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Polygalacturonase Gene Expression in Rutgers, *rin*, *nor*, and *Nr* Tomato Fruits[1]

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ABSTRACT

Polygalacturonase (PG) gene expression was studied in normally ripening tomato fruit (*Lycopersicon esculentum* Mill, cv Rutgers) and in three ripening-impaired mutants, *rin*, *nor*, and *Nr*. Normal and mutant fruit of identical chronological age were analyzed at 41, 49, and 62 days after pollination. These stages corresponded to mature-green, ripe, and overripe, respectively, for Rutgers. The amount of PG mRNA in Rutgers was highest at 49 days and accounted for 2.3% of the total mRNA mass but at 62 days had decreased to 0.004% of the total mRNA mass. In *Nr*, the amount of PG mRNA steadily increased between 41 and 62 days after pollination, reaching a maximum level of 0.5% of the total mRNA mass. The mutant *nor* exhibited barely detectable levels of PG mRNA at all stages tested. Surprisingly, PG mRNA, comprising approximately 0.06% of the mRNA mass, was detected in 49 day *rin* fruit. This mRNA accumulation occurred in the absence of elevated ethylene production by the fruit and resulted in the synthesis of enzymically active PG I. The different patterns of PG mRNA accumulation in the three mutants suggests that distinct molecular mechanisms contribute to reduced PG expression in each ripening-impaired mutant.

The ripening of tomato fruit is characterized by a series of coordinated biochemical and physiological changes which collectively contribute to the final texture, color, and flavor of the ripe fruit. The softening of tomato fruit during ripening has been studied extensively during the past 20 years and a large body of evidence has accumulated which suggests the cell wall enzyme PG[2] plays a major role in this process. Several lines of evidence which support this include: (a) a rough correlation between levels of extractable PG activity and the rate of softening for a number of tomato cultivars (6, 13); (b) a lack of correlation between the activity of other cell wall enzymes (*e.g.* cellulase and β-1,3-glucanase) and the rate of fruit softening (14, 29); (c) similarities between *in vivo* cell wall degradation products during ripening and *in vitro* cell wall products released by the action of purified PG on isolated fruit cell walls (15, 30); and (d) the demonstration that slow ripening cultivars and nonripening tomato mutants are deficient in PG activity (6, 22, 28). Although these lines of evidence suggest a major role for PG in softening, other factors such as the levels of free and bound Ca^{2+} in the cell wall and substrate accessibility may also be important (7).

Recently, several groups have studied changes in gene expression which occur during tomato fruit ripening (4, 11, 17, 20, 24, 26). PG is an obvious candidate for such studies because the protein is absent in green fruit and has been shown to be synthesized *de novo* during ripening (5, 9). Using PG cDNA clones, we have shown that there is a 2000-fold increase in PG mRNA levels during ripening (9) indicating that developmental regulation of PG gene expression is instrumental for its role in ripening. Recent studies analyzing *in vitro* translation products of RNA isolated from fruit of various ripening stages (4, 11, 26) and others employing differentially hybridizing cDNA clones (17, 20, 25) have identified numerous mRNAs, encoding proteins of unknown function, which also appear to be developmentally regulated during ripening. These studies lend further support to the hypothesis that ripening is regulated, at least in part, by the developmental regulation of gene expression. The long-term goal of our research is to understand the mechanisms which initiate and control this phenomenon.

In contrast to the sequence of events observed in normally ripening fruits there exist three well characterized, genetically distinct, ripening mutations *rin*, *nor*, and *Nr*, which show pleiotropic effects on the ripening process (28). Normal ripening processes such as Chl degradation, carotenoid biosynthesis, increased respiration, increased ethylene production, and the appearance of PG activity are all negatively affected to varying degrees in the three mutants (28). The recessive mutations *rin* and *nor* virtually inhibit these changes while the dominant mutation *Nr* retards and attenuates these changes. Other aspects of growth and development of the parent plant and fruit seem to be unaffected by the mutations. With so many physiological processes of ripening affected by these mutations, it is likely that the normal expression of developmentally regulated ripening genes may also be affected, a hypothesis readily tested with molecular probes we have recently developed (2, 9). These mutants have historically been important tools in comparative biochemical and physiological studies with normal ripening genotypes and it is our hope that they will prove to be equally useful in elucidating the molecular mechanisms governing gene expression during ripening. As an initial step towards this goal we have characterized PG expression in the three ripening-impaired mutants in relation to that observed in normally ripening fruit. Preliminary results have been presented previously (3).

MATERIALS AND METHODS

Plant Material. Tomato plants (*Lycopersicon esculentum* Mill, cv Rutgers) were grown in pots in a lathhouse during the summer of 1986. The *rin*, *nor*, and *Nr* mutants were near isogenic (backcrossed 4, 4, and 6 times to Rutgers, respectively). Seed of the mutant lines and background line Rutgers were the generous gift of Dr. E. C. Tigchelaar (Purdue University). Flowers of each genotype were hand-pollinated, tagged, and harvested at the fully developed mature-green stage, empirically determined as 41 d after pollination based on ethylene production rates and locular

[1] Supported by gifts from Chesebrough-Ponds, Beatrice/Hunt Wesson, and the Campbell Institute for Research and Technology.

