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Gene expression during tomato ripening

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[Plate 1]

The changes in mRNA content that occur during tomato ripening have been studied by translation *in vitro* and cDNA cloning. A number of ripening-related cDNA clones have been characterized by hybrid-select translation, Northern blotting and sequencing. Some of these clones appear to be ripening-specific, based on the results of hybridization to mRNA from roots, leaves, and unripe and ripe fruit. DNA and protein sequencing have identified a full-length clone for polygalacturonase, an enzyme synthesized *de novo* during ripening that plays an important role in the softening process. mRNAs for polygalacturonase and some other unidentified proteins are absent or reduced in amount in the *ripening inhibitor* mutant of tomato. The accumulation of new mRNAs in normal fruit at the mature green stage is stimulated by ethylene. The regulation of gene expression during ripening and the mechanism of ethylene action are discussed.

Introduction

Nearly 30 years ago Steward showed that a single differentiated plant cell can, under the appropriate conditions, be induced to regenerate a complete new plant (Steward *et al.* 1958). This work demonstrated what many botanists had believed for a long time, that plant cells are totipotent and, therefore, that development and differentiation must involve the selective expression of different subsets of genes present in each nucleus. Thus an important goal of research on plant development is to understand the mechanisms regulating the expression of particular genes in tissues and organs at different stages in the life cycle. We began a study of gene expression during ripening of tomato (*Lycopersicon esculentum* Mill) fruits with this objective in mind. The choice of this system offers a number of advantages. Tomato has a small genome and a good genetic map, and a number of ripening mutants are available (Rick 1980). The fruit pericarp provides large amounts of material for biochemical analysis and with controlled environment facilities material for study can be produced throughout the year.

Ripening of fleshy fruits is a terminal phase in the reproductive process, during which the cells undergo a series of changes in composition that make them attractive to eat. In tomato, ripening involves changes in the mitochondria, plastids, nucleus, cytosol, vacuole and cell wall (table 1). Since mature green fruit can ripen after picking, it follows that the process does not depend on the import of materials from the parent plant but involves the conversion of components already existing in the organ. Although the various ripening changes involve different cell compartments they are highly coordinated, both with respect to events within

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Table 1. Major changes that occur during the maturation and ripening of tomatoes

- degradation of starch
- loss of chlorophyll
- disappearance of thylakoids and photosynthetic enzymes
- accumulation of vitamins
- degradation of toxic alkaloids such as α-tomatine
- large increase in synthesis of ethylene
- stimulation of respiration
- metabolism of organic acids
- accumulation of lycopene and β-carotene
- partial solubilization and softening of cell walls
- production of volatile compounds contributing to aroma and flavour

the cell and in neighbouring cells. There are some similarities between ripening and leaf senescence, including the degradation of starch and loss of chlorophyll and photosynthetic enzymes (Roberts *et al.* 1985). Similarily, cell wall solubilization occurs both during ripening and after the activation of abscission layers of leaves and flowers (Sexton & Roberts 1982). In climacteric fruits (Biale & Young 1981) such as tomato, ethylene, which is synthesized at the start of ripening, appears to play an important role in regulating the process. Significantly, leaf senescence and abscission are also often regulated by ethylene (Roberts *et al.* 1985; Sexton & Roberts 1982). Notwithstanding these similarities with leaf senescence and abscission, ripening represents a unique combination of physiological and biochemical changes that occur together only in one organ at one stage in the life cycle. The study of ripening may therefore be justified both by its intrinsic interest and also by its economic importance. In this article we review the evidence that ripening requires the expression of specific genes and discuss the function and regulation of 'ripening genes'.

cDNA cloning and characterization of ripening-related mRNAs

The first evidence for changes in mRNA content related to fruit ripening was obtained from *in vitro* translation experiments by Rattanapanone *et al.* (1978). Subsequently it was shown that some mRNAs existing in green fruit disappeared and new ones accumulated during ripening (Spiers *et al.* 1984; Grierson *et al.* 1985*b*). Screening of a cDNA clone library generated from poly(A)-containing mRNA from ripe tomatoes produced 146 colonies containing sequences that hybridized preferentially to cDNA prepared from mRNA from ripe compared with that from unripe fruit. Related sequences in this library were classified by cross-hybridization, and the clone with the largest cDNA insert in each group was designated the type member (Slater *et al.* 1985). We identified 11 groups of cDNA clones containing more than one member, plus 8 unique clones.

Hybrid-release translation experiments identified seven clones homologous to mRNAs encoding polypeptides with molecular masses from 57 to 28 kDa (figure 1, table 2). Five of these seven polypeptides correspond to those detected by translation *in vitro* of total mRNA (Grierson *et al.* 1985*b*). We measured the sizes of the transcripts homologous to these clones by hybridization of ^{32}P-labelled cDNA probes to poly(A)-containing mRNA fractionated by agarose gel electrophoresis and transferred to membranes. Four of the clones hybridized to a single mRNA species and three others also showed homology to minor mRNAs smaller than the major transcript (table 2) (Maunders *et al.* 1986). In several cases the cloned insert was similar in size to the mRNA, indicating that several of the clones were almost full length. We measured

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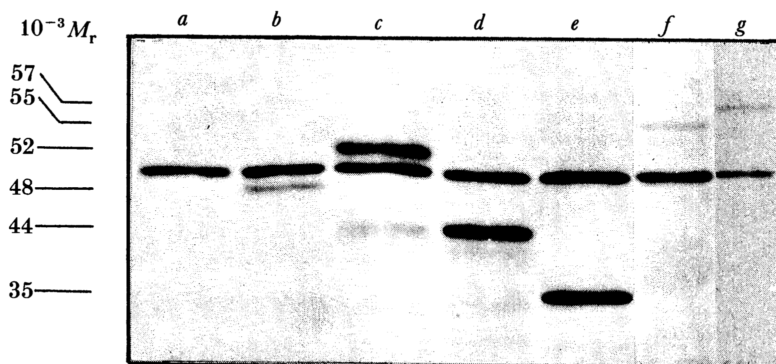

FIGURE 1. Translation *in vitro* of mRNA hybrid-selected by ripening-related cDNA clones. Tomato fruit mRNA was hybridized to DNA from each clone and the mRNA was recovered and translated in a rabbit reticulocyte lysate in the presence of [^{35}S]methionine. Radioactive proteins were fractionated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis and detected by fluorography. The sizes of the polypeptides, determined from the mobility of radioactive marker proteins in the same gel, are shown on the left. A polypeptide of approximately 50 kDa became labelled without the addition of mRNA (track *a*). Translation products of mRNAs homologous to pTOM 5 (track *b*), pTOM 36 (track *c*), pTOM 99 (track *d*), pTOM 13 (track *e*), pTOM 6 (track *f*), and pTOM 137 (track *g*) (see Slater *et al.* 1985).

TABLE 2. PROPERTIES OF SOME RIPENING-RELATED mRNAs FROM TOMATO

(The sizes of smaller RNAs that also hybridized to the cDNA probes are shown in parentheses.)

clone (pTOM)	cDNA (base pairs)	mRNA (nucleotides)	protein M_r	mRNA expressed in unripe fruit	ripe fruit	leaves	roots
5	1640	1960	48000	–	+	–	–
6	1618	1580 (1260, 960)	55000	–	+	–	–
13	1400	1400 (1050, 820)	35000	–	+	–	–
36	1300	1470 (1050, 725)	52000	–	+	–	–
75	950	1200	28000	+	+	+	+
99	900	1520	44000	–	+	–	–
137	1000	1680	57000	+	+	+	+

the expression in different organs of mRNAs homologous to the seven ripening-related cDNA clones by immobilizing poly(A)-containing RNA from ripe and unripe tomatoes, leaves and roots on membranes and hybridizing the dots to ^{32}P-labelled cDNA probes. The results indicate that five of the seven mRNAs (homologous to clones pTOM 5, 6, 13, 36 and 99) are expressed only in ripe tomatoes, whereas two (homologous to pTOM 75 and 137) are also expressed in unripe fruit, leaves and roots (figure 2).

To investigate the timing of appearance of these mRNAs in relation to ripening their expression was followed in fruit allowed to ripen while attached to the plant. The progress of ripening was monitored by measuring the lycopene content. The five ripening-specific mRNAs increased in concentration several hundredfold during ripening, and those homologous to pTOM 75 and pTOM 137, although not ripening-specific, also increased severalfold (figure 3). None of the mRNAs was particularly abundant, in each case representing less than 0.05% of the total mRNA (Maunders *et al.* 1986). All the mRNAs appeared at an early stage of ripening. Maximum expression was generally observed when tomatoes reached the fully orange stage, although the mRNAs persisted in soft red fruit.

