Beyond toxicity

A regulatory role for mitochondrial cyanide

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In non-cyanogenic plants, cyanide is a co-product of ethylene and camalexin biosynthesis. To maintain cyanide at non-toxic levels, *Arabidopsis* plants express the mitochondrial β-cyanoalanine synthase CYS-C1. *CYS-C1* knockout leads to an increased level of cyanide in the roots and leaves and a severe defect in root hair morphogenesis, suggesting that cyanide acts as a signaling factor in root development. During compatible and incompatible plant-bacteria interactions, cyanide accumulation and *CYS-C1* gene expression are negatively correlated. Moreover, *CYS-C1* mutation increases both plant tolerance to biotrophic pathogens and their susceptibility to necrotrophic fungi, indicating that cyanide could stimulate the salicylic acid-dependent signaling pathway of the plant immune system. We hypothesize that CYS-C1 is essential for maintaining non-toxic concentrations of cyanide in the mitochondria to facilitate cyanide's role in signaling.

Cyanide is a low molecular weight molecule that reacts with keto compounds and Schiff base intermediates to yield cyanohydrins and stable nitrile derivatives, respectively, and it chelates di- and trivalent metal ions in the prosthetic groups of several metalloproteins, affecting their function.[1] Cyanide is most lethal in mitochondria, where it binds to the heme iron of cytochrome *c* oxidase and blocks oxidative phosphorylation, thereby impairing vital functions.[2] Despite its toxicity, cyanide is present in a wide range of organisms of all kingdoms including bacteria, fungi, insects, and plants.[3-5] Cyanogenic plants synthesize cyanogenic compounds to poison herbivores by releasing cyanide.[6,7] In non-cyanogenic plants, cyanide is a co-product of ethylene and camalexin biosynthesis, both of which are molecules involved in plant defense against pathogens.[8-10]

Ethylene and camalexin are synthesized by the action of the 1-aminocyclopropane-1-carboxylic acid oxidase oxidase and the P450 enzyme PAD3 (CYP71B15), respectively, in the plant cell cytosol,[11-13] where cyanide is produced. HCN is gaseous but can partially dissolve in water. It is weakly acidic and can dissociate into H^+ and CN^- in aqueous solution, especially in more basic solutions. Because the cytosolic pH is close to 7, it is expected that cyanide would be mostly present as hydrogen cyanide (HCN) in this compartment, according to its pK_a of 9.3. The moderate lipid solubility and small size of the HCN molecule allow HCN to cross membranes rapidly and enter mitochondria, where the respiration-associated proton pumps transport protons from the matrix to the inner and outer mitochondrial membranes, thus raising the matrix pH to approximately 8. Hence, HCN would dissociate into H^+ and CN^- at a high level in the mitochondrial matrix, preventing its escape from this organelle, which is its main target. These physicochemical properties, together with its moderate reactivity and its ability to modify protein activity, make cyanide a candidate signaling molecule.

In plants, cyanide has been proposed to act as a regulator of biological processes, such as seed dormancy and germination,[4,14,15] and to play a role in resistance to viral and fungal pathogens.[16-18] The use of
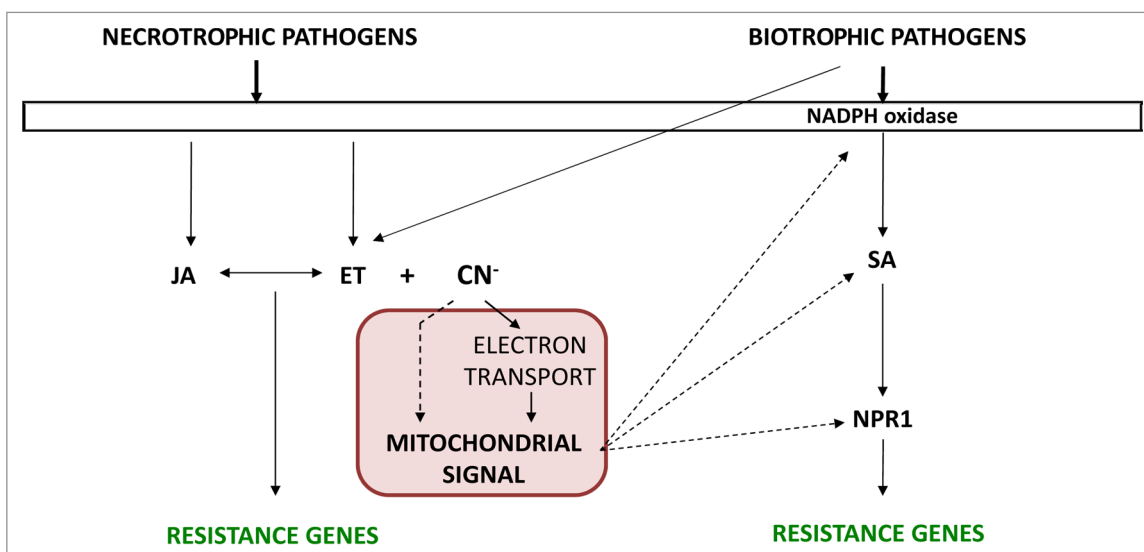
Figure 1. Cyanide and the plant immune response. Pathogen attack induces the biosynthesis of hormones, mainly jasmonic acid (JA) and ethylene (ET) in response to necrotrophic pathogens and salicylic acid (SA) in response to biotrophic pathogens, although biotrophes can also induce ET biosynthesis. In our model, cyanide, which is produced concomitantly to ethylene, enters mitochondria where it generates a direct and/or indirect a signal that stimulates the SA-dependent response pathway.

