

Sulfide as a signaling molecule in autophagy

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Hydrogen sulfide is already recognized as an important signaling molecule in mammalian systems, and emerging data suggest that H₂S is a signaling molecule just as important as nitric oxide (NO) and H₂O₂ in plants. Although sulfide is generated in chloroplasts and mitochondria, it is present predominantly in the charged HS⁻ form due to the basic pH inside both organelles, thus requiring an active transporter, which is yet to be identified, to be released. In *Arabidopsis*, we found that the cytosolic L-cysteine desulfhydrase DES1 is involved in the degradation of cysteine, and therefore responsible for the generation of H₂S in this cellular compartment. DES1 deficiency leads to the induction of autophagy. Moreover, we have demonstrated that sulfide in particular exerts a general effect on autophagy through negative regulation, in a way unrelated to nutrient deficiency. The mechanisms of H₂S action and its molecular targets are largely unknown, although in animal systems, protein S-sulfhydration has been proposed as a mechanism for sulfide-mediated signaling.

Hydrogen sulfide has increasingly been recognized as an important signaling molecule of comparable importance to NO and carbon monoxide in mammalian systems. Similarly, emerging data over recent years suggest that H₂S may be a new signaling molecule equally important to plants as NO and H₂O₂. It has been implicated, for example, in the protection against metal stress or the regulation of photosynthesis and stomata movement. We recently investigated DES1 from *Arabidopsis thaliana*, which is the only identified L-cysteine desulfhydrase located in the cytosol, and

is involved in the degradation of cysteine and the concomitant generation of H₂S in this cellular compartment. Mutations of the *DES1* gene impede H₂S generation in the cytosol and strongly affect plant metabolism. The detailed characterization of null mutants has provided insight into the role of sulfide generated from Cys in the cytosol as a signaling molecule regulating the process of autophagy.

DES1 deficiency leads to the accumulation and lipidation of ATG8 isoforms in *Arabidopsis*, a landmark of autophagy activation in different systems. In addition, transcriptomic data confirm the induction of autophagy in *des1* null mutants. Because mutation of the *DES1* gene disrupts H₂S generation in the *Arabidopsis* cytosol, restoring the capacity of H₂S generation eliminates the phenotypic differences of the null mutants from wild-type plants. Exogenous sulfide prevents ATG8 protein accumulation and lipidation in *des1* mutant plants, indicating that intracellular H₂S regulates autophagy. Interestingly, exogenous sulfide is also able to suppress autophagy induction caused by carbon starvation in wild-type plants, whereas exogenous ammonium, a second product of DES1 activity, has no effect on autophagy. Therefore, sulfide, in particular, exerts a general effect on autophagy through negative regulation, and in a way unrelated to nutrient limitation stress, as we have demonstrated in our plant system.

There are three subcellular compartments where sulfide is generated in the plant cell (Fig. 1). In the chloroplast, sulfide is produced by photosynthetic sulfate reduction to incorporate it into organic metabolites and cellular components. Although it has been proposed that H₂S

Keywords: *Arabidopsis*, ATG8 lipidation, carbon starvation, cysteine desulfhydrase, cytosolic sulfide, senescence

Submitted: 12/13/12

Revised: 12/27/12

Accepted: 01/02/13

<http://dx.doi.org/10.4161/auto.23460>

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Punctum to: Álvarez C, García I, Moreno I, Pérez-Pérez ME, Crespo JL, Romero LC, et al. Cysteine-generated sulfide in the cytosol negatively regulates autophagy and modulates the transcriptional profile in *Arabidopsis*. *Plant Cell* 2012; 24:4621–34; PMID:23144183; <http://dx.doi.org/10.1105/tpc.112.105403>

