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Interrelationship of Gene Expression, Polysome Prevalence, and Respiration during Ripening of Ethylene and/or Cyanide-Treated Avocado Fruit[1]

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ABSTRACT

Upon initiation of ripening in avocado fruit (*Persea americana* Mill. cv Hass) with 10 microliters/liter ethylene, polysome prevalence and associated poly(A)$^+$ mRNA increase approximately 3-fold early in the respiratory climacteric and drop off to preclimacteric levels at the peak of the respiratory climacteric. The increase in poly(A)$^+$ mRNA on polysomes early in the respiratory climacteric constitutes a generic increase in constitutive mRNAs. New gene expression associated with ripening is minimal but evident after 10 hours of ethylene treatment and continues to increase relative to constitutive gene expression throughout the climacteric. The respiratory climacteric can be temporally separated into two phases. The first phase is associated with a general increase in protein synthesis, whereas the second phase reflects new gene expression and accumulation of corresponding proteins which may be responsible for softening and other ripening characteristics. A major new message on polysomes that arises concomitantly with the respiratory climacteric codes for an *in vitro* translation product of 53 kilodaltons which is immunoprecipitated by antiserum against avocado fruit cellulase.

Cyanide at 500 microliters/liter fails to affect the change in polysome prevalance or new gene expression associated with the ethylene-evoked climacteric in avocado fruit. Treatment of fruit with 500 microliters/liter cyanide alone initiates a respiratory increase within 4 hours, ethylene biosynthesis within 18 hours, and new gene expression akin to that educed by ethylene within 20 hours of exposure to cyanide.

Several fruits show an increase in protein synthesis during the early stages of fruit ripening (23). In particular, Richmond and Biale (17) reported that, in avocado, amino acid incorporation into protein increased very early in the respiratory climacteric and diminished to less than preclimacteric levels at the peak of respiration. Although an increase in protein synthesis in several climacteric fruits has been demonstrated, its importance to the ripening process has been questioned (23). Brady and O'Connell (4) exposed 6-mm thick cross sections of banana fruit to 5 μl/l ethylene for 6 h and observed an increase in protein synthesis without concomitant ripening; however, when the sections were exposed to ethylene for 12 h, enhanced protein synthesis was accompanied by ripening. Upon analyzing *in vivo*-labeled protein samples on polyacrylamide gels, they found that the increase in protein synthesis with 24 h of ethylene treatment was not limited to a few ripening specific proteins, but reflected a generic increase in protein synthesis. They allowed, however, that continued exposure to ethylene might initiate ripening-specific proteins. Christoffersen *et al.* (6), based on *in vitro* translation of isolated poly(A)$^+$ RNA, showed that at least three new abundant to middle abundant class mRNAs appear with the climacteric rise in respiration of avocado. They concluded that ripening may be linked to the expression of specific genes.

The significance of the respiratory climacteric in relation to other ripening characteristics is not well understood. McGlasson *et al.* (12) and Frenkel *et al.* (8) found that they could inhibit ripening of thick banana slices and Bartlett pear fruit, respectively, with cycloheximide but not the respiratory climacteric. Quazi and Freebairn (16) found that bananas ripened normally under diminished O_2 tension, while having a much reduced respiratory climacteric. Romani and French (22) found that upon increasing the temperature of early climacteric pear fruit to 40°C, respiration increased dramatically, whereas the normal ripening-associated increase in polysome prevalence was prevented, polysome prevalence actually declining to less than preclimacteric levels. The fruit did not ripen until returned to lower temperatures.

Of further interest in regard to the significance of the respiratory climacteric is that cyanide, at concentrations which inhibit Cyt oxidase, initiates the respiratory climacteric and ripening of several fruits (27). Solomos and Laties (26) proposed that the alternative, cyanide-insensitive electron transport path was favored during the ripening process. Since the alternative electron transport path makes a single ATP when linked to the oxidation of mitochondrial NADH whereas the Cyt path makes three (30), evocation of the alternative path during ripening might be equated with a controlled uncoupling of oxidative phosphorylation. Experiments designed to determine the contribution of the alternative path to the respiration of slices of ripening fruit have shown, however, that the alternative path does not contribute to the respiration of 1-mm slices taken from either preclimacteric or climacteric avocado fruit (28) and is seemingly not necessary for ripening of 1.5-cm banana fruit sections (29).

In this study, the interrelationship of new gene expression, polysome prevalence, and respiration in avocado were studied by following the temporal development of each of these ripening-related indicators after initiation of ripening with ethylene and/or cyanide. Since, as noted, cyanide causes a full blown respiratory climacteric in many fruits (27), we particularly wished to know the effect of cyanide—and presumably the intracellular energy charge—on the underlying concomitants of the respiratory climacteric, namely the increase in polysome prevalence and new gene expression (6). To this end, cyanide was presented

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in the influent gas stream so as to attain rapid and complete inhibition of Cyt oxidase.

MATERIALS AND METHODS

Treatment of Plant Material. Mature avocado fruit (*Persea americana* Mill. cv Hass) were picked from a local tree in August and September. Within a few h of picking, individual fruit were placed in 4-L glass jars kept at 25°C with a continuous flow of water-saturated air passed through at 50 ml/min. CO_2 production was monitored automatically every hour by passing the effluent gas through an IR gas analyzer (Anarad, model AR500). Ethylene production was followed by GC (F&M Scientific Corp., model 810) using a Poropak N column (Waters Associates). Ethylene at 10 μl/l in air was administered as a mixture of compressed gasses. Cyanide was added to the gas stream by bubbling air or ethylene and air through 500 ml of buffered solution (Tes, 20 mM, pH 7.0) containing 5 mM KCN, which is in equilibrium with 500 μl/l HCN in air at 25°C (19).

Cyanide Measurement. Cyanide in the gas stream was measured by bubbling 25 ml of a gas sample through 8 ml of indicator solution (39 μM phenolphthalin, 0.1 mM $CuSO_4$, 4 mM Na_2HPO_4), adding 2 ml 20 mM KOH, and measuring the ensuing color spectrophotometrically at 552 nm (20). Cyanide in the tissue was measured as follows: avocado fruit was sliced into liquid N_2 immediately after removal from the jar. Five g of frozen tissue was homogenized at 4°C in 45 ml of 50 mM NaOH and 100 mg/l sodium 2-mercaptobenzothiazole as a polyphenol oxidase inhibitor. In an evacuated closed system, 2 ml of 1 M lactic acid was added to the homogenate and the mixture heated to distill cyanide into 8 ml of the designated indicator solution. After complete distillation, 2 ml of 20 mM KOH was added and the resulting color quantified spectrophotometrically at 552 nm (20).

