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Characterization and organization of gene families at the *Gli-1* loci of bread and durum wheats by restriction fragment analysis

P. A. Sabelli*, and P. R. Shewry**

Department of Agricultural Sciences, University of Bristol, AFRC Institute of Arable Crops Research, Long Ashton Research Station, Long Ashton, Bristol BS18 9AF, UK

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Summary. Probes related to γ-gliadins and to the LMW subunits of glutenin were used to determine the complexity of the *Gli-1* loci, by RFLP analysis of euploid and aneuploid lines of bread wheat cv Chinese Spring and durum wheat cv Langdon. The two probes hybridised to separate sets of fragments derived from chromosomes 1A, 1B and 1D. The fragments related to the LMW subunit probe had a total copy number in *Hin*dIII digests of about 35 in Chinese Spring and 17 in Langdon, with more fragments derived from chromosomes 1D. The fragments hybridising to the γ-gliadin probe could be divided into two classes, based on whether they hybridised to the whole probe at high stringency or to the 3′ nonrepetitive region at moderate stringency. The fragments that failed to hybridise under these conditions were considered to be related to ω-gliadins. The fragments related to γ- and ω-gliadins had total copy numbers of about 39 and 16, respectively, in *Hin*dIII digests of Chinese Spring, and about 24 and 12, respectively, in Langdon.

Key words: Wheat – *Gli-1* loci – Gliadins – LMW glutenins – RFLP

Introduction

Wheat gluten consists of over 50 individual proteins, which are traditionally classified into two groups: the monomeric gliadins, and the glutenins that are polymers consisting of individual subunits associated by interchain disulphide bonds. The gliadins are further classified into α-, β-, γ- and ω-gliadins on their electrophoretic mobility at low pH, and the glutenin subunits, into high-molecular-weight (HMW) and low-molecular-weight (LMW) types.

Although the traditional classification has provided an excellent basis for studies of gluten structure and functionality, recent molecular, biochemical and genetic analyses demonstrate that the gluten proteins form three groups (Shewry et al. 1986; Shewry and Tatham 1990). Two of these correspond to the ω-type gliadins and HMW glutenin subunits, and are called the sulphur-poor (S-poor) prolamins and HMW prolamins, respectively. The third group consists of the α-type (α + β) gliadins, the γ-type gliadins and the LMW glutenin subunits, and are called the S-rich prolamins.

Genetic analyses demonstrate that most gluten proteins are encoded by three sets of loci on the homoeologous group 1 and 6 chromosomes (Payne 1987). The *Gli-2* loci on the short arms of group 6 chromosomes encode all the α-type gliadins, while the *Glu-1* loci on the long arms of group 1 chromosomes encode the HMW glutenin subunits. Whereas these loci encode single groups of closely related proteins, the *Gli-1* loci on the short arms of group 1 chromosomes are more complex, encoding the S-poor ω-type gliadins and the S-rich γ-type gliadins and LMW glutenin subunits. In addition, a fourth series of loci present on the short arms of chromosomes 1A and 1B only (*Gli-3*) encode poorly characterized proteins which are probably related to ω-type gliadins (Payne et al. 1988).

Analysis of the complexity and organisation of the *Gli-1* loci is limited by the low frequency of the intralocus recombination and the presence of extensive sequence homology between the groups of encoded proteins, in

* Permanent address: Department of Agrobiology and Agrochemistry, University of Tuscia, Via de Lellis, 01100 Viterbo, Italy
** To whom correspondence should be addressed (Long Ashton Research Station)

particular between the ω-type and γ-type gliadins. Thus, previously published restriction analyses (Bartels et al. 1986) of the γ-gliadin gene family may include sequences encoding ω-type gliadins. We have tried to eliminate this problem by designing specific restriction fragment length polymorphism (RFLP) probes for the different families of genes at the *Gli-1* loci, and have used these probes to compare the complexity of the loci in hexaploid bread and tetraploid durum wheats.

Materials and methods

Plant material

Hexaploid wheat (2n = 6x = 42) (*Triticum aestivum*) cv 'Chinese Spring', the nullisomic-tetrasomic series for the homoeologous group 1 chromosomes and the ditelosomic lines 1AS, 1AL, 1BS and 1BL were all obtained from Mr. T. E. Miller (AFRC-IPSR, Cambridge Laboratory). Tetraploid wheat (2n = 4x = 28) (*Triticum durum*) cv 'Langdon' and the disomic substitution lines 1D (1A) and 1D (1B), in which the chromosomes 1D of 'Chinese Spring' substitute, respectively, the chromosomes 1A and 1B of 'Langdon,' were obtained from Dr. L. R. Joppa (North Dakota State University).