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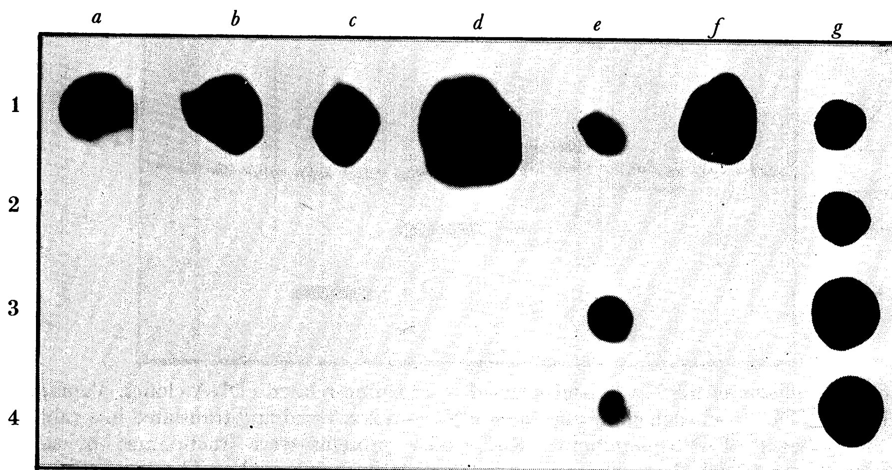

FIGURE 2. Expression in different organs of cloned ripening-related mRNAs. Poly(A)$^+$ RNA (1 μg) from ripe (1) and unripe (2) fruit, roots (3) and leaves (4) was fixed to membranes and probed with ^{32}P-labelled cDNA from pTOM 5 (*a*), pTOM 6 (*b*), pTOM 13 (*c*), pTOM 36 (*d*), pTOM 75 (*e*), pTOM 99 (*f*), and pTOM 137 (*g*). Hybridization was detected by X-ray film (see Maunders *et al.* 1986).

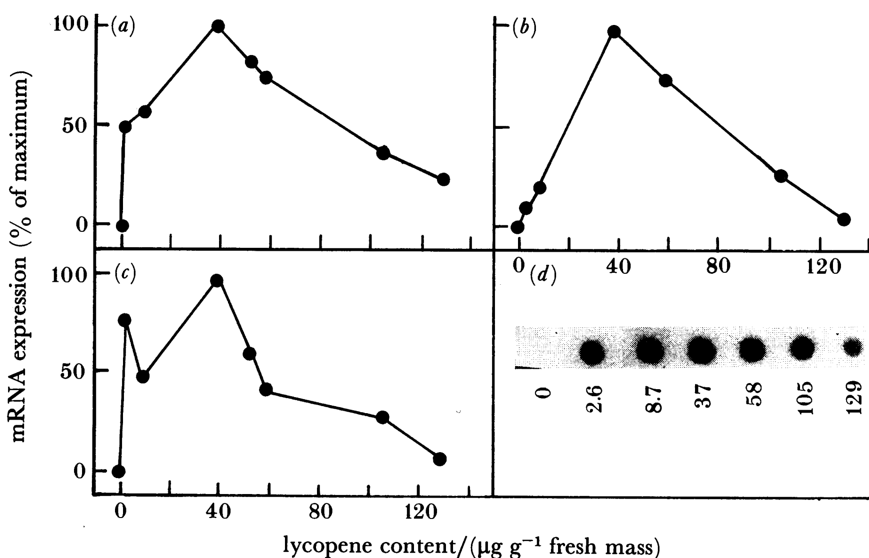

FIGURE 3. Expression of mRNA homologous to cDNA clones during ripening. RNA samples from mature green fruit and at different stages of ripening were fixed to membranes and hybridized to ^{32}P-labelled cDNA clones. Hybridization was detected by X-ray film. Results were quantified employing a range of dilutions and DNA standards and scanning the X-ray film with a densitometer. The stage of ripening is indicated by the lycopene content. Results are for mRNA homologous to pTOM 5 (*a*), pTOM 6 (*b*), and pTOM 13 (*c*). The dot hybridization to pTOM 5 is shown in (*d*), where the numbers beneath the dots refer to the lycopene content (see Maunders *et al.* 1986).

IDENTIFICATION OF cDNA CLONES FOR THE CELL WALL SOFTENING ENZYME POLYGALACTURONASE

Since ripening-specific mRNA sequences accumulate early during the ripening programme, it is reasonable to propose that they encode enzymes involved in some of the processes listed in table 1. One aspect that has received considerable attention is the softening process that occurs during ripening. Experiments by Hobson indicated that an increase in polygalacturonase

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activity is correlated with softening of tomato fruit (Hobson 1964, 1965). We showed that polygalacturonase (PG) is synthesized *de novo* during ripening (figure 4) (Tucker *et al.* 1980; Tucker & Grierson 1982). Purified PG releases soluble uronides from cell wall material *in vitro* (Themmen *et al.* 1982) and when added to unripe tomato tissue it produces similar

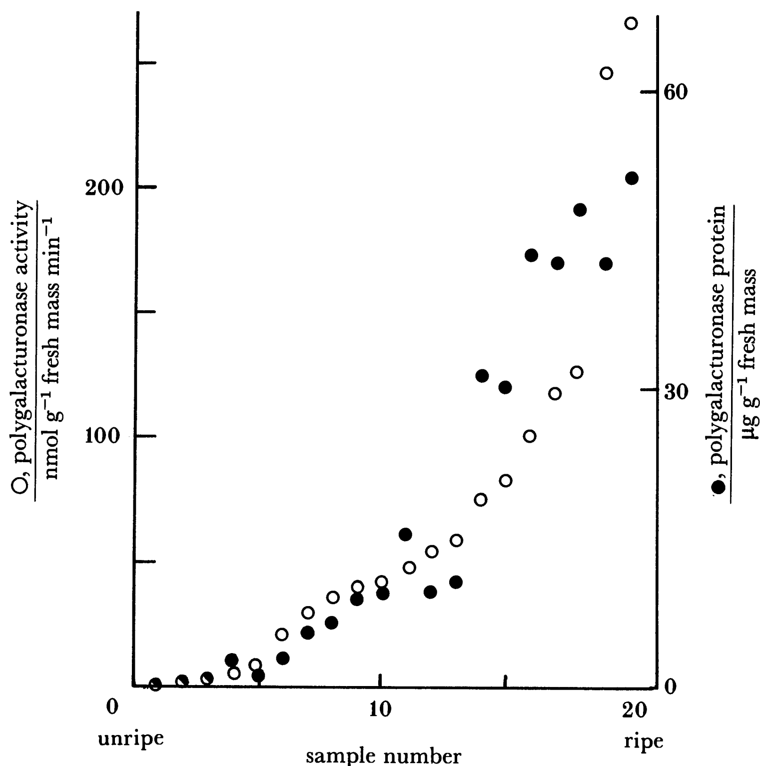

FIGURE 4. Appearance of polygalacturonase activity during tomato ripening measured by enzyme activity and radioimmunoassay. (Redrawn from Tucker & Grierson (1982).)

ultrastructural changes in the wall to those occurring during ripening (figure 5, plate 1) (Crookes & Grierson 1983). Tucker *et al.* 1980) showed that two isoforms of PG appear at different times during ripening: PG-1 accumulates early during colour change and PG-2 becomes the dominant form as the fruit become fully ripe. PG-1 and PG-2 are structurally and immunologically related (Tucker *et al.* 1980) and PG-2 can be converted into a form resembling PG-1 *in vitro* (Tucker *et al.* 1981). Zainon & Brady (1982) showed that PG-2 can be separated into two forms (PG-2a and PG-2b), which are also related immunologically. All isoforms are glycosylated (Zainon & Brady 1982). The main polypeptide has a molecular mass of approximately 46 kDa (Tucker *et al.* 1980; Tucker & Grierson 1982), although there may be other polypeptides associated with PG-1 (Moshrefi & Luh 1983; Pressey 1984). It is not yet clear whether the isoforms are the products of separate genes or are formed by post-translational modification of a single polypeptide. Experiments involving the immunoprecipitation of mRNA translation products *in vitro* indicated that PG mRNA accumulates during ripening. Accordingly, we set out to determine which of the ripening-specific cDNA clones encoded PG. Immunoprecipitation experiments suggested that the PG mRNA translation product *in vitro* had a molecular mass of either 54 kDa (Sato *et al.* 1984) or 48 kDa (Grierson *et al.* 1985*b*; Slater *et al.* 1985). Hybrid-select translation experiments showed that polypeptides

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of this size are encoded by pTOM 5 and pTOM 6 (table 2) (Slater *et al.* 1985), indicating that these were the prime candidates for PG clones. We sequenced both of these cDNAs and compared the predicted amino acid sequence with that determined for the N-terminus of purified polygalacturonase-2 (predominantly PG-2a). The results show that pTOM 6 contains the sequence for PG-2a (figure 6). This cDNA hybridizes to a mRNA encoding a polypeptide