[2] Abbreviations: PG, polygalacturonase (EC 3,2,1.15); PG 1 and PG 2 are high and low mol wt isozymes, respectively; *rin*, *nor*, and *Nr*, ripening inhibitor, nonripening, and never ripe tomato mutants.

development (27). Ripe and overripe stages for Rutgers fruit were empirically determined as 49 and 62 d, respectively, based on visual inspection and ethylene production rates. For these later time points, harvested mature-green fruit of each genotype were held at 20°C in a chamber ventilated with ethylene free air. At the appropriate age (49 and 62 d after pollination), fruit were sectioned, locular contents removed, and the pericarp tissue frozen in liquid N_2. The tissue was stored at −80°C until use.

Protein Extraction and Enzyme Assays. Soluble and cell wall protein extracts were prepared as follows. Thirty g of frozen pericarp tissue was homogenized in 40 ml of extraction buffer (17 mM Tris [pH 10.0] containing 5 mM β-mercaptoethanol) and centrifuged for 15 min at 15,000*g*. The resulting supernatant (containing soluble protein) was then filtered through Miracloth, dialyzed against 4 L of 100 mM NaCl, 1 mM DTT, 10 mM sodium acetate (pH 6.0), and frozen. The cell debris pellet was resuspended in 6 ml of 1.7 M NaCl, 15 mM EDTA, 50 mM sodium citrate (pH 5.5) with shaking at 4°C. After 1 h the slurry was centrifuged at 15,000*g* for 20 min and the supernatant (containing cell wall protein) was filtered through Miracloth and brought to 80% saturation with ammonium sulfate. The precipitated proteins were collected by centrifugation at 15,000*g* for 15 min, dissolved in 100 mM NaCl, 1 mM DTT, 10 mM sodium acetate (pH 6.0), then dialyzed overnight against 4 L of the same buffer and frozen at −20°C until later use. Protein was measured by the method of Lowry *et al.* (19). The assay mixture for PG activity was as described (1) except that the final concentration of polygalacturonic acid in the reaction mixture was 0.075% (w/v) and 150 mM NaCl was substituted for 150 mM NH_4Cl. Reducing sugars were measured by the arsenomolybdate method (21) using α-D-galacturonic acid as a standard.

Gel Electrophoresis and Western Blotting. The cationic system of Reisfeld *et al.* (23) was used with 10% polyacrylamide gels for nondenaturing activity gels. PG activity in these gels was detected with ruthenium red (18). For denaturing gels, protein extracts were separated in 10% polyacrylamide SDS gels (16) and silver-stained or blotted to nitrocellulose for Western analysis. Preparation of PG antibodies and biotinylated mol wt standards, and electrophoretic blotting and immunological detection methods were as described (8, 9).

RNA Isolation and Northern Analysis. Total RNA from 41, 49, and 62 d old fruit of all four genotypes was isolated and poly(A)$^+$ RNA selected as described (9). Poly(A)$^+$ RNA was either size-fractionated in a 1.2% agarose gel containing formaldehyde and blotted to nitrocellulose, or dot blotted directly to nitrocellulose. Purified PG mRNA, for use in estimating the abundance of PG mRNA in fruit poly(A)$^+$ RNA extracts, was produced using T7 RNA polymerase and a transcription vector following the instructions supplied by the manufacturer (Bluescribe vector, Stratagene, LaJolla, CA). Briefly, the full-length PG cDNA insert of pPG 1.9 (isolated and characterized elsewhere [2]) was subcloned into Bluescribe in such a way that sense PG mRNA was transcribed by T7 RNA polymerase. The transcribed PG mRNA was quantified by UV absorption and used as a standard on dot blots. Known amounts of transcribed PG mRNA and 1 μg of the various tomato poly(A)$^+$ RNAs were dot blotted onto the same nitrocellulose filter and probed with the nick-translated insert of pPG 1.9. For analysis of D21 mRNA expression, identical poly(A)$^+$ RNA dot blots were probed with the nick-translated insert of the tomato fruit cDNA clone D21. The cDNA clone D21 is derived from a constitutively expressed mRNA and was generously supplied by Dr. R. Fischer (University of California, Berkeley). Prehybridization, hybridization, and washing conditions for Northern blots were as described elsewhere (9). After autoradiography each dot was cut out and the associated radioactivity was determined by scintillation counting. Correlation coefficients (r) for transcribed polygalacturonase mRNA standards were consistently greater than 0.995.

Genomic DNA Analysis. Tomato genomic DNA was isolated by $CsCl_2$ centrifugation (10). Tomato DNA (10 μg) from each genotype was digested to completion with Eco RI, Bam HI, or Hind III, electrophoresed in a 0.8% agarose gel, blotted to nitrocellulose, and probed with the nick-translated insert of pPG 1.9. Prehybridization, hybridization, and washing conditions were as described (9) except that the final wash was at 60°C in 0.015 M NaCl, 0.1% SDS, 0.0015 M trisodium citrate (pH 7.0).

