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Characterization of Two Divergent Endo-β-1,4-Glucanase cDNA Clones Highly Expressed in the Nonclimacteric Strawberry Fruit

Immaculada Llop-Tous, Eva Domínguez-Puigjaner, Xavier Palomer, and Miquel Vendrell*

Departmento de Agrobiologia, Centro de Investigación y Desarrollo, Consejo Superior de Investigaciones Científicas, Jordi Girona, 18–26, 08034 Barcelona, Spain

Two cDNAs clones (*Cel1* and *Cel2*) encoding divergent endo-β-1,4-glucanases (EGases) have been isolated from a cDNA library obtained from ripe strawberry (*Fragaria x ananassa* Duch) fruit. The analysis of the amino acid sequence suggests that *Cel1* and *Cel2* EGases have different secondary and tertiary structures and that they differ in the presence of potential *N*-glycosylation sites. By in vitro translation we show that *Cel1* and *Cel2* bear a functional signal peptide, the cleavage of which yields mature proteins of 52 and 60 kD, respectively. Phylogenetic analysis revealed that the *Cel2* EGase diverged early in evolution from other plant EGases. Northern analysis showed that both EGases are highly expressed in fruit and that they have different temporal patterns of accumulation. The *Cel2* EGase was expressed in green fruit, accumulating as the fruit turned from green to white and remaining at an elevated, constant level throughout fruit ripening. In contrast, the *Cel1* transcript was not detected in green fruit and only a low level of expression was observed in white fruit. The level of *Cel1* mRNA increased gradually during ripening, reaching a maximum in fully ripe fruit. The high levels of *Cel1* and *Cel2* mRNA in ripe fruit and their overlapping patterns of expression suggest that these EGases play an important role in softening during ripening. In addition, the early expression of *Cel2* in green fruit, well before significant softening begins, suggests that the product of this gene may also be involved in processes other than fruit softening, e.g. cell wall expansion.

Fruit softening during ripening is a major factor contributing to postharvest deterioration. Loss of firmness in fruits is mainly due to cell wall disassembly, resulting in a significant increase in polyuronide and hemicellulose solubilization. The mechanisms by which this solubilization occurs are unclear and may differ between species. In tomato, the best-studied fruit, polyuronide solubilization occurs through its depolymerization by hydrolytic enzymes. In this fruit the enzyme PG plays an important role in pectin depolymerization during ripening (Themmen et al., 1982; Brady et al., 1983). However, it has been suggested that other pectolytic enzymes, such as pectate lyase, may also be involved in pectin metabolism accompanying fruit softening (Domínguez-Puigjaner et al., 1997). In contrast to tomato, solubilization of polyuronide in strawberry (*Fragaria x ananassa* Duch) fruit involves different mechanisms, because no reduction in pectin chain length is observed during softening (Huber, 1984). Huber suggested that increased levels of soluble polyuronide in strawberry fruit are due mainly to the synthesis of a more freely soluble form during ripening and that enzymic hydrolysis of polyuronide is not a likely cause for their solubilization. Accordingly, the activity of the pectolytic PG is found only at very low levels in strawberry fruit (Nogata et al., 1993). However, it has been reported recently that the expression of pectate lyase correlates with the softening of strawberry fruit, suggesting that the action of this enzyme in polyuronide solubilization cannot be excluded (Medina-Escobar et al., 1997).

Although polyuronide solubilization has been generally believed to be the major factor contributing to fruit softening, the expression of a chimeric PG in tomato mutant *rin* shows that polyuronide degradation and solubilization to near wild-type levels is not sufficient to cause fruit softening (Giovannoni et al., 1989; DellaPenna et al., 1990). This observation suggests that the metabolism of nonpectolytic cell wall polymers such as hemicellulose and cellulose may also play an important role in the decline of fruit firmness during ripening.

