Theor Appl Genet (1991) 82:44–53

Chromosomal control of wheat gliadin protein epitopes: analysis with specific monoclonal antibodies

J. H. Skerritt [1], O. Martinuzzi [1,*] and E. V. Metakovsky [2]

[1] CSIRO Wheat Research Unit, Division of Plant Industry, PO Box 7, North Ryde, NSW 2113, Australia
[2] Institute of General Genetics, Moscow B-333, USSR

Received August 23, 1990; Accepted November 8, 1990
Communicated by G. Wenzel

Summary. The genetic relationships between small clusters of monomeric alcohol-soluble wheat (*Triticum aestivum* L.) grain storage proteins (gliadins) were studied using a panel of monoclonal antibodies and immunoblotting, ELISA, and RIA methods. Use of Chinese Spring nullisomic-tetrasomic lines showed that several narrow-specificity antibodies bound specifically to gliadins encoded by genes located on a single chromosome. In at least one case, antibodies bound to genetic "blocks" of gliadins, indicating that these block members have structural homology. However, often not all gliadins of a block were recognized by an antibody. For broad-specificity antibodies and some narrow-specificity antibodies, structural genes on several chromosomes were important. Studies with several primitive wheat species indicated that, while antibodies usually bound gliadins from the same genome in bread and primitive wheats, antibodies sometimes bound proteins of quite differing mobilities in the two wheat types. Use of antibodies to identify gliadin blocks is simpler than block analysis based on performing crosses, and should be of value in monitoring genotype/end-use quality relationships.

Key words: Gliadin – *Triticum* – Chromosomal control – Blocks – Monoclonal antibodies

Introduction

The aqueous alcohol-soluble gliadin proteins form the monomeric portion of wheat (*Triticum aestivum* L.) gluten, which is the protein complex responsible for the unique rheological behavior of wheaten doughs. Over 30 different gliadin polypeptides, varying in net charge and size, can be separated by cathodic gel electrophoresis for any particular wheat variety (see Kasarda et al. 1976; Garcia Olmedo et al. 1982; Payne et al. 1982; Galili and Feldman 1983, for reviews). Bread wheat is an allohexaploid; genes encoding gliadin polypeptides occur in clusters on the short arms of chromosomes 1 (*1A*, *1B*, *1D*) and 6 (*6A*, *6B*, *6D*). Analysis of the progeny of large numbers of crosses has shown that specific clusters of genes on each of these chromosomes encode several gliadin components (termed a block) inherited together as a Mendelian character; recombination between genes within a cluster is either rare or not observed (Sozinov and Poperelya 1980; Metakovsky et al. 1984a; Payne et al. 1984).

Coincident with these genetic studies has been the development of monoclonal antibody probes for groups of gliadin proteins, to study their structure and function and to develop tests for the identification and quantification of markers of dough quality (Skerritt et al. 1987; Skerritt 1988; Dawood et al. 1989; Skerritt 1990). While several antibodies, specific for small groups of gliadins, have been produced, indicating that the gliadins bear regions of surface sequence or structural homology, the genetic relationships between these components have not been studied. In this study, the interaction of gliadin-binding monoclonal antibodies with the protein products of genes on specific wheat chromosomes is examined, using aneuploid lines of Chinese Spring hexaploid wheat and a number of diploid primitive wheats. Several antibodies were found to bind specifically with gliadins encoded by genes located on homoeologous arms; in at least one case, these groups of gliadins corresponded to a specific "block" of components.

* Present address: Department of Haematology, Royal Prince Alfred Hospital, Missenden Road, Camperdown, NSW 2050, Australia

Materials and methods

Wheat stocks

Chinese Spring hexaploid wheat (*Triticum aestivum* L.) and various nullisomic-tetrasomic lines derived by Sears (1954) were provided by Dr. K. W. Shepherd, University of Adelaide, Australia. In these lines, particular chromosome pairs have been deleted and substituted with an additional homoeologous chromosome pair. Bezostaya 1 was authenticated by one of us (EVM), while other varieties were obtained from authenticated stocks held by the Australian Winter Cereals Collection (Tamworth, NSW). Primitive wheats, two accessions each of *Triticum tauschii* var. *strangulata* and *T. tauschii* var. *meyen*, *T. monococcum*, *T. speltoides*, *T. bicorne*, *T. sharonesis*, and one accession of *T. longissima* were a gift of Dr. E. Lagudah, CSIRO Division of Plant Industry, Canberra.

Monoclonal antibodies and ELISA assays

The preparation, specificities, and isotypes of monoclonal antibodies used in this study have been described earlier (Skerritt et al. 1984; Skerritt and Underwood 1986; Donovan et al. 1989). In some cases, antibody binding to gliadin extracts of nullisomic-tetrasomic stocks and euploid Chinese Spring wheat was assessed using a indirect antigen-competition ELISA (Skerritt et al. 1987). Antibody concentrations were titrated to yield an absorbance of 1.0. In these assays, greater binding of gliadin antigen in a test sample is reflected by greater inhibition of binding to solid, phase-immobilized gliadin relative to an antigen-free control (Fig. 1). Where antibodies had insufficient affinity to function in competition ELISA, a nitrocellulose membrane radioimmunoassay was used (Skerritt et al. 1988a).

Polyacrylamide gel electrophoresis and immunoblotting

Gliadin proteins (prepared from extraction of flour or grain using 1 *M* urea) were fractionated by electrophoresis under acidic (4 m*M* sodium lactate buffer, pH 3.1) conditions, which fractionates them on the basis of both charge and size. Analysis of antibody binding to gliadins in nullisomic-tetrasomic lines (Fig. 2) or components of gliadin "blocks" (Figs. 3 and 4) was performed using large (19 × 16 cm) homogeneous (8.3% polyacrylamide, 3.3% cross-linked) gels run for 1800 Vh, while analysis of primitive wheats (Fig. 6) and other nullisomic-tetrasomic lines was performed using small (8 × 8 cm) 3–15% polyacrylamide gradient gels run for 500 Vh (Skerritt and Underwood 1986). The former system was used for block analysis as: (1) it provided higher resolution of α- and β-gliadins, and (2) earlier block identification had used homogeneous gels (Metakovsky et al. 1984a). Proteins were electroblotted (2.5 h at 36 V) to nitrocellulose membranes, using a Gradipore (Pyrmont, Australia) unit. Antibody binding to gliadin proteins on gradient-gel electroblots was detected by autoradiography, using biotinylated sheep antibodies to mouse immunoglobulins and ^{35}[S]-streptavidin or alkaline-phosphatase-labelled goat anti-mouse antibodies (Skerritt et al. 1988a).

For better identification of the chromosomal control of gliadins bound by antibodies, two controls were used: (1) for each set of electrophoretically resolved proteins blotted to nitrocellulose membranes, proteins on an identical (nonblotted) gel, run at the same time, were stained with 0.025% Coomassie Blue in 12% (w/v) trichloroacetic acid; (2) marker varieties, Bezostaya 1 and Chinese Spring (and its aneuploids), were run on each gel. The block patterns of each variety were confirmed in separate experiments (Metakovsky et al. 1990).

