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# Interaction between genes controlling a new group of glutenin subunits in bread wheat

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**Summary.** One-dimensional sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of reduced total protein extracts from the endosperm of hexaploid wheat revealed a new set of faintly-stained bands, having slower electrophoretic mobility than the high-molecular-weight (HMW) glutenin subunits. These new bands have been termed the E group of glutenin subunits. Analysis of aneuploid stocks of Chinese Spring wheat has shown that three of the E bands, in order of increasing electrophoretic mobility, are controlled by genes on the short arms of chromosomes 1B, 1A and 1D, respectively. The E bands are expressed only in the presence of the long arm of chromosome 1B indicating an interaction between two or more genes involved in their production in wheat endosperm. The gene on the short arm of chromosome 1D controlling an E subunit recombined freely with *Tri-D1* and the centromere but not at all with *Gli-D1,* indicating additional complexity at the *Gli-D1* locus in wheat.

**Key words:** Wheat – Glutenins – Genetic interaction – Linkage mapping – SDS-PAGE

## Introduction

Glutenin, the major determinant of the elasticity of wheat flour dough (Wall 1979), is a heterogeneous mixture of disulphide-linked aggregates of two major size classes of polypeptides, the high-molecular-weight (HMW = 80-140 kilodaltons) and low-molecular-weight (LMW = 35–51 kilodaltons) subunits (Bietz and Wall 1973, 1980). Collectively these have also been referred to as glutenin subunits A, B, C and D (Payne and Corfield 1979; Jackson et al. 1983). The HMW glutenin subunits (A subunits) are easily recognized after reduction and separation by SDS-PAGE and are controlled by genes located at the *Glu-1* loci on the long arms of group 1 chromosomes of wheat (Orth and Bushuk 1974; Bietz et al. 1975; Lawrence and Shepherd 1980, 1981; Payne et al. 1980). This locus has been shown to be closely linked with the centromere (Payne et al. 1982). The LMW glutenin subunits (B, C and D subunits) have been much more difficult to characterize biochemically and genetically because upon reduction they co-migrate with the classical gliadins in one-dimensional (1-D) SDS-PAGE. However, by employing 2-D electrophoretic procedures, it has been shown that some B and C subunits and all D subunits of LMW glutenins are controlled by genes located on the short arms of group 1 chromosomes (Jackson et al. 1983). Further, it has been shown that genes controlling some of these LMW subunits are closely linked with the *Gli-1* loci (Singh and Shepherd 1984b; Payne et al. 1984, 1986a) whereas some D subunits are controlled by another locus *Glu-B2* located on the short arm of chromosome 1B, equidistant between *Glu-B1* and *Gli-B1* (Jackson et al. 1985). Similarly, using a two-step SDS-PAGE procedure, Singh and Shepherd (1984b, 1987b) showed that some of the B subunits of LMW glutenin are coded by a locus *Glu-B3,* located very near to *Gli-B1* on the short arm of chromosome 1B.

Recently we observed that when reduced total protein extracts from the endosperm of many wheat cultivars, including Chinese Spring wheat, are separated by SDS-PAGE, some faint bands occur near the HMW subunits but closer to the origin. Since these bands are electrophoretically different from the triplet bands described by Singh and Shepherd (1985), the oligomeric bands of HMW glutenin subunits detected by Lawrence and Payne (1983) and the HMW glutenin subunits, we

believe that they represent a new class of wheat endosperm proteins. Furthermore, since they appear only after reduction with ME, it is likely that they occur as disulphide-linked aggregates in the endosperm and we have designated them as E subunits to distinguish them from the A, B, C and D glutenin subunits described earlier (Payne and Corfield 1979; Jackson et al. 1983). In this paper we report on the genetic control of the E subunits and an apparent genetic interaction which governs their expression.

