Molecular studies of fruit ripening

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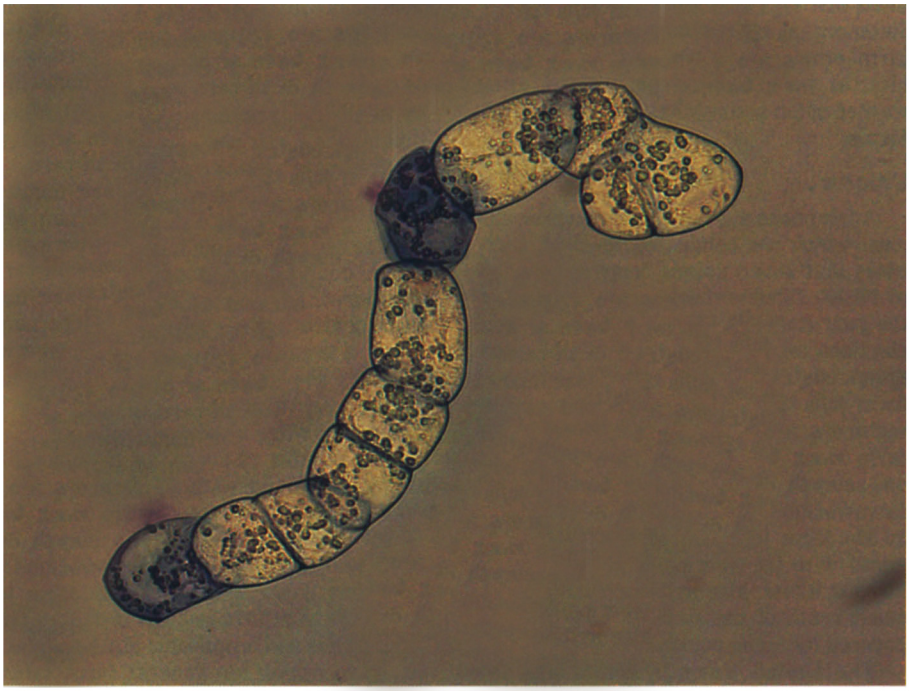
A primary objective in research leading to improvements in fruit quality and longevity is the control of cellular aging, or senescence. Detached fruit are composed of living cells that are not only deprived of nutrition from the parent tree but are genetically destined to age and die. The first phase, ripening, may be slowed but it must not be diverted from the normal course that results in the characteristic color, texture, and flavor associated with optimum market quality. The second phase, the one we wish to retard to accommodate transport and distribution, is a continuation of the first into overripening, breakdown, and increased susceptibility to disease. In physiological terms, the changes taking place in harvested fruit represent a continuous process of cellular senescence.

Even before the turn of the century, both refrigeration and the modification of storage atmospheres (lowered oxygen or increased carbon dioxide) had been found to suppress cellular metabolism and delay ripening — i.e., senescence. By the 1920s, it was also known that many ripening fruit produce ethylene gas, which further accelerates respiration and ripening. For the past several decades, temperature, relative humidity, atmospheric modification (oxygen, carbon dioxide, ethylene), and other **external** controls have been manipulated in seeking conditions that maximize fruit storage and market life. These studies have often been conducted with apparatus permitting maintenance of specific environmental conditions around each lot of fruit. Similar studies continue to provide valuable information on optimal storage and transit conditions for new fruit species and cultivars, altered cultural practices, or new market demands.

New breakthroughs for agriculture are anticipated from molecular biology, where attention is directed toward genetic manipulation and **internal** control of cellular events. We are hoping to use these techniques to genetically modify cultivars so that ripening of the fruit can be controlled. Some such fruit already exist — for example, the tomato cultivar 'Never-Ripe' and "slow-ripening" nectarine and plum genotypes, which apparently lack specific genes for senescence and therefore ripen very slowly, if at all. Successful application of molecular and recombinant DNA techniques to modify ge-

Cultured pear fruit cells growing in a liquid suspension provide a useful model



Suspension-cultured pear fruit cells magnified approximately 250 times. Dead cells are stained with Evans blue.

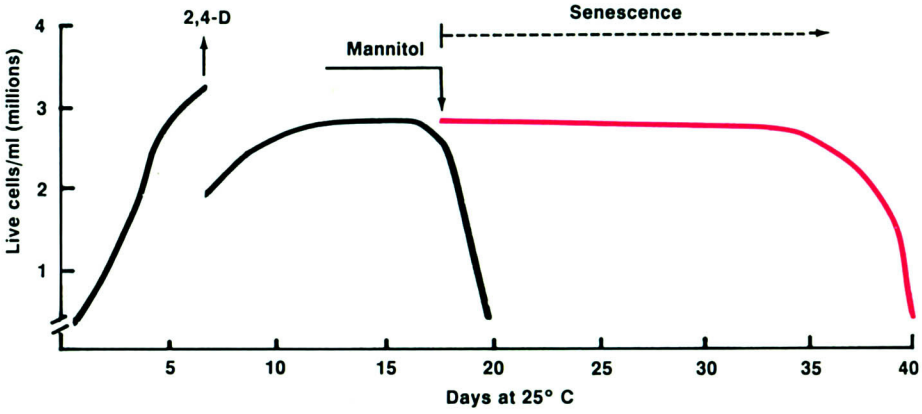


Fig. 1. Division of pear fruit cells in a suspension culture occurs about every 72 hours, leading to a density of 3 to 4 million cells per milliliter within a week or 10 days (line at left). When deprived of 2, 4-D, cells stop dividing after two or three days and begin to die (second line). Cells transferred to an aging medium, also lacking 2, 4-D, but containing mannitol, remain alive for an extended period (third line) and undergo changes associated with senescence, or ripening.