Results

A selection of monoclonal antibodies was chosen, representing differing specificity patterns for groups of gliadins (Skerritt 1988). Their specificities for gliadin proteins have been described earlier (Skerritt et al. 1984; Skerritt and Underwood 1986; Donovan et al. 1989), and they fit into four classes.

(1) Predominantly α- and/or β-gliadin-specific [221/23 (α), 222/5 (α, β, and γ), 227/22 (β), 230/9 (α), and 404/6 (β and γ)]. At the high antibody concentration required to produce dark staining for photography, weak reactions with other gliadins are seen: 221/23 (β), 227/22 (γ). The dependence of apparent antibody specificity on antibody concentration has been discussed elsewhere (Skerritt and Underwood 1986; Donovan et al. 1989).
(2) γ- and ω-gliadin-specific (218/17, 237/34, and 246/21). At the antibody concentration used, some binding of 218/17 to γ- and β-gliadins occurred.
(3) ω-gliadin-specific (122/24, 139/18, 304/13, and 401/21).
(4) Broad gliadin specificity (243/6, 243/11, and 245/4).

Both competition ELISA and immunoblotting techniques were used to identify the chromosomes bearing structural genes for gliadins that bound particular monoclonal antibodies. Blots were performed using all antibodies studied, while competition ELISAs were not performed with antibodies (1) which for kinetic reasons showed poor inhibition by gliadins (e.g., 122/24, 139/18, 237/24, 246/21), or (2) gave very clear chromosomal specificity results after immunoblotting (e.g., 218/17, 230/9, 304/13, 401/21, 404/6). Selected antibodies were then used in immunoblotting experiments to identify components of gliadin "blocks," while other antibodies

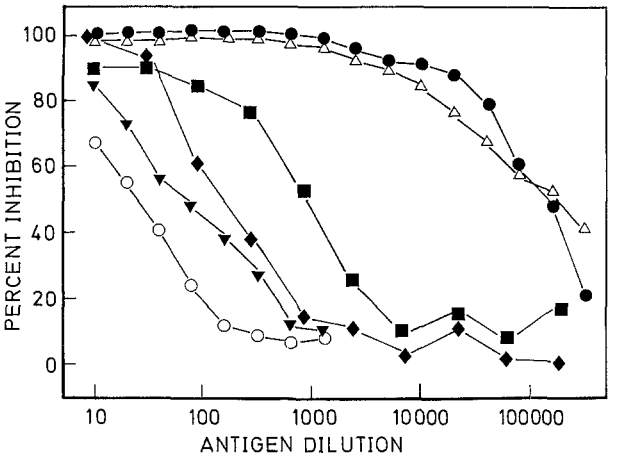

Fig. 1. Inhibition of antibody binding in competition ELISA. Chinese Spring gliadin-containing extract dilution-response curves for various monoclonal antibodies: (●) 221/23 subclone 8D11, (△) 222/5 9F9, (■) 227/22 12H12, (◆) 243/6 9E4, (▼) 243/11 7C10, (○) 245/4 1C4

