MOLECULAR ANALYSIS OF PLANT PROTEINS THAT ARE INVOLVED IN THE REGULATION OF TRANSCRIPTIONAL GENE SILENCING

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ABSTRACT

Metastable silencing and activation of gene transcription crucial for differentiation and environmental adaptation in multicellular eukaryotes are controlled by complex epigenetic mechanisms. Although the exact molecular mechanisms underlying epigenetic modifications are not yet known, they involve complex interplays between DNA methylation, chromatin remodeling, and histone modifications. Transcriptional gene silencing (TGS) is an example of epigenetic gene regulation.

This work aimed to study the role of proteins that are involved in maintenance of TGS. Previous study isolated the TGS regulator required for its maintenance in Arabidopsis, MOM1 (Morpheus' Molecule1), which seems to function independent of DNA methylation changes. The amino acid sequence analysis reveals two domains with homologies to the half-helicaes of SWI2/SNF2 ATPase and chicken tensin, respectively. This raises the possibilities of complex formation between MOM1 and yet unidentified partners. We identified MOM1 interacting partners by yeast two-hybrid screening of Arabidopsis cDNA expression library using MOM1 regions covering both conserved domains as baits. We examined two candidates, SWI3A and DAD1 in detail. SWI3A is a subunit of the SWI/SNF chromatin remodeling complex. DAD1 is an unknown protein with homology to proteins in other organisms across the kingdoms. We showed that both proteins play an essential role in Arabidopsis since homozygous mutant plants for each insertion were lethal. The specific interaction between MOM1 and DAD1 was further confirmed by in vitro pull-down assay. The functional relationship between MOM1 and the candidates was studied by the assessment of the release of TGS in lines carrying T-DNA insertions in the genes in question. Furthermore DNA methylation status and nuclear architecture were examined in these mutant lines. Currently, we can conclude that the two proteins interact with MOM1 and that both have functional importance during early development in Arabidopsis. The relevance of the two proteins in TGS requires further investigations using conditional mutant strains which are currently being constructed.

This investigation into proteins involved in TGS may provide important basic knowledge of the underlying the control of epigenetic regulation in plants.

KEY WORDS: GENE SILENCING/ DNA METHYLATION/CHROMATIN REMODELLING/MOM1

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