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Polyribosomes from Aging Apple and Cherry Fruit

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ABSTRACT

The sequence of events which occurs during the ripening of the Passe-Crassane pear fruit have been previously studied. In this work, we have investigated the ripening of another climacteric fruit (*Pyrus malus* L. cv Golden Delicious) and of a nonclimacteric fruit (*Prunus avium* L. cv Bigarreau Napoléon). We show that both climacteric fruits exhibit the same preclimacteric sequence of events. Differences exist, however, between the Golden Delicious apple and the Passe-Crassane pear in that the protein synthesis capacity of the two fruits is not the same during the over-ripening period. On the other hand, a nonclimacteric fruit, the Bigarreau Napoléon cherry, does not show an increase in its protein synthesis capacity during the over-ripening period.

The discovery by Kidd and West (14) of climacteric respiration in apples, has allowed fruits to be separated into two classes (1) on the basis of their respiratory behavior during ripening: climacteric fruits, such as apples, which undergo a large increase in respiration (climacteric rise) accompanied by marked changes in composition and texture, and nonclimacteric fruits such as cherries, which show no changes in respiration associated with distinct changes in the composition of the fruit.

Ripening fruit undergoes changes in RNA and protein metabolism (2, 8, 12, 19, 20). In previous papers (5, 6), the amount of polyribosomes was determined in Passe Crassane pear fruits tissues, at different ripening stages, allowing an estimation of the rate of protein synthesis. In this report, we have examined changes in the polyribosomes of Golden Delicious apple and Bigareau Napoléon cherry during ripening. These findings are discussed with respect to the control of ripening and senescence in fruits.

MATERIALS AND METHODS

Fruits. (*Pyrus malus* L. cv Golden Delicious) were harvested on September 30, 1981, 150 d from bloom, and placed in cold storage at 4°C. Samples of approximately 100 blemish-free apples were subsequently transferred to 15°C at three different dates: October 1, 1981, October 30, 1981, January 8, 1982.

Cherries (*Prunus avium* L. cv Bigarreau Napoléon) were harvested weekly 6 to 10 weeks from bloom.

Measurement of Respiratory Rates and Preparation of Samples. Rates of respiration were determined at 15°C by measuring CO_2 changes with an IR gas analyzer. At the indicated intervals, identical samples were peeled from six to seven apples. Peel and pulp tissues were separated and subsequently frozen in liquid N_2, lyophilized, and stored at low air pressure (about 10 Torr) in appropriate bottles until use. For cherries, after each harvest, the CO_2 emission at 15°C was measured before freezing and lyophilization of entire fruits.

Extraction of Polyribosomes. Procedures described elsewhere (5, 6), for the extraction and purification of polyribosomes from 5-g samples of lyophilized pear tissues were modified as follows to maximize polyribosomal yield. With apple pulp tissues, the pellet obtained after ultracentrifugation at 105,000g was resuspended in buffer up to 0.9 ml and aliquots (0.3 ml) of resuspended polyribosomes were layered on linear (15–60% w/v) sucrose gradients. With apple peel tissues, the extraction solution contained β-mercaptoethanol (55 mM) and the pellet obtained after ultracentrifugation at 105,000g was resuspended in buffer up to 0.6 ml. With cherries, extraction was performed on 4 g of pulverized material by homogenization in 25 ml of cold extraction solution containing 0.6 M Tris (pH 9), 55 mM β-mercaptoethanol, 3% PEG, 160 mM KCl, 35 mM $MgCl_2$, 7% sucrose, 5 mM EGTA, and 2.5 g insoluble PVP (Polyclar AT).

Polyribosomes Profile Analysis. After density-gradient centrifugation, the contents of the tubes were analyzed with an ISCO model 185 density gradient fractionator attached to an ISCO

