

Cloning and characterization of five cDNAs for genes differentially expressed during fruit development of kiwifruit (*Actinidia deliciosa* var. *deliciosa*)

Susan E. Ledger and Richard C. Gardner*

Centre for Gene Technology, School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand (*author for correspondence)

Received 31 January 1994; accepted in revised form 16 May 1994

Key words: *Actinidia deliciosa* var. *deliciosa*, ATP synthase, fruit development, metallothionein-like protein

Abstract

Five cDNAs for genes differentially expressed during fruit development of kiwifruit (*Actinidia deliciosa* var. *deliciosa* cv. Hayward) were isolated from a library made from young fruit, 8–10 days after anthesis. One gene (pKIWI503) has low levels of expression in young fruit but is induced late in fruit development and during fruit ripening, and has some homology to plant metallothionein-like proteins. The other four genes are highly expressed in young fruit with reduced expression in the later stages of fruit development. pKIWI504 has strong homology to plant metallothionein-like proteins and pKIWI505 exhibits homology to the β -subunit of the mitochondrial ATP synthase gene. The two other genes (pKIWI501 and 502) encode proteins with no significant homology to other known sequences.

Introduction

Fruit development involves a complex interaction of molecular, biochemical and structural changes that transforms a group of fertilized ovules into a mature fruit. Physical growth comprises two major cellular activities: cell division and cell enlargement. In kiwifruit, cell division commences at anthesis, presumably in response to fertilization, and continues at a high rate in the outer pericarp until 23 days after anthesis (DAA) and in the inner pericarp until 33 DAA [17]. There is

a high level of cell enlargement from 25 DAA until 40 DAA which then continues at a lower rate until fruit maturity [17].

Molecular investigations of fruit development have concentrated mainly on fruit ripening [15], although several genes have previously been recorded to have high levels of expression in young developing fruit. These include: (1) 2A11, a fruit-specific gene isolated from tomato with no homology to other sequences in the databanks [27, 36]; (2) 3-hydroxy-3-methylglutaryl-coenzyme A reductase, expressed during the stage of

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession numbers L27810 (pKIWI501), L27809 (pKIWI502), L27811 (pKIWI503), L27813 (pKIWI504) and L27812 (pKIWI505).

rapid cell division in tomato fruit development [24]; (3) TPRP-F1, a proline-rich protein that is highly expressed in tomato fruit one week after anthesis [31]; (4) metallocarboxypeptidase inhibitor which is highly expressed in tomato ovaries at anthesis [22]; (5) two uncharacterized cDNAs from peach fruit – pch 104 with a bimodal pattern of expression with peaks 30 and 71 days after bloom, and pch108 which is highly expressed in fruit 17 days after bloom [5].

In this paper we describe the isolation and characterization of five cDNAs for genes that are differentially expressed during fruit development of kiwifruit. Four are expressed early in fruit development while one is highly expressed late in development and also during fruit ripening.

Materials and methods

Plant material

Flowers, fruit and leaves were collected from mature kiwifruit (*Actinidia deliciosa* [A. Chev.] C.F. Liang et A.R. Ferguson var. *deliciosa* cv. Hayward) vines grown in the research orchards of The Horticulture and Food Research Institute of New Zealand Ltd. at Kumeu. Flowers were tagged at anthesis and the length and the diameter of the ovary were measured during fruit development. For RNA isolation whole ovaries were collected from flowers up to 6 days after anthesis (DAA) and for the later developmental stages the skin was removed from the fruit before the samples were frozen in liquid nitrogen. Young leaves ca. 1–2 cm in length were collected from the apex of shoots. Root tips were collected from 10-week old seedlings grown in a glasshouse.