## Materials and methods

*Wheat stocks analysed*

The following lines of Chinese Spring (CS) wheat, originally obtained from Dr. E.R. Sears and now maintained at the Waite Agricultural Research Institute, were used: euploid, nullisomic-tetrasomics (NT) 1A-1B, 1A-1D, 1B-1A, 1B-1D, 1D-1A and 1D-1B; ditelocentrics (Dt) 1AL, 1BL, 1BS and 1DL. Seeds of India-115, Chinese Spring-Imperial rye substitution line 1R (1B) and translocation lines 1BS-1RL, 1DS-1RL and 1DL-1RS were obtained from stocks produced at this Institute.

*Extraction of seed proteins*

Unreduced total protein extracts were obtained by treating crushed endosperm halves of single kernels of wheat with SDS in a Tris-HCl buffer pH 6.8 without mercaptoethanol (ME) as described by Singh and Shepherd (1985). When reduced proteins were required, 1.5% (v/v) ME was added to the above solvent buffer (Lawrence and Shepherd 1980). The proteins were extracted either overnight at 40 °C or for 2 h at 60 °C.

*One-dimensional SDS-PAGE*

The discontinuous system of SDS-PAGE was based on the method of Laemmli (1970) as modified by Lawrence and Shepherd (1980), except the gels (160 cm long × 150 cm wide × 1.5 mm thick) were more concentrated [8.75% (w/v) acrylamide]. Electrophoresis was carried out at a constant current of 25 mA/gel for about 4–5 h. The gels were stained as described by Lawrence and Shepherd (1980), but destaining was carried out in distilled water for 48 h following Singh and Shepherd (1985).

*Genetic analysis*

Test cross progeny were produced to map the genes controlling E bands on chromosome arm 1DS with respect to the *Tri-D1* and *Gli-D1* loci and the centromere, by using the translocation stock CS 1DS-1RL. Providing aneuploids can be excluded, just two types of progeny (parental and recombinant) are expected and the recombination fractions (p) can be calculated directly by dividing the observed number of recombinants with the total number of euploid progeny analysed (n).

The standard error ($s_p$) of the recombination fraction was calculated using the formula for the binomial distribution (c.f. Mather 1951)

$$s_p = [p(1-p)/n]^{1/2}$$

Where no recombinants were detected between the two protein markers in the test-cross progeny, the upper limit for the recombination value (at the 95% confidence level) between the genes controlling them was claculated using Stevens' (1942) table.

## Results

*Detection of E bands and their genetic control*

When the E bands were first detected they were very faint but it was found that they could be intensified by loading more protein sample in the gel (15–20 µl), electrophoresing them in a gel of lower concentration (8.75% w/v acrylamide), extracting for a longer period of time (16 h at 40 °C) or extracting at a higher temperature (60 °C for at least 2 h). Under these conditions, Chinese Spring wheat showed three clearly visible single bands and one diffuse double band in the E region. Analysis of aneuploid stocks of Chinese Spring wheat (Fig. 1) revealed that the band of intermediate mobility was absent when the short arm of chromosome 1A was removed (Fig. 1 b). Analysis of Dt 1BL and Dt 1DL stocks showed that the slowest and fastest moving bands were controlled by the short arms of chromosomes 1B (Fig. 1 c) and 1D (Fig. 1 d), respectively. In addition, there appears to be an E band controlled by the short arm of rye 1R, which overlaps the Chinese Spring 1D band because the wheat-rye translocation stock 1DL-1RS possesses a band in this position even though the 1DS arm is absent. This conclusion was supported by the observation that increasing the dose of the 1RS arm from two to four to six, resulted in a progressive increase in the staining intensity of this band (Gupta and Shepherd, unpublished).

The fourth band of the set was difficult to analyse because of its diffuse nature. However, there is an indication that it is in fact a double band, with the faster moving component controlled by 1AS and the slower one controlled by 1DS.

In addition to this set of bands, close inspection of these gels revealed another set of four bands (E′ zone, Fig. 1) nearer to the origin than the E bands. Although these bands are very difficult to analyse because of their diffuseness and low staining intensity, changes in the patterns of the E′ bands in the aneuploid stocks appeared to parallel the changes in the E band patterns, suggesting some connection between these two sets of bands.

