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"cAMP-SPONGE": A NEW GENETIC TOOL TO INVESTIGATE THE ROLE OF cAMP IN PLANTS

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Cyclic AMP is a well known second messenger which regulates a wide variety of cellular responses in living organisms such as bacteria, fungi, and animals. On the contrary, its presence and its role in plants have been debated for decades. The skepticism was finally overcome with the use of mass spectrometry that provided unequivocal evidence of its presence in higher plants. The information on the biological function of cAMP in plants remains very limited, mainly because its content in plant cells is significantly lower than in other organisms. To date, the cAMP involvement in several processes of higher plants, including cell cycle regulation, growth and reorientation of the pollen tube, seed germination and defense processes has been reported. However, in plants, the mechanisms involved in the cAMP-dependent signal transduction are yet unknown, especially for the failure to identify a kinase that responds specifically to cellular changes in cAMP concentration. Understanding both the biological events specifically attributable to cAMP, and the mechanisms by which these processes are regulated, through the combination of quantitative data with mathematical models, is a challenge for the study of plant signal transduction.

In order to obtain more information on the role of cAMP in plants we generated tobacco Bright yellow-2 (TBY-2) lines that constitutively express a non-invasive tool, the "cAMP-sponge", able to selectively perturb the 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). These domains have been engineered to be unable to interact with the catalytic subunit of PKA itself or to homodimerize. This construct binds with high affinity cAMP but not cGMP. The cAMP-sponge in frame with the reporter gene mCherry has been inserted in the binary vector pGreenII (kan) under the control of the strong constitutive promoter CaMV 35S.The construct has been transferred into *Agrobacterium tumefaciens* GV3101 strain through electroporation and mobilized into TBY-2 cells via *A. tumefaciens*-mediated transformation. Transgenic TBY-2 lines have been selected in the presence of kanamycin, and several independent transgenic lines obtained.

Transformed lines have been analyzed through PCR, RT-PCR and immunoblotting to assess trans-gene integration and mRNA and protein expression. Finally the effect of the lower levels of cAMP on the growth of TBY-2 cells has been analyzed.

REFERENCE

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