RNA isolation

RNA used in the construction of the cDNA library was isolated using a modification of protocols described previously [2, 28]. Frozen material was ground in liquid nitrogen, resuspended in TLES buffer (0.18 M Tris-HCl, 0.09 M LiCl,

4.5 mM EDTA, 1% SDS pH 8.2) containing 10% (w/v) insoluble polyvinylpyrrolidone and 5% (v/v) 2-mercaptoethanol. The samples were extracted three times with phenol/chloroform, once with chloroform and then precipitated with ethanol. The nucleic acids were recovered by centrifugation and resuspended in dimethyldicarbonate (DMDC)-treated water. RNA was selectively precipitated and purified using two sets of alternating LiCl precipitations (0.25 volumes of 10 M LiCl) and phenol/chloroform extractions. The RNA was precipitated again with LiCl, recovered by centrifugation and resuspended in DMDC-treated water. The RNA isolation protocol described above was not efficient for extracting RNA from the range of tissues used in this study. Therefore RNA used for differential screening and for northern analyses was isolated using a modification of the procedure described in Boedtker *et al.* [3]. Frozen material was ground in liquid nitrogen, resuspended in 5× Calvaria buffer and extracted with phenol:chloroform at 60 °C. The organic phase was back-extracted, the pooled aqueous phases extracted again with hot phenol:chloroform and then precipitated with ethanol. The nucleic acids were recovered by centrifugation, resuspended in sarcosyl-lysis buffer (1% sarcosyl, 50 mM Tris-HCl pH 7.5, 10 mM EDTA) and solid CsCl (0.5–0.7 g/ml) was added. The solution was layered on top of a 2.5 ml cushion of 5.7 M CsCl and centrifuged at 15000 rpm for 17 h at 4 °C. The pelleted RNA was resuspended in DMDC-treated water.

Poly(A)⁺ RNA was purified from total RNA using Pharmacia's mRNA Purification Kit following the manufacturer's instructions. A cDNA library was constructed from 8–10 DAA poly(A)⁺ RNA in pSPORT 1 using the SuperScript Plasmid System from GIBCO BRL and amplified five-fold by liquid growth of the transformed *Escherichia coli*.

Differential screening

Duplicate colony/Plaque Screen membranes (DuPont) of the amplified cDNA library were hybrid-

ized with cDNA probes synthesized from poly(A)⁺ RNA extracted from fruit 8–10 DAA or from leaf. The cDNA was ³²P-labelled with a Random Primers DNA Labelling System from GIBCO BRL. Hybridization was performed at 65 °C for 16 to 18 h in 1% SDS, 1 M NaCl, 10% dextran sulfate plus denatured salmon sperm DNA (100 mg/l). The membranes were washed twice with 2 × SSC, 1% SDS at 75 °C for 30 min. Putative differential clones from the initial screen (77 from 11000) were reduced to 21 clones through two subsequent rounds of differential hybridizations.

Northern analysis

Total RNA was separated by electrophoresis on formaldehyde-agarose gels [13] and transferred to Hybond-N⁺ membranes (Amersham) by capillary transfer with 0.05 M NaOH. cDNA inserts were isolated from agarose gels using Magic PCR Cleanup columns (Promega) or GeneClean DNA Purification Kit (Bio II Inc.) and labelled as described above. Membranes were hybridized with the labelled cDNA inserts, as described above, and then washed twice with 2 × SSC, 1% SDS at 65 °C for 30 min. The 25/26S ribosomal RNA probe was pTIP6 from *Asparagus officinalis* [19]. The sizes of the hybridizing mRNA bands (see Table 1) were estimated from their mobility relative to the RNA ladder bands (GIBCO BRL) on the original stained gel.

Autoradiographs were scanned using an Ul-

troscan XL Densitometer, and GelScan XL version 2.1 software (Pharmacia) was used for peak integration. Identical zero reference points were used for each lane within an autoradiograph. Three scans were performed on each lane and these values were averaged before peaks were analysed.

DNA sequencing

Plasmid DNA for sequencing was extracted using the Magic Minipreps DNA Purification System (Promega). DNA was sequenced using the dideoxynucleotide chain-termination method [32] with an Applied Biosystems Catalyst robotic workstation and 373A DNA sequencer. The Genetics Computer Group (GCG) programs version 7.1.1.-Unix [10] were used for the alignment and analysis of the sequence. Database searches used the BLAST programs [1].

