

MaXB3 Limits Ethylene Production and Ripening of Banana Fruits

Ripening of climacteric fruits such as banana (*Musa acuminata*), tomato (*Solanum lycopersicum*), and mango (*Mangifera indica*) is typically triggered and regulated by the gaseous plant hormone ethylene (Seymour et al., 2013; Hu et al., 2019). As a gas, ethylene levels and subsequent signal transduction in plants can be regulated by changes in biosynthesis and gas diffusion rates (Abeles et al., 2012; Hartman et al., 2019). In climacteric fruits, ethylene signaling is activated through an initial burst in ethylene biosynthesis at the onset of the ripening process (Seymour et al., 2013). Ethylene biosynthesis can be summarized as a simple two-step reaction, where 1-aminocyclopropane-1-carboxylic (ACC) synthase (ACS) controls the production of ACC, which in turn is converted to ethylene by ACC oxidase (ACO; Fig. 1A; Pattyn et al., 2020). While ACSs and ACOs are established regulators of ethylene biosynthesis and subsequent climacteric fruit ripening (Seymour et al., 2013), little is known about how these enzymes themselves are regulated to control ripening processes.

In this issue of *Plant Physiology*, Shan et al. (2020) report several genes involved in the transcriptional and posttranslational control of ethylene biosynthesis and banana fruit ripening. The authors previously uncovered ETHYLENE RESPONSE FACTOR11 (MaERF11) as a transcriptional repressor of the ethylene biosynthesis genes *MaACS1* and *MaACO1* (Xiao et al., 2013). In Shan et al. (2020), they reveal that *MaERF11* itself is also negatively regulated by the two NAC transcription factors, MaNAC1 and MaNAC2. Using several techniques, they demonstrate that both MaNAC1 and MaNAC2 directly repress *MaERF11* promoter activity. Together, these findings reveal that MaNAC1 and MaNAC2 positively regulate ethylene biosynthesis and banana fruit ripening through inhibition of the transcriptional repressor ERF11 (Fig. 1A).

Next, the authors used yeast two-hybrid screens to search for putative MaNAC1 and MaNAC2 interacting proteins and uncovered the E3 ligase XA21 Binding Protein3 (MaXB3) as a top candidate. Moreover, they found that *MaXB3* transcript and protein levels decreased during fruit ripening, while MaNAC1 and MaNAC2 levels increased (Fig. 1B). This negative association suggested that MaXB3 and MaNAC1 and MaNAC2 could potentially balance each other's levels to control fruit ripening. Previous research in rice and *Arabidopsis* classified MaXB3 orthologs as functional E3 ligases that flag their targets for ubiquitination, leading to proteasomal degradation (Wang et al., 2006; Prasad et al., 2010; Lyzenga et al., 2012). Therefore, the

