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Carboxylate clamp tetratricopeptide repeat (TPR) domain containing Hsp90 cochaperones in Triticeace: An insight into structural and functional diversification

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ABSTRACT

The molecular chaperones serve as surveillance molecules that mediates regulatory crosstalk between protein folding and degradation pathways under natural and stress conditions. In present study, we focused on the diversification and role of tetratricopeptide repeat (TPR) domain containing Hsp90 cochaperones. These co-chaperone were recognized by the presence of three motifs of TPR with the basic conserved residues often referred to as carboxylate clamp (CC). A total of 213 putative CC-TPRs were found in Triticeace, clustered into 16 groups, amongst which few CC-TPR families such as TPR-RPAP3 and TPR-SMYD were documented. Domain architecture and genomic organization revealed that CC-TPRs are very diverse in nature. Evolutionary analyses showed that CC-TPRs are conserved, stable and ubiquitous in nature. Analysis of available RNA-seq data revealed a high degree of tissue-specific expression of 1-TPR and TaTPR-FKBP family members at various developmental stages. The transcripts of *TaCC-TPRs* displayed differential expression in two contrasting wheat cultivars under abiotic stress conditions. Complementation and heterologous expression of *TaTPR-FKBP5* in yeast conferred abiotic stress tolerance. Together, these results provide a glimpse into the genetic diversity and evolution of CC-TPRs in Triticeace, which would help to better understand of how TPR-domain cochaperones function in plants.

1. Introduction

Plants, being sessile organisms, are exposed to extreme environmental conditions throughout their lifecycle, amongst which abiotic stress is the most crucial that limits plant growth and reduces crop yield. The tight regulation between the heat shock proteins (HSPs) and their cochaperones maintains cellular balance under abiotic stress, especially high temperature stress (HTS). The interactions of cochaperones and chaperonins are specific to different HSPs (Finka et al., 2011). Members of Hsp90 family and their cochaperones are the major components of Hsp-cochaperone machinery, which function together in ATP-dependent manner to prevent the formation of protein aggregates. The open state (v-shaped) of Hsp90 is responsible for binding to client protein with the help of ATP and various cochaperones. Hsp90 cochaperones are classified into two categories on the basis of presence and absence of tetratricopeptide repeat (TPR) (Schopf et al., 2017). In the present study, we focused on TPR containing Hsp90 cochaperones and their diversification. Hsp90 interacts electrostatically with its acidic EEVD chain to basic residues of TPR domain. Interaction between Hsp90 and TPR not only requires the electrostatic, but also the hydrophobic and van der Waals forces, which provides more specificity to the complex (Schopf et al., 2017). Each TPR motif is a 34 residue helical structure arranged head-to-tail manner (Zeytuni and Zarivach, 2012). A TPR domain is formed by three motifs and is the smallest functional unit of TPR proteins (Main et al., 2003). The three motifs that form the TPR domain are N-terminal TPR1, middle TPR2A and C-terminal TPR2B. TPR protein can have either single, double or triple TPR domain, and hence classified as 1-TPR, 2-TPR and 3-TPR, respectively. Intriguingly, TPR proteins are very diversified in nature, except size of functional unit and few conserved basic residues (Schopf et al., 2017). TPR domain contains the basic residues Lys_5 and Asn_9 in first, Asn_6 in second, and Lys_2 and Arg_6 in third motif. These conserved basic residues (K_5N_9-N_6-K_2R_6) are considered as carboxylate clamp TPR (CC-TPR), which is mainly responsible for binding to Hsp90. Few consensus residues (found on position 4, 7, 8, 11, 20, 24, 27 and 32) are hydrophobic, which also help in maintaining the interaction of Hsp90 and its cochaperones (Main et al., 2003). Previous investigation showed that TPR motifs are present in all three domains of life *i.e.* archaea, bacteria and eukaryotes (Schapire et al., 2006). It is one of the important family of tandem repeats (TR), which is distributed throughout the genome.

