PRIMARY STRUCTURE OF A C-HORDEIN GENE FROM BARLEY

by

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The nucleotide sequence of a 2065 base pair *Hind*III fragment, containing a gene (λ hor1-14) belonging to the *Hor1* locus in barley, has been determined. The fragment consists of 1044 bp of coding region interrupted by an amber codon at base 481, a 5' non-coding region of 428 bp and a 3' non-coding region with 593 bp. The deduced amino acid sequence of the mature protein (327 amino acids) is characterized by an octapeptide motif PQQPFPQQ which is repeated throughout the peptide chain between a unique 12 amino acid long NH_2 -terminal and an equally unique 10 amino acid long COOH -terminal end. The proline + glutamine content is 62% and the next three most abundant amino acids are leucine (9%), phenylalanine (8%) and isoleucine (3%). In the 5' non-coding region there is a TATA box at -98 bp from the start methionine. The 3' non-coding region has a polyadenylation signal 76 bp downstream from the TAA stop codon. The deduced amino acid sequences of the NH_2 - and COOH -terminals of λ hor1-14 are very similar but not identical to those known from the Edman degradation and carboxypeptidase Y analysis of C-hordein polypeptides. The 3' coding and non-coding region of λ hor1-14 is closely similar but different in detail to the known C-hordein cDNA clones. One polyadenylation signal is found in λ hor1-14 whereas two are present in each of the three known C-hordein cDNAs. These differences and the amber codon interrupting the open reading frame indicate that this gene is silent.

1. INTRODUCTION

The major seed storage proteins of barley are the alcohol-soluble prolamins, usually termed hordein. The four main groups of hordein, namely the B-, C-, D- and γ -hordeins, account for approximately 50% of the total protein in the mature grain. The B- and C-hordeins are the two major groups and together form 95% of the total hordein fraction. The structural genes encoding the B-, C- and D-hordein polypeptides are located in separate complex loci on chromosome 5,

designated *Hor*-2, *Hor*-1 and *Hor*-3, respectively (3, 24, 44, 47). SDS-PAGE analyses indicate that C-hordein can be separated into 5 polypeptide groups, C1-C5, with an apparent molecular weight range of 45-80 kD. Two-dimensional analyses using IEF and SDS-PAGE resolve the 5 classes into at least 10 component polypeptides thus demonstrating charge polymorphism in each size class (22, 27). Southern blot analysis of barley DNA indicates that the *Hor*-1 gene family is comprised of 3-8 members (39).

Abbreviations: bp = base pair; IEF = iso-electric focusing; kbp = kilo base pair; kD = kilodalton; SDS-PAGE = sodium dodecyl sulphate polyacrylamide gel electrophoresis.

Edman degradation of C-hordein polypeptides indicates some polymorphism in the amino-terminal end of the polypeptide (42, 45, 46). Carboxypeptidase Y digestion and the deduced amino acid sequences of partial C-hordein cDNA clones indicate that C-hordein polypeptides have conserved carboxy-terminals (17, 39, 41). Chymotryptic peptide sequences and the deduced amino acid sequences of partial cDNA clones indicate that C-hordein polypeptides are made up of a repeating octapeptide motif, PQQPFPQQ (17, 39, 50) encoded by a 24 nucleotide repeat having a consensus of CCA CAA CAA CCA TTC CCC CAG CAA.

Hordein polypeptides are synthesized on the endoplasmic reticulum of the endosperm cells and are deposited in protein bodies from 10-30 days after fertilization (5, 6, 7, 10, 11). C-hordein polypeptide synthesis is preferentially enhanced when increasing nitrogen fertilization is applied to the developing endosperm (18), indicating a differential regulation of the hordein gene families.

Recently two different genes encoding B-hordein polypeptides and one encoding a γ -hordein polypeptide have been isolated and sequenced (8, 12, 15). These analyses have provided information about the primary structure of different hordein polypeptides as well as possible 5' upstream regulatory sequences. As the next step towards understanding the organization and tissue-specific expression of different members of the hordein gene families, a C-hordein gene has been isolated and sequenced.

2. MATERIALS AND METHODS

2.1. Chemicals

Restriction endonucleases, T4 DNA ligase, DNA polymerase I (Klenow fragment) and the M13 universal primer were purchased from Boehringer Mannheim (FRG). $\alpha^{32}\text{P}$ dATP was from New England Nuclear (USA). The SequenaseTM kit was from the United States Biochemical Company (USA). Nitrocellulose filters were from Schleicher and Schuell (FRG).

