

REVIEW

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Biochemical and Molecular Characterization of Gliadins*

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Abstract—Gliadins account for about 40–50% of the total proteins in wheat seeds and play an important role in the nutritional and processing quality of flour. Usually, gliadins can be divided into α - (α/β), γ -, and ω -groups, whereas the low-molecular-weight (LMW) gliadins are novel seed storage proteins. The low-molecular-weight glutenin subunits (LMW-GSs) are also designated as gliadins in a few publications. The genes encoding gliadins are mainly located on the short arms of group 6 and group 1 chromosomes, and not evenly distributed. Repetitive sequences cover most of the uncoding regions, which attributed greatly to the evolution of wheat genome. The primary structure of each gliadin is divided into several domains, and the long repetitive domains consist of peptide motifs. Conserved cysteine residues mainly form intramolecular disulfide bonds. The rare potential intermolecular disulfide bonds and the long repetitive domains play an important role in the quality of wheat flour. There is a general idea that gliadin genes, even prolamins genes, have a common origin and subsequent divergence leads to gene polymorphism. The γ -gliadins are considered to be the most ancient of the wheat prolamins family. Several elements in the 5'-flanking (e.g., CAAT and TATA box) and the 3'-flanking sequences have been detected, which has been shown to be necessary for the proper expression of gliadins.

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INTRODUCTION

Of all cereal grains, wheat plays an important role in human diet, and a wide diversity of food has been developed to take advantage of the properties of wheat flour. The unique properties of wheat flour primarily depend on seed storage proteins, which are the most important source of protein for human beings. Gliadins are one of the two main groups of seed storage proteins in wheat, accounting for about 40–50% of the total storage proteins, which are due to the expression of genes and their interaction with the environment [1–3].

With the intention of improving flour quality by wheat breeding, for a perspective life, extensive attention has been focused on the seed storage proteins and their new application. For example, gliadins could be employed in the development of nanoparticles, which could act as a vector for delivering certain medicine into target tissue within the human body [4]. In this paper, the properties of wheat gliadins with emphasis on the classification, gene location, biological roles, and their evolutionary origins were reviewed.

CLASSIFICATION

Because of their abundance and economic importance, wheat endosperm proteins were among the earliest proteins to be characterized. A systematic study was carried out by Osborne in the early years of the 20th century to develop a classification for cereal seed proteins on the basis of their extraction and differential solubility [5]. Four groups were classified, and prolamins (including gliadins and glutenins) were defined as soluble in 70–90% ethanol. Gliadins are monomeric proteins of 30–78 kD with poor solubility in dilute salt solutions and good solubility in alcohol : water mixtures (70% aqueous alcohol). Gliadins were successfully separated into four subfractions: α - (the fastest), β -, γ -, and ω -gliadins (the slowest) in accordance with the mobility in A-PAGE analysis [6, 7]. However, later investigation of genetic and chemical studies suggested that only three types of gliadins (α -, γ -, and ω -type or α/β -, γ -, and ω -type) exist [8, 9], which could be due to the fact that differences in net charge (e.g., electrophoresis at a low pH) did not always reflect the structural relationships of gliadins [10, 11]. The gliadins were originally identified according to their relative mobility values [7]. However, the relative mobility values of gliadins would be affected by several factors [12]. Bietz et al. [8] and Kasarda et al. [9] reclassified gliadins into the predominant sulfur-

* The text was submitted by the authors in English.

rich α/β - and γ -gliadins, and the less abundant sulfur-poor ω -gliadins. Using the N-terminal sequences in mature proteins and mobility in lactate gel electrophoresis, Kasarda et al. [9] classified the ω -gliadins into three types: the ω -1 type begin with KEL; the ω -2 type begin with ARQ, ARE or RQ; and the ω -5 type begin with SRL. Some LMW-GSs are similar to gliadins in their biochemical characteristics [13, 14] and even in their amino acid sequences [15–19]. Metakovsky et al. [20] and Anderson et al. [21] characterized gliadins as four families: α -, γ -, ω -gliadins and LMW glutenins, although there was not a general idea in the literature. Salcedo et al. [22] described ten proteins with molecular weights less than 20 kD, which were significantly smaller than that of the traditional gliadin family members, having similar alcohol-solubility and electrophoretic properties to the known gliadin groups, and suggested that these proteins be designated as LMW-gliadins. Anderson et al. [23] and Clarke et al. [24] reported that several LMW-gliadin clones were determined to be related to wheat gliadin sequences, but not similar enough to any known gliadin family. On the basis of complete sequence comparison, LMW-gliadins could be defined as four classes [23]. In conclusion, more and more gliadins have been characterized, and the gliadin family is apparently much larger and more diverse than previously thought. Classification of gliadins is very complex, and further investigations are needed.