Results

Fruit development

The early developmental stages of kiwifruit from flowers two days before anthesis (–2 DAA) up to fruit 8–10 DAA are shown in Fig. 1A. Longitudinal cross sections of fruit 8–10 DAA and 102 DAA are shown in Fig. 1B and C respectively, showing the gross morphological changes that occur during fruit development. Length and diameter measurements of floral or fruit ovaries were recorded from –2 DAA up to 147 DAA. Kiwifruit ovary development followed a sigmoidal growth curve (Fig. 2) with the most rapid period of growth occurring between 13 and 44 DAA.

Isolation of cDNA clones

A cDNA library was constructed using poly(A)⁺ RNA from kiwifruit harvested at 8–10 DAA. Fruit at 8–10 DAA should be actively expressing genes involved in the cell division stage of fruit

Table 1. Characteristics of the pKIWI differential cDNA clones. Insert sizes (excluding poly(A) residues) are from sequence data and mRNA sizes are estimated from northern hybridizations. Homologies were identified by BLAST searches of the databanks.

Clone	Insert size (bp)	mRNA size (nucleotides)	Homology
pKIWI501	836	850	– (Glu, Ala, Pro-rich)
pKIWI502	1199	1500	~
pKIWI503	473	500	metallothionein-like protein
pKIWI504	595	700	metallothionein-like protein
pKIWI505	729	2100	β-subunit of ATP synthase

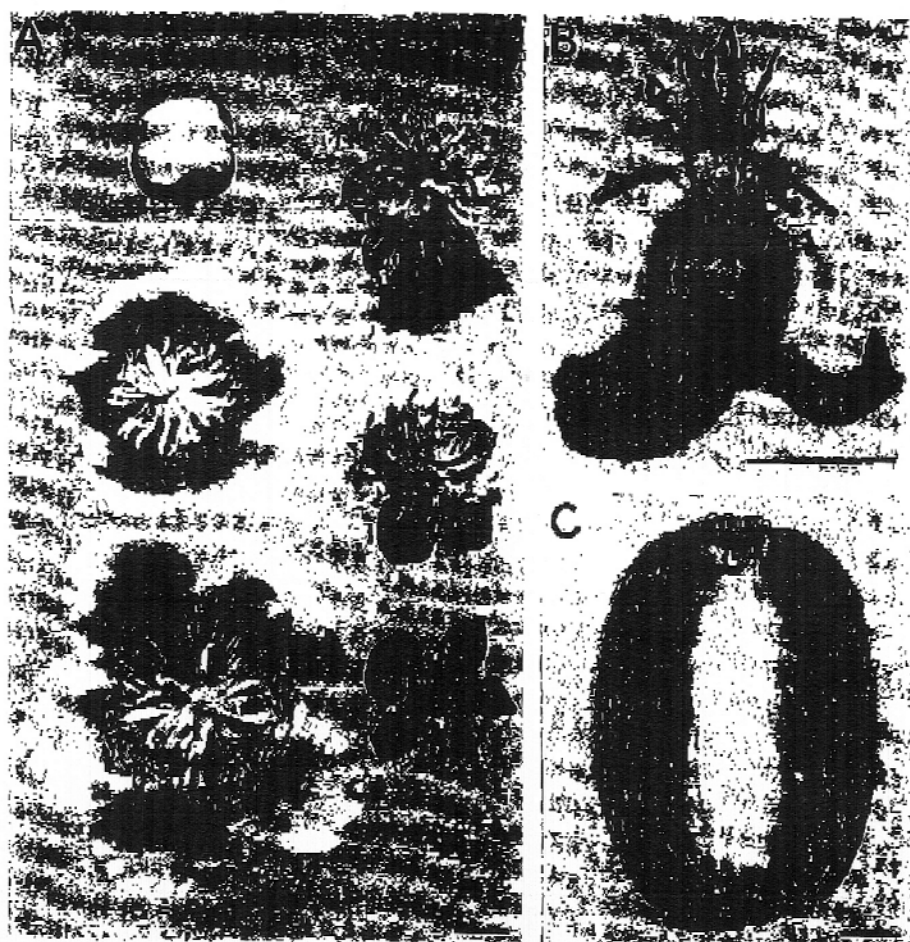


Fig. 1. Stages of flower and fruit development of kiwifruit. A. Whole flowers and fruit at 2 days before anthesis (top left), anthesis (centre left), and 2 (bottom left), 4 (top right), 6 (centre right) and 8-10 DAA (bottom right). Longitudinal cross-section of fruit 8-10 DAA (B) and 102 DAA (C). Scale bar = 1 cm.