Xyloglucans, the predominant hemicellulose in dicotyledonous plants, are thought to play a pivotal role in cell wall architecture, because they can form extensive cross-links between cellulose microfibrils, locking them together (Brett and Waldron, 1996). Enzymes such as xyloglucan endotransglycosylases (Arrowsmith and Silva, 1995), expansins (Rose et al., 1997), and EGases have been proposed as allies cooperating in the modification of the hemicellulose network during fruit ripening. However, the specific contribution of each of these enzymes in fruit softening remains unclear.

Egases (EC 3.2.1.4), commonly referred to as cellulases, are usually assayed by their capacity to degrade the artificial substrate carboxymethylcellulose. Although the natural substrate for plant EGases is unknown, it has been shown (Brummell et al., 1994) that they hydrolyze β-1,4-

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* Corresponding author; e-mail mvmagr@cid.csic.es; fax 34–3–204–5904.

Abbreviations: EGase, endo-β-1,4-glucanase; PG, polygalacturonase.

linked glucans in vitro, suggesting that xyloglucan is a likely substrate for EGases in vivo. In addition, although plant EGases are unable to degrade crystalline cellulose, they may be able to attack noncrystalline regions of the cellulose microfibrils, modifying the nature of fibril organization (O'Donoghue et al., 1994). EGase activity is associated with several processes that require cell wall weakening, including cell elongation, organ abscission, and fruit softening. Brummell et al. (1994) reported that the softening of fruits such as tomato, avocado, and strawberry accompanies an increase in EGase activity and temporally correlates with a decrease in the average molecular size of xyloglucan.

Ripening-related EGase cDNAs have been characterized from avocado (Tucker et al., 1987; Cass et al., 1990), tomato (Lashbrook et al., 1994), and pepper (Ferrarese et al., 1995; Harpster et al, 1997). However, little effort has been exerted to characterize EGase genes in strawberry fruit, and to date only a partial cDNA clone has been isolated from this fruit (Manning, 1998).

In an effort to understand the essential features of the softening of nonclimacteric strawberry fruit, we have focussed our research on the isolation and characterization of genes encoding EGases that accumulate during ripening. We have found two divergent EGase transcripts expressed in ripe strawberry fruit; each of these shows a different pattern of accumulation during ripening.

MATERIALS AND METHODS

Plant Material

Our experiments used strawberry (*Fragaria x ananassa* Duch cv Selva) fruits harvested at different stages of ripening, as assessed by the coloration of the fruit surface. We selected the following stages: green (S0), mature-green with a white surface (S1), one-fourth (S2) or three-fourths (S3) surface with red pigmentation, and fully red (S4) fruits. For RNA extraction achenes and the central fibrous core were removed, and the remaining receptacle tissue was immediately frozen with liquid nitrogen and stored at −80°C until needed.

For construction of a cDNA library, we used ripe fruit that had been stored at 4°C for 2 d under a continuous flow of humidified air.

RNA Preparation and Analysis

Total RNA was extracted from strawberry fruits or leaves as described earlier by Domínguez-Puigjaner et al. (1997). RNA (20 μg) was fractionated on 1.5% agarose/2.2 M formaldehyde denaturing gels and capillary blotted onto a membrane (Hybond N, Amersham) as described by Ausubel et al. (1992). Northern blots were hybridized with random-primed DNA probes synthesized with the Ready-to-Go system (Pharmacia) using full-length *Cel1* and *Cel2* cDNAs as the templates. Hybridization was carried out at 42°C for 16 h, as described by Amasino (1986). Filters were washed three times for 15 min in 3× SSC and 0.5% (w/v) SDS at 65°C.

