Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 2793–2797, May 1987
Developmental Biology

Regulation of gene expression by ethylene during *Lycopersicon esculentum* (tomato) fruit development

(hormone sensitivity/fruit ripening/proteinase inhibitor I gene/polygalacturonase gene)

JAMES E. LINCOLN, SABINE CORDES, EVAN READ, AND ROBERT L. FISCHER*

Division of Molecular Plant Biology, University of California, Berkeley, CA 94720

Communicated by J. E. Varner, December 23, 1986 (received for review September 12, 1986)

ABSTRACT **We have investigated the regulation of gene expression by the plant hormone ethylene by cloning mRNAs that accumulate in unripe tomato fruit (*Lycopersicon esculentum*) exposed to exogenous ethylene. The response to exogenous ethylene is rapid; within 30–120 min we detect an increase in the cloned mRNA concentrations. DNA sequence analysis indicates that one of the ethylene-inducible genes is related to a gene encoding wound-inducible proteinase inhibitor I. We have measured ethylene production during fruit development and detect low basal levels in unripe fruit and much higher levels in ripening fruit. Blot hybridization experiments show that expression of the cloned genes is developmentally regulated by ethylene during fruit ripening: the mRNAs produced by these genes are more abundant in ripe fruit than in unripe fruit, and this mRNA accumulation is repressed by a competitive inhibitor of ethylene action, norbornadiene. However, during fruit development some of the cloned mRNAs begin to accumulate when ethylene production is at a basal level, whereas other mRNAs begin to accumulate later when the endogenous ethylene concentration increases, suggesting that gene expression during fruit development can be activated by ethylene in two ways. In some cases gene expression is primarily activated by an increase in sensitivity to basal ethylene levels, whereas in other cases it may be regulated by an increase in ethylene concentration.**

The plant hormone ethylene has a profound influence on plant development. Active in trace amounts, it affects seed germination, seedling growth, root and leaf growth, many stress phenomena, plant senescence, and fruit development (1, 2). In particular, ethylene has been implicated in controlling fruit ripening. This is because removal of ethylene from fruits or exposure of fruits to specific inhibitors of ethylene biosynthesis greatly retards ripening, and the onset of ripening is hastened when unripe mature green (MG) fruits are exposed to exogenous ethylene (3–5). Thus, ethylene induces a complex ripening process. Associated with ripening is an accumulation of carotenoid pigments, conversion of chloroplasts to chromoplasts, increased expression of genes encoding cell wall degradation enzymes, and an autocatalytic surge in ethylene biosynthesis (4–8).

Recent efforts to understand the regulatory role of ethylene during fruit ripening have focused on the activation of gene expression in unripe fruit exposed to elevated levels of exogenous ethylene for relatively long periods of time, 10 (8) to 24 hr (6). However, due to these long time intervals, it is not known whether such changes in gene expression represent a primary response to ethylene or are the result of a complex series of metabolic events initiated by ethylene. Moreover, these experiments do not address studies showing that fruits become increasingly responsive to ethylene as they develop and mature (9). Sensitivity of fruit tissue to ethylene may be an important factor in the regulation of ripening (10).

To understand the sequence of events linking ethylene to the onset of fruit ripening, we have analyzed rapid genetic responses to elevated levels of this hormone. To this end we have exposed MG tomato fruit to exogenous ethylene for short periods of time and have cloned mRNAs that increase in concentration. Our results show that the cloned mRNAs accumulate rapidly in response to exogenous ethylene. Increases in their concentration are detected within 30–120 min. Furthermore, the developmentally regulated expression of each cloned gene is repressed by exposure to an inhibitor of ethylene action. However, we find that during fruit ripening some of the cloned mRNAs begin to accumulate when ethylene production is at a basal level, whereas others begin to accumulate later when the ethylene concentration increases. This suggests that in some cases gene expression during ripening is primarily activated by increased sensitivity to basal ethylene levels, while in other instances it may be regulated by an increase in ethylene concentration.

MATERIALS AND METHODS

Plant Material. *Lycopersicon esculentum* cv. VFNT Cherry plants were grown under standard greenhouse conditions. Fruit maturity stage was determined as follows. Immature fruits were 50% full size. Full size MG stages were identified by the extent of locular tissue breakdown resulting in the formation of a viscous gel (7). In MG1 fruit the locular tissue was firm, in MG2 fruit a small amount of gel was present, and in MG3 fruit the formation of the gel was complete. In MG4 fruit pigmentation was just detectable in the interior of the fruit, whereas later stages of fruit development were defined by further pigment accumulation.

