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ATG8H ROLE IN CADMIUM-DEPENDENT PEXOPHAGY INDUCTION

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Autophagy is a self-degradation mechanism involved in removing damaged or obsolete cellular constituents including organelles under starvation and stress conditions. While the role of autophagy in metal starvation has been thoroughly studied (1), its role in heavy metal toxicity has been little explored. Recently, we observed that, in *Arabidopsis* plants treated with 100 μ M CdCl₂ for short periods (30 min-24 h), selective peroxisome autophagy (pexophagy) occurs, with autophagy marker *ATG8h*'s transcript levels being slightly up-regulated. To determine whether ATG8h plays any specific role in Cd-induced autophagy and whether it specifically targets peroxisomes for autophagy, we characterized *Arabidopsis* T-DNA mutants lacking ATG8h (*atg8h*) in response to Cd.

Arabidopsis atg8h grown on vertical plates showed a smaller Cd-dependent reduction in root growth than WT (Col 0). Moreover, *atg8h* plants grown hydroponically showed a delayed Cd effect, while similar results were obtained in both WT and *atg8h* plants after 3d of Cd treatment. Therefore, chlorophyll and carotenoid content was higher in *atg8h* than in Col 0 plants. Lipid peroxidation and H₂O₂ content, which showed no differences in leaves between either line, increased considerably in *atg8h* in roots after 3 d of treatment. This could be explained by changes observed in catalase and peroxidase activity, particularly in *atg8h* roots. However, no statistically significant changes were observed in Cd uptake or translocation in either line. In *atg8h*, electron microscopic ultrastructural analysis of leaves showed larger peroxisome areas and numbers under control conditions, and peroxisomes in a crescent formation surrounding the vacuole after 15 h of Cd treatment. This suggests that ATG8h is involved in pexophagy and that the absence of ATG8h induces alternative pathways to regulate peroxisomal protein content. However, Cd does not appear to induce bulk autophagy after 3 d of treatment, with longer treatments being required to analyse Cd-induced autophagy.

Key words: autophagy, cadmium, oxidative stress, peroxisome, pexophagy

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