Reverse Transcriptase-PCR

We used reverse transcriptase-PCR to amplify EGase transcripts from total RNA extracted from ripe strawberries. First-strand cDNA synthesis used Moloney murine leukemia virus reverse transcriptase (Promega), following the manufacturer's instructions. Approximately 50 ng of the obtained cDNA was used for the PCR amplification with degenerate primers (5′-GGNTAYTAYGAYGCNGGNGAY-AAY-3′ and 5′-CCWACCATRTANSACAT-3′), designed on the highly conserved amino acid sequences GYYDAGDN and MSYMVG. The reaction was carried out in a 40-μL volume containing 0.2 μM each primer, 0.2 mM dNTP, and 2.5 mM $MgCl_2$. The template was denatured at 95°C for 1 min, annealed at 60°C for 2 min, and extended at 72°C for 2 min. We repeated this cycle 35 times and followed it by one additional cycle in which the template was extended for 7 min. Under these conditions the PCR reaction generated two bands. The expected 980-bp band was recovered from the agarose gel, purified, and used as a probe to screen a cDNA library.

cDNA Library Construction and Screening

We obtained poly(A^+) mRNA from ripe fruit kept for 2 d at 4°C, using an mRNA-isolation system (PolyATtract, Promega) according to the instructions provided. We recovered double-stranded cDNA from fruit poly(A^+) RNA using a cDNA synthesis kit (λ-ZAP, Stratagene). The cDNA was ligated to Uni-ZAP XR vector arms and packaged in vitro using a Gigapack-II packaging extract (Stratagene). Using as a probe the cDNA fragment obtained by reverse transcriptase-PCR, we screened approximately 10^5 clones of the resulting cDNA library. Plaques hybridizing with the probe were purified in a secondary and tertiary screening. Ten plaques were selected and resuspended in 500 μL of buffer (0.1 M NaCl, 8 mM $MgSO_4$, 50 mM Tris-HCl, pH 7.5), from which 2 μL were used to amplify the insert by PCR with the T3 and T7 primers present in the Uni-ZAP XR vector. The obtained PCR fragment was digested by restriction enzymes and the corresponding restriction map analyzed. Our analysis then used clones with different restriction patterns after the in vivo excision of the corresponding pBluescript.

Genomic DNA Isolation and Southern Analysis

We obtained genomic DNA from ripe fruit as described by Prat et al. (1989). DNA extracted from isolated nuclei was purified through a gradient of CsCl containing 1% (w/v) sarcosyl. Total DNA (5 μg) was digested with *Eco*RI or *Hin*dIII and separated by electrophoresis on a 0.8% (w/v) agarose gel. After denaturing, the DNA was transferred to a nylon membrane and hybridized with a random-primed DNA probe prepared from the 3′-untranslated region of *Cel1* or *Cel2* cDNA. Hybridization and washing conditions were the same as for northern-analysis procedures.

In Vitro Transcription and Translation of *Cel1* and *Cel2*

Plasmid DNA (1 μg) containing *Cel1* or *Cel2* cDNAs was linearized with restriction enzymes and transcribed using T3 or T7 RNA polymerase (Promega) following the manufacturer's instructions. The resulting mRNA was purified and in vitro translated in a rabbit reticulocyte lysate (Promega) system, using [^{35}S]Met as an amino acid precursor. To obtain the mature protein from which the signal peptide had been excised, canine pancreatic microsomal membranes (Promega) were added to the translation reaction according to the manufacturer's protocol.

Protein Analysis

The in vitro translation products were mixed with an equal volume of sample buffer (120 mM Tris-HCl, pH 6.8, 20% [v/v] glycerol, 4% [w/v] SDS, and 10% [v/v] 2-mercaptoethanol). The ^{35}S-labeled protein was fractionated on a one-dimensional 12.5% SDS-PAGE gel, as described by Laemmli (1970).

Gels were stained with Coomassie blue to show nonradiolabeled molecular mass markers. After destaining in 30% methanol/7% acetic acid, gels were washed exhaustively with water and incubated afterward with 1 M sodium salicylate, pH 6.0, for 15 min before drying under a vacuum. The dried gels were exposed to preflashed Kodak film.