RESULTS

Genomic Southern Analysis. All four genotypes contain genomic sequences which hybridize to pPG 1.9 (Fig. 1). The banding patterns of Rutgers and *rin* are identical with all three restriction enzymes indicating that at this level of analysis the *rin* gene(s) appears to be structurally intact and indeed indistinguishable from Rutgers. The mutants *nor* and *Nr* also contain the normal Rutgers bands but in addition contain several other unique bands which hybridize with pPG 1.9. With this type of analysis it is impossible to determine whether these restriction length polymorphisms represent alterations in gene structure or additional polygalacturonase genes.

Quantification of PG mRNA. Because it appears that all four genotypes contain genomic sequences hybridizing to our PG cDNA clone we proceeded to analyze the steady state PG mRNA levels in each genotype during ripening. To quantify the amount of PG mRNA in each sample, pure PG mRNA was obtained by *in vitro* transcription, quantified by UV absorption, dot blotted to nitrocellulose, hybridized with the ^{32}P-labeled insert of pPG 1.9 and analyzed by autoradiography (Fig. 2, upper panel). The radioactivity hybridized to each dot was determined and used to construct a standard curve (Fig. 2, lower panel). In subsequent experiments (*i.e.* Fig. 3) pure PG mRNA was dot blotted on the same nitrocellulose filter as poly(A)$^+$ RNA samples and used as an internal standard to quantify the amount of PG mRNA in each poly(A)$^+$ RNA sample.

Polygalacturonase mRNA in Rutgers Fruit. Based on ethylene production rates and visual scoring, the time points of 41, 49,

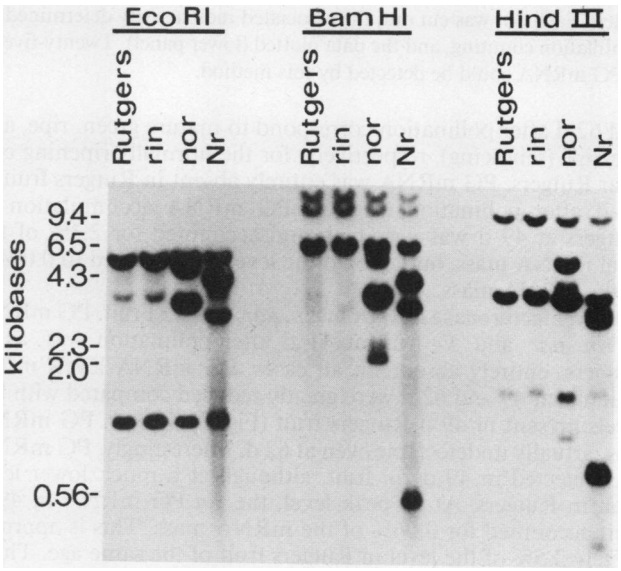

FIG. 1. Genomic Southern analysis of restriction endonuclease digested tomato DNA from Rutgers, *rin*, *nor*, and *Nr* genotypes. Tomato DNA (10 μg) was digested to completion with Eco RI, Bam HI, or Hind III, electrophoresed in a 0.8% agarose gel, blotted to nitrocellulose, and probed with the ^{32}P-labeled cDNA insert of pPG 1.9. The autoradiogram was exposed 48 h at −70°C.

