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Molecular cloning of tomato fruit polygalacturonase: Analysis of polygalacturonase mRNA levels during ripening

(cDNA/mRNA induction/ethylene/pectic enzyme)

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ABSTRACT **The expression of a gene encoding the cell wall-degrading enzyme polygalacturonase [poly(1,4-α-D-galacturonide) glucanohydrolase, EC 3.2.1.15] was characterized during tomato fruit ripening. Polygalacturonase was purified from ripe tomato fruit and used to produce highly specific antiserum. Immunoblot analyses detected a 45- and a 46-kDa protein in ripe fruit but immunoprecipitation of *in vitro* translation products of mRNA from ripe tomato fruit yielded a single 54-kDa polypeptide, suggesting post-translational processing. A plasmid cDNA library was prepared from poly(A)$^+$ RNA isolated from ripe tomato fruit. The cDNA library was inserted into a λ-based expression vector, and polygalacturonase cDNA clones were identified by immunological screening. Hybrid-select translation experiments indicated that the cDNAs encode a 54-kDa *in vitro* translation product that is specifically immunoprecipitated with polygalacturonase antiserum. RNA-blot analysis indicated that the 1.9-kilobase polygalacturonase mRNA was virtually absent from immature-green fruit, accumulated steadily during the ripening process, and was at its highest level in red-ripe fruit. There was at least a 2000-fold increase in the level of polygalacturonase mRNA between immature-green and red-ripe tomato fruit. These studies show that the levels of polygalacturonase mRNA are developmentally regulated during tomato fruit ripening.**

The ripening behavior of tomato fruit is characterized by a series of coordinated changes in the biochemistry and physiology of the tissues involved. Some of these changes include degradation of chlorophyll, synthesis of lycopene and aromatic compounds, alterations in organic acid metabolism, and a softening of the fruit tissue (1). These events and others are all considered part of the ripening process and occur in conjunction with an increase in CO_2 production (the respiratory climacteric) and an increase in ethylene production by the fruit. Ripening tomato fruit is actively synthesizing RNA and proteins (2–4). *In vitro* translation products of mRNA isolated from tomatoes at various ripening stages have shown that ripening is accompanied by changes in mRNA populations (5, 6). Similar results have been shown for ripening avocado fruit (7). Further studies employing differential hybridization to screen tomato fruit cDNA libraries have identified numerous cDNA clones, encoding proteins of unknown functions, that represent genes expressed predominantly during the unripe or ripe stages of fruit development (8, 9). These observations support the hypothesis that ripening involves, at least in part, developmental regulation of gene expression.

In tomatoes, the best studied enzyme related to ripening is the pectin-hydrolyzing enzyme polygalacturonase [poly(1,4-α-D-galacturonide)glucanohydrolase, EC 3.2.1.15]. This enzyme's role in cell wall degradation has been firmly established (10–14). Three structurally and immunologically related polygalacturonase isozymes have been characterized from ripe fruit (15–17). Polygalacturonase activity and immunologically detectable protein are entirely absent from mature-green fruits, first become detectable as the fruits initiate color change, and continue to increase dramatically as ripening proceeds (17–19). These studies provide good evidence that polygalacturonase is synthesized *de novo* during tomato fruit ripening. To further study the expression of polygalacturonase during ripening, we have identified polygalacturonase cDNA clones and examined the accumulation of polygalacturonase mRNA during tomato fruit ripening.

MATERIALS AND METHODS

Plant Materials. Ripe fruit (stage six; ref. 20) from field-grown tomato plants (*Lycopersicon esculentum* Mill, cv. Castlemart) were used for protein purification. Other ripening stages for mRNA purification were determined as follows. Mature-green fruit were harvested from the field, surface-sterilized, and ripened at 20°C. Individual fruits were scored daily by visual inspection (20) and by measurement of ethylene production rates (21).

Purification of Polygalacturonase Protein and Antibody Production. Polygalacturonase was purified from ripe tomato fruits by the method of Ali and Brady (15) with the following modifications. Following CM-Sepharose chromatography, fractions containing polygalacturonase activity (22) were applied to a Con A-Sepharose column, and, after extensive washing, polygalacturonase was eluted with 10 mM α-methylmannoside (15). At this stage, purification of polygalacturonase was complete as judged from $NaDodSO_4$/polyacrylamide gels stained with Coomassie blue. An additional purification step of Sephacryl S-200 gel filtration chromatography was employed to prepare the purified protein for antibody production. Protein concentration was determined by the method of Lowry *et al.* (23).