exogenously applied cyanide has led to these conclusions; however, our work has clarified the role of endogenously produced cyanide.

Cyanide must be rapidly detoxified and metabolized by the plant to maintain the concentration below toxic levels. *Arabidopsis* plants carry the mitochondrial β-cyanoalanine synthase (CAS) CYS-C1,[19] which, together with *O*-acetylserine (thiol) lyase (OASTL), belongs to the family of β-substituted alanine synthase enzymes. CAS is a pyridoxal phosphate-dependent enzyme that uses cysteine to detoxify cyanide by converting cyanide and cysteine into hydrogen sulfide (H_2S) and β-cyanoalanine, which is later converted to Asn, Asp, and ammonia by NIT4 nitrilases, recycling the nitrogen for the plant.[20,21] The CAS activity and its role in cyanide detoxification have also been described in other plants, such as rice and soybean.[22-24] Mutants lacking CAS activity would therefore allow the investigation of the role of endogenously produced cyanide.

The *CYS-C1* loss-of-function mutation is not toxic for the plant and leads to an increased level of cyanide in the *cys-c1* mutant as well as a root hairless phenotype, suggesting that endogenously produced cyanide plays a signaling role in root development.[25] The root hair defect is phenocopied in wild-type plants by the exogenous addition of cyanide to the growth medium and is reversed by the addition of hydroxocobalamin, which is the most commonly used antidote for severe acute cyanide poisoning in humans.[25]

Because cyanide production is closely linked to the biosynthesis of metabolites involved in the immune response, the effect of cyanide in the plant response to pathogens has been analyzed.[26] In particular, it has been observed that *cys-c1* plants present an increased susceptibility to the necrotrophic fungus *Botrytis cinerea* and an increased tolerance to the biotrophic bacterium *Pseudomonas syringae* pv *tomato DC3000* as well as the beet curly top virus. The *cys-c1* mutation reduces the respiration rate in leaves, causes the accumulation of reactive oxygen species, and induces the expression of alternative oxidase *AOX1a* and the pathogenesis-related *PR1*. Interestingly, no patches of dead cells are observed when this mutant is grown under either a long or short photoperiod. We hypothesize that cyanide, which is transiently accumulated during avirulent bacterial infection and constitutively accumulated in the *cys-c1* mutant, generates a mitochondrial signal, the nature of which is yet to be identified. The fact that uncoupling of oxidative respiration,

induction of alternative oxidase activity, and accumulation of reactive oxygen species (ROS) are observed in *cys-c1* plants suggests that ROS generated in mitochondria could induce a signal that generates a series of events that modulate the salicylic acid-dependent signaling pathway of the plant immune system. ROS generation is essential for the establishment of the signaling pathway leading to resistance to biotrophic pathogens in the initial steps of the interaction,[27] and ROS modulate late steps in the SA-dependent response pathway, such as the import of NPR1 into the nucleus or SA biosynthesis and accumulation.[28,29] A direct role for cyanide in the generation of this mitochondrial signal cannot be excluded, although this is very unlikely (**Fig. 1**).

In conclusion, mitochondrial β-cyanoalanine synthase activity is essential for maintaining non-toxic concentrations of cyanide; however, the transient repression of the *CYS-C1* gene and the accumulation of cyanide in response to specific pathogens suggest the existence of a signaling mechanism that is dependent on cyanide, probably through the controlled accumulation of ROS in this organelle. The mechanisms underlying this modulation and, especially, the mode of action and specific targets of cyanide

are open questions and are the focus of our present research.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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