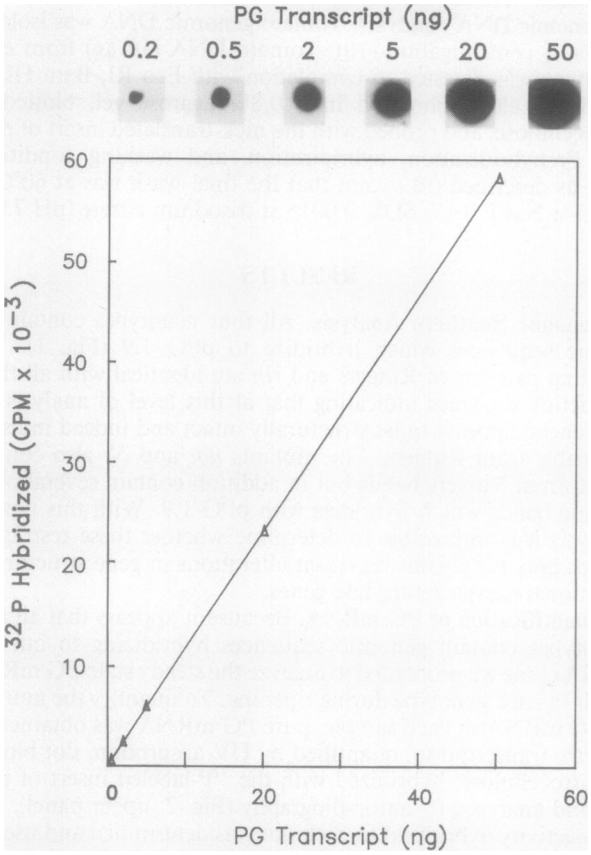

FIG. 2. Autoradiogram and standard curve of T7 transcribed PG mRNA used to quantify the relative levels of PG mRNA in Rutgers, *rin*, *nor*, and *Nr* fruit. The full length 1.9 kb cDNA insert of pPG 1.9 was subcloned into a transcription vector and the resulting plasmid (pBS 1.9) was used as a template to transcribe pure PG mRNA. After UV quantitation, pure transcribed PG mRNA was dot blotted to nitrocellulose, probed with the ^{32}P-labeled pPG 1.9 insert, and autoradiographed (upper panel). Each dot was cut out, the associated radioactivity determined by scintillation counting, and the data plotted (lower panel). Twenty-five pg of PG mRNA could be detected by this method.

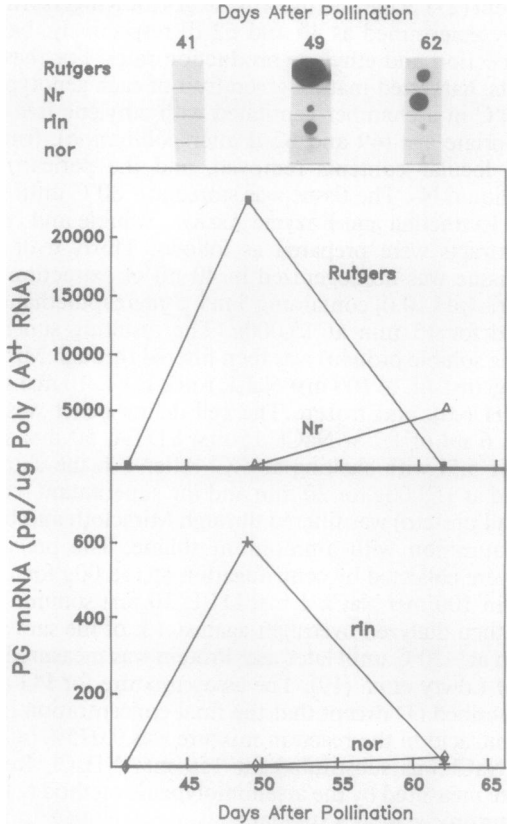

FIG. 3. Dot blot analysis of steady state PG mRNA levels in Rutgers, *rin*, *nor*, and *Nr* fruit at various developmental ages. One μg of poly(A)$^+$ RNA from each genotype at the indicated developmental age was dot blotted to nitrocellulose. Known amounts of transcribed PG sense mRNA were also dot blotted on the same filter. The filter was hybridized to the ^{32}P-labeled insert of pPG 1.9 and exposed to x-ray film. An autoradiogram is shown in the upper panel. Each dot was cut out and radioactivity determined by scintillation counting. The absolute amount of PG mRNA present in each sample was determined using the transcribed PG sense mRNA as an internal standard (lower panels). Note that the vertical scales of the two panels are not the same. The autoradioagram shown was exposed 16 h at −70°C.

and 62 d after pollination correspond to mature green, ripe, and overripe (senescing), respectively, for the normally ripening cultivar Rutgers. PG mRNA was entirely absent in Rutgers fruit at 41 d after pollination (Fig. 3). PG mRNA accumulation in Rutgers at 49 d was very high and accounted for 2.3% of the total mRNA mass, but by 62 d the level had dropped to 0.004% of the mRNA mass.

Polygalacturonase mRNA in *rin*, *nor*, and *Nr* Fruit. PG mRNA in *rin*, *nor*, and *Nr* fruit at 41 d after pollination was, as in Rutgers, entirely absent. In all cases, PG mRNA levels in the mutants at 49 and 62 d were greatly reduced compared with the levels present in 49 d Rutgers fruit (Fig. 3). In *nor*, PG mRNA was virtually undetectable even at 62 d. Interestingly, PG mRNA was detected in 49 d *rin* fruit, although at a much lower level than in Rutgers. At its peak level, the *rin* PG mRNA in 49 d fruit accounted for 0.06% of the mRNA mass. This is approximately 2.5% of the level in Rutgers fruit of the same age. Three separate RNA isolations of fruit from different plants confirmed this result. By 62 d the amount of PG mRNA in *rin* fruit had decreased below the level of detection. PG mRNA in *Nr* fruit was detected at both 49 and 62 d and appeared to be progressively accumulating, though at a much slower rate than in Rutgers. At 62 d the level of PG mRNA in *Nr* was 22% of the maximal level seen in Rutgers and accounted for 0.5% of the total mRNA mass. As shown in Figure 4 the PG mRNA produced in both *rin* and *Nr* is identical in size to that produced by Rutgers fruit.