Antibodies to polygalacturonase were produced in female New Zealand rabbits by subcutaneous injection of purified protein (1.0 mg) in Freund's complete adjuvant followed 4 weeks later by 0.5 mg in Freund's incomplete adjuvant. Antiserum yielded a single precipitation band when tested by Ouchterlony double diffusion against total protein extracts of ripe tomato fruit. However, development of protein blots using polygalacturonase antiserum at a dilution of 1:100 and peroxidase-conjugated second antibody detected several other faint contaminating protein bands in addition to polygalacturonase. Contaminating antibodies were eliminated by preabsorption of the antiserum (24) with immobilized proteins extracted from immature-green tomato fruit. IgG was then purified from the preabsorbed serum by protein A-Sepharose chromatography (25). The IgG concentration

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Abbreviation: kb, kilobase(s).

was estimated by absorption at 280 nm, and the volume was adjusted to give a concentration of 5.0 mg of IgG per ml. Antibodies purified in this manner were used for all experiments except immunological screening of the cDNA library for which preabsorbed serum was used without purifying IgG by protein A chromatography.

RNA Isolation, *in Vitro* Translation, and Immunprecipitation. Frozen pericarp tissue at various stages of ripeness was lyophilized at 0°C. RNA was isolated following the method of Cathala *et al.* (26) with the following modification. Ten grams of lyophilized tomato pericarp tissue was homogenized in 100 ml of lysis buffer [5.0 M guanidine monothiocyanate/10 mM EDTA/300 mM Tris·HCl, pH 7.5/8% (vol/vol) 2-mercaptoethanol]. Total RNA was dissolved in 10 mM Tris·HCl, pH 7.6/1 mM EDTA/0.1% $NaDodSO_4$/500 mM NaCl and passed over a cellulose column (Whatman CF-11) equilibrated with the same buffer to remove polysaccharide contaminants (27). Poly(A)$^+$ mRNA was purified by two rounds of oligo(dT)-cellulose chromatography (28).

A cell-free protein synthesizing system was prepared from fresh wheat germ by the method of Anderson *et al.* (29). Poly(A)$^+$ RNA was translated as described (29) except for the inclusion of 10 units of RNasin (Promega Biotec, Madison, WI) per 25 μl of translation mixture. Translation products used for immunoprecipitations were brought to 3 mM EDTA to release polysomes. The products were then centrifuged for 5 min in an Eppendorf microfuge to remove insoluble material. Preimmune and immune sera were also centrifuged for 5 min immediately prior to use. Four tubes, each containing 8 μl of the centrifuged translation products, were diluted to 100 μl with sterile immunoprecipitation buffer [10 mM NaH_2PO_4, pH 7.4/135 mM NaCl/1% (vol/vol) Nonidet P-40/1 mM EDTA/bovine serum albumin at 5 mg/ml], the appropriate amounts of serum or protein were added (see Fig. 2), and the reaction mixtures were placed at 4°C. After 12 hr, 20 μl of protein A-Sepharose in immunoprecipitation buffer was added to each tube and shaken at room temperature. After 1 hr, 1 ml of immunoprecipitation buffer was added, the mixture was briefly Vortex mixed and centrifuged in a microfuge for 2 min, and the supernatant was removed. The immunoprecipitate was washed a total of five times with immunoprecipitation buffer and of two times with 1.0 ml of 10 mM NaH_2PO_4, pH 7.4/135 mM NaCl. The resulting pellet was resuspended by Vortex mixing in 100 μl of 62 mM Tris·HCl, pH 6.8/2% (wt/vol) $NaDodSO_4$/10% (vol/vol) glycerol/5% (vol/vol) 2-mercaptoethanol, placed in a boiling water bath for 3 min, and centrifuged. The supernatant was analyzed by $NaDodSO_4$/PAGE.