GENETICS AND POLYMORPHISM

The evidence for close genetic linkage of some gliadin genes is consistent with a physical clustering in wheat genome [25–27], although others are more distant from one another. The genes encoding gliadins are mainly located on the short arms of group 1 chromosomes at three homologous loci (i.e., *Gli-A1*, *Gli-B1*, and *Gli-D1*) and on the short arms of group 6 chromosomes at *Gli-A2*, *Gli-B2*, and *Gli-D2* loci. Gene loci on the short arms of chromosome 1 from *Triticum monococcum* are in the relative order *Gli-A3-Glu-A3-Gli-A1* [28]. The α -gliadins are encoded by the group 6 chromosomes, where some of the γ -gliadin genes also locate. The majority of γ -gliadins and all the ω -gliadins are encoded by the *Gli-1* loci on the short arms of chromosome 1 and are tightly linked to the LMW-glutenin genes on the *Glu3* loci [29–31]. It was reported that the gliadin-like LMW-GS presumably result from *Gli-1* and *Gli-2*. Genes controlling gliadins and C-type and D-type LMW-GS are interspersed inside the *Gli-1* locus [16, 19, 20, 32]. The ω -5 type gliadins locate on the 1B arm, whereas the 1A and 1D arms encode both the ω -1 and ω -2 types of gliadins [9, 31, 33, 34]. It was speculated that ω -1 and ω -2 gliadins were encoded by the separated genes [9]. Considering the well-established importance of LMW-GS in flour quality, the close linkage between

specific gliadin genes and LMW-GS genes was useful for identifying the valuable traits that were introduced into a new cultivar, because the γ - and ω -gliadin composition could be screened more readily than specific LMW-GS, and these gliadins were potentially useful as indicators of LMW-GS alleles [35, 36]. LMW-gliadins are the new class of seed endosperm proteins [22–24], and less attention has been paid. Only a few LMW-gliadins have been assigned to the 4B, 7A, and 7D chromosomes by aneuploid analysis [37]. Each type of gliadins is encoded by members of a multigene family. There was a discrepancy between the α -gliadin gene copy number estimated on the basis of genomic Southern analysis and the protein spots on 2-D PAGE [26, 38, 39]. Possible explanations for this discrepancy are that a large percentage of pseudogenes are involved and the single spot contains proteins originated from multiple genes [39]. The discrepancy might also exist in ω -type gliadins, since many pseudogenes are present [40, 41]. It was found that the distribution of loci controlling γ - and ω -gliadins on group 1 chromosomes was more complex than originally supposed [42]. Theoretically, it is now possible to determine the exact number and sequences of the ω -gliadin genes by sequencing the tightly linked ω -gliadin loci on the short arm of chromosome 1 [41]. Gu et al. [26, 27] suggested that α -gliadin genes are not evenly dispersed in the *Gli-2* locus regions. The *Gli-2* loci represent complex genomic regions, which have undergone dynamic evolution. The existence of multiple alleles in the multigenic family accounts for a specific combination of alleles of the gliadin-coding genes in each genotype, which would result in a unique gliadin pattern [43]. The gliadin polymorphism in the wheat collections might be useful for the refinement of the geographical origin of samples [36, 43, 44].

GENE ISOLATION, STRUCTURE, AND AMINO ACID COMPOSITION

Most of the gliadin amino acid sequences were not directly obtained from protein sequencing, but rather from the analysis of cDNA and genomic DNA sequences [2, 21, 23, 25, 39, 45–47]. Gliadin genes could be isolated from cDNA and genomic libraries. However, cloning gliadin genes from genomic libraries are relatively time consuming, although it allows flanking DNA to be obtained. A general problem with the characterization of large gene families is that the identification of additional family members would strongly depend on the initial probe. PCR is rapid, but use of PCR to avoid library screening depends on knowledge of previously studied genes. Another method of gene isolation utilizes EST. A large number of random cDNA clones have been sequenced. This method is relatively fast and has the advantage of avoiding those potential biases [21, 23].

