MOLECULAR AND CELLULAR BIOLOGY, June 1985, p. 1370–1378
0270-7306/85/061370-09$02.00/0

Vol. 5, No. 6

Structure and Developmental Regulation of a Wheat Gene Encoding the Major Chlorophyll a/b-Binding Polypeptide

GAYLE K. LAMPPA,† GIORGIO MORELLI, AND NAM-HAI CHUA*

Laboratory of Plant Molecular Biology, The Rockefeller University, New York, New York 10021-6399

Received 2 January 1985/Accepted 7 March 1985

A genomic clone for a major chlorophyll a/b-binding polypeptide of the light-harvesting complex has been sequenced from wheat. This gene, whAB1.6, encodes a 70-nucleotide 5′-nontranslated spacer, a 34-amino-acid NH_2-terminal extension, i.e., the transit peptide, and a mature coding protein of 232 amino acid residues. The exact molecular weight of the precursor polypeptide is 28,560. The transit peptide is basic and is rich in serines. No intervening sequences are found in this gene. The transcription start site of the whAB1.6 gene occurs at AAAC as determined by S1 nuclease analysis. Putative regulatory sequences occur upstream of the gene at −25 (TTTAAATA) and at −72 (CCAACCA). Northern blots show a single RNA species estimated to be 1,100 nucleotides. Heterogeneity of the RNA population is demonstrated in S1 nuclease analyses with a 5′-end-labeled fragment that extends 191 nucleotides into the mature protein coding sequence. At least seven different transcripts can be recognized. The highest levels of RNA transcribed from the whAB1.6 gene are found in the basal segments of the wheat leaf, whereas other chlorophyll a/b-binding transcripts in the cell show a different pattern of abundance. As a control, we show that roots do not contain chlorophyll a/b-binding RNA. The most abundant RNA species shows an interrupted homology with the whAB1.6 gene at the start of the mature protein coding sequence; another species shows homology beginning at the start of the transit peptide and does not include the nontranslated region. Chlorophyll a/b-binding polypeptides accumulate toward the tip of the leaf as shown by Western blot analysis of total thylakoid proteins.

One of the most abundant classes of transcripts found in light-grown plant shoots codes for the chlorophyll a/b-binding (cab) polypeptides of the light-harvesting complex (LHC), as shown by in vitro translation of polyadenylated [poly(A)$^+$] RNA from peas (9), barley (48), and *Lemna gibba* (46). The proteins of LHC play a key role in the thylakoid membranes where they orient the chlorophylls for maximal photosynthetic efficiency (for a review see references 3, 25, and 42). The cab polypeptides are heterogeneous in size with molecular weights of 25,000 to 30,000 in green plants. Two major proteins of the LHC, originally designated 15 and 16 on the basis of their decreasing electrophoretic mobility on sodium dodecyl sulfate gels, are encoded by the nuclear genome, synthesized on cytoplasmic free polysomes as precursor polypeptides, and posttranslationally imported into the chloroplast (19, 39, 40).

Light stimulates an increase in the steady-state levels of RNA for the cab proteins in peas (17, 45), barley (1, 49), and *L. gibba* (43, 46), and the response is mediated by phytochrome. Nuclei isolated from light-grown pea leaves contain about ninefold more cab RNA than do nuclei from dark-grown tissue (23). In barley, a monocotyledon, the translatable RNA for the cab proteins changes along the length of the leaf, with its highest concentration at the leaf base (48, 49).

We are interested in the regulation and expression of nuclear genes required for photosynthetic functions in wheat. We chose to investigate this monocotyledon for two reasons. First, wheat is the most important cereal in the world, accounting for approximately 20% of the food intake (35). Second, the physiology and pattern of development of the wheat leaf have been well characterized (7, 18, 22). Cell division stops at the base of the leaf; beyond this zone a gradient exists in which cells become photosynthetically competent. In fact, individual segments of the wheat leaf from the base to the tip represent progressive stages in plant cell development and chloroplast biogenesis and consequently offer an opportunity to study selective gene expression.

Previously, a wheat genomic clone for the small subunit (rbcS) of ribulose-1,5-bisphosphate carboxylase was characterized (10). In this paper we present results on the expression of the wheat cab genes at both the RNA and the protein level. The cab polypeptides are encoded by a multigene family in peas (15), petunias, barley, and tobacco (20). To explore selective gene expression, we isolated a wheat genomic clone (whAB1.6) for the major cab polypeptide and determined its nucleotide sequence and transcription initiation site. The structural information allowed us to predict the amino acid sequence of the cab polypeptide and its NH_2-terminal extension, the transit peptide. Based on S1 nuclease analyses, in which a 5′ fragment of whAB1.6 extending into the coding sequence was used as a probe, we present evidence suggesting that transcripts from different cab genes contain sequence divergence in both the 5′-nontranslated leader region as well as the transit peptide. This observation allowed us to describe the relative abundance of RNA transcribed from the cab gene we characterized.

MATERIALS AND METHODS

Growth of plants. Wheat seeds were germinated in vermiculite and grown for 8 days in a diurnal light-dark (16:8) cycle with 18°C days and 22°C nights before the leaves and roots were harvested.

Isolation of the genomic clone. Total wheat DNA was partially digested with *Bam*HI and cloned into the phage vector Charon 30 as previously described (10). A pea cDNA clone pAB96, which codes for the major cab polypeptide

* Corresponding author.
† Present address: Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL 60637.

(15), was used as a hybridization probe to screen the recombinants.

Preparation of RNA. Wheat leaves were cut into 2-cm segments, beginning at the seed. Leaf sections or roots (8-day plants) were frozen in liquid nitrogen upon harvesting and ground in a coffee grinder after prechilling the apparatus with dry ice. This method produces a fine powder with most cell walls broken and a high nucleic acid yield. A solution of 5 M guanidine thiocyanate, 25 mM sodium citrate, 0.5% sarcosyl, 2 mM EDTA, and 5% mercaptoethanol was added (2 ml/g of fresh-weight tissue) to the frozen powder, and the RNA was isolated as described previously (14). Poly(A)$^+$ RNA was isolated by poly(U)-Sepharose chromatography (27).

Analysis of transcripts. Total RNA denatured with glyoxal (11) was separated on agarose gels and blotted onto nitrocellulose papers (44). ^{32}P-labeled DNA probes (10^8 cpm/μg) were prepared by nick translation (New England Nuclear Corp.) and hybridized with filters at 42°C in 50% formamide–0.3 M NaCl–0.02 M sodium citrate–50 mM $NaPO_4$–0.04% Ficoll–0.04% polyvinylpyrrolidone–0.04% bovine serum albumin–100 μg of sonicated salmon sperm DNA per ml. To determine the transcription initiation site, isolated restriction enzyme fragments were 5′ end labeled with T4 kinase and reassociated with 1 μg of poly(A)$^+$ RNA for 8 to 16 h in 40 mM PIPES (piperazine-*N*-*N*′-bis(2-ethanesulfonic acid; pH 6.4)–1 mM EDTA (pH 8.0)–0.4 M NaCl–80% formamide at 50°C. The DNA-RNA hybrids were digested at 37°C with S1 nuclease (Bethesda Research Laboratories) at a concentration of 30 U per reaction, in 0.3 M NaCl–4.5 mM $ZnCl_2$–5 mM sodium acetate (pH 4.6)–20 μg of salmon sperm DNA per ml. In the development studies, a typical hybridization reaction contained 0.2 μg of labeled DNA fragment and 10 μg of total RNA.