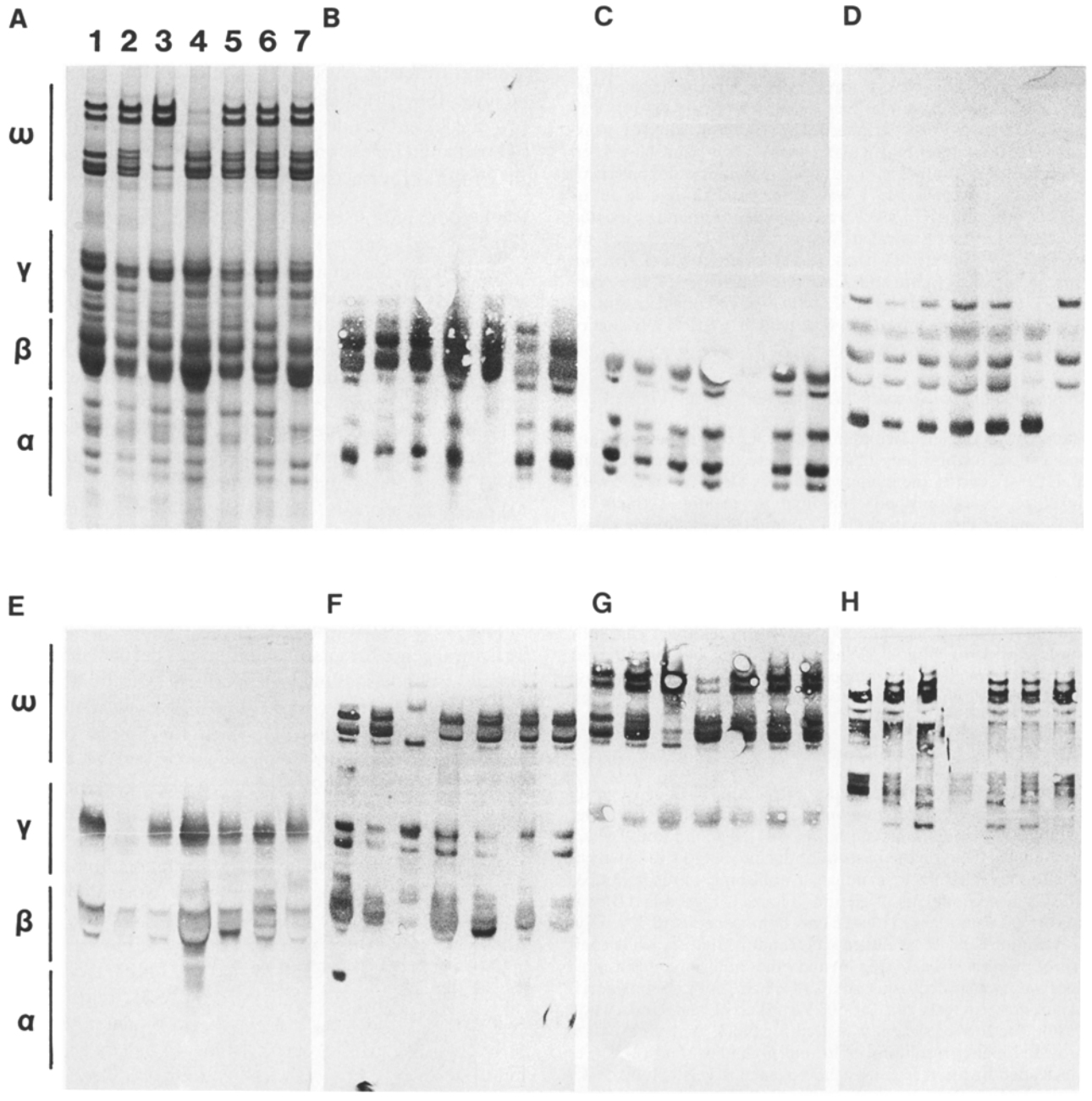

Fig. 2A–H. Homogeneous PAGE and immunoblotting of gliadin-containing extracts of Chinese Spring aneuploids. Lanes as follows: *1* Chinese Spring, *2* nulli (n) 1A-tetra (t) 1D, *3* n1B-t1D, *4* n1D-t1A, *5* n6A-t6D, *6* n6B-t6D, *7* n6D-t6A. Gliadin clusters (α, β, γ, and ω) are shown. **A** Stained electrophoresis gel. **B** Immunoblot probed with antibody 221/23, 8D11, 0.05 µg/ml purified antibody. **C** 0.13 µg/ml 230/9 1A5. **D** 0.23 µg/ml 404/6 1A2. **E** 0.44 µg/ml 227/22 12H12. **F** 0.02 µg/ml 218/17 7E8. **G** 10 µg/ml 122/24 1A9. **H** 304/13 1B2, supernatant 1/400

were used in ELISA and immunoblotting experiments to study antibody binding to gliadin extracts of primitive wheats.

Chromosome identification

Antibody 221/23 bound primarily to α-gliadins encoded by chromosome *6A*, although on the immunoblots, weak binding to *6B* (β) and *6D* (α and β) gliadins can also be seen (Fig. 2A, B). Extracts of Chinese Spring wheat lacking *6A* bound considerably less well to this antibody (Table 1). Thirty to thirty-five percent inhibition of antibody binding was produced by extracts of grain lacking chromosome *6A*, and 92% inhibition was found with extracts of euploid Chinese Spring wheat in this experiment (Fig. 1). From Fig. 1, the differences in ELISA re-

Table 1. Binding of monoclonal antibodies to extracts of Chinese Spring wheatmeal-bearing chromosomal deletions: competition ELISA

Chinese Spring	Antibody						
	Narrow specificity			Broad specificity			
	221/23	227/22 (A)	227/22 (B)	222/5	243/6	243/11	245/4
Experiment 1							
Euploid	74	39	77	55	44	50	66
n1A-t1B[a]	n.t.[b]	22	54**	62	56	59	77
n1A-t1D	65	17**	32**	49	42	43	68
n1B-t1A	n.t.	23	42**	57	58	63	75
n1B-t1D	71	27	35**	52	46	49	74
n1D-t1A	n.t.	78*	99*	62	65	66	65
n1D-t1B	79	20	52**	54	54	65	71
Experiment 2							
Euploid	92	40	77	55	44	50	66
n6A-t6B	30**	25	37**	57	55	72*	59
n6A-t6D	35**	16**	40**	60	54	64	68
n6B-t6A	95	31	64	55	56	59	65
n6B-t6D	96	20**	42**	59	49	62	75
n6D-t6A	92	40	55**	41	51	69	53
n6D-t6B	92	9**	39**	35	47	66	69

[a] n1A-t1B = Chinese Spring nulli 1A-tetra 1B, etc.
[b] n.t. = not tested
Data shown are percent inhibition of binding assessed by competition ELISA. In these assays, greater inhibition of antibody binding is produced by higher concentrations of gliadin antigens. Thus, the higher the number, the greater the inhibition. Statistical indications of errors are omitted for brevity (mean standard deviation = 5%, maximum standard deviation = 10%). Values greater (*) or lower (**) than euploid by 20% or more are indicated

Table 2. Gliadin blocks identified in wheats used in this study (data from Metakovsky et al. 1990)

Wheat variety Standards	Block number					
	1A	*1B*	*1D*	*6A*	*6B*	*6D*
Chinese Spring	14	6	3	7	3	6
Bezostaya 1	4	1	1	1	1	1
Set 1						
Gamenya A	9	1	2	24	15	18
Gamenya B	11	1	2	24	15	18
Timgalen	9	1	2	24	15	18/19
Cook	9	1	2	3	15	19
Oxley	9	1	1	3	15	19
Gambee	9	1	3	24	15	18
Falcon	1	1	2	24	c?	12
Set 2						
Tincurrin	3	10	3	2	d?	12
Gluclub	1	10	3	2	c?	12
Halberd	1	11	6	7	a?	12
Pinnacle	3	11	6	7	6	8
Millewa	11	15	1	3	15?	4
Eagle A	1	1	5	24	g?	9
Suneca	2	2	2	20	15?	15

"?" in the block designation indicates that the blocks appeared novel, by true designation could not be provided without an inheritance study

sponse arise from a 30-fold difference in the amounts of specific gliadin antigens. Deletion of various chromosome 1 pairs did not affect antibody binding. Therefore, 221/23 recognizes identical epitopes in certain α- and β-gliadins encoded by chromosomes of the sixth homoeological group.

Another antibody, 230/9, bound specifically to chromosome *6A*-encoded α- (and some β-) gliadins. On immunoblots of gliadin extracts of Chinese Spring aneuploids (Fig. 2C), binding was specifically abolished in the nullisomic chromosome *6A* flour extracts. Earlier studies using electrophoretic analyses of a number of F_2 seeds have shown how complex gliadin electrophoretic patterns can be simplified by separating out the electrophoretic bands encoded by genes at each chromosomal locus (Metakovsky et al. 1984). Therefore, the patterns obtained following probing of gliadin blots from varieties with the same block pattern will be identical. For example, those for Chinese Spring, Halberd, and Pinnacle, which all had block 6A7, were identical with 230/9, and those for Tincurrin and Gluclub (block 6A2) were identical to one another, and so on (Fig. 4B).

Two antibodies that primarily bound β-gliadins were investigated. Antibody 404/6 (which also bound certain γ-gliadins and weakly to α-gliadins) bound to nearly all of the components encoded by the *6B* block and some *6D*-controlled components (Fig. 2D). Antibody 227/22 bound to β- and weakly to γ-gliadins, but analysis of its binding to aneuploid wheat extracts by both ELISA and blotting methods (Fig. 2E, Table 1) revealed unexpected complexity of the chromosomal origin of genes bearing its epitope. Removal of chromosome *1A* or *1B* decreased antibody binding in the ELISA and also on blots, while removal of chromosome *1D* did not have a consistent effect. Removal of any of the chromosomes 6 also decreased binding; on immunoblots, different β-gliadins, controlled presumably by *6A*, *6B*, and *6D*, are bound (Fig. 2E).

