ACTA
PHYSIOLOGIAE
PLANTARUM
Vol. 19. No. 4. 1997: 577-579

Is tobacco response to TMV infection modulated by catalase activity?

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Abstract

Previous studies argue that salicylic acid (SA) plays an important role in the plant signal transduction pathway(s) leading to disease resistance. It has been proposed that one of its modes of action is inhibition of catalase and elevation of H_2O_2 level in the tissue. To verify the role of SA and H_2O_2 during pathogenesis, transgenic tobacco plants expressing *Saccharomyces cerevisiae CTA1* gene coding for peroxisomal catalase were constructed. These plants possess 2-4-fold higher total catalase activity under normal growth conditions. No symptoms of chlorosis and/or necrosis were observed. Levels of pathogenesis-related proteins (PR) and their respective mRNAs were significantly reduced in the infected leaves of the transgenic plants. No change in PR expression was detected in uninfected leaves of both CTA1 and control plants challenged with TMV.

These results suggest that elevation in catalase activity and resulting reduction of H_2O_2 level results in more severe local disease symptoms, apparently due to alteration of the hypersensitive response mechanism and does not influence systemic acquired resistance after viral infection.

Plants which permanently face various kind of stresses in their environment evolved complex responses. Pathogen assault can activate general plant defense mechanisms consisting of several processes such as: production of various antimicrobial compounds (phytoalexins); lignification of the cell wall, cross-linking of cell wall proteins and callose deposition; synthesis of proteins with potential pathogen-degrading activity (Figure). These or additional triggered mechanisms lead to the localization of the pathogen to the site of entry and surrounding cells. Various factors such as Ca^{2+}, ethylene, jasmonic acid and salicylic acid (SA) have been proposed to be signal transducers involved in establishing successful defense against pathogen attack (Klessig and Malamy 1994). SA has focused particular attention because of its pharmaceutical properties and its universal function in both animal and plant defense against disease (Cutt and Klessig 1992). Exogenous application of SA induces Pathogenesis Related (PR) gene expression and leads to development of systemic acquired resistance (SAR) (Bi *et al.* 1995). The mode of SA action in

