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Evolution of *FW2.2-like (FWL)* and *PLAC8* genes in eukaryotes

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The tomato FW2.2 quantitative trait locus, which regulates tomato fruit size, was genetically and physically mapped around 15 years ago. Subsequently, the *FW2.2* gene was cloned and shown to contain a PLAC8 domain, originally identified in mammalian placental proteins. Data suggest that FW2.2 likely controls tomato cell size, perhaps by direct interaction with casein kinase II. Several *FW2.2-like* (*FWL*) genes have now been identified from a variety of plant species, but until recently only the tomato *FW2.2* gene had been the subject of detailed investigation. Recently, soybean and maize *FWL* genes were identified and shown to have a role in plant organogenesis. It is now apparent that the *FWL* genes in plants are a large gene family, which is even larger given inclusion of genes for the various eukaryotic PLAC8-domain proteins. Although overall the protein sequence identity/similarity among the family members is relatively low, there is strong conservation of key domains, suggesting a conservation of the core biochemical function of these proteins. In this Addendum Article, we highlight the similarities and differences exiting between plant *FWL* genes and enlarge this comparison to the mammalian *PLAC8* genes. These comparisons suggest the possible conservation of biological function for FWL proteins.

Key words: FW2.2, PLAC8, cys-rich domain, soybean, tomato, maize, plant organ development, nodule

Abbreviations: Aa, amino acids; FWL, FW2.2-like

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The *FWL/PLAC8* Gene Family

The genome sequences of 14 plant species were mined based on similarity to the tomato *FW2.2* gene to identify 103 *FWL* genes.[1] Using similar criteria, Guo et al.[2] identified 136 *FWL* genes in 25 animal, plant and fungal genomes. Although most plant FWL proteins range from 100 to 150 amino acids, some are >400 amino-acids. The comparison of the amino acid sequences between FW2.2 homologs led to the identification of a highly conserved core domain. The strong stabilization of the *FWL* gene structure and core domain nucleotide sequences[1] logically lead to the strong conservation of the amino acid sequence (**Fig. 1**). Based on the comparison of the FWL amino-acid sequences, the core domain is divided into three sub-domains: one or two transmembrane domains surrounded by two cysteine/proline-rich domains (**Fig. 1**). The conservation of cysteine and proline residues, amino acids known to affect protein structure, supports the conservation of the tertiary structure of the FWL core domain that is probably essential to its functionality.

Despite their similarities, striking differences exist between FWL proteins. First, the core domain is surrounded by various N and C-terminal extensions leading to the heterogeneous FWL protein sizes reported earlier.[1,2] Second, FWL proteins carry one to two predicted transmembrane domains.[1,2] Because several FWL proteins were previously identified as plasma membrane proteins,[1,3] the variation of the number of transmembrane domains between FWL proteins may lead to a different organization of the core domain relative to the membrane and, ultimately, to their biological functionality.

Similarities between the FWL proteins and the mammalian placenta-specific PLAC8 proteins[4] were recently highlighted. Based on the conservation

