Plant Physiol. (1989) 90, 1372–1377
0032-0889/89/90/1372/06/$01.00/0

Received for publication December 19, 1988
and in revised form March 10, 1989

# Transcriptional Analysis of Polygalacturonase and Other Ripening Associated Genes in Rutgers, *rin*, *nor*, and *Nr* Tomato Fruit[1]

**Dean DellaPenna, James E. Lincoln, Robert L. Fischer, and Alan B. Bennett***

*Mann Laboratory, Department of Vegetable Crops, University of California, Davis, California 95616* (D.D.P., A.B.B.) *and Division of Molecular Plant Biology, 305 Hilgard Hall, University of California, Berkeley, California 94720* (J.E.L., R.L.F.)

## ABSTRACT

**We have studied the transcription of polygalacturonase (PG) and several other ripening-associated genes in wild-type tomato (*Lycopersicon esculentum*) fruit and three ripening-impaired mutants, *rin*, *nor*, and *Nr*. In wild-type fruit, the PG gene becomes transcriptionally active early in ripening and remains transcriptionally active during the ripening process. Fruit of the three ripening-impaired mutants, which have reduced levels of PG mRNA, have correspondingly reduced PG transcription rates. Other ripening-associated genes showed diverse patterns of expression in the ripening-impaired mutant backgrounds. These results indicate that transcriptional activation of the PG gene is an important control point regulating the expression of PG during ripening in wild-type fruit and that PG expression in *rin*, *nor*, and *Nr* fruit is blocked at the level of transcription. A comparison of PG transcription rates and mRNA levels with those of other ripening-associated genes suggests that posttranscriptional processes may also contribute to the large accumulation of PG mRNA during ripening.**

Fruit ripening is a complex, developmentally regulated process resulting from the coordination of numerous biochemical and physiological changes within the fruit tissue (6, 7). In ripening tomato fruit, these changes include increased ethylene biosynthesis, lycopene production, Chl degradation, alterations in acidity and aroma, and softening of the fruit tissue. The softening of tomato fruit during ripening has been the focus of considerable research and a single cell wall enzyme, PG,[2] has been implicated as the primary enzyme mediating this process (for review, see ref. 4). PG activity and immunologically detectable protein increase dramatically during ripening (8), and studies with PG cDNA clones have shown that during this period the steady state levels of PG mRNA increase more than 2000-fold, accounting for as much as 2% of the mRNA mass in ripe fruit (3, 9, 10).

Further studies of gene expression in tomato fruit using *in vitro* translation and RNA blot analysis have shown increases in the steady state levels of other mRNAs during ripening (5, 12–14, 19, 23, 25, 26). Application of exogenous ethylene to mature green tomato fruit, a treatment that hastens the onset of ripening, stimulates the accumulation of some of these ripening-associated mRNAs (19, 24) and also increases the transcription rates of several of the corresponding genes (20). Conversely, exposure of tomato fruit to norbornadiene, an inhibitor of ethylene action and ripening, represses the accumulation of several ripening-associated mRNAs (19). These observations support the hypothesis that alterations in gene expression play an important role in the ripening process.

One advantage in using tomato fruit as a system for studying gene expression during ripening is the existence of several single-gene mutations which impair the ripening process. Normal changes such as lycopene production, increased ethylene production, and the appearance of PG activity are all repressed to varying degrees by the mutations (28). These mutations have been useful tools in comparative studies with normally ripening genotypes (10, 20). We have previously characterized the steady state PG mRNA levels in wild-type and three ripening-impaired mutant genotypes of tomato and found that, in contrast to the high level of PG mRNA expression in wild-type fruit, fruit of the mutant genotypes showed greatly reduced but distinct patterns of PG mRNA accumulation (10). The regulation of PG gene expression during fruit ripening may occur at many levels including gene transcription, mRNA processing and transport, mRNA stability, and translation (11). To extend our understanding of the mechanisms regulating gene expression during tomato fruit ripening we have determined the relative transcription rates and steady state mRNA levels of PG and other ripening-associated genes in wild-type and mutant genotypes.

## MATERIALS AND METHODS

### Plant Materials

Two tomato (*Lycopersicon esculentum*) genotypes were used in our analysis of transcriptional activity of PG. VFNT cherry fruit were used to examine early induction of PG expression since this genotype has been previously characterized with regard to gene expression in the earliest stages of ripening (19). Rutgers fruit were used to examine PG transcription in wild-type and ripening-impaired mutant fruit because isogenic mutants are available in this genetic back-

[1] Supported by U.S. Department of Agriculture Competitive Research Grants Office grant 87-CRCR-2525.

[2] Abbreviations: PG, polygalacturonase (EC 3.2.1.15); MG, mature green; [$^{32}$P]nRNA, $^{32}$P-labeled *in vitro* nuclear RNA; DAPI, 4′,6′-diaminido-2-phenylindole; bp, base pair; kb, kilobase pair.

ground and because PG mRNA levels had been previously characterized in these lines (10).