DNA sequence determination. In the initial analysis double digests with *Pst*I-*Hin*cII and *Pst*I-*Xho*I fragments of the genomic clone were sequenced by the method of Maxam and Gilbert (29). After new restriction enzyme sites were identified *Hpa*II and *Bgl*II subclones were made in the M13 phage vectors (31) mp10 and mp11 with JM101 as a host. The sequences of these subclones were determined by the dideoxynucleotide method (37) with [^{35}S]dATP (900 Ci/mmol; New England Nuclear Corp.).

Protein analysis. Leaf sections were ground with a mortar and pestle in a buffer containing 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid)–KOH (pH 7.5)–0.15 M NaCl–10 mM EDTA–1 mM phenylmethylsulfonyl fluoride. The homogenate was passed through eight layers of cheesecloth, and the filtrate was adjusted to 10% trichloroacetic acid for precipitation of total protein. Protein pellets were processed for electrophoresis on 7.5 to 15% sodium dodecyl sulfate gradient gels as described by Piccioni et al. (34). Western blots were performed by the method of Blake et al. (6).

RESULTS

Structure of a wheat gene for the cab polypeptide and its amino acid sequence. A wheat genomic clone for the cab polypeptide was isolated from a *Bam*HI partial genomic library of wheat nuclear DNA in phage Charon 30. A labeled cDNA clone from peas, pAB96, previously shown to encode for most of the major cab polypeptide (15), hybridized with phage carrying a *Bam*HI 8.7-kilobase (kb) fragment from the wheat genome. Labeled 5′ and 3′ *Bgl*I subfragments of pAB96 were used in restriction fragment analyses to identify a 1.6-kb *Pst*I fragment within the *Bam*HI fragment, which encoded the mature cab polypeptide and was large enough to also code for a transit peptide with an estimated molecular weight of 4,000 and contain 5′- and 3′-flanking sequences.

Figure 1 presents the sequence of the wheat cab gene (whAB1.6) located in the *Pst*I 1.6-kb fragment and summarizes its structural features. The first ATG codon found in the PstI 1.6-kb fragment establishes an open reading frame that extends for 798 nucleotides (n) before the first termination (ochre, TAA) codon. The amino acids deduced from this reading frame are shown in a single-letter code above each codon. Beginning at amino acid 46 with four consecutive serines, almost complete homology (90%) exists between the whAB1.6 amino acid sequence and that sequence deduced previously from the pea cDNA clone pAB96 for the mature cab polypeptide (15). The first amino acid of the mature cab polypeptide has not yet been definitively established for any plant species. However, tryptic digests of pea thylakoids release a peptide containing the amino acids arg-lys-ser-ala-thr-thr-lys-lys (32). This octapeptide is located at the NH_2 terminus of the mature cab polypeptide, as initially established from a comparison with the sequence of pAB96 (15). Upstream of the four serines at position 46 in whAB1.6 there is an arg-lys sequence that aligns with the NH_2-terminal arg-lys of the tryptic released peptide (see Fig. 7B); it recently was found in a pea cab genomic clone (12). This information suggests that the wheat transit peptide most likely ends with a methionine and that the mature protein begins with arginine. If this is the case, the cab transit peptide of wheat would contain 34 amino acid residues, with a molecular weight of 3,332, and the mature protein would contain 232 amino acids, with a molecular weight of 25,229. In total, the major cab polypeptide from wheat would be 28,560 daltons before processing by the chloroplast envelope or amino acid modification.

S1 analysis of transcripts for the cab protein. The transcription initiation site of the wheat cab gene, whAB1.6 (Fig. 1), was established by S1 nuclease mapping (5). Poly(A)$^+$ RNA was hybridized with a 492-n 5′-end-labeled *Pst*I-*Xho*I fragment that begins 200 bases before the first ATG in the *Pst*I 1.6-kb clone (Fig. 1) and extends into the mature protein coding sequence (Fig. 2). Seven S1-resistant products were found (Fig. 2B). Two lines of evidence indicate that these products are not S1 artifacts but instead reflect heterogeneity between the gene-specific probe and transcripts from other cab genes. First, hybridization temperatures between 32 and 42°C have little effect on the presence of these S1-resistant products (data not shown). Second, we find that the relative intensity of the bands depends on the source of RNA used in the hybridization reactions. The labeled *Pst*I-*Xho*I fragment was strand separated, and each strand was hybridized with either total poly(A)$^+$ RNA or one fraction of poly(A)$^+$ RNA from a sucrose gradient in the region enriched for the major cab polypeptide. When total poly(A)$^+$ RNA is used, band a is less intense than bands b and c (Fig. 2C, lanes 1 and 3). In contrast, when the selected fraction of RNA is used, band a is the strongest in the profile (Fig. 2C, lanes 2 and 4), indicating that the whAB1.6 transcripts are more abundant in this fraction.

Two of the S1 products start upstream of the first ATG of the 798-n open reading frame in the *Pst*I 1.6-kb clone (Fig. 2), and thus either one could be the transcription initiation site of whAB1.6. The longest one (Fig. 2B, band a) begins 360 bases from the labeled *Xho*I end and corresponds to a transcript starting at AAAC, which would yield a nontranslated leader sequence of 70 n. The second S1 product is 302 n in length (Fig. 2B, band b) and coincides with an AGTG

A

```
                                                                    TGCAGATTTC TTTTCACCAC
ACCGTCTCTC TTGTCAGCTG TCACCAAGCA CCAACCAATT AATTTCTCTA TATTCTTGTT CCCTCAAGGC TCAAGCTCTT TAAATAGCTT
                                 -72                                                    -25
CCATACAATC TTCCTCTTAA ACCATCTTCC ACACACTCAA GCCACACTAT TGGAGAACAC ACAGGGACAA CACACCATAA GTGCAGCGCA
                      * *  * *

 M  A  A  T  T  M  S  L  S  S  S  S  F  A  G  K  A  V  K  N  L  P  S  S  A
ATG GCG GCC ACC ACC ATG TCT CTT TCC TCT TCG TCC TTC GCC GGC AAG GCC GTC AAG AAC TTG CCC TCG TCG GCT

 L  I  G  D  A  R  V  N  M  R  K  T  A  A  K  A  K  Q  V  S  S  S  S  P  W
CTC ATT GGT GAC GCA CGT GTG AAC ATG CGC AAG ACT GCG GCG AAG GCC AAA CAA GTT TCG TCG AGC AGC CCG TGG

 Y  G  S  D  R  V  L  Y  L  G  P  L  S  G  E  P  P  S  Y  L  T  G  E  F  P
TAC GGC TCT GAC CGT GTG CTC TAC CTC GGC CCG CTT TCC GGC GAG CCC CCC AGC TAC TTG ACT GGT GAG TTC CCC

 G  D  Y  G  W  D  T  A  G  L  S  A  D  P  E  T  F  A  K  N  R  E  L  E  V
GGT GAC TAT GGG TGG GAC ACC GCC GGG CTC TCG GCT GAC CCT GAG ACC TTC GCC AAG AAC CGG GAG CTC GAG GTG

 I  H  C  R  W  A  M  L  G  A  L  G  C  V  F  P  E  L  L  A  R  N  G  V  K
ATC CAC TGC CGA TGG GCC ATG CTC GGT GCG CTT GGT TGT GTC TTT CCC GAG TTG CTT GCC CGC AAT GGT GTG AAG

 F  G  E  A  G  W  F  K  A  G  S  Q  I  F  S  D  G  G  L  D  Y  L  G  N  P
TTC GGC GAG GCC GGT TGG TTC AAG GCT GGC TCT CAG ATC TTC AGC GAT GGT GGC CTC GAC TAC CTT GGC AAC CCC

 S  L  V  H  A  Q  S  L  L  A  I  W  A  C  Q  V  V  L  M  G  A  V  E  G  Y
AGC CTC GTC CAC GCC CAG AGC CTC CTC GCC ATA TGG GCT TGT CAA GTT GTG CTC ATG GGG GCC GTT GAG GGG TAC

 R  I  A  G  G  P  L  G  E  I  V  D  P  L  Y  P  G  G  S  F  D  P  L  G  L
CGC ATT GCT GGT GGT CCG CTC GGC GAG ATC GTC GAC CCA CTC TAC CCT GGT GGC AGC TTT GAC CCA CTT GGC CTG

 A  E  R  P  Q  A  F  A  E  L  K  V  K  E  I  K  N  G  R  L  A  M  F  S  M
GCC GAG CGA CCC CAG GCA TTT GCG GAG CTC AAG GTT AAG GAG ATC AAG AAT GGC CGC CTC GCC ATG TTC TCC ATG

 F  G  F  F  V  Q  A  I  V  T  G  K  G  P  L  G  D  L  A  D  H  I  A  D  P
TTT GGC TTC TTT GTG CAG GCC ATT GTC ACC GGC AAG GGC CCA TTG GAG GAC CTT GCC GAC CAC ATT GCC GAC CCT

 V  N  N  N  A  W  L  I  A  T  N  F  V  P  G  K
GTC AAC AAC AAC GCG TGG CTC ATT GCC ACC AAC TTC GTG CCC GGG AAA TAAAGCAGGACTT GATGGATGCA AATTGTATGT

 GAGTGTCAGT TGAATTGTAA CACCAACATG TAAACTATGA GAATTGTGTG CAAAGACATT GTTTGATTAG TCCAATCCGG TGACAATGGC

 CTGCACAAGA AGCCAACATG GAGAACATGG CGAGGCGGCC ATTATTGATC TCCTTAACTG AGCTCCGCAA
```