Exposure of Fruit to Gasses. One kilogram of mature green fruit was placed in a 25-liter chamber and exposed to 4.5 liters per min of humidified ethylene in air (10 μl per liter) or to air alone.

mRNA Isolation. Pericarp tissue was frozen in liquid nitrogen and stored at −80°. Polysomal, poly(A)$^+$ mRNA was isolated using procedures described elsewhere (11) except that the EDTA-release step was omitted, and the polysome extraction buffer was adjusted to 160 mM KCl and 70 mM $MgCl_2$.

Constructing and Screening an Enriched cDNA Library. A cDNA library enriched for MG4-specific sequences was constructed using subtraction hybridization procedures (12). Seven micrograms of ^{32}P-labeled MG4 cDNA and 70 μg of MG1 mRNA were hybridized for 16.5 hr at 70°C [equivalent R_0t 4000 (13)] in a 12-μl reaction containing 33 mM Pipes, pH 6.9/1 M NaCl/0.2 mM EDTA/0.2% $NaDodSO_4$. Unreacted cDNA (12% of the total cDNA mass) was isolated by passing the reaction over a 1-ml hydroxyapatite column at 68°C

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: MG, mature green.

*To whom reprint requests should be addressed.

equilibrated in 0.12 M NaH_2PO_4/0.12 M Na_2HPO_4/0.2% $NaDodSO_4$. Enriched for MG4-specific sequences, the unreacted cDNA was used to construct a cDNA library (12). To screen the library, a probe enriched for ethylene-inducible sequences was isolated. ^{32}P-labeled cDNA was synthesized using template mRNA isolated from MG1 fruit treated with exogenous ethylene for 8 hr. Two micrograms of this labeled cDNA was hybridized for 18 hr (equivalent R_0t 3000) to 24 μg of MG1 mRNA in a 6-μl reaction, and the unreacted cDNA (10% of the total cDNA mass) was isolated by hydroxyapatite chromatography. Although the unreacted cDNA represented a 10-fold enrichment for ethylene-inducible sequences, it still contained a substantial amount of nonspecific sequences. We therefore constructed a control probe that represented only the nonspecific sequences. Two micrograms of ^{32}P-labeled MG1 cDNA was hybridized to 24 μg of its own template mRNA, and the unreacted cDNA (9% of the total cDNA mass) was isolated by hydroxyapatite chromatography as described above. The MG4-enriched library was differentially screened (14) by hybridizing replica clones to both probes. Ethylene-inducible clones hybridized intensely to the probe enriched for ethylene-inducible sequences and hybridized weakly or not at all to the control probe.

RESULTS

Changes in Ethylene Production and Gene Expression Occur During Tomato Fruit Ripening. Ethylene production at different stages of fruit development was measured. Immature and early mature green stage (MG1 and MG2) fruit evolved ethylene at a low, basal rate. At the MG3 stage a small increase in ethylene evolution rate was detected (data shown in Fig. 6*A*). However, at the MG4 stage when overt signs of ripening were observed, the ethylene evolution rate sharply increased. As fruit ripening progressed further, the ethylene evolution continued to increase (data not shown). To determine whether changes in gene expression occurred at approximately the same time as the observed increase in ethylene production rate, mRNAs were isolated from different stages of fruit development, translated *in vitro*, and the labeled proteins were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis. Fig. 1 shows that at least eight mRNAs were more abundant in MG4 stage fruit than in MG1 fruit. In contrast, these changes in the mRNA population were not evident when MG1 stage fruit were exposed to exogenous ethylene for 8 hr, but the sensitivity of this experiment is limited to the detection of mRNAs that are at least moderately abundant. These findings demonstrate that tomato fruit ripening is associated with an increase in ethylene evolution rate and changes in gene expression.

