the (approximately known) mature peptide is 91% homologous to tomato CP29 type I. The barley gene has five introns in the same positions as the five introns in the tomato gene.
(C + G) Content:
Coding regions 63.5%; introns 43.5%.
Regulation:
Approximately coordinated with PSII type I *cab* genes in the developmental gradient in 7-d-old barley leaves.
Feature of Gene Structure:
A region in the promoter with 21/24 bp identity with a region in the tomato *cab9* gene promoter (coding for CP29) is located approximately −160 from the transcription start site in both genes. The 5′-nontranslated leader is 88 nucleotides. The gene has five introns. There are at least three polyadenylation sites. The longest 3′-nontranslated region contains a 70-nucleotide perfect direct repeat that overlaps by four nucleotides.
Structural Features of the CP29 Precursor:
Open reading frame 286 amino acids; M_r 30,749. Transit peptide processing site is not known.
Location of Protein:
Chloroplast thylakoid membrane.
EMBL Accession No.:
X63052