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Molecular analysis of γ-gliadin gene families at the complex *Gli-1* locus of bread wheat (*T. aestivum* L.)

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Summary. A cDNA clone (pTag1436) carrying a complete coding sequence for a γ-gliadin polypeptide has been identified and sequenced. By hybridisation to size fractionated poly A^+ RNA from wheat nullisomic-tetrasomic lines, homologous transcripts from the *Gli-A1*, *Gli-B1* and *Gli-D1* loci were identified. These mRNAs differed from those complementary to a low molecular weight (LMW) glutenin cDNA clone. Hybridization of pTag1436 to digested wheat DNA produced a pattern of fragments unrelated to that obtained using a LMW glutenin cDNA probe. These results indicate that the γ-gliadin and LMW glutenin families, although both located at the *Gli-1* loci, are distinct by hybridisation.

Key words: Gliadins – Glutenins – DNA sequence – Genes – *Gli-1* locus

Introduction

The major endosperm proteins of wheat have been classified by genetic analysis into the products of nine complex gene loci, three in each of the diploid genomes of hexaploid wheat.

These are *Glu-A1*, *Glu-B1*, and *Glu-D1*, on the long arm of group 1 chromosomes, coding for the high molecular weight (HMW) subunits of glutenin, *Gli-A2*, *Gli-B2*, and *Gli-D2*, on the short arm of group 6 chromosomes, coding for α+β gliadins ('α-type' of N-terminal; Kasarda et al. 1984) and *Gli-A1*, *Gli-B1*, and *Gli-D1*, on the short arm of group 1 chromosomes, coding for ω-gliadins, γ-gliadins and LMW glutenins. The γ-gliadins, coded for by *Gli-1*, have N-terminal amino acid sequences corresponding to the γ-2 and γ-3 gliadin components sequenced by Bietz et al. (1977) and Kasarda et al. (1984). LMW glutenins have a distinct N-terminal amino acid sequence, the 'aggregated gliadin' type (Bietz et al. 1977; Shewry et al. 1981), and the ω-gliadins have a third distinct N-terminal amino acid sequence type (Kasarda et al. 1984). Sequence comparisons between these groups of proteins, however, reveal low-level homologies which suggest that they have arisen via duplication and divergence of a common ancestral gene (Kasarda et al. 1984; Harberd et al. 1985). LMW glutenins and γ-type gliadin polypeptides, which are of similar molecular weights, have recently been resolved into two separate groups each comprising about ten polypeptides using 2-Dimensional (2-D) gel systems (Jackson et al. 1983). When this technique is used to compare polypeptides encoded by allelic variants at *Gli-1*, the results indicate that the two groups of polypeptides are the products of separate genes.

In this paper we present the complete coding sequence of a γ-gliadin polypeptide as deduced from a cDNA clone. The sequence is very similar to the incomplete sequence reported by Scheets et al. (1985) but with the addition of a C-terminal region which distinguishes it from all gliadin or other prolamin sequences so far reported, and establishes LMW glutenin and γ-gliadin genes as two distinct sequence types. Under conditions of moderate stringency γ-gliadin and LMW glutenin cDNA probes are shown to hybridise to different mRNA species on Northern filters and to different DNA fragments in digests of size-fractionated wheat DNA. Further classification within the γ-gliadin gene family has been attempted using non-repeating 5′ and 3′ segments of the gene as probes, and by using as probes DNA fragments derived from γ-gliadin-related genomic clones.

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Materials and methods

Plant material

RNA and DNA used in these experiments was isolated exclusively from the wheat variety 'Chinese Spring' or from nullisomic-tetrasomic lines derived from that variety. These lines were generously provided by Dr. C. N. Law and colleagues at the Plant Breeding Institute, Cambridge.

cDNA clone construction

Methods followed for synthesis and cloning of cDNA were as described in Bartels and Thompson (1983), with the addition of RNasin (Biotec, Madison, WI, USA) at 1 unit per µl in the cDNA synthesis reaction.

DNA sequencing

The DNA sequence of the cDNA clone was determined by forced cloning of restriction enzyme digest fragments into M13mp18 and 19 (Messing and Vieira 1983) followed by dideoxynucleotide sequencing according to Sanger et al. (1980). In order to complete the DNA sequence, three fragments were sequenced using the chemical degradation procedure of Maxam and Gilbert (1977) to degrade DNA fragments end-labelled using γ-^{32}P ATP and polynucleotide kinase.

Analysis of protein products

Methods for poly A^+ RNA preparations and cDNA cloning are as described in Bartels and Thompson (1983). Conditions for in vitro translation, using reticulocyte lysates, gel electrophoresis and fluorographic analysis of translation products are described in Thompson et al. (1983). Hybrid release translation was performed as described in Forde et al. (1981) with the modifications described in Thompson et al. (1983).

Size fractionation of RNA and hybridisation

'Northern' analysis: 1 µg of poly A^+ RNA was separated on a 1.5% agarose gel containing 2.2 M formaldehyde (Lehrach et al. 1977) using 40 mM MOPS (morpholinopropanesulfonic acid) pH 7.0, 10 mM sodium acetate and 1 mM EDTA (pH 8.0) (1 × MOPS buffer), as gel and electrophoresis running buffer (Maniatis et al. 1982). The RNA was dissolved in 5 µl H_2O and 15 µl loading buffer (0.2 ml 5 × MOPS buffer, 0.35 ml formaldehyde, 1 ml formamide and 5 mg of Bromophenol blue) were added. The sample was heated for 5 min at 65 °C, cooled on ice and immediately loaded on the gel. The gels were run at 7.5 V cm^{-1} for 4–5 h. Immediately after the run the gels were transferred to nitrocellulose filters by blotting with 20 × SSC. After a 14 h transfer the filters were baked in vacuo at 80 °C, and then prehybridised in 50% formamide, 5 × SSC or 1 × SSC, 10 mM Pipes (pH 6.8), 0.1% SDS, 0.02% BSA, Ficoll and PVP (polyvinylpyrrolidone) at 50 °C for 2 h. The prehybridisation buffer was replaced by a fresh sample of the same buffer containing the nick-translated probe. Conditions for hybridisation and washing were as described in the figure legends.