Antibody 218/17 bound strongly to certain *1B* ω-gliadins and, at the concentration used, less well to some β- and γ-gliadins (Fig. 2F). Most genes encoding polypeptides binding another γ-/ω-gliadin-binding antibody (401/16) were also located on chromosome *1B*, although chromosomes *1A* and *1D* encoded minor polypeptides. Major genes for antibodies 237/24 and 246/21 (also in this specificity group) were encoded by chromosomes *1D* and *1B* (not shown). The genes encod-

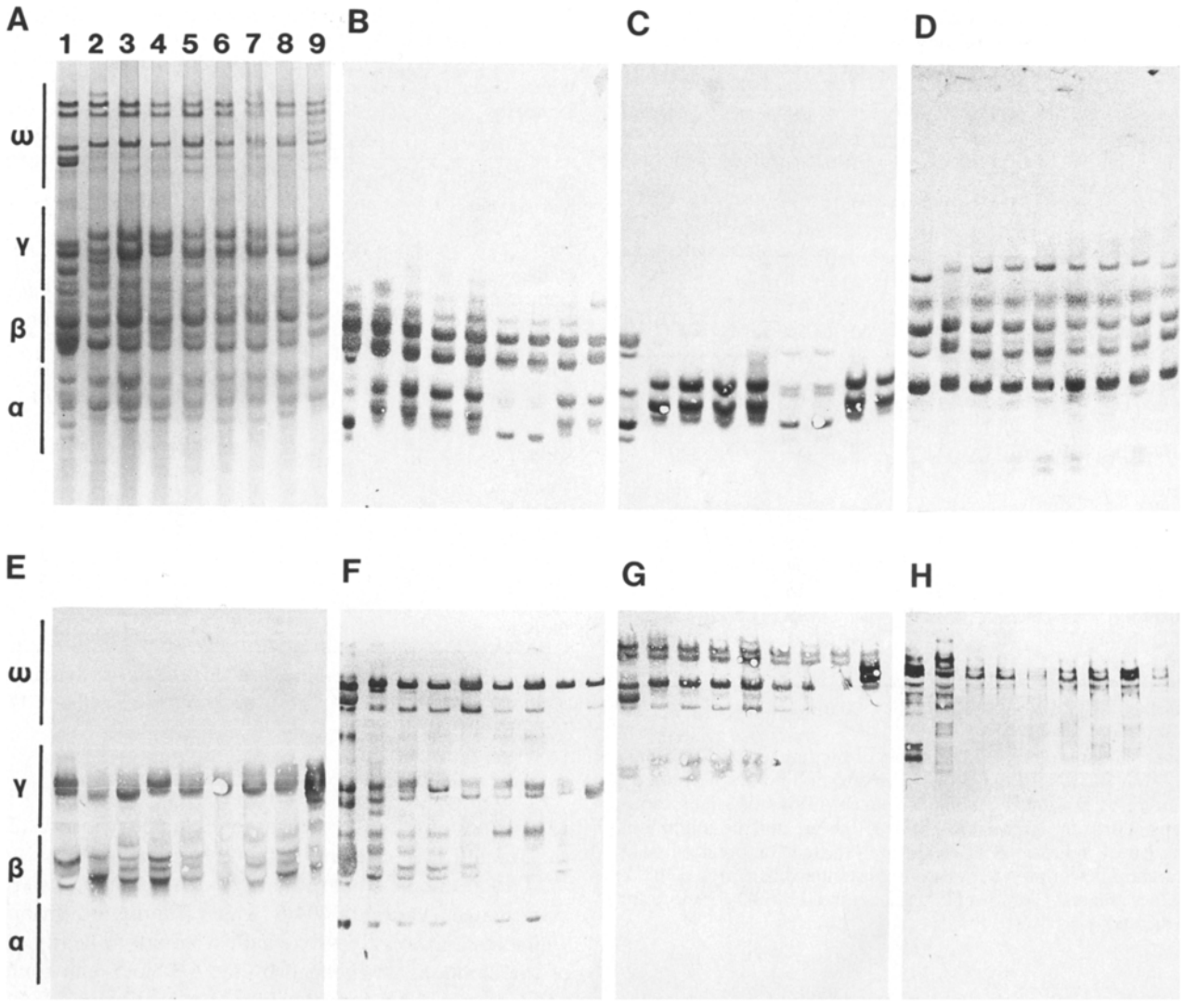

Fig. 3A–H. Identification of gliadin blocks: homogeneous PAGE and immunoblotting of gliadin-containing extracts of different wheat varieties. Lanes as follows: *1* Chinese Spring, *2* Bezostaya 1, *3* Gamenya A, *4* Gamenya B, *5* Timgalen, *6* Cook, *7* Oxley, *8* Gambee, *9* Falcon. These wheats have block formulae shown in set 1 of Table 2. Antibodies as for legend to Fig. 2

ing gliadins bound by antibodies 122/24 (Fig. 2G) and 139/18, which bound slow and fast ω-gliadins, were controlled by *1D* and less so by *1B*. Here again, antibodies only recognized certain bands in the *1D*- and *1B*-controlled blocks (Metakovsky et al. 1984a). Antibodies 304/13 (Fig. 2H) and 405/7 both mainly bound to ω- but not to γ-gliadins encoded by chromosome *1D*. Antibody 304/13 bound some fast-moving ω-gliadins on the blot that were very weakly stained by Coomassie Blue (compare Fig. 2A and 2H).

As may be predicted, the broad-specificity antibodies (222/5, 243/6, 243/11, 245/4; Table 1) bound to gliadins encoded by genes on several chromosomes. Thus, since compensated nullisomic-tetrasomic lines were used for ELISA analysis, antibody binding was not decreased by the removal of specific chromosome arms. The aneuploid Chinese Spring lines had higher grain protein contents (nulli-chromosome 1 lines: mean 140%, range 132–145%; nulli-chromosome 6 lines: mean 144%, range 133–165%) than euploid wholemeal as determined by Kjeldahl nitrogen analysis, probably resulting from lower grain yields (K. W. Shepherd, personal communication). However, protein in the 1 *M* urea [gliadin-containing extracts, analysis method of Bradford (1976)] extracts was not usually elevated as much, relative to similar extracts of euploid Chinese Spring (nulli-chromosome 1 lines: mean 114%, range 83–150%; nulli-chromosome 6 lines: mean 111%, range 87–126%). This elevation,