Probes

Clone pKAP1a (γ-gliadin) was obtained from Dr. Kathryn A. Pratt (Rothamsted Experimental Station) by subcloning the *Hpa*I/*Hin*dIII fragment of pTag1436 (Bartels et al. 1986) into the *Sma*I site of pUC19, and pLMWTG2 (LMW subunit) was obtained by subcloning the fragment from nucleotides 31 to 999 of the LMWG-1D1 clone (Colot et al. 1989) into the same plasmid and site. pSc503 (γ-secalin) (Kreis et al. 1985a), pB11 (B1 hordein) (Forde et al. 1985) and pcP387 (C hordein) (Forde et al. 1985) were as described by the original authors. All clones are cDNAs except pLMWTG2 (genomic DNA), and include the complete coding regions and short sequences of the 3′ untranslated regions, except pB11 and pcP387, which are incomplete at the 5′ end. Only 714 bp out of a total of about 1,100 bp of pSc503 have been sequenced. The probes obtained by excision from the above clones will, for convenience, be referred to by the names of the clones. Probes pKAP1a and pLMWTG2 were obtained by double digestion with *Eco*RI and *Bam*HI (~1,049 and ~990 bp, respectively), and pSc503, pB11 and pcP387 by digestion with *Hin*dIII (~1,100, ~870 and ~475 bp, respectively). Figure 1 shows alignments of the probes and homologous regions in the encoded proteins.

DNA isolation

Genomic DNA was prepared from young shoots by banding twice in a CsCl/ethidium bromide density gradient (Kreis et al. 1983). The activity of residual DNases was assayed by incubation of aliquots of DNA in digestion buffer, without enzyme, for 2 h at 37 °C, and subsequent agarose gel electrophoresis. The DNA concentration was estimated by comparison with known quantities of undigested λ DNA in a 0.4% agarose gel. Plasmid DNA was isolated by the alkaline-lysis method, followed by banding in a CsCl/ethidium bromide density gradient.

Restriction enzyme cleavage

To optimise digestion conditions, control DNA was digested with enzyme quantities of up to 30 units/μg, in reaction volumes of up to 500 μl, and for times of up to 24 h. Routinely, 8 μg of DNA was digested overnight with 50 units of endonuclease in a 50-μl reaction volume containing 4 m*M* spermidine (Pingoud 1985).

Gel-transfer hybridisation analysis

The method used was based on that of Southern (1975). Samples were electrophoresed on 0.8% agarose gels in 1 × TBE buffer (90 m*M* TRIS, 90 m*M* boric acid, 2.5 m*M* Na_2EDTA), pH 8.3, with 0.5 μg/ml ethidium bromide in the gel, buffer and samples. When necessary, the electrophoresis time was increased to about 50 h (long gels) to improve resolution in the high-molecular-size range. The DNA was denatured, neutralised, transferred by capillarity and bonded to a Hybond-N nylon membrane (Amersham Int. plc. UK) according to standard protocols. Depurination by HCl was avoided because of subsequent loss of signal for smaller fragments. Plasmid inserts were purified from agarose gel either by electroelution or by using low-gelling temperature agarose. Approximately 100 ng of DNA template, suitable for hybridisation of six (20 × 20 cm) membranes, was oligolabelled (Feinberg and Vogelstein 1983) according to the modification by Hodgson and Fisk (1987). It was then separated from unincorporated nucleotides by gel filtration and hybridised in the buffer described by Boulnois (1987) at 65 °C for ca. 15 h to the pre-hybridised membranes, at approx. 5×10^5 dpm/ml activity (usually corresponding to a concentration of ca. 0.5 ng/ml) in heat-sealed plastic bags (Hybaid Ltd., UK). The membranes were then washed at 65 °C with increasing stringency, from 2 × SSC (low stringency, LS) (1 × SSC = 0.15 *M* NaCl, 0.015 *M* Na-citrate, pH 7.0) to 0.5 × SSC (medium stringency, MS) and finally to 0.2 × SSC (high stringency, HS), always in 0.1% (w/v) SDS. The criterion values were calculated according to the equation of Schildkraut and Lifson (1965) and are equivalent to ~27 for hybridisation and LS-washing, ~18 for MS-washing and ~8 for HS-washing (values do not significantly change with the probe used). This equation was derived from studies on hybridisation in solution, and therefore provides only an approximate estimate for filter hybridisation experiments.

Gene copy number reconstruction

γ-Type and LMW subunit gene copy numbers were estimated by comparing hybridisation signals between genomic restriction fragments and linearised plasmids. The amount of haploid unreplicated DNA (1C value) in 'Chinese Spring' is 17.3 pg (Bennett and Smith 1976); therefore, about 4.62×10^5 haploid genome copies per lane were loaded on the gel. The autoradiographic signal was compared to those of amounts of linearised pKAP1a and pLMWTG2 equivalent ot 0.5, 2, 6 and 10 copies per haploid genome. Correction was necessary for durum wheat because of the smaller genome size. Preliminary dot-matrix "Diagon" homology analyses (Staden 1982) revealed that neither the pKAP1a nor pLMWTG2 inserts show homology to the vector pUC19 (not shown).