*Effect of chromosome arm 1BL on the expression of E subunits*

The one-dimensional SDS-PAGE patterns of reduced proteins extracted from NT 1A-1B, 1A-1D, 1D-1A and 1D-1B (data not shown) confirmed the results obtained with the Dt 1AL and Dt 1DL stocks. However, the electrophoretic patterns obtained with NT 1B-1A and

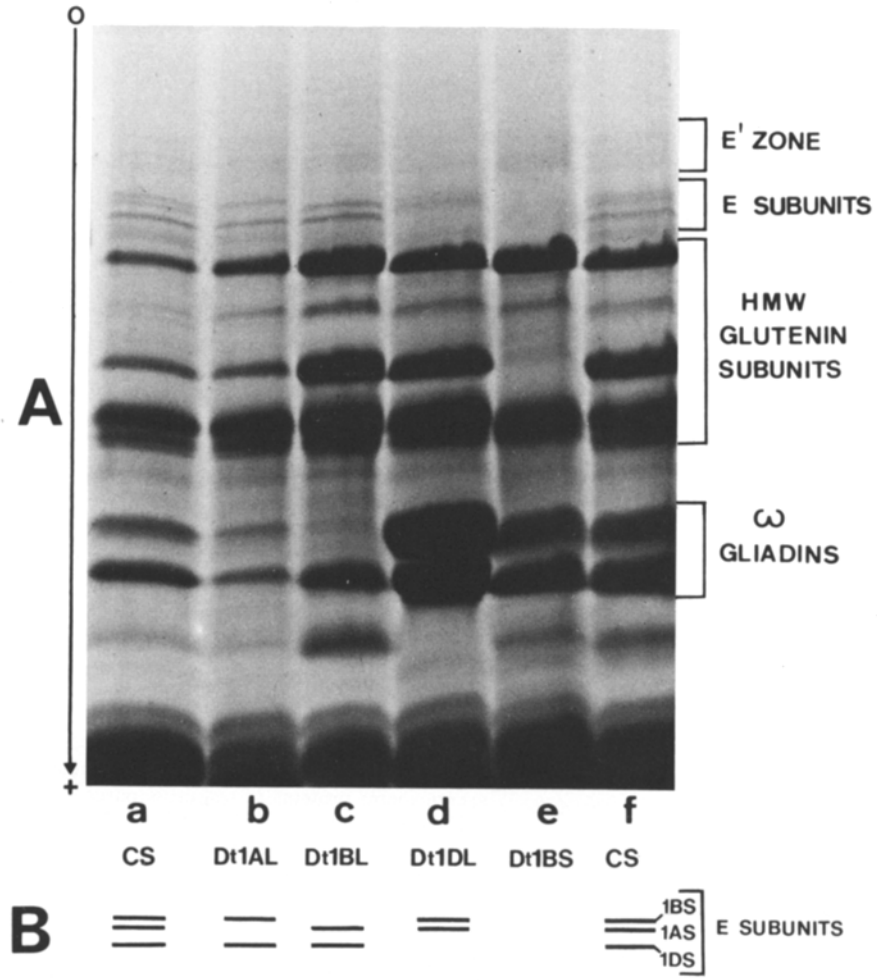


**Fig. 1 A, B.** (**A**) SDS-PAGE patterns of reduced endosperm proteins of Chinese Spring (CS) euploids and ditelocentric (Dt) stocks, and (**B**) diagrammatic representation of E subunits. The *arrow* indicates direction of protein migration

1B-1D and the substitution line 1R (1B) (Fig. not shown) were very different from those observed with Dt 1BL, since none of the E bands were present in these stocks. Furthermore, stocks Dt 1BS (Fig. 1 e) and translocation 1BS-1RL (data not shown) lacking only the long arm of 1B, showed the same effect on these E subunits. From these results, it is concluded that the expression of the E group of subunits is dependent on the presence of the long arm of chromosome 1B. It is possible that this arm carries gene(s) having a regulatory effect on their expression. In contrast, the removal of chromosome arm 1BL did not have any obvious effect on the pattern of bands in the E′ zone.