Polysome Preparation and Poly(A)$^+$ Isolation. A 20-g cross section of mesocarp tissue was homogenized in an Oster vegetable juicer with 66 ml of cold (4°C) polysome extraction buffer (0.5 M sucrose, 200 mM Tris-HCl, pH 9.0, 400 mM KCl, 35 mM $MgCl_2$, 25 mM EGTA, 5 mM 2-mercaptoethanol). The coarse debris was removed by filtration of the homogenate through a strip of Miracloth which lined the basket centrifuge of the juicer. Further isolation and purification of polysomes and associated poly(A)$^+$ RNA were accomplished essentially as described by Christoffersen and Laties (7). The procedure for poly(A)$^+$ RNA isolation, however, entailed two additional steps to remove contaminating polysaccharides from the ethanol precipitate of extracted polysomal RNA. The ethanol precipitate was suspended in 3.6 ml of elution buffer (10 mM Tris-HCl, pH 7.6; 1 mM EDTA; 0.1% SDS). The suspension was heated to 80°C for 3 min, rapidly cooled to room temperature, and 0.4 ml 5 M NaCl added. The suspension was then centrifuged at 10,000 *g* for 5 min producing a pellet which was discarded. To the supernatant was added 2.5 volumes of cold (−20°) ethanol to precipitate RNA. The RNA precipitate was suspended again in elution buffer, heated to 80°C for 3 min, cooled, 0.1 volume of 5 M NaCl was added, and the suspension was passed through a cellulose column equilibrated with 0.5 mM NaCl in elution buffer (13). The eluate was then passed once through an oligo(dT)-cellulose affinity column, and poly(A)$^+$ RNA subsequently released as previously described (7).

***In Vitro* Translation.** A wheat germ *in vitro* translation extract was prepared as described by Roberts and Paterson (21). The extract was not nuclease treated as has become traditional (15) because nuclease treatment inhibited [^{35}S]methionine incorporation in all samples. The ratio of [^{35}S]methionine incorporation with 0.5 μg exogenous mRNA to that with no exogenous RNA was 12:1. The final volume of the *in vitro* translation mixture

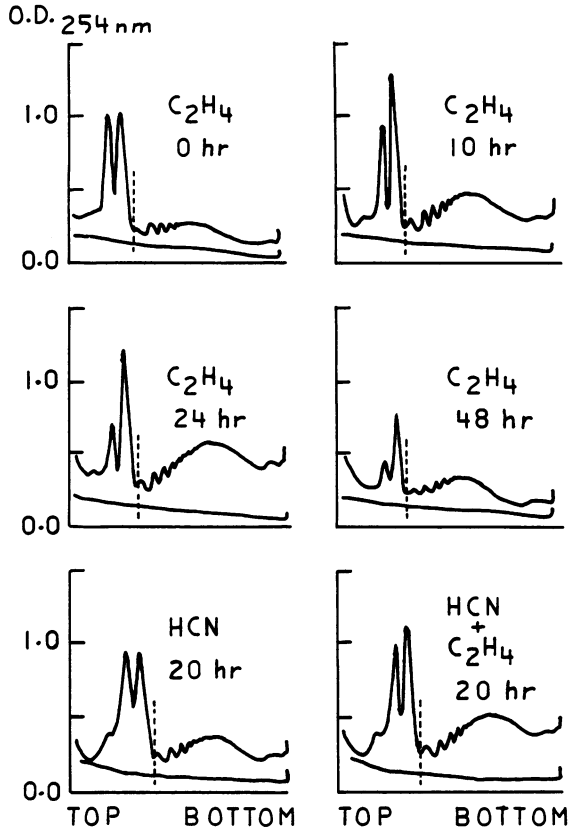

FIG. 2. Effect of 10 μl/l ethylene and/or 500 μl/l cyanide on polysome profiles of avocado fruit. Hours refer to hours of exposure to ethylene and/or cyanide. RNA associated with peaks to the left of the dashed lines were excluded from calculations for polysome prevalence in Figures 1 and 7.

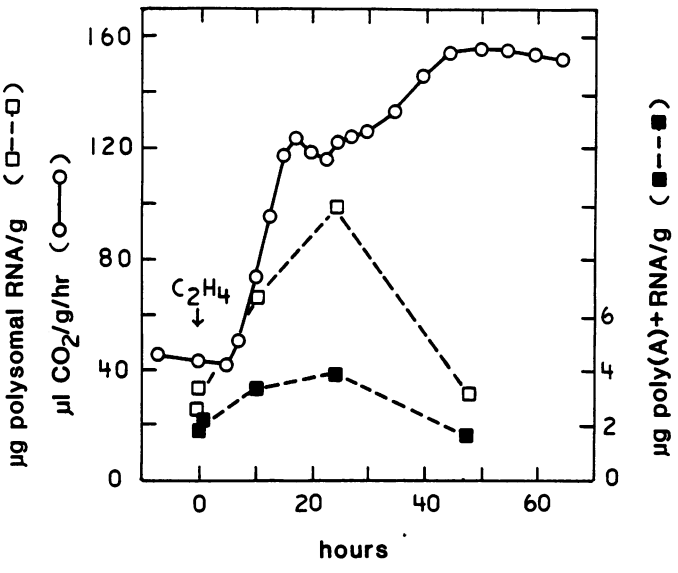

FIG. 1. Effect of 10 μl/l ethylene treatment of avocado fruit on respiration, polysome prevalence, and poly(A)$^+$ RNA extracted from polysomes. CO_2 curve is an average of three separate samples. All fruit were picked and kept at 25°C in a continuous flow of water-saturated air for 24 h prior to treatment with ethylene. Individual fruits were homogenized and RNA extracted after 0, 10, 24, and 48 h of ethylene treatment. The two 0-h samples shown for RNA quantification were homogenized for RNA extraction 24 and 72 h, respectively, after detachment from the tree.