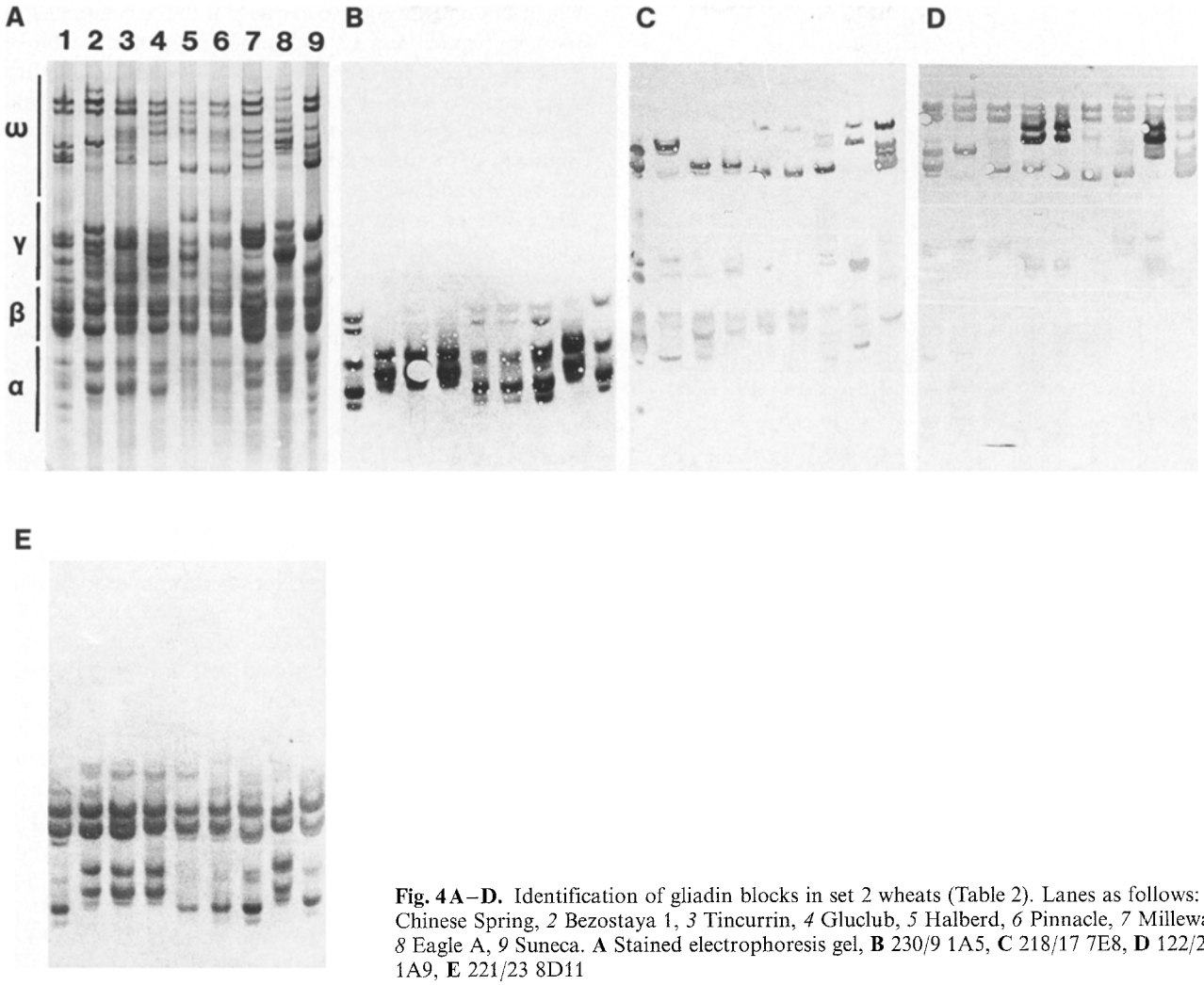

Fig. 4A–D. Identification of gliadin blocks in set 2 wheats (Table 2). Lanes as follows: *1* Chinese Spring, *2* Bezostaya 1, *3* Tincurrin, *4* Gluclub, *5* Halberd, *6* Pinnacle, *7* Millewa, *8* Eagle A, *9* Suneca. **A** Stained electrophoresis gel, **B** 230/9 1A5, **C** 218/17 7E8, **D** 122/24 1A9, **E** 221/23 8D11

although minor, led to slightly greater inhibition with most aneuploid extracts being observed in ELISA assays (Table 1).

Identification of gliadin blocks

Antibody binding to blotted gliadins was initially examined using Chinese Spring and Bezostaya 1, as these wheats have been widely used for genetic studies, and each of their gliadin blocks differed (Table 2, Akhmedov and Metakovsky 1987). In different varieties, narrow-specificity antibodies labelled groups of gliadin bands of similar overall mobilities, but the precise mobilities of components labelled and their intensities differed. These studies were extended to the analysis of two sets of seven Australian wheat varieties or biotypes (Table 2, Metakovsky et al. 1990). The sets were chosen such that pairs of wheats that differed in only one block were present; exceptions were Millewa, Eagle A, and Suneca, which had differing pedigrees (Metakovsky et al. 1990).

Results of the block analysis generally confirmed those obtained with immunoblot and ELISA analysis of extracts of nullisomic-tetrasomic lines of Chinese Spring wheat. That is, antibodies binding gliadins encoded by genes on a particular chromosome arm or arms bound to groups of polypeptides of similar mobilities in varieties with the same "block" code (Table 2). The most striking example is 230/9, which bound to all and only *6A*-controlled allelic variants of blocks – note the difference between 6A7 (Chinese Spring, Halberd, Pinnacle), 6A3 (Cook, Oxley Millewa), and 6A20 (Suneca). Allelic variants of blocks of this type differ in mobility and number of components in the middle α- and fast β-regions of the gliadin spectrum (Metakovsky et al. 1984a, 1990).

Antibody 218/17 (Figs. 3F and 4C) bound mainly *1B*-controlled ω-gliadins and, at high concentrations, some γ- and β-components controlled by other chromosomes, but not *1B*-controlled γ-gliadins in any of the cultivars studied. Antibody 404/6 (Fig. 3D) bound specifically to some *6D*- and *6B*-controlled gliadins. An-

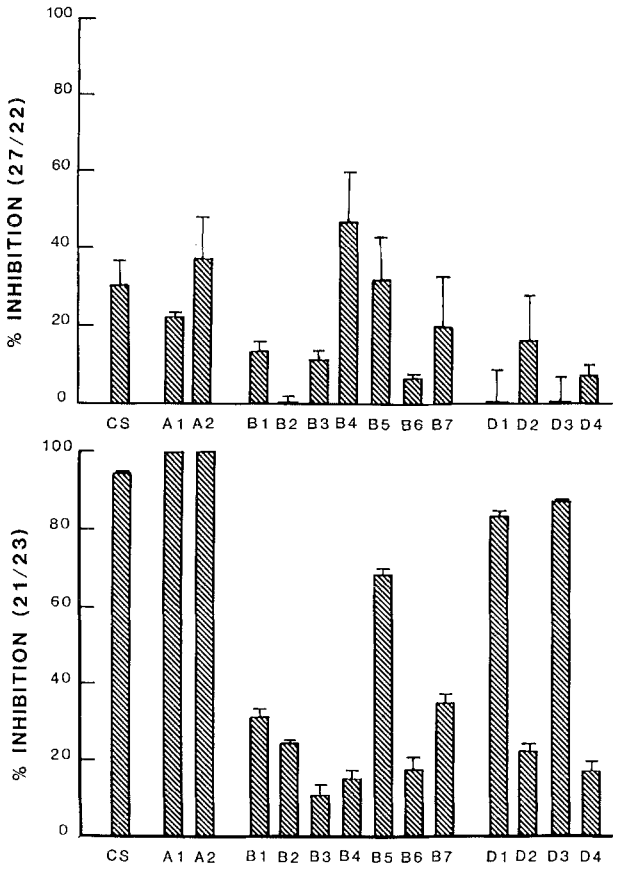

Fig. 5. Binding of an α-gliadin-specific antibody 221/23 (21/23, *lower panel*) and a β-gliadin-specific antibody 227/22 (27/22, *upper panel*) to gliadin-containing extracts of different species determined by inhibition ELISA. Wheats: CS (Chinese Spring), A1 and A2 (*T. monococcum* accessions), B1 and B2 (*T. speltoides*), B3 and B4 (*T. bicorne*), B5 and B6 (*T. sharonesis*), B7 (*T. longissima*), D1 and D2 (*T. tauschii* ssp. *strangulata*), and D3 and D4 (*T. tauschii* ssp. *meyer*)

