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Functional characterization of rice CW-domain containing zinc finger proteins involved in histone recognition

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ABSTRACT

Histone recognition is important for understanding the mechanisms of histone modification, which play a pivotal role in transcriptional regulation during plant development. Here, we identified three cysteine-tryptophan (CW)-domain containing zinc finger (ZF) proteins involved in histone recognition, namely OsCW-ZF3, OsCW-ZF5 and OsCW-ZF7. Protein sequence analysis showed that they have two unknown motifs in addition to the CW domain. All three *OsCW-ZFs* were expressed in aerial tissues, with relatively high levels in developing panicles. Subcellular localization revealed that the OsCW-ZFs target the cell nucleus and CW domains are not necessary for their nuclear localization. In contrast to OsCW-ZF3 and OsCW-ZF5 where the CW domains bind histone H3 lysine 4 with different methylated forms (H3K4me), the CW domain from OsCW-ZF7 recognizes only tri-methylated histone H3 lysine 4 (H3K4me3). Analysis of mutant suggested that three conserved tryptophan residues in the CW domain are essential for binding to H3K4me. Further study found that OsCW-ZF7 interacts with TAFII20, a transcription initiation factor TFIID 20 kDa subunit. Knockout of *OsCW-ZF7* caused defective development of awns. This study provides new insights into our understanding of the CW domain and lays a foundation for further investigation of its roles in rice.

1. Introduction

Chromatin structure is modulated dynamically by posttranslational modifications [1–3]. Histone lysine methylation, a type of posttranslational modification in N-terminal histone tails, has an important role in controlling chromatin dynamics and gene expression [1–3]. Histone lysine residues are subject to different degrees of methylation including mono, di- and trimethylated forms, which have distinct effects on gene expression [1,2,4]. In general, methylations of histone H3 lysine 9 (H3K9) and H3K27 residues are associated with gene repression, whereas H3K4 and H3K36 methylations are thought to serve as active chromatin marks [1,4]. These modifications associated with states of gene expression are considered to be histone codes [5]. Hence the mechanism of histone modification of chromatin has two aspects: one is to write histone codes by histone-modifying enzymes and the other is to read these codes by histone recognition modules.

Accumulating evidence has revealed that histone lysine methyltransferases involve histone lysine modifications, and that they are mainly constituted of SET domain group (SDG) proteins. Based on the homology of their SET domains they are distributed into four families: SU(VAR)3–9 (suppressor of variegation3-9), E(Z) (enhancer of zeste), TRX (trithorax), and ASH1 (absent, small, or homeotic discs 1) [1,3,4,6]. Among them, SU(VAR)3–9 and E(Z) respectively catalyze H3K9 and H3K27 methylation, whereas TRX and ASH1 are responsible for H3K4 and/or H3K36 methylation [1,2]. Different codes of histone modification contain different information that recruits special readers/modules to translate them into specific effects on chromatin dynamics and gene expression. Compared to histone-modified enzymes much less information was available to decipher codes of histone modification, but several special readers/modules that recognize modified histones were recently reported in plants [3,7]. It was shown that the chromodomain can bind methylated H3K27 to maintain or alter chromatin structure in *Arabidopsis* LHP1, a homologue of *Drosophila* heterochromatin protein 1, and rice CHD3, a member of the chromodoamian,

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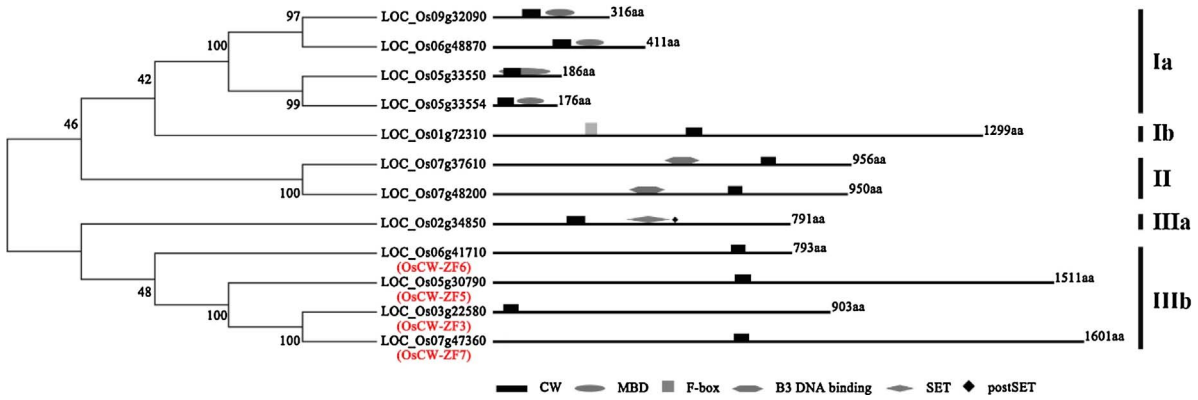

Fig. 1. Phylogenic analysis of CW-domain containing proteins in rice. Twelve different CW-domain containing proteins were identified, and classified into five subgroups. Different domains are shown schematically. Four CW-type zinc finger proteins denoted as OsCW-ZFs are shown in red color. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