*Linkage mapping*

The CS 1DS-1RL translocation stock was employed to map the gene controlling the E subunit on chromosome arm 1DS with respect to the *Tri-D1* and *Gli-D1* loci and the centromere. As discussed by Singh and Shepherd (1984a), the translocation method of mapping can be used for determining gene-centromere distances in wheat in a way analogous to the telocentric mapping procedure developed by Sears (1962). The following test-cross was used to map the E subunit controlled by 1DS;

[(Chinese Spring 1DS-1RL × India-115) ×
Chinese Spring NT 1D-1A]

This parental combination was chosen because each parent has a contrasting phenotype with respect to Tri-D1, Gli-D1 and E bands. The Tri-D1 band of India-115 is recognizably faster than that of CS (Fig. 2 b) and India-115 does not have any bands which overlap those labelled Gli-D1cs and E-D1cs in CS 1DS-1RL (Fig. 3 A, cf. slots b and c). Also, the genes on the long arm of 1D in India-115 produced two widely-separated HMW glutenin subunits (Fig. 3 A, slot c), while the equivalent genes on 1RL in CS 1DS-1RL produced two narrow bands (Fig. 3 A, slot b). The protein bands controlled by these different genes could be easily recognized in the test-cross progeny because the male parent used (CS NT 1D-1A) was deficient for chromosome 1D and did not contribute any bands

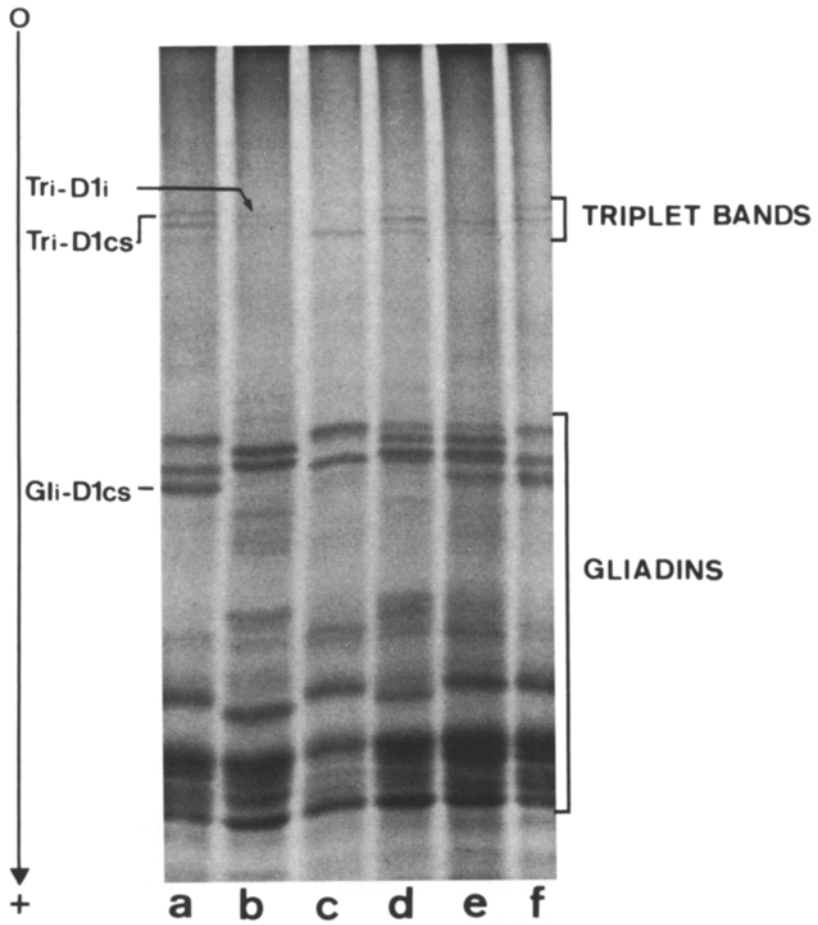


**Fig. 2.** SDS-PAGE patterns of unreduced endosperm proteins of test cross parents *a* CS 1DS-1RL, *b* India-115, *c* CS NT 1D-1A and progeny (*d, e, f*). The *arrow* indicates direction of protein migration

which overlapped those of interest in the $F_1$ heterozygote. Thus, the presence of recognizably different protein markers on each of the four arms of the 1D/1DS-1RL bivalent allowed test-cross progeny to be scored in gels for parental and recombinant types and also for aneuploid variants.