development, and not those involved with fertilization [16] or cell enlargement [17]. To avoid obtaining genes that are related to general cell division, for example histones [21], the differential screen used cDNA from very young, actively dividing leaf tissue. A total of 21 clones were selected for further characterization after three rounds of screening.

Sixteen of the clones corresponded to a single gene family, as determined by comparison of the nucleotide sequence from the 3' termini or by hybridization to each other on a dot blot. pKIWI504 is a member of this gene family and was selected as a representative of the group for further characterization. The other five clones

represented five independent genes. The transcript of one clone was not able to be detected on a northern and this clone was not further characterized. In total, five clones (pKIWI501-505) were sequenced and their expression level during fruit development were examined in northern analyses. The sizes of the clones and their corresponding mRNAs are listed in Table 1.

Sequence analysis of pKIWI clones

The nucleotide sequence of the five differential clones was determined on both strands. The putative protein encoded by pKIWI501 has a

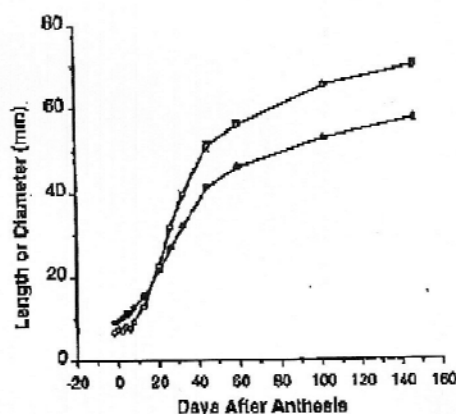


Fig. 2. Fruit growth of kiwifruit. The length (open circles) and diameter (closed circles) of the ovaries were measured during the course of development. Values are means ($n = 30-100$) and bars indicate the standard error.

calculated molecular mass of 18.9 kDa and a pI of 3.5. The protein is rich in Glu (47 residues out of 184), Ala (42 residues) and Pro (31 residues). pKIWI502 encodes a 35.2 kDa protein with a calculated pI of 8.5. pKIWI501 and 502 show no significant homology with sequences currently in the database.

pKIWI503 encodes a 6.5 kDa protein with a calculated pI of 4.2. The putative protein encoded by pKIWI504 has a molecular mass of 7.8 kDa and a pI of 4.4. Both pKIWI503 and 504 have homology to plant metallothionein-like genes. A comparison of the predicted proteins of pKIWI503 and 504 and eleven plant metallothionein-like proteins is shown in Fig. 3. pKIWI504 appears to have the typical structure of metallothionein-like proteins, with three Cys pairs in both the N- and C-terminal domains, separated by a hydrophobic region. However, pKIWI503 and two random cDNA sequences from rice and *Arabidopsis* appear to form a subgroup that has only four Cys residues in the N-terminal domain.

pKIWI505 encodes the C-terminal portion of a gene which has homology to the β -subunit of

Fig. 3. Comparison of the deduced amino acid sequences of pKIWI503 and 504 with other known plant metallothionein-like proteins. The conserved Cys residues are shown in bold. Dots indicate gaps that have been inserted for optimal alignment.