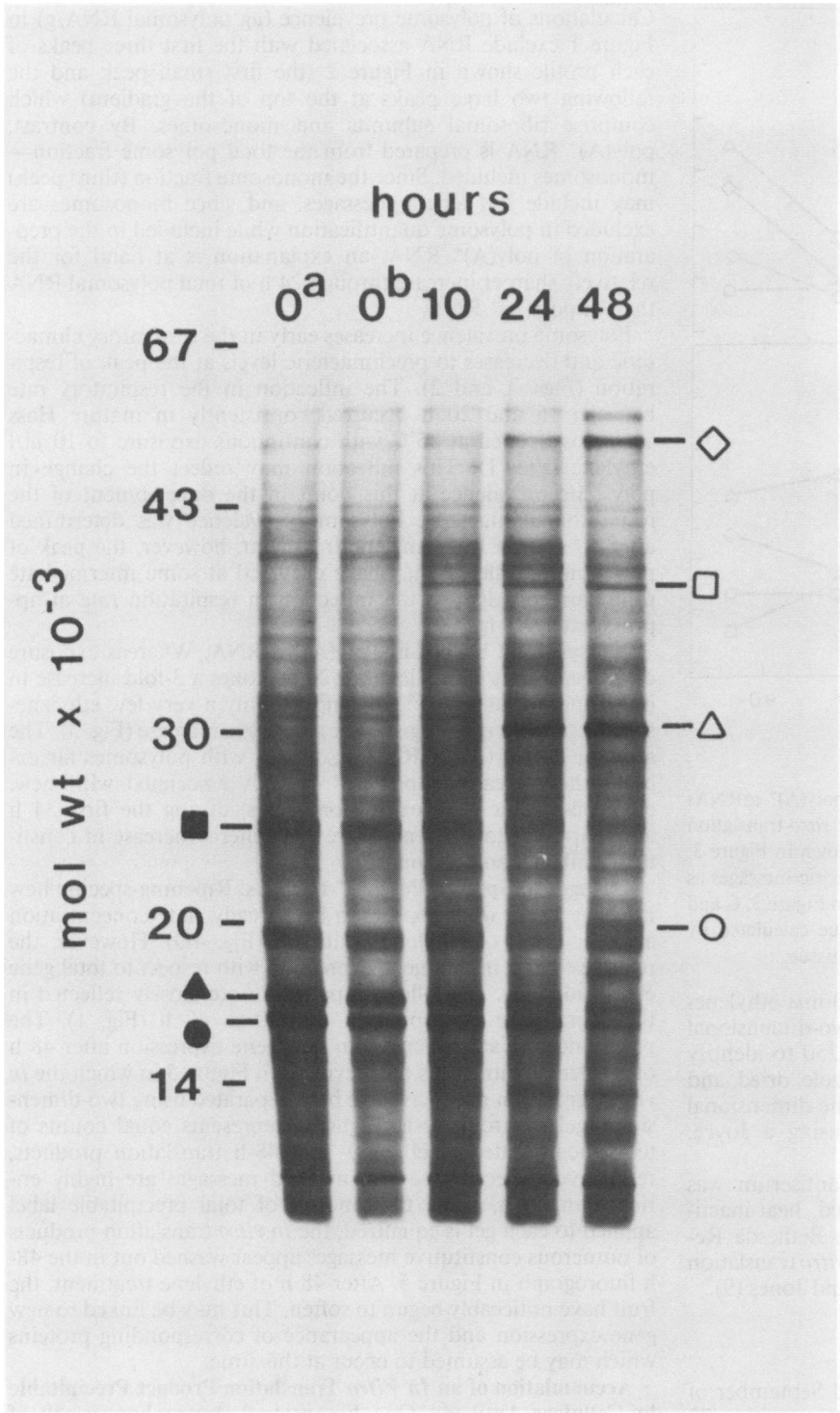

FIG. 3. Fluorograph of electrophoretically separated *in vitro* translation products of avocado fruit polysomal poly(A)$^+$ RNA. The number above each lane refers to hours of ethylene treatment. 0^a and 0^b refer to translation products of mRNA extracted from untreated fruit 24 and 72 h, respectively, after detaching fruit from the tree. Symbols refer to selected translation products which change in relative abundance with time. Decreasing with time: (■), 25 kD; (▲), 18 kD; (●), 17 kD. Increasing with times: (◇), 53 kD; (□), 40 kD; (△), 31 kD; (○), 20 kD.

was 50 μl, including 20 μl wheat germ extract. Final concentrations were 20 mM Hepes (pH 7.6), 50 mM potassium acetate, 0.25 mM magnesium acetate, 0.25 mM spermidine, 1 mM ATP, 50 μM GTP, 8 mM phosphocreatine, 50 μg/ml creatine phosphokinase, 2 mM DTT, 30 μM each of a combination of amino acids lacking methionine, 60 μg placental ribonuclease inhibitor (Biotec), and 15 μCi (5.6×10^5 Bq) [^{35}S]methionine (>600 Ci/mmol, Amersham). Translation was started upon bringing the volume to 50 μl with 0.5 μg poly(A)$^+$ mRNA in water. Samples were incubated for 1 h at 25°C. The reaction was stopped on ice and the mixture stored at −20°C.

Electrophoresis of *In Vitro* Translation Products. For one-dimensional electrophoresis, aliquots of translation products (approximately 3 μl/sample) containing 2×10^5 cpm TCA-precipitable [^{35}S]methionine were suspended and boiled 3 min in 25 μl of SDS sample buffer (63 mM Tris-HCl pH 6.8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol), and electrophoresed on a 10 to 15% polyacrylamide gradient gel containing 1% SDS (10). For two-dimensional electrophoresis, an aliquot containing 2×10^5 cpm of TCA-precipitable [^{35}S]methionine was suspended in 20 μl of 9 M urea, 4% Nonidet P-40, 2% ampholytes, 2% 2-mercaptoethanol. Samples were electrophoresed essentially as described by O'Farrell (14). Buffers used for isoelectric focusing in the first dimension of two-dimensional gels were 10 mM