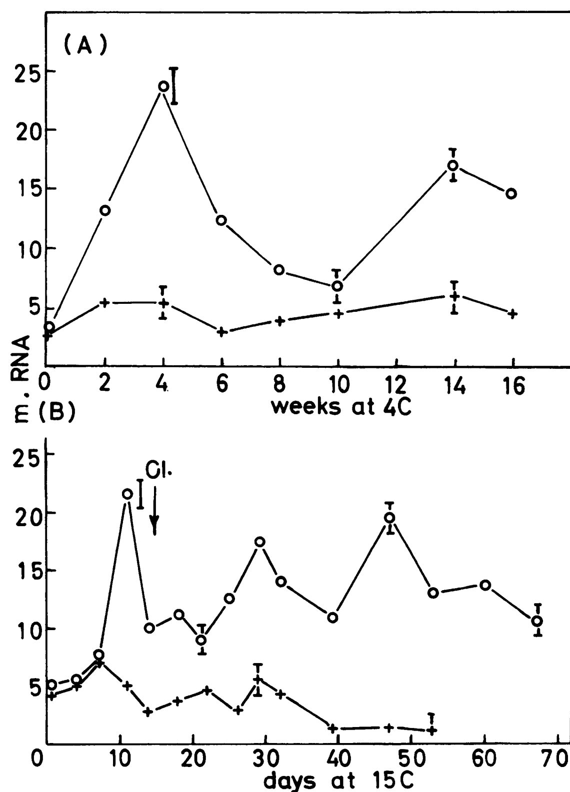

FIG. 1. Changes in estimated amount of available mRNA of peel and pulp tissues during ripening and senescence of apple fruit. A, Changes at 4°C; B, changes at 15°C; (O), pulp tissues; (+), peel tissues; Cl, climacteric peak. Vertical bars represent 2 × SE.

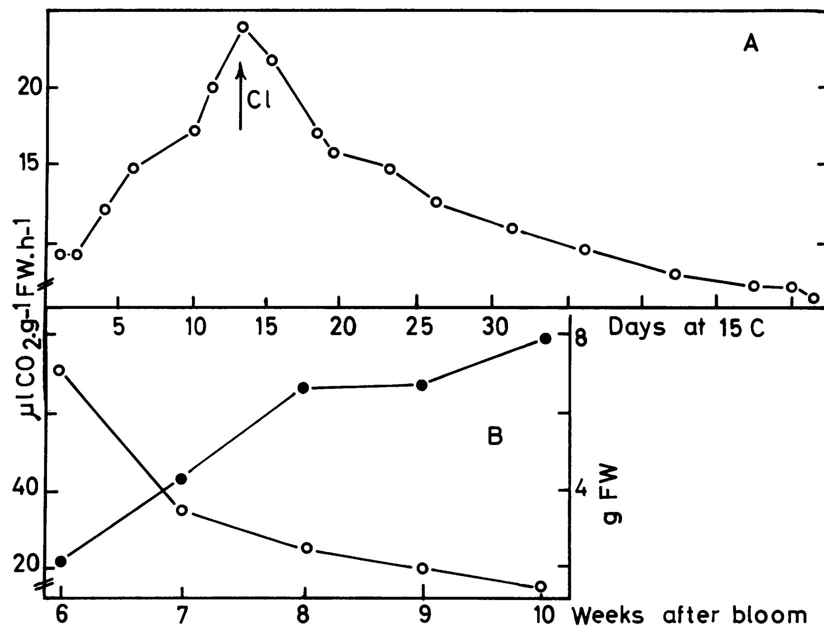

FIG. 2. Respiratory activity and fresh wt of fruits. A, Respiratory activity of apple fruit harvested 150 d from bloom and held continuously at 15°C in air. Cl, Climacteric peak. B, Respiratory activity (○) and fresh weight (●) of cherry fruit harvested at different times after bloom.