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The TR proteins generally show variation among their members as they are more prone to duplication events during evolution as compared to non-TR protein (Jernigan and Bordenstein, 2015). There has been great deal of speculation that TPR motifs might have evolved through convergent evolution or horizontal gene transfer, but it has recently been proved that they evolved from common ancestor (Schapire et al., 2006). Interestingly, consensus sequences of CC-TPR between prokaryotes and eukaryotes are found to be highly conserved. Unlike animals, TR families are higher in number in plants (Sharma and Pandey, 2016).

The extrinsic feature of CC-TPR domain is that it can associate with an additional domains. TPR proteins *viz.*, PP5 (phosphoprotein phosphatases), immunophilin, Tom70, U-box and HOP (Hsp70-Hsp90 organizing protein) harboring additional domains were previously investigated in animal system (D'Andrea and Regan, 2003; Smith, 2004). Interestingly, model plant Arabidopsis has many more additional domains such as HSCB (heat shock chaperonin-binding), TL (thioredoxin-like) TOC64 (Amidase), ANK (ankyrin), DnaJ, PB1/Phox (octicosapeptide/Phox/Bem1p) and SET (Suppressor of variegation, Enhancer of Zeste, Trithorax) in combination with TPR domain. However, few additional domains are specific to rice TPR proteins such as FBD, F-box, STYKc (Ser/Thr/Tyr kinase) and multiple repeats of ankyrin (Bhangoo et al., 2007; Prasad et al., 2010).

The TPR domains not only serve as a protein-protein interaction module, but are also involved in the regulation of diverse cellular functions. In plants, TPR proteins have been implicated in diverse cellular functions such as ABA responses and osmotic stress tolerance (Rosado et al., 2006). Several TPR proteins have been demonstrated to be responsive to other phytohormones *viz.*, auxin, cytokinin, gibberellins and ethylene in Arabidopsis (Sharma and Pandey, 2016). Furthermore, TPR proteins have also been shown to be involved in photosystem I assembly (Stockel et al., 2006), mRNA processing and stability (Boudreau et al., 2000; Vaistij et al., 2000), effector-triggered immunity against pathogens (Kwon et al., 2009), protein trafficking (Sparks et al., 2016) and abiotic stress tolerance (Lee et al., 2009). A recent report suggested a key role of TPR proteins in transcriptional and posttranscriptional gene regulation in plastids, suggesting its involvement in chloroplast development and hybrid sterility (Yu et al., 2016; Ma et al., 2017).

Bread wheat is the most important staple crop in temperate region with high global production. It has three homoeologous sub-genome and is hexaploid in nature. The evolution of polyploids in wheat was generated through hybridization. The intergenomic and revolutionary changes thus resulted in gain and loss of coding and non-coding region, duplication and transponsons, causing genetic diversity in wheat (Feldman and Levy, 2012). We sought to analyze CC-TPR proteins in Triticeace. Unlike Arabidopsis, CC-TPRs are more in number in Triticeace *viz.*, *T. aestivum*, *A. tauschii* and *B. distachyon*. Next, we analyzed the evolutionary relationship of *T. aestivum* and its relatives based on evolution of CC-TPRs. *In silico* analysis of major evolutionary lineages of plants revealed that the CC-TPRs are ubiquitous in plant kingdom. Tissue-wise expression profiling revealed the functional redundancy of *CC-TPR* genes in *T. aestivum*. Heterologous expression of *TaTPR-FKBP5* in yeast showed multifuctional role in cell defense and abiotic stress responses. The findings may provide new insights into molecular mechanism of evolution and diversification of CC-TPRs, and their biological relevance in plants.