DNA filter hybridisations

Hybridisation of nick-translated inserts of cDNA clones to size-fractionated wheat DNA digests was performed as described in Thompson et al. (1983) and Harberd et al. (1985).

Results

DNA sequence

A cDNA clone pTag1436 with an insert size of 1,350 bases was isolated from a cDNA library made to endosperm poly A^+ RNA from the wheat variety 'Chinese Spring', using conditions as described in Bartels and Thompson (1983). pTag1436 hybridised strongly to wheat endosperm poly A^+ RNA and also to cDNA clones encoding γ-gliadin-type sequences (Harberd et al. 1985) but only weakly to a cDNA clone (pTag53) encoding an α/β gliadin (Harberd et al. 1985). The cloned cDNA insert was subcloned and sequenced. Figure 1 shows the DNA sequence and a restriction enzyme site map. During the course of sequencing, an 83 bp non-contiguous duplication was detected preceding the 5′ end of the reverse transcript, which has been omitted from Fig. 1. Similar artefacts have been noted by others (Forde et al. 1985; Shyam et al. 1985).

The sequence possesses the characteristic features of a prolamin polypeptide. These are divided into segments A to F in Fig. 1 b. The mature amino terminus is preceded by a 19 residue leader sequence. The amino terminal region and the block of internal repeats show a high degree of homology to those of the gene sequenced by Scheets et al. (1985) and match the γ-type N terminal sequence (Kasarda et al. 1984). Comparison of the coding sequence with that of a LMW glutenin (Okita et al. 1985) (aggregated gliadin) and with an α-gliadin (Rafalski et al. 1984) shows clearly the presence of the sequence blocks designated A, B and C (Kreis et al. 1985) and possessed by many other storage proteins. The A block resides between bases 493–582, B between bases 637–744 and C between bases 862–906. (NB. These do not coincide with A–C in Fig. 1 b.) The carboxyl terminal sequence is not closely related to that found in other prolamins but most resembles that determined for a γ-secalin (Kreis et al. 1985). In the 3′ untranslated sequence two possible polyadenylation sequences have been noted. Presumably the second one was utilised in the synthesis of the mRNA template from which this clone was derived.

The predicted coding sequence specifies a mature polypeptide of M_r 31,629, with an amino acid composition consistent with that determined for γ-gliadins (Bietz et al. 1977). This is slightly smaller than the usual estimates for γ-gliadins (35–45 kD) and may reflect the retardation of apparent mobility observed for prolamin polypeptides. This also suggests that the clone was derived from an mRNA coding for the smallest size class of γ-gliadin polypeptide.

DIAGON homology plots (Staden 1982) with other γ-gliadin sequences (Fig. 1 c) indicate that pTAG1436 is very nearly identical to pw10 of Scheets et al. (1985)

A

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                                                                  M  K  T  L
CCAGACCATCATCCATCCTTCACACAACTAGAGCACAAGCATCAAATCCAAGTACGTAGTAGTTAACGCAAATCCACTATGAAGACCTTA
      10        20        30        40        50        60        70        80        90

 L  I  L  T  I  L  A  M  A  I  T  I  G  T  A  N  M  Q  V  D  P  S  S  Q  V  Q  W  P  Q  Q
CTCATCCTAACAATCCTTGCGATGGCAATAACCATCGGCACCGCCAATATGCAGGTCGACCCTAGCAGCCAAGTACAATGGCCACAACAA
      100       110       120       130       140       150       160       170       180

 Q  P  V  P  Q  P  H  Q  P  F  S  Q  Q  P  Q  Q  T  F  P  Q  P  Q  Q  T  F  P  H  Q  P  Q
CAACCAGTCCCACAGCCTCACCAACCATTCTCCCAGCAACCACAACAAACATTTCCCCAACCCCAACAAACATTCCCCCATCAACCACAA
      190       200       210       220       230       240       250       260       270

 Q  Q  F  P  Q  P  Q  Q  P  Q  Q  Q  F  L  Q  P  Q  Q  P  F  P  Q  Q  P  Q  Q  P  Y  P  Q
CAACAATTTCCCCAGCCTCAGCAACCACAACAACAATTTCTCCAGCCCCAACAACCATTCCCCCAACAACCACAACAACCATATCCCCAG
      280       290       300       310       320       330       340       350       360

 Q  P  Q  Q  P  F  P  Q  T  Q  Q  P  Q  Q  L  F  P  Q  S  Q  Q  P  Q  Q  Q  F  S  Q  P  Q
CAACCACAACAACCATTCCCCCAGACTCAACAACCCCAACAACTATTTCCCCAGTCCCAGCAACCACAACAACAATTTTCTCAGCCCCAA
      370       380       390       400       410       420       430       440       450

 Q  Q  F  P  Q  P  Q  Q  P  Q  Q  S  F  P  Q  Q  Q  P  P  F  I  Q  P  S  L  Q  Q  Q  V  N
CAACAATTCCCGCAGCCCCAACAACCGCAACAATCATTCCCCCAACAACAACCACCGTTCATTCAGCCATCTCTACAACAACAGGTGAAC
      460       470       480       490       500       510       520       530       540

 P  C  K  N  F  L  L  Q  Q  C  K  P  V  S  L  V  S  S  L  W  S  M  I  W  P  Q  S  D  C  Q
CCATGCAAGAATTTCCTCTTGCAGCAATGCAAACCTGTGTCACTGGTGTCATCCCTCTGGTCAATGATCTGGCCACAAAGCGATTGCCAA
      550       560       570       580       590       600       610       620       630

 V  M  R  Q  Q  C  C  Q  Q  L  A  Q  I  P  Q  Q  L  Q  C  A  A  I  H  T  V  I  H  S  I  I
GTGATGCGGCAACAATGCTGCCAACAACTAGCACAGATTCCTCAGCAGCTCCAGTGTGCAGCCATCCATACCGTCATACATTCCATCATC
      640       650       660       670       680       690       700       710       720