pKIWI503	MSDKCGNCD	ADSSQCVKKG	NS...I....
arab-EST	MSBNCSCDC	ADKTQCVKKG	TSYTF....
rice-EST	MSDKCGNCD	ADKSQCVKKG	TSYGV....
barley	MS....CSC	GSSCGCGSNC	NGRMYPDL.
maize	MS....CSC	GSSCGCGSSC	KCGKYPDL.
wheat	MS....CNC	GSGCSCGSDC	KCGRMYPDLT
castor bean	MSCCGGNCGC	GSGCKCGNGC	GGCKMYPDM.
soybean	MSCCGGNCGC	GSSCKCGNGC	GGCKMYPDL.
pKIWI504	MSCCGGKCGC	GSSCSCGSGC	GGCGMYPDL.
arabidopsis	MSCCGGNCGC	GSGCKCGNGC	GGCKMYPDL.
pea	MS....GCGC	GSSCNCGDSC	KCNKRSSGL.
mimulus	MS....SGSC	GSGCKCGDNC	.SCSMYPDM.
barley2	MSCCGGKCGC	GAGCQCGTGC	GGCKMFPDV.
consensus	MSCCGGNCGC	GSSC-CGSGC	GGCKMYPDL-

pKIWI503	DIVETDKSYI	EDVVMGVPA	
arab-EST	DIVETQESYK	ZAMIMDVGA	
rice-EST	VIVEAEKSHF	EEV...AAGX	
barley	EKSGATMQV	TVIVLGVGSA	KVQ...FEE
maize	EETSTAAQ..	PTVVLGVAP	KAAPEFVEA
wheat	EQGSAAQAQA	AVVVLGVAP	NKAGQ...FEV
castor bean	.SFS.EKTTT	ETLVLGVGA	.KAHFEGGEM
soybean	.SYT.ESITT	ETLVMGVAPV	.KAQFEGAEM
pKIWI504	.SYS.EMTTT	ETLIVGVAPQ	.KTYFEGSEM
arabidopsis	.GFSGETTTT	ETPVLGVAPA	MKNQYEASG.
pea	.SYS.EMETT	ETVILGVCPA	.KIQFEGAEM
mimulus	ETNTT	VTMLGVAP.	LKMYSEGSEK
barley2	EATAGA	AAMVMTASH	.KGSSEGFEN
consensus	ES-STETTTT	ETVVLGVAP-	KKAQFEG-EM

pKIWI503	...ESGG...R	CKCGTSCPCV	NCTCD*
arab-EST	...ENNANCK	CKGSSCSCV	NCTCCPN*
rice-EST	...ENGG...	CKCGTSCCST	DCCKGK*
barley	AAEFGEAAHG	CSCGANCNCN	PCNC*
maize	AAESGEAAHG	CSCGSGCKCD	PCNC*
wheat	AA...GQSGEG	CSCGNCCKCN	PCNC*
castor bean	GVVGAEEG.G	CKCGDNCTCN	PCTCK*
soybean	G.VPAEND.G	CKCGPNCSCN	PCTCK*
pKIWI504	GV...AAEN.G	CKCGSDCKCD	PCTCK*
arabidopsis	ESNNAESD.A	CKCGSDCKCD	PCTCK*
pea	SA.ASEEG.G	CKCGDNCTCD	PCNCK*
mimulus	SFGAEGGN.G	CKCGSNCKCD	PCNC*
barley2	AGGETGG...	CDC ATCKCG	TRAA....
consensus	AA-EAEG-HG	CKCGSNCKCD	PCTCK*--

ment. The consensus sequence was determined using the PRETTY program. The sequences obtained from the GenBank and SwissProt databases are: arab-EST (*Arabidopsis thaliana*-expressed sequence tag), accession number T12945 (T. Newman, unpublished); rice-EST (*Oryza sativa*-expressed sequence tag), accession number D21979 (M. Yuzo and S. Takuji, unpublished); barley (*Hordeum vulgare*), accession number P26571 [26]; maize (*Zea mays*), accession number P30571 [8]; wheat (*Triticum aestivum*), accession number L11879 [34]; castor bean (*Ricinus communis*), accession number P30564 (A. Weig and E. Komor, unpublished); arabidopsis (*A. thaliana*), accession number P25860 (K. Takahashi, unpublished); pea (*Pisum sativum*), accession number P20830 [11]; mimulus (*Mimulus guttatus*), accession number X51993 [9]; barley2, accession number S16534 [20]. The published soybean (*Glycine max*) sequence [18] is also included.

