

Waking up for defense! Melatonin as a regulator of stomatal immunity in plants

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Melatonin is a tryptophan-derived compound discovered in the late 1950s as a molecule produced by the pineal gland of most vertebrates. Melatonin gained notoriety in the 1970s when it was demonstrated that its production increased in humans during the night in a rhythmic fashion associated with sleep synchronization (Xie et al., 2017). Melatonin is a ubiquitous molecule produced by bacteria, fungi, plants, and animals. In plants, melatonin regulates a wide range of processes, such as seed germination, root and shoot growth, and production of secondary metabolites, and is involved in circadian cycle regulation (Arao and Hernández-Ruiz, 2019). The recent identification of a melatonin receptor named PHYTOMELATONIN RECEPTOR1 (AtPMTR1) in the plant model *Arabidopsis thaliana* demonstrated that melatonin also governs an AtPMTR1-dependent mechanism of stomatal closure (Wei et al., 2018). As stomatal closure is a well-known process utilized by plants to restrain the invasion of pathogens, usually referred to as stomatal immunity (Melotto et al., 2006), melatonin is now gaining attention for its capacity to mediate responses to biotic stress in plants (Moustafa-Farag et al., 2020). Unfortunately, the underlying molecular mechanisms of melatonin regulation of plant immunity remain largely unknown.

In this issue of *Plant Physiology*, Yang et al. (2021) uncover aspects in the signaling pathway associated with melatonin-induced stomatal immunity in *Arabidopsis* and in the Chinese medicinal plant *Panax notoginseng*. The authors treated leaves of *P. notoginseng* with melatonin, followed immediately or 2 h later by inoculation with bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*)

DC3000. In both cases, melatonin treatment significantly reduced the bacterial growth in *P. notoginseng* leaves compared to control plants (mock treatment), indicating that melatonin modulates immunity. This result was partially explained by melatonin-induced stomatal closure observed in *P. notoginseng* leaves by 2 h after treatment, thus limiting the capacity of *Pst* to invade and infect the plant. As in a chemical arms race, *Pst* can produce coronatine, a phytotoxin that reverts the melatonin-induced stomatal closure, reopening these pores by 4 h after bacterial inoculation. Indeed, co-treatment of melatonin and coronatine inhibits the melatonin-induced stomatal closure in *P. notoginseng* leaves.

One of the key processes associated with stomatal immunity involves the activity of NADPH oxidases, enzymes involved in the production of reactive oxygen species (ROS) that function as secondary messengers to induce stomatal closure (Kwak et al., 2003; Li et al., 2020). To evaluate whether NADPH oxidases and ROS are involved in the melatonin-induced stomatal closure in *P. notoginseng*, the authors treated leaves with melatonin and catalase (a ROS scavenger) or melatonin and diphenyleioidonium chloride (an inhibitor of NADPH oxidase). Both treatments decreased the stomatal aperture when compared to control plants treated only with melatonin, indicating that NADPH oxidase activity and ROS production are involved in stomatal closure induced by melatonin.

To further dissect the molecular machinery involved with this process, the authors used immunoblot experiments to identify two MAPK kinases, MPK3 and MPK6, whose melatonin-induced activation promotes stomatal closure in *P. notoginseng*. These results were confirmed using MPK3- and

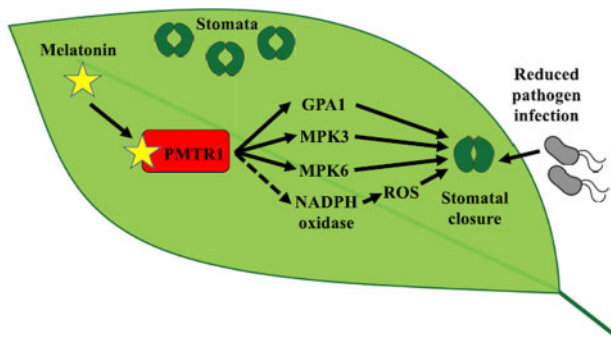


Figure 1 Melatonin as a regulator of stomatal immunity in plants. In this issue of *Plant Physiology*, Yang et al. (2021) dissect the molecular machinery involved with melatonin-induced stomatal closure in *Arabidopsis* and *P. notoginseng*. The authors demonstrate that melatonin binds to the melatonin receptor PMTR1 to activate a signaling cascade that includes GPA1 and activation of MAPKs MPK3 and MPK6 to induce stomatal closure and increase resistance to bacterial pathogen infection. They also demonstrate that melatonin-mediated stomatal closure depends on the activity of NADPH oxidase and ROS. The dashed line indicates a PMTR1-NADPH oxidase connection previously identified by the same research group (Wei et al., 2018).

MPK6-inducible knockout mutants in *Arabidopsis*, where inactivation of the two MAPKs hinders the capacity of melatonin to induce stomatal closure and to mediate bacterial resistance. Previous studies demonstrated that melatonin binding to the AtPMTR1 receptor is required for melatonin-mediated stomatal closure in *Arabidopsis* (Li et al., 2020). Using an *Arabidopsis pmtr1* knockout mutant, the authors demonstrated that a functional melatonin receptor is necessary to activate MPK3 and MPK6 and consequently to promote stomatal closure upon melatonin perception. The role of PMTR1 in stomatal immunity was confirmed by pathogen infection assays using *Pst*, where the receptor knockout showed reduced bacterial resistance and reduced stomatal closure when compared to melatonin-treated control plants. By contrast, overexpression of PMTR1 in *Arabidopsis* led to enhanced bacterial resistance, even in the absence of melatonin. Their experiments with PMTR1 also led to the identification of an MPK3- and MPK6-independent molecular component that is necessary for melatonin-mediated stomatal immunity, the G protein α -subunit (GPA1). GPA1 is an important component of numerous signal transduction pathways, and it has been associated with stomatal closure before (Zhang et al., 2008). However, work by Yang et al. (2021) allowed evaluating how GPA1 participates in a more intricate signaling pathway that is activated upon melatonin treatment to induce stomatal immunity in plants (Figure 1).

Melatonin is currently one of the most popular medicines to treat sleep disorders in humans, but its effects on plant

physiology are just now being uncovered. Work by Yang et al. (2021) provides a comprehensive overview on the signaling cascade activated by melatonin to induce stomatal immunity in *P. notoginseng* and *Arabidopsis* (Figure 1). Although additional components of the melatonin signaling pathway remain to be identified and more studies are necessary to understand how this molecular machinery is conserved in other plant species, this work already offers powerful insights for reanalyzing previous studies relating to the circadian clock and the induction of the defense response against pests and pathogens at different times of the day (Wang et al., 2011; Goodspeed et al., 2012). The potential role of melatonin keeping plants “awake” to biotic attack might be another path to enhance plant resilience in the near future.

Conflict of interest statement. None declared.

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