 M  Q  Q  E  Q  Q  Q  G  M  H  I  L  L  P  L  Y  Q  Q  Q  Q  V  G  Q  G  T  L  V  Q  G  Q
ATGCAGCAAGAACAACAACAAGGCATGCATATCCTGCTGCCACTATATCAGCAGCAACAGGTGGGTCAAGGTACTCTCGTCCAGGGCCAG
      730       740       750       760       770       780       790       800       810

 G  I  I  Q  P  Q  Q  P  A  Q  L  E  A  I  R  S  L  V  L  Q  T  L  P  T  M  C  N  V  Y  V
GGCATCATCCAACCCCAACAACCAGCTCAATTGGAGGCGATCAGGTCATTGGTGTTGCAAACTCTTCCAACCATGTGCAACGTGTATGTC
      820       830       840       850       860       870       880       890       900

 P  P  E  C  S  I  I  K  A  P  F  S  S  V  V  A  G  I  G  G  Q  *
CCACCTGAGTGCTCCATCATCAAGGCACCATTTTCCAGCGTAGTCGCCGGCATTGGTGGCCAATGAAAAATGCAAGAGCCATACTAATAG
      910       920       930       940       950       960       970       980       990

GTAGATGGATCATCGTTGCTTAGTTGATTTACCCATCGATGTAACGATGATAGAATAAACTGGCGTGCACCATCATGTGTGACCCCGACC
     1000      1010      1020      1030      1040      1050      1060      1070      1080

AGTGCAATTTCAAGCTTGGGAATAAAAGACAAAGAAAGTTCAAGTTTGCGAAAAAAAAAAAA
     1090      1100      1110      1120      1130      1140
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B

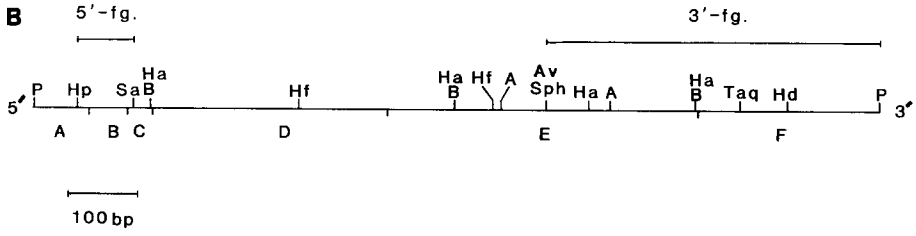

Fig. 1. A DNA sequence of pTag-1436. The one letter code translation of the predicted coding sequence is shown. The tandem repeat motif and putative poly A^+ addition sites are overlined. **B** Restriction enzyme site map of pTag-1436. Sites present for enzymes are indicated above the line as follows: A = AluI, Av = AvaIII, B = BalI, Ha = HaeIII, Hd = HindIII, Hf = HinfI, Hp = HpaI, P = PstI, Sa = SalI, Sph = SphI, Taq = TaqI. Below the line, segments of the sequence are indicated as follows: A = 5′ end of clone to methionine start-codon, B = 'leader' sequence, C = non-repeated amino terminal portion, D = region of tandem repeats, E = non-repeated carboxy terminal portion, F = 3′ untranslated portion. Bars labelled 5′ fg. and 3′ fg. refer to DNA fragments used as probes in hybridisation to size-fractionated RNA. **C** DIAGON homology matrices of sequences indicated. The comparison was carried out using the proportional matching option, with all matches of 9 bases in a 'window' of 13 being printed as a dot. Coordinates refer to base pairs from 5′ end of clone sequence. pW10 sequence was obtained from Scheets et al. (1985) and the p11–33 sequence from Okita et al. (1985)

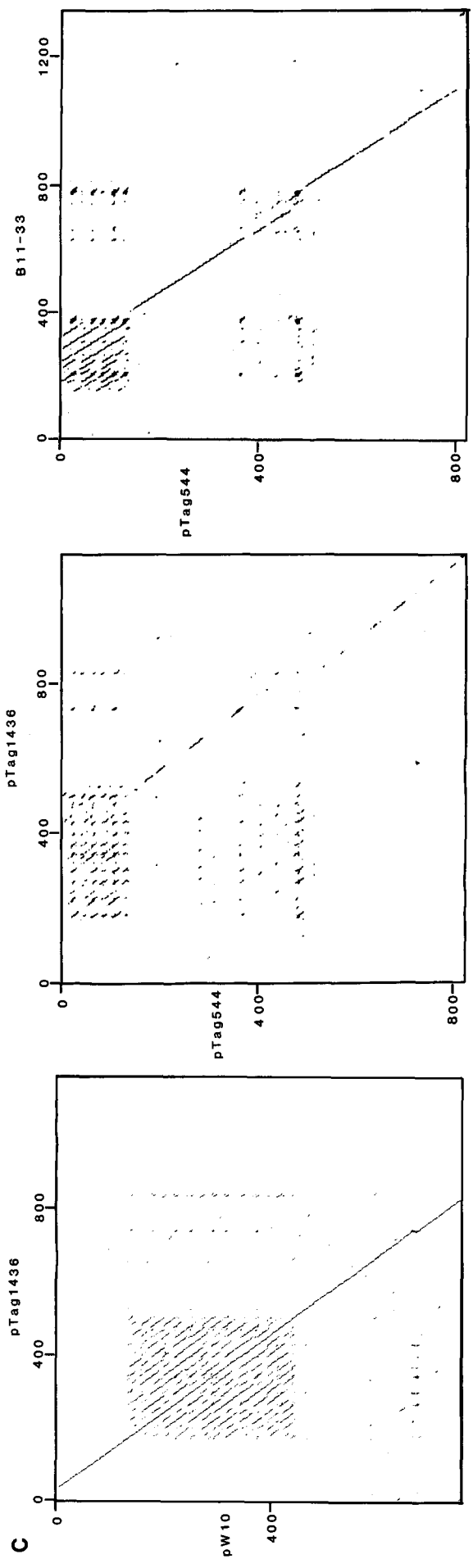

Fig. 1C

but much less closely related to pTag544 (Thompson et al. 1983), or B-11-33 (Okita et al. 1985). DIAGON comparisons indicate the latter two clones are very similar.