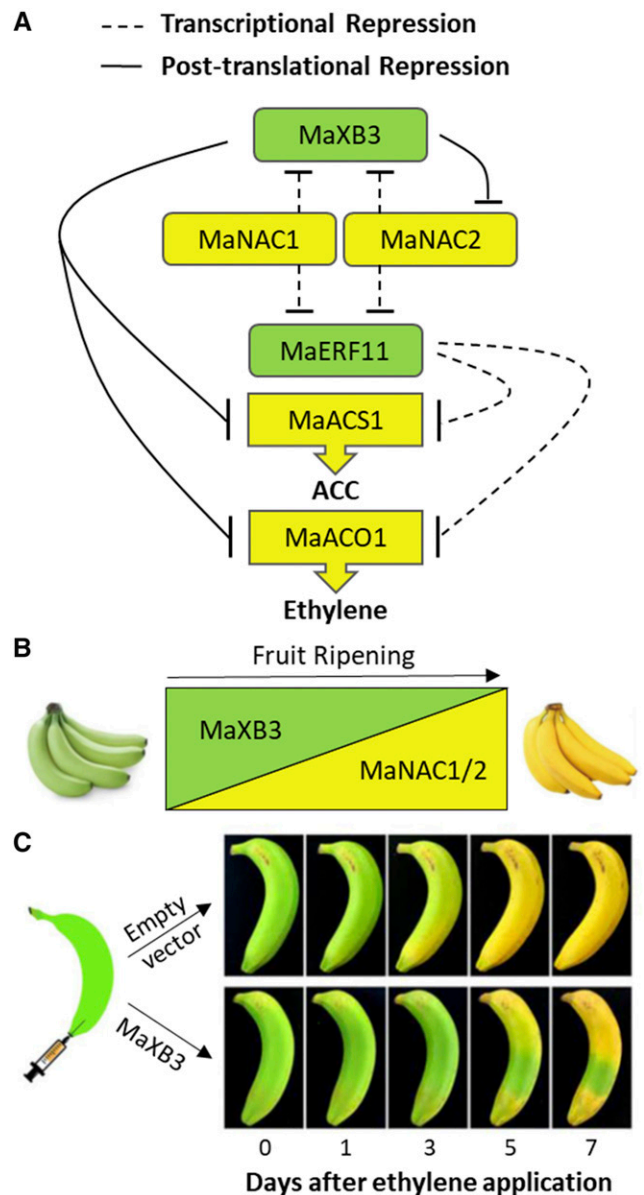


Figure 1. A, Proposed model for how E3 ligase MaXB3 negatively regulates ethylene biosynthesis and banana fruit ripening, showing positive (yellow) and negative (green) regulators of ethylene biosynthesis. MaACS1 and MaACO1 regulate ethylene biosynthesis and control climacteric fruit ripening. MaXB3 targets positive regulators of ethylene biosynthesis MaNAC2, MaACS1, and MaACO1 for ubiquitination and degradation. MaNAC1 and MaNAC2 transcriptionally repress *MaXB3* and *ERF11*, while *ERF11* inhibits transcriptional activation of *MaACS1* and *MaACO1*. B, MaXB3 (green) protein and transcript levels decrease, while MaNAC1 and MaNAC2 (yellow) protein and transcript levels increase during banana fruit ripening. C, Inoculation of *Agrobacterium tumefaciens* solution (causing overexpression of MaXB3 or an empty vector) into banana pulp through the fruit distal end. Fruits injected with MaXB3 exhibited a reduction in ethylene biosynthesis and fruit ripening. Images were adapted from figures 7, A and B, and 10 of Shan et al. (2020).

¹Author for contact: j.g.w.hartman@uu.nl.

²Senior author.

www.plantphysiol.org/cgi/doi/10.1104/pp.20.01140

authors tested whether MaNAC1 and MaNAC2 levels are under MaXB3-mediated proteolytic control and found that MaXB3 indeed ubiquitinates and degrades MaNAC2 but not MaNAC1. Additionally, they showed that MaXB3 interacts with and ubiquitinates the essential ethylene biosynthesis enzymes MaACS1 and MaACO1 and causes their subsequent proteasomal degradation. Collectively, the results of Shan et al. (2020) show that MaXB3 promotes MaACS1, MaACO1, and MaNAC2 degradation and potentially limits ethylene biosynthesis and fruit ripening in banana (Fig. 1A).

Since MaXB3 levels typically decline as the fruits ripen (Fig. 1B), the authors wondered if ectopic overexpression of *MaXB3* would lead to a delay in ethylene production and subsequent ripening. To test this, MaXB3 was transiently overexpressed in unripe bananas. MaXB3 overexpression indeed led to a local reduction in MaNAC2, MaACS1, MaACO1, ethylene biosynthesis, ethylene-responsive gene expression, and a delay in ripening (Fig. 1C). Finally, the authors heterologously expressed *MaXB3* in tomato plants and again found that elevated MaXB3 levels led to a reduction of ethylene biosynthesis, signaling, and ripening in tomato fruits. These experiments suggest that MaXB3 function is also conserved in other climacteric fruit species. Taken together, the work of Shan et al. (2020) identifies MaXB3 as an essential negative regulator of ethylene biosynthesis and climacteric fruit ripening.