tibody 122/24 bound slow-moving *1D*- and *1B*-controlled ω-gliadins in each of the cultivars, and in Falcon, Gluclub, Halberd, and Eagle it also bound to two minor bands controlled by 1A1 and, in Suneca, two 1A2 bands (Figs. 3A and 4A). Therefore, 122/24 antibodies are very specific to the position of protein in the spectrum, but not to its chromosomal control.

Primitive wheats

In further studies on the genome specificity of some gliadin-binding antibodies, several accessions each of *T. monococcum* (related to the A-genome of modern hexaploid wheat), *T. bicorne*, *T. sharonesis*, *T. speltoides*, and *T. longissima* (B-genome related) and *T. tauschii* ssp. *typica* and *T. tauschii* ssp. *strangulata* (D-genome related) were analyzed, as well as durum (*T. turgidum*, A- and B-genome related) and bread wheats. In ELISA assays, 221/23 (which bound to *6A*-encoded α-gliadins in bread wheat), bound strongly to extracts of the A-genome relatives, but much less to the putative B-genome relative extracts (except for one *T. sharonesis* extract). Gliadins from one accession of each *T. tauschii* subspecies bound strongly to this antibody (Fig. 5). Similar results were obtained in immunoblotting studies (not shown).

The β- (and weakly γ-) gliadin-binding antibody, 227/22, exhibited a different pattern, binding mainly to gliadins present in *T. monococcum*, and in one *T. bicorne*, one *T. sharonesis*, and one *T. longissima* extract (Fig. 5). No binding to extracts of *T. tauschii* or *T. speltoides* accessions was seen on immunoblots with 227/22 (Fig. 6A, B). These findings are in keeping with the complex specificity properties of 227/22 and, like the results obtained with 221/23, also suggest that some of the high-mobility gliadins of *T. tauschii* studied differ from those in bread wheat, since the antibody bound D-genome-encoded proteins in bread wheat.

Analysis of binding of the γ- and ω-gliadin-binding antibody, 246/21, to Chinese Spring aneuploids indicated weak binding to chromosome *1B*-encoded gliadins and strong binding to *1D*-encoded gliadins. In radioimmunoassay, this antibody bound better to bread wheat gliadins than to durum wheat gliadins. The antibody bound to gliadins from each of four accessions of *T. tauschii*, and very strongly to both *T. bicorne* and *T. sharonesis* accessions, but weakly to the other putative B-genome donors, *T. speltoides* and *T. longissima*. No binding was seen to gliadins from two of four *T. monococcum* accessions, while one accession showed strong antibody reaction and another, moderate reaction.

As may be predicted, two antibodies (122/24 and 139/18) that bind low-mobility ω-gliadins, and the broad-specificity antibody, 243/11, bound to gliadins from each of nine *T. tauschii* accessions studied (not shown); the former antibodies especially labelled clusters of chromosome *1D*-encoded ω-prolamins. Intriguingly, while 122/24 bound ω-mobility gliadins in *T. tauschii*, several prolamins of β- or γ-gliadin mobility were labelled in extracts of certain *T. sharonesis* and *T. longissima* accessions (Fig. 6B). The very high concentration of 139/18 that was used (Fig. 6D) labelled ω- and γ-gliadins most strongly in most species, except two *T. monococcum*, one *T. speltoides*, and a *T. longissima* accession, where only higher-mobility gliadins were labelled.

Discussion

The complex gliadin composition of wheat arises from the presence of structural genes for gliadins in highly linked clusters on each of six chromosomes (Wrigley and Shepherd 1973; Galili and Feldman 1983). The specificities of selected monoclonal antibodies have been further characterized by the identification of the chromo-

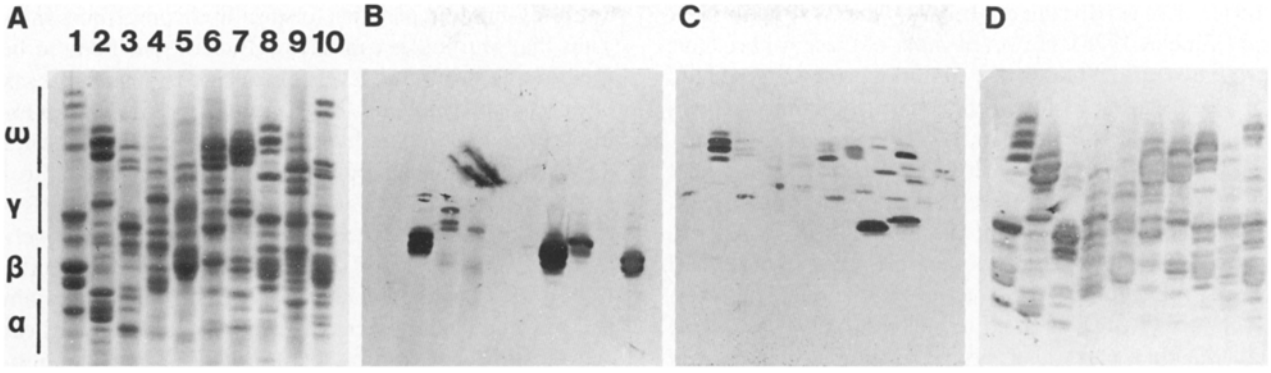

Fig. 6A–D. Specificities of monoclonal antibodies for gliadins in different primitive wheat species determined by polyacrylamide gradient gel electrophoresis and immunoblotting. Wheat symbols as for legend to Fig. 5, from left: D1, A1, A2, B1, B2, B3, B4, B5, B7, and CS. **A** Stained electrophoresis gel. **B** 227/22 12H12 supernatant 1/2000, **C** 122/24 1A9 ascites 1/1000, **D** 139/18 2A1 ascites 1/5000

some arm or arms bearing structural genes for each particular group of gliadin antigens. In some cases, antibodies bound to a set of gliadins encoded by a small family of genes on a single arm of one of the homoeologous chromosomes 1 or 6. Earlier studies (Sozinov and Poperelya 1979; Metakovsky et al. 1984) had shown that litte recombination occurs between these gene families, i.e., that the gliadins were inherited as a "block." Results from this study, using antibodies such as 122/24, 139/18, 230/9, and 304/13, provide immunochemical evidence for structural and/or sequence homology between gliadin members of a block, irrespective of which allele was present. These members may have arisen from gene duplication and divergence, following mutation to yield components with differing charges and possibly slightly different molecular masses (Metakovsky et al. 1984). However, other antibodies bound preferentially to only some members of two or more blocks (e.g., 227/22, 404/6), possibly indicating both mutation or divergence within a block, yet homology between gliadins in different blocks.