2.2. Construction and screening of a partial HindIII genomic library

Barley DNA was isolated from immature

endosperms of *Hordeum vulgare* L. cv. Bomi according to the method of HOPP et al. (23). The DNA was partially digested with HindIII restriction enzyme and ligated into bacteriophage λ L47.1 DNA (29) and in vitro packaged (21) and plated using *E. coli* BJ5183 rec BC⁻ strain as host (48). The yield of recombinant phage, determined by dilution plating onto a P2 host, was estimated to be 1.8×10^6 . The library was screened using a nick-translated C-hordein cDNA, pc hor1-3, as a probe (39). Hybridizing clones were plaque purified and phage DNA was prepared (30).

2.3. Nucleotide sequencing

A 2 kb HindIII fragment from λ hor1-14 was subcloned into pBR328 (13) using *E. coli* HB101 as host. Large scale preparation of plasmid pBR328 was made according to the method of BIRNBOIM and DOLY (2) and purified by banding in a caesium chloride gradient. Restriction fragments generated from these subclones were separated by agarose gel electrophoresis and inserted into the bacteriophage vectors M13mp18 and mp19 (33) using *E. coli* JM101 as host cells. Sequencing was carried out by the dideoxy chain termination method of SANGER (40), using *E. coli* polymerase I (Klenow fragment) or modified T7 bacteriophage DNA polymerase, as supplied in the SequenaseTM kit. The universal M13 primer was mainly used, except for 3 cases, where specific 15mer oligonucleotides were synthesized on an applied Biosystem 380A DNA synthesizer using the phosphoramidite tri-ester method. The sequence data were assembled and analysed on an IBM computer equipped with the Beckman micro-genie programme.

3. RESULTS

Screening of 1.8×10^6 recombinant phages resulted in the isolation of 80 clones containing sequences related to the C-hordein cDNA probe (39). HindIII restriction enzyme and Southern blotting analyses identified three size classes of hybridizing fragments of 1.9, 2.0 and 4.0 kbp in the 80 clones. The same three size classes of HindIII fragments can be identified when total

Figure 1. (a) Restriction map of the 2065 base pair HindIII fragment containing the C-hordein gene λ hor1-14. The shaded area indicates the coding region. ATG = start methionine, TAG and TAA = stop codons. (b) Restriction map and sequencing strategy used to determine the nucleotide sequence of the 2065 base pair HindIII fragment.

barley DNA is analysed. The 2.0 kbp fragment represents the strongest hybridizing size class (39). One clone, λ hor1-14, was chosen from this size group for further analysis. λ hor1-14 contains 4 HindIII fragments of 7.0, 2.0, 1.5 and 0.5 kbp. The 2.0 kbp hybridizing fragment was cloned into pBR328 and mapped with a variety of restriction enzymes. Figure 1 shows the restriction map of the 2.0 kbp fragment of λ hor1-14 and the strategy used for the nucleotide sequence determination.

3.1. The deduced amino acid sequence

The nucleotide sequence and the deduced amino acid sequence of the coding region of λ hor1-14 is shown in Figure 2. The open reading frame of the coding region is composed of 327 amino acids interrupted by an amber codon at base 481. The recognition of the in-frame amber

codon will produce a truncated polypeptide of only 140 amino acids. The sequence R Q L N P S begins at residue 21 and is identical to the N-terminal sequences determined by Edman degradation of isolated C-hordein polypeptides (42). The preceding 20 amino acids are likely to be a signal peptide which is cleaved after the alanine residue. The amino acid sequence of residues 1-20 are highly hydrophobic and there is a charged lysine residue next to the initiation methionine, both of which are characteristics of signal peptides (20). The occurrence of a signal peptide is also consistent with the observation that C-hordein polypeptides are synthesized as larger sized precursors (7, 32). The first 25 amino acid residues of the deduced NH_2 -terminus of λ hor1-14 are highly homologous with those found in C-hordein polypeptides. However, λ hor1-14 contains two amino acid changes and an insert of six amino acids when compared to the NH_2 -terminal sequence of a C_2 -hordein polypeptide:

1)	RQLNPSSQELQSPQQP.....YLQQPYQPQNXYL
2)	H FLKQQS QP
1)	C2 Amino acid sequence (42)
2)	λ hor1-14 Deduced amino acid sequence

The main body of the deduced amino acid sequence is characterized by a repeating octapeptide with the basic structure of PQQPF-PQQ (cf. Figure 3). The same octapeptide repeat motif is found in the deduced amino acid sequences of two partial cDNA clones, Figure 4 (17, 39). While the deduced amino acid sequence of the λ hor1-14 clone and the cDNA clones pc hor1-3 (39) and pc P387 (17) are similar polymorphisms in the form of insertions, deletions and amino acid substitutions are present.