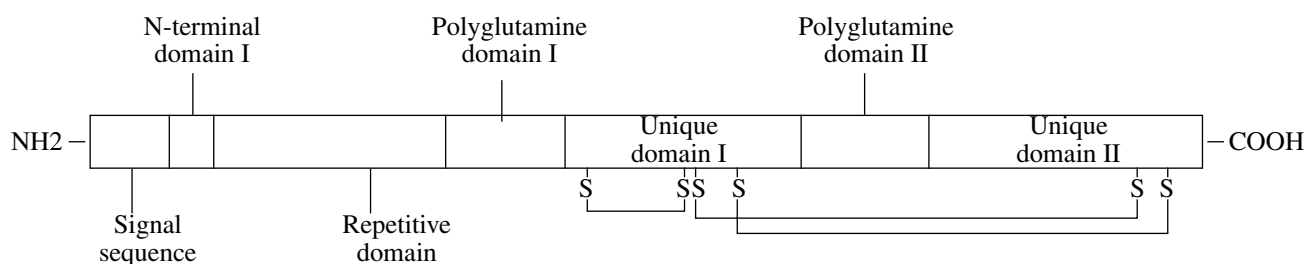


Fig. 1. Model of α -gliadin polypeptides based on Anderson et al. [39] and Müller and Wieser [49].

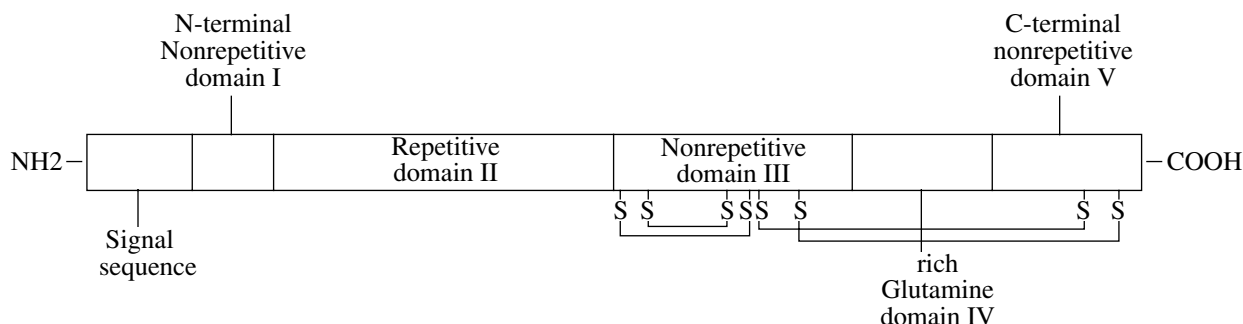


Fig. 2. Structure of γ -gliadins based on Anderson et al. [21] and Müller and Wieser (1995) [49].

α -Gliadin sequences (Fig. 1) could be divided into a 19/20-amino-acid signal peptide, absent from mature proteins, plus five distinct sections or domains of primary structure: a repetitive domain, the first polyglutamine stretch, the first region of unique sequence, the second polyglutamine stretch, and the second unique sequence [39, 45, 48, 49]. However, an N-terminal, which is a short domain with 5–14 amino acid residues, is separated from the repetitive domain [42]. The main regions of variability are centered within the two polyglutamine stretches. The repetitive domain is composed of repeat units, which have been suggested to be PQQPFP and PQQPY on the basis of protein sequences [50]. Most α -gliadins contain six conserved cysteine residues in two unique domains, which form intramolecular disulfide bonds. The α -gliadins with odd numbers of cysteine residues also have been reported [39].

The amino acid sequence of γ -gliadin (Fig. 2) starts with a 20-residue signal peptide, followed by a short N-terminal nonrepetitive domain (I), a highly variable repetitive domain (II), a nonrepetitive domain containing most of the cysteine residues (III), a polyglutamine region (IV), and the C-terminal nonrepetitive domain containing the final two conserved cysteine residues (V) [21, 51]. All the eight conserved cysteine residues form intramolecular disulfide bonds. Anderson et al. [21] reported that approximately a quarter of the γ -gliadins contain an uneven number of cysteine residues. After the formation of intramolecu-

lar disulfide bonds, free-SH would be left and could be used to form intermolecular disulfide bonds. Therefore, these gliadins could contribute to the glutenin polymer formation. In γ -gliadins, the repetitive domain is rich in β -reverse turns and might form an extended structure, while the nonrepetitive domain is rich in α -helices. The feature is also presented in α -gliadins, but the β -turns have an irregular distribution when compared with γ -gliadins [42].

