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CaM-binding transcriptional activator, OsCBT, is regulated by calmodulin in rice (*Oryza sativa L.*)

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Objectives

We have tried to understand the cellular function of calmodulin (CaM) in the gene expression.

Materials and Methods

1. Material

Plant - rice(*Oryza sativa L.*),

2. Methods

Expression of the fusion constructs was monitored at 24 h after transformation by fluorescence microscopy using a Zeiss Axioplan fluorescence microscope (Jena, Germany), and the images were captured with a cooled CCD camera. The filter sets used were XF116 (exciter, 474AF20; dichroic, 500DRLP; emitter, 510AF23), and XF33/E (exciter, 535DF35; dichroic, 570DRLP emitter, 605DF50) (Omega, Inc., Brattleboro, VT) for green fluorescent protein, and red fluorescent protein, respectively.

Results and Discussion

Calmodulin (CaM) regulates diverse cellular functions by modulating the activities of a variety of enzymes and proteins. However, direct modulation of transcription factors by CaM has been poorly understood. In this study, we isolated a putative transcription factor by screening a rice cDNA expression library using CaM:HRP as a probe. This factor, which we have designated *OsCBT* (*Oryza sativa CaM binding transcription factor*), has structural features similar to *Arabidopsis AtSRs/AtCAMTAs*and encodes a 103 kDa protein in that it contains a CG-1 homology DNA-binding domain, three ankyrin repeats, a putative transcriptional activation domain, and five putative CaM-binding motifs. Using a gel overlay assay, gel mobility shift assays, and site-directed mutagenesis, we showed that OsCBT has two different types of functional CaM-binding domains (CaMBDs), an IQ motif and a Ca2+-dependent motif. To determine the DNA binding specificity of OsCBT, we employed a random binding site selection (RBSS) method. This analysis showed that OsCBT preferentially binds to the sequence 5′-TWCG(C/T)GTKKKKTKCG-3′(W and K represent A or C and T or G, respectively). OsCBT was able to bind this sequence and activate -*glucuronidase* (GUS) reporter gene expression driven by a minimal promoter containing tandem repeats of these sequences in *Arabidopsis* leaf protoplasts. GFP fusions of two putative nuclear localization signals of OsCBT, a bipartite and a SV40 type, were predominantly localized in the nucleus. Interestingly, the transcriptional activation mediated by OsCBT was inhibited by co-transfection with a *CaM* gene. Taken together, our results suggest that OsCBT is a transcription activator modulated by CaM.