These results indicate that gliadins encoded by genes on the short arm of a particular chromosome usually bear greater homology to one another than to gliadins encoded by genes on other chromosomes. In contrast, similar attempts to identify groups of structurally related low- and high-molecular-weight glutenin subunits have shown that glutenins encoded by genes on different chromosomes are often cross-reactive with particular antibodies. For example, 218/17 bound selectively to *1B*-encoded gliadins, but equally well to low-molecular-weight glutenins encoded by each chromosome 1 genome (Skerritt and Robson 1990).

Identification of most blocks of gliadins, following one-dimensional polyacrylamide gel electrophoresis, requires prior analysis of electrophoretograms produced, using progeny from a large number of crosses with a line having a previously established block formula. As gliadin blocks are useful markers of pedigree and perhaps quality (Sozinov et al. 1974; Branlard and Dardevet 1985; Sasek et al. 1986), antibody methods offer a simple means of identifying specific blocks.

In several cases, the chromosomes bearing genes encoding specific antibody-binding gliadins could readily be predicted from the electrophoretic mobilities of the respective antigens, e.g., "slow" ω-gliadins, bound by 122/24. However, genetic control of the expression of gliadins bearing some other epitopes was very complex. While antibody 227/22 bound a tight cluster of gliadins of β-mobility and only weakly to γ-gliadins, both group 1 and group 6 chromosomes regulated expression of these polypeptides. These studies also suggest that gliadins of similar electrophoretic mobility, but encoded by genes on homoeologous chromosomes, can differ significantly in sequence or structure. In contrast, antibodies binding various low- or high-molecular-weight glutenin subunits usually bound to glutenins encoded by each of the three genomes (Skerritt and Robson 1990).

Several research groups have used immunochemical methods as an adjunct to electrophoretic techniques in the study of the nature and origin of the genomes of cultivated polyploid wheats. From serological studies, Konarev and coworkers (1979) identified two forms of the A-genome; A^b from *T. monococcum* and A^u from *T. urartu* and durum wheats. Similarly, two B-genomes were noted: B^{sp} from *A. speltoides* and B^l from *A. longissima*. The D-genome of bread wheats was most closely related to *A. squarrosa* ssp. *strangulata*. Others have identified individual gliadin bands in different varieties or species which had identical electrophoretic mobilities but were controlled by genes on different chromosomes (Metakovsky et al. 1984; Maistrenko et al. 1986). Therefore, blocks of gliadin components instead of single bands are much more valuable for the study of the origin of genomes of cultivated polyploid wheats. Immunochemical methods have been used over a number of years to study genome relationships between species in the sub-

tribe Triticinae (Bozzini et al. 1970; Aniol 1974; Konarev and Chmelev 1986). However, most of these studies have used antisera to albumin and globulin proteins (which do not show varietal polymorphism) rather than to prolamin storage proteins (Ewart 1969).

A number of putative donors of the modern wheat A-, B-, and D-genomes were also analyzed in this study. While, in many cases, antibodies bound most strongly to proteins encoded by the same genome active in hexaploid wheats, exceptions were found. For example, 221/23, which most strongly bound to chromosome *6A*-encoded α-gliadins in bread wheat, bound to some B-genome and D-genome donor (*T. sharonesis* and *T. tauschii*) gliadins, and 246/21, which bound only *1B*- and *1D*-encoded bread wheat gliadins, bound significantly to gliadins from two A-genome donor (*T. monococcum*) accessions. Indeed, we have found in immunoblotting studies that proteins in *T. tauschii* accessions with ω-gliadin-like immunoreactivity (reaction with 122/24) may have α- or β-gliadin-like electrophoretic mobilities. Odintsova and Egorov (1989) isolated a number of gliadins from the D-genome progenitor *T. tauschii*, and found that certain ω-gliadins had N-terminal sequences corresponding to B-genome-encoded gliadins in hexaploid wheat. On the other hand, Reeves and Okita (1987) found that the sequences of the α/β-gliadin genes from bread wheat and the diploid A-genome progenitor *T. uratu* differed only by a few point mutations. With these considerations in mind, antibody probes may still be of use in the analysis of alien transfers in wheat breeding (Konarev et al. 1979; Porceddu et al. 1983).

Recently, restriction fragment length polymorphism analysis has been found to be a most valuable additional approach for varietal identification and genome analysis, complementing the use of isozyme and storage protein genetic markers. Use of immunoblotting and block-specific monoclonal antibody probes may make the use of the protein markers a more powerful technique, and is technically simpler and less expensive than molecular genetic techniques. Harberd and coworkers (1985) used nullisomic-tetrasomic Chinese Spring lines to identify the chromosomal localization of genomic sequences hybridizing to a number of gliadin cDNAs. In each case, hybridization of similar intensity was seen in DNA prepared from each of the three genomes, but hybridization of the same cDNA probe to fragments from both group 1 and group 6 chromosomes was not observed. Similarly, Sharp et al. (1989) have obtained RFLP markers for most of the sets of homoeologous bread wheat chromosomes. In contrast, both monoclonal (Skerritt and Underwood 1986; this study) and polyclonal (Vu and Popineau 1987) antibodies exhibited a wider variety of hybridization patterns.

In conclusion, the results have several important implications. First, the localization of structural genes for antibody-binding proteins to specific chromosomes indicates that antibodies can have sufficient specificity to be used in measuring the levels of specific protein products of quality-related genes. For example, genetic studies using Australian wheats have indicated that the presence or absence of allelic blocks of gliadins encoded by chromosomes *6A* and *6D* was significantly related to differences in dough resistance and extensibility, respectively (Metakovsky et al. 1990). Other work has led to the development of monoclonal antibodies specific for certain gliadins encoded by genes on the short arm of wheat chromosome *1B* (Howes et al. 1989, Skerritt, unpublished), which can be used to screen for wheats bearing the *1B/1R* chromosomal translocation. Second, studies of the relationship between copy number of a particular gene family, which (as assessed by molecular biological methods) can vary between different cultivars (Reeves and Okita 1987), and the levels of gene product (protein) should be quite feasible using immunological methods. Third, it should be possible to examine more thoroughly, with quantitative immunochemical methods, other aspects of prolamin gene expression, e.g., during grain development and under environmental stresses (Skerritt et al. 1987, 1988). Other implications of antibody specificity for small groups of gliadins may be the use of a collection of antibody probes in varietal identification tests and in the purification of groups of gliadins by immunoaffinity chromatography, to enable studies of their functionalities using dough reconstitution techniques.