Hybrid-selected translation products

The pattern of polypeptides synthesized in vitro when wheat endosperm mRNA selected by hybridisation to pTag1436 at high stringency was translated in the reticulocyte lysate system is shown in Fig. 2. This pattern was the same as that observed previously for γ-type sequences, for example pTag64 (Harberd et al. 1985), with clear hybridisation to mRNAs coding for four polypeptide size classes, M_rs between 35 and 45 kD being the most abundant.

'Northern' RNA-filter hybridisations

1) Chromosome assignment of transcripts. Poly A^+ RNA was prepared from developing endosperms of 'Chinese Spring' wheat and from 'Chinese Spring' lines lacking group 1 or group 6 chromosomes. The RNA was fractionated on agarose-formaldehyde gels and transferred to nitrocellulose filters for probing with nick-translated cDNA inserts (Fig. 3). The γ-gliadin probes hybridised to mRNAs at 3 discernible sizes in the range 1,250–1,350 bases using rRNA and DNA fragments as standards. It is possible to identify some of the RNA size components as the products of individual chromosomes. The smallest hybridising band cannot be assigned to a single chromosome. The next largest band is derived from chromosome 1D, as it is absent in NIDTIB and more prominent in NIBTID and NIATID lines. The next largest band is derived from chromosome 1A (elevated in NIBTIA) and the largest hybridising mRNA is derived from chromosome 1B (absent in NIBTID and NIBTIA). A summary of these assignments is given in Fig. 3.

The use of RNA from nullisomic-tetrasomic wheat lines showed that the broad region of hybridisation to a γ-gliadin cDNA probe could be divided into a number of separate mRNA bands. This pattern of hybridisation was compared to that obtained by hybridisation of a LMW glutenin cDNA probe to euploid and aneuploid lines (Fig. 4). The result indicated that the two probes hybridised to different mRNAs. pTag544, the LMW glutenin cDNA probe, hybridised to a single broad band, larger than the smallest γ-gliadin mRNA, and not resolved into subcomponents, in contrast to the result obtained by hybridisation with pTag1436. When the experiment was repeated at higher stringency, Tm–3 °C, the same result was obtained (data not shown).

2) Subgroups of γ-gliadin mRNAs. The homologous genes complementary to pTag1436 may differ in the 5′ region as suggested by N-terminal sequence differences (Bietz et al. 1977) or in the 3′ untranslated segments as has been shown for other gene families (Ordahl and Cooper 1983). If this is the case, the use of probes from these regions at high stringency may distinguish a subset of gliadin genes. Size-fractionated euploid 'Chinese Spring' RNA was bound to nitrocellulose and probed with nick translated restriction fragments, as indicated in Fig. 1 b. Probes were derived from the 5′ end (HpaI–SalI, 62–145 bases) or from the 3′ end (SphI–PstI, 743 bases to the end), and each hybridisation was carried out under two different stringency conditions, low stringency, Tm–12 °C, or high stringency, Tm–3 °C, as estimated according to Beltz et al. (1983). The results of these experiments (data not shown) showed that these probes hybridised to all the separated γ-gliadin mRNA bands, i.e. we could not identify sequence types absent from one or more homeologous chromosomes using these probes.

Another approach taken to identify a subset of γ-gliadin genes involved the use of genomic clones as probes. A library was produced by cloning wheat DNA fragments in λCh34 (Loenen and Blattner 1983) and screened with pTag647, another closely related γ-gliadin cDNA probe. The resulting positive plaques were purified and classified according to strength of hybridisation to pTag1436. It was reasoned that weakly hybridising clones might represent related possibly minor gliadin sequence families. Alternatively, such clones might be homologous to part of a γ-gliadin gene. One such clone, λ6.13 was used as a probe in hybridisations to size-fractionated RNA (Fig. 5) and showed no hybridisation to the two higher molecular weight 1D and 1A mRNAs although hybridising to the 1B mRNA and to the smallest RNA band. This observation suggests that λ6.13 has a region of homology which is present only in a subset of the γ-gliadin gene family.

Wheat DNA filter hybridisations

The isolated insert of pTag1436 was used to probe digests of wheat DNA (Fig. 6) in order to investigate the organization and complexity of DNA fragments carrying γ-gliadin genes. (Certain digests were probed with pTag647, which is a closely related γ-gliadin cDNA clone, with indistinguishable hybridisation properties to pTag1436 (compare (c) with 'Euploid').) When the result was compared with that obtained using pTag544 insert (LMW glutenin cDNA), the hybridising fragments were seen to be different (compare Fig. 6 (a) versus (b)). Certain fragments, indicated by arrowheads, could be assigned to group 1 chromosomes, consistent with the location of the γ-type sequences at *Gli-1*.

Discussion

The use of cloned cDNA probes (Okita et al. 1984, 1985; Harberd et al. 1985; Scheets et al. 1985) has demonstrated that the α/β and γ gliadins and low molecular weight glutenins comprise an 'extended' multigene family of sequences of varying degrees of relatedness. Previous studies have distinguished between α/β and γ-type sequences, but the distinction between clones for γ-type and LMW glutenin genes has been more difficult to establish, due to the lack of a full-length coding sequence in a γ-gliadin cDNA. This paper presents such a sequence and shows that this sequence type corresponds to the γ-2/γ-3 type polypeptides of Kasarda (by N-terminal sequence comparison) and to γ-gliadin polypeptides by hybrid release translation. LMW glutenin cDNA clone sequences differ in sequence from γ-gliadins and in hybrid release translation products (Bartels and Thompsön 1983; Harberd et al. 1985) although the latter result is weak due to the underrepresentation of LMW glutenin polypeptides amongst in vitro translation products (Bartels; Jackson, unpublished). That the two clone types hybridise to different mRNA species is shown here by different patterns of hybridisation to size fractionated filter-bound RNA samples from aneuploid lines.