Analysis of D21 mRNA in Normal and Mutant Fruit. The cDNA clone D21 hybridizes to a message that is constitutively expressed from early in fruit development to the 50% red stage in the normally ripening cultivar VFNT cherry (R. Fischer, unpublished results). We used this cDNA clone as a hybridization probe to test whether constitutive mRNA levels might also be affected by the nonripening mutations. In the four genotypes we examined, the amount of D21 mRNA decreased during ripening (Fig. 5). All four genotypes showed a near identical pattern of D21 mRNA levels at 41 and 49 d with the 49 d values in all cases being approximately 50% of the 41 d values (Fig. 5). It was not until 62 d that differences between the genotypes were observed. The D21 mRNA level of *nor* remained essentially constant between 49 and 62 d while the 62 d levels of D21 mRNA in *rin*, *Nr*, and Rutgers had decreased 2.5-, 4-, and 27-fold from their 49 d levels, respectively.

Total Protein Changes in Normal and Mutant Fruit. Soluble proteins (Fig. 6) and cell wall bound proteins (Fig. 7) were extracted from all four genotypes at 41 and 49 d and subjected to SDS-PAGE. Protein profiles of 41 d wall and soluble proteins for the four genotypes were very similar. At 49 d, differences in

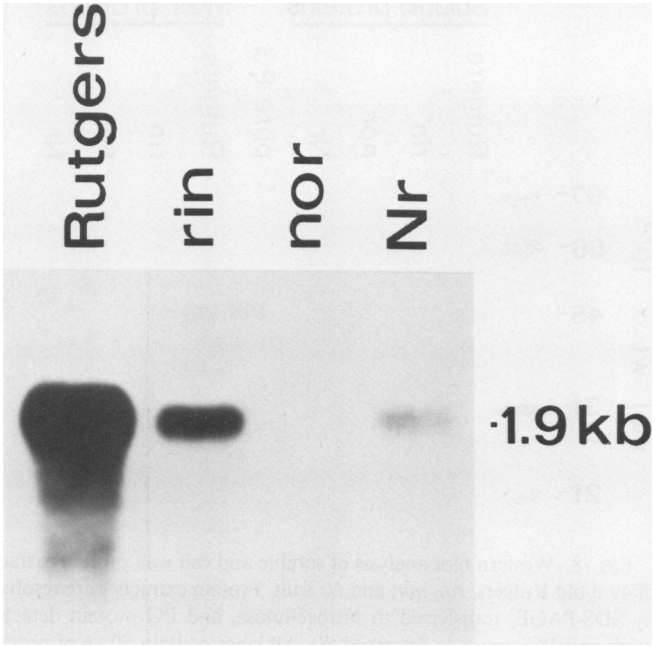

FIG. 4. Northern blot analysis of the size of PG mRNA in normal and mutant tomato fruit. Poly(A)$^+$ RNA from 49 d old fruit of Rutgers (1 μg), *rin* (5 μg), *nor* (5 μg), and *Nr* (5 μg) was resolved in a 1.2% agarose-formaldehyde gel, blotted to nitrocellulose and probed with the ^{32}P-labeled insert of pPG 1.9. The autoradiogram shown was exposed 2 h at −70°C.

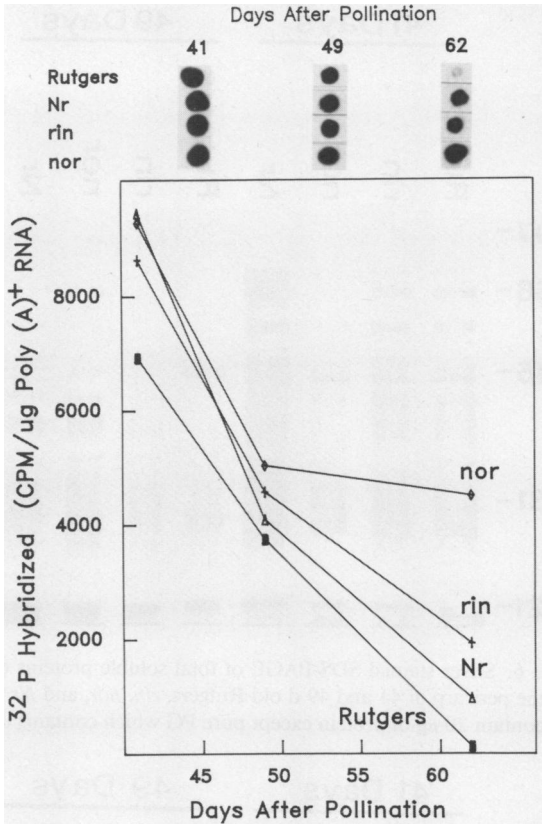

FIG. 5. Dot blot analysis of D21 levels in Rutgers, *rin*, *nor*, and *Nr* fruit of various developmental ages. One μg of poly(A)$^+$ RNA from each genotype at the indicated developmental age was dot blotted to nitrocellulose and hybridized with the ^{32}P-labeled insert of the cDNA clone D21. The autoradiogram shown in the upper panel was exposed 20 h. After autoradiography each dot was cut out, the associated radioactivity determined by scintillation counting, and plotted as a function of fruit age (lower panel).

protein profiles of the four genotypes became apparent. The most obvious difference between Rutgers 49 d cell wall extracts and those of the three mutants is the apparent lack of a prominent 45 kD band (corresponding to polygalacturonase). Upon closer inspection of the 49 d cell wall protein profiles other less prominent differences between Rutgers and the mutants become apparent. Similar observations have been made and cataloged in detail elsewhere (4).