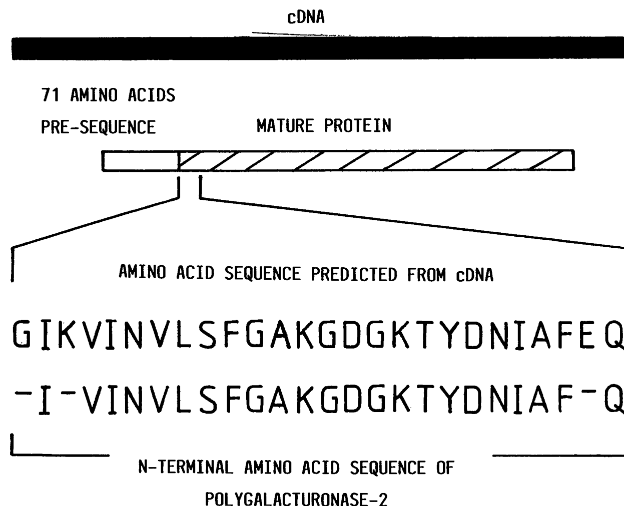

Figure 6. pTOM 6 codes for polygalacturonase. The DNA sequence of pTOM 6 predicts the amino acid sequence shown. This corresponds exactly to the N-terminal amino acid sequence determined for PG-2a (courtesy of Dr J. Findlay, University of Leeds). The gaps in the protein sequence indicate residues where no clear signal was obtained during the sequencing run. The cDNA sequence also predicts a pre-sequence of 71 amino acids.

of molecular mass 55 kDa (figure 1), approximately 9 kDa heavier than the mature PG polypeptide. This discrepancy is due, at least partly, to the presence of a long pre-sequence of 71 amino acids. The pre-sequence may function as a signal, directing the polypeptide through the cell endomembrane system and across the plasmalemma, although no direct test of this possibility has yet been performed. The present evidence, based on the N-terminal amino acid sequence of PG-2a, does not allow us to conclude which isoform of PG is encoded by pTOM 6. We have isolated 12 other cDNA clones that cross-hybridize to pTOM 6 (Slater *et al.* 1985) and are investigating the possibility that some of these show sequence variation. The sequence of pTOM 5 is not related to that of pTOM 6 and we conclude that it does not code for PG-2a. A cDNA clone for tomato PG has also been identified by Penna *et al.* (1986) by hybrid select translation *in vitro* and immunodetection of the translation product expressed in *Escherichia coli*.

Absence of PG and its mRNA in the ripening inhibitor (*rin*) mutant

The *ripening inhibitor* (*rin*) mutation of tomato is one of several mutations affecting the ripening process (Rick 1980). It shows mendelian inheritance and is located on chromosome 5, tightly linked to *macrocalyx*. Mature fruit of *rin*/*rin* plants turn yellow extremely slowly but are deficient in several processes associated with ripening (table 3). Very little softening occurs, owing, at least in part, to the failure to synthezise polygalacturonase (figure 7) (Tucker &

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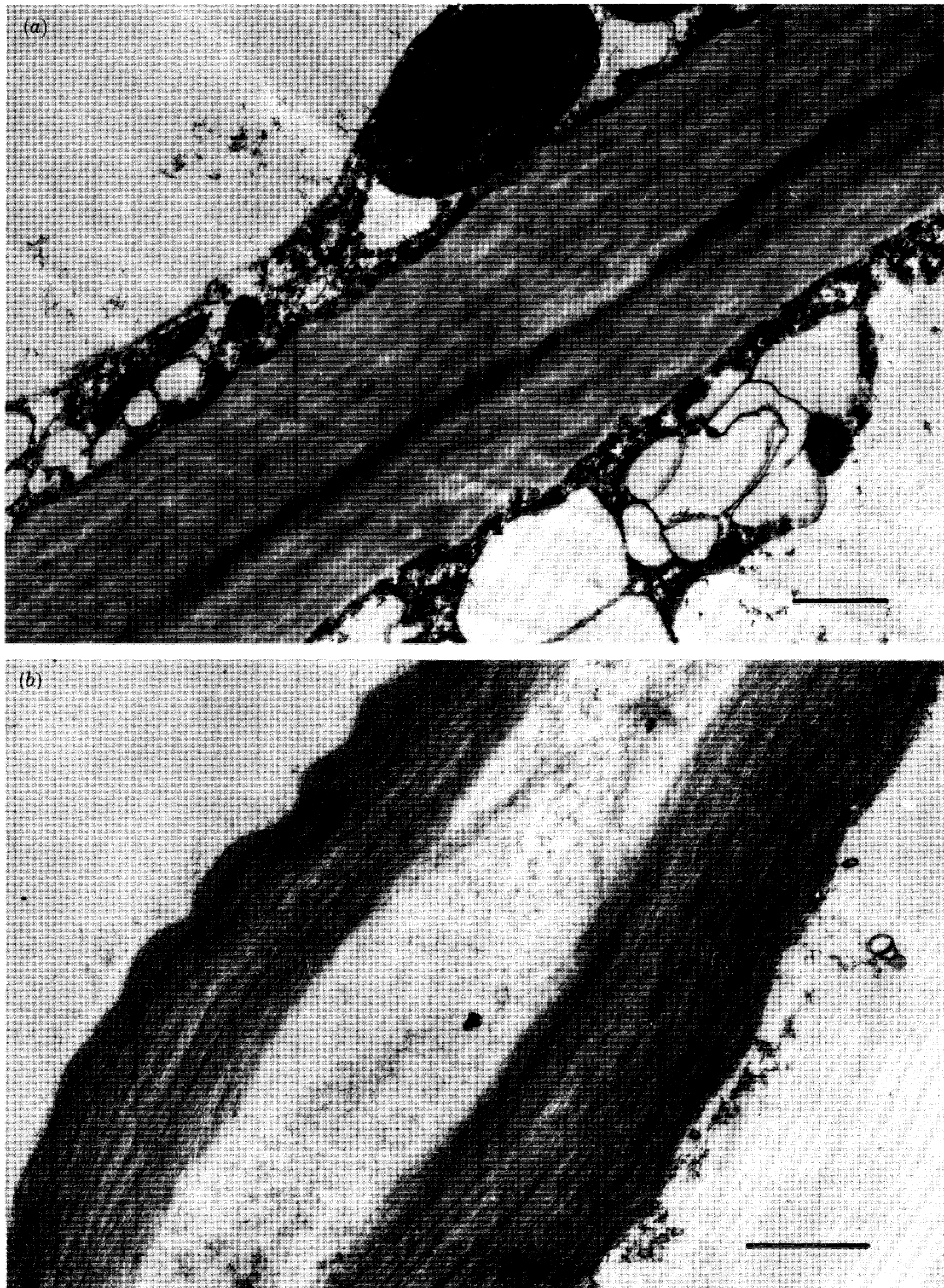

FIGURE 5. Effect of purified polygalacturonase on ultrastructure of cell walls from unripe tomatoes. Cell walls from (*a*) green fruit and (*b*) after incubation in purified polygalacturonase before preparation for electron microscopy. The bar represents 1 μm (see Crookes & Grierson 1983). Figure 7 shows a coomassie blue-stained polyacrylamide gel of wall-associated proteins from unripe and ripe fruit, together with a sample of purified PG-2 used in (*b*).

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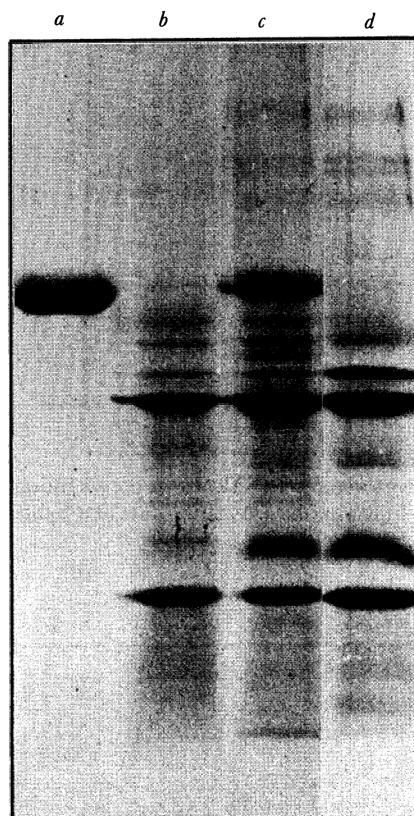

Figure 7. Absence of polygalacturonase in the *rin* mutant. Cell wall associated proteins were prepared from unripe and ripe normal fruit and from yellow fruit of *rin/rin* plants. Proteins were fractionated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis and stained with coomassie blue. Purified PG-2 (*a*), proteins from unripe fruit (*b*), ripe fruit (*c*), and *rin/rin* fruit (*d*) (See Tucker & Grierson (1982).)