*L. esculentum* cv VFNT cherry plants were grown as described (19). Immature fruit were 50% full size; MG1 fruit were full size with firm locules; MG2 fruit had a small amount of locule gel present; in MG3 fruit the locule gel was fully formed and a rise in ethylene production was detectable, whereas in MG4 fruit carotenoid pigment development was visible on the fruit interior and a sharp rise in ethylene production was observed.

*L. esculentum* cv Rutgers plants were grown, hand-pollinated, and tagged, and the fruit were harvested as described previously (10). The *rin*, *nor*, and *Nr* mutants were near isogenic (backcrossed to Rutgers 8, 4, and 6 times, respectively) and were obtained from Dr. E. Tigchelaar (Purdue University). Fruit were harvested at the fully developed mature green stage. This stage was determined to be 45 d after pollination based on locular development and ethylene production rates. Forty-five d-old fruit had at least one but not all locules fully gelled, corresponding to a VFNT cherry stage of MG2. Ripe and overripe Rutgers fruit were determined as 55 and 67 d, respectively, based on ethylene production rates and visual inspection. For these later time points, mature green fruit (45 d after flowering) of each genotype were harvested and ripened as described (10), and at the appropriate ages fruit pericarp tissue was harvested, frozen in liquid nitrogen, and stored at −80°C.

### mRNA Isolation and Northern Analysis

Polysomal poly(A)$^+$ RNA was isolated from VFNT cherry fruit pericarp tissue as described elsewhere (19). Poly(A)$^+$ RNA from pericarp tissue of Rutgers, *rin*, *nor*, and *Nr* genotypes was isolated as described (9, 10). Plasmids used for RNA hybridization analysis were pPG1.9, E4, E8, E17, J49, and D21. pPG1.9 contains a 1.7 kb full-length cDNA insert encoding the tomato cell wall enzyme polygalacturonase (3, 4, 10). E4, E8, E17, and J49 are full-length tomato cDNA clones (750, 1600, 600, and 950 bp inserts, respectively) corresponding to mRNAs that are expressed in fruit in response to ethylene (19). D21 is a 650 bp full-length cDNA expressed at relatively constant levels from the immature stage to the 50% ripe stage in VFNT cherry tomatoes.

The full-length inserts of D21, pPG1.9, E8, and E17 were subcloned into a transcription vector (Bluescribe vector, Stratagene, LaJolla, CA) in such a way that sense mRNA could be synthesized using T7 or T3 RNA polymerase *in vitro*. The sense mRNA made from each clone was purified, quantified by UV absorption, and used as a standard on RNA dot blots as described (3, 10). Poly(A)$^+$ mRNA and a dilution series of purified sense mRNA standards were dot-blotted to nitrocellulose (27) and probed with the respective nick-translated plasmid. Prehybridization and hybridization conditions were as described (10). After autoradiography each dot was cut out and the associated radioactivity determined by scintillation counting. This allowed an estimation of the abundance of each mRNA in the various poly(A)$^+$ RNA preparations. Correlation coefficients (r) for RNA standards were greater than 0.98.

### Nuclei Isolation and *in Vitro* [$^{32}$P]nRNA Synthesis

Nuclei from VFNT cherry fruit were isolated, and [$^{32}$P]nRNA synthesis was carried out as described (20). Nuclei from Rutgers, *rin*, *nor*, and *Nr* genotypes were isolated as described (20–22), except that the Percoll gradient sucrose pad was increased from 2.0 to 2.2 M sucrose. [$^{32}$P]nRNA synthesis was carried out for 25 min at 30°C as described by Walling *et al.* (30). Of the [$^{32}$P]UTP incorporating activity, 50 to 85% was found at the 80% Percoll/2.2 M sucrose interface. Nuclei from this interface were used for all experiments. [$^{32}$P]UTP incorporation was inhibited on average 60% in the presence of 2 μg/mL α-amanitin. Transcript sizes ranged from 0.1 to 3.0 kb. Nuclear DNA concentration in isolated nuclei preparations was determined by DAPI fluorescence as described (1) using cesium chloride-purified tomato leaf DNA as a standard.

