The Role of EXPORTIN1A and EXPORTIN1B in Nuclear Export of AGO4 in Arabidopsis thaliana

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Epigenetics is the study of how genes are regulated at a level above the DNA nucleotide sequence. Methylation, the attachment of methyl groups onto DNA to upregulate or down-regulate a gene, is one epigenetic marker that can be influenced by environmental factors such as diet, drought or disease. Methylation is passed through generations and can have lasting effects, which is why epigenetics is important to understand. The RNA directed DNA Methylation pathway (RdDM) that occurs in Arabidopsis thaliana is a methylation pathway involving proteins in the cytoplasm and nucleus. The current research focuses on the protein AGO4 that binds to siRNAs which guide methylation onto genomic loci. The research question is: Are EXPORTIN1A, EXPORTIN1B, or both necessary for proper function and nuclear export of AGO4? It is predicted that these proteins are responsible for nuclear export of AGO4 because they both recognize nuclear export sequences (NESs) found on the surface of the AGO4 protein and share 86% identity. Four lines of A. thaliana- XPO1A knockout, XPO1B knockout, positive wild type control, and negative ago4-3 control- were planted. DNA collected from each line underwent genotyping PCR to confirm mutation and chop PCR to test whether RdDM was functioning properly. After mutations were confirmed in both knockout lines, the chop PCR showed methylation present on loci methylated by RdDM for both XPO1A and XPO1B lines, indicating EXPORTIN1A and EXPORTIN1B do not need the other to export AGO4. The next step in this research is to create a double mutant that produces neither EXPORTIN1A nor EXPORTIN1B. Genotyping and chop PCR will then be used to narrow the results even further to confirm that the EXPORTIN proteins are the only mechanisms exporting AGO4 from the nucleus.