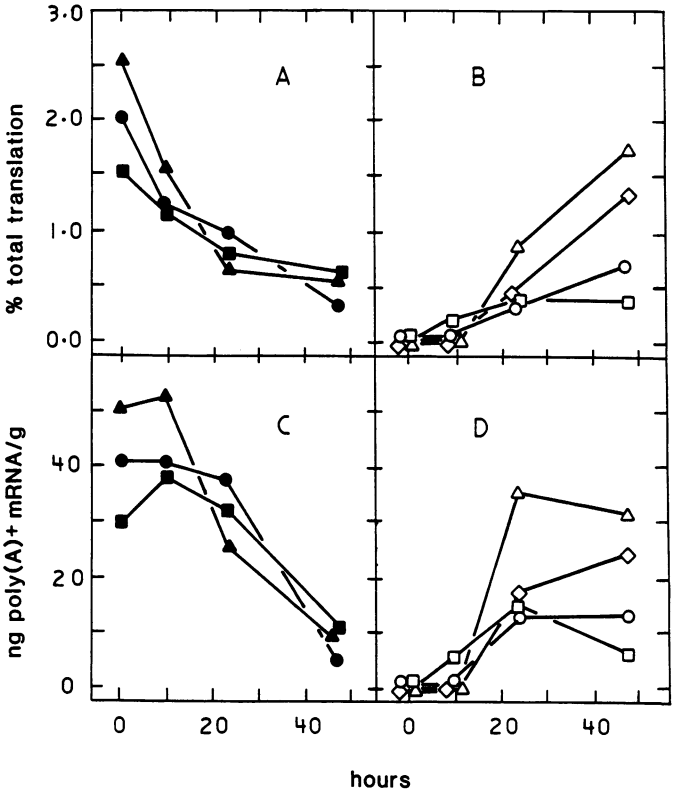

FIG. 4. Temporal change in relative abundance of poly(A)$^+$ mRNAs coding for polypeptide(s) of a specific mol wt in an *in vitro* translation assay. Symbols refer to *in vitro* translation products shown in Figure 3. A and B show percentage of relative abundance of specific messages as determined by a densitometry scan of the fluorograph in Figure 3. C and D shows absolute amount of specific messages/g tissue calculated by multiplying relative abundance by μg poly(A)$^+$ RNA/g tissue.

iminodiacetic acid in the anode chamber and 10 mM ethylenediamine in the cathode chamber. Both one- and two-dimensional gels were fixed, stained with Coomassie blue R-250 to identify mol wt markers, infiltrated with 2,5-diphenyloxazole, dried, and exposed to Kodak XAR-5 x-ray film (11). One-dimensional fluorographs were scanned for quantification using a Joyce, Loebl & Co. Ltd. microdensitometer.

Immunoprecipitation of Cellulase. Cellulase antiserum was prepared by Awad and Lewis (2). Formalin-fixed, heat-inactivated *Staphylococcus aureus* was obtained from Bethesda Research Laboratories. Immunoprecipitations of *in vitro* translation products were performed as described by Ivarie and Jones (9).

RESULTS

Mature avocado fruit harvested in August and September of 1982 showed no initiation of a respiratory climacteric within 72 h after picking unless treated with ethylene and/or cyanide (Figs. 1 and 7). Polysome prevalence in untreated fruit was essentially the same at 24 and 72 h after picking (Fig. 1). Furthermore, *in vitro* translation of polysomal poly(A)$^+$ RNA extracted from untreated avocados at 24 and 72 h after picking gave essentially the same translation products as determined by one-dimensional (Fig. 3) and two-dimensional (data not shown) gel electrophoresis. Hence, fruit were homogenized for RNA extraction between 24 and 72 h after picking, and the reported changes in respiration, polysome prevalence, and gene expression are a result of ethylene and/or cyanide treatment.

Changes in Polysome Prevalence. Polysome profiles shown in Figure 2 represent equal amounts of tissue on a wet weight basis. Calculations of polysome prevalence (μg polysomal RNA/g) in Figure 1 exclude RNA associated with the first three peaks of each profile shown in Figure 2 (the first small peak and the following two large peaks at the top of the gradient) which comprise ribosomal subunits and monosomes. By contrast, poly(A)$^+$ RNA is prepared from the total polysome fraction—monosomes included. Since the monosome fraction (third peak) may include full length messages, and since monosomes are excluded in polysome quantification while included in the preparation of poly(A)$^+$ RNA, an explanation is at hand for the relatively sharper increase through 24 h of total polysomal RNA than of poly(A)$^+$ RNA.

Polysome prevalence increases early in the respiratory climacteric and decreases to preclimacteric levels at the peak of respiration (Figs. 1 and 2). The inflection in the respiratory rate between 15 and 20 h occurred consistently in mature Hass avocados ripened at 25°C with continuous exposure to 10 μl/l ethylene (Fig. 1). This inflection may reflect the change in polysome prevalence at this point in the development of the respiratory climacteric. Polysome prevalence was determined after 10 and 24 h of ethylene treatment; however, the peak of polysome prevalence may have occurred at some intermediate time corresponding to the inflection in respiration rate at approximately 18 h.

Nonspecific Changes in Poly(A)$^+$ mRNA. Whereas exposure of avocado fruit to ethylene for 24 h evokes a 3-fold increase in polysome prevalence (Figs. 1 and 2), only a very few ethylene-specific (and/or ripening-specific) messages increase (Fig. 3). The amount of poly(A)$^+$ mRNA associated with polysomes far exceeds the increase in poly(A)$^+$ mRNA associated with new, ethylene-specific gene expression. Thus, during the first 24 h after exposure to ethylene, there is a generic increase in constitutive mRNA on polysomes.

Changes in Specific Poly(A)$^+$ mRNAs. Ripening-specific new mRNAs attain what appears to be a steady state concentration at 20 to 24 h of ethylene treatment (Fig. 4D). However, the relative change in new gene expression with respect to total gene expression (Fig. 4B) follows a pattern more closely reflected in the overall rise in respiration from 0 to 48 h (Fig. 1). The pronounced relative increase in new gene expression after 48 h of ethylene treatment is most evident in Figure 5 in which the *in vitro* translation products have been separated using two-dimensional gel electrophoresis. Figure 5 represents equal counts of total incorporated label for 0- and 48-h translation products, respectively. Because several induced messages are highly enriched in 48 h, while the amount of total precipitable label applied to each gel is equalized, the *in vitro* translation products of numerous constitutive messages appear washed out in the 48-h fluorograph in Figure 5. After 48 h of ethylene treatment, the fruit have noticeably begun to soften. This may be linked to new gene expression and the appearance of corresponding proteins which may be assumed to occur at this time.