Several questions remain about how MaXB3 is normally regulated during fruit development and subsequent ripening. Shan et al. (2020) show that *MaXB3* is transcriptionally repressed by increasing levels of MaNAC1, MaNAC2, and ethylene (Fig. 1A), which could function as a feedback loop to stimulate the ripening process once MaNAC1, MaNAC2, and ethylene levels increase (Fig. 1, A and B). Future research could aim to make the *MaXB3* promoter less sensitive to this repression by MaNAC1 and MaNAC2 through binding-site deletion, with the potential to slow down fruit ripening. Conversely, what if MaXB3 could already be experimentally repressed during fruit development? Would this lead to premature and/or accelerated fruit ripening? Finally, how are high levels of MaXB3 initially maintained during fruit development (Fig. 1B)? And can they be altered by developmental and

environmental signals? Currently, inadequate control over fruit ripening processes contributes to large amounts of food waste globally (Hu et al., 2019). One promising approach may therefore be to identify regulators of MaXB3 in the future, as increased control over MaXB3 levels could ultimately allow breeders, retailers, and consumers to delay ripening and increase the shelf life of our beloved fruits.

Sjon Hartman^{1,2}

ORCID ID: 0000-0002-6709-6436

Plant Ecophysiology,
Institute of Environmental Biology,
Utrecht University, 3584 CH Utrecht,
The Netherlands

LITERATURE CITED

- Abeles FB, Morgan PW, Saltveit ME (2012) Ethylene in Plant Biology, 2nd ed. Academic Press, New York
- Hartman S, Sasidharan R, Voeselek LACJ (2019) The role of ethylene in metabolic acclimations to low oxygen. *New Phytol* doi:10.1111/nph.16378
- Hu B, Sun DW, Pu H, Wei Q (2019) Recent advances in detecting and regulating ethylene concentrations for shelf-life extension and maturity control of fruit: A review. *Trends Food Sci Technol* **91**: 66–82
- Lyzenga WJ, Booth JK, Stone SL (2012) The Arabidopsis RING-type E3 ligase XBAT32 mediates the proteasomal degradation of the ethylene biosynthetic enzyme, 1-aminocyclopropane-1-carboxylate synthase 7. *Plant J* **71**: 23–34
- Pattyn J, Vaughan-Hirsch J, Van de Poel B (2020) The regulation of ethylene biosynthesis: A complex multilevel control circuitry. *New Phytol* doi:10.1111/nph.16873
- Prasad ME, Schofield A, Lyzenga W, Liu H, Stone SL (2010) Arabidopsis RING E3 ligase XBAT32 regulates lateral root production through its role in ethylene biosynthesis. *Plant Physiol* **153**: 1587–1596
- Seymour GB, Østergaard L, Chapman NH, Knapp S, Martin C (2013) Fruit development and ripening. *Annu Rev Plant Biol* **64**: 219–241
- Shan W, Kuang J, Wei W, Fan Z, Deng W, Li Z, Bouzayen M, Pirrello J, Lu W, Chen J (2020) MaXB3 modulates MaNAC2, MaACS1, and MaACO1 stability to repress ethylene biosynthesis during banana fruit ripening. *Plant Physiol* **184**: 1153–1171
- Wang YS, Pi LY, Chen X, Chakrabarty PK, Jiang J, De Leon AL, Liu GZ, Li L, Benny U, Oard J, et al (2006) Rice XA21 binding protein 3 is a ubiquitin ligase required for full Xa21-mediated disease resistance. *Plant Cell* **18**: 3635–3646
- Xiao YY, Chen JY, Kuang JF, Shan W, Xie H, Jiang YM, Lu WJ (2013) Banana ethylene response factors are involved in fruit ripening through their interactions with ethylene biosynthesis genes. *J Exp Bot* **64**: 2499–2510