DNA Sequencing and Sequence Homology Analysis

We used standard dideoxy sequencing (Sanger et al., 1977) to sequence both strands of the *Cel1* and *Cel2* cDNAs. An automated laser-fluorescent DNA sequencer (Pharmacia) generated the sequence data. The nucleotide sequence data obtained and the amino acid sequence derived were compared with other sequences in the database, using a Genetics Computer Group (Madison, WI) program. The Megalign Program, Clustal method, with a PAM250 residue weight table, generated the phylogenetic tree shown in Figure 2.

RESULTS

Isolation of Strawberry EGase cDNA Clones

EGase transcripts expressed in ripe strawberry fruit were amplified from total RNA by means of reverse transcriptase-PCR using degenerate primers. We obtained a band of the expected molecular mass (980 bp) and used it as a probe to screen a ripe strawberry fruit cDNA library. A large number of hybridizing phage plaques were captured, accounting for the 0.6% of the total clones screened. We selected and purified 10 plaques after two rounds of screening. Restriction endonuclease mapping of the inserts amplified by PCR revealed that the clones could be classified into two groups, from which the longest clones, *Cel1* and *Cel2*, were chosen for sequencing.

Sequence Analysis

Sequence analysis of *Cel1* and *Cel2* cDNA revealed that they were 1690 and 2503 bp, respectively, in length, including an open reading frame of 1488 and 1683 bp that encoded two proteins homologous to EGases. Comparison of *Cel1* and *Cel2* sequences revealed that the EGases shared only 46% identity at the amino acid level. When compared with other EGases in the database, the protein encoded by *Cel1* showed 78% amino acid identity with the tomato ripening-related *Cel2* (Lashbrook et al., 1994). In contrast, the deduced *Cel2* protein showed only 49% amino acid identity to the same tomato EGase and similar low values when compared with other plant EGases. Optimal alignment of the two strawberry EGases and tomato *Cel2* (Fig. 1) revealed that the *Cel2* protein bore several insertions and deletions in its sequence. The longest insertion was found in the C terminus of *Cel2* protein, which showed 67 more residues than strawberry *Cel1* and 66 more than tomato *Cel2*. Comparison of this sequence with proteins in the database did not reveal any close homology.

The analysis of the deduced amino acid sequence indicated that both EGases contained a hydrophobic N-terminal sequence characteristic of secreted proteins. As assessed by

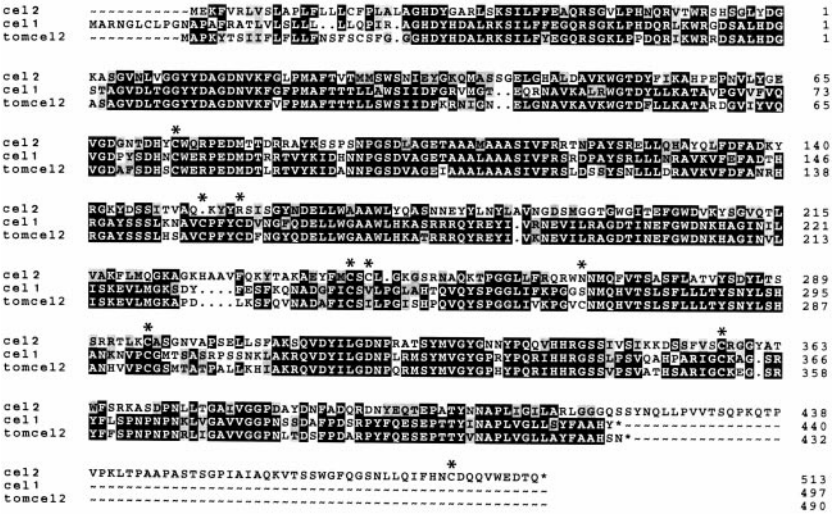

Figure 1. Alignment of strawberry *Cel1* (cel 1) and *Cel2* (cel 2) predicted proteins with tomato cellulase *Cel2* (tomcel2). Black boxes identify the residues shared by at least two of the cellulases. Conservative amino acid substitutions are represented by gray boxes. Gaps are introduced to optimize alignment. The Cys residues are indicated by asterisks.

the rules of von Heijne (1983), strawberry *Cel1* and *Cel2* proteins possessed predicted signal sequences of 32 and 24 amino acid residues, respectively. After the signal sequence was processed, *Cel1* and *Cel2* mature proteins had a calculated molecular mass of 51.5 and 59.6 kD, with basic predicted pIs of 9.4 and 8.6, respectively.