Accumulation of an *In Vitro* Translation Product Precipitable by Cellulase Antibody. One-dimensional electrophoresis gels of *in vitro* translation products of poly(A)$^+$ mRNA extracted from preclimacteric and climacteric fruit show an increase in a protein with a mol wt of 53 kD. This 53-kD protein can be partially immunoprecipitated by immune serum prepared against purified avocado fruit cellulase (Fig. 6). Two-dimensional gel electrophoresis of *in vitro* translation products made from poly(A)$^+$ mRNA shows an increase upon ripening of at least four 53-kD polypeptides having slightly different isoelectric points clustered around pH 5.7 (Fig. 5).

Cyanide Effects. Table I shows that cyanide in the gas stream remains high throughout the experiment. Whereas avocado does not attain equilibrium with the gas phase within 24 h, the tissue exposed to approximately 500 μl/l cyanide attains a minimum

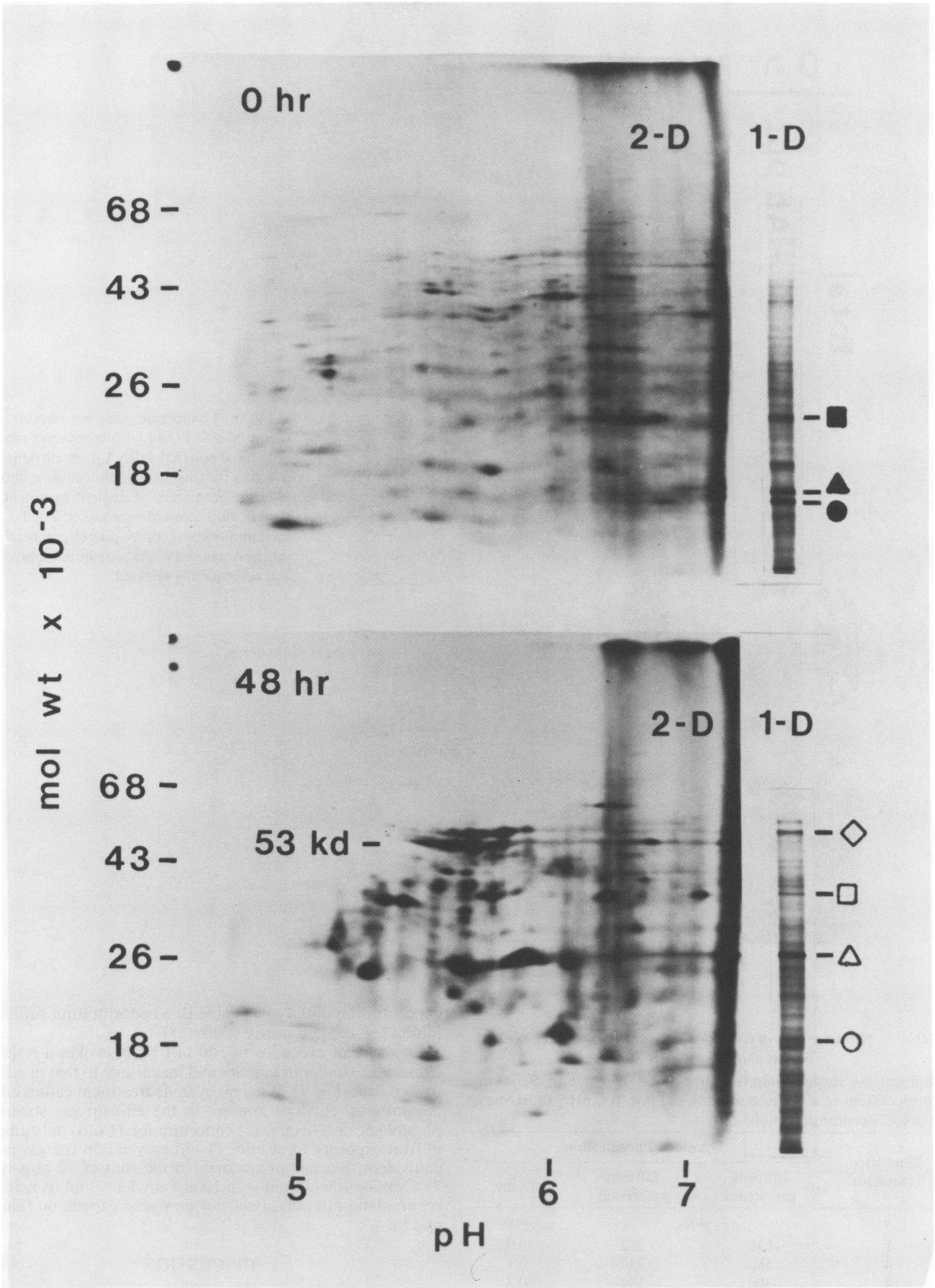

FIG. 5. Fluorograph of two-dimensional (2-D) and one-dimensional (1-D) gel electrophoresis of *in vitro* translation products of polysomal poly(A)$^+$ RNA extracted from untreated (0 h) avocado fruit and fruit treated with ethylene for 48 h. Symbols refer to selected *in vitro* translation products which change with ethylene treatment as shown in Figure 3.