The triplet and gliadin bands were scored after SDS-PAGE of unreduced protein extracts (Fig. 2), whereas the HMW glutenin and E subunits were scored after the same extracts had been reduced and re-run on SDS-PAGE (Fig. 3 B). Although the Gli-D1cs band was more easily scored in the unreduced gels, it could also be seen in the reduced gels and this provided a cross check on the first scoring. Progeny resulting from recombination between genes *Tri-D1* and *Gli-D1* would have phenotype Tri-D1cs + Gli-D1i (Fig. 2 d) or Tri-D1i + Gli-D1cs (Fig. 2 e), and as shown in Table 1 these recombinants occurred in high frequency. In contrast, no recombinants were detected between the genes controlling Gli-D1 and E-D1 and all progeny had the parental phenotypes, Gli-D1i + E-D1i or Gli-D1cs + E-D1cs (Fig. 3 B, slots a, b, c, Table 1).

The recombination value between loci *Tri-D1* and *Gli-D1* (including the genes controlling E bands) and the centromere, was calculated using the *Glu-1* locus as a marker for the centromere. This approach will be valid if there is no pairing and crossing over between the homoeologous arms 1DL and 1RL as expected because of the inhibitory effect of the *Ph1b* gene on chromosome 5B on homoeologous pairing. The progeny resulting from recombination between *Gli-D1* and the centromere would have the phenotype Gli-D1cs + Glu-D1a (Fig. 3 B, slot, c) or Gli-D1i + Glu-R1 and those between *Tri-D1* and the centromere would be Tri-D1cs + Glu-D1a (Fig. 2, slots, d, f, and their corresponding slots, a, c, in Fig. 3 B) or Tri-D1i + Glu-R1 (Fig. 2, slot, e, and its corresponding slot, b, in Fig. 3 B). The recombination percentages calculated from these data are included in Table 1.

These results indicate that the genes controlling E subunits are loosely linked with the *Tri-D1* locus and the centromere. On the other hand, no recombinants were detected between genes controlling Gli-D1 and E-D1 among 192 euploid test cross progeny giving an upper limit to the recombination value of 1.5% (95% level of probability).

Overall, 10.2% of the test cross progeny were aneuploids but these were ignored in the estimation of the