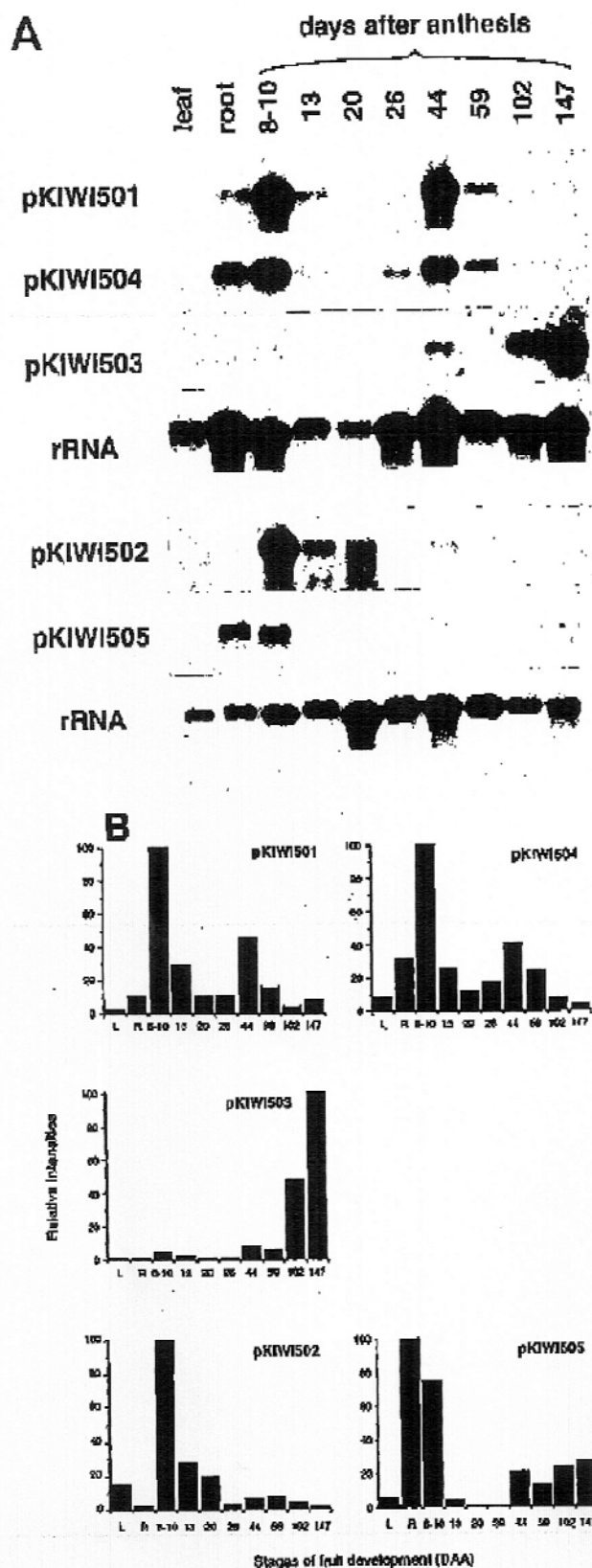


Fig. 4. Northern analysis of pKIWI501-505 during fruit development. Total RNA was extracted from leaf (L), root (R)

mitochondrial ATP synthase from both plants and animals. Comparison of the predicted amino acid sequence of pKIWI505 to sequences in the databank indicates 100% homology to para rubber tree (*Hevea brasiliensis*, accession number P29685 [6]), 99% to curled-leaved tobacco (*Nicotiana glauca*, accession number P17614 [4]), 99% to maize (*Zea mays*, accession number P19023 [39]), 98% to rice (*Oryza sativa*, accession number Q01859 [30]), 95% to *Chlamydomonas reinhardtii* (accession number S20804, L. Franzen and G. Falk unpublished), 91% to man (accession number X03559 [25]) and 91% to rat (accession number M19044 [14]).