Electrophoresis and Immunoblotting. All protein extracts, total translation products, immunoprecipitated translation products, and hybrid-select translation products were separated by $NaDodSO_4$/PAGE (30). Preparation of biotinylated protein molecular weight standards, electrophoretic blotting, and detection methods were as described (31).

cDNA Library Production and Immunological Screening. A vector-primed cDNA library was prepared in the cloning vector pARC7 from ripe tomato fruit [stage six, 15.7 nl of C_2H_4 per g (fresh weight) per hr] poly(A)$^+$ RNA by the method of Alexander *et al.* (32). A cDNA reaction with 2 μg of vector (1 pmol vector ends) and 5 μg of poly(A)$^+$ RNA yielded 4.4×10^6 independent transformants. Total library plasmid was isolated (33) and used for construction of an expression library in λ Charon 16. The plasmid preparation was linearized with *Sst* I and inserted into the λ vector so that the cDNA inserts were ligated to the phage β-galactosidase gene. Primary screening of 150,000 plaques (53% containing inserts) was performed at high density with polygalacturonase antiserum (1:100 dilution) on nitrocellulose "filter lifts" as described (‡, 34). Randomly selected immunological-positive plaques were plated at low density and subjected to further rounds of immunological screening. Clonal lines yielding 100% positive signals after three rounds of screening were recovered from the λ screening vector and transformed as ampicillin-resistant plasmids into *Escherichia coli* cells (‡).

DNA Hybridization. cDNA inserts isolated after agarose gel electrophoresis and purified using a NACS minicolumn (Bethesda Research Laboratories) were nick-translated to a specific activity of 10^7–10^8 cpm/μg using DNase I, DNA polymerase I (Bethesda Research Laboratories), and [^{32}P]dCTP (Amersham). Plasmids, isolated from the clones identified by antibody screening, were digested with *Sma* I and electrophoresed in a 1.5% agarose gel. Tomato genomic DNA was isolated by cesium chloride gradient centrifugation (35), digested with the appropriate restriction enzymes, and electrophoresed in a 0.7% agarose gel. The gels were blotted to nylon membranes (Bio-Rad), prehybridized, and hybridized by the method of Southern (36). After hybridization, the filters were washed twice with 0.3 M NaCl/0.03 M trisodium citrate, pH 7.0/0.1% $NaDodSO_4$ for 15 min at room temperature, twice with 0.015 M NaCl/0.0015 M trisodium citrate, pH 7.0/0.1% $NaDodSO_4$ for 15 min at room temperature, and finally twice for 30 min in 0.015 M NaCl/0.0015 M trisodium citrate, pH 7.0/0.1% $NaDodSO_4$ at 42°C. The blots were autoradiographed at −70°C using Kodak XAR-5 film and an intensifying screen (Cronex, Dupont).

Hybrid-Select Translations. Twenty micrograms of plasmid DNA from selected clones was dissolved in 10 μl of sterile water, spotted onto sterile nitrocellulose discs, dried overnight in a covered Petri dish, and denatured as described (35). The nitrocellulose discs were then baked for 2 hr at 90°C under vacuum. Prehybridization was performed for 1 hr at 42°C in 1 ml of 50% (vol/vol) formamide/400 mM NaCl/10 mM Pipes, pH 6.4/4 mM EDTA containing yeast tRNA at 0.5 mg/ml. The hybridization solution was identical to the prehybridization solution except for the inclusion of 40 μg of poly(A)$^+$ mRNA isolated from ripe tomato fruits [15.7 nl of C_2H_4 per g (fresh weight) per hr] and 150 units of RNasin. Hybridization was performed for 5 hr at 42°C. The nitrocellulose discs were washed as described (35). Bound mRNA was released into 300 μl of sterile H_2O by heating to 100°C for 90 sec and quickly removing the nitrocellulose filter. The hybrid-selected mRNA was precipitated by the addition of 0.1 volume of 3.0 M sodium acetate, pH 5.5/5 μg of "carrier" yeast tRNA/2.5 vol of 100% ethanol and placed overnight at −20°C. The hybrid-selected mRNA was heated to 68°C for 5 min and cooled on ice immediately prior to being translated in a wheat germ translation system.

RNA Gel Blotting. One microgram of poly(A)$^+$ RNA isolated from various ripening stages was separated by electrophoresis in a 1.2% agarose gel containing formaldehyde and blotted to nitrocellulose (37). The filter was prehybridized and hybridized to denatured nick-translated cDNA probes as described (38). The filter was washed and autoradiographed as described for Southern blots. After autoradiography, radiolabeled bands from RNA gel blots were cut out, and the associated radioactivity was determined by scintillation counting.