In the longer reading frame the repeat motif does not extend to the COOH-terminus as the last 10 amino acids are unique. The deduced COOH-terminal region of λ hor1-14 is different from those found by carboxypeptidase Y analysis of C-hordein polypeptides or by deducing the amino acid sequences of three C-hordein cDNA clones. The most frequently occurring amino acids are glutamine (122 residues) and proline (95 residues) which together account for 62% of the total. The next 3 most abundant amino acids

Figure 2. Nucleotide sequence and deduced amino acid sequence of the gene λ hor1-14. Horizontal arrows indicate the '-300' region, direct and inverted repeats. The vertical arrow after position 60 indicates the most likely cleavage point of the signal peptide. The TATA box (TATAAATA), stop codons (TAG and TAA) and the polyadenylation signal are boxed.

J. ENTWISTLE: C-hordein gene

550 560 570 580 590 600
 CAACAACCAATTGGACAGCAACCAACCAACCACTCCTGCAGCAACCACAACAACAATT
 GlnGlnProIleGlyGlnGlnProLysGlnProLeuLeuGlnGlnProGlnGlnThrIle

610 620 630 640 650 660
 CCCCAGCAACCACAACAACCAATTCCCCCTGCAGCCGCAACAACCAATTCCCCCAACAACCA
 ProGlnGlnProGlnGlnProPheProLeuGlnProGlnGlnProPheProGlnGlnPro

670 680 690 700 710 720
 CAACAACCACTTCCCCAACAACCCCAACAAATAATTTCCCAGCAACCCCAACAACCAATTC
 GlnGlnProLeuProGlnGlnProGlnGlnIleIleSerGlnGlnProGlnGlnProPhe

730 740 750 760 770 780
 CCTCTACAACCTCAACAACCAATTCCCCCAACCCCAACCAATTCCCCGAGGAGCAACCCCAA
 ProLeuGlnProGlnGlnProPheProGlnProGlnProPheProGlnGluGlnProGln

790 800 810 820 830 840
 CAAGCATTCCCCTACAACCGCAACAACCAATTCCCCGAGGAATCAGAACAATAATTACC
 GlnAlaPheProLeuGlnProGlnGlnProPheProGluGluSerGluGlnIleIleThr

850 860 870 880 890 900
 CAACAACCACTCCCTCTACAACCACAACAACCTGTTCCCCAGCAACCACAACAACCACTT
 GlnGlnProPheProLeuGlnProGlnGlnLeuPheProGlnGlnProGlnGlnProLeu

910 920 930 940 950 960
 CCCCAGCCCCAACAACCAATTCCGCCAACTACCAAAATATATAATTCCCCAGCAACCTCAA
 ProGlnProGlnGlnProPheArgGlnLeuProLysTyrIleIleProGlnGlnProGln

970 980 990 1000 1010 1020
 CAACCATTCTTCTGCAACCACCAACCTCAGCAACCTTATGCACAACAAGACATCTGG
 GlnProPheLeuLeuGlnProHisGlnProGlnGlnProTyrAlaGlnGlnAspIleTrp