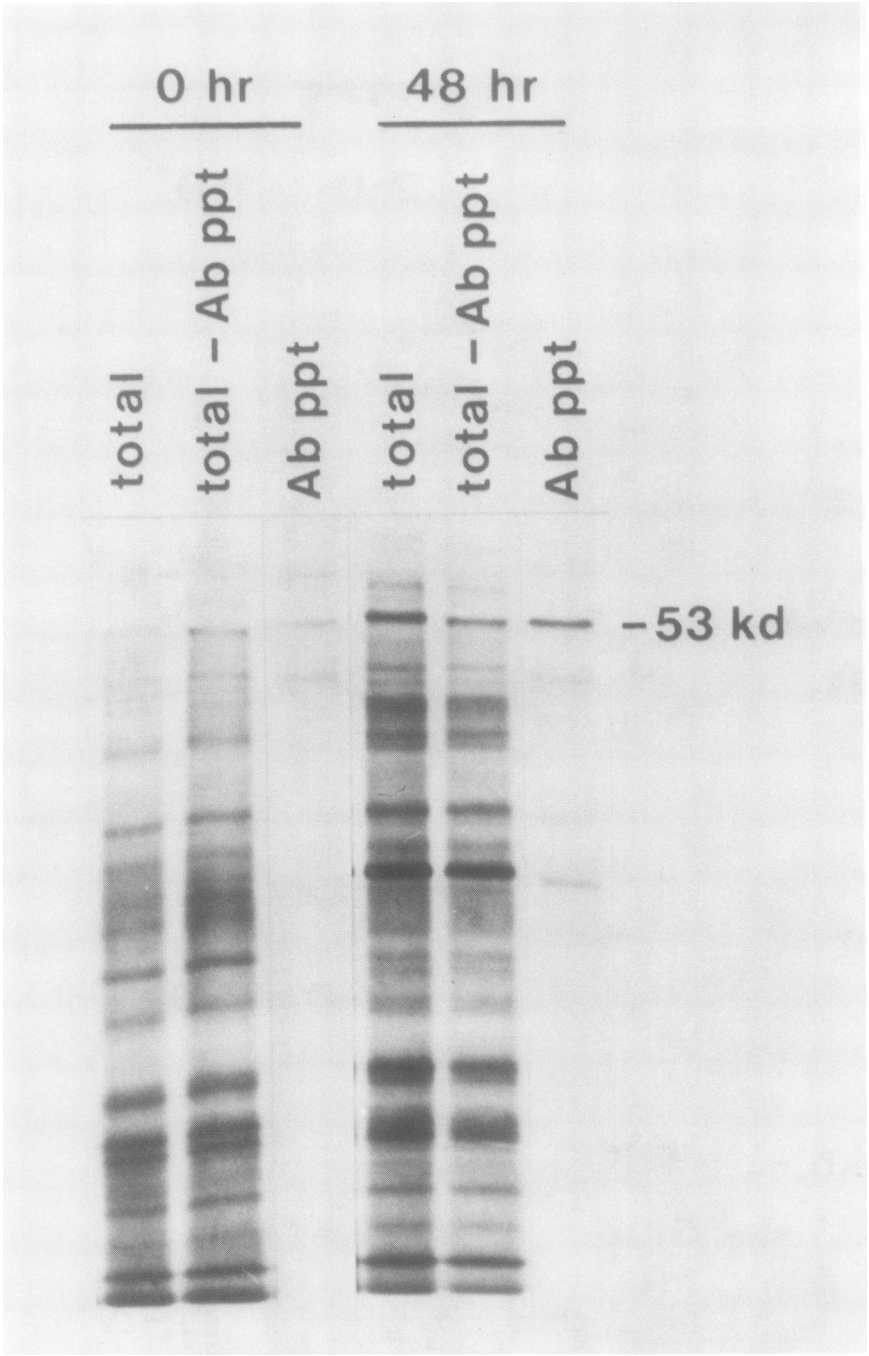

FIG. 6. Fluorograph showing immunoprecipitation of a 53-kD *in vitro* translation product of polysomal poly(A)$^+$ mRNA from ethylene-treated avocado fruit using avocado cellulase antiserum. Hours refer to hours of ethylene treatment. Total, total *in vitro* translation products; Total—Ab ppt, total minus immunoprecipitated polypeptides; Ab ppt, polypeptides which were immunoprecipitated and subsequently released.

Table I. *Measurements of Cyanide Concentration in the Tissue and Influent and Effluent Gas Stream*

Influent gas stream comprises air bubbled at a rate of 50 ml/min through 500 ml of a buffered solution (20 mM Tes, pH 7.0) having an initial KCN concentration of 5 mM.

Time after Treatment	Cyanide Concentration		
	Influent gas stream	Effluent gas stream	Tissue
h	*μl/l*		*mM*
1	636	372	0.02
4	560	332	0.1
24	300	144	0.3

concentration of 0.3 mM (Table I), a concentration sufficient to inhibit Cyt oxidase nearly 100% (28).

Exposure of avocados to 500 μl/l HCN evokes a respiratory climacteric similar in kinetics and magnitude to that in ethylene-treated fruit (Fig. 7). Whereas cyanide treatment causes ethylene biosynthesis, ethylene appears in the effluent gas stream at a physiologically effective (5) concentration (1 μl/l) only after 18 h of fruit exposure to cyanide (1 μl/l ethylene in the gas phase is equivalent to a rate of synthesis in the fruit of 17 nl/g·h) (Fig. 7). Cyanide when given with 10 μl/l ethylene inhibits neither the accumulation of polysomes nor new gene expression (Figs. 1, 7 and 8).

DISCUSSION

It is well established that ethylene initiates the ripening process in avocado (3) and several other fruits (23). Accordingly, in order

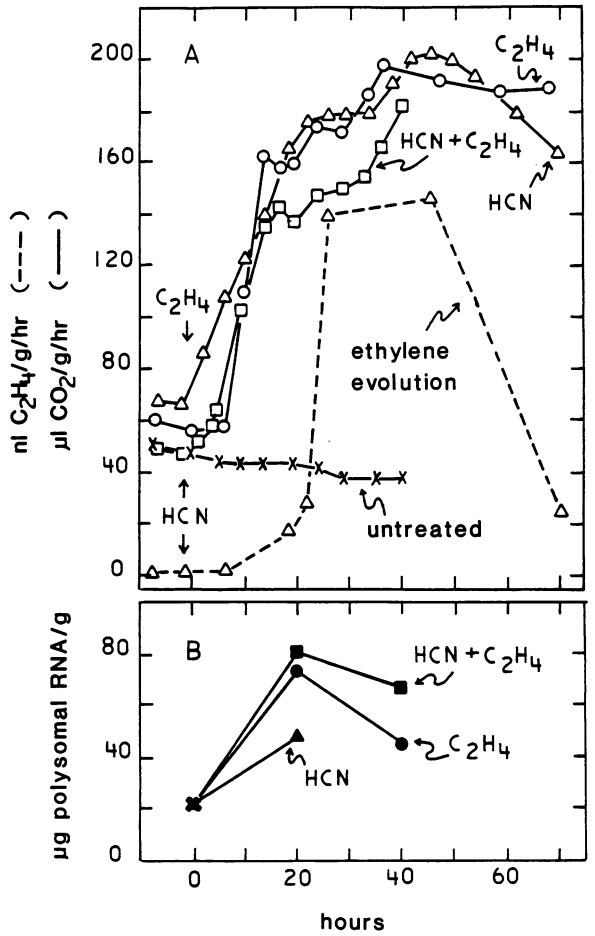

FIG. 7. A, effect of 10 µl/l ethylene and/or 500 µl/l cyanide on respiration of avocado fruit, and the effect of 500 µl/l cyanide on the endogenous synthesis of ethylene in avocado fruit. B, effect of 10 µl/l ethylene and/or 500 µl/l cyanide on polysome prevalence in avocado fruit. (X), untreated fruit; (△, ▲), HCN; (□, ■), HCN and C_2H_4; (○, ●), C_2H_4.

to make consistent comparisons and conclusions about the temporal development of ripening in avocado, ripening was synchronized by initiation with ethylene, except when cyanide was used alone.