Results

RFLP probes and restriction enzymes

Two cDNAs encoding γ-type prolamins were obtained: pKAP1a, which encodes a complete γ-type gliadin of wheat, and pSc503, which encodes part of a γ-secalin of rye. The encoded proteins are highly homologous, with N-terminal repetitive domains based on a heptapeptide motif (PQQPFPQ) and C-terminal nonrepetitive do-

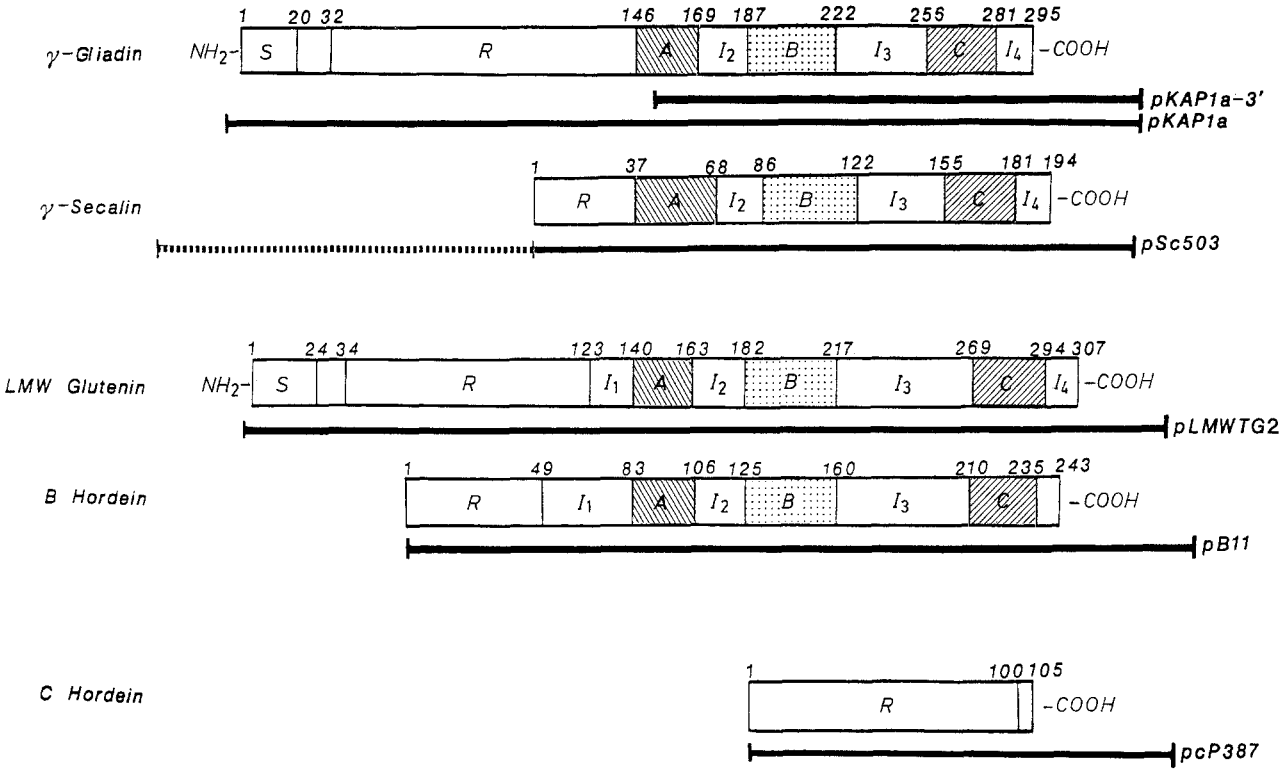

Fig. 1. Schematic comparisons of the clones and subclone used as probes and the structures of the encoded proteins. The S-rich prolamins consist of repetitive N-terminal domains (R) and nonrepetitive C-terminal domains consisting of three-conserved regions (A, B, C), flanked and separated by less conserved sequences (I_1, I_2, I_3, I_4). The γ-gliadin and LMW glutenin clones also encode signal peptides (S). The γ-secalin clone contains an unsequenced region (broken line), while the B1 hordein clone is incomplete. The incomplete clone for the S-poor C hordein encodes a protein consisting almost entirely of repeats (R)

mains consisting of three conserved regions (A, B and C) flanked and separated by intervening regions (I_2, I_3, I_4) (see Kreis et al. 1985 b). Dot-matrix (Staden 1982) analyses showed that the pairs of probes pKAP1a/pLMWTG2, pKAP1a/pSc503, pKAP1a/pcP387 and pLMWTG2/pB11 share extensive regions of homology at low-level stringency, and that this homology is retained at medium-level stringency in all cases except pKAP1a/pLMWTG2 (not shown).

Probes for ω-type gliadins are not available, so a partial cDNA (pcP387) encoding the S-poor C hordein of barley was used. This consists almost entirely of tandemly repeated octapeptides, the consensus motif (PQQPFPQQ) being closely related to the repeats present in γ-gliadins (Fig. 1). The ω-gliadins encoded by chromosomes 1A and 1D of bread and pasta wheats have similar amino acid compositions to C hordein and presumably have a similar repeat structure, whereas the ω-gliadins encoded by chromosome 1B may differ (Kasarda et al. 1983). Because of the high degree of homology, pSc503, pcP387 and pKAP1a would all be expected to hybridise to sequences encoding γ-type and at least some ω-type gliadins. We therefore also used a subclone encoding only the 3′ nonrepetitive end of pKAP1a (called pKAP1a-3′) as a specific probe for γ-type sequences (see Fig. 1).