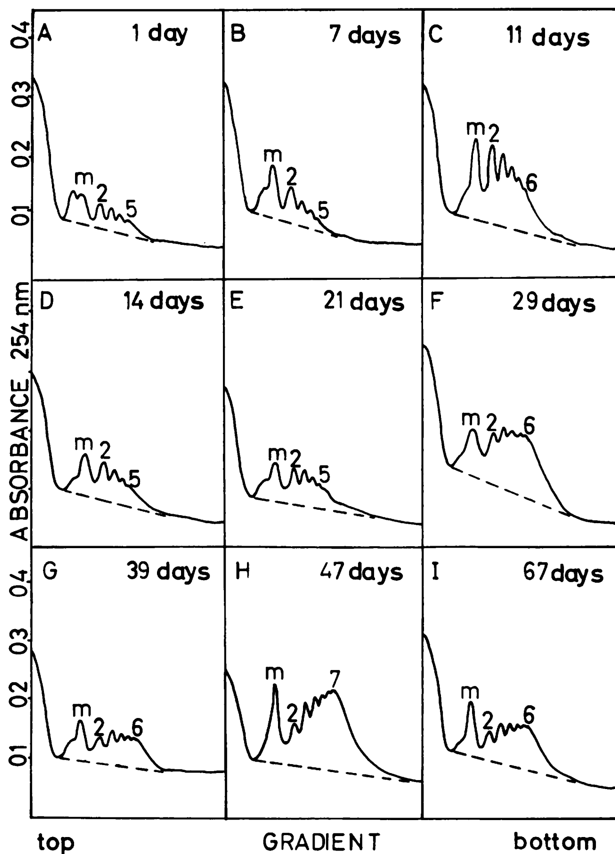

FIG. 3. Sucrose density gradient profiles of polyribosomal populations extracted from pulp tissues of apple fruit held for different numbers of days at 15°C after the harvest, *i.e.* A, 1 d; B, 7 d; C, 11 d; D, 14 d; E, 21 d; F, 29 d; G, 39 d; H, 47 d; I, 67 d. The periods examined represented specific stages of ripening and senescence, *i.e.* A–C, preclimacteric period; D, climacteric peak; E–I, postclimacteric period. The different regions of the polyribosomal profiles are: monosomes, m; dimers, 2; pentamers, 5; hexamers, 6; heptamers, 7.

model UA 5 absorbance monitor. The areas in different regions of the polyribosomal profiles were determined from the average of three measurements of two different curves by counting the number of squares under each profile peak; the SD of the measurements was 2.5%. The different regions analyzed were: subunits, monosomes, polyribosomes, and large polyribosomes sedimenting faster than pentamers. Equilibrated blank gradients were always monitored in order to subtract the base line area for each figure. The areas under different peaks were divided by the number of ribosomes in polyribosomes of that size class as described previously (5). Thus, the values represent the relative contents of mRNA strands in each polymeric class (4). The area of absorbance of each size class of unresolved (larger) polyribosomes was approximated as done by Davies and Larkins (4) except that 10-mers were used as the average size class.

RESULTS

Polyribosomal Changes during Apple Ripening. *Effect of the Post-Harvest Storage Temperature.* The patterns of change in amount of mRNA over the post harvest period are illustrated in Figure 1. At 4°C (Fig. 1A), mRNA in pulp tissues was detected at a low but measurable level, and increased about 8-fold during the following 4 weeks. The amount of mRNA increased very slowly during the same 4-week period in peel tissue. In both tissues, a second increase in rate of mRNA synthesis was noted as the fruits reached 14 weeks of storage. At 15°C (Fig. 1B), before the climacteric rise, which occurred on day 14, (Fig. 2A), a very sharp increase in the rate of mRNA synthesis was noted on day 11 in pulp tissues, while it remained at a low level in peel tissues. During the postclimacteric phase, two sharp increases in mRNA synthesis occurred in pulp tissues on days 29 and 47, while only one, on day 29, was visible in the peel.

Figure 3 shows typical polyribosomal profiles from pulp of fruit held at 15°C for the indicated number of days. Preclimacteric fruits (profiles A–C) show an increase in ribosome production, with the proportion of polyribosomes to total ribosomes increasing from 50% to 72%, and the proportion of large polyribosomes (≥5 mers) to total polyribosomes increasing from 11% to 19%. After the climacteric peak, a decrease of ribosomes till day 21 was accompanied by a gradual loss of polyribosomes and