RESULTS

Antibody Production. Fig. 1 illustrates the antibody specificity for polygalacturonase. At a dilution of 1:1000 preabsorbed polygalacturonase antiserum detected purified polygalacturonase 2A and 2B (Fig. 1, lane 6) and specifically

‡Genez, A. L., Alexander, D. C., Rejda, J. M., Williamson, V. M. & Williams, B. G. (1985) First International Congress of Plant Molecular Biology, Oct. 28, 1985, Savannah, GA, p. 87 (abstr.).

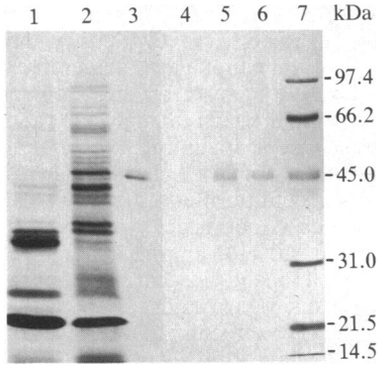

FIG. 1. $NaDodSO_4$/polyacrylamide gel and immunoblot of tomato proteins. Lanes 1 and 4: total proteins (50 and 10 μg, respectively) isolated from immature-green tomato fruit (no detectable C_2H_4). Lanes 2 and 5: total proteins (50 and 10 μg, respectively) isolated from ripe tomato fruit (15.7 nl C_2H_4/g/hr). Lanes 3 and 6: purified polygalacturonase (2.5 μg and 150 ng, respectively) isolated from ripe tomato fruit. Lane 7: biotinylated protein molecular weight standards. Lanes 1–3 were stained with Coomassie blue; lanes 4–7 were blotted to nitrocellulose and visualized as described (31).

detected corresponding 45- and 46-kDa protein bands in total protein extracts from ripe fruit (Fig. 1, lane 5). No protein bands were immunodetected in total protein extracts from immature-green tomato fruits (Fig. 1, lane 4).

***In Vitro* Translation of Polygalacturonase.** Poly(A)$^+$ RNA isolated from ripe tomato fruit was translated *in vitro* using a wheat germ translation system, and the resulting polygalacturonase translation product was immunoprecipitated with polygalacturonase antiserum (Fig. 2). In contrast to the 45- and 46-kDa proteins identified *in vivo*, the immunoprecipitated *in vitro* translation product had a molecular weight of 54 kDa (Fig. 2, lane 3). As shown in Fig. 2, we verified that the immunoprecipitated translation product was a polygalacturonase precursor by competition experiments with either 5 μg or 20 μg of purified polygalacturonase protein (Fig. 2, lanes 4 and 5, respectively). The ability of purified polygalacturonase to compete for antibody binding with the 54-kDa *in vitro* translation product suggests that the 54-kDa polypeptide is a polygalacturonase precursor.

Identification of Polygalacturonase cDNA Clones. Polygalacturonase cDNA clones were identified by immunological screening of a ripe tomato fruit cDNA library incorporated into the β-galactosidase gene of λ Charon 16 (‡). Of 75,000 phage cDNA insert-containing plaques screened, half of which were in the proper orientation for expression, 50 plaques showed a positive reaction with polygalacturonase antiserum. A group of these immunologically selected cDNAs strongly cross-hybridized and ranged in size from 550 to 900 base pairs. To determine whether this group of cDNAs was derived from polygalacturonase mRNA, a representative cDNA (pPG16) was used in hybrid-select translation experiments. This cDNA (pPG16) hybrid selected an mRNA that codes for a 54-kDa *in vitro* translation product (Fig. 3, lane 3) that comigrated with the polygalacturonase precursor immunoprecipitated from total translation products (Fig. 3, lane 2). Furthermore, the 54-kDa hybrid-select translation product was specifically immunoprecipitated with polygalacturonase antiserum and not with preimmune serum (Fig. 3, lanes 4 and 5, respectively). These results indicate that pPG16 was indeed derived from polygalacturonase mRNA.