The general protein structure of ω -gliadin (Fig. 3) is simple: a 19-residue putative signal peptide followed by a 10- to 11-residue nonrepetitive N-terminus, a repetitive region encompassing 90–96% of the protein, and a 10- to 11-residue C-terminus. No cysteine or methionine has been observed [9, 40]. In a consequence, no disulfide bonds exist. Therefore, a compact structure could not be observed in ω -gliadins. Tatham and Shewry [31] and Hisa and Anderson [40] noted that ω -gliadins have few charged amino acids, consistent with other gliadins [52]. For example, gliadins contain low levels of lysine [53]. Possibly, this is a feature essential for efficient self-assembly of gliadins into protein bodies [54]. The sulfur-rich gliadins have polyglutamine homopolymers or glutamine-rich regions separate from the repeat domains, while all glutamine residues in the ω -gliadins are found within the repeat units that make up the repetitive domain [40]. For ω -gliadins, proline, glutamine, and phenylalanine residues cover approximately 80% of the total amino acids compared to

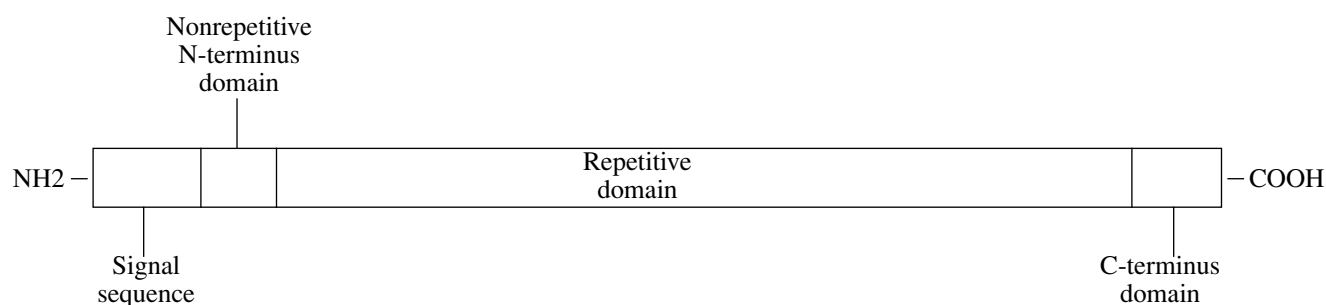


Fig. 3. Schematic of ω -gliadins based on Hisa and Anderson [40] and Shewry and Tatham [50].

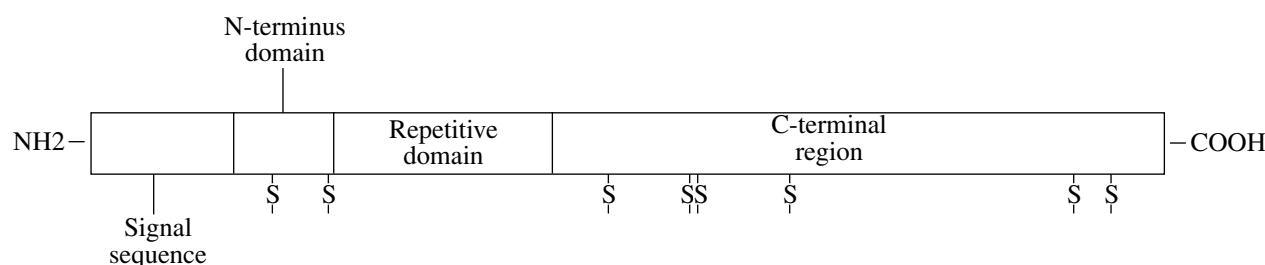


Fig. 4. Model structure of LMW-gliadins based on Anderson et al. [23] and Clarke et al. [24].

50–60% for the other gliadins [9, 40]. The ARQ/ARE-type ω -gliadins have a 4 : 3 : 1 ratio of Q : P : F for total amino acids, which is similar to the S-poor prolamins of rye (ω -secalins) and barley (C-hordeins). The SRL type has a different Q : P : F ratio (5 : 2 : 1) [31, 33, 34].