1030 1040 1050 1060 1070 1080
 AGTGATATAGCCCTCTTGGGCTAATGAAGCGAAAAGTTGTAATACTAGATGGGTGTATCA
 SerAspIleAlaLeuLeuGlyEnd

1090 1100 1110 1120 1130 1140
 TCGTTGTTTACTCAGTGAGTGTTCCAGTGATTGATCATGAATAAAGTGATGTGCCACCAT

1150 1160 1170 1180 1190 1200
 CATGTGTAATCCCGACCTATACTAGTTCAAGCATGAGAAAAGGACAAGAAGTTGTCACAA

1210 1220 1230 1240 1250 1260
 GGACAATGATGGTAATTTTTTACCATTGATGCCATATTTAGATTTTTTCCCTCAACTATA

1270 1280 1290 1300 1310 1320
 ATTTGAGTCATATGCATTATCTACCTATTTATGAAGTGAAGCCCTATGAGCCAAACATTA

1330 1340 1350 1360 1370 1380
 TTAATGGTTGTCAAACCTTGGTTGAATTTGATTATTAGCAAATAAGGAGTATGAAGTAAT

1390 1400 1410 1420 1430 1440
 TGCAAATTATCTCTCGGTGGCTAGTCGTATGAACTGTGTGACTTGCAAGTTATTCAAAT

1450 1460 1470 1480 1490 1500
 GGTTCCTTTAATGGACCGGTGCTCATGTTAGAAAATATGGGCTTGGCCCGTATTGATTT

1510 1520 1530 1540 1550 1560
 CTTGCAAATGGACAATATGAAGGGTAGATTGAATTTATTCCTTCTATTTCTCCTCCACAT

1570 1580 1590 1600 1610 1620
 GTATTACGAGTTACTTAGAGAAGTTCACCAGACTGATATGGTCCAATTGGTATAGCTAC

1630
 CCAATGCTCGGAAGCTT

HKTFLTFVLLAMAMSI VTTA	signal
RQLNPSHQELQS	amino-terminus
PQQPFLKQ	CCA CAA CAA CCA TTT CTG AAA CAA
..QSYLQQ	. . CAA TCA TAT CTG CAA CAA
...PYPQQ	. . . CCA TAT CCA CAA CAA
...PYL..	. . . CCA TAT CTA . .
PQQPFPT.	CCG CAG CAA CCA TTC CCC ACA .
PQQFFPYL	CCC CAA CAA TTT TTC CCC TAT CTA
PQQTFF.P	CCA CAG CAA ACA TTT CCC . CCA
SQQPNPLQ	TCC CAA CAA CCA AAC CCC CTA CAA
PQQPFPLQ	CCA CAA CAA CCA TTC CCC CTG CAA
PQ.P....	CCC CAA . CCA . . .
PQQPFPPQ.	CCA CAA CAA CCT TTT CCT CAG .
PQQPNPQQ	CCC CAA CAA CCA AAT CCC CAG CAA
PQQPFPRQ	CCA CAA CAA CCT TTC CCC CGG CAA
PQQIVPQQ	CCA CAA CAA ATA GTA CCC CAG CAA
PQQPFPPQ	CCA CAA CAA CCA TTC CCT CAG CAA
PQQPFPPQ.	CCA CAA CAA CCT TTT CCT CAG .
PQQPFWSQ	CCC CAA CAA CCA TTC TCT TGG CAA
PQQPFLQ.	CCA CAA CAA CCA TTT CTC CAG .
PLQL+PLQ	CCC CTA CAA CTA TAG CCC CTG CAA
AQQPFPLQ	GCA CAA CAA CCA TTC CCC TTG CAA
PQLFFP.Q	CCT CAA CTA CCA TTT CCG . CAA
PQQPIGQQ	CCC CAA CAA CCA ATT GGA CAG CAA
PKQPLLQ	CCA AAA CAA CCA CTC CTG CAG CAA
PQQTIPQQ	CCA CAA CAA ACA ATT CCC CAG CAA
PQQPFPLQ	CCA CAA CAA CCA TTC CCC CTG CAG
PQQPFPPQ	CCG CAA CAA CCA TTC CCC CAA CAA
PQQPLPQQ	CCA CAA CAA CCA CTT CCC CAA CAA
PQQIISQQ	CCC CAA CAA ATA ATT TCC CAG CAA
PQQPFPLQ	CCC CAA CAA CCA TTC CCT CTA CAA
PQQPFP.Q	CCT CAA CAA CCA TTC CCC . CAA
P.QPFPQ.	CCC . CAA CCA TTC CCC CAG .
.....EQ	 GAG CAA
PQQAFFPLQ	CCC CAA CAA GCA TTC CCC CTA CAA
PQQPFPEE	CCG CAA CAA CCA TTC CCC GAG GAA
SEIITQQ	TCA GAA CAA ATA ATT ACC CAA CAA
...PFPLQ	. . . CCA TTC CCT CTA CAA
PQQLFPQQ	CCA CAA CAA CTG TTC CCC CAG CAA
PQQPLPQ.	CCA CAA CAA CCA CTT CCC CAG .
PQQPFRQL	CCC CAA CAA CCA TTC CCG CAA CTA
PKYIIPQQ	CCA AAA TAT ATA ATT CCC CAG CAA
PQQPFLQ	CCT CAA CAA CCA TTC CTT CTG CAA
...P..HQ	. . . CCA . . CAC CAA
PQQPYAQQ	CCT CAG CAA CCT TAT GCA CAA CAA
DIWSDIALLG	carboxy-terminus