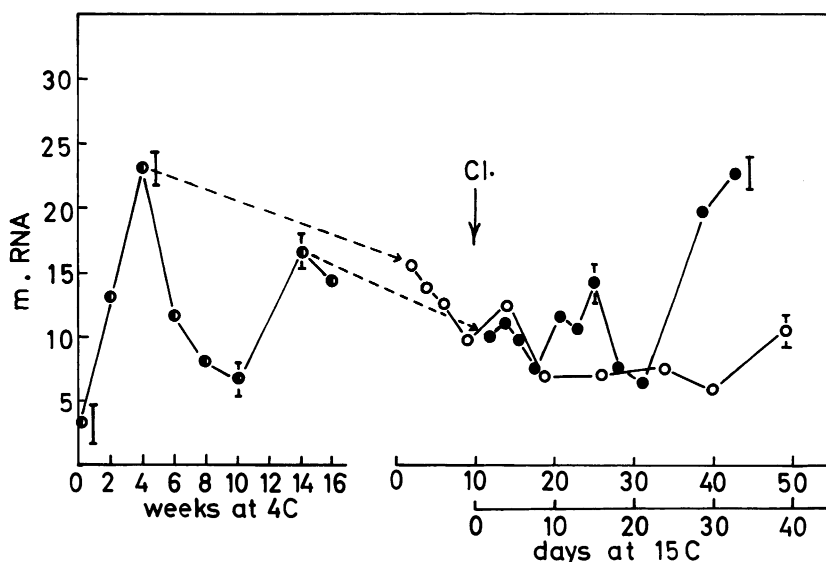

FIG. 4. Changes in estimated amount of available mRNA of apple pulp tissues during ripening and senescence. (◐), Changes at 4°C; (○), changes at 15°C following 4 weeks of cold storage; (●), changes at 15°C following 14 weeks of cold storage. Cl, Climacteric peak. Vertical bars represent 2 × SE.

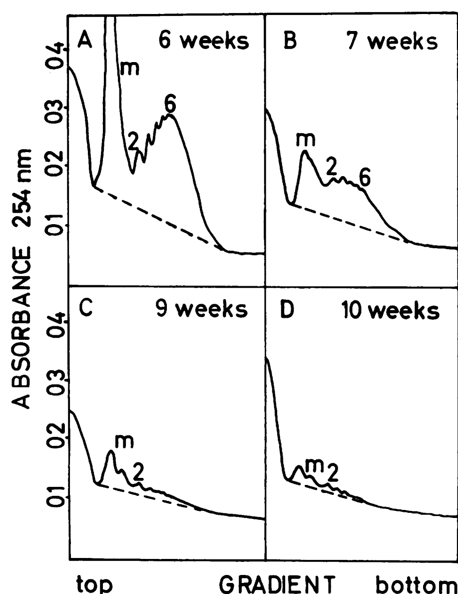

FIG. 5. Sucrose density gradient profiles of polyribosomes populations extracted from cherry harvested weekly. A, 6 weeks from bloom; B, 7 weeks from bloom; C, 9 weeks from bloom; D, 10 weeks from bloom. The different regions of the polyribosomes profiles are: monosomes, m; dimers, 2; hexamers, 6.

large polyribosomes. Two marked increases of ribosomes were further noted on day 29 and 47; the proportion of polyribosomes and of large polyribosomes was estimated on day 29 at 75% and 33%, and on day 47, at 81% and 43%, respectively.

Effect of Transfer to 15°C after Storage at 4°C. As shown in Figure 4, pulp of apple fruit undergoing ripening at 4°C exhibited two peaks of mRNA synthesis after 4 and 14 weeks of storage. Upon transfer to 15°C after 4 weeks at 4°C, the amount of mRNA declined before the climacteric rise on day 9. With prolonged storage at this temperature, two peaks of mRNA occurred on day 14 and at the end of senescence. After 14 weeks at 4°C, fruits transferred to 15°C were postclimacteric, *i.e.* the respiratory activity declined with prolonged storage at this temperature and fruits were becoming yellow. Pulp tissues exhibited three peaks of mRNA: the first one on day 2, corresponding to the end of the mRNA increase noted after 14 weeks at 4°C; the second one on day 17; and the third one on day 30, at the end of the storage at 15°C.

Polyribosomes Changes during Cherry Ripening. Ribosomal profiles from cherry fruit harvested after the specified number of weeks are shown in Figure 5. The amount of ribosomes and mRNA decreased strongly between each harvest, whereas the respiration curve decreased in a similar way (Fig. 2B). On the contrary, the weight of each cherry increased an average of 2 g/week when fruits change their color from green to red (Fig. 2B). The proportions of polyribosomes and of large polyribosomes declines, respectively, from 65% and 57% at 6 weeks from bloom to 40% and 28% at 10 weeks from bloom.

DISCUSSION

As previously noted by French and Romani (8) and Drouet and Hartmann (5, 6) with pear fruits, this type of data suggest variations in the protein synthesizing capacity of aging apple and cherry fruit cells. Three major points can be drawn from this work.