Isolation of cDNA Clones. We initially attempted to isolate mRNAs that accumulate in response to exogenous ethylene by constructing a cDNA library derived from MG1 fruit exposed to ethylene for 8 hr. The library was differentially screened with labeled mRNA probes isolated from MG1, MG1 fruit treated with ethylene for 8 hr, and MG4 fruit. We found a homologous class of cDNA clones represented by J49 hybridized more intensely to the latter two probes. In order to isolate additional ethylene-induced mRNAs, a cDNA library enriched for MG4-specific sequences was constructed. The library was then screened with a probe enriched for sequences that accumulate when MG1 fruit are exposed to ethylene for 8 hr (see *Materials and Methods*). Using these procedures we obtained three additional classes of ethylene-induced cDNA clones represented by E4, E8, and E17. In addition, we also selected from the MG4-enriched library a control cDNA clone, E41, that did not hybridize to the probe enriched for ethylene-inducible sequences. Fig. 2 shows that each cDNA clone represents a distinct size mRNA. These results indicate that exposing MG1 tomato fruit to exogenous ethylene for 8 hr induces the expression of at least four different genes.

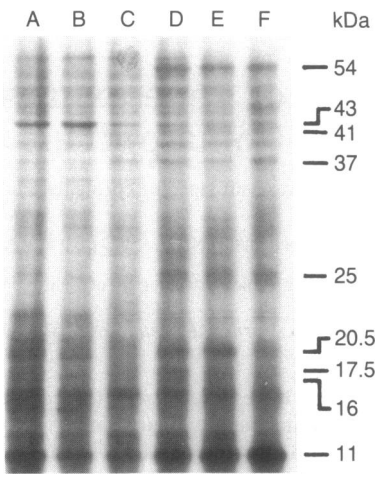

FIG. 1. *In vitro* translation of mRNA isolated from fruits at different stages of development. Polysomal, poly(A)$^+$ mRNA was isolated, translated *in vitro* using a wheat germ extract containing ^{35}S-labeled methionine, and the labeled proteins were fractionated by $NaDodSO_4$/polyacrylamide gradient (12–16%) gel electrophoresis (15). Molecular masses of proteins are expressed in kDa. Proteins encoded by mRNAs that display changes in abundance during fruit development are indicated. Fruit used for mRNA isolation were immature (lane A), MG1 (lane B), MG1 exposed to 10 μl of ethylene per liter for 8 hr (lane C), MG4 (lane D), 50% red (lane E), and red (lane F).

Proteins Encoded by Cloned mRNAs. To identify proteins encoded by the cloned genes, we compared characteristics of the cloned mRNAs and their *in vitro* translated polypeptides to those of known plant genes. The molecular mass of the *in vitro* translated polypeptides encoded by the cloned mRNAs is shown in Fig. 3. The size of the E41 mRNA and its *in vitro* translated polypeptide suggested that it might encode polygalacturonase (17, 18), an important cell wall degradation enzyme that accumulates during fruit ripening (10). This was confirmed by demonstrating that E41 mRNA hybridized with a polygalacturonase cDNA clone (17) and that the E41 mRNA and polygalacturonase DNA sequences (19) were 99% homologous (data not shown). The small size of the E17 mRNA (Fig. 2) suggested that it might encode a protein related to the proteinase inhibitors, a diverse class of low molecular weight proteins that accumulate in wounded leaves

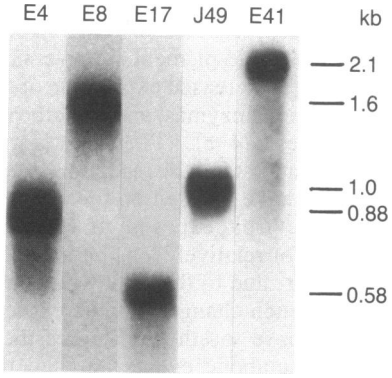

FIG. 2. Size of mRNAs encoded by ethylene-inducible genes. MG4 polysomal poly(A)$^+$ mRNA was denatured with glyoxyl, subjected to agarose gel electrophoresis, blotted, and hybridized to the ^{32}P-labeled DNAs designated at the top of each lane. mRNA sizes are expressed in kilobases (kb) and were calculated relative to RNA standards.