An increase in polysome prevalence early in the climacteric is consistent with previous results demonstrating an increase in protein synthesis during this period in avocado (17), banana (4), pear (8), and other fruits (23). Moreover, the finding that the greatest part of the increase in polysome prevalence in early climacteric avocado is due to a generic increase in constitutive mRNAs on polysomes, is corroborated by Brady and O'Connell's (4) results for banana which showed no significant differences between the proteins synthesized in preclimacteric banana fruit and during the peak of protein synthesis early in the climacteric.

Whereas Brady and O'Connell (4) found no evidence of new gene expression upon ripening of banana fruit, they nevertheless speculated that changes in gene expression might occur and might be responsible for normal ripening. Christofferson *et al.* (6) showed that the ripening process in avocado was associated with an increase in at least three mRNAs. It has been demonstrated in this study that several changes in gene expression occur, but that the relative enrichment of new mRNAs becomes evident only late in the respiratory climacteric when polysome prevalence, and presumably protein synthesis, have begun to decline (Fig. 4B). It may be that the new gene products are responsible for softening and other ripening characteristics which become evident late in the respiratory climacteric.

A provocative case in point would seem to be the appearance with avocado fruit ripening of a polysomal message which upon translation yields a 53-kD polypeptide that is immunoprecipitable by antibody raised to avocado cellulase (Fig. 6). Avocado fruit ripening is accompanied by a dramatic increase in cellulase activity (1). The mol wt of avocado fruit cellulase is between 50 and 55 kD, and the isoelectric point of the undenatured protein is approximately 4.7 (2). Figure 5 shows a marked increase in a group of four 53-kD *in vitro* translation products specific to ripe avocado. The isoelectric points of the denatured polypeptides are clustered between pH 5.4 and 6.0 (Fig. 5). One or more of these 53-kD *in vitro* translation polypeptides may in the undenatured and/or processed state have isoelectric points around pH 4.7. We have not established how many of these 53-kD polypeptides are immunoprecipitated by avocado fruit cellulase antiserum. It is possible that these four ripening-specific polypeptides represent a family of cellulase genes evoked by ethylene, and experiments are under way to investigate this point.

Ethylene-treated avocado fruit maintained at 25°C have an inflection in the respiration rate between 15 and 20 h (Fig. 1). If it is assumed that a change in polysome prevalence corresponds to a change in the rate of protein synthesis *in vivo*, it might be expected that the respiration rate would reflect this change in protein synthesis in response to its energy requirements for biosynthesis. The inflection may thus reflect the true peak, and subsequent decline, in polysome prevalence and protein synthesis in response to ethylene. Thus, polysome prevalence at 24 h after ethylene treatment (Fig. 1) may be less than an earlier peak value.

Figure 9 shows that the respiratory climacteric at 25°C can be separated into two components which together give the experimentally observed climacteric. The first phase in Figure 9 linked to a rise in gross polysome prevalence may be a result of energy demand for increased protein synthesis. The second phase may be associated with the expression of new genes, and accumulation of corresponding protein which in turn may be responsible for changes in membrane permeability (23) and cell wall degradation (1). This may explain Brady and O'Connell's (4) finding that a 6-h treatment of bananas with ethylene evoked an increase in protein synthesis and a short burst of respiration, the first phase of the respiratory climacteric, without concomitant ripening. By contrast, a longer 12-h treatment with ethylene elicited a complete ripening response attributable to the expression of new genes necessary for ripening and may be responsible for the appearance of the second phase of respiration.

An interesting question is the basis of the generic increase in polysomal poly(A)$^+$ mRNA. Whereas the generic increase early in the respiratory climacteric might be due to the mobilization of a stored pool of mRNA, Richmond and Biale (18) found that ^{32}P incorporation into an mRNA fraction increased early in the climacteric of avocado and declined to less than preclimacteric levels at the climacteric peak, while Christoffersen *et al.* (6) reported an increase in poly(A)$^+$ RNA isolated from total cellular RNA extracts. These results make it unlikely that a sequestered pool of mRNA accounts for the observed increase. Accordingly, a generic increase in poly(A)$^+$ mRNA on polysomes may be due to: (a) an overall increase in the transcription rate, (b) processing of mRNA and/or (c) a nonspecific inhibition of mRNA degradation. No evidence exists at present to choose among these possibilities.

Of further interest is that new ripening-specific mRNAs appear to attain a steady state concentration after 20 to 24 h of ethylene treatment, and do not decline with constitutive mRNAs between 24 and 48 h. There appears to be some favoring with time of ripening-specific gene expression over constitutive gene expression. This selection may occur at transcription, translation, and/or degradation of mRNA.