Immunological and Enzymic Analysis of PG. Because we had detected the presence of PG mRNA in 49 d old *rin* fruit it was of interest to determine if it resulted in production of PG protein and if so, whether this protein was normal with respect to the protein present in Rutgers. Even though silver-staining showed little, if any, protein in mutant cell wall extracts which co-migrated with purified PG (Fig. 7), we chose to use more sensitive immunological and enzymic assays to identify the presence of PG. As shown on the immunoblot in Figure 8, cell wall proteins isolated from 49 d old *rin* fruit contain a polypeptide of 45 kD that co-migrates with purified PG and is recognized by PG antiserum. Although the level of this protein in 49 d *rin* fruit is greatly reduced compared with Rutgers fruit of the same age, the size and immunological reactivity is consistent with normal polygalacturonase protein. The mutants *nor* and *Nr* do not contain immunologically detectable levels of PG protein in 49 d cell wall extracts (Fig. 8). The three mutants also do not contain immunologically detectable PG protein in their soluble protein extracts. This suggests that the lack of PG is probably not the result of a failure to secrete the enzyme. Rutgers extracts do contain an immunologically reactive protein in the soluble extract but this is most likely released from the cell wall during the extraction process (1). Enzymic analysis of 49 d cell wall protein extracts show a high level of PG activity in Rutgers, a reduced level in 49 d *rin*, and a lack of activity in 49 d *nor* and *Nr* (data not shown). Analysis of 49 d wall proteins in nondenaturing gels (Fig. 9) showed that *rin* fruit did produce enzymically active PG which comigrated with PG I. PG 2A and 2B were not present in 49 d *rin* wall extracts but accounted for the bulk of activity in 49 d Rutgers wall extracts. No activity was observed in 49 d wall extracts of *nor* or *Nr* fruit.

DISCUSSION

We have characterized the expression of polygalacturonase mRNA and protein in normal and mutant fruit of the same chronological age. The ages selected for analysis (41, 49, and 62 d) were chosen to reflect three very different physiological states in the normal ripening cultivar Rutgers (mature green, red ripe, and overripe, respectively). Although 49 and 62 d mutant fruit do not attain red ripe or overripe stages, for the purpose of comparing mutant gene expression with normal ripening gene expression, we chose to analyze fruits of identical chronological age.

Rutgers, *rin*, *nor*, and *Nr* fruit at 41 d after pollination were nearly identical with respect to size, color, morphology, and locular development. This similarity was further supported by: (a) a lack of PG mRNA for all four genotypes (Fig. 3); (b) similar levels of the constitutively expressed mRNA D21 (Fig. 5); and (c) a high degree of similarity in both soluble and wall protein profiles from all four genotypes (Figs. 6 and 7). The overall pattern observed for protein and mRNA levels at 41 days is consistent with the observation that the mutations have little or no effect on growth and development during preripening stages, but rather exert their influence during the period that normal fruit undergo ripening.

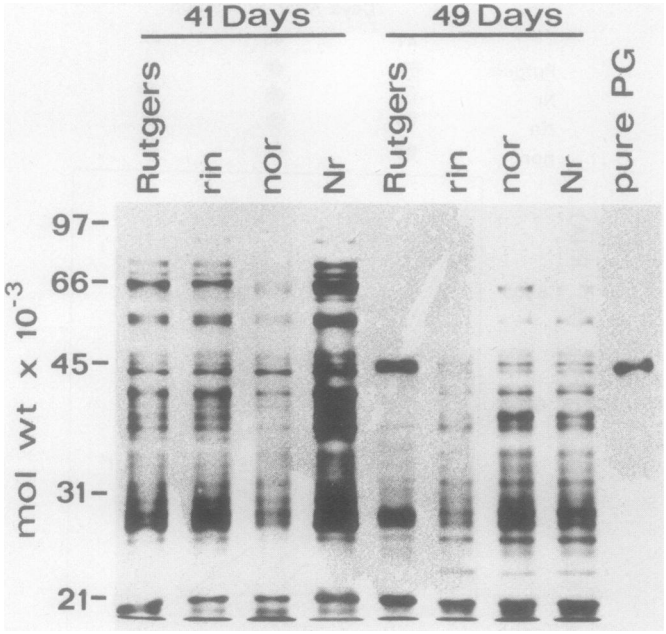

FIG. 6. Silver stained SDS-PAGE of total soluble proteins extracted from the pericarp of 41 and 49 d old Rutgers, *rin*, *nor*, and *Nr* fruit. All lanes contain 20 μg of protein except pure PG which contains 0.5μg.