Two probes for LMW subunit genes were used, pB11, which encodes the homologous B1 hordein of barley, and pLMWTG2, which encodes a LMW glutenin subunit and is derived from chromosome 1D of bread wheat. Both proteins have N-terminal repetitive and C-terminal nonrepetitive domains. The C-terminal domains are closely related to each other, but also have limited homology with γ-type gliadins in regions A, B and C only (Fig. 1). The repetitive domains of both proteins are also related to those in γ-type gliadins and in C hordein, but neither probe would be expected to cross-hybridise to γ-type or ω-type genes at medium or high stringency.

Three restriction endonucleases were used: *Hin*dIII, *Eco*RI and *Bam*HI. None of the probes contained sites for these enzymes, with the exception of a single *Bam*HI site at nucleotide 696 of pB11. Also, a gene bank search showed the absence of *Hin*dIII, *Eco*RI and *Bam*HI restriction sites within the coding regions of published sequences of other cDNAs and genes for γ-type gliadins and LMW subunits. Although the probes described here include short sequences at the untranslated 3′ end and the occurrence of *Gli-1* genes with restriction sites in this

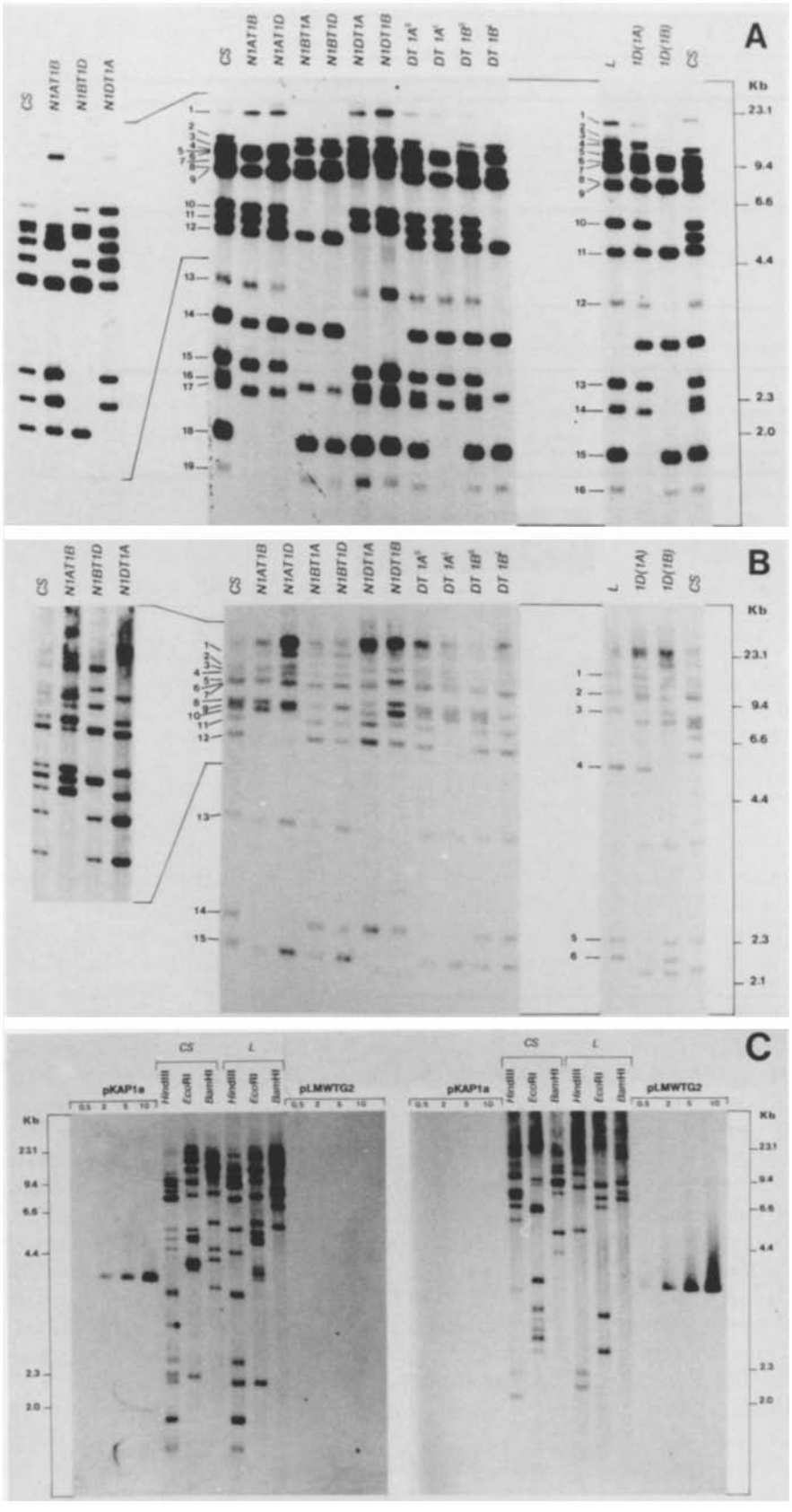

Fig. 2 A, B. Southern blot analysis of bread and durum wheat DNAs digested with *Hin*dIII and probed with the γ-gliadin probe pKAP1a (**A**) and with the LMW subunit of glutelin probe pLMWTG2 (**B**). The *left* and *central parts* of the figures show analyses of bread wheat 'Chinese Spring' (CS) and its aneuploid lines on long and normal gels, respectively. The *right part* shows analyses of durum wheat 'Langdon' (L), bread wheat 'Chinese Spring' (CS) and disomic substitution lines of chromosomes 1D of CS into L, separated on a normal gel. Restriction fragments in CS and L are numbered. **C** Estimation of the copy numbers of sequences related to the γ-gliadin probe pKAP1a (*left*) and the LMW subunit probe pLMWTG2 (*right*) in genomic DNAs of 'Chinese Spring' (CS) and 'Langdon' (L), digested with three endonucleases. Linearised plasmids corresponding to 0.5, 2, 5 and 10 copies per haploid genome are used as standards

Table 1. Total number of fragments and copy numbers per type of sequence and chromosome in 'Chinese Spring'

		*Hin*dIII		*Eco*RI[a]		*Bam*HI[a]	
		No. of fragments	Total copy no.	No. of fragments	Total copy no.	No. of fragments	Total copy no.
γ/ω Gliadin sequences							
Total	1A	7	16	5	11	4	10
	1B	7	19	7	18	5	10
	1D	5	20	4	19	3	12
	1A+1B+1D	19	55	16	48	12	32
γ-type	1A	3	7	2	5	3	6
	1B	5	14	6	16	4	7
	1D	4	18	3	9	2	4
	1A+1B+1D	12	39	11	30	9	17