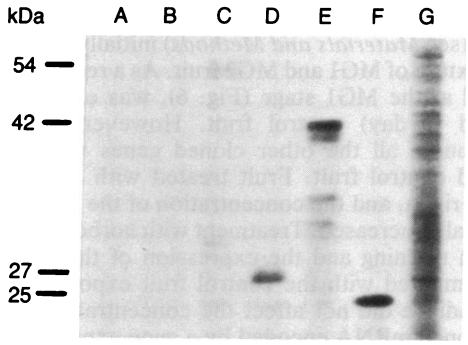

FIG. 3. *In vitro* translation of mRNAs that accumulate in response to ethylene. Specific mRNAs were purified by hybridizing MG4 mRNA to plasmids bound to nitrocellulose filters (16), translated *in vitro* using a wheat germ extract, and subjected to NaDodSO$_4$/polyacrylamide gel electrophoresis as described in Fig. 1. In lane A no mRNA was added to the wheat germ extract. The plasmid DNA used to purify mRNAs: B, pUC9 vector control; C, E41; D, J49; E, E8; F, E4. In lane G MG4 mRNA was translated *in vitro*.

(20). This was confirmed by determining 132 nucleotides of DNA coding sequence from the 3′ portion of the E17 cDNA clone. As shown in Fig. 4, the sequence is 67% homologous to the DNA sequence of a proteinase inhibitor I cDNA clone isolated by Graham *et al.* (20).

A Subset of the Developmentally Regulated Fruit Ripening Genes Responds Rapidly to Exogenous Ethylene. To determine how rapidly the cloned mRNAs accumulate in ethylene-treated fruit, we hybridized the labeled cDNA clones to mRNA isolated from MG1 fruit exposed to either ethylene or air for 0.5–8 hr. As shown in Fig. 5, mRNAs represented by E4, E8, E17, and J49 accumulated rapidly following exposure to ethylene. Within 2 hr the level of each mRNA had increased significantly over that of the air control. In contrast, the mRNA encoded by the control *E41* gene did not accumulate in response to ethylene for up to 8 hr. As shown in Figs. 5 and 6, expression of the ethylene-inducible genes and the control *E41* gene was developmentally regulated

```
    70                  75                  80
aAA tAT CCA aaa tTA ACa AAT gTT gaA Act cTA cTG AaT GGT TCa
lys tyr PRO lys leu THR ASN val glu thr LEU LEU asn GLY SER

    85                  90                  95
gCt tTT ACA gat GAT Ttg agA Tcg cAT aGA GTT CGT CTT TTT GAT
ala phe THR asp ASP leu arg ser his ARG VAL ARG LEU PHE ASP

    100                 105                 110
AAt tTa TTG GaC aTT GTT GTA CAc Act CCc aaa GTt ggT TgA
ASN leu LEU asp ile VAL VAL his thr PRO lys VAL gly STOP
```

FIG. 4. DNA sequence homology between E17 cDNA and a proteinase inhibitor I gene. E17 DNA was subcloned into the M13 phage vector mp19 (21) and a portion of its DNA sequence was determined using the dideoxy chain-termination method (22). Nucleotides and amino acids identical to those of the proteinase inhibitor I gene (20) are uppercase, whereas divergent sequences are lowercase. Amino acids conserved during the evolution of proteinase inhibitor I proteins are underlined. Numbers refer to the position of proteinase inhibitor I amino acids (20).

during fruit ripening. That is, their mRNAs were more abundant in ripe fruit than in unripe fruit. Taken together, these results indicate that some, but not all, of the developmentally regulated fruit ripening genes respond rapidly when MG1 fruit are exposed to exogenous ethylene.

Activation of Gene Expression and Ethylene Production During Fruit Development. The ethylene evolution rate and the concentration of each cloned mRNA were measured at different stages of fruit development. As shown in Fig. 6, the E4, E8, and the control E41 mRNAs began to accumulate at the MG3 stage, coincident with the increase in ethylene production. In contrast, increases in J49 and E17 mRNA concentration were detected at the MG1 and MG2 stages, respectively, when low basal levels of ethylene production were detected. Because the activation of *E17* and *J49* gene expression preceded the increase in ethylene biosynthesis, we conclude that their expression is not primarily regulated by an increase in ethylene concentration during fruit ripening.