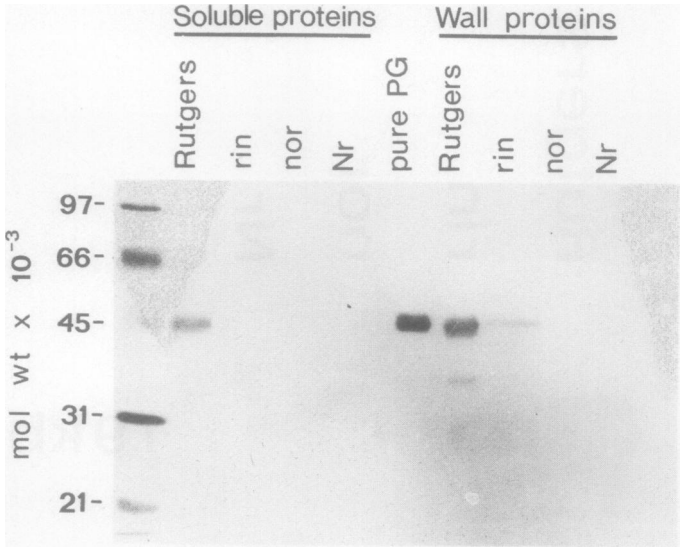

FIG. 8. Western blot analysis of soluble and cell wall protein extracts of 49 d old Rutgers, *rin*, *nor*, and *Nr* fruit. Protein extracts were resolved by SDS-PAGE, transferred to nitrocellulose, and PG protein detected using anti-PG serum as described (8). All lanes contain 50 μg of protein except the Rutgers soluble and Rutgers wall lanes which contain 10 and 2 μg, respectively. The first lane contains 10 μg of biotinylated mol wt standards prepared and detected as described (8).

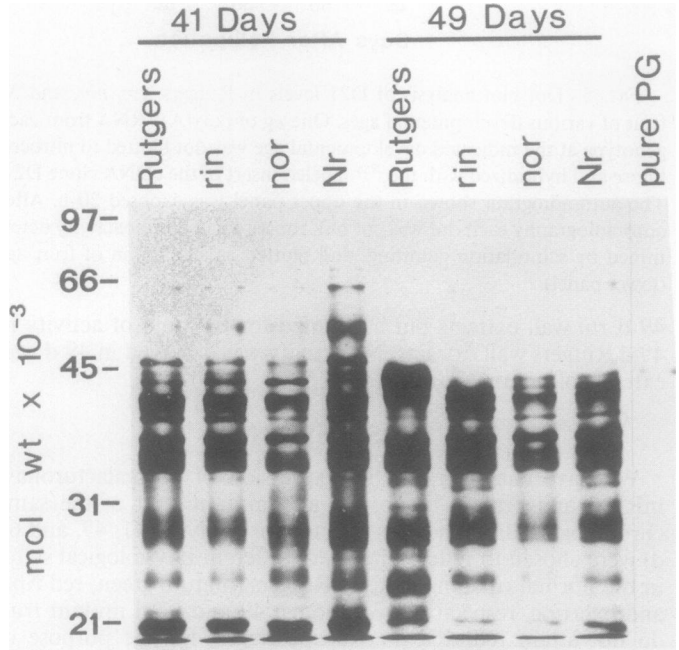

FIG. 7. Silver stained SDS-PAGE of total cell wall proteins extracted from the pericarp of 41 and 49 d old Rutgers, *rin*, *nor*, and *Nr* fruit. All lanes contain 20 μg of protein except pure PG which contains 250 ng.

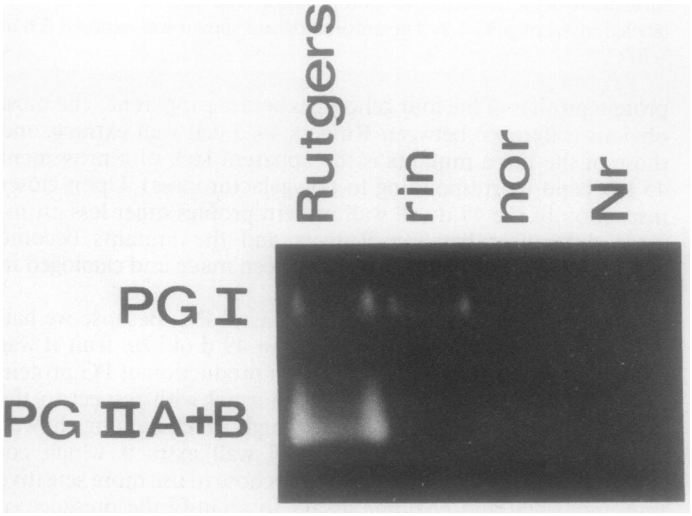

FIG. 9. Nondenaturing PAGE of 49 d old normal and mutant wall protein extracts stained for PG activity. Total wall protein extracts from 49 d Rutgers (2 μg), *rin* (100 μg), *nor* (100 μg), and *Nr* (100 μg) fruit were separated in a nondenaturing gel system and PG activity was detected by negative staining with Ruthenium Red. The positions of migration for the three polygalacturonase isozymes (PGI, PG-2A, PG-2B) determined using purified isozymes are indicated.