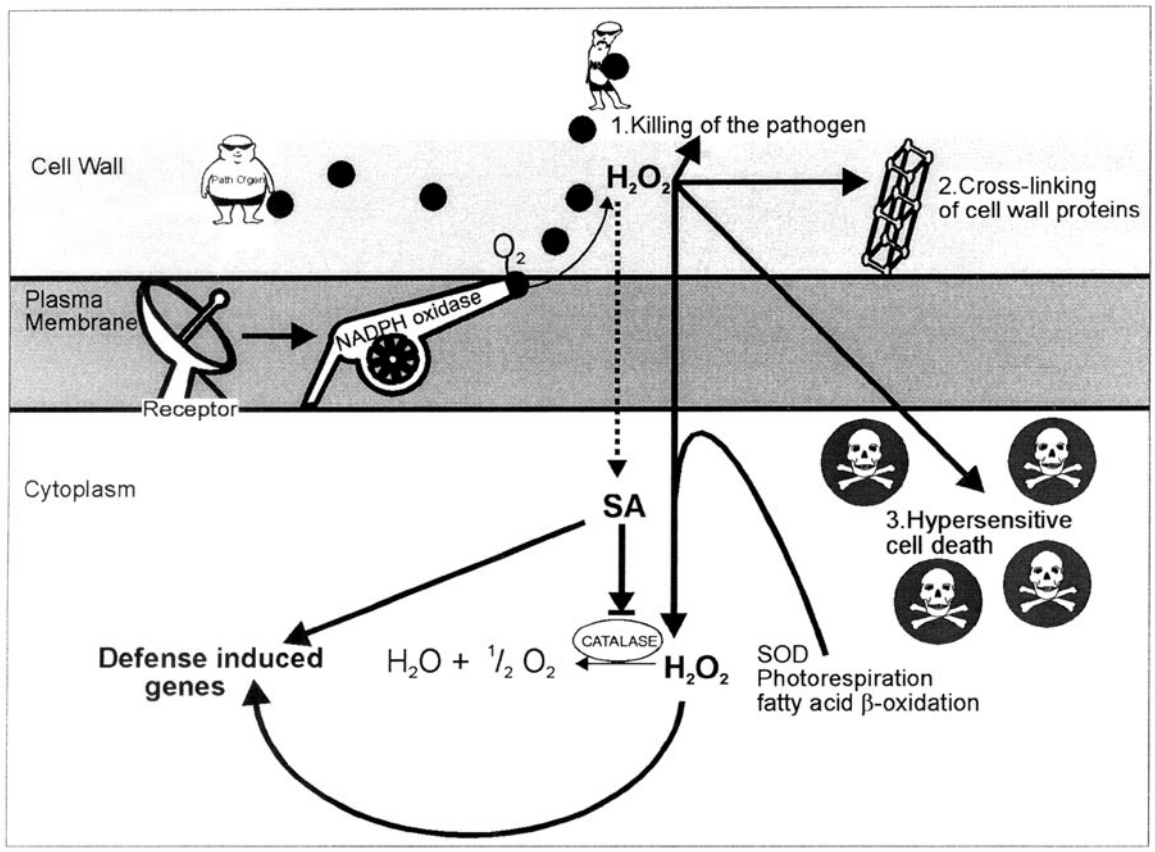

Figure. A simplified model for signal transduction in plant defense responses.

inducing resistance to plant pathogens is not completely known. The observation that protein that specifically binds SA in tobacco (Chen *et al.* 1993, Du and Klessig 1997) and in several other plant species (Sánchez-Casas and Klessig 1994) has catalase activity led to the suggestion that SA acts by elevating level of reactive oxygen species (ROS) such H_2O_2 (Figure) (Chen *et al.* 1993). Inhibition of catalase activity by SA *in vitro* and in tobacco cell cultures (Sánchez-Casas and Klessig 1994, Contrath *et al.* 1995) provides support for this hypothesis. Additionally, plants treated with SA accumulate H_2O_2 and show enhanced PR gene expression, whereas antioxidants suppress the induction of PR genes by SA (Contrath *et al.* 1995).

However, a growing body of evidence has accumulated suggesting that physiologically relevant concentrations of SA, detected in plant tissues, do not inhibit catalase activities *in vivo* (Willekens *et al.* 1994, Bi *et al.* 1995, Summermater *et al.* 1995). On the other hand, it is plausible that SA may reach high concentration within particular cell compartments. Moreover, SA may bind specifically not only to catalase but also to several Fe-containing enzymes such as lipoxidase or peroxidase (Ruffer *et al.* 1995). High concentration of H_2O_2 have been shown to induce biosynthesis of SA (Léon *et al.* 1995, Neuenschwander *et al.* 1995, Summermatter *et al.* 1995). This suggests that H_2O_2 may act upstream of SA in the process of SAR induction. Although it is not clear how they act, SA and/or H_2O_2-derived signals are considered to have a regulatory role in developing resistance to pathogens (Hammond-Kosack and Jones 1996).

To elucidate the role of H_2O_2 in the defense response, we used transgenic tobacco plants that express yeast catalase gene (CTA1). Total catalase activity in extracts prepared from these plants was over twice higher as compared to wild-type plants (Talarczyk *et al.* manuscript in preparation). We examined the influence of elevated levels of catalase on the defense response to tobacco mosaic virus (TMV) infection. Intensity of the hypersensitive response (HR), PR gene expression and SAR after infection with TMV were investigated. We have found that the yeast CTA1 catalase, was only partially inhibited by SA *in vitro*, retaining around 50 % of the initial activity at the concentration as high as 5 mM, while the activity in wild-type plants was almost completely inhibited in these conditions.

In the next experiments we have found that reaction to TMV infection was altered in CTA1-expressing plants. All transgenic plants were able to establish HR in response to infection with TMV but the average size of necrotic lesions on leaves of the CTA1-expressing plants was significantly bigger compared with those on leaves of the control plants. Additionally, we have found that expression of acidic (but not basic) isoforms of PR genes was reduced in leaves of the transgenic plants infected with TMV as compared to the controls. The reduction in lesion size and level of expression PR genes in upper leaves upon secondary infection allow us to conclude that CTA1 plants were able to establish SAR.

These results suggest that elevated level of catalase activity influences intensity of the hypersensitive response upon pathogen challenge.

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This research was supported by a grant from Komitet Badań Naukowych (project no. 6P20302006).

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