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Arabidopsis thaliana native and domesticated GAG protein interactions network

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Motivation and aim: Separate analysis of protein-protein interactions of native EVD retrotransposon GAG and domesticated copia-like dGAG proteins.
Materials and methods: *Arabidopsis thaliana* GAG:3xFLAG and dGAG:3xFLAG protein fusion overexpression lines were obtained via floral-dip agrobacterial transformation. GAG:3xFLAG and dGAG:3xFLAG proteins presence was confirmed via SDS-PAGE and Western blot analysis. Co-immunoprecipitation (Co-IP) procedure was performed in order to purify target proteins (GAG:3xFLAG and dGAG:3xFLAG) and their possible cooperators. Co-IP samples were subjected to shotgun orbitrap mass spectrometry (MS). MS data was analysed and quantified by MaxQuant tool. *In silico* analysis of protein interaction was performed via AlphaFold3 and PyMOL tools.
Results: Long Terminal Repeat Retrotransposons (LTR-RTs) are mobile genetic elements that make up a substantial fraction of the genome in most plant species. Their life cycle closely resembles that of retroviruses, involving the formation of virus-like particles composed of GAG proteins, which bind and encapsulate LTR-RT RNA. In animal cells, this process occurs within specialized organelles called retrosomes, but it remains unknown whether such structures exist in plant cells. Over evolutionary time, GAG proteins can be domesticated, acquiring new functions that benefit the host. A recent study identified an example of a domesticated GAG (dGAG) in the *A. thaliana* genome. However, comparative analyses between wild-type GAG and dGAG have not yet been conducted in plants.
In this work we obtained *A. thaliana* GAG:3xFLAG and dGAG:3xFLAG protein fusion overexpression lines which were further used for Co-IP and MS analysis. Analysis revealed dozens of co-purified proteins for both types of protein fusions including SERRATE, IIL1, HERK2, APR1, BAS1A proteins co-purified with dGAG. Later data is of great importance for understanding possible functions of domesticated RT GAG protein. Obtained results were compared with previously obtained *in silico* protein-protein interaction prediction data. We also performed comparative analysis of plant EVD GAG protein with retroviral (HIV) and other retrotransposon GAG proteins with known protein structures and key aminoacids residues responsible for GAG-GAG interaction. We used AlphaFold3 tool for EVD GAG multimer protein structures (T = 3 and T = 4) prediction. Obtained GAG protein polymers resembled known structures for HIV GAG capsid polymers. Key aminoacids responsible for HIV GAG polymer formation were also present in sites of EVD GAG-GAG interaction.
Conclusions: This study identified potential dGAG-interacting partners through Co-IP/MS analysis and validated structural similarities between EVD GAG and retroviral GAG proteins using AlphaFold3. The findings provide insights into the functional evolution of native and domesticated GAG proteins, bridging plant retrotransposons and viral systems.
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