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INVESTIGATION ON THE ROLE OF cAMP IN PLANTS USING THE "cAMP-SPONGE"

SABETTA W.*, SGOBBA A.**, VIGGIANO L.**, DE PINTO M.C.**, BLANCO E.*

*) Institute of Plant Genetics – CNR, Research Division Bari, Via Amendola 165/A, 70126 Bari (Italy)
**) Department of Biology, University of Bari "Aldo Moro", Via Orabona 4, 70126 Bari (Italy)

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Cyclic AMP is a well known second messenger involved in different cellular responses in all living organisms. In higher plants, its role as second messenger has been widely debated, due to its low content and to the difficulty of measuring it. However, its natural occurrence and the existence of adenylyl-cyclases and cAMP phosphodiesterases, that constitute the on-off switches needed for its action as second messenger, have been demonstrated. Data accumulated in the last three decades show the involvement of cAMP in several processes of higher plants, including cell cycle regulation, growth and reorientation of the pollen tube, seed germination and defense responses. However little is known on the mechanisms involved in the cAMP-dependent signal transduction in plants. To shed light on cAMP role in plant signaling pathways, Arabidopsis plants, whose genome has been completely sequenced, and tobacco Bright Yellow-2 (BY-2) cells, that are highly synchronizable, have been chosen as model systems. For this purpose, both Arabidopsis plants and tobacco BY-2 cells were transformed with the "cAMP-sponge", a non invasive tool able to selectively reduce cAMP concentration (Lefkimmiatis et al, 2009). The cAMP-sponge is composed of two high-affinity cAMP binding domains of the regulatory subunits I beta of human protein kinase A (PKARIbeta) that specifically bind cAMP and not cGMP.

The construct containing the cAMP-sponge in frame with the reporter gene mCherry were mobilized into TBY-2 cells and Arabidopsis via *A. tumefaciens*-mediated transformation. Transgenic TBY-2 calli and Arabidopsis plants were selected in the presence of appropriate antibiotics, and several independent transgenic lines were obtained. Trans-gene integration and its expression in Arabidospsis and tobacco transformed lines were verified by PCR, RT-PCR and immunoblotting analyses. The low levels of cAMP negatively affected the growth of TBY-2 cells whereas no distinctive phenotype was observed in Arabidobsis plants. However, a stress condition was evidenced for both tobacco BY-2 cells and Arabidopsis plants, as shown by the alteration of their cellular redox state, analysed by ascorbate and glutathione measurements.

Reference:

Lefkimmiatis K, Moyer MP, Curci S, Hofer AM (2009) "cAMP Sponge": A Buffer for Cyclic Adenosine3',5'-Monophosphate. PLoS ONE 4(11): e7649, doi:10.1371/ journal.pone.0007649.