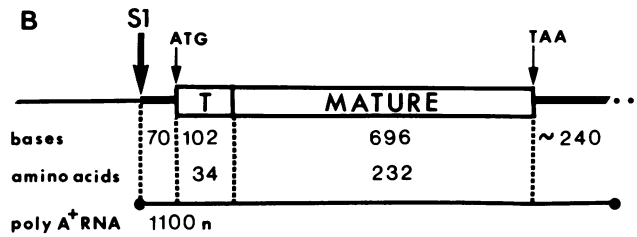

FIG. 1. (A) Sequence of the wheat cab polypeptide gene whAB1.6. The single-letter code for each amino acid is given above each codon. The transcription start site as determined by S1 nuclease analysis of DNA-RNA hybrids (Fig. 2) is marked by asterisks. The termination codon TAA and the TATA and CAAT boxes at −25, and −72, respectively, are underlined. An arrow points to the putative cleavage site at the transit and mature protein junction. An inverted repeat containing the TATA box is indicated by inverted arrows. S1-sensitive sites are indicated by dashed lines. (B) Structural features of the whAB1.6 gene. The transcription start site was established by S1 nuclease mapping. The first ATG and the TAA codons are indicated by arrows. The lengths of the different regions (transit sequence [T] and mature protein sequence [MATURE]) are given. The length of the 3′-nontranslated region is estimated from the size of the RNA species (1,100 n), as determined by Northern analysis (Fig. 6).

sequence 7 bases before the start of the transit peptide sequence. The continuity of both of these S1-resistant products through the junction of the transit peptide-mature protein coding sequence demonstrates the absence of an intron at that position in this gene. We find putative regulatory signals recognized by RNA polymerase II (for a review, see references 8 and 33) upstream of the AAAC site corresponding to band a. A TATA box, TTTAAATA, occurs at −25, and a CAAT box, CCAAC, exists at −72. These sequences are not found 5′ to the site corresponding to the shorter S1-resistant product (Fig. 2, band b). We conclude that band a represents the transcription start of whAB1.6, and the other S1-resistant products seen in Fig. 2B and C reflect the n sequence divergence of other cab RNAs in the cell when compared to the whAB1.6 gene-specific probe and the heterogeneity of the genes from which they are transcribed.

The whAB1.6 gene is located within a cluster of short repeated sequences of 5 to 7 bases at both its 5′ and 3′ ends (Fig. 3). At the 5′ end the repeats can be divided into three groups: one is CT rich, another is CA rich, and the third is comprised of three copies of CTCAAG. One of the CTCAAGs (at −36) is immediately upstream of the TATA box, TTTAAA, in a region capable of forming a 9-base-pair repeat (Fig. 1, arrows). At the 3′ end there are four copies of AATTG and three copies of (C/G)AACATG repeats which

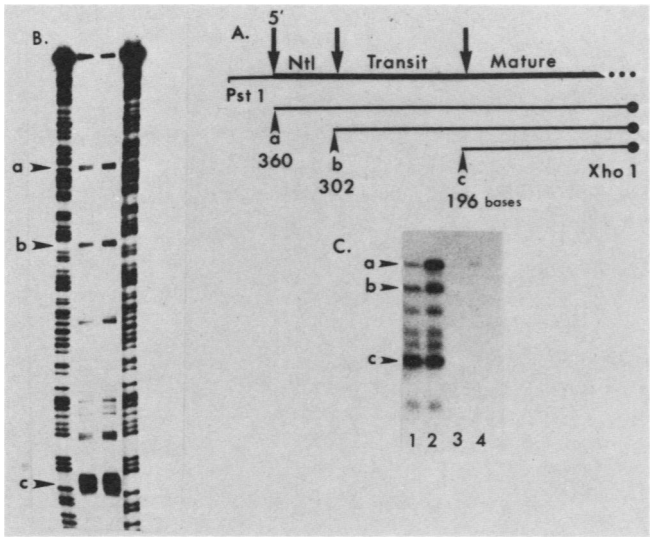

FIG. 2. Analysis of cab transcripts by S1 nuclease mapping. (A) Schematic diagram showing the 5′ positions of the S1-protected fragments. The distance from the *Xho*I site is given in n for bands a, b, and c. Ntl designates the nontranslated leader of the transcript, and the positions of the transit and mature coding sequences are labeled. (B) A *Pst*I-*Xho*I fragment from whAB1.6 was used for the S1 nuclease protection experiments. The fragment labeled at its 5′ end was hybridized with 2 μg of wheat poly(A)$^+$ RNA for 16 h at 50°C. This fragment is 492 n in length and extends 190 n into the mature coding sequence. The DNA-RNA hybrids were digested with S1 nuclease (see the text) and analyzed on an 8% sequencing gel (middle two lanes). A G-sequencing reaction of the *Pst*I-*Xho*I fragment was run as a marker (outer lanes). The arrows (a, b, and c) point to the S1-resistant bands that correspond to the positions shown in panel A. (C) The separated single strands of the *Pst*I-*Xho*I fragment were hybridized with total poly(A)$^+$ RNA (lanes 1 and 3) or a subfraction of poly(A)$^+$ RNA enriched in cab RNA by sucrose gradient fractionation (lanes 2 and 4). The experiments with coding and noncoding strands are shown in lanes 1 and 2 and in lanes 3 and 4, respectively.

both overlap with more complex arrays. In contrast, these repetitive sequences are not found within the coding region of the gene. They constitute 60% of the 5′- and 64% of the 3′-flanking sequences. It is not surprising that the whAB1.6 gene is situated within repetitive sequences since the wheat genome is comprised of 75% repetitive DNA, and they are interspersed with single-copy sequences (36). Short repeats with an average length of 200 bases account for about 20% of the wheat genome; however, the studies were not able to resolve repeats of less than 200 bases (36). Our sequence data of the whAB1.6 gene reveal that the short clusters of overlapping repeats give rise to complex sequence patterns that may themselves be part of long repetitive or unique DNA. The distribution of these repeats in the genome and their interaction with expressed, unique DNA remain to be explored.

