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Signaling unmasked

Autophagy and catalase promote programmed cell death

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Autophagy contributes to the removal of harmful cellular refuse, whereas catalase plays an important protective role by detoxifying reactive oxygen species. We recently found that autophagy and catalase are also required for promoting programmed cell death induced during plant immune responses. Here we discuss the difficulties in identifying cell death effectors, which are also required to maintain cellular homeostasis, and how their prodeath roles were unmasked using an unbiased forward genetics approach.

Keywords: cell death, autophagy, catalase, plant defense, plant-microbe interactions

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In plants, autophagic components have mainly been identified based on similarity to their yeast counterparts. Because of this extensive conservation of the autophagic machinery, limited efforts have been invested in developing screening strategies for the identification of autophagy-related (*ATG*) genes in plants. Instead, available *atg* mutants have been used in reverse genetics approaches to characterize the roles of autophagy in plant cells.

Our road into autophagy signaling was a different one. We initially sought to identify cell death signaling components in an *Arabidopsis* forward screen for suppression of cell death induced by the antineoplastic drug hydroxyurea. We recovered many catalase loss-of-function alleles and showed that the apparent catalase specificity could be explained by a direct interaction between catalase and hydroxyurea. While this discovery was informative with respect to understanding the mode of action of the drug, it seemed at first to reveal little about endogenous cell death signaling mechanisms in plants. To identify cell death effectors acting downstream of drug toxification by catalase, we then screened for hydroxyurea-resistant mutants with normal catalase activity and recovered a loss of function allele of the autophagy gene *ATG2*. In our screening system, hydroxyurea kills wild-type plants at a very early stage during germination, between the emergence of the embryonic root and the unfolding of the cotelydons. As a result of the unbiased screening of very young plants for suppression of cell death followed by map-based cloning of the causal gene, we could conclude that autophagy is unequivocally required for promotion of cell death induced by hydroxyurea.

This prodeath role of autophagy was in agreement with previous results implicating autophagy in the mediation of cell death induced during plant immune responses, but seemed at odds with reports of autophagy being required for limiting the spread of cell death during the same type of hypersensitive immune response (HR). When we tested immunity-associated cell death upon bacterial infection in the *atg2* mutant, we found it strongly attenuated, mirroring the prodeath role indicated by the hydroxyurea screen. So how can autophagy both attenuate and promote HR cell death? The explanation most likely hinges on the role of autophagy in cell homeostasis. A hallmark of plant autophagy mutants is premature senescence, which, depending on the severity of the autophagy deficiency, sets

in with clearly visible cell death symptoms in 5- to 6-wk old plants. Since cell death assays that depend on leaf infiltration with bacterial suspensions require a certain leaf size, they cannot be performed much earlier than 3–4 wk post germination and are frequently performed using 5-wk-old plants. We think that quantifying cell death in older autophagy mutants carries with it the risk that accumulating cellular stresses could mask autophagy-dependent cell death signaling, because the tissue is already on the brink of senescing. This is likely the reason that we see such clear suppression of cell death for the *atg2* mutants in the relatively young tissues, germinating seedlings and 3–4-wk-old leaves, used in our study.

With the role of autophagy in cell death signaling firmly established, we were intrigued by the dual loss of sensitivity of the autophagy mutants to hydroxyurea-induced and immunity-triggered cell death. Revisiting the catalase-deficient mutants recovered from the same screen, we found that they shared this dual loss of sensitivity. Moreover, while the catalase mutants displayed normal basal and starvation-induced autophagy, they showed impaired immunity-triggered autophagy. This places catalase upstream of autophagy in immunity-associated cell death and highlights catalase as a putative interpreter of reactive oxygen species signaling during the HR. While autophagy mutants display a premature senescence phenotype due to accumulation of cellular refuse, catalase mutants develop lesions under conditions that promote production of hydrogen peroxide through photorespiration. In the same way as assaying older tissues from autophagy mutants could mask the involvement of autophagy in cell death signaling, the analysis of catalase mutants under stress-inducing light conditions appears to have masked the role of catalase in promotion of cell death.

Our study has placed autophagy and catalase in the same immunity-triggered cell death signaling pathway. The key to unmasking their new roles was an unbiased genetic screen performed under conditions where the well-described roles of autophagy and catalase in cellular homeostasis were not of critical importance compared with the strong selective pressure of the cell death-inducing signal. Further studies are now needed to reach a more detailed understanding of autophagy-mediated regulation of plant cell death.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.