ω-type	1A	4	9	3	6	1	4
	1B	2	5	1	2	1	3
	1D	1	2	1	10	1	8
	1A+1B+1D	7	16	5	18	3	15
LMW subunit sequences							
	1A	4	10	3	8	4	8
	1B	4	12	5	11	2	3
	1D	7	17	5	15	5	11
	1A+1B+1D	15	39	13	34	11	22

[a] Full details of hybridisation patterns are available from the authors

region cannot be excluded, efficient and stable hybridisation between the probes and short sequences at the 3′ end is unlikely. In addition, prolamin genes appear not to contain introns. On this basis, fragments produced by digestion of genomic DNA with these enzymes can be expected to contain whole rather than parts of genes.

RFLP analysis of gene families at the Gli-1 loci

γ-Type sequences. DNAs of the euploid and aneuploid lines of bread and durum wheats were digested with three restriction enzymes (*Hin*dIII, *Eco*RI and *Bam*HI) and probed with pKAP1a. The hybridisation patterns of *Hin*dIII digests are shown in Fig. 2A, while the results with the other two enzymes are summarised in Table 1. The patterns of restriction fragments are more complex in 'Chinese Spring' (19 with *Hin*dIII, 16 with *Eco*RI and 12 with *Bam*HI) than in 'Langdon' (16, 14 and 8 respectively), with sizes ranging from about 24.5 to 1.6 kb. The complexity of the patterns, especially in the high-molecular-size range, required further analysis on long gels (shown on the left of Fig. 2A), which revealed that some apparently single bands on normal gels consisted of several fragments with the same or different chromosomal locations (e.g. 'Chinese Spring' fragments 4, 5, 6, 8 and 9 in Fig. 1A). Comparison of the restriction patterns of the euploid and aneuploid lines allowed all the restriction fragments in 'Chinese Spring' and most of those in 'Langdon' to be assigned to the short arms of the homoeologous group 1 chromosomes (Fig. 1A, Table 1). In 'Chinese Spring,' seven *Hin*dIII fragments were located on chromosome 1A, seven on 1B and five on 1D; five *Eco*RI fragments were on 1A, seven on 1B and four on 1D; four *Bam*HI fragments were on 1A, five on 1B and three on 1D. The ditelosomic 1DS and 1DL lines were not available, and the arm locations of the 1D restriction fragments were assumed from previous results (Bartels et al. 1986). It was not possible to conclusively assign the chormosomal locations of some restriction fragments in 'Langdon' because of the masking effect of fragments from 'Chinese Spring' in the disomic substitution 1D (1A) and 1D (1B) lines. However, comparisons of the intensities of the bands revealed probable locations. Heterologous probing with the γ-secalin probe pSc503 revealed similar patterns to those with pKAP1a (not shown).