From a comparison of the deduced coding sequence of B11–33 with the N-terminal sequence of an 'aggregated gliadin' fraction, which showed 4 amino acid differences between the two sequences, of which 3 could be due to single base changes, Okita et al. (1985) concluded LMW glutenins and γ-gliadins are related, and that LMW glutenins are a variant form of γ-gliadins. We propose in the light of the data presented here that B11–33 and other clones of Okita's Class I and II groups, and also pTag544 (Bartels and Thompson 1983) code for LMW glutenins rather than γ-gliadins. When the hybridisation pattern of pTag544 (LMW glutenin sequence type) and pTag1436 (γ-sequence type) to digested wheat DNA are compared, it can be concluded that the two types of genes are not located on the same fragments. The recent demonstration by Shewry et al. (1985) that minor barley hordein polypeptides possess γ-type N-terminal sequences suggests that the divergence of γ-gliadin and LMW glutenin/B1 hordein sequence types occurred prior to the divergence of the ancestors of wheat and barley.

In view of the chromosomal location of γ-gliadin and LMW glutenin genes at the same genetic locus, it is possible that recombinant or other intermediates between the two sequence types may arise to give hybrid genes with LMW glutenin type N-terminal sequences or repeats linked to γ-gliadin C-terminal sequences or vice versa, although we have not isolated such clones. Such clones would be expected to give rise to polypeptides for both sequence types in hybrid release translation, something we have not observed with the 4

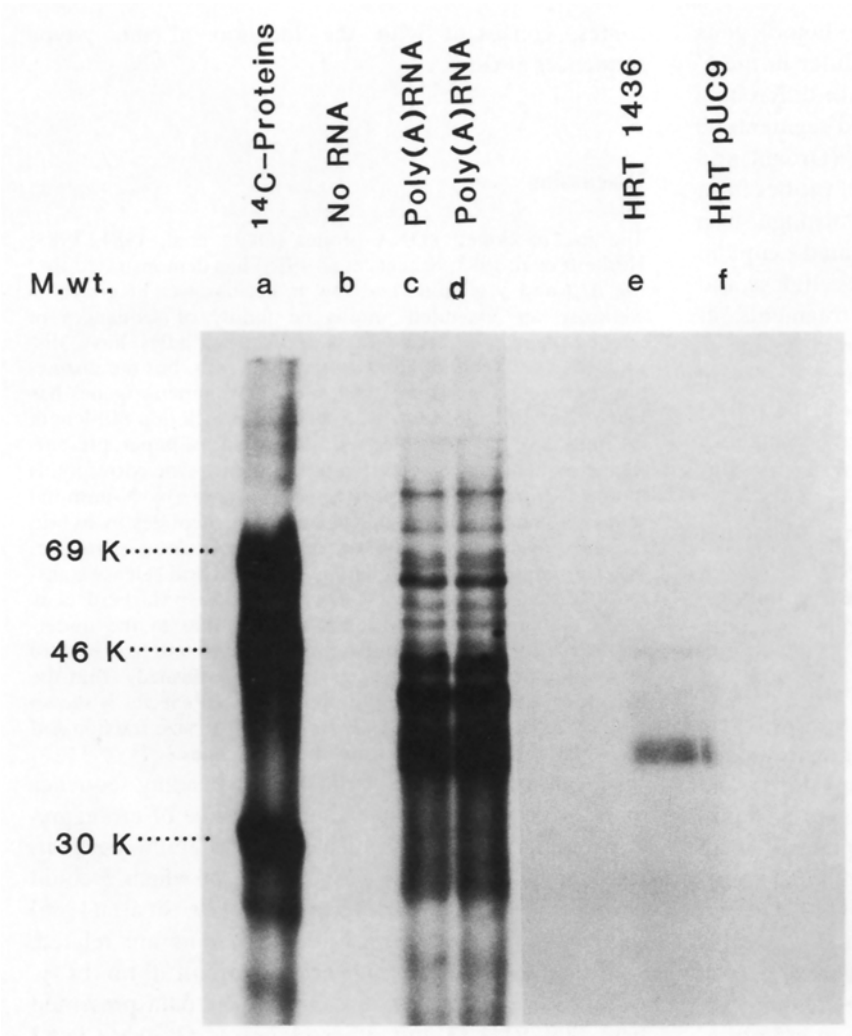

Fig. 2. Hybrid release translation. The hybridisation of mRNA to pTag1436 was carried out in 0.6 M Na^+, 65% formamide at 50 °C. The non-hybridised RNA was washed off with several washes at 1 × SSC, 0.1% SDS at 65 °C, and two final washes with 2 mM EDTA at room temperature before the hybridised mRNA was released by boiling in H_2O. The in vitro translation products were extracted with 50% isopropanol, alkylated and separated on a 15% acrylamide-urea gel

