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Elucidation of the biochemical and functional properties of an unknown novel protein from Arabidopsis thaliana

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Adenylate cyclases (ACs) are a special group of enzymes capable of catalyzing the conversion of adenosine 5-triphosphate (ATP) into the signaling molecule cyclic 3,5-adenosine monophosphate (cAMP), which in turn acts as a second messenger in various cellular and metabolic pathways. Apparently, while the presence of ACs and their functional roles in animals and prokaryotes have since been well-documented, their presence and/or functional roles in higher plants has somewhat remained a matter of serious debates and controversy. Notably and in a recent BLAST search of the Arabidopsis genome using a 14-mer motif with specificity for ATP binding and catalysis, an AC-like protein coded for by the At3g21465 gene has been identified. However, even though the AC-like protein does contain the AC catalytic core motif, it notably has not yet been shown to possess any known putative AC catalytic function and/or share any similarities with any annotated and/or experimentally confirmed ACs, but instead, it only appears to be transcriptionally up-regulated in response to biotic stress factors. Therefore in an attempt to test and determine whether this putative protein candidate has any functional AC activity, total mRNA of the 4-6 weeks old *Arabidopsis thaliana* plants was extracted and used as a template for the complementary synthesis and amplification of a 384 bp AC-like gene fragment via a specialized Reverse Transcriptase - Polymerase Chain Reaction (RT-PCR) system. The amplified fragment was then cloned into a pTrcHis2-TOPO expression vector and the resultant recombinant expression vector eventually transformed into chemically competent *E. cloni* express BL21 (DE3) pLysS expression host cells. Positive clones were determined by confirmatory PCR and further validated by nucleotide-specific sequencing. The 18.0 kDa C-terminus His-tagged recombinant AC-like protein was then over-expressed following an induction with isopropyl-β-D-1-thiogalactopyranoside (1 mM, IPTG) and purified over a nickel-nitrilotriacetic acid (Ni-NTA) affinity matrix system. The endogenous and in vitro AC activities of the resultant recombinant AC-like protein were then tested via a cAMP-linked enzyme immunoassaying system while its inherent in vivo AC activity was also concurrently tested via a complementation testing system using the cyaA SP850 mutant Escherichia coli cells. Results from these three independent assays collectively indicated that the AC-like protein encoded for by the At3g21465 gene from A. thaliana possesses the endogenous, in vitro and in vivo AC activities, and thus unequivocally confirming it as a bona fide higher plant AC molecule with a possible cAMP-mediated signaling system.

Biography

S K Sehlabane is currently working in North-West University, South Africa

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