MASTER'S THESIS

Functional Characterization of the Arabidopsis mRNA Cap Methyltransferase AtCMT

HUA, Jing Min

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Abstract

An important feature of eukaryotic messenger RNA is the presence of a methylguanosine (m^7G) moiety at the 5′ end, which contributes to mRNA stability and efficient processing and translation. mRNA capping and decapping are believed to be exclusive to eukaryotes. In the recent years, non-canonical caps, such as the NAD^+ cap, have been found in prokaryotic and eukaryotic RNAs. The DXO/RAI 1 family of proteins is widely present in eukaryotes. DXO proteins possess multiple activities, including decapping of m^7G- and NAD-capped RNAs and abnormally decapped mRNAs and 5′ to 3′ exoribonuclease activity. Previous study in *Arabidopsis thaliana* shows that DXO1 possesses both NAD-RNA decapping and 5′ to 3′ exoribonuclease activity. Previous work in our lab shows that DXO1 interacts with the mRNA cap methyltransferase (AtCMT) through its plant-specific N-terminus. Sequence analysis indicates that AtCMT contains a conserved AdoMet (S-adenosylmethionine)-dependent methyltransferase domain and has a 35-39% sequence identity to mRNA cap methyltransferases, which catalyses methylation of the guanosine cap to form the m^7G cap. Despite these recent studies, mRNA cap structures and the enzymes involved in the capping process have been little studied in *Arabidopsis.* This study is focused on understanding the biological function of AtCMT.

Analysis of the publicly available *Arabidopsis* transcriptome data and of expression profiles by quantitative polymerase chain reaction (PCR) showed that *AtCMT* is a ubiquitously expressed gene. Using a AtCMT-GFP reporter assay, the AtCMT protein was found to be localized in the nucleus. A mutant line containing a T-DNA insertion in the *AtCMT* gene was obtained, although a homozygous mutant line was not, indicating that the homozygous mutation is lethal. Reciprocal crosses between the heterozygous *Atcmt*/*AtCMT* plants with wild-type plants indicated that the *Atcmt* mutation does not affect the function of male or female gametophytes. However, the *Atcmt* mutation caused arrest of embryogenesis at the globular stage. This study indicates that *AtCMT* is an essential gene and a defect in forming the m^7G cap can lead to early embryonic lethality.

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