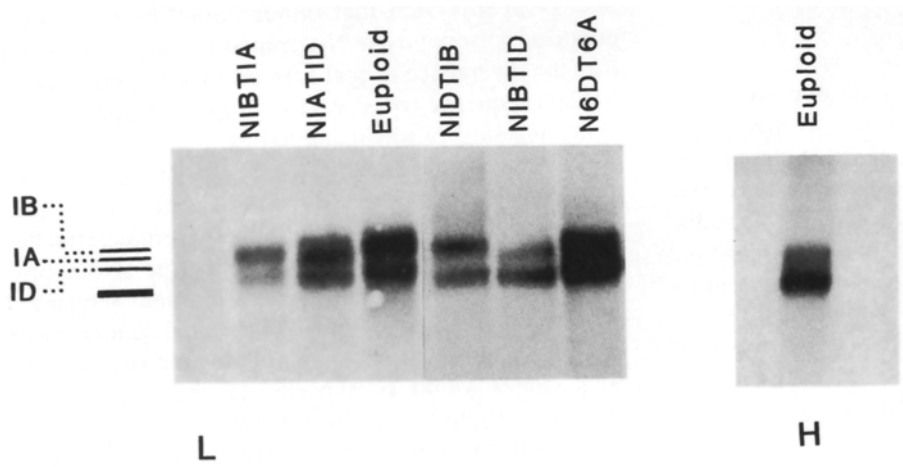

Fig. 3. Hybridisations of pTag1436 and subfragments to RNAs. Poly A^+ RNA from developing endosperms of euploid and nullisomic-tetrasomic lines was fractionated on formaldehyde agarose gels transferred to nitrocellulose filters and probed with the PstI insert of pTag1436 (NIDTIB, NIBTID, N6DT6A) or with the Pst insert of pTag647, a closely related γ-gliadin cDNA clone (NIBTIA, NIATID, Euploid). L = Low stringency conditions = 50% formamide, 5 × SSC = 0.75 M Na^+ at 50 °C; washing was at 0.5 × SSC, 0.1% SDS, 50 °C. H = High stringency = 50% formamide, 1 × SSC = 0.15 M Na^+, at 50 °C; washing was at 0.1 × SSC, 0.1% SDS, 50 °C

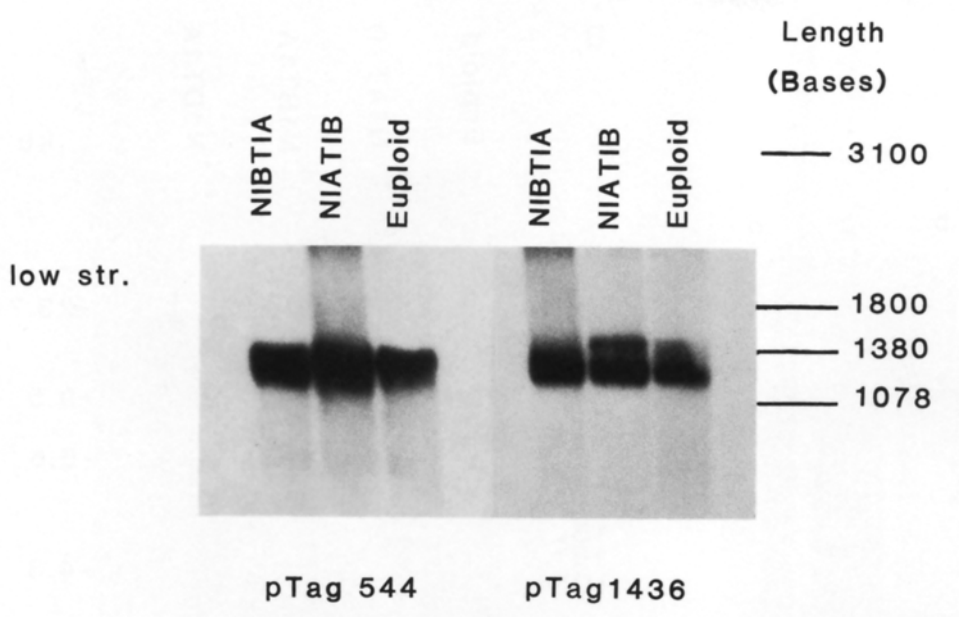

Fig. 4. Hybridisations of pTag1436 and pTag544 to wheat RNA. Poly A^+ RNAs from developing endosperms of euploid and nullitetra lines of wheat (cv. 'Chinese Spring') were separated on formaldehyde-agarose gels and transferred to nitrocellulose. Two filters were prepared, each carrying two identical sets of RNAs. Each filter was hybridised with the insert of pTag544 (removed by digestion with PstI) or with the PstI-removed insert of pTag1436. After hybridisation for 15 h the filters were washed with several buffer changes at 0.5×SSC, 0.1% SDS at 50°C. Size markers: the positions of the wheat cytoplasmic rRNAs are indicated (3,100 bases and 1,800 bases) as are the positions of DNA fragments derived from a HaeIII digest of Φ 174 RF DNA

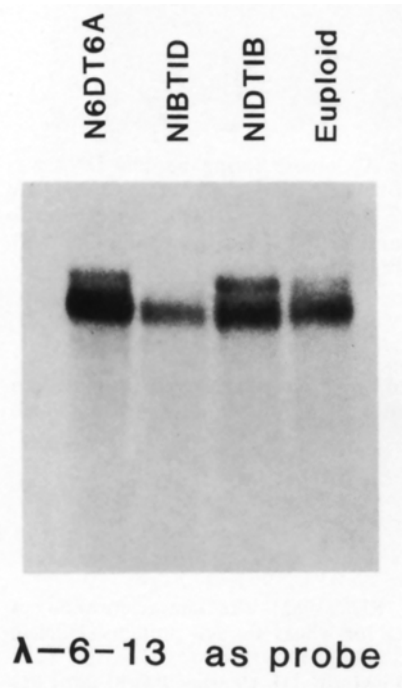

Fig. 5. Hybridization of λ6.13 to wheat RNA. Poly A^+ RNA size fractionated on formaldehyde agarose gels was probed with 2.0 kb HindIII–PstI DNA fragment derived from the γ-gliadin-related genomic clone λ-6-13. Hybridisation conditions were 50% formamide, 5×SSC, 50°C and washing conditions were 0.5×SSC, 50°C 0.1% SDS

different γ-gliadin clones tested (Harberd 1985 and unpublished).

To investigate sequence complexity within the γ-gliadin gene family, labelled DNA fragments from the amino terminal end of the coding sequence and the unique C-terminal sequence were used to probe size-fractioned, filter-bound RNA. Using this technique, we could not identify mRNA subsets, indicating that γ-gliadin gene family members are closely related in these regions.