Repression of Gene Expression by an Inhibitor of Ethylene Action. We reasoned that if *E17* and *J49* gene expression was not regulated by an increase in ethylene concentration, then perhaps their expression was activated by an increased

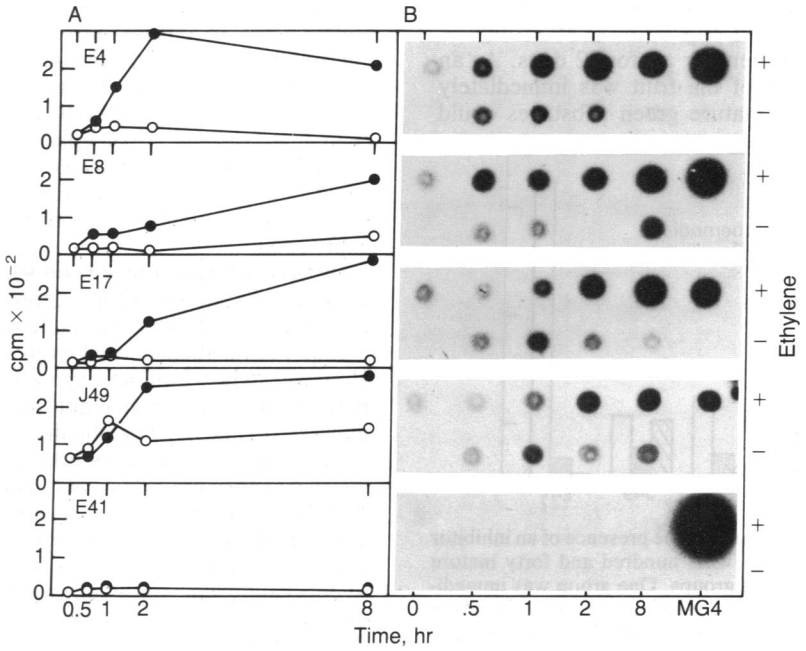

FIG. 5. Accumulation of specific mRNAs in fruit exposed to ethylene. mRNA was isolated from MG1 fruit treated with either ethylene (●) or air (○) for the indicated period of time, or from MG4 fruit. One microgram of each mRNA was then dotted (23) onto nitrocellulose and hybridized to the indicated ^{32}P-labeled DNA probes. Following autoradiography (*B*), each dot was excised, and the extent of hybridization was determined by liquid scintillation spectrometry (*A*).

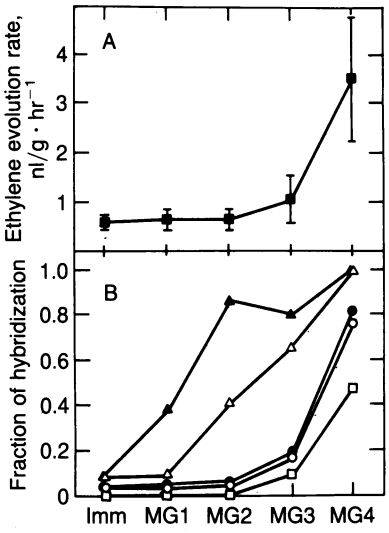

FIG. 6. Ethylene production rate and the accumulation of specific mRNAs during fruit development. (*A*) Individual fruits were placed in 50-ml containers that were sealed and incubated 1 hr. A 1-ml sample from the closed atmosphere was removed, and the ethylene content was determined by gas chromatography (Varian 500). Results represent the mean ± SD. Sample size for each group was as follows: immature (Imm), 5; MG1, 39; MG2, 57; MG3, 15; MG4, 26. (*B*) One microgram of mRNA isolated from fruit at the indicated stages was dotted onto nitrocellulose filters and hybridized to the cloned ^{32}P-labeled DNA probes. Following autoradiography, each dot was excised, and the extent of hybridization was determined by liquid scintillation spectrometry. Maximum cpm hybridized for each ^{32}P-labeled DNA probe are as follows: E4 (○), 6625 cpm at red stage; E8 (●), 2876 cpm at 50% red stage; E17 (△), 559 cpm at MG4 stage; J49 (▲), 3848 cpm at MG4 stage; and E41 (□), 12,352 cpm at red stage; results are reported as the fraction of that maximum hybridization.