### [$^{32}$P]nRNA Purification

[$^{32}$P]nRNA was isolated from VFNT cherry fruit nuclei as described (20). [$^{32}$P]nRNA from Rutgers, *rin*, *nor*, and *Nr* nuclei was isolated following published procedures (20, 30) with the following modifications. After phenol extractions, the aqueous phases were pooled, and an equal volume of 10% (w/v) TCA containing 30 mM $NaH_2PO_4$ was added and the solution incubated on ice for 30 min. The precipitated [$^{32}$P]nRNA was collected by vacuum filtration onto a 0.45 μm Millipore filter and washed twice with 5 mL of ice-cold, 5% (w/v) TCA containing 30 mM $NaH_2PO_4$. The filter was then placed into a silanized glass liquid scintillation vial containing 60 μg of RNase-free DNase in 1 mL of 10 mM Tris (pH 7.6), 10 mM $MgCl_2$ and was incubated at 37°C. After 30 min, EDTA and SDS were added to 15 mM and 1% (w/v), respectively, and incubation continued at 65°C. After 10 min the liquid (containing released [$^{32}$P]nRNA) was removed and replaced with 1 mL of 10 mM Tris (pH 7.6), 10 mM EDTA containing 1% SDS and incubated at 65°C. After 10 min the liquid was removed. This process was repeated a total of three times after which the aqueous samples were pooled and the [$^{32}$P]nRNA was ethanol precipitated. The [$^{32}$P]nRNA was then extracted with cetyltrimethylammonium bromide to remove polysaccharides, ethanol precipitated, and used for hybridizations.

### DNA Gel Blotting and [$^{32}$P]nRNA Hybridization

Each plasmid was digested with the appropriate restriction enzyme to liberate the cDNA insert. Five μg of DNA from each digestion were separated by agarose gel electrophoresis and blotted to nitrocellulose. Prehybridization and hybridization were for 24 and 48 h, respectively, as described (20). Each hybridization contained $6 \times 10^7$ cpm of purified [$^{32}$P]nRNA. Under these conditions of DNA excess, the amount of [$^{32}$P]nRNA hybridized reflects the rate of transcription of the respective gene (16, 17). After hybridization and washing, blots were treated with RNase as described (30). After autoradiography the radioactivity associated with each cDNA insert was determined by scintillation counting. Hybridization to vector sequences was undetectable.

## RESULTS

### Run-Off Transcription in Isolated Tomato Fruit Nuclei

The yield of nuclei was similar for all genotypes and ages and, assuming a DNA content of 1.5 pg/nucleus, ranged from $4 \times 10^5$ to $1 \times 10^6$ nuclei per g fresh tissue (Table I). Nuclei prepared as described incorporated [$^{32}$P]UTP into nRNA for up to 90 min, and incorporation was proportional to the number of nuclei in the reaction (results not shown). The nuclei were nearly identical in their ability to incorporate [$^{32}$P]UTP when expressed as a function of DNA content (Table I). These results indicated that nuclei isolated from all developmental stages and all genotypes were transcriptionally active and were comparable to other isolated plant nuclei (2, 15, 18, 20, 21).

### Transcription of the PG Gene in Normal and Ripening-Impaired Mutant Fruit

We have previously shown that during tomato fruit ripening there is a dramatic increase in steady state PG mRNA levels with PG mRNA first becoming detectable early in ripening after a slight but detectable rise in ethylene production by the fruit (9). To determine whether this mRNA increase results from activation of PG gene transcription, we analyzed the relative transcription rates and steady state PG mRNA levels during the early stages of ripening in VFNT cherry fruit, the genotype in which gene expression at the earliest stages of ripening has been well characterized (19). These data, as well as those for the constitutively expressed gene D21, are shown in Figure 1. Immature green fruit were approximately 20 d old, MG1 fruit approximately 35 d old, and the transition from MG1 to MG4 occurs over a period of approximately 3 to 5 d. We did not detect PG gene transcription in immature, MG1, or MG2 fruit. Transcription of the PG gene first became detectable at the MG3 stage, when a rise in ethylene production occurred, and continued to increase in MG4 fruit,

**Table I.** *Yield of Nuclei and [$^{32}$P]UTP Incorporation for the Indicated Tomato Genotypes and Fruit Developmental Stages*

| Genotype | Developmental Stage | Yield of Isolated Nuclei | Incorporation of [$^{32}$P]UTP[a] |
|---|---|---|---|
| | *DAF* | *μg DNA/g tissue* | *CPM/μg DNA × 10$^6$* |
| Rutgers | 45 | 1.05 | 3.8 |
| | 55 | 0.9 | 5.0 |
| | 67 | 1.26 | 2.6 |
| *rin* | 45 | 0.58 | 7.0 |
| | 55 | 0.76 | 3.4 |
| | 67 | 1.36 | 2.0 |
| *nor* | 45 | 0.86 | 3.7 |
| | 55 | 1.56 | 2.0 |
| | 67 | 1.04 | 3.3 |
| *Nr* | 45 | 1.32 | 3.3 |
| | 55 | 1.08 | 2.7 |
| | 67 | 1.22 | 2.2 |

[a] Incorporation analysis was performed in a 20 μL reaction volume containing 40 μCi of [$^{32}$P]UTP (>3000 Ci/mM) using the conditions of Walling *et al.* (30).