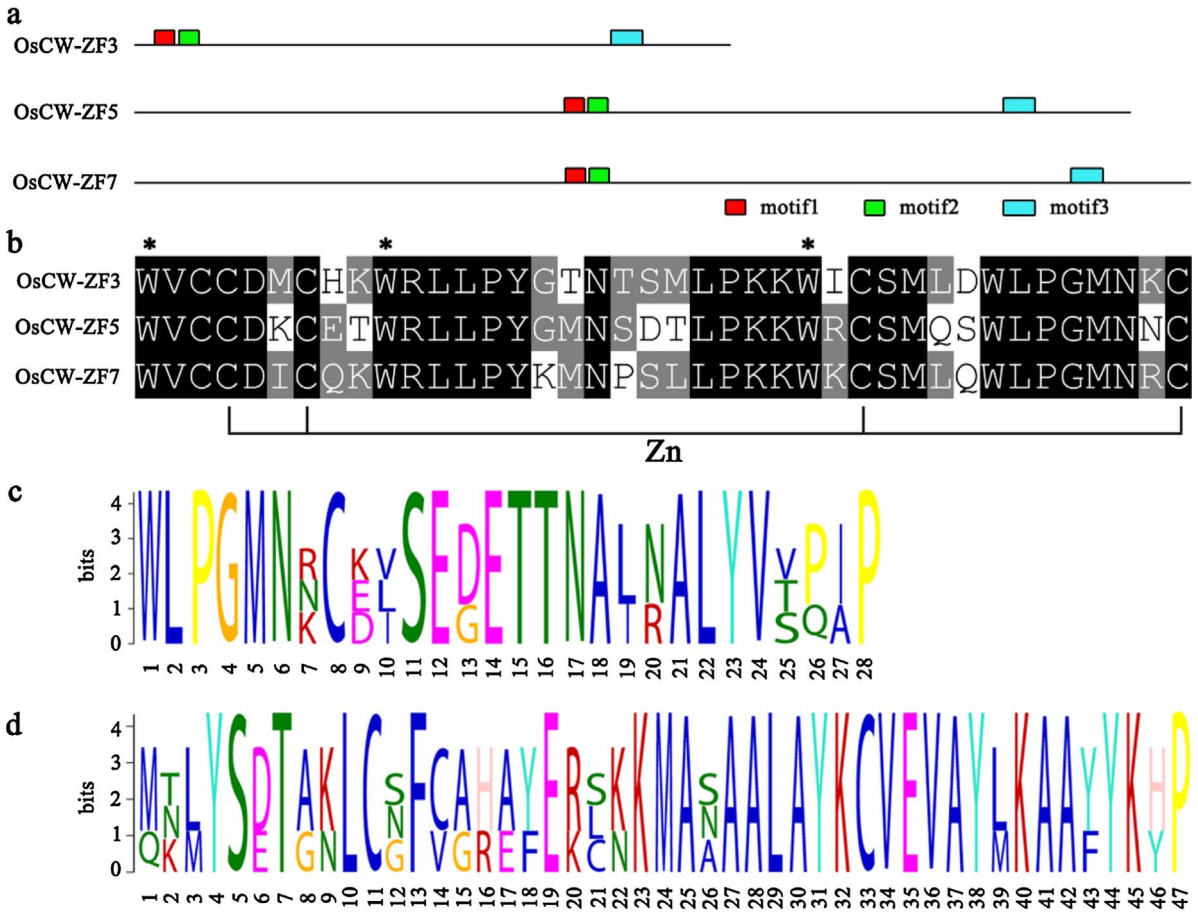

Fig. 2. Structures of rice CW-type zinc finger proteins. (a) Motif patterns of different CW-type zinc finger proteins. Motif 1 is the CW domain. (b) Sequence alignment of CW domains from rice CW-type zinc finger proteins. (c-d) WebLogo plots of consensus motifs in each CW-type zinc finger protein, including motif 2 (c) and motif 3 (d).

helicase/ATPase and DNA-binding domain (CHD) family [8–10]. Further studies indicated that rice CHD3 also interacts with methylated H3K4 via its PHD (plant homeodomain) [10]. The PHD as a histone recognition module was also identified in *Arabidopsis* PHD-domain containing proteins including ORC1 (the large subunit of the ORIGIN RECOGNITION COMPLEX) [11] and ING (INHIBITOR OF GROWTH) [12]. The WD40 repeat domain was also identified as a histone reader by binding H3K4 methylations in *Arabidopsis* WD40-repeat protein WDR5 [13].

In addition to the above-mentioned histone readers, the cysteine-tryptophan (CW) domain, initially identified in searching for protein domains containing four conserved cysteine and two to four tryptophan residues, was reported as a new histone recognition module in plants [14,15]. Among the four cysteine residues the first two are separated by two to four other residues and identified as classical zinc fingers, while the last two cysteine residues are arranged disorderly and separated by a variable number of additional residues [15]. Previous studies revealed that two CW-domain containing proteins from the human microrchida (MORC) family, MORC3 and MORC4 were able to bind methylated H3K4 [16]. In *Arabidopsis*, a well-known CW domain protein is SDG8, mutation of which produced pleiotropic phenotypes, including bushy, early flowering plants with impaired fertility [17,18]. Biochemical evidence showed SDG8 plays dual roles in chromatin histone modification: not only recognizing different methylated H3K4 peptides, but