capacity of fruit tissue to respond to low basal levels of ethylene. Alternatively, it was possible that *E17* and *J49* gene expression during fruit ripening was not regulated by ethylene. To investigate the role played by ethylene in regulating their expression, fruit were exposed to norbornadiene, a highly specific competitive inhibitor of ethylene action (24). As shown in Fig. 7, a population of MG fruit was treated with either norbornadiene or air for 12 days. As an additional control, a portion of the fruit was immediately harvested (0 day). Because mature green substages could only be determined by examining internal morphological markers (see *Materials and Methods*) initially the population was a mixture of MG1 and MG2 fruit. As a result, *J49*, a gene activated at the MG1 stage (Fig. 6), was expressed in the untreated (0 day) control fruit. However, as expected, expression of all the other cloned genes was low in the untreated control fruit. Fruit treated with air for 12 days began to ripen, and the concentration of the cloned mRNAs dramatically increased. Treatment with norbornadiene inhibited both ripening and the expression of the cloned genes when compared with the control fruit exposed to air. The norbornadiene did not affect the concentration of an additional cloned mRNA encoded by a gene expressed constitutively throughout fruit development (data not shown).

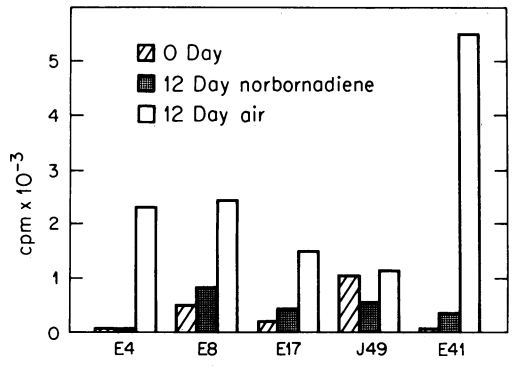

FIG. 7. Expression of cloned genes in the presence of an inhibitor of ethylene action, norbornadiene. One hundred and forty mature green fruit were divided into three groups. One group was immediately harvested. The other two groups were put in 60-liter chambers for 12 days. One chamber contained air plus 1000 μl of norbornadiene per liter, and the other contained only air. Ripening (accumulation of carotenoid pigments) was observed in the air controls, but not in the norbornadiene-treated fruit. One microgram of mRNA isolated from each group was dotted and hybridized to the indicated ^{32}P-labeled probes.

These results show that ethylene is needed for the maximal accumulation of all of the cloned mRNAs, and, in particular, they suggest that low basal levels of ethylene play a role in regulating *E17* and *J49* gene expression. These results support the hypothesis that *E17* and *J49* gene expression is activated by an increase in sensitivity to low basal levels of ethylene.

DISCUSSION

Ethylene has been shown to be intimately involved in the initiation of ripening of tomatoes and many other fruits (1, 4, 5, 10). For this reason, tomato fruit ripening represents an excellent paradigm for studying the effect of ethylene on plant development. In order to better understand the relationship between ethylene, gene expression, and the onset of fruit ripening, we have studied an early set of genetic responses to this important plant hormone.

Exogenous Ethylene Rapidly Induces Gene Expression. Others have shown that prolonged exposure (10–24 hr) to exogenous ethylene induces the expression of developmentally regulated fruit ripening genes (6, 8). We find, however, that ethylene affects gene expression in fruits earlier. As shown in Fig. 5, specific mRNAs rapidly accumulate in ethylene-treated fruit. Within 0.5–2 hr we detect increases in the concentration of four different mRNAs. A similar rapid induction has been observed when soybean hypocotyls were exposed to ethylene (25). Adjusting for the time it takes for ethylene gas to diffuse into fruit [at least 15 min for the internal ethylene concentration to achieve 50% of its final steady-state level (26)], ethylene-induced gene expression is nearly as rapid as auxin-induced gene expression that has been demonstrated in pea epicotyls and soybean hypocotyls (27, 28). These results suggest that plant hormones such as auxin and ethylene are capable of inducing changes in the physiology of plants by rapidly altering patterns of gene expression.

Differential Response of Fruit Ripening Genes to Exogenous Ethylene. As shown in Fig. 7, the expression of each cloned gene is inhibited by norbornadiene, suggesting that ethylene is required for their normal expression during fruit ripening. However, when fruit are exposed to exogenous ethylene for periods of time <8 hr, not all of the cloned genes are expressed (Fig. 5). Specifically, *E4*, *E8*, *E17*, and *J49* gene expression is activated by ethylene while *E41* (polygalacturonase) gene expression is not. The difference in the capacity of MG1 stage tomato fruit to rapidly respond to exogenous ethylene may reflect differences in the mechanism of ethylene-induced gene expression. That is, perhaps relatively few events catalyzed by ethylene result in rapid *E4*, *E8*, *E17*, and *J49* gene expression, whereas a greater number of events following ethylene exposure are required for polygalacturonase gene expression. Accordingly, others have shown (6) that polygalacturonase gene expression is detected only after exposure of fruit to ethylene for several days. Alternatively, ethylene-responsiveness may be a reflection of developmen-

tal state. That is, perhaps exogenous ethylene rapidly induces polygalacturonase gene expression, but only at a later stage of fruit ripening. The effect of exogenous ethylene on gene expression at both earlier and later stages of fruit development deserves further examination.