Figure 3. Repetitive motif of 24 nucleotides in the coding region of λ hor1-14 and the deduced octapeptides. Shading highlights the proline and glutamine codons. The amino- and carboxy-terminal regions are unique. The TAG stop codon interrupting the open-reading frame is boxed.

λ hor1-14	pc P387	pc hor1-3
PQQPFP.Q	PQSYVPVQ	
P.QPFPQ.	PQQPFPQ.	
.....EQ	PQ.PVPQQ	
PQQAFLQ	PQASPLQ	
PQQPFPEE	PQQPFPQG	FPQ.
SEQIITQQ	SEQII...	PQQPFPQQ
...PFPLQ	PQQPFPPLQ	PQQPFPPLQ
PQQLFPQQ	PQ.PFPQQ	PQQPFPQQ
PQQPLPQ.	PQQPLPQ.	PQQPFPQ.
PQQPFRQL	PQQPFRQQ	PQQPFRQQ
PKYIIPQQ	AELIIPQQ	AELIIPQQ
PQQPFLLO	PQQPLPLQ	PQQPFPLO

Figure 4. A comparison of the deduced amino acid sequence from λ hor1-14 and the two C-hordein cDNA clones, pc P387 and pc hor1-3. The alignment was made amino terminal to the leucine and glutamine residues encoded by the PstI site, CTGCAG, in the two cDNA clones.

are leucine (8.9%), phenylalanine (7.8%) and isoleucine (3.4%). In agreement with the amino acid analysis of mature C-hordein polypeptides there are no cysteine residues. There is a good agreement between the amino acid composition of the deduced sequence of λ hor1-14 and that of purified C2-hordein polypeptides, with the

exception of lysine. λ hor1-14 has an overall content of 1.1% mole of lysine whereas the C2 polypeptide was determined to have a lysine content of only 0.2% (41). There are 4 lysine residues in λ hor1-14, one being in the signal peptide. The codon usage is not biased except in the case of glutamine in which CAA is used 103 times whereas CAG is only used 19 times.

3.2. Nucleotide analysis of the coding region

With the exception of the regions encoding the signal peptide, the first 12 amino acids of the N-terminus and the last 10 amino acids of the C-terminus a tandemly repeated nucleotide sequence dominates the *λhor1-14* gene (Figure 3) in the longer open reading frame. The 24 nucleotide motif of the repeating sequence has the following consensus:

Frequently, the repeats are degenerate as a result of nucleotide substitutions, deletions and insertions as shown in Figure 3. The TAG stop codon interrupting the open-reading frame, is located in a 5th codon position of the respective element and, therefore, probably originated by two point mutations from a phenylalanine codon (TTT).

An alignment of λ hor1-14 with the cDNA

A CTGCAACCCACCAACCTCAGCAACCTTATGCACAACAAGAC.ATCTGGAGT..GATATAGCCCTC.T.TGGG..CTAATGA
B CTGCAGCCACACCAACCA.....TATACACAACAA.ACCATCTGGAGTATGGTCTAG...TCATCAGGGGCGCTA.TGA

A AGCGAAAAGTTGTAATACTAGATGGGTGTATCATCGTTGTTTAGTCAG.TGAGTGTTCAGTGTTATGATCATGAATAAAG
B AGCGACAAGTTGTAATACTAAATGGGTGGATCATCATCTTTTAGTCAACGGAGTGTTAA.TGTAATGATGATCAATAAAG

A TGATGTGCACCATCATGTGTAATCCCGACCTATACTAGTTCAAGCATGAG...AAAAGGA.CA.AGA.AG...TTGTCACAA
B TGATGTGCACCATCATGTGTAACCCCGCCTATACTAGTTCAA..ATGAGATAAAA.GACCAAAGAAAGTTCCTGTG(A_n)