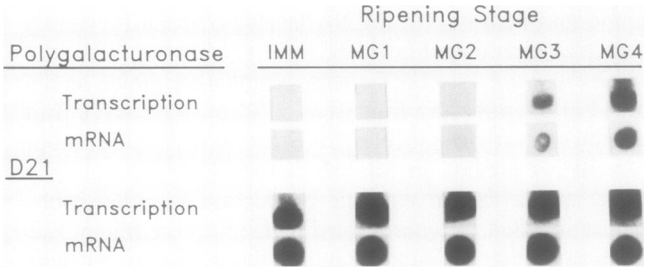


**Figure 1.** Transcription rates and mRNA levels of PG and D21 during the early stages of ripening of VENT cherry tomato fruit. Nuclei were isolated from fruit at the indicated developmental stage and used for *in vitro* transcription reactions. [$^{32}$P]nRNA was isolated and hybridized to DNA gel blots of PG and D21 cDNA inserts. mRNA was isolated from fruit of each stage, dot-blotted, and hybridized with the indicated $^{32}$P-labeled cDNA clone. Transcription autoradiograms were exposed 20 h at −70°C.

a stage when lycopene synthesis first becomes detectable. As shown in Figure 1, the steady state PG mRNA levels closely paralleled this increase in PG transcription. In contrast, both the transcription rate and mRNA level of the constitutively expressed cDNA clone D21 were relatively constant during this period. These results indicate that transcriptional activation of the PG gene is an important factor in the induction of PG gene expression during the early stages of tomato fruit ripening.

PG gene transcription was also studied during later stages of ripening in Rutgers fruit, a genotype in which well characterized isogenic lines of the ripening-impaired mutants are available (Fig. 2). At 45 d after flowering (maturity stage equivalent to MG2 of VFNT cherry) PG gene transcription and mRNA in Rutgers were near background levels. At 55 d the fruit were fully ripe and showed maximal ethylene production rates. PG transcription rates at this time had increased at least 50-fold over their level at 45 d, while PG mRNA levels had increased over 2000-fold. By 67 d Rutgers fruit were overripe and showed decreased ethylene production rates. The PG transcription rate had decreased 90% by this time while PG mRNA levels decreased approximately 80%. D21 transcription levels varied less than 2-fold during the same 3-week period while D21 mRNA levels showed a slow and steady decline. These results suggest that transcriptional processes play an important role in both the initiation (Fig. 1) and maintenance (Fig. 2) of PG expression throughout the ripening process.

We have previously shown that PG mRNA levels in the ripening mutants *rin*, *nor*, and *Nr* are greatly reduced compared to the levels in wild-type fruit of similar age (10). As shown in Figure 2, the relative transcription rates of PG in the mutants were also greatly reduced relative to wild-type levels. PG transcription rates in *rin* and *nor* at 55 d were less than 3% of the corresponding wild-type transcription rates, while PG mRNA levels in these mutants were less than 1% of wild-type levels. *Nr* fruit showed a delayed and attenuated PG transcription rate reaching 20% of the maximal wild-type rate by 67 d. PG mRNA in *Nr* fruit reached 8% of the maximal wild-type level by this time. D21 transcription rates