The major cab protein is encoded by a multi-gene family. The minimum number of genes coding for the major cab polypeptide can be estimated from S1 nuclease analysis (Fig. 2; see Fig. 7). At least seven bands are resolved by gel electrophoresis (Fig. 2), each with specific homology to whAB1.6 in the 5′ region. The presence of band b indicates the existence of a class of genes in which homology with whAB1.6 begins at the start of the transit peptide. The 5′ - nontranslated leader region is not highly conserved between these genes. Heterogeneity also exists in the sequence coding for the transit peptide of the precursor, where six S1-sensitive sites can be resolved (Fig. 2; see Fig. 7).

To investigate further the complexity of the gene family coding for the cab polypeptide, we performed a Southern analysis (41). A labeled *Pst*I-*Sma*I fragment containing 875 n of whAB1.6 gene and 125 of its 5′-flanking sequences was hybridized to total wheat DNA cut with *Xho*I, *Pst*I, *Bgl*I, and *Bam*HI. In the *Pst*I profile the 1.6-kb fragment which we sequenced and characterized in this work is easily identifiable (Fig. 4, arrow). Numerous, unresolved bands also hybridize with the *Pst*I-*Sma*I fragment. A considerable portion of the wheat genome that hybridizes with the *Pst*I-*Sma*I fragment is not digested by these enzymes, in particular *Xho*I, an enzyme that recognizes a GC-rich, hexanucleotide sequence and is inhibited by methylation. The complexity of the hybridization pattern makes it difficult to establish the absolute number of genes in wheat coding for the cab polypeptide. With this in mind, it is important to recall that the wheat genome is large, equal to 36 pg, and hexaploid, comprised of three ancestral genomes (AA, BB, and DD). We would anticipate at least three genes for the major cab polypeptide without allele heterozygosity or gene duplication. An alternative possibility is that the complex pattern is due to the short repetitive sequences located in the 125 n upstream of the cab gene as described above. However, these sequences are unusually short (5 to 7 n), making it unlikely that individually they would hybridize when standard conditions were used (see above). We do not know whether the longer sequences generated by these short repeats are reiterated and scattered throughout the wheat genome.

Expression of cab genes in the wheat leaf. Many of the physiological parameters of wheat leaf development have been described previously (7, 18). The gradient of cell development that exists in the leaf provides an ideal in vivo system to investigate the changes in gene expression and protein accumulation that occur as cells become photosynthetically competent. We estimated the changes in the steady-state levels of the major cab polypeptide along the length of the wheat leaf by Western blot analysis (6). Total protein was isolated from segments of wheat leaves cut at 2-cm intervals from the base to the apex of the leaves and then analyzed by sodium dodecyl sulfate-gel electrophoresis. Figure 5 shows the protein profile before (left panel) or after (right panel) transfer to nitrocellulose and incubation with monospecific antibodies to the mature cab polypeptide. Almost no mature cab polypeptide is detected in segment 1 of the leaf, but it increases toward the tip, with almost equal levels in the last three leaf segments.

To determine whether a parallel increase in cab RNA occurs along the length of the leaf, total RNA was isolated from a subset of the same leaf segments used in the protein analysis. The RNA samples were analyzed by Northern blots (44), with a *Pst*I-*Sma*I fragment from the whAB1.6 gene as a hybridization probe. The *Pst*I-*Sma*I fragment begins 200 bases before the first ATG and contains almost the entire coding sequence (Fig. 6). cab RNA is not detected in roots (lane R). The different samples of leaf RNA (lanes 1 to 5) contain only one cab RNA species with a size of 1,100 n. The level of cab RNA is lowest in segment 1 of the leaf, highest in segment 2, and declines toward the leaf tip. This pattern contrasts with the accumulation of cab protein that increases apically (Fig. 5). We interpret this to mean that the cab polypeptide is quite stable; once the photosynthetic apparatus is assembled high levels of cab RNA are no longer required.

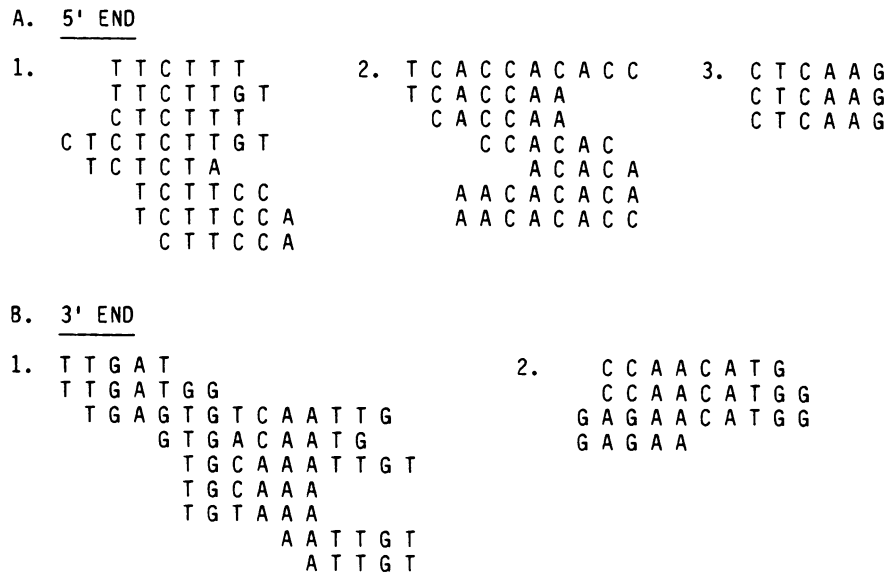

FIG. 3. Repeated sequences flanking whAB1.6. Repeated sequences are listed with the most overlap.

In wheat, as in several other higher plants studied (15, 20), the cab polypeptide is encoded for by a multigene family, as described in the previous section. Therefore, the levels of RNA found by Northern analysis reflect the expression of all of the chromosomal genes and do not discriminate among the genes. To establish whether the wheat gene we sequenced, whAB1.6 (Fig. 1), is expressed in the different leaf segments, a gene-specific probe was used in the S1 nuclease analysis. The pUC13 clone containing the *Pst*I 1.6-kb wheat DNA insert was linearized with *Xho*I, labeled at its 5′ termini, and then restricted with *Pst*I. This liberated a 500-n *Pst*I-*Xho*I fragment containing 360 n of the whAB1.6 gene, labeled only at the *Xho*I 5′ terminus, which was then hybridized with total leaf or root RNA. If the whAB1.6 gene was expressed, a DNA fragment of 360 n was expected after S1 nuclease digestion of the DNA-RNA hybrid. The longest protected fragment is the expected size and is found in RNA isolated from leaf sections 1, 2, and 3 (Fig. 7A). Only a trace of RNA is found in segments 4 and 5, and no evidence of expression of the whAB1.6 gene is found in root RNA (lane R). In addition to the S1-resistant DNA band which corresponds to transcripts from whAB1.6, other bands which are the same as those seen in our earlier S1 analysis are present and are leaf specific. Band c, with a length of 196 n, corresponds to those RNA species which are homologous to whAB1.6, beginning near the start of the sequence coding for the mature cab protein. Band b coincides with the start of the transit peptide sequence. The other bands in the S1 nuclease analysis profile most likely represent hybrids be-