The first point concerns the similarity in the sequence of events of preclimacteric apples and pear fruits. Preclimacteric apples stored at 4 or 15°C show a burst in mRNA and ribosome production only in pulp tissues, which at 15°C is followed by ethylene synthesis and a climacteric peak. This behavior matches that of the Passe-Crassane fruit in which ethylene biogenesis is regulated by two systems. The first system is inactive at 15°C, while the second one, which is related to a burst in mRNA and ribosome production in pulp tissues, is active in a wider range of temperatures and is followed by ethylene synthesis and by the respiratory crisis (6).

The similarity in the evolution of maturation in the two climacteric fruits, pear and apple, suggests that ripening and senescence of these two fruits are governed by the same sequence of events. As a result, the commercial harvest of apples (150 d after bloom) must take place between the two periods of ethylene synthesis (Fig. 6).

When apples are harvested 100 d after bloom and stored in air at 15°C, they do not ripen (16). The respiration curve always

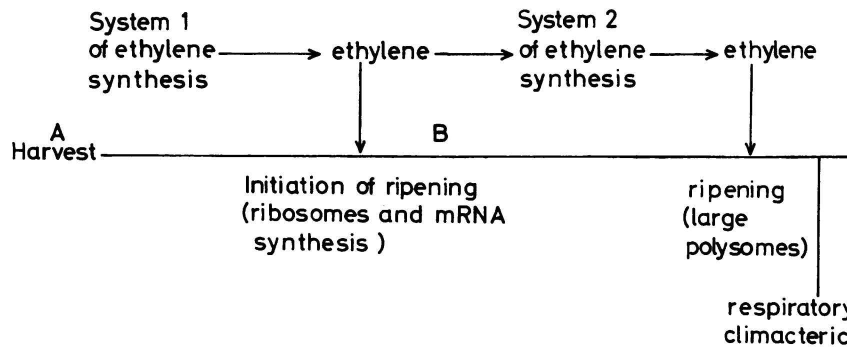

FIG. 6. Sequence of events which occur before the climacteric peak of Passe-Crassane pear and Golden Delicious apple. A, Early harvest (100 d from bloom); B, commercial harvest (150 d from bloom).

decreases and there is no measurable quantity of ethylene in fruits. Yet, an ethylene (16), an ethephon (18), or an O_2 treatment (16) restores ripening. Here again, there is similarity between the evolution of apple and pear ripening. The pear, unable to ripen at 15°C, can ripen at this temperature after a cold storage or after an ethylene or ethephon treatment (6). Early harvested apples may well require a cold storage before ripening at 15°C. Indeed, fruits that were harvested 100 d after bloom and stored at 4°C ripened afterwards at 15°C (21). Thus, an early harvest of apples places them at the very beginning of the sequence of events postulated for pear (Fig. 6A). However, difference between the species does occur when the fruits are still on the tree: system I of ethylene synthesis is inactive in pear but active in apple.

The second point is the fact that the postclimacteric evolutions of Golden Delicious apples and Passe-Crassane pear fruits do not appear to be governed by the same sequence of events. After the climacteric peak, apples exhibit two syntheses of mRNA while pears exhibit only one (6). It should be noted, moreover, that the postclimacteric phase is longer in apple than in pear. Lastly, in Passe-Crassane pear fruit, the preclimacteric phase has been correlated with malic enzyme synthesis, whereas during the postclimacteric phase malic enzyme activity decreases strongly. In contrast, in Golden Delicious apple malic enzyme activity does not decrease after the climacteric peak but increases regularly during the over ripening period. Regarding this point, we have recently shown, using deuterium oxide labeling, that the enzyme is synthesized in apples during both the pre and postclimacteric phases (unpublished results).

Finally, concerning nonclimacteric fruits, MacMurchie *et al.* (17) reported a total absence of the ethylene biogenesis system II. For cherry fruit, growth and ripening are concurrent (10). Consequently, we do not know if the amount of polyribosomes found in cherries harvested 6 weeks from bloom is connected with growth or ripening or both growth and ripening. In complete agreement with the scheme of MacMurchie *et al.* (3), ethylene synthesis remains low and the respiration rate decreases progressively (10; Fig. 2). Besides, cherry maturation can hardly be a part of senescence (as a matter of fact, senescence begins after physiological maturity). Thus, to compare apple, pear and cherry senescence, it may be important to elucidate the sequence of events which rule the senescence of cherries harvested at different times after bloom and stored at 15°C.

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