Function of Ethylene-Inducible Genes During Fruit Ripening. The function of the E4, E8, and J49 gene products is not yet known. Hybridization experiments and DNA sequence analysis (data not shown) indicate that they are not homologous to other ethylene-inducible genes, cellulase (8) or chitinase (29). Recently it has been shown that the exposure of unripe fruits to exogenous ethylene rapidly increases the activity of enzymes that are involved in ethylene production (30, 31), and it is possible that the cloned ethylene-responsive genes encode these enzymes.

As shown in Fig. 4, the *E17* gene is related to a tomato proteinase inhibitor I gene. Comparing the partial E17 DNA sequence to that of the proteinase inhibitor I gene reveals that approximately 67% of the nucleic acids and 50% of the amino acids are identical. Moreover, many of the amino acids that are conserved during the evolution of proteinase inhibitor I genes are present in the *E17* gene. These results suggest that the *E17* and proteinase inhibitor I genes may represent distantly related members of a multigene family. Proteinase inhibitor I is a powerful inhibitor of animal and microbial endopeptidases and is thought to defend plant tissue from insect predators. It is possible that the E17-encoding protein performs a similar function in tomato fruit.

Regulation of Gene Expression by Changes in Ethylene Concentration and by Changes in Sensitivity to Ethylene. Our results suggest that changes in ethylene concentration can activate gene expression. As shown in Fig. 5, exposure of fruits to elevated levels of exogenous ethylene activates *E4, E8, E17,* and *J49* gene expression. Furthermore, the onset of *E4* and *E8* gene expression is approximately coincident with the increase in ethylene evolution rate during fruit ripening (Fig. 6). These results are consistent with the idea that increasing ethylene concentration leads to the onset of gene expression. Similar results have been obtained in studies of the regulation of cellulase gene expression during avocado fruit ripening (8).

Our results also suggest that during development gene expression may be activated by an increased capacity of tissue to respond to low levels of ethylene. As shown in Figs. 6 and 7, E17 and J49 mRNAs accumulate before the increase in ethylene production, and these low levels of ethylene appear to be required for maximal gene expression. These results suggest that during fruit development E17 and J49 gene expression is activated by an increase in sensitivity to basal, low levels of ethylene. This is consistent with physiological evidence that most fruits become more sensitive to ethylene as they mature and approach the onset of ripening (9, 24). Furthermore, it has been shown that other important ethylene-mediated processes such as leaf abscission (32) and flower senescence (33) involve changes in sensitivity to ethylene. Thus, activation of gene expression by changes in sensitivity to ethylene may be an important general phenomenon during plant development.

The interaction between a growth substance and plant tissue has been expressed (34) by the relationship:

$$\text{growth substance} + \text{receptor} \leftrightharpoons \text{active complex} \rightarrow \rightarrow \text{biological response.}$$

In this case the growth substance is ethylene, and the biological response is the activation of gene expression. Depending on whether ethylene or the putative receptor is limiting, the relationship predicts that activation of gene expression may result from increasing ethylene concentration, by a change in receptor concentration, or by a change in receptor activity. It is possible that activation of *E4* and *E8* gene expression results from changes in ethylene concentration, whereas activation of *E17* and *J49* gene expression results from a change in receptor concentration or activity. This information underscores the importance of identifying the cellular factors that regulate ethylene-inducible gene expression during plant development.

This research was supported by National Institutes of Health grant (GM33856). Computer resources used to carry out our studies were provided by the National Institutes of Health-sponsored BIONET National Computer Resource for Molecular Biology.