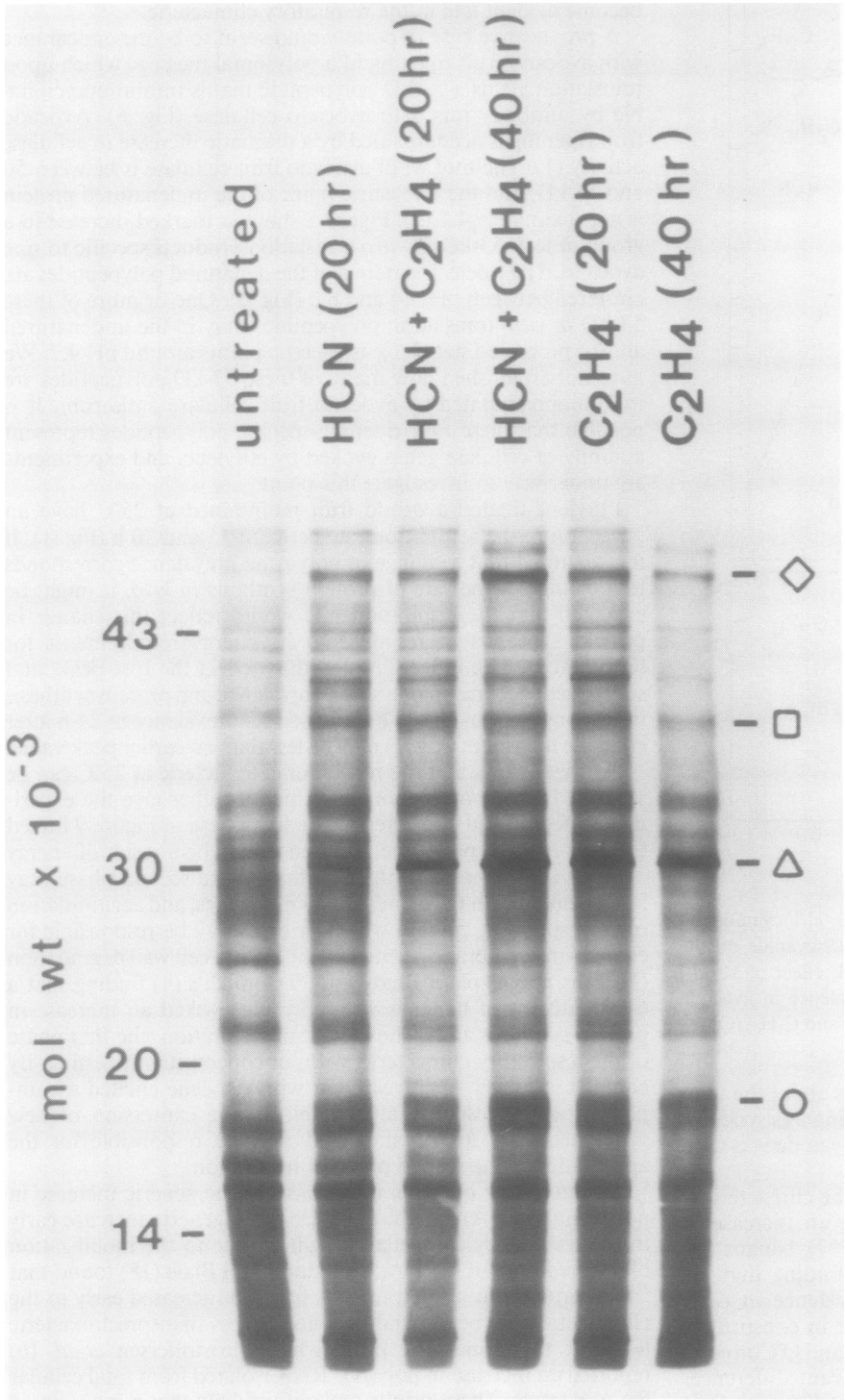

FIG. 8. Fluorograph of electrophoretically separated *in vitro* translation products of poly(A)$^+$ RNA from avocado fruit treated with ethylene and/or cyanide. Hours refer to hours of exposure to indicated treatments. Symbols as per Figure 3.

Cyanide has been demonstrated to cause a respiratory rise and ripening response in avocado fruit very similar to that evoked by ethylene (26). Cyanide has several of the chemical characteristics required of an ethylene analog (24). Moreover, in an *in vitro* assay for ethylene-binding sites, cyanide at 2,000 μl/l inhibited binding of ethylene by 85% (25). However, initiation of ripening by cyanide binding *per se* to ethylene receptor sites cannot unequivocably be proven for avocado fruit because cyanide evokes ethylene synthesis following exposure of the fruit to cyanide, and therefore may elicit ripening by initiation of ethylene synthesis. Nevertheless, in our experiments it was some 18 h before physiologically active ethylene levels were discerned in the cyanide-containing air stream passing over avocado fruit, and a case can accordingly be made for cyanide as a primary climacteric evocator.

In another context, as an inhibitor of Cyt oxidase, cyanide, at a minimum tissue concentration of 0.1 mM after only 4 h (Table I), inhibits neither the increase in polysome prevalence (Fig. 7) nor the change in gene expression due to ethylene (Fig. 8). A concentration of 0.1 mM cyanide is adequate to fully inhibit Cyt oxidase in avocado fruit (28). Thus, while the alternative path is seemingly quite capable of fulfilling the energy needs of ripening avocado, it remains to be established whether or not the alternative path contributes to the respiration of uninhibited intact fruit.

Theologis and Laties (28) concluded that the alternative path

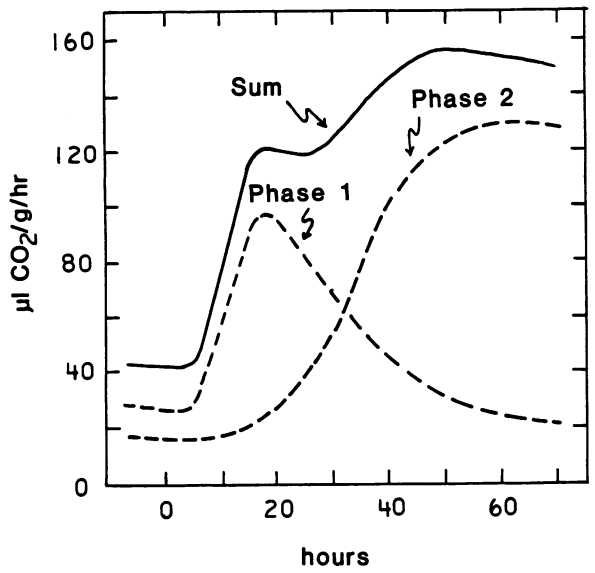

FIG. 9. Hypothesized separation of the respiratory climacteric of avocado fruit into two separate respiratory phases. Curves for phases 1 and 2 add together to give the sum curve which approximates the observed respiratory curve in Figure 1. Respiratory curve for phase 1 corresponds with the change in polysomal RNA in Figure 1.

does not contribute to the respiration of 1-mm fresh slices taken from preclimacteric and climacteric avocado fruit, respectively. However, the specific respiration rate of 1-mm fresh slices was higher in slices from preclimacteric fruit than in slices from climacteric fruit. Accordingly, slices may not be a good system for drawing conclusions about the respiration of the intact organ. The alternative path might indeed contribute to the respiration of intact avocado yet fail to contribute to the respiration of slices taken therefrom.

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