Acknowledgements. The authors wish to acknowledge maintenance from the Australian Wheat Research Council and the technical assistance of Ms Lisa Robson.

References

Akhmedov MG, Metakovsky EV (1987) Inheritance of gliadin components in hybrids from crossing bread wheat varieties Bezostaya 1 and Chinese Spring. Genetika 23:1478–1489

Aniol A (1974) A serological investigation of wheat evolution. Z Pflanzenzuecht 73:194–203

Bozzini A, Cantagelli P, Piazzi E, Sordi S (1970) An immunochemical approach to species relationships in *Triticum* and some related species. Theor Appl Genet 40:300–304

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Branlard G, Dardevet M (1985) Diversity of grain proteins and bread wheat quality. 1. Correlation between gliadin and quality characteristics. J Cereal Sci 3:329–343

Dawood MR, Howes NK, Bushuk W (1989) Preparation of monoclonal antibodies against specific gliadin proteins and preliminary investigation of their ability to discriminate cereal cultivars. J Cereal Sci 10:105–112

Donovan GR, Skerritt JH, Castle SL (1989) Monoclonal antibodies used to characterize cDNA clones expressing specific wheat endosperm proteins. J Cereal Sci 9:97–111

Ewart JAD (1969) Comparison of seed proteins of some Gramineae. J Sci Food Agric 20:221–224

Galili G, Feldman M (1983) Genetic control of endosperm in proteins in wheat. 1. The use of high resolution one-dimensional gel electrophoresis for the allocation of genes coding for endosperm protein subunits in the common wheat cultivar "Chinese Spring." Theor Appl Genet 64:97–101

Garcia-Olmedo F, Carbonero P, Jones BL (1982) Chromosomal locations of genes that control wheat endosperm proteins. Adv Cereal Sci Technol 5:1–47

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

Howes NK, Lukow OM, Dawood MR, Bushuk W (1989) Rapid detection of the *1BL/1RS* chromosome translocation in hexaploid wheats by monoclonal antibodies. J Cereal Sci 10:1–4

Kasarda DD, Bernardin JE, Nimmo CC (1976) Wheat proteins. Adv Cereal Sci Technol 1:158–236

Konarev VG, Chmelev VM (1986) Heterogeneity of the genome-specific proteins – serological markers of *Triticum* and *Aegilops* B- and D-genomes. Dokl Vses Akad Skh Nauk 5:7–9

Konarev VG, Gavrilyuk IP, Gubareva NK, Peneva TI (1979) Seed proteins in genome analysis, cultivar identification, and documentation of cereal genetic resources: a review. Cereal Chem 56:272–278

Maistrenko OI, Pel'tele SE, Pshenichnikova TA (1986) Study of the genetic control of gliadin biosynthesis in three unrelated varieties of wheat with the aid of ditelosomic lines. Sov Genet 22:479–485

Metakovsky EV, Novoselskaya AY, Kopus MM, Sobko TA, Sozinov AA (1984) Blocks of gliadin components detected by one-dimensional polyacrylamide gel electrophoresis. Theor Appl Genet 67:559–568

Metakovsky EV, Wrigley CW, Bekes F, Gupta RB (1990) Gluten polypeptides as useful genetic markers of dough quality in Australian wheats. Aust J Agric Res 41:289–306

Odintsova TI, Egorov TsA (1989) Isolation and characterization of gliadins from *Aegilops squarrosa*. Biochemistry USSR 54:301–310

Payne PI, Holt LM, Lawrence GJ, Law CN (1982) The genetics of gliadin and glutenin, the major storage proteins of the wheat endosperm. Qual Plant Plant Foods Hum Nutr 31:229–241

Payne PI, Holt LM, Jackson EA, Law CN (1984) Wheat storage proteins; their potential for manipulation by plant breeding. Philos Trans R Soc Lond Ser B 304:359–371

Porceddu E, Lafiandra D, Scarascia-Mugnozza GT (1983) Genetics of seed proteins in wheat. In: Gottschalk W (ed) Seed proteins, biochemistry, genetics, and nutritional value. Nijhoff and Junk, The Hague, pp 77–141

Reeves CD, Okita TW (1987) Analyses of the α/β-type gliadin genes from diploid and hexaploid wheats. Gene 52:257–266

Sasek A, Kubanek J, Cerny J, Maly J, Plocek J (1986) Parallel effects of gliadin and glutenin markers during evaluation of baking quality in common wheat. Sci Agric Bohemoslov 18:87–95

Sears ER (1954) The aneuploids of common wheat. Research Bull 572, Missouri College of Agriculture, Agricultural Experimental Station, Columbia/MO

Sharp PJ, Chao S, Desai S, Gale MD (1989) The isolation, characterization, and application in the Triticeae of a set of wheat RFLP probes identifying each homoeologous chromosome arm. Theor Appl Genet 78:342–348

Skerritt JH (1988) Immunochemistry of cereal grain storage proteins. Adv Cereal Sci Technol 9:263–338

Skerritt JH (1990) Simple antibody-based test for dough strength. In: O'Brien L, Ellison FW, Hare RA, Mackay MC (eds) In: Proc 6th Assembly Wheat Breeding Soc, Australia, pp 253–265

Skerritt JH, Robson LG (1990) Wheat low-molecular-weight glutenin subunits – structural relationship to other gluten proteins analyzed using specific antibodies. Cereal Chem 67:250–257

Skerritt JH, Underwood PA (1986) Specificity characteristics of monoclonal antibodies to wheat grain storage proteins. Biochim Biophys Acta 874:245–254

Skerritt JH, Smith RA, Wrigley CW, Underwood PA (1984) Monoclonal antibodies to gliadin proteins used to examine cereal grain protein homologies. J Cereal Sci 2:215–229

Skerritt JH, Martinuzzi O, Wrigley CW (1987) Monoclonal antibodies in agricultural testing: quantitation of specific wheat gliadins affected by sulfur deficiency. Can J Plant Sci 67:121–129

Skerritt JH, Lew PY, Castle SL (1988) Accumulation of gliadin and glutenin polypeptides during development of normal and sulfur-deficient wheat seed: analysis using specific monoclonal antibodies. J Exp Bot 39:723–737

Sozinov AA, Poperelya FA (1979) Genetic classification of prolamines and its use for plant breeding. Ann Technol Agric 29:229–245

Sozinov AA, Poperelya FA, Stakanova AI (1974) Use of electrophoresis of gliadin for selection of wheat by quality. Vestn Skh Nauki Moscow 7:99–110

Vu AT, Popineau Y (1987) An immunochemical study of two γ-gliadin components from common wheat. J Cereal Sci 6:15–25

Wrigley CW, Shepherd KW (1973) Electrofocusing of grain proteins from wheat genotypes. Ann NY Acad Sci 209:154–162