Classic experimental apparatus still being used effectively to measure fruit respiration and ethylene production and the response to modified atmospheres, temperature changes, or other "external" variables.

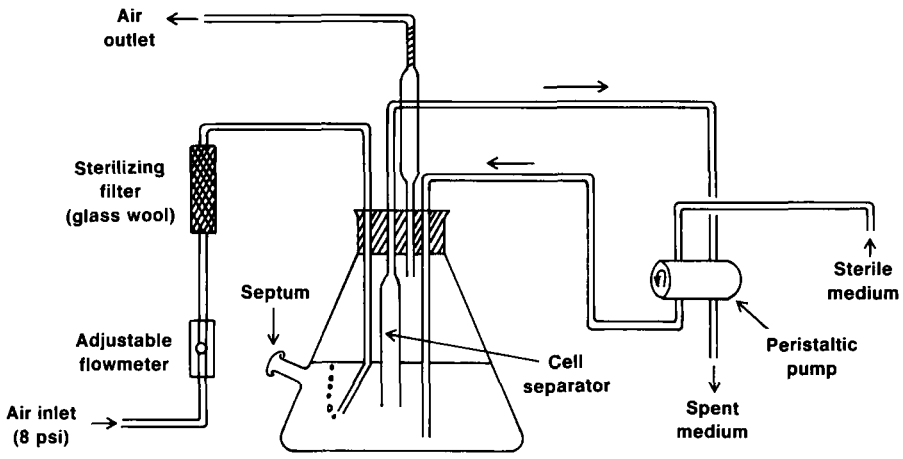


Fig. 2. Schematic view of the system for growing and aging pear fruit cells, showing one culture flask with lines for the introduction of sterile air and renewal of medium and access septum for cell sampling.

TABLE 1. Cellular functions and physiological changes characteristic of ripening pear fruit and aging, suspension-cultured pear fruit cells

	Ripening pear fruit	Aging, cultured pear fruit cells	Comments
1.	Large increase in respiration (the climateric respiratory rise).	Respiration rise in response to added substrate or stress.	Although a natural increase does not occur in culture, the potential is there to respond.
2.	Transient increase in protein and RNA synthesis coincident with onset of ripening.	Similar increase in protein and RNA synthesis early in the aging phase.	The changes are closely analogous, and in both cases lead to cell death.
3.	Increase in cell leakage with ripening.	Increase in cell leakage of serine with aging.	Phenomena are similar but not extensively studied in cultured cells.
4.	Increase in cyanide-resistant respiration (CN-R) with ripening.	CN-R induced by treatment with cycloheximide.	Cells in culture have potential for CN-R but it must be induced.
5.	Increased ethylene production coincident with onset of ripening.	Increase in inducible ethylene production after transfer to aging medium.	Ethylene production capability exists and increases in early aging phase in both systems.
6.	Low levels (2-10 ppm) of ethylene cause a 200-300% increase in respiration, more ethylene production, faster ripening.	Similar low levels of ethylene cause a modest (10-30%) increase in respiration, faster rate of senescence, earlier death.	Although cells do not show autocatalytic ethylene production, their responses to ethylene are qualitatively similar to those of fruit.
7.	Upon transfer to 40°C (106°F) ripening pears stop producing ethylene and polysomes break down to ribosomes.	Identical response.	Rate of change is faster in cells because of faster heat transfer in liquid culture as opposed to intact fruit.

netic information, however, requires identification of the specific cellular functions to be modified. In postharvest terms, this means first identifying the physiological and biochemical events that drive cellular senescence.

Scientists in the United States, Australia, France, England, and Israel, where fruit export is an important component of the agricultural economy, have been studying the biochemistry of ripening and cellular senescence. Progress is being made, but often the biological material itself hinders the research. Fruit have no circulatory system to facilitate the injection and retrieval of marker chemicals, they are bulky and difficult to infiltrate, and most often they are seasonally available for only a few months out of each year. It is here that suspension-cultured fruit cells are proving useful.

Many types of plant and fruit cells have been successfully grown in liquid culture. When properly handled, the cells keep growing and dividing indefinitely, but conditions that do not support growth quickly lead to cell death. In the mid 1970s, French scientists found that cultured pear cells transferred to a medium lacking the synthetic growth hormone 2,4-D but containing mannitol, a nonmetabolizable sugar, stopped dividing but remained alive and continued to age for two weeks or longer. The cells passed through the phases of growth, hormone-deprivation, aging, and death (fig. 1). Questions now are whether subtle changes occur during the protracted aging phase and, if so, whether they resemble the senescence changes in ripening fruit.

For the past few years we have examined cultured pear fruit cells using a system for growing and aging the cells that allows monitoring and adjustment of environmental gases and media and provides access for injection of chemicals or removal of cell samples under sterile conditions (fig. 2). We have compared several cellular functions and physiological transitions characteristic of ripening pear fruit with corresponding phenomena observed in aging, suspension-cultured, pear fruit cells (table 1). The extent of similarity is very encouraging.

Cells aging in liquid culture will never completely resemble their counterparts in ripening fruit. This limitation, however, is compensated for by the cultured cells' responsiveness, ease of manipulation, and year-round availability. With suspension-cultured cells as a model system, scientists can more readily examine fruit senescence at the cellular level and explore the biochemical basis for improved postharvest technologies.

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