Table 3. Properties of fruit homozygous for the *ripening inhibitor* (*rin*) mutation

fruit turn yellow very slowly
very little softening occurs
fruit store for 6–12 months but have poor flavour
there is no
  respiratory climactic
  increase in ethylene synthesis
  accumulation of lycopene
  synthesis of polygalacturonase
adding ethylene leads to increased respiration, induction of the 'ethylene-forming enzyme', but normal ripening is not restored
ethylene synthesis occurs in response to wounding
polygalacturonase is synthesized in flower abscission zones

Grierson 1982). Hybridization of poly$(A)^+$ RNA from *rin/rin* fruit to pTOM 6 cDNA showed that PG mRNA is absent from this fraction (figure 8). The possibility that the mRNA is present but not polyadenylated was investigated by translating total RNA *in vitro*. The results indicate a lack of translatable mRNA for polygalacturonase in *rin/rin* (figure 9). This is not the only deficiency, however, and several other mRNAs are affected. Dot hybridization experiments indicate that pTOM 5 mRNA is absent and there is a reduction in mRNA homologous to

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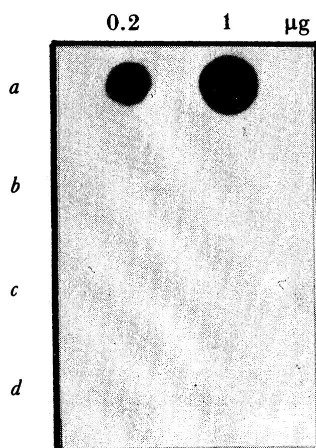

FIGURE 8. Lack of polyadenylated polygalacturonase mRNA in the *rin* mutant. Poly(A)$^+$ RNA from unripe (*b*, *c*) and ripe (*a*, *d*) normal and *rin*/*rin* fruit was fixed to membranes and hybridized to ^{32}P-labelled pTOM 6 DNA. Hybridization was detected by X-ray film.

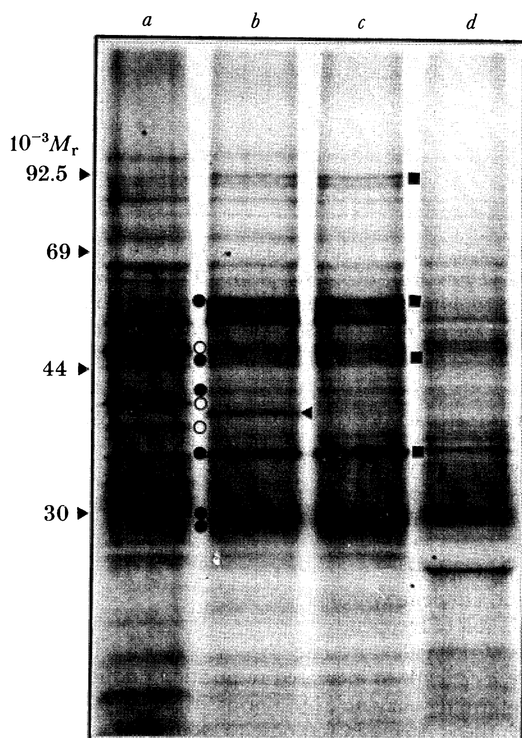

FIGURE 9. Changes in tomato mRNA in response to ethylene and reduction or absence of mRNAs in the *rin* mutant. Total RNA was translated in a rabbit reticulocyte lysate in the presence of [^{35}S]methionine and the products fractionated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis. Radioactive proteins were detected by fluorography. RNA from (*a*) mature green fruit; RNA from fruit after incubation in ethylene (10 μl l^{-1}) for (*b*) 30 h and (*c*) 48 h; (*d*) RNA from *rin*/*rin* fruit incubated in ethylene for five days. The sizes of radioactive marker proteins included in the same gel are shown on the left. Translation products that appear or accumulate in response to ethylene are indicated by solid circles, and those that disappear by open circles. Major differences between *rin* and normal are indicated by solid squares. The PG mRNA encoding a translation product of molecular mass 55 kDa (figure 1) is absent from the *rin*/*rin* sample.

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pTOM 13. Preliminary experiments involving the hybridization of the ripening-specific cDNA clones to nuclear DNA indicate that the genes are present. Thus it seems probable that *rin* affects some part of the regulatory apparatus leading to the expression of ripening genes.

Ethylene and the control of ripening

A large increase in the synthesis of ethylene occurs during the ripening of climacteric fruits (Biale & Young 1981). Several lines of evidence indicate that this is important in stimulating ripening (figure 10). For example, adding ethylene to unripe fruit can hasten the onset of ripening (McGlasson *et al.* 1978; Proctor & Caygill 1985). Inhibiting ethylene synthesis with aminoethoxyvinyl glycine retards ripening, as does removing the gas under hypobaric pressure (Knee 1985). Furthermore, silver ions, which are believed to interfere with either the perception of or the response to ethylene (Beyer 1976) inhibit ripening (Saltveit *et al.* 1979; Hobson *et al.* 1984).

In tomato, increased ethylene synthesis occurs quite early during ripening (Sawamura *et al.* 1978). Adding ethylene to mature green fruit stimulates the synthesis of PG, and during natural ripening the rise in endogenous ethylene production occurs before PG synthesis begins (Grierson & Tucker 1983). The enhanced synthesis of ethylene is correlated with an increase in activity of 1-aminocyclopropane-1-carboxylate (ACC) synthase, which occurs before PG synthesis (Su *et al.* 1984). One intriguing feature is that ethylene promotes its own synthesis by increasing the activity of the two enzymes, ACC synthase and the 'ethylene-forming enzyme', specific to the ethylene biosynthetic pathway (Yang & Hoffman 1984). It is possible

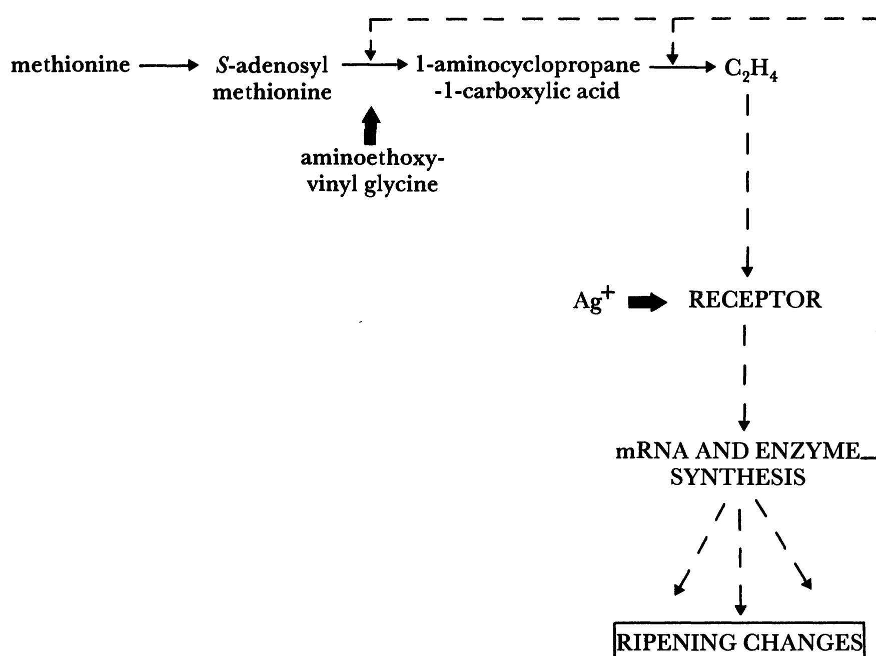

Figure 10. Ethylene and ripening. Ethylene is synthesized from *S*-adenosyl methionine by ACC synthase, which is inhibited by aminoethoxyvinyl glycine, and the 'ethylene-forming enzyme'. The gas stimulates its own synthesis and also promotes ripening. The details of ethylene perception and the response mechanism leading to the expression of ripening genes are not understood. Either ethylene perception or the response processes is inhibited by silver ions.

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that this occurs by *de novo* synthesis of proteins but there is no clear evidence on this point. Once formed, the ethylene is believed to interact with a specific receptor. Ethylene-binding sites have been detected in plant tissues and partly characterized (Sisler 1984; Smith & Hall 1985; Beyer 1985), but our knowledge of the structure and function of the receptors involved in ripening is poor. Ethylene metabolism may be important for its function but this is also unclear (Beyer 1985).

Treating mature-green fruit with ethylene stimulates the disappearance of some mRNAs and the accumulation of others. These changes can be detected within 30 h by translation of total mRNA *in vitro* (figure 9). The major mRNAs that accumulate during ethylene treatment encode proteins with molecular masses of approximately 55, 48, 44, 38, 35, 33 and 28 kDa (figure 9). The mRNA for the 38 kDa protein is especially interesting because it occurs transiently in response to ethylene (figure 9) (Grierson *et al.* 1985*a*). Unfortunately we have not identified a cDNA clone for this mRNA. However, the other major mRNAs encode polypeptides similar to those found by hybrid-select translation with our cDNA clones (figure 1). The mRNAs homologous to all these clones accumulate in green fruit in response to ethylene and are present in significant quantities many hours before ripening changes become visible (figure 11).