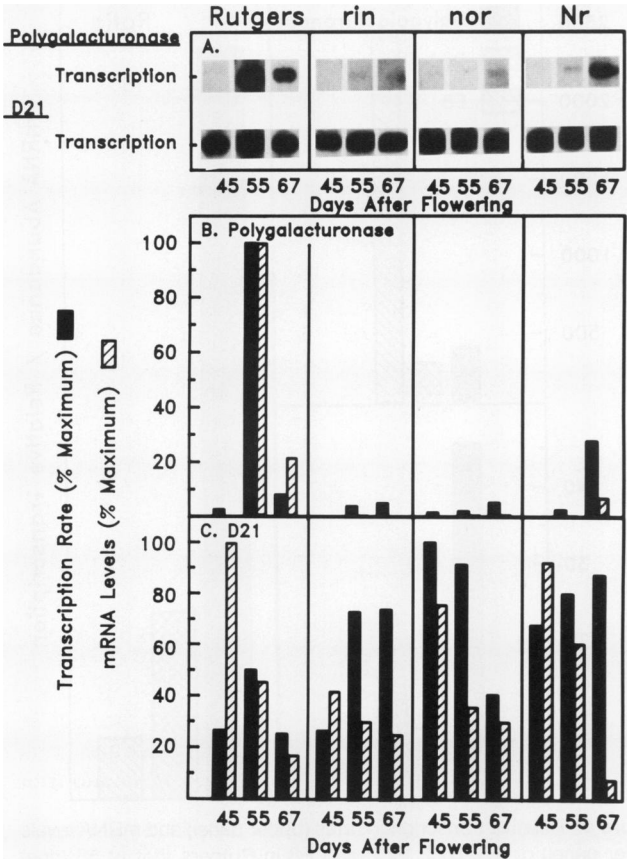


**Figure 2.** Transcription rates and mRNA levels of PG and D21 in Rutgers, *rin*, *nor*, and *Nr* genotypes at 45 (mature green), 55 (ripe), and 67 (overripe) DAF. After autoradiography of transcription (panel A) and RNA blots, the associated radioactivity was determined by scintillation counting and plotted (panel B). Data (after correcting for background) are plotted as a percentage of the maximal cpm hybridized for each clone. Maximum cpm for PG and D21 transcription were 379 and 507 cpm, respectively. Maximum cpm for PG and D21 mRNA were 26,900 and 12,875 cpm/$\mu$g poly(A)$^+$ RNA, respectively. Transcription autoradiograms were exposed 7 d at −70°C, mRNA autoradiograms were exposed 16 h at −70°C.

in mutant fruit were similar to those observed in wild type, varying less than 2-fold, while D21 mRNA levels decreased with age.

### Transcription Rates and mRNA Levels of Other Ripening-Associated Genes in Normal and Mutant Fruit

To extend our understanding of the mechanisms controlling gene-expression during ripening, we analyzed the transcription and mRNA accumulation of several other ripening-associated genes in wild-type and mutant genotypes. The four ripening-associated clones E4, E17, E8, and J49 have been characterized elsewhere (19, 20). Transcriptional analysis of these genes has only been performed during early ripening stages (MG1 through MG4) in wild-type and *rin* fruit (20). The data presented here have extended this analysis to later ripening time points and have been expanded to include wild-type, *rin*, *nor*, and *Nr* genotypes.

The expression of the ripening-associated genes, E4, E17, E8, and J49 was affected differently by each of the ripening mutations (Fig. 3). E4, like PG, showed near complete transcriptional repression in all three ripening-impaired mutants (Fig. 3, panel A). However, E4 was the only gene examined whose expression was affected by the mutations in the same manner as PG.

E8 mRNA levels in wild-type fruit closely paralleled transcription rates indicating that, as with PG, transcriptional

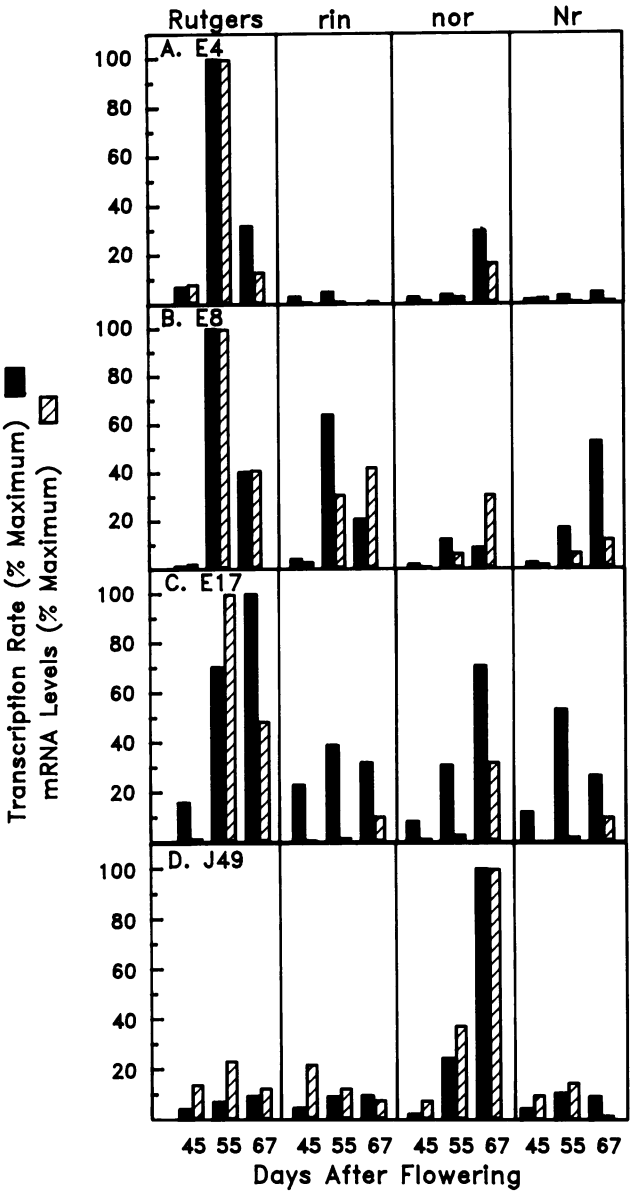


**Figure 3.** Transcription rates and mRNA levels of several ripening-associated genes in normal and ripening-impaired mutant genotypes at 45, 55, and 67 DAF. After autoradiography of transcription and RNA blots (not shown), the associated radioactivity was determined by scintillation counting and plotted. Data (after correcting for background) are plotted as a percentage of maximal cpm hybridized for each clone. Maximum cpm for E4, E8, E17, and J49 transcription were 399, 2383, 75, and 523, respectively. Maximum cpm for E4, E8, E17, and J49 mRNA were 7775, 17305, 596, and 1335, respectively.