The model structure of LMW-gliadins (Fig. 4) has not been clearly defined. The amino acid sequences of these proteins consist of a 20-amino-acid signal peptide, followed by a short N-terminal sequence of 12 amino acids, containing the first two cysteine residues. However, these sequences lack the long and regular repeat motif structure in the repeating sequence domains found in traditional gliadins. Instead, reminiscent of the gliadin repeat domain, there appears a domain near the N-terminal region, followed by a C-terminal region with high homology to the γ -gliadins. The repeat domain is a proline-glutamine (PQ)-rich domain, which is the major sequence variation that discriminates the different family members. The eight cysteines involved in the LMW-gliadins are also conserved at specific positions; meanwhile, a lower content of glutamine and proline is present [23, 24]. The C-terminal region of the γ -gliadins is known to assume a compact structure with a marked surface hydrophobicity [55]. Considering the four cysteines in the C-terminal region of LMW-gliadins (only two in γ -type), Clarke et al. [24] proposed that the structure of LMW-gliadins is more compact than that observed in γ -gliadins.

The general structure of a typical LMW-GS has been reviewed by D'Ovidio and Masci [56]. The LMW-glutenin consensus motif of repetitive sequence is the least similar to the three (α -, γ -, and ω -types) consensus motifs [21]. The γ -gliadin and ω -gliadin motifs are the most similar [40]. The consensus repeat of α -gliadin is similar to, but distinct from, those of the wheat γ -gliadin [39].

In conclusion, the gliadin family now includes three classes of proteins, one class with both a large repetitive domain and a conserved set of cysteine residues (α -gliadins, γ -gliadins), one class with a repetitive domain but no cysteine (ω -gliadins), and the other class with conserved cysteine pattern but no clear repetitive domain (LMW-gliadins).

CONTRIBUTIONS TO FLOUR QUALITY

It is the primary structure of peptides which finally determines very specific properties of the ending biomaterials [57]. Unique protein sequences of the wheat storage proteins would give rise to structures that are of interest in terms of protein chemistry, modes of protein structure, and structural-function relationships. The structures are important to dough rheology and other aspects of food technology [41]. Temperature is one of the most important factors in process performance, since high temperature would result in protein unfolding and loss of functionality [58]. In the baking process, temperature is above the peak endotherm temperature [58]; thus, denaturation takes place.

Therefore, conformational changes are promoted owing to the formation of new intermolecular disulfide bonds, resulting in the setting of the larger disulfide-linked gluten protein aggregates, which contributes significantly to the characteristics of baked products [58, 59].

The proteins encoded by LMW-GS and gliadin alleles vary in structure and level of expression and influence the flour quality [30]. LMW-GS play an important role in the formation of gluten polymer. Shewry et al. [60] reported that two features of HMW-GS, which are the major determinants of wheat flour quality, might be relevant to participation as gluten elastomers in the baking quality of dough, affecting (1) the number and distribution of cysteines forming inter- and intramolecular disulfide bonds, and (2) the properties and interactions of the repetitive domain. High levels of glutamine present in the repetitive domain means that high levels of $-OH$ groups are available to form hydrogen bonds and contribute to the elasticity of the proteins [61–63]. Masci et al. [47] suggested that these long repetitive regions in LMW-GS contribute to the flour quality. Some gliadins also contain long repetitive domains, which would be relevant to flour quality. Specific cysteines of LMW-GS could form intermolecular disulfide bonds, leading to a disulfide-linked network frame [64]. The LMW-GS with only one cysteine residue available for intermolecular disulfide act as a chain terminator [64], which would presumably have a negative effect on flour quality [15]. The other type of polymer-building subunits is the chain extenders (subunits with more than one cysteine residue that form intermolecular disulfide bonds), allowing the formation of stronger dough. The significance of gliadins to the functionality of wheat flour has been the subject of considerable debate in the literature. Negative, positive, and/or no correlation were reported in functional tests [31, 63, 65–67]. Much of this discrepancy probably relates to the complex composition of the gliadin fractions, together with the sensitivity of the functional properties of the gliadins to the method of preparation [68, 69]. Gliadins were previously reported as monomeric proteins without intermolecular disulfide bonds. However, Kasarda [70] proposed that gliadins with an odd number of cysteines could form intermolecular disulfide bond and thus participate in the gluten polymer. Anderson et al. [21, 39] and Müller and Wieser [49] found that certain α - and γ -gliadins contain an odd number of cysteine residues. Changes in position of cysteine residues might affect the pattern of disulfide bond formation, resulting in a failure of two cysteine residues in a protein. Two such cysteine residues would then be available for intermolecular disulfide bond formation [19]. Lew et al. [16] and Masci et al. [18] reported that a substantial portion of the lower molecular weight polypeptides in the glutenin polymer were α - and γ -gliadins. Khatkar et al. [63] sug-