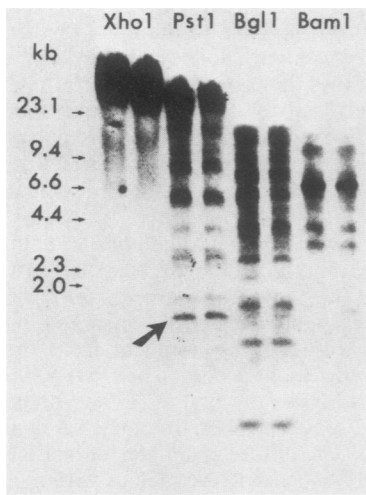

FIG. 4. Southern analysis of total wheat DNA. Wheat DNA was digested with restriction enzymes *Xho*I, *Pst*I, *Bgl*I and BamHI, as indicated. Two enzyme concentrations were used: 2 or 4 U/μg of DNA (left and right). Digestions were for 18 h. The DNA blot was hybridized with a labeled *Pst*I-*Sma*I fragment from whAB1.6. Size markers (lambda *Hin*dIII) are given in kb. The arrow points to the *Pst*I 1.6-kb fragment containing gene whAB1.6 sequenced in Fig. 1.

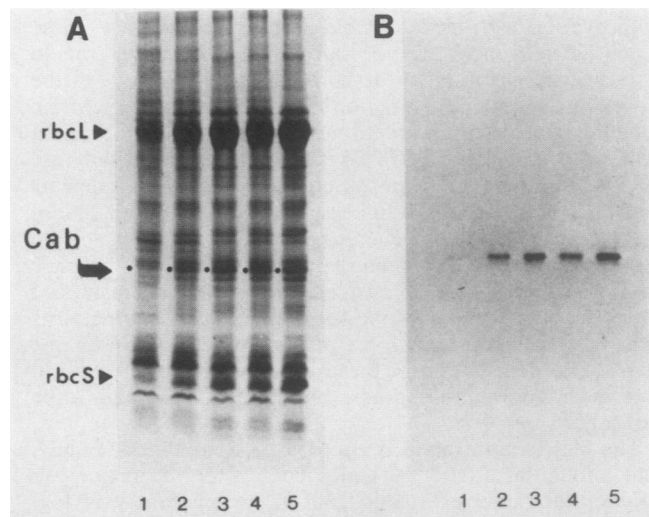

FIG. 5. Western blot of total wheat leaf polypeptides with monospecific antibodies to the apoprotein of the LHC cab protein. (A) Coomassie blue-stained polypeptide profiles from different wheat leaf segments; 50 μg was loaded per lane. (B) Western blot of total wheat leaf polypeptides. Western blot was performed at an antibody concentration of 54 ng/ml and a total protein concentration of 250 ng per lane.

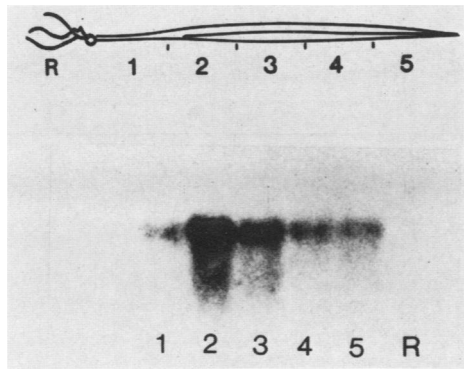

FIG. 6. Wheat leaves from an 8-day-old plant were cut into five segments (1 to 5) beginning from the basal region as depicted in the upper panel. RNAs were prepared from pools of the leaf sections and from roots (R). Northern blot analysis of the RNA samples (10 μg per lane on a 1% agarose gel) was performed with a ^{32}P-labeled *Pst*I-*Sma*I fragment of the wheat cab gene as a probe. Size markers included RNAs for wheat rbcS, yeast 28S and 18S, and *Escherichia coli* 23S and 16S species.

tween the whAB1.6 gene and different RNAs in the cell which show interrupted homologies at different positions. As argued earlier, these bands appear not to be S1 nuclease artifacts, as demonstrated by two observations. First, band a becomes most intense when a subfraction of cab protein RNA is used in the hybridization reactions. Second, none of the bands are found when root RNA is used (Fig. 7). Finally, the intensity of each band changes independently along the cell developmental gradient, i.e., from the base to the apex of the leaf. In particular, the expression of the whAB1.6 gene (band a) is low in sections 4 and 5, but the other bands, indicating the expression of other genes, are present in these segments. In fact, band 3 shows its lowest level at the leaf base, and the level increases toward the leaf apex.

To verify that the highest level of expression of whAB1.6 occurs in the first three segments of the wheat leaf, a fragment which traverses the transcription start site but contains only 47 n of the coding sequence was used in the S1 nuclease analysis. In this experiment, the whAB1.6 transcripts would not be competing with other cab RNAs for the DNA probe. A *Bam*HI-*Bgl*I fragment was 5′ end labeled and then cut with *Pst*I to release a *Pst*I-*Bgl*I fragment labeled at the 5′ *Bgl*I terminus which was hybridized with total RNA. The highest levels of expression of the whAB1.6 gene again are observed in segments 1, 2, and 3 of the leaf (Fig. 7B). Although this autoradiogram is overexposed, little RNA is found in leaf segments 4 and 5. At this time we have not established whether the whAB1.6 gene is actually expressed at its highest level in segment 1, but we can conclude that the whAB1.6 gene is expressed early in the gradient of cell development in a wheat leaf.

DISCUSSION

Ribulose-1,5-bisphosphate carboxylase and the LHC cab protein are the most abundant proteins in leaves, accounting for up to 50% of the soluble protein (21) and 10 to 20% of the membrane proteins (26), respectively. Evidence accumulated thus far supports the notion that these two proteins are rate-limiting factors in leaf photosynthesis (28, 42). The structure of an rbcS gene that is highly expressed in wheat leaves was established previously (10). We have extended our investigation of wheat nuclear genes involved in photosynthesis, and here we report the structure and expression of a wheat genomic clone for the major cab polypeptide of the LHC.

Structural analysis of flanking sequences. Several cDNA clones for the cab polypeptides have been isolated and characterized from two dicotyledon plants, peas (15) and petunias (20), and the n sequence of a pea cab genomic clone has been determined (12). The results reported in this paper on the structure and expression of a wheat cab gene represent the first case among monocotyledon plants. Because of the potential importance of 5′-flanking sequences in gene regulation we compared these regions of the wheat and pea cab genes. The transcripts of both genes have the same starting sequence, ACCAT, although their remaining nontranslated spacers are not conserved (Fig. 8A). At −9, we find that both contain the sequence ATCTT. The TATA boxes of these two genes are different in only one base, TTTAAATA and TATAAATA, as underlined for wheat and peas, respectively. Both plants have a CAAT box sequence at about −72, CCAAC. At the 3′ end of the cab gene there is a TTGTTT sequence 88 bases beyond the stop codon, which has been recognized as a conserved sequence in four petunia cDNA clones for cab polypeptides (20).

Structural analysis of the transit peptide. The complete n sequence of whAB1.6 has allowed us to predict the amino acid sequence of the transit peptide. A comparison of the transit peptides of the major cab polypeptide and the rbcS polypeptide from wheat (10) reveals little sequence relatedness (not shown), although both of the transit peptides begin with MA---M-(-)SS. Furthermore, both contain centrally located basic amino acids: lysine and arginine in the cab and the rbcS transit peptides, respectively, at about 16 amino acids from the junction of the transit and mature coding sequences. Basic amino acids also occur in the mature protein close to the cleavage site.