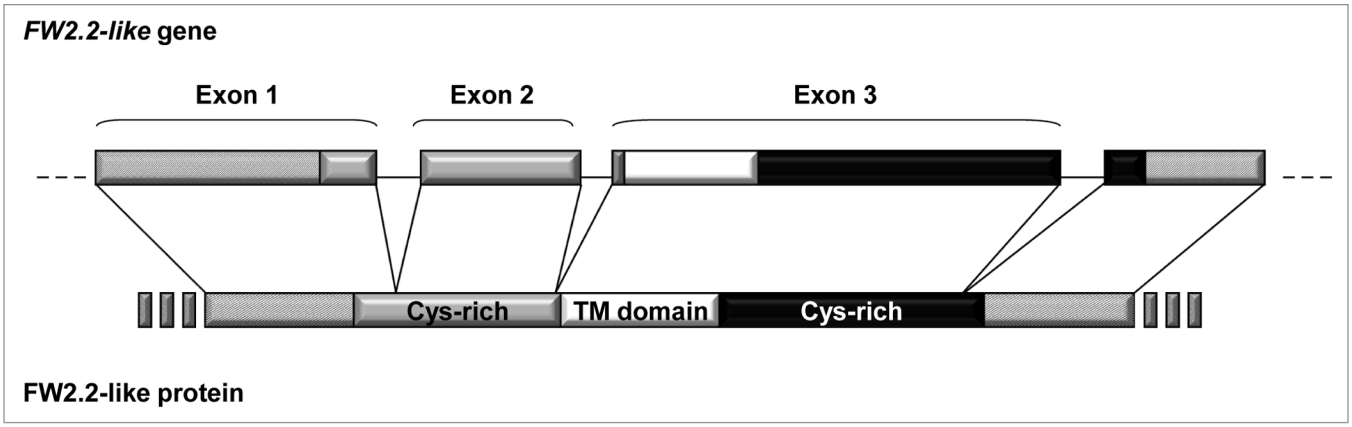

Figure 1. Schematic organization of *FWL* gene exon-intron structure encoding the protein core motif. FWL proteins share highly conserved cysteine-rich motifs [40 aa (grey) and 51 aa (black), respectively] usually separated by a transmembrane domain (white). These motifs are mainly encoded by three exons. Exons 1 and 2 encode 15 and 24 aa of the first cysteine-rich domain, respectively. Exon 3 encodes the last amino acid of the first cysteine-rich domain, the transmembrane domain and 47 aa of the second cysteine-rich domain.

of the number and location of cysteine residues, FWL and PLAC8 core domains share similar tertiary structures and potentially similar biochemical functions.[1,2] Altogether, these data support the hypothesis that *FWL* and *PLAC8* genes share a common ancestor before the divergence between plants and animals.[1,2] During their evolution, the number of plant *FWL* genes expanded faster than the number of animal PLAC8 genes. This feature suggests that each plant *FWL* gene may function in a unique tissue or cellular location, but carry out similar biochemical functions. Indeed, the expression patterns of different plant *FWL* genes can vary considerably. In some cases, *FWL* expression is restricted to a very limited number of cells. For example, At1g68610 is mainly expressed in pollen in contrast to other At*FWL* genes that have a more ubiquitous expression pattern (**Sup. Fig. 1**). In other cases; *FWL* gene expression is dependent on the response to specific treatments. For example, the soybean Gm*FWL1* and *3* are expressed specifically in root hair cells in response to inoculation with symbiotic bacteria.[1]

Biological Function of *FWL* Genes in Plant Development

FWL genes are currently emerging as essential regulators of plant organ development. For example, Le*FW2.2*, Zm*CNR1* and *2* and Gm*FWL1* regulate tomato fruit size, maize seedling and fruit development and soybean nodule development, respectively.[1,2,5] The Tanksley group at Cornell University originally identified a QTL for fruit size in studies of key domestication loci. The FW2.2 (Fruit Weight 2.2) QTL locus was shown to control 30% of the variation in tomato fruit size. Phenotypic observations of a Le*FW2.2* gene dosage series in tomato plants[6] indicated an inverse relationship between fruit size and *FW2.2* expression. Subsequently, yeast two-hybrid studies showed a direct interaction between LeFW2.2 and the β sub-unit of casein kinase II,[3] a broad specificity protein kinase previously shown to regulate the cell cycle.[7-9] These findings led to the hypothesis that LeFW2.2 controls fruit size through regulation of the cell cycle, perhaps mediated through direct interaction with casein kinase II.

More recently, the maize Zm*CNR1* and two were identified and shown to potentially regulate maize cell division,[2] or at least affect plant seedling and fruit development. The soybean *GmFWL1*,[1] gene was shown to be essential for nodule development, which arises due to infection by the symbiotic nitrogen fixing bacterium, *Bradyrhizobium japonicum*. Interestingly, silencing of *GmFWL1* expression resulted in greater condensation of cellular chromatin. Other, more distantly related *FWL* gene family members include Pa*FWL*,[10] shown to regulate avocado fruit development. Other family members have been implicated as regulators of calcium influx (At*MCA1* [11,12]), as well as conferring cadmium resistance (At*PCR1* [13]). In those cases studied (i.e., LeFW2.2 and GmFWL), the proteins appear to be localized to the plasma membrane. This suggests that these proteins may monitor the extracellular milieu of the cell and then signal to the cytoplasm, perhaps through interaction with other proteins (e.g., casein kinase II). By extension, the PLAC8 domain, of unknown function, may help mediate protein-protein interactions or be involved in the cellular signaling process.

Conclusion and Perspectives

It is now clear that the plant FWL gene family and the larger related family of proteins with the PLAC8 domain are critical components of the cellular regulatory schema to control cell division and organogenesis. Although a specific biochemical function cannot be attributed to the FWL proteins or to the PLAC8 domain, the conservation of the core domain sequences among the proteins suggests that the general biochemical function of these proteins is also likely conserved. Hence, a comparative approach may be useful to unravel the biochemical functionality of these protein families.

Note

Supplementary materials can be found at: www.landesbioscience.com/journals/psb/article/12808

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