

Plant Gene Register

High Conservation among Sequences Encoding Type-V Thionins in Wheat and *Aegilops*¹

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Thionins are Cys-rich plant polypeptides of about 5 kD that are synthesized as larger precursors, which include a typical signal peptide and a posttranslationally processed, C-terminal peptide, and are thought to be involved in plant defense (García-Olmedo et al., 1989, 1991, 1992). We have previously reported on a novel neutral thionin type in wheat (*Triticum aestivum* L.), designated type V, which has diverged extensively from type I by a process of accelerated evolution specially affecting the mature protein domain of the precursor (Castagnaro et al., 1992). It was further shown that type-V genes were located within a few kilobases of type-I genes in the long arms of group-1 chromosomes in the three genomes (ABD) of allohexaploid wheat and that both types were simultaneously expressed in endosperm (Castagnaro et al., 1992). During the transition between the two types, the nonsynonymous substitution rate in the mature protein domain of the precursor was equal to the synonymous rate, a rather singular situation, as it is now well known that nonsynonymous nucleotide substitutions, which do cause amino acid changes, occur at rates that are between 2 and 20 times slower than those of synonymous substitutions (Li et al., 1984; Graur, 1985; Graur and Li, 1988). Accelerated changes at functionally relevant sequences have been explained in terms of positive Darwinian selection (Hill and Hastie, 1987) or, alternatively, in connection with the neutral theory of evolution (Graur, 1985; Graur and Li, 1988). According to the second alternative, the relative mutability of a given sequence depends on the proportion of certain amino acid residues rather than on their specific positions, and can be predicted using an empirical index of mutability, *Im* (Graur, 1985). Because the *Im* value for the only known nucleotide sequence of type V (*Im* = 2.693) was greater than those found for type-I sequences (*Im* < 0.946), the observed divergence was compatible with a neutral hypothesis (Castagnaro et al., 1992). We have now determined two additional type-V sequences, which allow us to conclude that contrary to what would be expected from their higher *Im* values, evolution among type-V thionins is as slow as among those of type I.

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Table I. Characteristics of genomic sequences encoding type-V thionins from hexaploid wheat (*TthV2*) and *A. squarrosa* (*AthV1*)

Organisms:	<i>Triticum aestivum</i> L. cv Chinese Spring; <i>Aegilops squarrosa</i> AP1.
Loci and Products:	<i>TthV2</i> , thionin V2 from wheat; <i>AthV1</i> , thionin V1 from <i>A. squarrosa</i> .
Relevant Feature of Products:	Possible activity against plant pathogens.
Location in Genome:	Long arms of group-1 chromosomes within a few kilobases of type-I thionin genes.
Techniques:	Dideoxynucleotide sequencing of both DNA strands.
Method of Isolation and Subsequent Identification:	PCR amplification from genomic DNAs and cloning in phage M13; alignment with <i>TthV1</i> sequence.
Expression Characteristics:	Developmentally regulated, endosperm specific, synchronous with type-I genes (8–25 d after pollination).
Features of Gene Structures:	Sequences encode thionin precursors (signal peptide, mature protein, and C-terminal acidic protein) plus two introns that interrupt the acidic protein region.

The previously sequenced *TthV1* gene was associated with the D genome (Castagnaro et al., 1992), and the two new sequences, *AthV1* and *TthV2*, respectively, correspond to *Aegilops squarrosa* (DD), the diploid donor of the D genome, and to the A or B genome of hexaploid wheat. The ratio of nonsynonymous to synonymous mutation rates in the mature-protein domain of the *TthV1/TthV2* pair (*KA/kS* = 0.29) did not differ from the average for type I (Castagnaro et al., 1992). Percentages of sequence divergence for binary comparisons of equivalent domains within and between types I and V have been calculated. Coding sequences of *AthV1* and *TthV1* have been absolutely conserved during the approximately 10,000 years that the D genomes of the diploid and the allopolyploid have been evolving separately, whereas the introns have diverged, especially the larger one, which has suffered one major and several minor deletions. Percentages of divergence between types I and V at the mature-protein domain (59–69%) are about twice as great than those occurring at the other domains, namely signal peptide, C-terminal acidic peptide, and introns (21–36%). In contrast, divergence at the mature protein domain of type V is equal to or

lower than within type I, and certainly much lower than that of the corresponding introns.

Type I thionins have four disulfide bridges, whereas those of type V have only three. It is possible that a temporary loss of function due to mutation of one Cys (gain or loss) in the duplicated gene might suggest a period of accelerated evolution. Mutation of a second Cys (loss or gain) would have then led to a mature thionin with an even number of Cys's (a common feature of all known thionins) and to a recovery of function that would in turn impose a slower rate of evolution. The extreme divergence in the proportion of charged residues suggests that the functions of the two types, although similar, might be exerted in different contexts or in different cellular environments.

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