regulation is an important control point in the expression of E8 in wild-type fruit. E8 transcription was partially repressed in all three mutants (Fig. 3, panel B). In *rin* and *nor*, E8 transcription, though reduced, followed the same pattern as in wild-type fruit (*i.e.* peaking at 55 d), while in *Nr* E8 transcription steadily increased with age. E8 mRNA continued to accumulate with time in the three ripening-impaired mutants and did not follow transcription rates closely. E8 mRNA continued to accumulate in 67 d *rin* and *nor* fruit despite decreased transcription rates, suggesting that posttranscriptional processes may contribute to determining E8 mRNA levels in 67 d *rin* and *nor* fruit.

Lincoln and Fischer (20) reported that E17 transcription was only slightly reduced by the *rin* mutation whereas E17 mRNA levels were significantly reduced. They concluded that posttranscriptional processes were responsible for the failure of E17 mRNA to accumulate in the *rin* mutant. Our results for E17 expression in 45 and 55 d old *rin* fruit agree with this conclusion. Furthermore, E17 expression in *nor* and *Nr* also appeared to be posttranscriptionally affected, resulting in a failure of E17 mRNA to accumulate in 55 d *nor* and *Nr* fruit, despite transcriptional activation of the gene (Fig. 3, panel C). By 67 d all three mutants had partially overcome this post-transcriptional block in expression and had begun to accumulate E17 mRNA, though at a reduced level relative to wild-type fruit.

The *rin* and *Nr* mutations had little effect on the expression of J49 (Fig. 3, panel D). J49 transcription rates and mRNA levels in *rin* and *Nr* were similar to wild-type levels. In contrast, the *nor* mutation had a dramatic effect on J49 expression at both the transcriptional and steady state mRNA levels. J49 was transcriptionally activated in *nor* fruit, reaching a rate 10-fold higher than maximal wild-type levels with its mRNA accumulating to similarly elevated levels.

### Relationship of Relative Transcription Rates to mRNA Abundance

The relative transcription rates of PG and two other genes in ripe (55 d) Rutgers fruit were analyzed and compared to the abundance of their corresponding mRNAs (Fig. 4). At this developmental time point the PG mRNA level was 6-fold greater than that of D21, yet the transcription rates of the PG and D21 genes were approximately equal. Comparison of PG and E8 mRNA levels and transcription rates is even more striking. The PG mRNA level was 3.5-fold higher than the E8 mRNA level despite the transcription rate of the E8 gene being over 6-fold greater than that of the PG gene (Fig. 4). The relationship between mRNA levels and the relative transcription rates of each gene can most readily be seen by calculating the ratio of the mRNA levels to the relative transcription rate (Fig. 4). The high PG mRNA level, relative to its moderate transcription rate, suggests that posttranscriptional processes (29), such as mRNA stability or cytoplasmic entry rates, also contribute to the relatively high accumulation of PG mRNA during tomato fruit ripening.

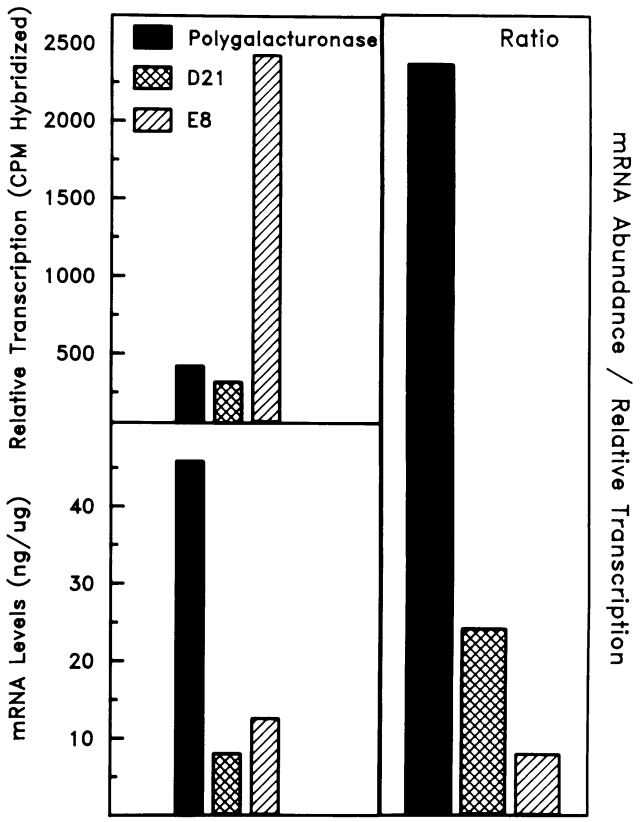


**Figure 4.** Relative transcription rates (upper panel) and mRNA levels (lower panel) of PG, D21, E17, and E8 in Rutgers fruit at 55 (ripe) DAF. The percentage of [$^{32}$P] nuclear RNA corresponding to each gene was calculated as [(cpm hybridized) (*R*) (100)]/[(Input cpm) (*H*)] where *R* is the ratio of mRNA to plasmid insert size and *H* is the hybridization efficiency, assumed to be 0.2 (Walling *et al.*, 1986). mRNA levels were determined by reference to *in vitro* transcribed mRNA as described in "Materials and Methods."