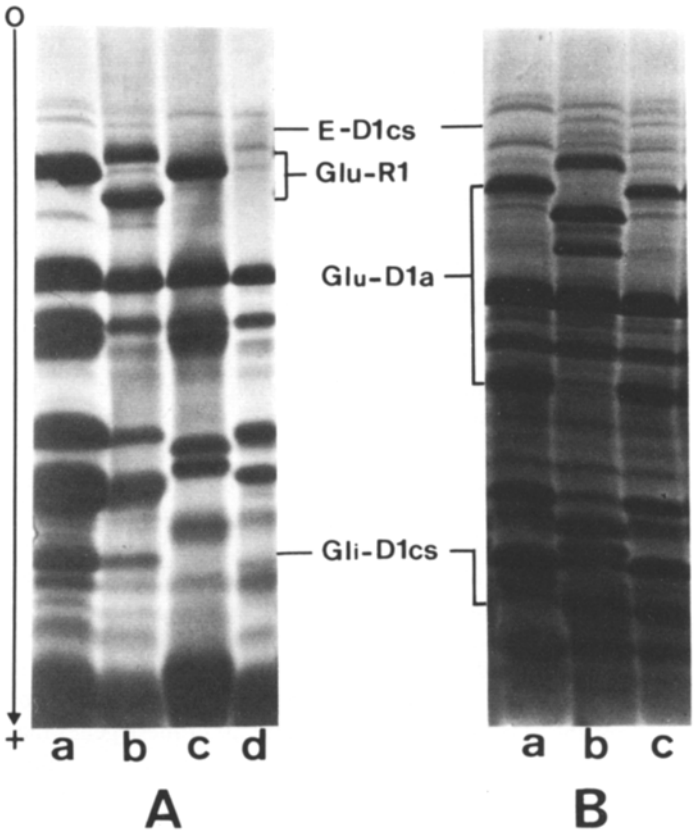


**Fig. 3 A, B.** SDS-PAGE patterns of reduced endosperm proteins of test cross parents and progeny. **A** *a* Chinese Spring Test cross parents, *b* CS 1DS-1RL, *c* India-115, *d* CS NT 1D-1A. **B** Test cross progeny (*a, b, c*). The *arrow* indicates direction of protein migration

recombination frequencies as it was shown earlier that they do not have a major effect on such estimates (Singh and Shepherd 1984a, 1987b).

## Discussion

The E subunits, constitute a minor but distinctive group of proteins in wheat endosperm. Their electrophoretic mobilities are different from that of all previously described wheat endosperm proteins and, therefore, they are considered to be a new class of proteins. These subunits are thought to be capable of aggregating through disulphide linkage as they do not appear in gels until after extracts have been treated with a disulphide cleaving reagent. Using the two-step SDS-PAGE procedure of Singh and Shepherd (1984b), the E bands appeared in the second step of SDS-PAGE, along with the other disulphide-linked HMW and LMW glutenin subunits (A, B and C subunits), suggesting that they may also be a part of the disulphide-linked aggregates making up the glutenin complex. However, before classifying them as glutenin subunits, it is necessary to consider their solubility characteristics.

When wheat flour was sequentially extracted at room temperature using the classical solvents of Osborne (1907), viz, distilled water, 0.04 M NaCl solution and 70% aqueous ethanol solution, the E bands did not appear in SDS-PAGE. However, when flour was treated with 70% ethanol solution at 60 °C these proteins were extracted along with HMW glutenin subunits (Gupta and Shepherd, unpublished). Although glutenins, as defined by Osborne, are not extractable in aqueous alcohols in the sequential extraction procedure, there is evidence that they are soluble in aqueous ethanol after reduction and alkylation (Bietz and Wall 1973) and, at low pH, after reduction only (Shewry et al. 1986). Furthermore, it has been shown that they are directly soluble in the higher alcohols e.g. n-propanol (Field et al. 1982) at room temperature. Therefore, their solubility is consistent with that of the classical glutenins.

The storage proteins of wheat, including HMW and LMW glutenin subunits, gliadins and triplet bands, are known to be deposited in protein bodies during endosperm development (Payne et al. 1986b; Singh and Shepherd 1987a). To determine whether the E subunits are also located in protein bodies, the sucrose density gradient fractions isolated by Singh and Shepherd (1987a) from the developing endosperm of Chinese Spring wheat, were re-analysed by SDS-PAGE.

**Table 1.** Observed frequency of Gli-D1, E-D1, Tri-D1 and Glu-1 protein phenotypes among test-cross progeny, grouped according to inferred meiotic events

| Progeny class | Protein phenotypes | | | Observed frequency[b] |
|---|---|---|---|---|
| | Gli-D1/E-D1[a] | Tri-D1 | Glu-1 | |
| Parentals: | | | | |
| | cs | cs | R1 | 32 |
| | i | i | D1a | 53 |
| Recombinants: | | | | |
| c.o. region 1 | | | | |
| | cs | i | D1a | 41 |
| | i | cs | R1 | 39 |
| c.o. region 2 | | | | |
| | cs | cs | D1a | 8 |
| | i | i | R1 | 7 |
| double c.o. | | | | |
| | cs | i | R1 | 5 |
| | i | cs | D1a | 7 |
| Aneuploids: | | | | |
| hypoploid | – | – | – | 18 |
| hyperploid | cs+i | cs+i | R1+D1a | 4 |
| misdivision product | i | i | – | 1 |
| Total | | | | 215 |

c.o. region 1 = between *Gli-D1*/E-D1 gene and *Tri-D1;* c.o. region 2 = between *Tri-D1* and centromere; – protein phenotypes absent