Accumulation of Polygalacturonase mRNA During Ripening. Using the polygalacturonase cDNA (pPG16), it was possible to directly examine the appearance of polygalacturonase mRNA during tomato fruit ripening. The level of ethylene production and the corresponding visual stage of ripeness of the fruit are displayed in the *Inset* of Fig. 4. Poly(A)$^+$ RNA isolated from these tomatoes was probed with the ^{32}P-labeled cDNA insert from pPG16 (Fig. 4 *Lower*). The 1.9-kilobase (kb) polygalacturonase mRNA first becomes detectable at an early stage of ripening (mature-green-5), progressively increases throughout ripening and is at its maximum level in red-ripe fruit. The levels of polygalacturonase mRNA in immature-green and mature-green-2 fruits were consistently less than 5 cpm above background levels indicating that constitutive expression of polygalacturonase mRNA during these developmental stages is extremely low. Based on this maximum value of 5 cpm for the immature-green stage and the value for red-ripe fruit shown in Fig. 4, our minimum estimate of the increase in polygalacturonase mRNA between the immature-green and red-ripe stages is 2000-fold.

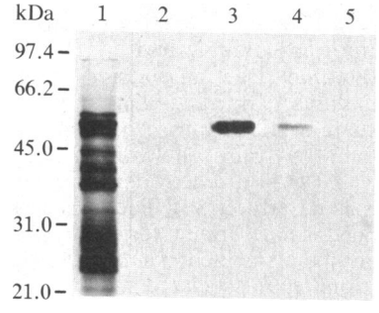

FIG. 2. Immunoprecipitation of the polygalacturonase *in vitro* translation product. Total *in vitro* translation products of poly(A)$^+$ RNA isolated from ripe tomato fruit were used for immunoprecipitations, separated by $NaDodSO_4$/PAGE, and visualized by autoradiography. Lane 1: total translation products of poly(A)$^+$ RNA isolated from ripe tomato fruit. Lane 2: immunoprecipitation with 2 μl of preimmune serum. Lane 3: immunoprecipitation with 2 μl of polygalacturonase antiserum. Lane 4: immunoprecipitation with 2 μl of polygalacturonase antiserum in the presence of 5 μg of purified polygalacturonase. Lane 5: immunoprecipitation with 2 μl polygalacturonase antiserum in the presence of 20 μg of purified polygalacturonase.

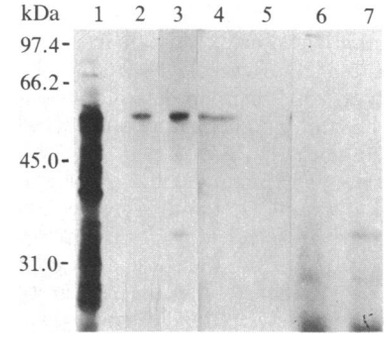

FIG. 3. Characterization of polygalacturonase cDNA clone pPG16 by hybrid-select translation and immunoprecipitation. pPG16 was bound to nitrocellulose and used in hybrid–select translation experiments with poly(A)$^+$ RNA isolated from ripe tomato fruit. The hybrid-selected mRNA was translated, immunoprecipitated, and analyzed by $NaDodSO_4$/PAGE followed by autoradiography. Lane 1: total translation products of poly(A)$^+$ RNA isolated from ripe tomato fruit. Lane 2: immunoprecipitation of lane 1 using 2 μl of polygalacturonase antiserum. Lane 3: pPG16 hybrid-select translation product from ripe tomato fruit poly(A)$^+$ RNA. Lane 4: immunoprecipitation of lane 3 using 2 μl of polygalacturonase antiserum. Lane 5: immunoprecipitation of lane 3 using 2 μl of preimmune serum. Lane 6: hybrid-select translation products using pARC 7 (cloning vector with no insert). Lane 7: *In vitro* translation products with no added RNA.

