This Week's Citation Classic

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Chrispeels M J & Varner J E. Gibberellic acid-enhanced synthesis and release of α-ribonuclease by isolated aleurone layers. *Plant Physiol.* **42**:398-406, 1967.

Gibberellic acid enhances (he synthesis and secretion of α-amylase by isolated aleurone layers. The isolated layers responded as well as the half seeds used in earlier studies. This hormonal effect was shown to depend on continuous protein and RNA synthesis. [The *SCI*® indicates that this paper has been cited over 215 times since 1967.]

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"In the late 1950s and early 1960s, Haraguro Yomo[1] working in Japan, and Leslie Paleg[2] working in Australia, observed independently that treatment of barley endosperm with the plant hormone gibberellic acid causes the endosperm to release sugar as a result of enhanced amylase activity. The significance of that discovery was not lost on others who realized that the cereal endosperm constituted an excellent system to study the molecular mechanism of hormone action in plants. In the fall of 1964 I had the good fortune of becoming associated with Joe Varner[3] who had just demonstrated that gibberellic acid triggered the *de novo* synthesis of α-amylase and that RNA synthesis was required for the expression of this hormonal effect.

"The difficulty of getting metabolic inhibitors and radioactive precursors into the endosperm prompted me to perfect a system based on isolated aleurone layers. Indeed, Haberlandt[4] had shown in 1890 that aleurone tissue could be removed from germinating cereal grains and that it was the aleurone layer which produced the diastase during germination. Furthermore, in 1964 both Paleg[5] and Varner[6] showed independently that the aleurone layer was the target tissue for the hormone. Aided by Nancy Joseph, who cheerfully stripped thousands of aleurone layers from de-embryonated barley seeds, I made the chance discovery that the addition of 20 mM $CaCl_2$ to the incubation medium prevented the breakdown of the α-amylase secreted into the medium. Indeed, treatment of aleurone layers with gibberellic acid elicits the synthesis and secretion not only of α-amylase, but also of a potent protease as well as of other hydrolases such as ribonuclease, β-glucanase, phosphatase and xylanase.

"Our observation made it possible to study the effect of gibberellic acid on isolated aleurone layers. Many experiments which would have been quite difficult with de-embryonated half seeds now became feasible. Isolated layers have since been the system of choice to study the mechanism of hormone action and of enzyme secretion. Once the secretion of α-amylase starts (8 -10 hours after the addition of hormone) the synthesis of this enzyme accounts for nearly half of the total protein synthesized by the cells.

"The system remains one of the best to study the mechanism of actions of a plant hormone, yet we are not much closer now than we were in 1964 in understanding how this hormone works. Recent experiments show that the hormone brings about an increase (synthesis?) in the level of translatable mRNA for α-amylase in the tissue.[7] However, the hormone still needs to be present after α-amylase synthesis is refractory to inhibitors of RNA synthesis.[8] These experiments show that gibberellic acid may control enzyme synthesis at the transcriptional as well as the translational level.

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