[a] No recombination was recorded between *Gli-D1* and E-D1 genes

[b] Recombination percentages: Between *Gli-D1*/E-D1 gene and *Tri-D1* = 47.91 ± 3.60%; between *Tri-D1* and centromere = 14.06 ± 2.51%; between *Gli-D1*/E-D1 gene and centromere = 49.48 ± 3.61%

It was found that the E subunits were specifically associated with the fractions containing the protein bodies (Gupta and Shepherd, unpublished). Thus, it is concluded that these proteins are also part of the true storage protein complex of wheat, and from their solubility and aggregation behaviour described above they are considered to be another group of glutenin subunits.

The genetic control of these proteins was determined to find more about their possible relationship with the other storage proteins of wheat. Since the genes controlling these subunits occur on the short arms of each of the group 1 chromosomes (1A, 1B, 1D and 1R) of wheat and rye, it is assumed that they belong to a homoeologous series. The gene mapping experiment verified the linkage relationships between *Tri-D1* and *Gli-D1* and the centromere, as determined by Singh and Shepherd (1984b, 1987b). However, the most interesting result from this mapping experiment was the failure to detect any recombination between the gene controlling an E band and the *Gli-D1* locus in Chinese Spring, indicating that these genes occur at adjacent loci or as members of the same complex locus, as do genes for some of the LMW glutenin subunits (Singh and Shepherd 1984b; Payne et al. 1984, 1986a). This conclusion will not be valid, however, if the null phenotypes Gli-D1i and E-D1i in India-115 are due to a deletion in the chromosome segment corresponding to that coding for Gli-D1cs and E-D1cs in CS 1DS-1RL, since this would prevent crossing over between these genes even if they were physically far apart. Because the $F_1$ heterozygote between CS 1DS-1RL and India-115 showed a high degree of meiotic pairing between the 1DS arms (>90% at diakenesis) and India-115 possesses low-molecular-weight glutenin and other gliadin bands controlled by the short arm of chromosome 1D (Singh and Shepherd 1987b), it is unlikely that the null phenotypes in India-115 are due to any major deletion. However, the possibility of a minor deletion cannot be ruled out.

The apparent close linkage between the genes controlling gliadins, LMW glutenin subunits and E subunits adds to our knowledge of the complexity of this region of the 1DS arm and suggests that all of these genes may have evolved from a common ancestral gene by duplication and subsequent mutation. Clearly, this hypothesis needs to be further tested by comparing the amino acid composition and sequence of these three groups of proteins.

The remaining features of interest with the E bands are their dependence on the long arm of 1B for coordinate expression and their relationship with the E′ bands. Examples of possible gene interactions affecting the expression of storage proteins in wheat endosperm are rare (Shepherd 1968; Galili et al. 1986) and it is important to investigate this effect of 1B further. There are many possible explanations for the observed interaction between gene(s) on 1BL and the genes on the short arm controlling E bands, but they can be grouped into two broad categories. Either the interaction occurs up to the stage of polypeptide synthesis e.g. there may be a direct effect of a product of the 1BL gene(s) on the transcription of structural genes for E subunits, or the interaction may be a post-translational phenomenon. Post-translational cleavage of larger precursor protein into smaller polypeptides in the case of other cereals such as oats and rice is well documented (Matlashewski et al. 1982; Brinegar and Peterson 1982; Yamagata et al. 1982). Although at this stage we have no conclusive evidence to decide between these possibilities with the E bands, however, because of the apparent relationship between the two groups of bands, it is tempting to speculate that the E bands are derived from the E′ bands by post-translational cleavage controlled by gene(s) on 1BL.

Obviously much more work is required to resolve this question, and this information could lead to a better understanding of the evolutionary and functional relationships between the diverse proteins produced by this segment on the short arm of group 1 chromosomes.

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