LMW subunit sequences. The same membranes were also hybridised with pLMWTG2, as shown in Fig. 2B and summarised in Table 1. In 'Chinese Spring' 15 hybridising fragments were detected in DNA digested with *Hin*dIII, 13 with *Eco*RI and 11 with *Bam*HI; 'Langdon' had six hybridising fragments with *Hin*dIII, five with *Eco*RI and six with *Bam*HI. The molecular sizes ranged from 22.4 kb to 2.1 kb. These fragments were also located on the short arms of the homoeologous group 1 chromosomes. In 'Chinese Spring,' four *Hin*dIII fragments were assigned to chromosome 1A, four to 1B and

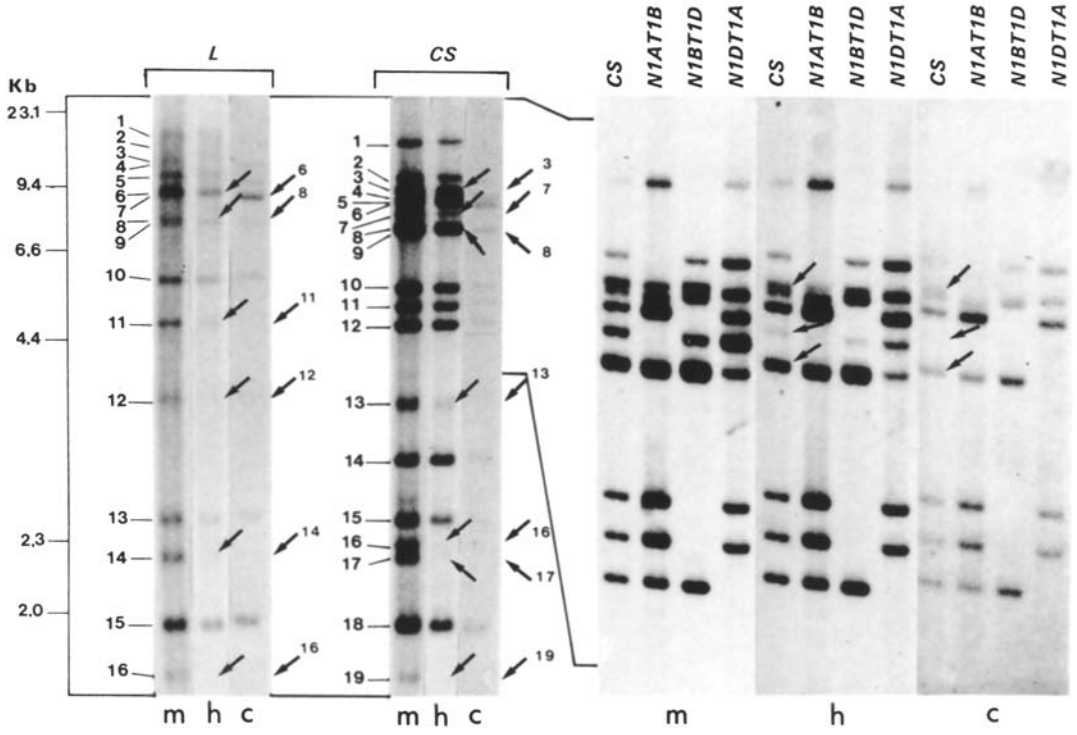

Fig. 3. Hybridisation of the γ-gliadin probe pKAP1a to *Hin*dIII-digested DNAs of 'Chinese Spring' (CS) and 'Langdon' (L) under conditions of medium (*m*) and high (*h*) stringency. Tracks *c* show the patterns of the hybridization of 3′ (nonrepetitive) region of pKAP1a (pKAP1a-3′) under conditions of medium stringency. *Arrows* (*h*) and *numbered arrows* (c) indicate fragments that hybridise only weakly or not at all

seven to 1D; three *Eco*RI fragments were assigned to 1A, five to 1B and five to 1D; and four *Bam*HI fragments were assigned to 1A, two to 1B and five to 1D (Table 1). As with the γ-type sequences, it was not possible to conclusively assign all the hybridising fragments in 'Langdon' due to the masking effect of fragments present on chromosome 1D of 'Chinese Spring'. Heterologous probing with the B1 hordein probe pB11 showed identical patterns to those with pLMWTG2 (not shown).

Copy number

The approximate copy number of each restriction fragment per haploid genome was estimated for γ-type and LMW subunit type sequences (Fig. 2C) as summarised in Table 1. For pKAP1a-related sequences, the copy number appeared to vary from one to ten in 'Chinese Spring' and from one to five in 'Langdon', and for pLMWTG2-related sequences, from one to four in 'Chinese Spring' and from one to five in 'Langdon'. The total copy numbers per chromosome and haploid genome are summarised in Table 1.

Identification of sequences related to S-poor prolamins

In order to discriminate between sequences differing in their degree of homology with the probes, high-stringency washes were used with both pKAP1a and pLMWTG2. The latter did not reveal any differences, which suggests that the probe was specific for LMW subunit genes. In contrast, considerable differences were observed with pKAP1a, with certain fragments hybridising less strongly or not at all (these are indicated by arrows in Fig. 3).

Some fragments also showed reduced hybridisation or no hybridisation at all when the 3′ (nonrepetitive) end of pKAP1a (pKAP1a-3′) was used as a probe (Fig. 3). The hybridisation of these fragments with the whole pKAP1a probe was presumably due to homology with the 5′ repetitive domain.

The membranes were also probed with pcP387, corresponding to the repetitive domain of C hordein. This showed a generally low level of signal, but some fragments hybridised relatively more strongly, as shown in Fig. 4.