## DISCUSSION

We have previously shown (9) that during ripening of wild-type tomato fruit PG mRNA first becomes detectable after a small rise in ethylene production and continues to accumulate to high levels in ripe fruit. Polygalacturonase activity and immunologically detectable protein closely parallel this rise in PG mRNA indicating that PG expression during ripening is primarily regulated by the level of PG mRNA rather than by a translational or posttranslational mechanism. To determine the role that transcription plays in controlling this dramatic increase in PG mRNA during normal fruit ripening, we have analyzed transcription of the PG gene in nuclei isolated from wild-type fruit of various ripening stages. We have shown that the PG gene is transcriptionally inactive in immature, MG1, and MG2 fruit and becomes transcriptionally activate in MG3 fruit, the stage at which ethylene production by the fruit increases. Transcription of the PG gene increases by the MG4 stage to 20% of the transcription rate of D21 (Fig. 1) and continues to increase at later stages in ripening to a rate approximately equal to that of D21 (Fig. 2, panel B, 55 d Rutgers). The transcription rate of D21 remains relatively constant during this time. The kinetics and magni-

tude of the changes in PG mRNA accumulation during ripening parallel the changes in transcriptional activity of the PG gene, indicating that transcriptional control plays an important role in both the initiation (Fig. 1) and maintenance (Fig. 2) of PG expression during ripening in wild-type fruit.

In the ripening mutants *rin*, *nor*, and *Nr*, PG steady state mRNA levels are greatly reduced relative to wild-type (10). The greatest reduction is observed in *rin* and *nor* (approximately 100-fold less than wild-type) while *Nr* fruit eventually accumulate PG mRNA to 10 to 20% of wild-type levels (see Fig. 2). We have shown here that the rate of PG gene transcription in all three mutant genotypes is greatly reduced relative to wild-type fruit of the same age. *Rin* and *nor* fruit show the most severe reduction in PG gene transcription while *Nr* fruit show a delayed activation and reduced rate of PG gene transcription relative to wild type. The steady state PG mRNA levels in the ripening mutants parallel PG transcription rates, providing further evidence for the importance of transcriptional control in the regulation of PG mRNA levels during ripening. While transcriptional activation of the PG gene is apparently important in determining the developmental timing of PG expression, the high level of PG mRNA (2–4% of the mRNA mass) relative to its modest transcription rate suggests that posttranscriptional processes may also contribute to the high accumulation of PG mRNA in ripening fruit.

The transcription of other ripening-associated genes in the mutant genotypes did not, in general, parallel that of PG. This indicates that the mutations do not generally repress transcription of ripening-associated genes, but rather, they affect the transcriptional activity of these genes in diverse manners. In addition, the expression of some ripening-associated genes in the mutant genotypes was also affected at the posttranscriptional level. Overall the results presented here suggest that multiple mechanisms operate to regulate gene expression during ripening in wild-type fruit and that the three ripening mutations, *rin*, *nor*, and *Nr* exert distinct effects on these mechanisms.

## ACKNOWLEDGMENT

We would like to thank Ms. Betty Rumbaugh for preparation of the manuscript.