gested that the interactive behavior of gliadins (α -, γ - and ω -type) with gluten protein (LMW-GS and HMW-GS) through covalent and noncovalent bonding might be important in determining bread-making quality. It seems likely that α -, β - and γ -gliadins have a greater tendency to interact with gluten than ω -gliadins because of the presence of cysteines in these types of gliadins. Furthermore, ω -gliadins probably affect gluten viscoelastic properties by interfering with or modifying the interaction of other gluten proteins, for example, by modifying hydrogen bonds between glutenin polymers [67]. In terms of dough strength, α -, γ - and ω -gliadins might not have a direct effect on wheat quality, which might result from the participation of LMW-GS, because of tight linkage between them and the difficulty in separating these proteins [42, 70]. The abundance of the LMW-gliadin gene transcripts in endosperm and its similarity to genes encoding the seed-storage proteins suggest that they might play an important role in the quality attributes of wheat flour. A high content of cysteine in LMW-gliadins means a more compact structure, while the lack of a large repetitive domain indicates that the attribute of high levels of hydrogen bonds would be negligible in LMW-gliadins. The addition of these proteins into bread flours would provide a significant increase in loaf volume without the accompanying increase in mixing energy that would normally be required for stronger dough in all proteins tested [24]. Furthermore, a correlation seems to exist between gliadin surface hydrophobicity and loaf volume [65, 71]. The interfacial behavior of the gliadins and glutenins might influence the quality of baked products [68]. Functional studies of single gliadin by reduction and oxidation flours containing that type proteins are useful in assessing the effect of a particular protein on functional properties of dough [24, 41, 72]. A low content of lysine is observed in gliadins, which is a major negative factor affecting the nutritional quality of wheat flour [53]. Threonine and tryptophan are involved in seed at a low level, due to the low proportions of these amino acids in the gliadins [45, 52]. Adding gliadin classes to flour is to alter the relative proportions of different components in flour. Environment, e.g., temperature, could lead to the same result [73]. However, storage proteins are expressed coordinately in the developing endosperm [74], which might indicate the presence of similar controlling sequences in the genes [25, 48]. Gene-dosage compensation of endosperm proteins in hexaploid wheat is reported as well [75].

DYNAMIC EVOLUTION OF GLIADIN GENE FAMILY AND THEIR EXPRESSION PATTERNS

It was reported that the prolamin storage proteins have a common evolutionary origin [25, 52]. Prolamins are unique to the grass family of flowering

plants, which appeared late in the evolutionary process. Therefore, the genes coding for these proteins might be a recently evolved family [9]. Gliadin polymorphism in common wheat is due to genetic variation at multigenic families, which originated by successive duplication, subsequent divergence, and recombination of the ancestor genes within the families [41, 44, 52]. It was suggested that recombination has played a central role in the evolution of wheat genome structure, and gradients of recombination rates along chromosome arms promote more rapid rates of genome evolution in distal, high-recombination regions than in proximal, low-recombination regions, while mutagenic loci tend to be more frequent in distal, high-recombination regions [76]. In some cases, the wheat genome is unstable, which would result in rapid genome evolution, resulting from clusters of homologous genes that frequently undergo imperfect pairing and recombination, or unequal crossover [27]. For example, the estimated number of α -gliadin genes ranges from 25 to 150 copies [2]. The combining genomes from different species also could lead to chromosomal instability [77], although the wheat genome could tolerate major chromosomal rearrangements and deletions [78]. Therefore, the existence of multiple alleles in the multigenic family accounts for a specific combination of alleles in the gliadin-coding genes in each genotype [44]. In addition, gene conversion plays an important role in the evolutionary process [21]. Retrotransposable elements also contribute significantly to the dynamic changes of gliadin gene loci, for example, the localized expansion, which leads to an uneven distribution of genes and expansion of genomes by rapid amplification of themselves and illegitimate recombination [26, 27, 79, 80].