In all cases the fragments that showed little or no hybridisation at high stringency and with pKAP1a-3′ were those that hybridised more strongly with pcP387. It is probable that these correspond to genes encoding ω-gliadins, which are thought to consists almost entirely of repeats. Degenerate 14-mer olignonucleotides, corresponding to the N-terminal sequences determined for the ω-5 gliadin from *Triticum aestivum* and for an ω-gliadin

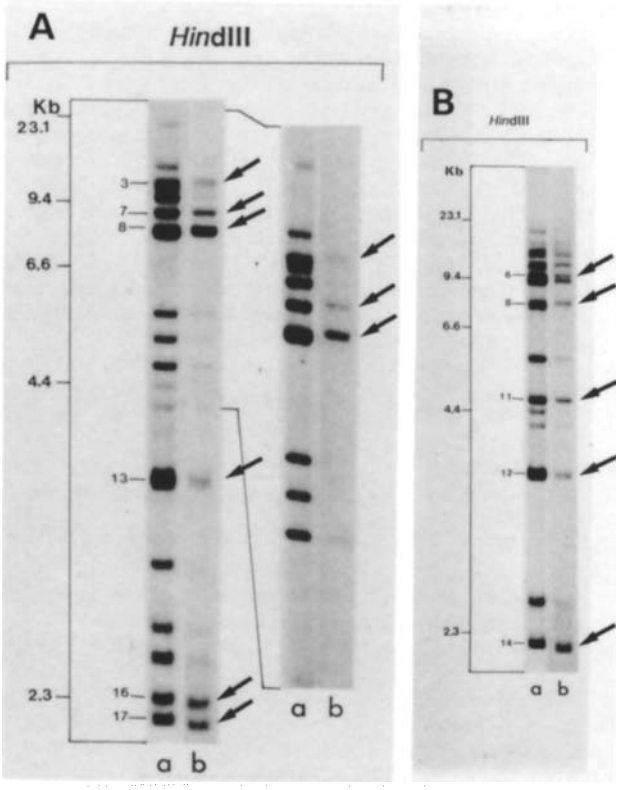

Fig. 4 A and B. Comparisons of the hybridisation patterns of the γ-gliadin probe pKAP1a (*a*) and the C hordein probe pcP387 (*b*) to *Hin*dIII-digested DNAs of 'Chinese Spring' (CS) (part **A**) and 'Langdon' (L) (part **B**). The restriction fragments that show strong hybridisation with pcP387 are *numbered* in *a* and *arrowed* in *b*. The membranes were washed with 1 × SSC, 0.1% (w/v) SDS at 65 °C

from *T. monococcum* (Kasarda et al. 1983), were used in an attempt to provide support for this hypothesis, but a high level of background hybridisation was observed, presumably because of the short and highly degenerate nature of the probes (not shown).

Discussion

Because the fragments hybridising to the various probes were only mapped to the short arms of the group 1 chromosomes by analysis of genetic stocks, it is not possible to conclude that all are present at the *Gli-1* loci, or to rule out the possibility that some are encoded by *Gli-3* or by additional minor loci, as described by Metakovsky et al. (1986). However, published studies indicate that the vast majority of γ-gliadin, ω-gliadin and LMW subunit genes are present at the *Gli-1* loci (see Payne 1987 for a review). Although the analysis of crosses indicates that the genes at these loci are tightly linked, only one genomic fragment containing two genes (both encoding γ-gliadins) has been isolated (Rafalski 1986).

Hybridisation with pKAP1a and pLMWTG2 under conditions of moderate stringency (allowing ~18% base mismatching in the duplex) showed that γ-type and LMW sequences represent different hybridisation classes (see Fig. 2C) (Bartels et al. 1986; Colot et al. 1989). The sensitivity of the method allowed us to resolve approximately twice as many restriction fragments as in previously reported studies (Bartels et al. 1986), with a total of 30–55 copies of pKAP1a-related sequences and 20–40 copies of pLMWTG2-related sequences per haploid genome of 'Chinese Spring,' and ca. 15–35 copies of pKAP1a-related sequences and 10–15 copies of pLMWTG2-related sequences in 'Langdon'. These figures can only be taken as approximate estimates, as variation in the degree of homology between gene and probe and multiple hybridisation of probes to repetitive sequences could affect the signal intensity. In addition the fragments might include inactive genes (Rafalski 1986). Both groups of hybridising fragments were present on all three genomes, with a slightly higher proportion on chromosome 1D of 'Chinese Spring'.

Heterologous probing with the γ-secalin-related pSc503 and the B hordein-related pB11 revealed identical patterns to pKAP1a and pLMWTG2, respectively (not shown), as expected from their homology at the protein and nucleic acid levels (Fig. 1) (Kreis et al. 1985a; Colot et al. 1989).

The fragments hybridising to pKAP1a were further resolved into two groups using hybridisation at high stringency. The fragments that showed reduced hybridisation under these conditions also failed to hybridise to the 3′ end of pKAP1a, but hybridised to the C hordein-encoding probe pcP387. We suggest that these fragments include genes encoding the S-poor ω-gliadins. They are present on all three genomes, with total copy numbers per haploid genome of about 15 in 'Chinese Spring' and five to ten in 'Langdon'. If this is correct, the copy numbers of the γ-gliadin genes must be adjusted to 15 to 40 in 'Chinese Spring' and 10 to 25 in 'Langdon' (Table 1). These "ω-type" sequences may correspond to those defined as class III γ-type sequences by Okita et al. (1985).