Figure 5. Alignment of λ hor1-14 (A) and the C-hordein cDNA clone, pc hor1-3 (B) in the carboxy-terminal and 3' non-coding regions. Gaps introduced for maximum alignment are indicated by dots. Asterisks indicate nucleotide differences between the two sequences. Stop codons are underlined and polyadenylation signals are boxed.

clone pc hor1-3 (39) shows close similarity but also microvariation in the form of insertions, deletions and point mutations (Figure 5). The PstI site, CTGCAG, present in the three previously studied cDNA clones (17, 39) is not found in the gene λ hor1-14, because the glutamine codon CAA is used instead of CAG, in the position where a PstI site would be expected. The stop codon TAG is used in the cDNA clones whereas the genomic clone has a TAA stop codon 12 nucleotides further downstream. There is one polyadenylation signal in the 3' non-coding region of λ hor1-14 whereas the C-hordein cDNA clones contain two polyadenylation signals. The alignment of the λ hor1-14 and pc hor1-3 sequences reveals a three nucleotide deletion in λ hor1-14 whereby the second polyadenylation signal has been eliminated (Figure 5).

3.3. The 5' non-coding region of λ hor1-14

Coordinate induction of genes by *cis* interaction with a common regulatory molecule requires that homologous sequences are present in the vicinity of the genes. Shared sequence elements have been found in genes that are functionally related and that are induced in concert (14). Sequences in the 5' non-coding region are thus expected to control tissue specific and temporal gene expression of hordein genes. Some of the regulatory sequences may be specific for a Hor locus while others may be shared by members of different Hor loci. For a comparative analysis the 5' non-coding region of the C-hordein gene λ hor1-14 was aligned with that of the B-hordein gene λ hor2-4 (8), a γ -hordein gene (12) and the α/β -gliadin gene pW8233 (37) from wheat. Although the α/β -gliadins are similar to B-hordeins in terms of size and amino acid composition they are considered to represent two of the most divergent groups of S-rich prolamins (27) and it was thus considered of interest to include the wheat gene in this comparison. The alignment of the four sequences was made partly by computer and partly by eye, introducing gaps where appropriate. The most highly conserved regions of the four genes are shown in Figure 6.

Region A is the '-300 region' which has been

identified in most prolamins genes and is thought to act as a transcriptional enhancer. In λ hor1-14 the '-300' region is located between -294 and -324 base pairs from the start methionine (cf. Figure 2). This sequence contains a portion which is homologous to the core sequence of the SV40 enhancer (53), namely TGTAAGT which is seen in all four genes. A closely related 8 base pair sequence with this motif in the form GTAAAGTA is present at position -206, in the λ hor1-14 gene. The 8 adenine nucleotides immediately downstream of the enhancer sequence at -310 in λ hor1-14 are replaced in the B- and γ -hordeins and the α/β -gliadin gene by the nucleotide sequence ^{GAATAAGA}_G. Regions B and C may represent CAAT boxes and region D is a typical TATA box which may act as a transcriptional promotor (25, 43). The TATA box in λ hor1-14 is located at -98 base pairs from the start methionine. It is noteworthy that there is an 8 base pair inverted repeat bracketing the TATA box (Figure 2). Analysis of the nopaline synthase promoter has shown that maximum efficiency of transcription requires the interaction between the binding capacities of both the CAAT and TATA box regions (43). Nuclease S1 protection of messenger RNA start points with probes from the α/β -gliadin gene pW8233 (38) and the γ -hordein gene (12) has identified the 5' end of region E, indicated by an arrow in Figure 6, as the transcriptional start of the two genes. On the basis of sequence similarity the transcriptional starting points for the B-hordein and C-hordein are also considered to be located at the 5' end of region E. The numbers of nucleotides separating the conserved regions of the C-hordein, γ -hordein and α/β -gliadin genes are very similar. However, the B-hordein gene has three times as many nucleotides spacing region B from C and only half as many nucleotides between regions C and D as compared to the other three genes.