The use of nullisomic-tetrasomic wheat lines permitted the assignment of several of the mRNA size classes and DNA fragments to chromosomal loci. Relative intensities of the hybridizing mRNA size classes were related to gene dosage in the nullisomic-tetrasomic lines. The assignments, to group 1 chromosomes, are consistent with the identification of γ-gliadin polypeptides as products of *Gli-1* on the short arm of group 1 chromosomes (Payne et al. 1985). It will be of interest to establish whether the hybridising sequences are restricted to *Gli-1*, as other loci for gliadins have been mapped to group 1 chromosomes (Jackson et al. 1985; Singh and Shephard 1984).

In the case of the *Glu-D1* locus encoding HMW glutenins, subloci differences, for example differences between *Glu-D1x* and *Glu-D1y* genes, are greater than homoeoallelic differences, for example the difference

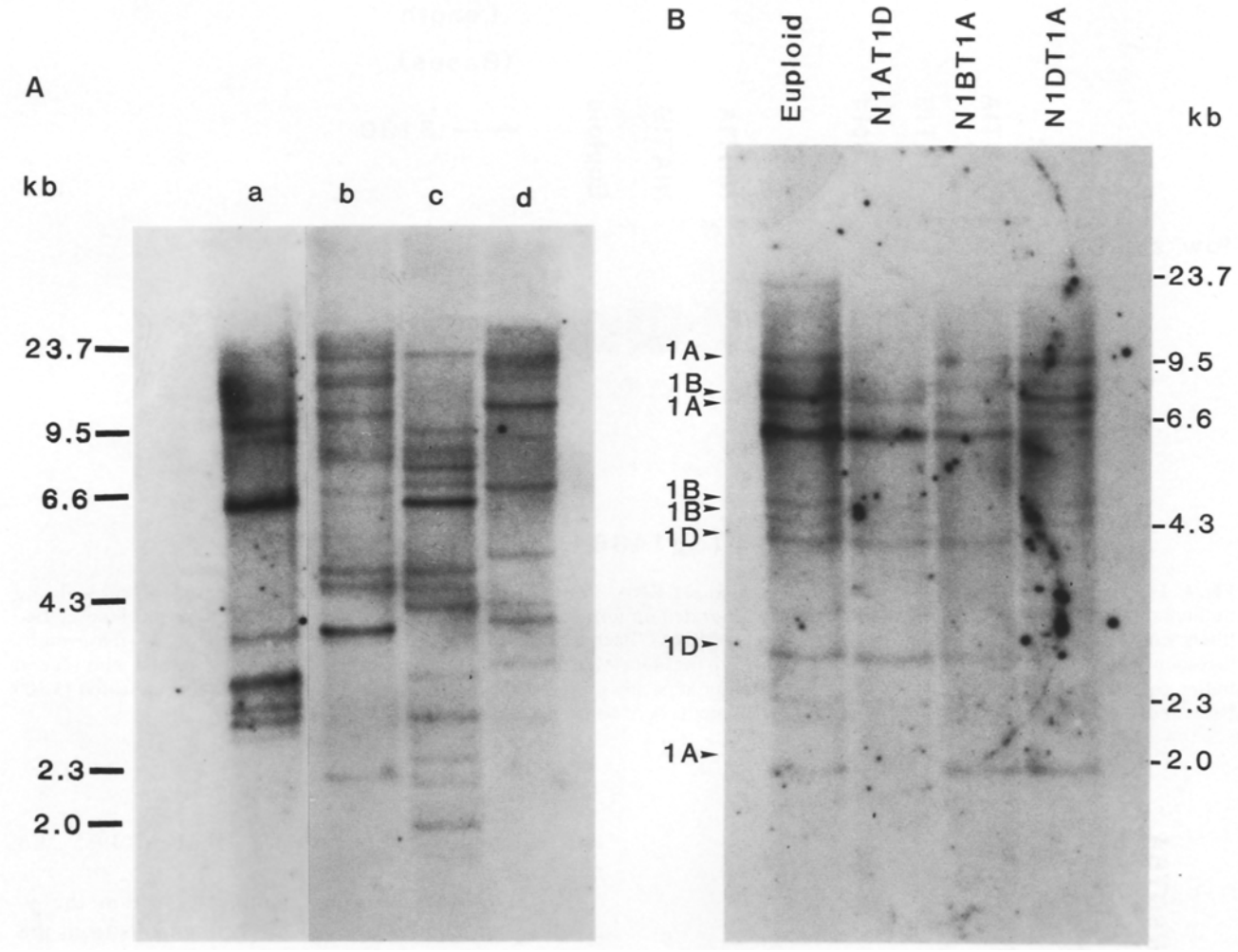

Fig. 6A, B. Hybridisation of γ-gliadin and LMW glutenin cDNAs to wheat DNA fragments. **A** 'Chinese Spring' euploid DNA was fractionated on agarose gels and transferred to nitrocellulose for hybridisation. (*a*) EcoRI digest, probed with pTag544, a LMW glutenin cDNA; (*b*) EcoRI; (*c*) HindIII and (*d*) BamHI digests, all probed with pTag647, a γ-gliadin cDNA closely related to pTag1436. **B** HindIII-digested DNA from 'Chinese Spring' euploid, Nullisomic, Tetrasomic 1D (NIATID), Nullisomic 1B, Tetrasomic 1A (NIBTIA) and Nullisomic 1D, Tetrasomic 1A (NIDTIA) lines, probed with pTag1436

between *Glu-D1y* and *Glu-A1y* (Thompson et al. 1985; Forde et al. 1985; Sugiyama et al. 1985). The homoeoallelic comparison, made recently, showed differences mainly of short deletion/insertion changes within the block of repeats. This may also be the case for the *Gli-1* locus. The λ6.13 probe used here also detected a subset of closely related genes within the γ-gliadin sequences at the *Gli-B1* and *Gli-D1* loci. Further experiments are required to establish whether this subset of genes is also present at or expressed from *Gli-A1*, and to identify the sequence which distinguishes the subset from γ-gliadin genes.