In addition to the different expected molecular masses of *Cel1* and *Cel2* mature proteins, other interesting differences were deduced from their amino acid sequences. The lack of alignment of some of the Cys residues suggested that the two strawberry EGases had different secondary and tertiary structures. Moreover, they also differed because of the presence of motifs for *N*-glycosylation, which are frequently found in secreted proteins. Whereas *Cel1* protein contained an Asn at position 460 that suits the consensus motif for *N*-glycosylation (Asn-X-Ser/Thr), the *Cel2* EGase did not bear this consensus sequence.

Figure 2 illustrates the evolutionary relationship between plant EGases from which the entire amino acid sequences are available. The phylogenetic tree shows that strawberry *Cel2* belongs to a new evolutionary branch that diverged early from other known plant EGases, including strawberry *Cel1*. Based on this dendogram, the strawberry *Cel1* EGase is closely related to the tomato *Cel2* expressed during ripening, as well as to other EGases that are involved in cell expansion or abscission processes.

In Vitro Translation of the EGases Encoded by *Cel1* and *Cel2*

Linearized plasmids containing *Cel1* and *Cel2* cDNA were in vitro transcribed, and the obtained RNA was used to translate the corresponding EGases in a rabbit reticulocyte system. To assess the functionality of the signal peptide, the translation reaction was performed simultaneously in the presence or absence of canine pancreatic microsomal membranes, which caused the cleavage of the signal peptide.

Analysis by one-dimensional SDS-PAGE of the product translated without microsomal membranes indicated that the immature *Cel1* mRNA was translated into a 55-kD protein, whereas the *Cel2* protein had an apparent molecular mass of 62 kD (Fig. 3, lanes I). The differences in molecular mass are in agreement with the observation that *Cel2* cDNA had an open reading frame 195 bp longer than that of *Cel1*.

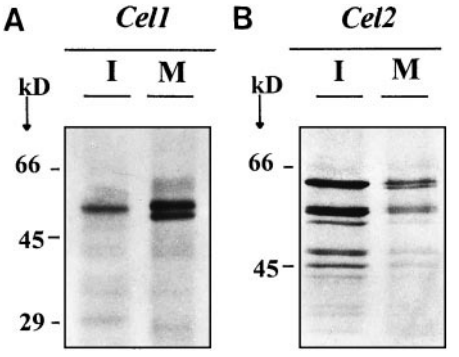

Figure 3. In vitro translation of *Cel1* (A) and *Cel2* (B) in a rabbit reticulocyte system using [^{35}S]Met as the amino acid precursor. The mature proteins were obtained by including canine pancreatic microsomal membranes in the translation reaction. The translated products were fractionated by SDS-PAGE. Lanes I, Immature protein, prior to the cleavage of the signal peptide; lanes M, mature protein.

The incorporation of microsomal membranes in the translation reaction resulted in the cleavage of the signal peptide and the appearance of the mature protein, which possessed an apparent molecular mass of 52 kD for *Cel1* and 60 kD for *Cel2* (Fig. 3, lanes M).

Analysis of EGase Gene Expression by Northern Analysis

Northern-blot assay was used to assess the accumulation of *Cel1* and *Cel2* transcripts in leaves and throughout strawberry fruit development and ripening. Full-length EGase cDNAs were used to obtain the random-primed probes.

