REGULATION OF THE TRANSCRIPTION LEVEL AND PHOSPHORYLATION STATUS OF CREB BY cAMP MODULATORS AND SALICYLIC ACID

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In animal cells, the cAMP-responsive element binding protein (CREB), a b-ZIP transcription factor, mediates the effect of cytosolic cAMP accumulation on the level of transcription of several genes. The activity of CREB depends on the phosphorylation of a Ser133 by a protein kinase A. Phosphorylation of CREB leads to protein dimerization, exposure of two transactivating domains (Q1 and Q2) and binding of the dimerized protein on cAMP-responsive elements (TGACGTCA) located upstream of cAMP-dependent genes (Fiol *et al.*, 1994).

Several experimental data suggest that cAMP plays also an important physiological and signalling role in plant cells. Cyclic AMP modulates potassium-channel gating in Vicia faba (Li *et al.*, 1994), pollen tube growth in Arachis hypogea (Tezuka *et al.*, 1993) and auxin action in Nicotiana tabacum (Ichikawa *et al.*, 1997). However, if the cAMP-mediated signal transduction pathway is well established in animal cells, it is not the case in plants. Although adenylate cyclase (Ichikawa *et al.*, 1997), phosphodiesterases (Calvert *et al.*, 1996), protein kinase A (Li *et al.*, 1994) and CREB-like proteins (Miao *et al.*, 1994) have been identified in plants, the physiological links (if any) between these effectors are unknown.

In this study, we have used semi-quantitative RT-PCR and previously produced anti-CREB antibodies (Messiaen et al., 1998) in order to investigate the impact of cAMP modulators and salicylic acid (SA) on the transcription level and phosphorylation status of CREB respectively.

Practically, suspension-cultured BY-2 tobacco cells were treated with dibutyryl-cAMP (100 µM), forskolin (100 µM) and salicylic acid (250 µM) under mild agitation in the dark. Total RNA and proteins were extracted during time. CREB mRNAs were analyzed by semi-quantitative RT-PCR using the level of ubiquitin transcripts as control. The phosphorylation status of the CREB protein was assessed by western blotting and autoradiography.

Dibutyryl-cAMP, forskolin and SA induced the transcription of the CREB gene within 5 minutes after treatment. At the protein level, only SA was able to induce the phosphorylation of CREB 5 minutes after treatment. These results clearly establish a direct link between elevated cytosolic cAMP concentrations and the transcription of the CREB gene. They also suggest a possible cross-talking between the cAMP and SA signalling pathways at both the transcriptional and post-translational level.

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