Expression patterns during fruit development

Figure 4A shows the results of northern hybridizations with the five pKIWI clones, using RNA isolated from fruit at different stages of development. The amount of RNA loaded in each lane was not identical, as can be seen from the hybridization to the rRNA probe. This variability was the result of contamination of the samples with polysaccharides, so that duplicate samples varied in their concentration. The autoradiographs of the rRNA hybridizations were scanned to determine the relative amounts of RNA loaded per lane, and these values were used to normalize the signals obtained from the pKIWI501-505 probes. The highest value for each autoradiograph was designated 100% and all other signals were calculated relative to that sample. The values are displayed graphically as relative intensities in Fig. 4B.

and fruit at the indicated number of days after anthesis. Two separate gels were electrophoresed and alkali blotted. The membranes were successively hybridized to the pKIWI probes as well as the 25/26S rRNA probe (from pTIP6). A. Autoradiographs of the northern hybridizations. B. Relative expression levels determined by densitometry. The autoradiographs from the 25/26S rRNA probing were scanned to determine the relative RNA loadings. Each autoradiograph shown in A was scanned and the expression levels were corrected for loading differences as described in Materials and methods. The highest value was designated 100% and other samples were calculated relative to that value.

There is a bimodal pattern of expression of pKIWI501, 502 and 504 during fruit development with the highest level of expression in fruit at 8–10 DAA, and a second lower peak of expression at 44 DAA. The expression levels of all three clones is very low at the later stages of fruit development (2–8% of the 8–10 DAA values). There is also a low level of expression in the leaf (2–15%) and roots (1–31%).

pKIWI505 is highly expressed in root tips and also in young fruit 8–10 DAA. Low levels of expression were detected in leaf and fruit 13 to 26 DAA. In the later stages of fruit development, from 44 to 147 DAA, there was an increase in the expression levels to 13–26% of the levels in roots.

pKIWI503 has a very different pattern of expression during fruit development compared to the other four differential clones isolated. The transcript was present at very low levels in fruit up to 59 DAA but at 102 DAA there was a sharp increase in the level of expression with the highest level in this range of samples at 147 DAA. A northern filter with RNA extracted from ripening fruit of *A. chinensis*, a close diploid relative of *A. deliciosa*, was also probed with pKIWI503 (data not shown). pKIWI503 showed a further increase in mRNA during ripening, with the maximum level detected in fruit with a firmness of 9.8 N. In the fruit sample with a firmness of 2.9 N (approximately eating ripeness), there was a decrease in pKIWI503 transcript to 30% of the maximum levels.

The expression patterns of pKIWI501–504 were investigated in more detail at developmental stages closer to anthesis (Fig. 5). All clones showed low expression in flowers two days before anthesis. There was an increase in expression of pKIWI501, 503 and 504 at anthesis while the first detected increase of pKIWI502 was in fruit at 6 DAA. The highest expression level of pKIWI504 was in fruit 6 DAA while the highest levels of pKIWI501 and 502 were present in fruit 8–10 DAA. In the early developmental series long exposure times (up to 10 times those required for the other clones) were required to detect pKIWI503 transcripts, which showed an increase