As shown in Figure 4, we detected no hybridization signal in the leaves, whereas both EGase transcripts were highly expressed in the fruit of the strawberry. However, *Cel1* and *Cel2* EGases showed distinct temporal patterns of accumulation in the strawberry fruit. *Cel2* mRNA was expressed in green fruit and its levels rose as the fruit turned from green to white, remaining at an elevated, constant level until the fruit reached their full-red color. In contrast, expression of the *Cel1* gene occurred at a later stage than that of *Cel2* and increased as the fruit ripened. The *Cel1* transcript was not observed in the green fruit and it was

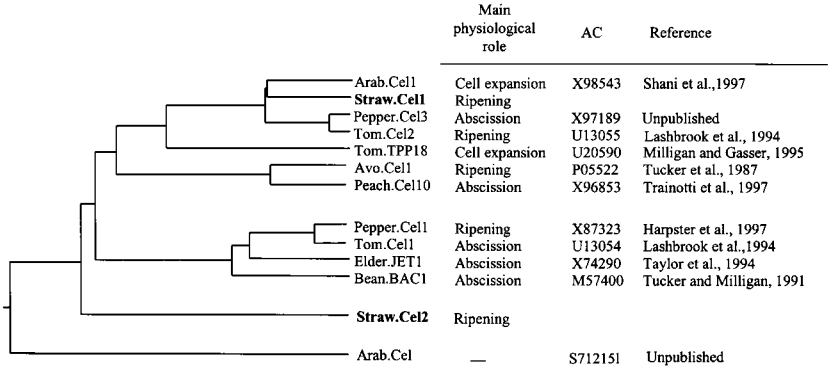

Figure 2. Phylogenetic analysis of the plant cellulases from which the complete amino acid sequence is available. The phylogenetic tree was obtained as described in the text. Arab., Arabidopsis; Straw, strawberry, Tom, tomato; Avo, avocado; AC, accession number.

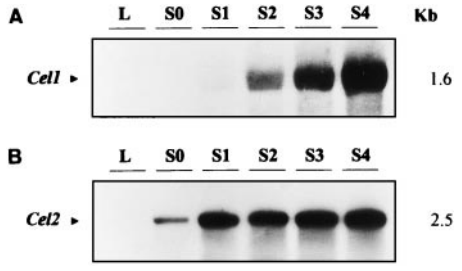

Figure 4. Northern analysis of the expression of *Cel1* (A) and *Cel2* (B) in leaves (lanes L) and fruit at different stages of growth and ripening. Lanes S0, Green fruit; lanes S1, white fruit; lanes S2, fruit with one-fourth red surface; lanes S3, fruit with three-fourths red surface; and S4, fully red fruit. Each lane contained 20 μg of total RNA. Northern blots were hybridized with random-primed probes obtained from full-length cDNAs. Both Northern blots were exposed overnight at −80°C.

only just detectable in the white fruit. Expression of this EGase was clear at the onset of ripening, when the synthesis of anthocyanins was evident. *Cel1* mRNA accumulated gradually as ripening proceeded, reaching maximum levels in fully ripe fruit.

Southern Analysis

Southern blots of genomic DNA digested with the restriction enzymes *Eco*RI and *Hin*dIII were hybridized with random-primed probes obtained from either *Cel1* or *Cel2* 3′-untranslated regions (Fig. 5). Comparison of the two Southern blots indicated that *Cel1* and *Cel2* probes did not cross-react, each probe hybridizing to a different array of fragments. Both probes hybridized preferentially to a single restriction fragment in each lane, although additional faintly hybridizing bands could be seen.