It previously was proposed that the transit peptide of the precursor facilitates the transport of proteins into the chloroplast (39). The mechanism of targeting a precursor polypeptide to the chloroplast envelope, proteolytic processing, and movement through the membranes to the correct compartment of the organelle have not been elucidated. The lack of homology between the cab and rbcS transit peptides within a species suggests that different pathways, or modes, of transport exist for those proteins which ultimately reside in the two different compartments of the chloroplast, i.e., the thylakoids or stroma.

Of equal importance is a comparison of functionally homologous transit peptides, that is, the transit peptides of the cab polypeptides from two different plants. Figure 8B compares the transit peptides of wheat and peas, representing two divergent higher plant groups, the monocotyledons and dicotyledons, respectively. The wheat transit peptide of the cab polypeptide is three amino acids shorter than that found in peas (12). Despite the length difference, regions of amino acid conservation stand out with potential functional importance. Both the wheat and pea cab transit peptides begin with MAA; a conserved M-LSS occurs within several residues. Two additional conserved features are of primary interest: (i) the positions of the basic amino acids, lysine and arginine, and (ii) the abundance of serines. Basic amino acids appear to be a characteristic of the transit peptides of both the cab polypeptide as shown herein and the rbcS polypeptide. Seven serines occur in the wheat cab transit peptide, equal to 20% of its residues. Only 14, or 6%, of the amino acids of the mature protein are serine, and 4 of these are found immediately beyond the cleavage site. The same is true in the pea polypeptide (12, 15). The large number of

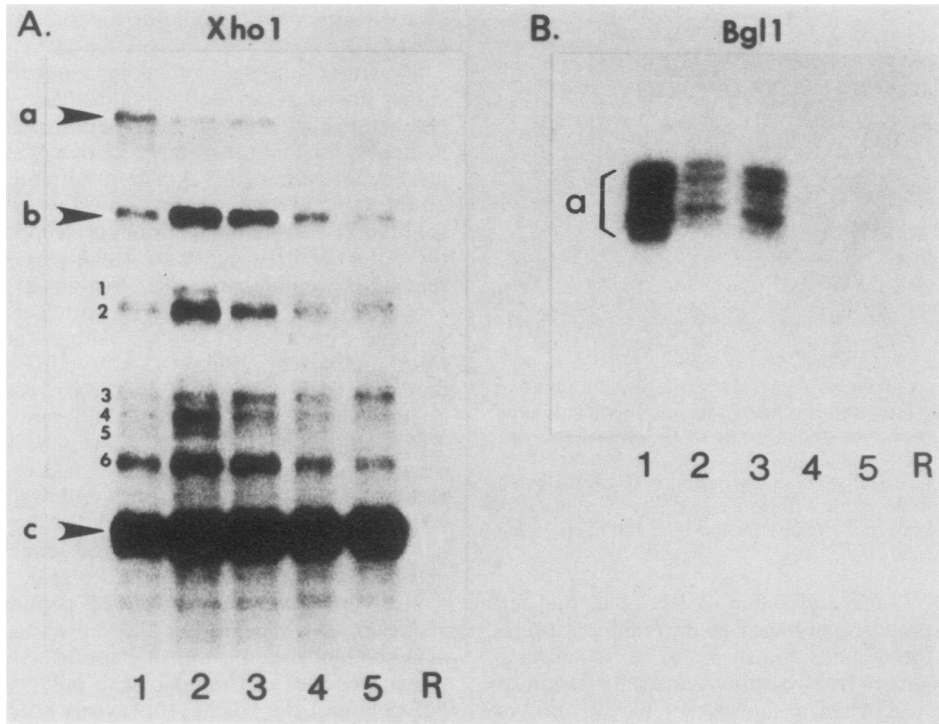

FIG. 7. S1 nuclease analysis of transcripts in different segments of the wheat leaf with a gene-specific probe. (A) A 5′-end-labeled *Pst*I-*Xho*I fragment was hybridized with the same total RNA analyzed by the Northern method (Fig. 6) and then run on a 6% sequencing gel. The numbers below each lane refer to the leaf segments, beginning at the base; R denotes root RNA. The arrows (a, b, and c) point to the three bands described in Fig. 2. The intensity of the longest S1-resistant product, band a, indicates the level of expression of whAB1.6 (Fig. 1). Bands 1 to 6 reflect transcripts with S1-sensitive sites in the transit sequence when compared to the DNA probe as described in the text. (B) A 5′-end-labeled *Pst*I-*Bgl*I fragment was hybridized with total RNA and then analyzed on an 8% sequencing gel. The four bands designated a correspond to the starting 4 n of the transcripts coming from whAB1.6 and equal the single band a in panel A.

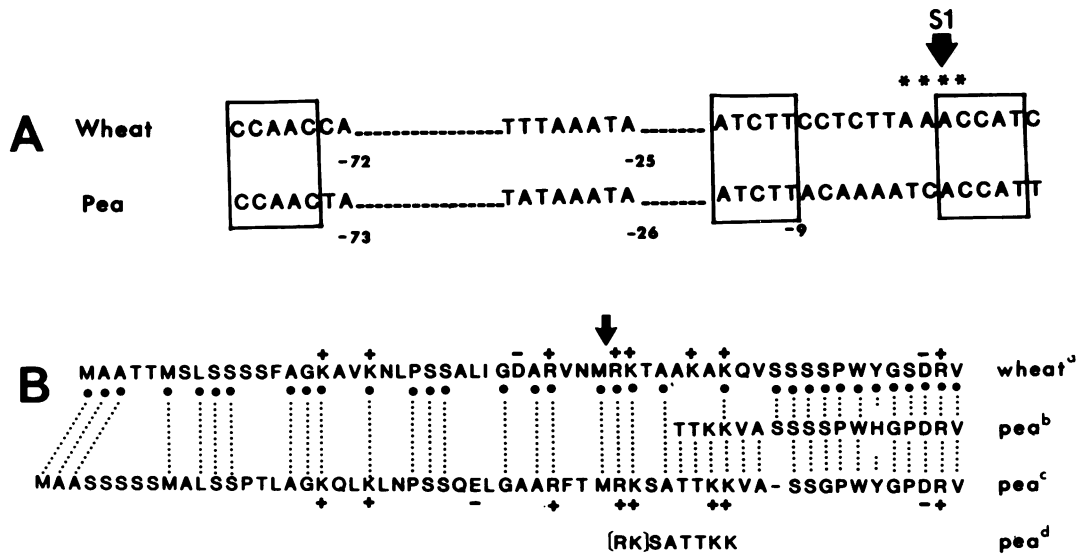

FIG. 8. (A) Comparison of the 5′-flanking sequences of wheat and pea cab genes. The top line gives the sequences flanking the transcription start site of the wheat gene whAB1.6. The bottom line presents sequences upstream of a cab gene from peas sequenced by Cashmore (12). The asterisks show the position of longer transcripts identified by S1 nuclease mapping (Fig. 4). Three conserved regions are blocked, including a CAAT consensus sequence at −72. The TATA consensus sequences also are shown. (B) Comparison of the amino terminus of the cab precursor polypeptide from wheat and peas. Wheat[a] shows the amino acids of the wheat protein, pea[b] shows the sequence of the NH_2 terminus of the mature protein determined from a pea cDNA clone (15), pea[c] shows the sequence deduced from a pea genomic clone (12), and pea[d] shows a peptide released by tryptic digestion of purified cab polypeptide from peas (32). The basic (+) and acidic (−) amino acids are marked.

serines at the NH_2 terminus of the cab precursor polypeptide raises the question of their function. The OH groups of serine and threonine are often modified by phosphorylation (24, 38). In peas, an analysis of the mature cab polypeptide in the thylakoids has shown that threonine, and not serine, is phosphorylated (2, 4, 32). We do not know whether this is also true for the transit peptide. It is striking that at four positions in the NH_2 terminus of the wheat and pea cab precursors, serine and threonine substitute for one another, suggesting the functional interchangeability of these amino acids.