1. Abeles, F. B. (1973) *Ethylene in Plant Biology* (Academic, New York), pp. 1–302.
2. Lieberman, M. (1979) *Annu. Rev. Plant Physiol.* **30,** 533–591.
3. Lyons, J. M. & Pratt, H. K. (1964) *Proc. Am. Soc. Hortic. Sci.* **84,** 491–500.
4. Rhodes, M. J. C. (1980) in *Senescence in Plants*, ed. Thimann, K. V. (CRC, Boca Raton, FL), pp. 157–205.
5. Biale, J. B. & Young, R. E. (1981) in *Recent Advances in the Biochemistry of Fruit and Vegetables*, eds. Friend, J. & Rhodes, M. J. C. (Academic, London), pp. 1–39.
6. Grierson, D. (1985) *CRC Crit. Rev. Plant Sci.* **3,** 113–132.
7. Su, L.-Y., McKeon, T., Grierson, D., Cantwell, M. & Yang, S. F. (1984) *Hortic. Sci.* **19,** 576–578.
8. Tucker, M. L., Christoffersen, R. E., Woll, L. & Laties, G. G. (1985) in *Ethylene and Plant Development*, eds. Roberts, J. A. & Tucker, G. A. (Butterworths, London), pp. 163–171.
9. McGlasson, W. B., Wade, N. L. & Adato, I. (1978) in *Phytohormones and Related Compounds: A Comprehensive Treatise*, eds. Letham, D. S., Goodwin, P. B. & Higgins, T. J. V. (Elsevier, Amsterdam), Vol. 2, pp. 447–493.
10. McGlasson, W. B. (1985) *Hortic. Sci.* **12,** 315–316.
11. Goldberg, R. B., Hoschek, G., Tam, S. H., Ditta, G. S. & Breidenbach, R. W. (1981) *Dev. Biol.* **83,** 201–217.
12. Davis, M. M., Cohen, D. I., Nielsen, E. A., Steinmetz, M., Paul, W. E. & Hood, L. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2194–2198.
13. Van Ness, J. & Hahn, W. E. (1982) *Nucleic Acids Res.* **10,** 8061–8077.
14. Grunstein, M. & Hogness, D. S. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3961–3965.
15. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
16. Parnes, J. R., Velan, B., Felsenfeld, A., Ramanathan, L., Ferrini, U., Appella, E. & Seidman, J. G. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2253–2257.
17. DellaPenna, D., Alexander, D. C. & Bennett, A. B. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 6420–6424.
18. Sato, T., Kusaba, S., Nakagawa, H. & Ogura, N. (1984) *Plant Cell Physiol.* **25,** 1069–1071.
19. Grierson, D., Tucker, G. A., Keen, J., Ray, J., Bird, C. R. & Schuck, W. (1986) *Nucleic Acids Res.* **14,** 8595–8603.
20. Graham, J. S., Pearce, G., Merryweather, J., Titani, K., Ericsson, L. & Ryan, C. A. (1985) *J. Biol. Chem.* **260,** 6555–6560.
21. Norrander, J., Kemp, T. & Messing, J. (1983) *Gene* **26,** 101–105.
22. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
23. Thomas, P. S. (1983) *Methods Enzymol.* **100,** 255–266.
24. Yang, S. F. (1985) *Hortic. Sci.* **20,** 41–45.
25. Zurfluh, L. L. & Guilfoyle, T. J. (1982) *Plant Physiol.* **69,** 338–340.
26. Cameron, C. C. & Yang, S. F. (1982) *Plant Physiol.* **70,** 21–33.
27. Hagen, G. H., Kleinschmidt, A. & Guilfoyle, T. (1984) *Planta* **162,** 147–153.
28. Theologis, A., Huynh, T. & Davis, R. W. (1985) *J. Mol. Biol.* **183,** 53–68.
29. Broglie, K. E., Gaynor, J. J. & Broglie, R. M. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 6820–6824.
30. Liu, Y., Hoffman, N. E. & Yang, S. F. (1985) *Plant Physiol.* **77,** 407–411.
31. Liu, Y., Su, L.-Y. & Yang, S. F. (1985) *Plant Physiol.* **77,** 891–895.
32. Abeles, F. (1967) *Physiol. Plant.* **20,** 442–454.
33. Kende, H. & Hanson, A. D. (1976) *Plant Physiol.* **57,** 523–527.
34. Trewavas, A. J. (1982) *Physiol. Plant.* **55,** 60–72.