

Is MAP65-1 phosphorylation related to Cr(IV) effects on the microtubules of *Arabidopsis thaliana*?

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Chromium (Cr) is a heavy metal, occurring in both terrestrial and aquatic habitats. It is found in two oxidation states, the trivalent Cr(III) and the hexavalent Cr(VI). Cr (VI) is a toxic and non-essential element for plants. When encountered at high concentrations, almost all physiological, biochemical and cellular processes of plants are negatively affected. Microtubules (MTs) in particular, have been found to be prone to Cr(VI) in plant cells, and constitute a universal target of Cr(VI) toxicity. MAP65-1 (the most abundant plant structural microtubule-associated protein) modulates microtubule stability since, it binds and bundles them by forming stabilizing cross-bridges between neighboring MTs. This ability is affected by its phosphorylation status, and when MAP65-1 becomes phosphorylated it becomes unbound from MTs during the cell cycle phases. In the present study, the effects of Cr(VI) on MAP65-1 presence on cortical MTs of *Arabidopsis thaliana* roots and hypocotyls were investigated. *A. thaliana* lines expressing GFP:TUA, CFP:AtMAP65-1 and the line expressing the non-phosphorylatable AtMAP65-1, AtMAP65-1^{9A} (CFP:AtMAP65-1^{9A}) have been used. Four-day-old seedlings were transplanted to Petri dishes with ½ MS solid medium supplemented with 100 µM potassium dichromate (K₂Cr₂O₇, Cr(VI) for), and left to grow for 24 or 48 h. Confocal laser scanning microscopy (CLSM) revealed that, already after 24 h, Cr(VI) variously affected cortical MTs. Moreover, MAP65-1 presence was substantially reduced as revealed by the CFP:AtMAP65-1 signal intensity measurements. However this was not the case for CFP:AtMAP65-1^{9A} where its signal was retained when Cr(VI) was applied. When examining MTs of the CFP:AtMAP65-1^{9A} line, Cr(VI) did not affect them. The above observations show that the influence of Cr(VI) on MTs is related to MAP65-1 phosphorylation.