Early synthesis of cab RNA in leaf cell development. Northern analysis indicates a major increase in the steady-state levels of cab mRNA between the first and second segments of the wheat leaf; thereafter a gradual decline in synthesis (or more rapid turnover) occurs as cells mature. The amount of chloroplast rRNA per cell increases dramatically during wheat leaf development (18), and this increase could create an apparent decrease of cab mRNA between segments 3 and 5 due to a dilution of mRNAs in total cell RNA. However, Dean and Leech (18) reported that the increase in chloroplast rRNA as a percentage of total cellular RNA occurs between segments 1 and 2 of the leaf, i.e., within the first 4 cm. Our acridine orange-stained RNA gels confirm their results (not shown). Rather than finding a decrease in cab transcripts in this zone as a consequence of chloroplast rRNA synthesis, the largest increase occurs. In barley the highest levels of translatable poly(A)$^+$ RNA for the cab polypeptides are found in segments 2 and 3 of the leaf (48, 49), which correspond to segment 2 of the wheat leaf as analyzed here. We conclude that there is an increase in the levels of total cab mRNA at the base of the wheat leaf, which is probably due to a burst of RNA synthesis since it is not diluted by the rapid accumulation of chloroplast rRNA. Activation of cab genes appears to accompany the cellular changes required for photosynthesis that occur within this zone. The levels of cab mRNA then decline as cab polypeptide becomes stably incorporated into the thylakoid membranes.

We used a gene-specific probe to refine our analysis of the levels of cab mRNA in the wheat leaf. The results of the S1 nuclease study provide evidence that whAB1.6 transcripts represent only a fraction of poly(A)$^+$ RNA (Fig. 2B and C, band a) and total RNA (Fig. 7, band a). The intensity of band c in Fig. 2 and 7 indicates that either a class of cab genes, heterologous to whAB1.6 near the start of the mature protein sequence, is selectively expressed or its transcripts are more stable. We do not know the nature of the genes represented by band c. One possibility is that they represent a group of genes from one of the three genomes of hexaploid wheat. Although the nucleus of common wheat (*Triticum aestivum*) is comprised of three ancestral genomes, AA, BB, and DD, the cytoplasm originates from only one parent BB, as established by isozyme studies of the large subunit of ribulose-1,5-biphosphate RUBISCO (13) and by chloroplast DNA restriction enzyme digests (47). Perhaps nuclear-cytoplasmic genome compatibility determines the relative expression of nuclear genes in a polyploid species. An alternative, but not exclusive possibility, is that the strength of the promoters of different classes of genes establishes their transcriptional rates. These hypotheses are now testable in wheat with gene-specific probes for either the cab gene described herein or an rbcS gene that was recently characterized (10).

The S1 nuclease study suggests that not all of the cab genes follow the same pattern of expression along the length of the leaf. Band a represents the contribution of transcripts from the whAB1.6 gene; it starts at a high level and falls to almost undetectable levels in segments 4 and 5. Differential expression of a family of actin genes in the genus *Dictyostelium* was investigated earlier by an S1 analysis approach (30). It recently was shown for peas that two S1 products are distinguishable by using an rbcS genomic DNA probe. One of the S1 products corresponded to the transcription initiation site of the cloned gene, and the other corresponded to the start of the transit sequence of rbcS. The relative intensity of these products changed from tissue to tissue, indicating differential expression of at least one rbcS gene in a multigene family (16). For the cab RNAs we detect considerable divergence not only in the 5'-nontranslated region but also in the transit sequence, yielding a particularly complex pattern of S1 products. We wish to emphasize that four of the S1-sensitive sites in the transit peptide occur at codons for either serine or proline, two amino acids that typify the degeneracy of the genetic code, i.e., at these sites six different codons would not affect the transit peptide structure. The changes in pattern of each band along the length of the leaf support our interpretation that the S1 fragments reflect sequence divergence between different cab RNAs and the whAB1.6 gene-specific probe and that these RNAs change in relative abundance during leaf development. Final confirmation of this interpretation awaits the isolation and complete characterization of other cab genes from wheat.

ACKNOWLEDGMENTS

We thank Susan Fiteni Love and Sara Trillo Adams for their excellent technical assistance.

G.L. is a National Institutes of Health postdoctoral fellow, and G.M. is a fellow of the Instituto Pasteur-Fondazione Cenci Bolognetti (University of Rome). This work was supported by Public Health Service grant GM-30726 from the National Institutes of Health and by a grant from Monsanto Co., St. Louis, Mo.

LITERATURE CITED

1. **Apel, K.** 1979. Phytochrome-induced appearance of mRNA activity for the apoprotein of the light-harvesting chlorophyll a/b protein of barley (*Hordeum vulgare*). Eur. J. Biochem. **97**:183–188.
2. **Bennett, J.** 1977. Phosphorylation of chloroplast membrane polypeptides. Nature (London) **269**:344–346.
3. **Bennett, J.** 1983. Regulation of photosynthesis by reversible phosphorylation of the light harvesting chlorophyll a/b protein. Biochem. J. **212**:1–13.
4. **Bennett, J., K. E. Steinbach, and C. J. Arntzen.** 1980. Chloroplast phosphoproteins: regulation of excitation energy transfer by phosphorylation of thylaoid membrane polypeptides. Proc. Natl. Acad. Sci. U.S.A. **77**:5253–5257.
5. **Berk, A. J., and P. A. Sharp.** 1977. Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 nuclease-digested hybrids. Cell **12**:721–732.
6. **Blake, M., K. H. Johnston, G. J. Russell-Jones, and E. C. Gotschlich.** 1984. A rapid, sensitive method for dilution of alkaline phosphatase-conjugated anti-antibody on Western blots. Anal. Biochem. **136**:175–179.
7. **Boffey, S. A., J. R. Ellis, G. Sellden, and R. M. Leech.** 1979. Chloroplast division and DNA synthesis in light-grown wheat leaves. Plant Physiol. **64**:502–505.
8. **Breathnach, R., and P. Chambon.** 1981. Organization and expression of eucaryotic split genes coding for proteins. Annu. Rev. Biochem. **50**:349–384.
9. **Broglie, R., G. Bellemare, S. Bartlett, N.-H. Chua, and A. Cashmore.** 1981. Cloned DNA sequences complementary to mRNAs encoding precursors to the small subunit of ribulose-1,5-bisphosphate carboxylase and a chlorophyll a/b binding

