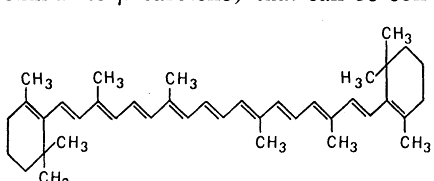
Bioregulators: Alteration of Gene Expression in Citrus Fruit

A substantially different approach to the problem of improving the nutritional quality and physical characteristics of food crops may be emerging from studies at the Pasadena, California, laboratories of the U.S. Department of Agriculture's Agricultural Research Service (ARS). Such improvements have in the past been obtained by crossbreeding different genetic varieties of a particular crop, but preliminary experiments by Henry Yokoyama of ARS suggest that it might be possible to make some types of improvements by manipulating the genetic expression of individual plants. The key to this possibility, Yokoyama last month told the 167th national meeting of the American Chemical Society, is the discovery of bioregulators that appear to derepress specific genes of citrus fruits, thereby inducing the production of additional quantities of components that enhance their color, flavor, and nutritional quality.

Yokoyama and his associates Wan J. Hsu, Stephen M. Poling, Ernest Hayman, and Charles DeBenedict were initially interested in improving the color and provitamin A content of oranges, the most important citrus crop. A substantial fraction of the U.S. orange crop, for example, does not possess the deep orange color most attractive to consumers. These oranges have in the past been colored with synthetic dyes, but questions about the toxicity and carcinogenicity of the dyes have largely halted this practice.

The citrus industry is also interested in increasing the provitamin A content of citrus juices to compete more effectively with artificial fruit drinks that have a high provitamin A concentration. The term provitamin A encompasses a group of carotenoids (naturally occurring terpenes with structures similar to β-carotene) that can be con-



β-Carotene

verted by the liver into vitamin A, which plays a crucial role in night vision and in the functioning of the immune system. Color and provitamin A content are linked because carotenoids are also pigments; not all carotenoids are provitamins, however.

The ARS group has identified three primary classes of bioregulators that induce the formation of carotenoids when applied in low concentrations to the surface of ripe citrus fruit. Group one consists principally of chalcone derivatives of triethylamine, such as 2-(*p*-diethylaminoethoxybenzal)-*p*-methoxyacetophenone. These chemicals stimulate the production of the orange provitamin A γ-carotene and its precursor, lycopene, a red pigment that is the primary coloring agent of tomatoes. Color development begins within hours and is essentially complete within 10 days. The group 1 agents give a 10- to 16-fold increase in total carotenoid content, but produce a deep red color inappropriate for commercial citrus.

Three Orange Carotenoids

The group 2 bioregulators, which include diethyloctylamine and diethylnonylamine, cause citrus to produce three orange provitamin A carotenoids (α-, β-, and γ-carotene) as well as lycopene. The two- to fivefold increase in provitamin A carotenoids produces a deep orange color that persists for as long as 5 weeks before the red color develops. These agents may be useful for commercial processors since color development can be stopped when the juice is separated from the fruit. The group 3 bioregulators, whose identities Yokoyama is shielding pending application for a patent, induce as much as a fivefold increase in the concentration of two orange carotenoids that have not yet been identified, These were the first bioregulators that do not induce formation of lycopene. Yokoyama has also preliminarily identified several other bioregulators that increase the concentration of flavor constituents. One agent, for example, when applied to ripe lemons induces a 3.5-fold increase in their content of citral, a constituent of essential oil that is an important contributor to flavor.

These results might be of only limited interest except that the phenomenon appears to be a general one. A bioregulator that induces formation of carotenoids will do so in any species that contains genes coding for the enzymes that synthesize those carotenoids. These include all citrus fruits; other fruits, such as apricots, peaches, and tomatoes; vegetables, such as sweet potatoes and carrots; molds, such as *Phycomyces blakesleeanus*; and the photosynthetic bacterium *Rhodospirillum rubrum*. This universality of effect suggests that the bioregulators act through a common route, derepression of a specific gene. This hypothesis is supported by experiments which show that the bioregulators can reverse the effects of chemicals that are known to repress the genes that code for formation of carotenoids in *Phycomyces*. Beyond that, however, little is known about the specific mechanism of action of the bioregulators, although the group 1 agents also appear to block further metabolism of lycopene and γ-carotene, causing those two chemicals to accumulate in the fruit.

The proposed mechanism of action of the bioregulators, Yokoyama argues, is unique. The only process that might be considered roughly comparable is the use of ethylene to promote ripening of citrus fruits and tomatoes. Ethylene, however, is a broadly acting stimulant that accelerates all metabolic activity in immature fruit. The bioregulators stimulate only specific metabolic pathways in fully ripened fruit.

Yokoyama is confident that the bioregulators will be found to be safe for human consumption. Many of the most promising bioregulators are, in fact, naturally occurring components of citrus and other foods. Toxicological studies on a number of the agents are now being conducted by Albert N. Booth of the Department of Agriculture's Western Regional Research Center in Albany, California.

If the speculation that the bioregulators act by gene derepression is confirmed, several potentially important avenues of research will be opened. It might be possible, for example, to increase the concentration of essential amino acids that occur at low levels in some varieties of corn. It might also be possible to increase the protein concentration of important crops such as rice without the necessity for the large amounts of extra fertilizer required for current hybrids. And, perhaps most important, the bioregulators may provide a new tool for learning more about the mechanisms for control of genes.

—Thomas H. Maugh II