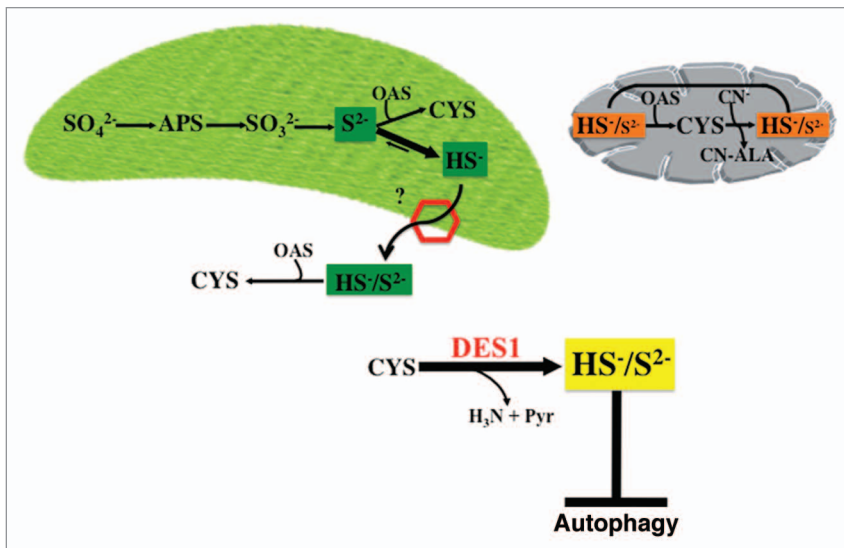


Figure 1. Cysteine-generated sulfide in the cytosol regulates autophagy. In the Arabidopsis plant cell there are different subcellular compartments where sulfide is generated: chloroplast, mitochondria and cytosol. In the chloroplast, sulfide is produced by photosynthetic sulfate reduction and its predominant charged HS^- form requires an active transporter (unidentified to date) to cross the envelope. In the mitochondria, sulfide is recycled for cyanide detoxification and is also predominantly present in the charged HS^- form. In the cytosol, DES1 is responsible for the generation of sulfide. Irrespective of nutrient conditions, the sulfide generated in the cytosol behaves as a signaling molecule acting as a repressor of autophagy.

reaches the cytosol via diffusion through the chloroplast envelope membrane, hydrogen sulfide is weakly acidic and dissociates in aqueous solution, and, at the stromal pH of 8.5, sulfide is predominantly in the charged HS^- form, which cannot permeate the chloroplast envelope and requires an active transporter that has yet to be identified in plant cells. In the mitochondrion, sulfide is produced in the detoxification of cyanide, which is a potent inhibitor of cytochrome c oxidase. But sulfide is also an inhibitor of this enzyme and is necessary to be consumed to produce cysteine, which in turn, is used to detoxify cyanide, thus generating a cyclic pathway (Fig. 1). Similar to the chloroplast, in metabolically active cells, the pH of the mitochondrial stroma is about 8, due to the pump of protons out of the matrix, and consequently the sulfide is predominantly present in the charged HS^- form and its release out of the mitochondria is very limited. In the cytosol, DES1 is involved in the production of sulfide generated to act as a signaling molecule. Therefore, in the plant cell DES1 should be responsible for modulating the generation of sulfide for the repression of autophagy in a manner unrelated to nutrient

limitation. The mechanisms underlying this modulation and the specific conditions in which it takes place are still an open question. However, experimental evidence can provide some clues about the conditions in which sulfide acts as a signaling molecule regulating autophagy.

Autophagy is involved in normal plant development processes even in nutrient-rich conditions. Thus, autophagy occurs before or during senescence irrespective of the plant nutrient status, and sulfide might have a signaling role in this process. Accordingly, DES1 deficiency promotes a significant reduction in endogenous sulfide together with premature leaf senescence and autophagy activation. Moreover, increasing the level of endogenous sulfide eliminates premature leaf senescence and reverses autophagy induction. Autophagy has also been shown to play diverse roles in response to abiotic and biotic stresses. Under abiotic stress conditions autophagy is generally considered to be a response for cell survival, and, curiously, the *des1* null mutants with lower endogenous sulfide content and induced autophagy show an increased tolerance to Cd and H_2O_2 stresses. Recently autophagy has been implicated in plant immunity through

controlling programmed cell death associated with microbial effector-triggered immunity, although its exact role is still unclear, since autophagy acts with a pro-survival or prodeath role. However, it is accepted that autophagy suppresses the spread of pathogen-induced cell death to uninfected tissue from the infection site that is the hypersensitive response (HR) in addition to the activation of systemic acquired resistance (SAR). Thus again, the *des1* null mutants behave as constitutive SAR mutants upon pathogen infection, suggesting an inverse correlation between endogenous sulfide content and autophagy.

The knowledge about the mechanisms of H_2S action and its molecular targets is largely deficient in animal systems. Protein S-sulfhydration has been proposed as a mechanism for sulfide-mediated signaling in analogy with S-nitrosylation. For this post-translational modification to occur, the cysteine residues should be in an oxidized state as sulfenic acid or disulfide, being then attacked by the hydrosulfide anion to produce a persulfide product. It has been suggested that the thiol redox state can profoundly influence autophagy, especially during the initiation and completion of the autophagosomes. Proteins susceptible to modification by sulfhydration are the E1 and E2 enzymes involved in the ubiquitination processes, which are regulated through a reversible S-thiolation. Another possible target of sulfide could be the cysteine protease ATG4 that has been characterized in detail in mammalian systems to be redox regulated, and a cysteine residue located near the catalytic site is critical for this regulation. Finally, autophagy is a process involved in the proteolytic degradation of cellular macromolecules and requires the activity of proteases. The *A. thaliana* genome has over 550 protease sequences, and recent findings highlight the potential of proteases to participate in many aspects of plant growth and development. In particular, the C1A papain-like cysteine proteases contain a catalytic Cys that can be a target of S-sulfhydration.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

This work was funded by the European Regional Development Fund and the Ministerio de Economía y Competitividad, Spain (Grants BIO2010-15201, BFU2012-35913 and CSD2007-00057).