CLONING OF CDNA-GENE OF *ARABIDOPSIS THALIANA* RIBOSOMAL PROTEIN S6, ITS EXPRESSION IN *ESCHERICHIA COLI* AND PURIFICATION OF *AT*RPS6B1 RECOMBINANT PROTEIN

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Living organisms must adapt their growth and development in response to external factors like stress and nutrient availability throughout their lifespan. Consequently, they have evolved various regulatory pathways to enhance environmental perception and facilitate necessary metabolic adjustments. These pathways involve key proteins that, under stress and nutrient limitation, initiate anabolic and catabolic cellular processes. One critical pathway present in all eukaryotes involves the protein kinase TOR (Target of rapamycin), a large kinase that governs numerous biological processes, including the activation of S6K kinase, which phosphorylates the S6 protein (RPS6). Crystal structures have revealed the precise location of RPS6 within the 40S ribosomal subunit, highlighting structural features such as the C-terminal helix containing multiple phosphorylation sites. RPS6 primarily regulates mechanisms controlling cell growth and division. In plants, eS6 is encoded by two conserved genes, and its activation and phosphorylation are closely linked to S6Ks. Recent studies emphasize the significance of S6 protein phosphorylation as a key event in signaling pathways related to cell growth and survival factors, crucially regulating translation initiation and protein synthesis. S6 protein can be phosphorylated on various serine and threonine residues by S6K kinase, and further research shows its interaction with other molecules and proteins, expanding its functional roles in cell signaling.

Further exploration of ribosomal protein S6 could unveil new insights into its molecular interactions, roles in cellular physiology and pathophysiology, and potential applications in enhancing plant biomass and yield. This study involved cloning and site-directed mutagenesis of cDNA for the second isoform of *At*RPS6 protein *(At*RPS6B), followed by expression of natural and phosphomimetic forms of the protein in *E. coli* ArcticExpress (DE3) cells. The proteins were then purified using metal-chelate affinity chromatography (IMAC), and their presence and degree of purification were confirmed via Western blotting.

These findings contribute to the broader research context surrounding ribosomal protein S6. RPS6, a key effector in the TOR signaling pathway, plays a crucial role in regulating ribosome biosynthesis by controlling transcription and translation processes. Phosphorylation of RPS6, indicative of S6K activity, is associated with actively dividing cells. However, the precise role of RPS6 in ribosome biosynthesis regulation remains a subject of ongoing investigation.

The data obtained from the study on AtRPS6B expand our understanding of the molecular mechanisms underlying ribosomal biosynthesis regulation. These insights may significantly contribute to our knowledge of the interplay between signaling pathways, ribosomal proteins, cellular metabolism, and offer new perspectives for therapeutic interventions targeting cellular processes associated with ribosomes.