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This Week's Citation Classic

Jacobsen J V & Varner J E. Gibberellic acid-induced synthesis of protease by isolated aleurone layers of barley. *Plant Physiol.* **42**:1596-600, 1967.
[MSU/AEC Plant Research Laboratory, Michigan State University. East Lansing. MI]

Isolated aleurone layers of barley secreted protease in response to gibberellic acid. Density labelling studies showed that the protease arose by new synthesis. This and comparative kinetic and inhibitor studies of protease and α-amylase induction indicated that the enzymes arose in a unified way. [The *SCI*® indicates that this paper has been cited over 125 times since 1967.]

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"On completion of a research program at the University of California, Davis, in 1965 on the production of volatile compounds in *Allium,* the pressures to sacrifice the good life in California and return to Australia were strong, particularly those exerted by the immigration authorities. However, an eleventh hour reprieve came when I had the good fortune of being offered the chance to work with Joe Varner and thus to pursue a long held interest in hormone physiology. My interest had developed in the peach orchards of South Australia many years before when my misuse of plant hormones as potential fruit thinning compounds led to the destruction of many acres of prime trees.

"At the newly formed laboratory at Michigan State University, Joe, Maarten Chrispeels, and Ram Chandra were able to make isolated aleurone respond well to GA_3 in terms of α-amylase production and they had amassed a body of information showing that at least part of the amylase was newly synthesized and that RNA synthesis was required for this to occur.[1] For the sake of further defining the response of aleurone to GA_3, we chose to examine in some detail another enzyme known to respond to the hormone. Protease was the activity of choice, having been already reported by A. MacLeod's group in Britain and H. Yomo and colleagues in Japan, to rise in aleurone in response to GA_3.[2,3]

"We examined protease induction in the light of what was known of α-amylase induction and found that the two enzymes behaved similarly in many ways. At that time Phil Filner and Joe Varner were devising a density labelling technique which would give an answer to the question of whether all or only some of the induced a-amylase was made de *novo.*[4,4] We applied the technique to protease as well as α-amylase and the results in both cases were that all of the enzyme appearing in response to GA_3 was newly synthesized. The possibility that enzyme induction involved activation of preexisting (inactive) protein seemed no longer tenable. This work not only told us something about aleurone physiology but also boosted confidence in the applicability and validity of the technique. The results led us to the thought that protease and α-amylase were probably produced in unison although the data did not permit a critical answer to the question.

"The protease work is often cited together with the paper on α-amylase by Filner and Varner and they are frequently cited probably for two reasons. Firstly, the studies were the first in plant biology to employ density labelling technology in protein synthesis studies and secondly they provided the first evidence of complete synthesis of hormone-induced enzymes in plant tissues."

1. **Chrispeels M J & Varaer J E**. Gibberellic acid-enhanced synthesis and release of α-amylase and ribonuclease by isolated barley aleurone layers. *Plant Physiol.* **42**:398-406, 1967.
2. **MacLeod A M, Duffus J H & Johnston C S.** Development of hydrolytic enzymes in germinating grain. *J. Inst. Brew.* **70**:521-8, 1964.
3. **Yomo H & Iinuma H.** The enzymes of the aleurone layer of barley endosperm. *Amer. Soc. Brewing Chemists Proc.* **1964**:97-102.
4. **Filner P & Varner J E.** A test for de novo synthesis of enzymes: density labeling with H_2O^{18} of barley α-amylase induced by gibberellic acid. *Proc. Nat. Acad. Sci. US* **58**:1520-6, 1967.