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References

Bartels D, Thompson RD (1983) The characterisation of cDNA clones coding for wheat storage proteins. Nucleic Acids Res 11:2961–2977

Beltz GA, Jacobs KA, Eickbush TH, Cherbas PT, Kafatos FC (1983) Isolation of multigene families and determination of homologies by filter hybridisation methods. Methods Enzymol 100:266–285

Bietz JA, Huebner FR, Sandersen JE, Wall JS (1977) Wheat gliadin homology revealed through N-terminal amino acid sequence analysis. Cereal Chem 54:1070–1083

Bietz JA, Wall JS (1980) Identity of high molecular weight gliadin and ethanol-soluble glutenin subunits of wheat: relation to gluten structure. Cereal Chem 57:415–421

Forde BG, Kreis M, Bahramian MB, Matthews JA, Miflin BJ, Thompson RD, Bartels D, Flavell RB (1981) Molecular cloning and analysis of cDNA sequences derived from poly A^+ RNA from barley endosperm: identification of B hordein related clones. Nucleic Acids Res 9:6689–6707

Forde BG, Kreis M, Williamson MS, Fry RP, Pywell J, Shewry PR, Bunce N, Miflin BJ (1985) Short tandem repeats shared by B- and C-hordein cDNAs suggest a common evolutionary origin for two groups of cereal storage protein genes. EMBO J 4:9–15

Forde J, Malpica J-M, Halford NG, Shewry PR, Anderson OD, Greene FC, Miflin BJM (1985) The nucleotide sequence of a HMW glutenin subunit gene located on chromosome 1A of wheat (*Triticum aestivum* L.). Nucleic Acids Res 13:6817–6832

Harberd NP, Bartels D, Thompson RD (1985) Analysis of the gliadin multigene loci in bread wheat using nullisomic-tetrasomic lines. Mol Gen Genet 198:234–242

Jackson EA, Holt LM, Payne PI (1983) Characterisation of the high molecular weight gliadin and low molecular weight glutenin subunits of wheat endosperm by two-dimensional electrophoresis and the chromosomal localisation of their controlling genes. Theor Appl Genet 66:29–37

Jackson EA, Holt LM, Payne PI (1985) Glu-B2, a storage protein locus controlling the D group of LMW glutenin subunits in bread wheat (*Triticum aestivum*). Genet Res 46:11–17

Kasarda DD, Lafiandre D, Morris R, Shewry PR (1984) Genetic relationships of wheat gliadin proteins. Kulturpflanze 32:41–60

Kreis M, Forde BG, Rahman S, Miflin BJ, Shwry PR (1985) Molecular evolution of the seed storage proteins of barley, rye and wheat. J Mol Biol 183:499–502

Lehrach H, Diamond D, Woxney JM, Boedtker H (1977) RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. Biochemistry 16:4743–4751

Loenen WAM, Blattner FR (1983) Lambda Charon vectors (Ch32, 33, 34 and 35) adapted for DNA cloning in recombination-deficient hosts. Gene 26:171–179

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor NY

Maxam AM, Gilbert WD (1977) A new method for sequencing DNA. Proc Natl Acad Sci USA 560–564

Messing J, Vieira J (1982) A new pair of M13 vectors for selecting either DNA strand of double-digest restriction fragments. Gene 19:269–276

Okita TW (1984) Identification and DNA sequence analysis of a γ-type gliadin cDNA plasmid from winter wheat. Plant Mol Biol 3:325–332

Okita TW, Cheesbrough V, Reeves CD (1985) Evolution and heterogeneity of the α/β type and γ-type gliadin DNA sequences. J Biol Chem 260:8203–8213

Ordahl CP, Cooper TA (1983) Strong homology in promoter and 3′ untranslated regions of chick and rat α-actin genes. Nature 303:348–349

Payne PI, Holt LM, Jarvis MG, Jackson EA (1985) Two-dimensional fractionation of the endosperm proteins of bread wheat (*Triticum aestivum*): biochemical and genetic studies. Cereal Chem 62:319–326

Rafalski JA (1984) Developmentally regulated plant genes: the nucleotide sequence of a wheat gliadin genomic clone. EMBO J 3:1409–1415

Sanger F, Coulson AR, Barrell BG, Smith AGH, Roe BA (1980) Cloning in single-stranded bacteriophage as an aid to rapid DNA sequencing. J Mol Biol 143:161–178

Scheets K, Rafalski JA, Hedgcoth C, Soll DG (1985) Heptapeptide repeat structure of a wheat γ-gliadin. Plant Sci Lett 37:221–225

Shewry PR, Miflin BJ, Lew EJL, Kasarda DD (1983) The purification and characterisation of an aggregated gliadin fraction from wheat. J Exp Bot 34:1403–1410

Shewry PR, Kreis M, Parmar S, Lew EJ-L, Kasarda DD (1985) Identification of γ-type hordeins in barley. FEBS Lett 190:61–64

Shyam E, Reddy P, Rao V (1985) A simple method for the detection of poly A-containing cDNAs and a possible mechanism for the generation of rearrangements in cDNAs. Gene Anal Tech 2:46–49

Singh NK, Shepherd KW (1984) A new approach to studying the variation and genetic control of disulphide linked endosperm proteins in wheat and rye. In: Proc 2nd Int Workshop on Wheat Gluten Proteins. PUDOC, Wageningen, pp 129–136

Staden R (1982) An interactive graphics program for comparing and aligning nucleic acid and amino acid sequences. Nucleic Acids Res 10:2951–2961

Sugiyama T, Rafalski A, Peterson D, Kasarda DD (1985) A wheat HMW subunit gene reveals a highly repeated structure. Nucleic Acids Res 13:8729–8737

Thompson RD, Bartels D, Harberd NP, Flavell RB (1983) Characterisation of the multigene family coding for HMW glutenin subunits in wheat using cDNA clones. Theor Appl Genet 67:87–96

Thompson RD, Bartels D, Harberd NP (1985) Nucleotide sequence of a gene from chromosome 1D of wheat encoding a HMW-glutenin subunit. Nucleic Acids Res 13:6833–6846