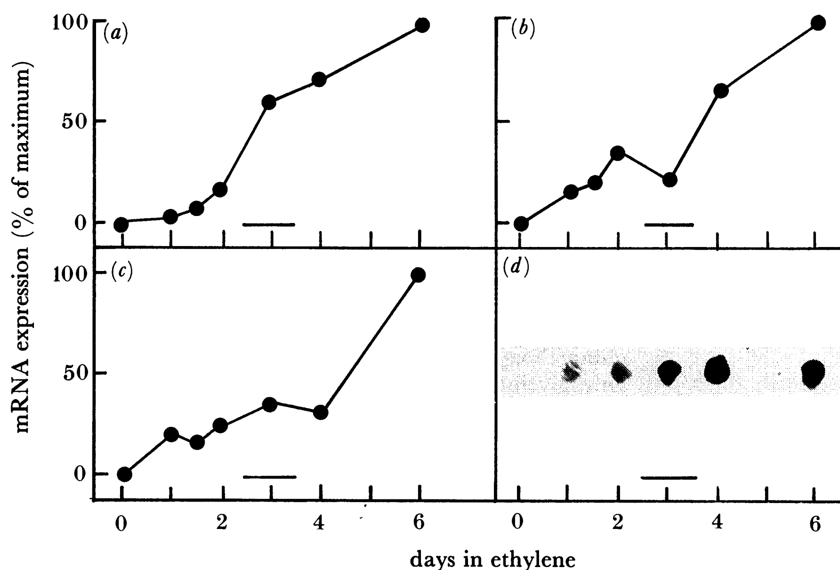

FIGURE 11. Appearance of ripening-specific mRNAs in response to ethylene. Mature green fruit were picked and incubated in ethylene (10 μl l^{-1}) in air. Poly$(A)^+$ RNA samples were prepared at different times, fixed to membranes, and the amount of RNA homologous to (*a*) pTOM 5, (*b*) pTOM 6, and (*c*) pTOM 13 was measured by hybridization and quantified. The dot hybridizations to pTOM 5 are shown in (*d*). The period when changes such as lycopene production and PG synthesis were first detectable is shown by the horizontal bar (see Maunders *et al.* 1986).

DISCUSSION

Our results show that tomato ripening is accompanied by the disappearance of some existing mRNAs and the accumulation of new ones. This is consistent with the recent demonstration that there are alterations in the pattern of proteins synthesized *in vivo* during tomato development and ripening (Baker *et al.* 1985). We have shown that one of the enzymes

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synthesized *de novo* is polygalacturonase, which is involved in cell wall solubilization and fruit softening. Studies with ripening-related cDNA clones indicate that of the 19 mRNAs that increase in amount, at least 5 exhibit a degree of organ specificity and temporal regulation in that they are expressed during ripening but are not present at significant levels in unripe fruit, leaves or roots. The early appearance of these mRNAs is consistent with the suggestion that they are related to changes initiated during ripening. This view is supported by the DNA and amino acid sequence data indicating that one of the clones (pTOM 6) encodes polygalacturonase. The availability of cDNA probes for ripening-specific mRNAs will permit us to study the corresponding genomic sequences. Since transformation and regeneration of plants can be achieved with tomato, by using vectors derived from *Agrobacterium tumefaciens* Ti plasmids, it should be possible to test whether *cis*-acting DNA sequencies are involved in regulating expression during ripening or in response to ethylene.

Studies of *rin*/*rin* tomatoes indicate that they fail to undergo a number of changes associated with normal ripening and are deficient in several mRNAs, including that for polygalacturonase. Several lines of evidence suggest that the mutation affects some part of the regulatory mechanism governing expression of ripening genes. Thus, although *rin*/*rin* fruit do not synthesize ethylene during ripening, they do produce the gas in response to wounding (McGlasson *et al.* 1975). Furthermore, although the fruits do not synthesize polygalacturonase or its mRNA, an immunologically similar enzyme is produced in the flower abscission zones (Tucker & Roberts 1984). Genomic blots indicate that there are no differences in the organization of the genes homologous to pTOM 6 in normal and *rin*/*rin* plants (P. Moureau, unpublished). It has been suggested that *rin* is a mutation affecting the ethylene receptor (McGlasson 1985). However, this seems unlikely because *rin*/*rin* tomatoes respond to ethylene by showing a rise in respiration (Herner & Sink 1973) and also there is an increase in activity of the 'ethylene-forming enzyme' (Liu *et al.* 1985). It seems more probable, therefore, that the mutation affects part of the response mechanism that switches on the 'ripening genes'.

Although ethylene stimulates the accumulation of ripening-specific mRNAs, we have no clear picture of the mechanisms involved. Further work is urgently required on the receptor and the subsequent signal transduction mechanism. Another intriguing aspect of the problem is how the production of ethylene itself is regulated. These questions are of practical importance, not only for the control of ripening, but also for related processes such as abscission and the senescence of flowers and leaves.

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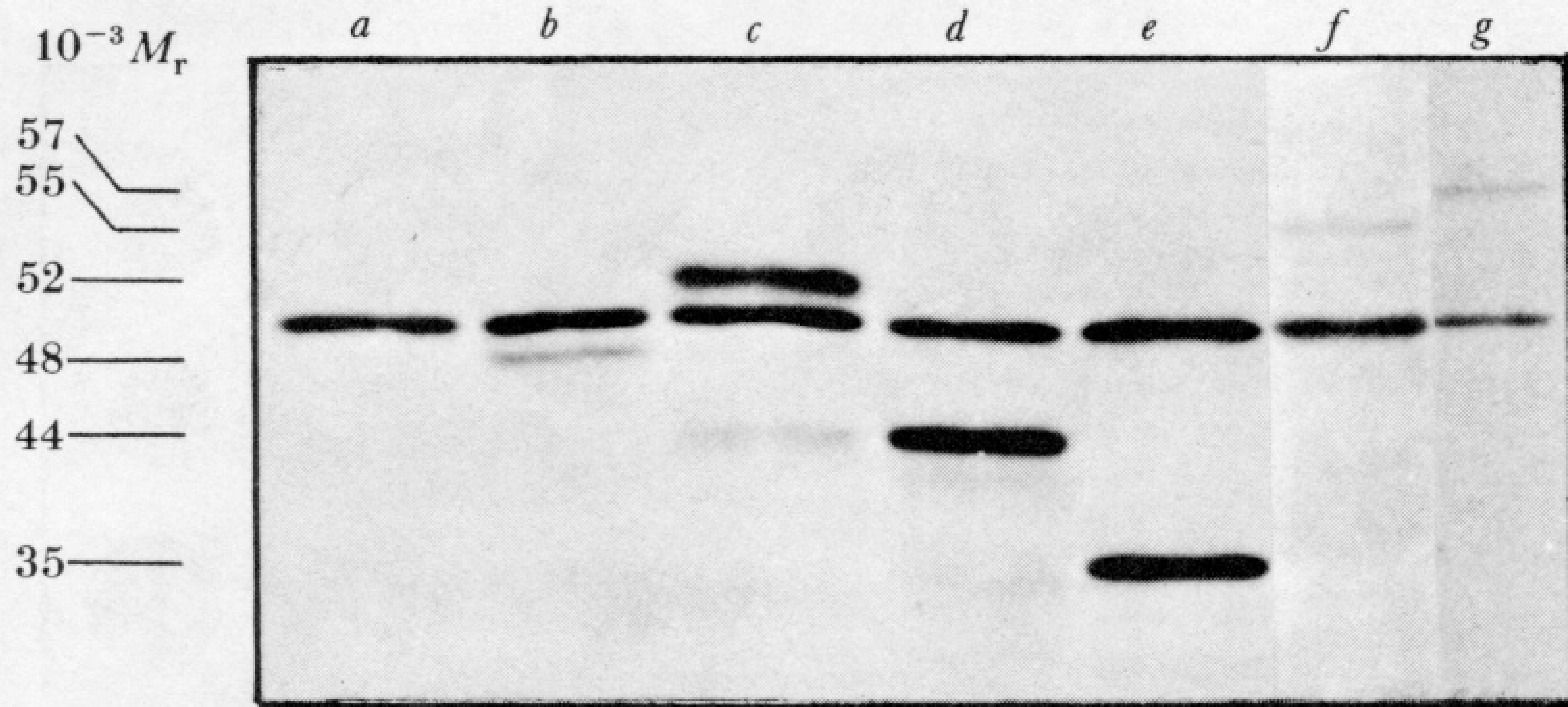

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GURE 1. Translation *in vitro* of mRNA hybrid-selected by ripening-related cDNA clones. Tomato fruit mRNA was hybridized to DNA from each clone and the mRNA was recovered and translated in a rabbit reticulocyte lysate in the presence of [^{35}S]methionine. Radioactive proteins were fractionated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis and detected by fluorography. The sizes of the polypeptides, determined from the mobility of radioactive marker proteins in the same gel, are shown on the left. A polypeptide of approximately 50 kDa became labelled without the addition of mRNA (track *a*). Translation products of mRNAs homologous to pTOM 5 (track *b*), pTOM 36 (track *c*), pTOM 99 (track *d*), pTOM 13 (track *e*), pTOM 6 (track *f*), and pTOM 137 (track *g*) (see Slater *et al.* 1985).