polypeptide. Proc. Natl. Acad. Sci. U.S.A. **78**:7304–7308.
10. **Broglie, R., G. Coruzzi, G. Lamppa, B. Keith, and N.-H. Chua.** 1983. Structural analysis of nuclear genes coding for the precursor to the small subunit of wheat ribulose-1,5-bisphosphate carboxylase. Biotechnology **1**:55–61.
11. **Carmichael, G. G., and G. R. McMaster.** 1980. The analysis of nucleic acids in gels using glyoxyl and acridine orange. Methods Enzymol. **65**:380–391.
12. **Cashmore, A. R.** 1984. Structure and expression of a pea nuclear gene encoding a chlorophyll a/b-binding polypeptide. Proc. Natl. Acad. Sci. U.S.A. **81**:2960–2964.
13. **Chen, K., J. C. Gray, and S. G. Wildman.** 1975. Fraction 1 protein and the origin of polyploid wheats. Science **190**: 1304–1305.
14. **Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Ruter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18**: 5294–5304.
15. **Coruzzi, G., R. Broglie, A. Cashmore, and N.-H. Chua.** 1983. Nucleotide sequence of two pea cDNA clones encoding the small subunit of ribulose-1,5-bisphosphate carboxylase and the major chlorophyll a/b-binding thylakoid polypeptide. J. Biol. Chem. **258**:1399–1402.
16. **Coruzzi, G., R. Broglie, C. Edwards, and N.-H. Chua.** 1984. Tissue-specific and light-regulated expression of a pea nuclear gene encoding the small subunit of ribulose-1,5-bisphosphate carboxylase. EMBO J. **3**:1671–1679.
17. **Cuming, A. C., and J. Bennett.** 1981. Biosynthesis of the light-harvesting chlorophyll a/b protein. Control of messenger RNA activity by light. Eur. J. Biochem. **118**:71–80.
18. **Dean, C., and R. M. Leech.** 1982. Genome expression during normal leaf development. Plant Physiol. **69**:904–910.
19. **Delepelaire, P., and N.-H. Chua.** 1981. Electrophoretic purification of chlorophyll a/b-protein complexes from *Chlamydomonas reinhardtii* and spinach and analysis of their polypeptide composition. J. Biol. Chem. **256**:9300–9307.
20. **Dunsmuir, P., S. M. Smith, and J. Bedbrook.** 1983. The major chlorophyll a/b binding protein of petunia is composed of several polypeptides encoded by a number of distinct nuclear genes. J. Mol. Appl. Genet. **2**:285–300.
21. **Ellis, R. J.** 1981. The most abundant protein in the world. Trends Biol. Sci. **4**:241–244.
22. **Ellis, R. J., A. J. Jellings, and R. M. Leech.** 1983. Nuclear DNA content and the control of chloroplast replication of wheat leaves. Planta **157**:376–380.
23. **Gallagher, T. F., and R. J. Ellis.** 1982. Light-stimulated transcription of genes for two chloroplast polypeptides in isolated pea leaf nuclei. EMBO J. **1**:1493–1498.
24. **Gerken, S. C., and S. M. Arfin.** 1984. Threonyl-tRNA synthetase from Chinese hamster ovary cells is phosphorylated on serine. J. Biol. Chem. **259**:11160–11161.
25. **Glazer, A. N.** 1983. Comparative biochemistry of photosynthetic light-harvesting systems. Annu. Rev. Biochem. **52**: 125–157.
26. **Hiller, R. G., and D. J. Goodchild.** 1981. Thylakoid membrane and pigment organization. Biochem. Plants **8**:1–49.
27. **Lindberg, U., and T. Persson.** 1972. Isolation of mRNA from KB cells by affinity chromatography on polyuridylic acid covalently linked to Sepharose. Eur. J. Biochem. **31**:246–254.
28. **Makino, A., T. Mae, and K. Ohira.** 1983. Photosynthesis and ribulose-1,5-bisphosphate carboxylase in rice leaves. Plant Physiol. **73**:1002–1007.
29. **Maxam, A. M., and W. Gilbert.** 1977. A new method for sequencing DNA. Proc. Natl. Acad. Sci. U.S.A. **74**:560–564.
30. **McKeown, M., and R. A. Firtel.** 1981. Differential expression and 5′ end mapping of actin genes in *Dictyostelium*. Cell **24**:799–807.
31. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101**:20–65.
32. **Mullet, J. E.** 1983. The amino acid sequence of the polypeptide segment which regulates membrane adhesion (grana stacking) in chloroplasts. J. Biol. Chem. **258**:9941–9948.
33. **Nevins, J.** 1983. The pathway of eucaryotic mRNA formation. Annu. Rev. Biochem. **52**:441–466.
34. **Piccioni, R., G. Bellemare, and N.-H. Chua.** 1982. Methods of polyacrylamide gel electrophoresis in the analysis and preparation of plant polypeptides, p. 985–1014. *In* M. Edelman, R. Hallick, and N.-H. Chua (ed.), Methods in chloroplast molecular biology. Elsevier Biomedical Press., Amsterdam.
35. **Reitz, L. P.** 1973. World distribution and importance of wheat, p. 1–18. *In* K. S. Quisenberry and L. P. Reitz (ed.), Wheat and wheat improvement. The American Society of Agronomy, Inc., Madison, Wis.
36. **Rimpau, J., D. Smith, and R. Flavell.** 1978. Sequence organization analysis of the wheat and rye genomes by interspecies DNA/DNA hybridization. J. Mol. Biol. **123**:327–359.
37. **Sanger, F., S. Nicklen, and A. R. Coulsen.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. U.S.A. **74**:5463–5467.
38. **Scheidtmann, K.-H., B. Echle, and G. Walter.** 1982. Simian virus 40 large T antigen is phosphorylated at multiple sites clustered in two separate regions. J. Virol. **44**:116–133.
39. **Schmidt, G. W., S. Bartlett, A. R. Grossman, A. R. Cashmore, and N.-H. Chua.** 1980. *In vitro* synthesis, transport and assembly of the constituent polypeptides of the light-harvesting chlorophyll a/b protein complex, p. 337–351. *In* C. J. Leaver (ed.), Genome organization and expression in plants. Plenum Publishing Co., New York.
40. **Schmidt, G. W., S. G. Bartlett, A. R. Grossman, A. R. Cashmore, and N.-H. Chua.** 1981. Biosynthetic pathways of two polypeptide subunits of the light-harvesting chlorophyll a/b protein complex. J. Cell Biol. **91**:468–478.
41. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
42. **Staehelin, L. A., and C. J. Arntzen.** 1983. Regulation of chloroplast membrane function: protein phosphorylation changes the spatial organization of membrane components. J. Cell Biol. **97**:1327–1337.
43. **Stiekema, W. J., C. F. Wimpee, J. Silverthorne, and E. M. Tobin.** 1983. Phytochrome control of the expression of two nuclear genes encoding chloroplast proteins in *Lemna gibba* G-3. **72**:717–724.
44. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. U.S.A. **77**:5201–5205.
45. **Thompson, W. F., M. Everett, N. O. Polans, R. A. Jorgensen, and J. D. Palmer.** 1983. Phytochrome control of RNA levels in developing pea and mung-bean leaves. Planta **158**:487–500.
46. **Tobin, E. M.** 1981. Phytochrome mediated regulation of messenger RNAS for the small subunit of ribulose 1,5-bisphosphate carboxylase and the light harvesting chlorophyll a/b protein in *Lemna gibba*. Plant Mol. Biol. **1**:35–51.
47. **Tsunewaki, K., and Y. Ogihara.** 1983. The molecular basis of genetic diversity among cytoplasms of *Triticum and Aegilops* species. II. Genetics **104**:155–171.
48. **Viro, M., and K. Kloppstech.** 1980. Differential expression of the genes for ribulose-1,5-bisphosphate carboxylase and light-harvesting chlorophyll a/b protein in the developing barley leaf. Planta **150**:41–45.
49. **Viro, M., and K. Kloppstech.** 1982. Expression of genes for plastid membrane proteins in barley under intermittent light conditions. Planta **154**:18–23.
50. **Viro, M., and K. Kloppstech.** 1983. Gene expression in the developing barley leaf under varying light conditions. Planta **157**:202–208.