DISCUSSION

In this paper we report the characterization of two divergent EGase cDNA clones that have been isolated from strawberry fruit. The EGase encoded by *Cel1* cDNA shows a high degree of homology to other plant EGases, especially to tomato EGase *Cel2* (Lashbrook et al., 1994) to which it shares 78% identity at the amino acid level. In contrast, *Cel2* EGase exhibits a much lower homology with plant EGases in the database, with only 49% identity to tomato *Cel2* and 46% to strawberry *Cel1*. An analysis of the phylogenetic relations among the plant EGases from which the complete amino acid sequence is available shows that the strawberry EGase *Cel2* is the only representative of an evolutionary branch that diverged early from other plant EGases. In contrast, the EGase *Cel1* is phylogenetically related to EGases that are involved in various physiological events such as fruit ripening, cell elongation, or abscission processes (Fig. 2). In contrast to previous reports, the phylogenetic tree presented in this paper indicates a poor correlation between the physiological role of a particular EGase and the phylogenetic group to which it belongs.

An evident difference between *Cel1* and *Cel2* is the presence of several insertions and deletions along the *Cel2* amino acid sequence. The different molecular weight that is deduced from the amino acid sequence was confirmed by in vitro translation of *Cel1* and *Cel2* mRNA. By using this approach we also showed that both EGases possess a functional peptide signal that was excised to render mature *Cel1* and *Cel2* proteins with an apparent molecular mass of 52 and 60 kD, respectively. The strawberry EGase *Cel2* is larger than most of the known plant EGases, the molecular masses of which range from 49 to 56 kD. The presence of large EGases has also been described in pea, where a 70-kD native protein was found in epicotyls (Byrne et al., 1975), and in tomato, where a native 93-kD EGase was reported recently (Brummell et al., 1997b).

Another noticeable difference between *Cel1* and *Cel2* EGases was deduced from the lack of alignment of the Cys residues, which suggests different secondary and tertiary structures for these two proteins. Moreover, *Cel1* and *Cel2* also differ in the presence of motifs for *N*-glycosylation that is often found in proteins directed to the secretory pathway. Whereas one potential site for *N*-glycosylation is found in the *Cel1* mature protein (Asn-X-Ser/Thr), this motif is not present in *Cel2* EGase. Although most of the reported plant EGases appear to be glycosylated, the non-glycosylated nature of *Cel2* is shared by the EGase *BAC1*, expressed in bean abscission zones (Tucker and Milligan, 1991). TPP18, also named *Cel4*, was found in rapidly expanding tissues of tomato (Milligan and Gasser, 1995; Brummell et al., 1997a). Despite the differences discussed above, *Cel1* and *Cel2* share a basic pI, as deduced from the primary sequence of the mature proteins. The predicted pI of 9.4 for *Cel1* and 8.6 for *Cel2* is close to the 9.2 and 8.2 deduced for tomato *Cel4* and *Cel2* (Lashbrook et al., 1994; Milligan and Gasser, 1995) and to the 8.25 expected for the pepper EGase *Cel1* expressed in ripening fruit (Harpster et al., 1997). Other plant EGases with basic pIs are the bean

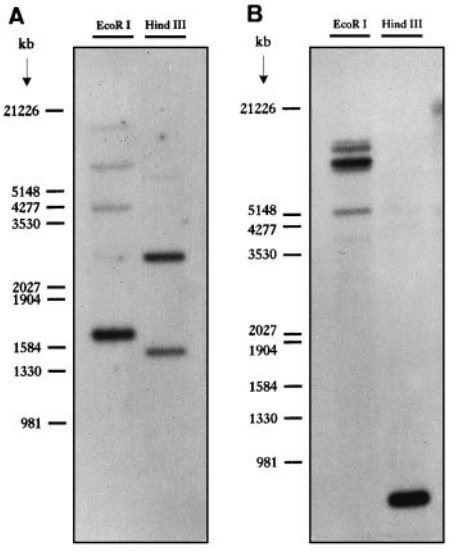

Figure 5. Southern analysis of *Cel1* (A) and *Cel2* (B). Approximately 5 μg of genomic DNA was digested with either *Eco*RI or *Hin*dIII. The Southern blots were hybridized with random-primed probes obtained from *Cel1* and *Cel2* 3′-untranslated regions.