2. Materials and methods

2.1. Identification of CC-TPRs in Triticeace

Three TPR motifs of *Arabidopsis* CC-TPRs were taken for HMM (Hidden Markov Model) profile. The profile was used to extract the putative CC-TPRs in wheat proteome database through HMMER 3.0 (http://hmmer.janelia.org/). The proteome information of *T. aestivum* were taken from Ensembl plant database (http://plants.ensembl.org/Triticum_aestivum/Info/Index). The proteins containing all three TPR motifs with e-value lower than 1e-3 were considered for downstream analysis. The putative TPRs were subjected to domain analysis in PFAM and InterPro. Presence of PF13414/IPR013026 domain in TPR is considered as must criterion in fulfillment of CC-TPR. A typical CC-TPR comprises one or more TPR domain along with the conserved residues (K_5N_9-N_6-K_2R_6), which were manually analyzed. Likewise, sequences of *T. urartu*, *A. tauschii, H. vulgare* and *B. distachyon* were also downloaded *via* Ensembl plant database and same strategy was carried out for sorting CC-TPRs in these species. The theoretical molecular weight and isoelectric point were computed by ExPASy tool (http://www.expasy.org/). The hydropathicity and protein disorder were calculated by GRAVY (grand average of hydropathicity) index (http://www.bioinformatics.org/sms2/protein_gravy.html) and RAPID software (http://biomine-ws.ece.ualberta.ca/RAPID/index.php), respectively. The prediction of subcellular localization was performed through LocTree3 web server (https://rostlab.org/services/loctree2/). The nomenclature was based on the presence of TPR-domain and an additional domain found in CC-TPRs as followed previously (Prasad et al., 2010) with few modifications.

2.2. Phylogenetic analysis of Triticeace CC-TPRs

Multiple alignments of protein sequences of TaCC-TPRs were carried out through MUSCLE algorithm, and the phylogram was constructed using Neighbour-Joining (NJ) method keeping bootstrap value 1000 in MEGA7 (Kumar et al., 2016). The phylogram was displayed through Interactive Tree of Life (version 3) (http://itol.embl.de). Similar approach was followed for phyloanalyses of Triticeace CC-TPRs.

2.3. Analysis of CC-TPR gene organization, domain, motif, and protein structure

Domain of CC-TPRs was searched using PFAM, InterPro, and CDD search web server, and the domain structure was visualized *via* Annotation Viewer (version 1) (http://www.nextgenetics.net/tools/anno_view/annotator.html). Protein motifs were analyzed using multiple EM for motif elicitation (MEME, http://meme.nbcr.net/meme3/meme.html) with the following parameters: maximum number of motifs was 25 and the optimum width was set between 5–200 amino acids. The three-dimensional structures of TaCC-TPRs were analyzed by Phyre2 (version 2) (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index). The exon/intron structure was visualized through GSDS (Gene Structure Display Server) (http://gsds.cbi.pku.edu.cn/index.php).

2.4. Chromosomal location, gene duplication and synteny analysis

The chromosomal location of TaCC-TPRs was extracted from genome annotation information and displayed through MapChart (Voorrips, 2002). Segmental and tandem duplicated pairs of CC-TPRs were detected in *T. aestivum* and *B. distachyon* through MCScanX (Wang et al., 2012). The orthologous gene pairs of *T. aestivum* chromosome A (TaA)-*T. urartu* and *T. aestivum* chromosome D (TaD)-*A. tauschii* were calculated as described previously (Qiao et al., 2015). The analysis was carried out for TaA-*T. urartu* CC-TPRs and the two genes from both species falling in the same branch of the phylogram were considered as orthologs (Figs. S3 and S4). The segmental duplication of CC-TPRs was considered when sequence coverage and similarity of aligned gene regions was found to be ≥70%. The tandem duplicates were separated by 5 or fewer genes in a 100-kb region. The same strategy was followed for extracting the CC-TPR orthologous pairs between TaD and *A. tauschii*. The paralogous and orthologous pairs were visualized using ClicO FS (http://codoncloud.com:3000/). To deduce evolution trend of CC-TPRs, proteome of representative members of major evolutionary lineage were downloaded from phytozome (https://phytozome.jgi.doe.