The restriction fragment analyses reported here also provide information on the minimum sizes of the *Gli-1* loci. In 'Chinese Spring', the ω-/γ-gliadin fragments at the *Gli-1A*, *Gli-1B* and *Gli-1D* loci add up to about 40–57 kb, 50–75 kb and 33–44 kb, respectively, and the LMW subunit fragments to about 12–31 kb, 35–43 kb and 20–76 kb, respectively. These figures represent only approximate values and it is probable that the loci are much larger due to the presence of intervening restriction fragments without any prolamin genes.

Comparison of the RFLP patterns of 'Chinese Spring' and 'Langdon' shows that some fragments present on chromosomes 1B and 1A appear to be conserved, having similar chromosomal locations, sizes, copy num-

bers and hybridisation poperties in the two species. A greater proportion of these are related to γ-/ω-type gliadins than to LMW glutenin subunits, even allowing for their higher total copy number. Thus, seven, six and two common γ/ω-gliadin-related fragments were present in *Hin*dIII, *Eco*RI and *Bam*HI digests, respectively, compared to two fragments in each digest hybridised with the LMW subunit probe. In the *Hin*dIII digest, the γ/ω-gliadin-related fragment 8 of 'Chinese Spring' corresponds to fragment 8 of 'Langdon', and fragment 10 to 10, 13 to 12, 15 to 13, 17 to 14, 18 to 15 and 19 to 16. Similarly the LMW subunit-related fragments 5 and 14 of 'Chinese Spring' correspond to fragments 2 and 5 of 'Langdon', respectively.

In conclusion, the analyses reported in this paper enable us to identify and quantify three families of prolamin genes located on the short arms of the group 1 chromosomes of bread and durum wheats, which appear to encode γ-type gliadins, ω-type gliadins and LMW glutenin subunits. They provide an excellent basis for further detailed analyses of the structure and organisation of the *Gli-1* loci using long-range mapping techniques, such as pulse field gel electrophoresis, chromosome walking and cloning into yeast artifical chromosomes.

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References

Bartels D, Altosaar I, Harberd NP, Barker RF, Thompson RD (1986) Molecular analysis of γ-gliadin gene families at the complex *Gli-1* locus of bread wheat (*T. aestivum*). Theor Appl Genet 72:845–853

Bennett MD, Smith JB (1976) Nuclear DNA amounts in angiosperms. Philos Trans R Soc Lond Ser B 274:227–274

Boulnois GJ (ed) (1987) University of Leicester gene cloning and analysis: a laboratory guide. Blackwell Scientific, Oxford

Colot V, Bartels D, Thompson R, Flavell RB (1989) Molecular characterization of an active wheat LMW glutenin gene and its relation to other wheat and barley prolamin genes. Mol Gen Genet 216:81–90

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

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

Hodgson CP, Fisk RZ (1987) Hybridization probe size control: optimized 'oligolabelling'. Nucleic Acids Res 15:6295

Kasarda DD, Autran J-C, Lew EJ-L, Nimmo CC, Shewry PR (1983) N-terminal amino acid sequences of ω-gliadins and ω-secalins implications for the evolution of prolamin genes. Biochim Biophys Acta 747:138–150

Kreis M, Shewry PR, Forde BG, Rahman S, Miflin BJ (1983) Molecular analysis of a mutation conferring the high lysine phenotype on the grain of barley (*Hordeum vulgare*). Cell 34:161–167

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

Kreis M, Shewry PR, Forde BG, Forde J, Miflin BJ (1985b) Structure and evolution of seed storage proteins an their genes with particular reference to those of wheat, barley and rye. Oxford Surv Plant Mol Cell Biol 2:253–317

Metakovsky EV, Akhmedov MG, Sozinov AA (1986) Genetic analysis of gliadin-encoding genes reveals gene clusters as well as single remote genes. Theor Appl Genet 73:278–285

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

Payne PI (1987) Genetics of wheat storage proteins and the effect of allelic variation on bread-making quality. Annu Rev Plant Physiol 38:141–153

Payne PI, Holt LM, Lister PG (1988) *Gli-A3* and *Gli-B3*, two newly designated loci encoding for ω-type gliadins and D subunits of glutenin. Proc 7th Int Wheat Genet Symp, IPSR, Cambridge, pp 999–1002

Pingoud A (1985) Spermidine increases the accuracy of type II restriction endonucleases. Eur J Biochem 147:105–109

Rafalski JA (1986) Structure of wheat gamma-gliadin genes. Gene 43:221–229

Schildkraut C, Lifson S (1965) Dependence of the melting temperature of DNA on salt concentration. Biopolymers 3:195–208

Shewry PR, Tatham AS (1990) The prolamin storage proteins of ceral seeds: structure and evolution. Biochem J 267:1–12

Shewry PR, Tatham AS, Forde J, Kreis M, Miflin BJ (1986) The classification and nomenclature of wheat gluten proteins: a reassessment. J Cereal Sci 4:97–106

Southern E (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

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