BAC1 and tomato *Cel1*, both expressed preferentially in abscission zones (Tucker and Milligan, 1991; Lashbrook et al., 1994).

Based on the pattern of expression and hormonal regulation, plant EGases have been classified into two different groups. One group contains EGases whose mRNA levels are stimulated by auxin. These EGases have been found in rapidly expanding tissues such as pea epicotyls (Wu et al., 1996) or poplar stems, roots, and leaves (Nakamura et al., 1995). The second group is formed by EGases whose expression is induced by exogenous ethylene and retarded by exogenous auxin (Tucker et al., 1988; Lashbrook et al., 1994; del Campillo and Bennett, 1996; Koehler et al., 1996). In general, EGases found in abscission zones and ripening fruit fall into this category. However, the extended belief that ripening-related EGases are regulated by ethylene does not take into account nonclimacteric fruits such as strawberry, which has been described to be nonresponsive to exogenous ethylene and insensitive to inhibitors of ethylene synthesis and perception (Given et al., 1988; Abeles and Takeda, 1990). In strawberry it has been proposed that auxin rather than ethylene plays a pivotal role in fruit development and ripening. For instance, it has been demonstrated that the growth of the strawberry receptacle is stimulated by the auxins provided by the achenes and the subsequent decline in the auxin content in achenes as they mature modulates the rate of ripening (Veluthambi and Poovaiah, 1984; Given et al., 1988). Manning (1994) showed that the accelerated strawberry ripening by achene removal involves a set of mRNAs that are qualitatively similar to those expressed in fruit ripened normally. Similarly, the analysis of the expression of an EGase transcript isolated recently from strawberry fruit shows that this gene is negatively regulated by auxin (Manning, 1998). These results, together with the finding that EGase activity increases during strawberry fruit ripening in an ethylene-independent manner (Abeles and Takeda, 1990), suggest that the expression of *Cel1* and *Cel2* strawberry EGases may be subject to a regulatory control different from the one described for EGases found in climacteric fruits.

The expression of *Cel2* mRNA is clearly detected in green fruit and increases substantially thereafter as fruit turn white. This early increase in *Cel2* levels is not associated with any apparent change in fruit texture. However, Huber (1984) reported that changes in hemicellulose molecular weight were first detected in fruits at the white stage, indicating that hemicellulose degradation is initiated in green fruit. The expression of the *Cel2* transcript in green fruit suggests that the product of this gene may be involved in the early changes in hemicellulose polymer that lead to fruit softening. On the other hand, the early expression of the *Cel2* EGase in fruit suggests that the product of this gene may also participate in other processes other that softening, for instance in cell expansion during fruit development. At the onset of ripening the levels of *Cel2* mRNA are high and *Cel1* starts to accumulate gradually, to reach a maximum in fully ripe fruit. Similar to strawberry, the ripening of tomato is accompanied by an increase in the expression of the EGases *Cel1* and *Cel2*. However, the level of expression of *Cel2* was 20-fold higher than that of *Cel1*, and *Cel1* transcript was found to accumulate to a higher level in abscising flowers than in fruit (Lashbrook et al., 1994; del Campillo and Bennett, 1996). In contrast, both strawberry EGases exhibit similar high levels of accumulation in fruit, as deduced from the short exposure time required to obtain a clear signal in the northern blot (Fig. 4). This result suggests that both *Cel1* and *Cel2* EGases play a significant role in fruit softening. On the other hand, the overlapping expression of *Cel1* and *Cel2* in fruit suggests that the products of these two genes cooperate in the metabolism of cell wall polymers that occurs during fruit ripening. The fact that these two EGases show distinct biochemical and structural features suggests that they could have different substrate specificity. Experiments to investigate the physiological function of *Cel1* and *Cel2* EGases are under way.

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The accession numbers for the sequences reported in this article are AF051346 for *Cel1* and AF054615 for *Cel2*.

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