It was found that hexa-, tetra-, and diploid wheat species, including the ancestral genomes which have contributed to the modern hexaploid wheat, all contain α -, γ - and ω -type gliadin proteins [81, 82]. Therefore, multiplication of the origin gliadin genes might have occurred in some ancestor common to the diploid strains [48]. Unlike other wheat gliadins, α -gliadins are present only in wheat and its closely related species, not in rye and barley [50]. Therefore, α -gliadin genes are likely to have evolved after the divergence of wheat and rye [26]. A close relationship with corresponding sequences of α -gliadin fractions from bread, spelt, durum, emmer, and einkorn wheats was established by Wieser [82]. Given the structural relatedness to other gliadins (e.g. γ - and ω -gliadin), the α -gliadin genes on chromosome 6 probably originated from the gliadin genes on chromosome 1 through a duplication and/or translocation event [50]. Frequent duplication events lead to a large number of α -gliadin genes in the genome [26]. The γ -gliadins are considered to be the most ancient of the wheat prolamins family [50]. Outside of the repetitive domain II, γ -gliadins are more

conserved than α -gliadins and LMW-GS [21]. Okita et al. [54] suggested that some highly homologous γ -gliadins are a product of a recent duplication and the LMW-GS are a variant of γ -gliadins. Some LMW-GS genes are gliadin-like. The presence of these subunits is very likely due to mutations that affect the number and/or the distribution of cysteine residues [17, 19]. It is likely that mutated genes acquired only one extra or lost cysteine, so that chain terminators with odd numbers of cysteine residues emerged [16, 18]. However, Tao et al. [15, 83] proposed that the gliadin subunits are physically entrapped in the gluten polymers, due to their entangled structures. The S-poor prolamins group was hypothesized to have evolved from the S-rich prolamins by further amplification of the repeated sequences and deletion of most of the nonrepetitive domain [52]. Gianibelli et al. [84] recharacterized a previous HMW-GS as a ω -gliadin. DuPont et al. [41] reported that the 1A and 1D ω -gliadins are closely related to each other and to the ω -secalins and C-hordeins, and differ from the exceptionally glutamine-rich 1B ω -gliadins.

LMW-gliadins were characterized into new classes by analyzing the complete sequences of the cDNA clones. The first class of LMW-gliadins is most similar to the LMW-GS. The second class shows N-terminal homologies to LMW-secalins from rye and has an amino acid composition similar to wheat and barley LMW proteins with extraction properties similar to prolamins. The third class is most similar to α -gliadins, and the fourth class has no close association to previously described wheat endosperm proteins [23]. As discussed above, there seems to be no obvious gap between these gliadin groups, and close linkages exist between them, consistent with their presumed divergence from a common ancestral gene. However, genes with limited homology to other wheat seed storage proteins are excellent candidates for wheat improvement [62].

SNPs and small insertions/deletions are the most abundant form of DNA sequence variation in the genome of most organisms [85]. Zhang et al. [86] reported that the SNP rate in wheat γ -gliadin sequences is similar to that observed in the human genome. And these point mutations are randomly distributed throughout the cDNA sequences of α - and γ -type gliadins [54]. Approximately 80% of the wheat genome consists of repetitive (noncoding) DNA sequences, interspersed amongst low-copy-number or singleton genes [78]. The gene regions are more conserved. The majority of DNA changes are base substitution, with either purine to purine and pyrimidine to pyrimidine transitions, or purine $\rightleftharpoons$ pyrimidine transversions. The C $\rightarrow$ T transition is the most common mutation, causing in-frame stop codons, which contributes significantly to the high percentage of pseudogenes in α -gliadins [39]. The ω -gliadin family

also contains many pseudogene members, whereas the γ -gliadins are similar to the LMW-GS, for which only a few pseudogenes have been reported [51, 87]. Among the plenty of base change sites, only a few could result in amino acid changes, since with the exception of Met and Trp, all amino acids are encoded by two to six synonymous codons. Even though no amino acid substitutions occur in mature proteins in most cases [54], because of codon bias within genes, the level of expression of the protein would be affected [88]. The strongly biased usage of codons CCA and CCG for proline is noteworthy [54], and the stop codon for published functional α -gliadins is only UGA. Neill et al. [89] suggested that the low levels of expression of α -gliadins might be in part due to codon usage bias, since α -gliadin codons for some amino acids, especially glutamine, did not correlate well with the abundant yeast isoacceptor tRNAs. The same cases might occur in wheat endosperm. In general, genes within a taxonomic group exhibit similarities in codon choice, regardless of the function of these genes [88], which could be observed within the gliadin gene family [2, 21, 23, 25, 39, 40, 48, 54]. Pseudogenes should be considered to be necessary, since one way to overcome gene redundancy is by genetic diploidization, which in wheat is achieved through genetic repression of mutation [75, 90]. The fact that many duplicated genes are inactive pseudogenes was also reported [91]. Another way to overcome redundancy is by gene-dosage compensation, which should regulate a desirable level of protein production independently of the number of active structural genes present [75]. It was confirmed by the fact that storage proteins are expressed coordinately in the developing endosperm [74]. It was reported that the ω -gliadins are partially cleaved by a legumain-like asparaginyl endoprotease [41]. Fewer genes are required to account for the number of proteins observed if some differences in size and N-terminal sequence are accounted for by posttranslational proteolysis at the N-termini [41].