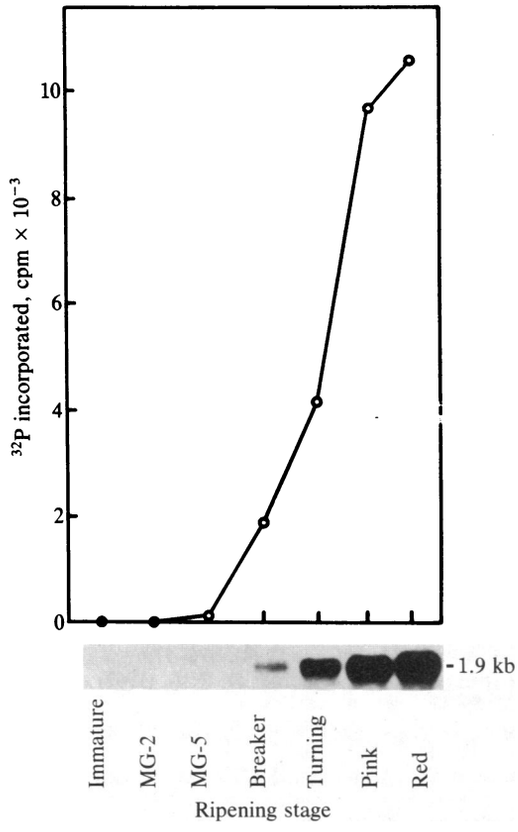

FIG. 4. RNA gel blot analysis of poly(A)$^+$ RNA isolated from fruits of various ripening stages. The indicated ripening stages are as follows: Immature-green: no jelly material in any of the locules. Mature-green-2 (MG-2): jelly material in at least one but not all locules. Mature-green-5 (MG-5): jelly material in all locules, no color change visible from the fruit exterior, but when cut open, a faint pink color is visible on the inner radial pericarp wall. Breaker: "star" of color visible from exterior on blossom end of fruit. Turning: fruit are 10–30% red. Pink: fruit are 30–60% red. Red: fruit are 100% red. Poly(A)$^+$ RNA (1 μg) from each stage was electrophoresed, blotted to nitrocellulose, and probed with the ^{32}P-labeled cDNA insert of pPG16. The autoradiograph shown below the graph was exposed 4 hr. Upon longer exposure (20 hr) a band became visible in the MG-5 lane. ^{32}P incorporation is corrected for background (40–50 cpm). Ethylene production (in nl per g per hr) at each ripening stage is as follows: immature, 0; MG-2, 0.08; MG-5, 0.28; breaker, 1.12; turning, 4.26; pink, 7.20; red, 15.76.

Polygalacturonase Genomic Fragments. Hybridization of the polygalacturonase cDNA to mRNA indicated the presence of a single-size class of mRNA (Fig. 4). Because three polygalacturonase isozymes have been identified in ripe tomato fruit (16–18), we examined the banding pattern of tomato genomic DNA fragments probed with the ^{32}P-labeled insert of pPG16 to evaluate whether the polygalacturonase isozymes may be derived from a multigene family (Fig. 5). The polygalacturonase cDNA hybridized to two bands when tomato genomic DNA was digested with *Bam*HI. Upon prolonged exposure a faint second band was also visible in *Eco*RI digests, while only one band was observed in *Hin*dIII digested DNA. pPG16 does not contain sites for *Eco*RI, *Bam*HI, or *Hin*dIII.

DISCUSSION

We have identified a 54-kDa protein precursor of polygalacturonase by immunoprecipitation of *in vitro* translation products using poly(A)$^+$ RNA isolated from ripe tomato fruit and a wheat germ translation system. Purified polygalacturonase

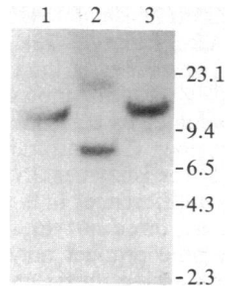

FIG. 5. Hybridization of polygalacturonase cDNA clone pPG16 to digested tomato DNA. Tomato DNA (10 μg) was digested to completion with *Eco*RI (lane 1), *Bam*HI (lane 2), or *Hin*dIII (lane 3), electrophoresed in a 0.7% agarose gel, transferred to nitrocellulose, and hybridized with the ^{32}P-labeled insert of cDNA clone pPG16. The filter was washed at 50°C in 0.15 M NaCl/0.015 M trisodium citrate, pH 7.0 containing 0.1% $NaDodSO_4$. Positions of molecular size standards in kilobase pairs are indicated.

competes for antibody binding with the 54-kDa *in vitro* translation product indicating that the two proteins are immunologically related and most likely represent differentially processed forms of the same polypeptide. Based on these competition experiments and the high specificity of our polygalacturonase antiserum, we conclude that the 54-kDa translation product is indeed the precursor form of at least one, and possibly all three, polygalacturonase isozymes found in ripe tomato fruit.