The significance of the similarities and differences between the 5' non-coding regions among the four genes, in terms of tissue specific and temporal expression, remains to be determined. It has recently been shown that 549 bp of 5' non-coding region of a B1 hordein gene are sufficient to direct the tissue specific and temporal expression of the chloramphenicol acetyl transferase gene in tobacco (31). However, func-

Figure 6. Diagram showing the conserved sequences in the 5' non-coding regions of a B-hordein gene (λ hor2-4), a γ -hordein gene, an α/β -gliadin gene (pW8233) and in the C-hordein gene λ hor1-14. The alignment was made partly by computer and partly manually. The percentage of homology between the conserved regions of the three genes with the corresponding regions in the C-hordein gene are indicated by the figures in the boxed areas. Dotted lines indicate where gaps have been introduced to give maximum sequence alignment. The number of nucleotides separating the conserved regions in the 4 genes are indicated.

tional mapping of the 5' non-coding region will be necessary to demonstrate the precise role of the highly conserved regions.

4. DISCUSSION

λ hor1-14 is the first C-hordein gene to be isolated and sequenced. The gene presented in this paper is identified as a member of the Hor-1

locus by the following criteria:

The gene encodes a polypeptide with a deduced amino acid sequence which has about 70% sequence identity to the NH_2 -terminal sequences of C-hordein polypeptides as determined by Edman degradation. The octapeptide repeat motif, apparent in the deduced amino acid sequence of λ hor1-14 has been determined

in chymotryptic peptides of C-hordein as well as in the deduced amino acid sequences of C-hordein cDNA clones. Finally, λ hor1-14 has a 5' non-coding region with sequences which are highly conserved in genes encoding other prolamins storage proteins.

The coding region of 1044 base pairs is interrupted by a TAG stop codon at base 481. If the amber codon is suppressed then the gene encodes a mature polypeptide of 327 amino acids, however, if the stop codon is functional then a mature truncated polypeptide of only 140 amino acids is translatable. The in-frame amber codon located at base 481 in the open reading frame is either due to a double mutation in a phenylalanine codon TTT or the result of a substantial insertion or deletion of nucleotides in this area. Since the motif has not been disrupted the latter is unlikely. Although introns have not been identified in prolamin genes the amber codon and surrounding area may be a small intron. However, typical intron/exon splice sites have not been identified in λ hor1-14 (9). Whether the gene, λ hor1-14 is expressed in the developing endosperm or silent remains to be determined.

In frame stop codons interrupting open reading frames of several other animal and plant genes belonging to multi-gene families have been reported. Examples of these are genes of the human α/β -globin gene families (35, 36), human leukocyte interferon genes (51, 54), a γ -gliadin gene from wheat (38), a high molecular weight glutenin gene from wheat (16), a legumin gene from pea (4) and three zein genes from maize (28, 49, 52). Two of the zein genes have one stop codon and one gene has four internal stop codons in an otherwise normal coding region.

Non expressed genes reported in the literature show either an absence of regulatory upstream and downstream sequences or the presence of mutations/deletions in these sequences. The '300 region' of a silent HMW glutenin is only partially present (16) and mutated polyadenylation signals have been reported in silent zein genes (49).

A comparison between the upstream regions of two γ -gliadin genes, one with two premature stop codons and one without a premature stop

codon, showed no appreciable differences in the 5' flanking regions. However, this does not preclude the possibility of differences between the two genes further upstream.

In some cases non-typical intervening sequences in the coding region or the occurrence of poly A tracts in the 3' downstream region caused by the incorporation of reverse transcribed mRNA has resulted in pseudogenes which show almost no similarity to their gene family counterparts (35, 54). In the case of λ hor1-14 the amber codon interrupts an otherwise normal coding region which does not contain any intervening or atypical sequences. The 5' non-coding region is highly similar to those of the genes encoding B- and γ -hordein polypeptides.

It is assumed that genes containing stop codons interrupting the coding regions are not expressed. Transcripts containing in-frame stop codons have been reported for a human leukocyte interferon gene, a class I major histocompatibility gene from rat and an SSU of ribulose biphosphate carboxylase gene from tobacco (19, 26, 34). Transcript levels, however, are low and subsequent translation into a protein has not been demonstrated. Exceptions may be some zein genes which produce truncated polypeptides (49, 52).

Suppression of an amber codon in the tobacco mosaic virus by a cytoplasmic tRNA^{try} isolated from lupin seeds produces a read through protein of 183 kD in *Xenopus* oocytes (1). Although the expression of λ hor1-14 is open to question, the coding sequence is assumed to be similar to that of expressed C-hordein genes. This is substantiated by the high degree of homology between the coding region and 3' non-coding region of λ hor1-14 and the C-hordein cDNA clones which have been analysed.

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