Sequence comparisons indicated that some regions within gliadin sequences are more variable. Small insertions/deletions occur frequently in those regions. Variation of the two polyglutamine domains in all α -gliadin proteins accounts for most of the difference in protein size [25, 39, 48]. These regions were encoded by microsatellite-like sequences [39]. A glutamine-rich region might have begun as a reiterant [92]. It appears that the polyglutamine stretch was initially generated by multiplication of a CAA codon and subsequently in some genes by multiplication of a CAG codon found immediately in front of the CAA stretch, and in some genes, the poly CAG stretch might have been reduced into a single codon [48]. In the genes for proteins containing the longer reiterants, the most common reiterants are those that contain poly (CAG); meanwhile, in polyglutamine domains and other cod-

ing regions of most α -gliadin sequences, the number of CAA is larger than that of CAG, which might account for the CAG to CAA transition [25, 48, 54, 92]. Green and Wang [92] confirmed that amino acid reiterants were not functional and that modification by nucleotide substitution might be important to the function of the protein. However, a large proportion of glutamine in gliadins acts importantly as a nitrogen source for seed regeneration. The rate of nucleotide substitution, the age of reiterated sequence, and selective pressure act against reiterants [92]; thus, the functional properties of polyglutamine domains could be partially explained. Sumner-Smith et al. [48] defined a P-box with a few mutational changes that were tandemly repeated four or five times within the coding region and thought it might represent the ancestral gene, which presumably arose in the ancestral α/β -gliadin gene and was multiplied prior to the extensive multiplication of the whole gene. The exact determination of what constitutes a repeat unit is partially subjective, and different motifs have been suggested by Shewry and Tatham [50] and Anderson et al. [39]. Presumably, the patterns of the repeats diverged subsequent to the separation of the gliadin gene families [39]. Anderson et al. [23] also reported that the internal repeat motifs in the repetitive domain of γ -gliadins are undergoing relatively rapid changes mainly by single base alterations and repeat duplication/deletions, and differences/similarities in repeat structure should not be used as a basis for gene-family relatedness since repeat domains are evolving relatively rapidly, and most likely independently, within each gliadin family. A clear repeating-sequence domain is absent from LMW-gliadins, due to a failure of the genes to evolve such a domain, but might also be the consequence of a loss of most or all of the DNA corresponding to the repetitive domains by the crossing-over/slip-mismatching mechanisms involved in prolamin repetitive-domain evolution [21].

No introns have been observed in gliadin genes. It was suggested that either the evolution of gliadin genes did not involve intron/extron structures, or these structures were eliminated during their evolution [25]. Wang et al. [93] indicated the important role of introns in the origin of new genes. Thus, it seems that the latter is more reasonable.

THE FLANKING DNA SEQUENCES

The flanking DNA information is mainly obtained by the sequences isolated from wheat genomic libraries and partly from cDNA sequences. This region contains promoter and other untranslated sequences. Several regulated elements have been recognized. In the 5' upstream sequence of α -gliadins, several sequences related to presumptive control elements are characterized. A "TATA" sequence of TATAAAT, a typical eukaryotic RNA polymerase II promoter sequence,

matching the consensus sequences of TATAA/TAA/T is found; further upstream is the sequence CAAAT-GCCAAT, which contains two potential "CAAT" like elements (CAAT-CAAT structures). The 3' regions contain two potential polyadenylation signals (AAATAAT) and (AATAAA). These sequences have been shown to be necessary for proper polyadenylation of mRNAs [25, 48]. TATA box and multiple polyadenylation signals have been reported for other gliadin genes [21]. Potentially significant inverted repeat structures occur in the noncoding regions of α -type sequences [25]. Several repeated sequences were also found in the common 5'-flanks [48]. Most available γ -gliadin flanking DNA has no significant homology to any known sequences, consistent with observations made with wheat prolamin gene families [21, 25]. Hisa and Anderson [40] described the divergence points in the flanking DNA sequences of members of the gliadins and suggested that conservation of flanking DNA is necessary for gene function. Heat shock element-like sequences were discovered in the promoters of gliadin genes [94], whereas there is no evidence of a significant effect on the gliadin accumulation in grains developing under heat stress [73].

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