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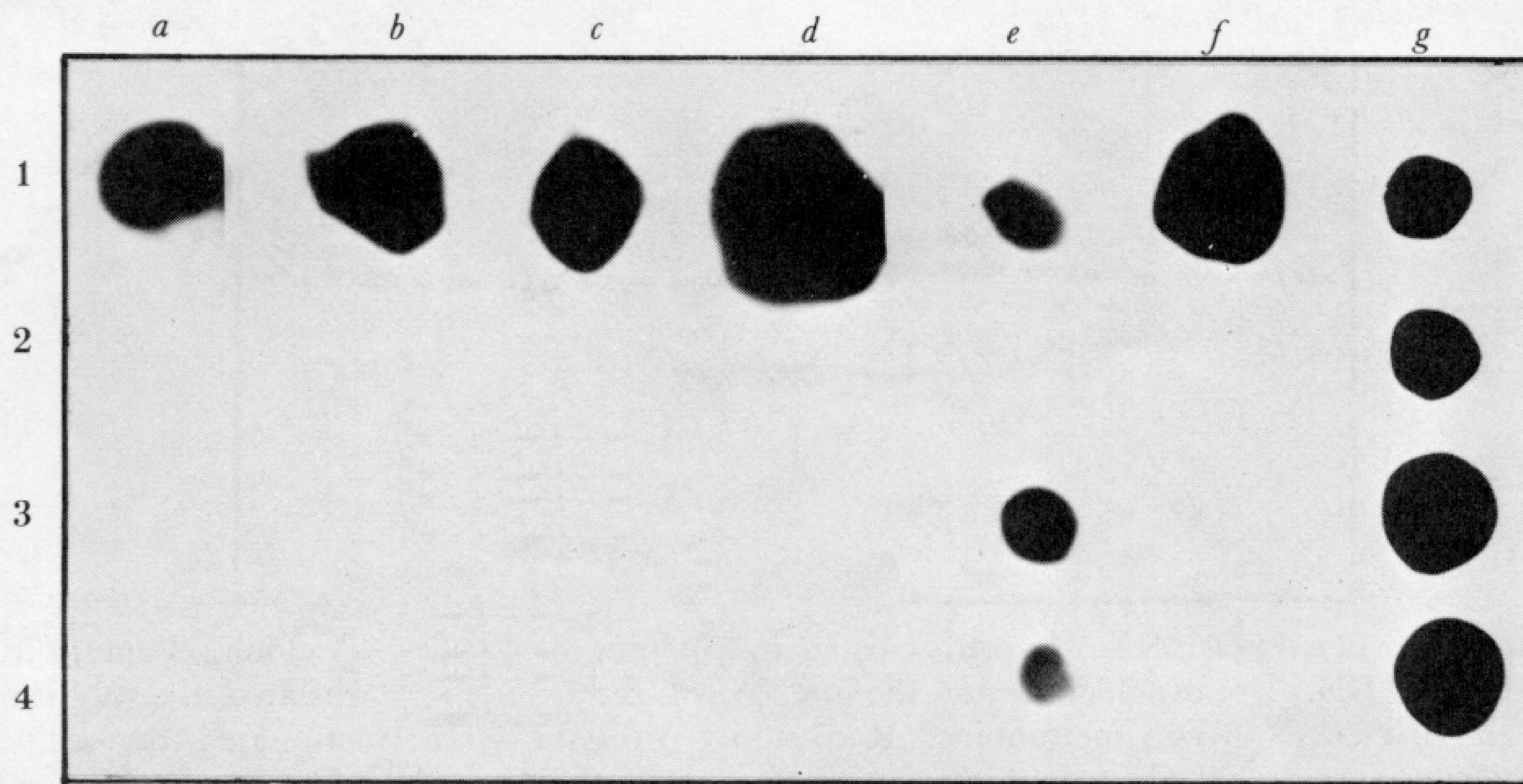

FIGURE 2. Expression in different organs of cloned ripening-related mRNAs. Poly(A)$^+$ RNA (1 μg) from ripe (1) and unripe (2) fruit, roots (3) and leaves (4) was fixed to membranes and probed with ^{32}P-labelled cDNA from pTOM 5 (*a*), pTOM 6 (*b*), pTOM 13 (*c*), pTOM 36 (*d*), pTOM 75 (*e*), pTOM 99 (*f*), and pTOM 137 (*g*). Hybridization was detected by X-ray film (see Maunders *et al.* 1986).

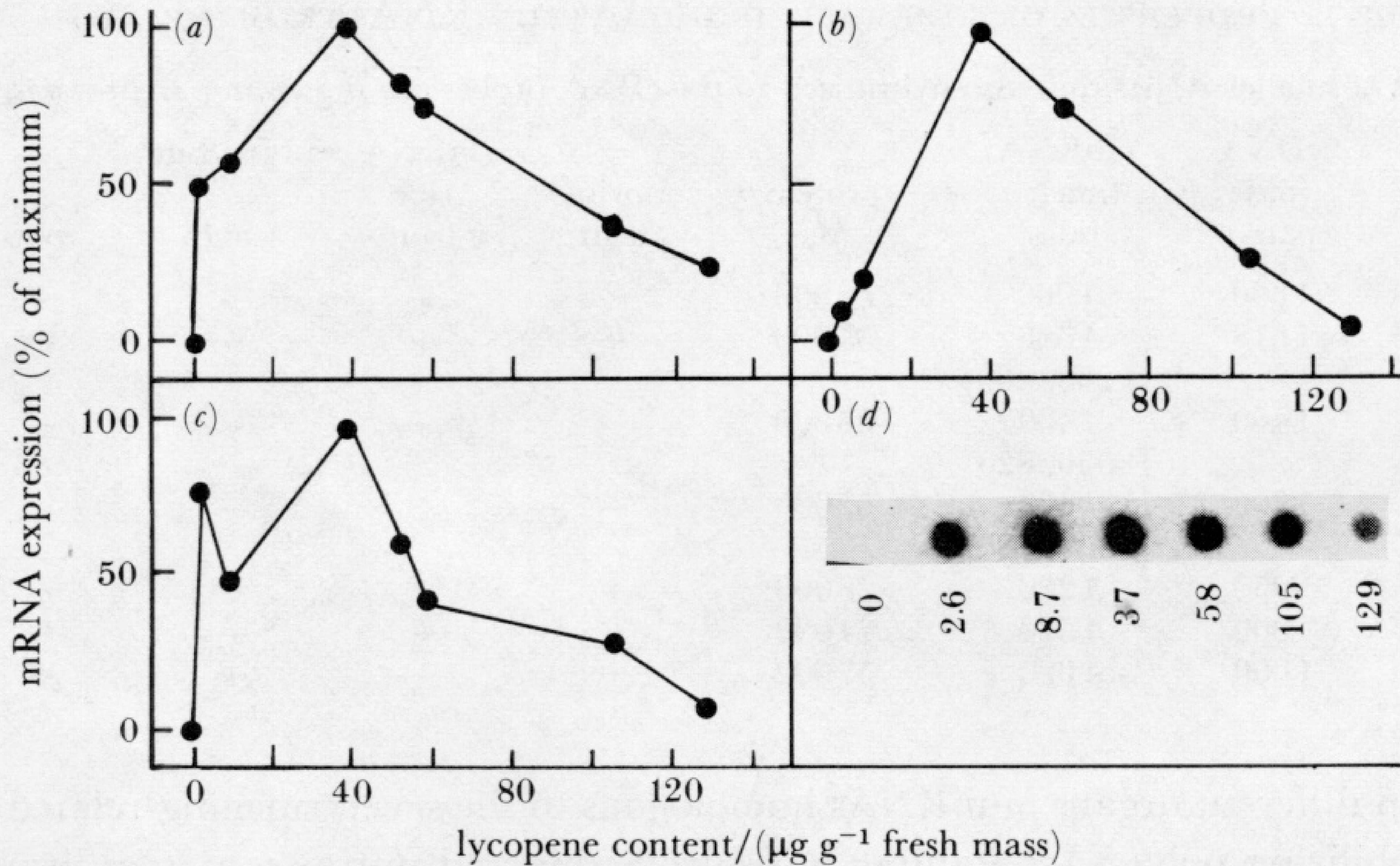

FIGURE 3. Expression of mRNA homologous to cDNA clones during ripening. RNA samples from mature green fruit and at different stages of ripening were fixed to membranes and hybridized to ^{32}P-labelled cDNA clones. Hybridization was detected by X-ray film. Results were quantified employing a range of dilutions and DNA standards and scanning the X-ray film with a densitometer. The stage of ripening is indicated by the lycopene content. Results are for mRNA homologous to pTOM 5 (*a*), pTOM 6 (*b*), and pTOM 13 (*c*). The dot hybridization to pTOM 5 is shown in (*d*), where the numbers beneath the dots refer to the lycopene content (see Maunders *et al.* 1986).