During the 7 to 10 d between the mature green and red ripe stages in Rutgers, a large number of biochemical and physiological changes take place which collectively determine the final quality of the fruit. Recently, several groups have shown that these changes are accompanied by alterations in the steady state levels of mRNAs encoding proteins which may have a role in the ripening process (4, 11, 17, 20, 24, 26). The single member of this group of ripening-induced mRNAs to which a definite function has been ascribed is the mRNA encoding the cell wall enzyme polygalacturonase. In Castlemart fruit PG mRNA first appears very early in ripening and accumulates to high levels, accounting for 1.2% of the mRNA mass in red ripe fruit (2, 9). In developmentally comparable red ripe Rutgers fruit the PG mRNA levels are nearly twice that found in Castlemart. Rutgers and Castlemart are considered relatively soft and firm cultivars, respectively. The difference in levels of PG mRNA in each cultivar raises the possibility that PG mRNA levels might be correlated with fruit softening, analogous to the observed correlation between PG protein and fruit softening (6, 13). Further work with other cultivars is needed to substantiate this correlation.

By 62 d after pollination Rutgers fruit had begun to senesce as evidenced by their greatly decreased ethylene production rates

(10–20% of the 49 d values) and an increase in their susceptibility to fungal attack. Biggs *et al.* (4) noted that by d 65 total RNA and poly(A)$^+$ RNA levels in Rutgers had decreased 75% from their maximal levels during ripening. This decrease is reflected in our results by the drastic decrease in the steady state levels of PG and D21 mRNA in Rutgers between 49 and 62 d (650-fold and 27-fold reductions, respectively). Similar results were reported for 65 d *Nr* fruit (4), and our D21 mRNA results for *Nr* are consistent with these observations. Interestingly, *nor* fruit showed almost constant steady state levels of D21 mRNA at 49 and 62 d postanthesis. If the decreased levels of PG and D21 mRNA in Rutgers between 49 and 62 d reflect the activity of a general system modulating steady state mRNA levels during senescence, *nor* fruit might prove interesting for studying this aspect of senescence at the molecular level.

In contrast to the high levels of PG mRNA observed in 49 d Rutgers fruit, all three mutants showed greatly reduced levels of PG mRNA. Although the levels were reduced, each mutant showed a distinct pattern of PG and D21 mRNA expression. The *nor* mutant showed the most drastic reduction in PG mRNA levels. In contrast, *rin* showed transient expression of PG mRNA while *Nr* fruit showed a steady and seemingly progressive increase in PG mRNA. Although the mutants are phenotypically similar, our results suggest that distinct mechanisms contribute to the reduced steady state levels of PG mRNA in each mutant. This is consistent with the observation that the three mutations map to distinct loci and suggests that each locus may code for a distinct regulatory factor involved in ripening.

The most significant result of this study is the observation that 49 d *rin* fruit do accumulate PG mRNA, but at a much lower level than Rutgers. The accumulation of PG mRNA in *rin* was coincidental with PG mRNA accumulation in Rutgers and resulted in detectable levels of enzymically active PG I isozyme. Furthermore, the size of the PG message in *rin* is identical to that found in Rutgers. Superficially, PG mRNA accumulation in *rin* appeared to be temporally coincident with PG mRNA accumulation in Rutgers. This result is surprising because expression of PG activity, PG protein, and PG mRNA in tomato fruit has always been associated with an increase in ethylene production by the tissue involved. The production of PG mRNA and protein by *rin* fruit, which do not produce elevated levels of ethylene (28), suggests that PG gene expression may be regulated, at least in part, by some developmental cue other than increased levels of ethylene. Our results do not support an earlier report of a lack of PG mRNA accumulation in *rin* fruit (12).

One of the most striking biochemical features of the ripening-impaired tomato mutants is their reduced levels of polygalacturonase and it was originally proposed that the basis of the mutations might be a structural alteration in the PG gene (28). It has recently been suggested, however, that the mutations are of a regulatory rather than structural nature. Genomic Southern analysis (Fig. 1) indicates that the ripening-impaired mutants possess polygalacturonase genes and that each mutant possesses restriction fragments which correspond to the fragments observed in Rutgers. This supports the view that a structural alteration in the polygalacturonase gene is not the primary mutation in the ripening-impaired mutants. This view is further supported by Northern analysis of the PG mRNA detected in *rin* and *Nr* fruit which indicates that the PG mRNA produced by these mutants is identical in size to that produced by normally ripening Rutgers fruit. Furthermore, the PG mRNA produced by *rin* and *Nr* results in the production of enzymically active PG protein. Thus, although the mutants produce lowered levels of PG mRNA, it appears that the pathway for translation, processing, and secretion of PG mRNA and protein is functional, suggesting that the mutations affect a regulatory step prior to translation. The probable sites where the mutations exert their effect are at the level of regulation of transcription and/or PG mRNA stability. We are currently performing experiments to determine what role transcription plays in the altered steady state levels of PG mRNA in *rin*, *nor*, and *Nr* fruit.

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