EDITORIAL: REFLECTIONS ON THE PLANT CELL CLASSICS

Behind the Screen: How a Simple Seedling Response Helped Unravel Ethylene Signaling in Plants

In 1901, Dimitry Neljubov demonstrated that ethylene caused altered growth of etiolated pea seedlings, a morphology that by 1913 came to be referred to as the “triple response”. This response was utilized by Guzman and Ecker in a genetic screen designed to identify mutants affected in various aspects of ethylene function in Arabidopsis thaliana, as described in their landmark paper in The Plant Cell (Guzmán and Ecker 1990). At the time, relatively little was known regarding the molecular aspects of ethylene action, or really for any of the plant hormones. Previously, Anthony Bleecker, while a post-doc with Hans Kende, had exploited the triple response of etiolated seedlings to identify the etr1 mutation (Bleecker et al., 1998), which later turned out to encode the founding member of the ethylene receptor family in Arabidopsis, and Sakis Theologis’ lab had just identified a gene from zucchini fruit encoding ACC synthase, the enzyme catalyzing the generally rate-limiting step in ethylene biosynthesis. The triple response provided a perfect tool to genetically dissect ethylene function. While biochemical analysis is facilitated by simple, specific assays for protein activity, geneticists dream of a simple screen directly targeting a pathway of interest in which mutants can be easily identified. The development of the triple response assay provided such a facile screen for mutants altered in ethylene action, which opened up the road to elucidation of the ethylene signaling pathway, as well as factors regulating ethylene biosynthesis and other aspects of ethylene function.

Guzman and Ecker (1990) formalized a description of the triple response in Arabidopsis, such responses to ethylene varying among seedlings of different plant species. Guzman and Ecker wrote: “After 3 days of incubation at 23°C in the dark, seedlings germinated in air are readily distinguished from seedlings germinated in 10 µL/L ethylene. Air-treated Arabidopsis seedlings are highly elongated and exhibit an apical hook at the terminal part of the shoot axis. Conversely, ethylene-treated seedlings show inhibition of root and hypocotyl elongation, exaggerated tightening of the apical hook, and swelling of the hypocotyl (i.e., a triple response).” These morphological features were documented in Figure 2 of their paper (shown above).

In the paper, Guzman and Ecker identified three different classes of mutants, each of which would ultimately lead to insight into different aspects of ethylene function. The first class were mutants that constitutively displayed an ethylene response. Similar mutants lead to the discovery of CTR1, a key element in the ethylene signaling pathway (Kieber et al., 1993). In the Guzman and Ecker paper, the eto1 mutation was identified, which was found to produce high levels of endogenous ethylene (hence the constitutive triple response). The identification of ETO1 ultimately lead to studies that demonstrated regulation of ACC synthase protein stability was a key input into controlling the level of ethylene biosynthesis (Chae et al., 2003). ETO1 was ultimately shown to affect the stability of ACC synthase by targeting it for poly-ubiquitination (Wang et al., 2004).

A second class of mutants that were identified specifically affected the formation of the apical hook of the Arabidopsis seedlings. This hookless1 (hls1) mutation, which alters a gene encoding a putative N-acetyltransferase, suggested that the apical hook could be exploited as a model developmental event to investigate differential growth in plants. Further, it is a useful system to explore signal crosstalk, as the formation of the hook integrates multiple signaling pathways, including ethylene, gibberellin, jasmonate, auxin and light, to promote asymmetrical growth of the cells on opposite sides of the hook. The identification of hls1 also indicated that mutants could be identified that affected only specific aspects of the triple response, an approach used in subsequent genetic screens that identified other key aspects of plant signaling, such as the root-specific ethylene-insensitive mutants that lead to the identification of genes involved in auxin biosynthesis.

The final class of mutants identified by Guzman and Ecker were ethylene-insensitive mutants (ein), which, like the prior Bleecker paper (Bleecker et al., 1988), identified elements of the ethylene signaling pathway. The ein1 mutation was subsequently shown to be allelic to etr1 identified by Bleecker et al. (1988), which was ultimately found to encode the ethylene receptor (Schaller and Bleecker, 1995), the first hormone receptor identified in plants. The second mutation identified by Guzman and Ecker, ein2, was later found to encode a protein with homology to the Nramp family of metal-ion transporters and demonstrated a role in ethylene signaling.

Morphological features of the triple response to ethylene in wild type (A) as compared to wild type incubated without ethylene (B). (Reprinted from Guzmán and Ecker [1990], Figure 2)
functioned at an intermediate step in the ethylene signaling pathway (Alonso et al., 1999). Similar screens using the triple response were used by multiple groups to identify nearly all of the elements involved in ethylene signaling.

The Guzmán and Ecker paper had a profound impact on the field of ethylene biology. It provided the foundation for the subsequent identification of elements involved in multiple aspects of ethylene action. Using these and similar mutants, ethylene signaling went from a black box to a well-characterized signaling pathway. Although genetic screens for new mutants exploiting the triple response have been employed by many labs and is likely nearly saturated, this by no means indicates that labs have foregone use of the triple response assay. Its simplicity and sensitivity allows for rapid quantification of ethylene responses, whether in characterizing mutants or in structure-function studies focused on key elements affecting ethylene action. Almost a century after Neljubov described the ethylene triple-response of pea seedlings, Guzmán and Ecker repopularized the term within the context of genetic analysis in Arabidopsis. There are over 4000 citations for the “triple response” to ethylene since 1990, and so one could say that Neljubov’s initial description of ethylene action in pea seedlings has far exceeded what he could have possibly imagined, and that is in large part thanks to this pioneering article by Guzmán and Ecker (1990).

REFERENCES
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