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(a)

(b)

FIGURE 5. Effect of purified polygalacturonase on ultrastructure of cell walls from unripe tomatoes. Cell walls from (a) green fruit and (b) after incubation in purified polygalacturonase before preparation for electron microscopy. The bar represents 1 μm (see Crookes & Grierson 1983). Figure 7 shows a coomassie blue-stained polyacrylamide gel of wall-associated proteins from unripe and ripe fruit, together with a sample of purified PG-2 used in (b).

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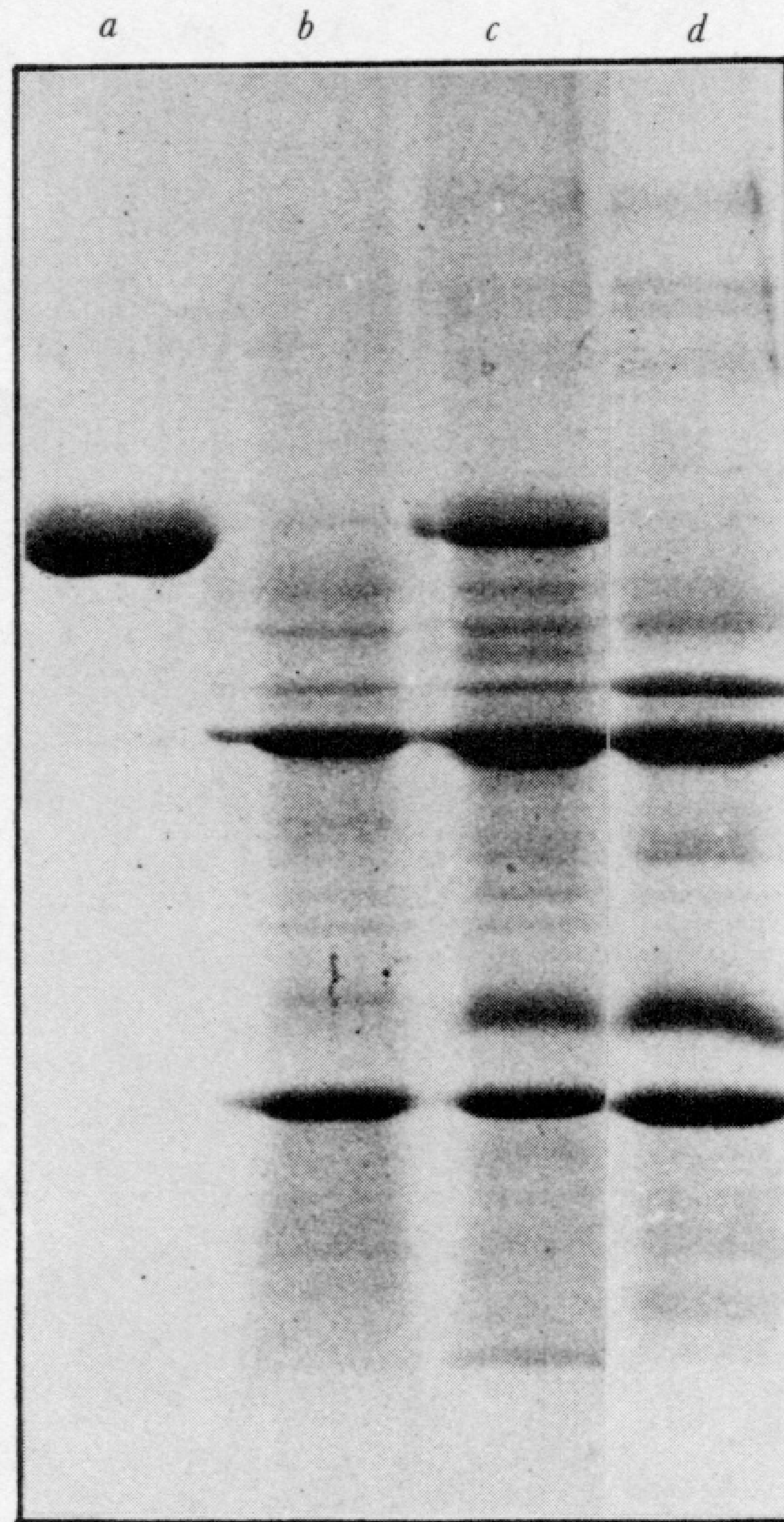

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'IGURE 7. Absence of polygalacturonase in the *rin* mutant. Cell wall associated proteins were prepared from unripe and ripe normal fruit and from yellow fruit of *rin*/*rin* plants. Proteins were fractionated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis and stained with coomassie blue. Purified PG-2 (*a*), proteins from unripe fruit (*b*), ripe fruit (*c*), and *rin*/*rin* fruit (*d*) (See Tucker & Grierson (1982).)

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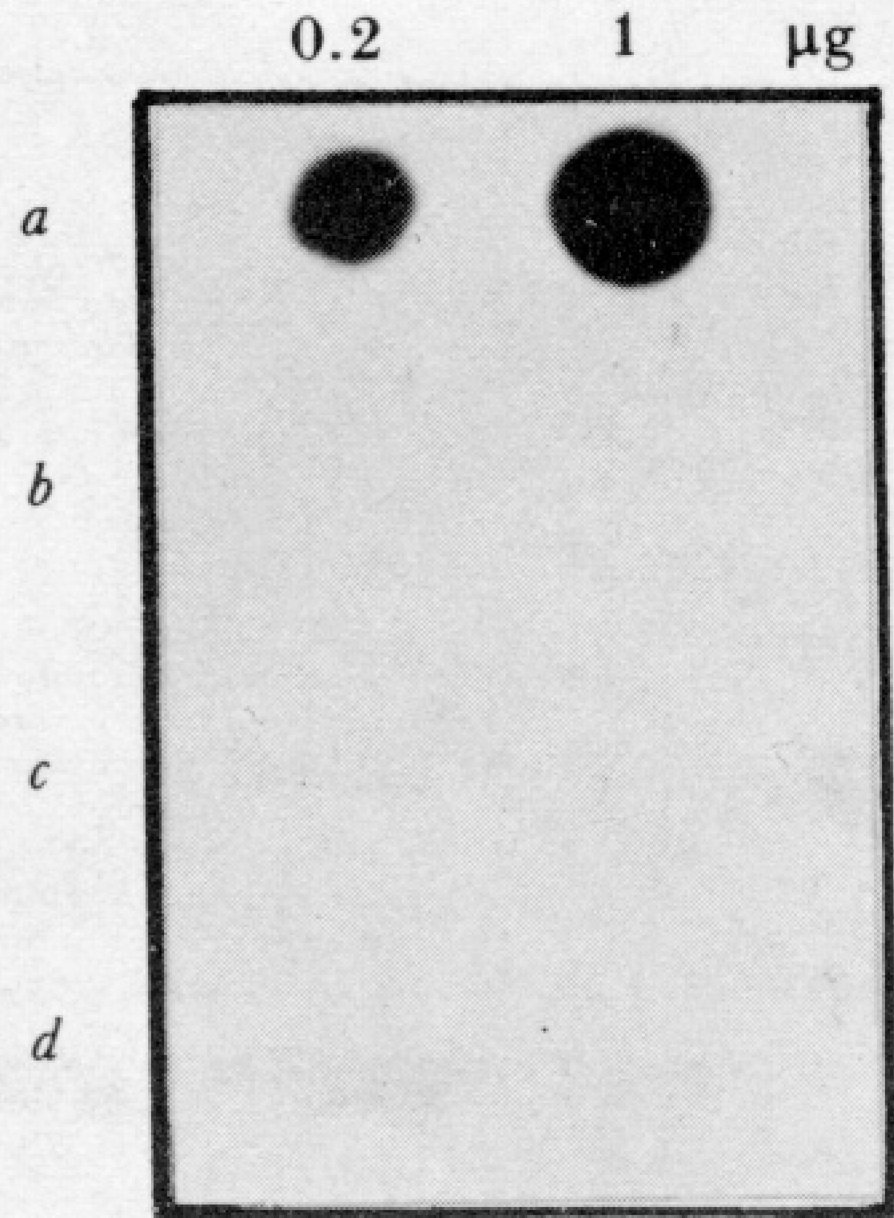

GURE 8. Lack of polyadenylated polygalacturonase mRNA in the *rin* mutant. Poly(A)$^{+}$ RNA from unripe (*b*, *c*) and ripe (*a*, *d*) normal and *rin*/*rin* fruit was fixed to membranes and hybridized to ^{32}P-labelled pTOM 6 DNA. Hybridization was detected by X-ray film.