## LITERATURE CITED

1. **Baer GR, Meyers SP, Molin WT, Schrader LE** (1982) A simple and sensitive DNA assay for plant extracts. Plant Physiol **70:** 999–1003
2. **Beach LR, Spencer D, Randall PJ, Higgins TJV** (1985) Transcriptional and post-transcriptional regulation of storage protein gene expression in sulfur-deficient pea seeds. Nucleic Acids Res **13:** 999–1013
3. **Bennett AB, DellaPenna D** (1987) Polygalacturonase gene expression in ripening tomato fruit. *In* D Nevins, R Jones, eds, Tomato Biotechnology. Alan R Liss, New York, pp 299–308
4. **Bennett AB, DellaPenna D** (1987) Polygalacturonase: Its importance and regulation in ripening. *In* WW Thompson, E Nothnagle, R Huffaker, eds, Plant Senescence: Its Biochemistry and Physiology. American Society of Plant Physiologists, Rockville, MD pp 98–107
5. **Biggs MS, Harriman RW, Handa AK** (1986) Changes in gene expression during tomato fruit ripening. Plant Physiol **81:** 395–403
6. **Brady CJ** (1987) Fruit ripening. Annu Rev Plant Physiol **38:** 155–178
7. **Brady CJ, McGlasson B, Spiers J** (1987) The biochemistry of fruit ripening. *In* D Nevins, R Jones, eds, Tomato Biotechnology. Alan R Liss, New York, pp 279–288
8. **Brady CJ, MacAlpine G, McGlasson WB, Ueda Y** (1982) Polygalacturonase in tomato fruits and the induction of ripening. Aust J Plant Physiol **9:** 171–178
9. **DellaPenna D, Alexander D, Bennett AB** (1986) Molecular cloning of tomato fruit polygalacturonase mRNA levels during ripening. Proc Natl Acad Sci USA **83:** 6420–6424
10. **DellaPenna D, Kates DS, Bennett AB** (1987) Polygalacturonase gene expression in Rutgers, *rin*, *nor*, and *Nr*. Plant Physiol **85:** 502–507
11. **Darnell JE** (1982) Variety in the level of gene control in eukaryotic cells. Nature **297:** 365–371
12. **Grierson D** (1985) Gene expression in ripening tomato fruit. CRC Crit Rev Plant Sci **3:** 113–132
13. **Grierson D, Slater A, Speirs J, Tucker GA** (1985) The appearance of polygalacturonase mRNA in tomatoes: one of a series of changes in gene expression during development and ripening. Planta **163:** 263–271
14. **Grierson D, Maunders MJ, Holdsworth MJ, Ray J, Bird C, Moureau P, Schuch W, Slater A, Knapp JE** (1987) Expression and function of ripening genes. *In* D Nevins, R Jones, eds, Tomato Biotechnology. Alan R Liss, New York, pp 309–323
15. **Hagen G, Guilfoyle TJ** (1985) Rapid induction of selective transcription by auxins. Mol Cell Biol **5:** 1197–1203
16. **Hofer E, Darnell JE** (1981) The primary transcription unit of the mouse Beta-major globin gene. Cell **23:** 585–593
17. **Hofer E, Hofer-Warbinek J, Darnell JE** (1982) Globin RNA transcription: a possible termination site and demonstration of transcriptional control correlated with altered chromatin structure. Cell **29:** 887–893
18. **Lawton MA, Lamb CJ** (1987) Transcriptional activation of plant defense genes by fungal elicitor, wounding and infection. Mol Cell Biol **7:** 335–341
19. **Lincoln JE, Cordes S, Read E, Fischer RL** (1987) Regulation of gene expression by ethylene during *Lycopersicon esculentum* (tomato) fruit development. Proc Natl Acad Sci USA **84:** 2793–2797
20. **Lincoln JE, Fischer RL** (1988) Diverse mechanisms for the regulation of ethylene-inducible gene expression. Mol Gen Genet **212:** 71–75
21. **Luthe DS, Quatrano RS** (1980) Transcription in isolated wheat nuclei I. Isolation of nuclei and elimination of endogenous ribonuclease activity. Plant Physiol **65:** 305–308
22. **Luthe DS, Quatrano RS** (1980) Transcription in isolated wheat nuclei II. Characterization of RNA synthesized *in vitro*. Plant Physiol **65:** 309–313
23. **Mannson PE, Hsu D, Stalker D** (1985) Characterization of fruit specific cDNAs from tomato. Mol Gen Genet **200:** 356–361
24. **Maunders MJ, Holdsworth MJ, Slater A, Knapp JE, Bird CR, Schuch W, Grierson D** (1987) Ethylene stimulates the accumulation of ripening-related mRNAs in tomatoes. Plant Cell Environ **10:** 177–184
25. **Slater A, Maunders MJ, Edwards K, Schuch W, Grierson D** (1985) Isolation and characterization of DNA clones for tomato polygalacturonase and other ripening-related proteins. Plant Mol Biol **5:** 137–147
26. **Speirs J, Brady CJ, Grierson D, Lee E** (1984) Changes in ribosome organization and mRNA abundance in ripening tomato fruit. Aust J Plant Physiol **11:** 225–233
27. **Thomas PS** (1983) Hybridization of denatured RNA transferred or dotted to nitrocellulose paper. Methods Enzymol **100:** 255–266
28. **Tigchelaar EC, McGlasson WB, Buescher RW** (1978) Genetic regulation of tomato fruit ripening. HortScience **13:** 508–513
29. **Tobin A** (1979) Evaluating the contribution of posttranscriptional processing to differential gene expression. Dev Biol **68:** 47–58
30. **Walling L, Drews GN, Goldberg RB** (1986) Transcriptional and post-transcriptional regulation of soybean seed protein mRNA levels. Proc Natl Acad Sci USA **83:** 2123–2127