The large size of the polygalacturonase precursor as compared to the mature protein (54 kDa versus 46 kDa) may be accounted for in part by the presence of a signal peptide required for secretion of the protein to the cell wall. Several other secreted proteins from plants, however, possess signal peptides of about 2.5 kDa (39, 40). The polygalacturonase precursor is much larger (7–9 kDa) suggesting that this protein may undergo more extensive post-translational processing.

We have identified a group of polygalacturonase cDNA clones that hybridize to a 1.9-kb polyadenylylated mRNA species. The size of the polygalacturonase mRNA is more than large enough to contain the structural information required to code for a 54-kDa protein (approximately 1.5 kb) and is in good agreement with the size (1.95 kb) determined from sucrose density gradient fractionation of RNA (41). We have used a representative member of this cDNA group as a probe to monitor changes in polygalacturonase mRNA levels during tomato fruit ripening. By this direct method we have shown that there is a large accumulation of polygalacturonase mRNA during ripening. The dramatic increase in polygalacturonase activity and immunodetectable polygalacturonase protein during tomato fruit ripening has been well documented (16–19), and our results indicate there is at least a 2000-fold increase in the level of polygalacturonase mRNA between the immature-green and red-ripe stages of fruit development. We have found no evidence for an inactive (stored) pool of polygalacturonase mRNA in unripe fruit. Overall this indicates that expression of the polygalacturonase enzyme is regulated by the level of polygalacturonase mRNA rather than by a translational or a post-translational regulatory mechanism. The exact mechanism by which the polygalacturonase mRNA level is regulated is at present unknown, but likely results from an increase in transcription and/or stabilization of the newly formed polygalacturonase mRNA.

As stated earlier, there are three polygalacturonase isozymes present in ripe tomato fruit. Isozyme 1 is the first to appear at the onset of ripening, but in ripe fruit it constitutes only a minor percentage of the total polygalacturonase protein (16–18). Isozymes 2A and 2B appear 1–2 days after

isozyme 1 and constitute the majority of polygalacturonase protein present in ripe fruit (16–18). Because the three polygalacturonase isozymes are immunologically related (15), it is likely that they share common structural properties. This is further substantiated by the similarity of tryptic digest patterns for the isozymes (17) and identical NH_2-terminal amino acid sequences for isozymes 2A and 2B (results not shown). However, the differences in the time of appearance and relative levels of the three isozymes raise the possibility that more than one gene product may be responsible for polygalacturonase expression during tomato fruit ripening. Although the research presented here does not directly address this question, our results have not eliminated the possible existence of more than one polygalacturonase gene. While only a single-size class of polygalacturonase mRNA was detected, it may consist of multiple gene products that cannot be resolved by RNA gel blot analysis or may only be distinguishable at the nucleotide sequence level. The presence of two bands on genomic Southern blots of tomato DNA probed with pPG16 suggests there may be more than one polygalacturonase gene. Similar results have been shown for other plant proteins encoded by more than one gene (42–44). Further experiments are being conducted to determine the number of genes responsible for expression of the three polygalacturonase isozymes.

During the final stages of the research reported here a paper appeared identifying a 48-kDa protein as the polygalacturonase precursor on the basis of *in vitro* translation and immunoprecipitation with polygalacturonase antiserum (6). A second paper from the same group reported the identification of a putative polygalacturonase cDNA clone that was shown by hybrid-select translation and immunoprecipitation to encode the 48-kDa protein (9). During the course of the research presented here, we found no evidence for the presence of a 48-kDa polygalacturonase *in vitro* translation product. In addition, several other groups have identified the polygalacturonase *in vitro* translation product as a 54-kDa polypeptide (45, §, ¶). In light of this emerging agreement that polygalacturonase is synthesized as a 54-kDa precursor protein, reports regarding the 48-kDa protein (6) and the putative polygalacturonase cDNA clone (9) may be incorrect.

§Biggs, M. S., Tieman, D. M. & Handa, A. V. (1985) First International Congress of Plant Molecular Biology, Oct. 28, 1985, Savannah, GA, p. 87 (abstr.).

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