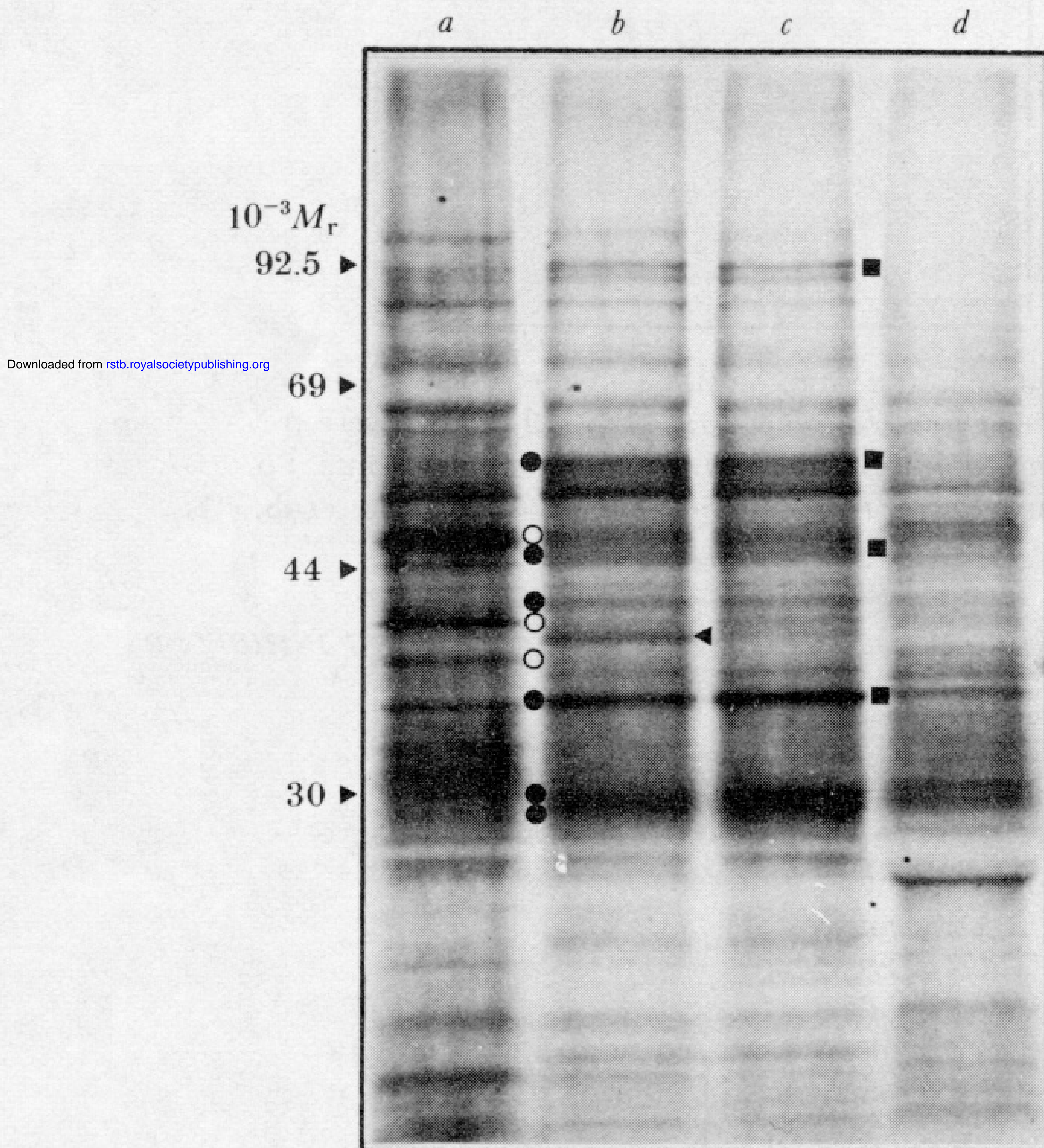

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FIGURE 9. Changes in tomato mRNA in response to ethylene and reduction or absence of mRNAs in the *rin* mutant. Total RNA was translated in a rabbit reticulocyte lysate in the presence of [^{35}S]methionine and the products fractionated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis. Radioactive proteins were detected by fluorography. RNA from (*a*) mature green fruit; RNA from fruit after incubation in ethylene (10 μl l^{-1}) for (*b*) 30 h and (*c*) 48 h; (*d*) RNA from *rin*/*rin* fruit incubated in ethylene for five days. The sizes of radioactive marker proteins included in the same gel are shown on the left. Translation products that appear or accumulate in response to ethylene are indicated by solid circles, and those that disappear by open circles. Major differences between *rin* and normal are indicated by solid squares. The PG mRNA encoding a translation product of molecular mass 55 kDa (figure 1) is absent from the *rin*/*rin* sample.

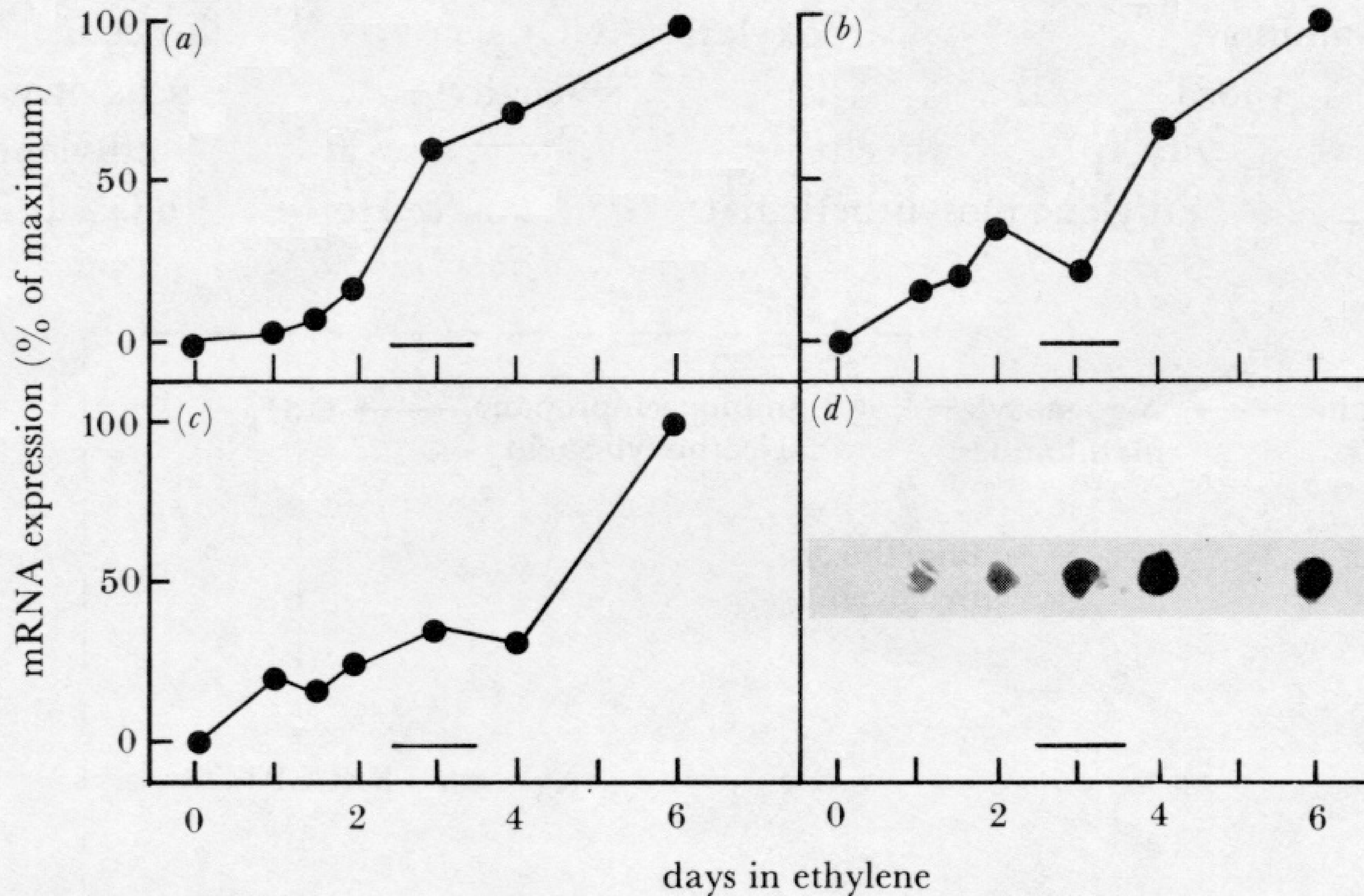

FIGURE 11. Appearance of ripening-specific mRNAs in response to ethylene. Mature green fruit were picked and incubated in ethylene (10 μl l^{-1}) in air. $Poly(A)^+$ RNA samples were prepared at different times, fixed to membranes, and the amount of RNA homologous to (*a*) pTOM 5, (*b*) pTOM 6, and (*c*) pTOM 13 was measured by hybridization and quantified. The dot hybridizations to pTOM 5 are shown in (*d*). The period when changes such as lycopene production and PG synthesis were first detectable is shown by the horizontal bar (see Maunders *et al.* 1986).

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