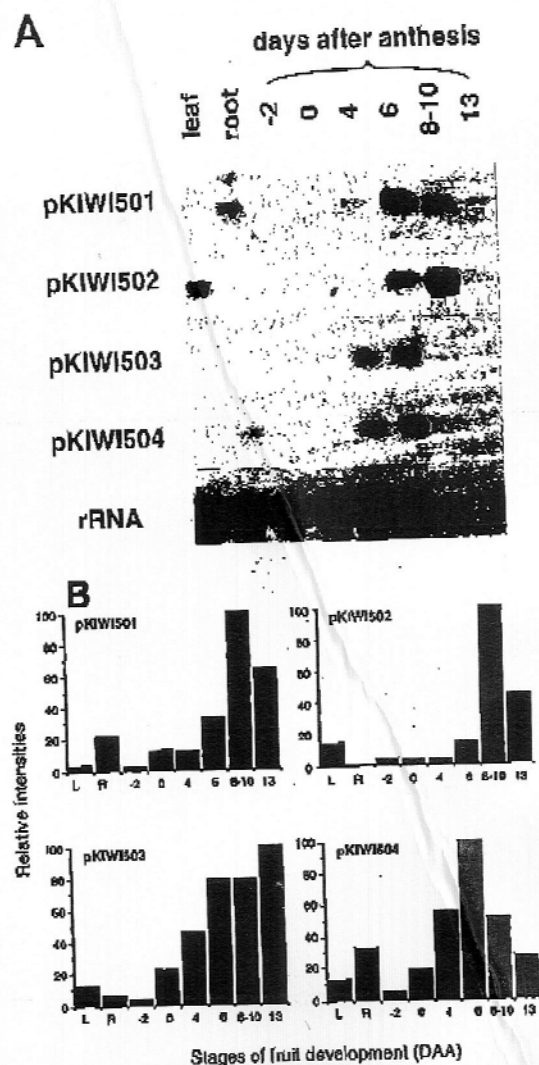


Fig. 5. Northern analysis of pKIWI501–504 during early fruit development. A. Autoradiographs of the northern hybridizations. B. Relative expression levels determined by densitometry (see Fig. 4 for details). L = leaf, R = root, numbers refer to developmental stage in days after anthesis (DAA) where 0 = anthesis.

in expression levels from anthesis through to 13 DAA.

The expression pattern of HMGR in tomato [24] and in maize [23] led us to investigate the expression of HMGR in kiwifruit development. Using the tomato HMGR clone as a probe, a kiwifruit homologue of HMGR was isolated from the cDNA library constructed from fruit 8–10

DAA. One positive clone was obtained from 2000 colonies screened. Northern analysis of kiwifruit leaf, root, 8–10 DAA and 147 DAA fruit showed expression of HMGR in the root sample but no detectable expression in either stage of fruit development or in the leaf (data not shown). The fact that the kiwifruit homologue of HMGR was isolated from the cDNA library confirms that HMGR is expressed in young fruit, although it was not at a sufficiently high level to be detected with northern analysis.

Discussion

Fruit growth in kiwifruit has previously been characterized by a single [38], a double [17] or a triple sigmoidal [29] growth curve. The data presented here shows the very dramatic initial growth phase which is typical of the sigmoidal growth curve of many fruit, such as tomato [37]. The second and third growth phases that have previously been recorded for kiwifruit, ca. 70 DAA and 150 DAA, may not have been identified in this study due to the large sampling intervals in the later stages of fruit development. Variation in the observed growth curves may also be due to cultivar differences; the cultivar Hayward has exhibited a single sigmoidal curve, this study and [38], while Monty [17] and Bruno [29] have exhibited double or triple growth curves respectively.

Two major patterns of expression during fruit development were observed for the differential clones isolated from the cDNA library. pKIWI501, 502, 504 and 505 are all highly expressed in fruit 8–10 DAA and have much reduced levels of expression in the later stages of fruit development, while pKIWI503 has very low levels of expression in young fruit and is induced to high levels of expression in the late stages of fruit development.

All of the five kiwifruit genes code for proteins that have not been previously reported to be highly expressed in the early stages of fruit development. The transcript size of pKIWI502, and its bimodal pattern of expression are both similar to those

observed for the peach cDNA pch104 [5], although the peak of expression of pKIWI502 is much earlier in development compared to pch104. Sequencing of the peach cDNA would determine if there is in fact any homology between the two genes. The pattern of expression of pKIWI503 is similar to that reported for 2A11 [27], with expression detectable around anthesis, increasing in level throughout fruit development and reaching a maximum level in ripe fruit. However, sequence comparison of 2A11 and pKIWI503 shows no homology between the cDNAs.

pKIWI503 and 504 have homology to a group of metallothionein-like proteins isolated from plants. The role of metallothionein-like proteins has not been clearly established in plants although, by analogy with animal metallothioneins, they have been suggested to play a role in metal ion detoxification or metal ion homeostasis. There is some indirect evidence that the plant metallothionein-like proteins bind cadmium and copper [12, 35]. Comparison of plant metallothionein-like proteins suggested that pKIWI503 and two other metallothionein-like proteins form a subgroup with a modified N-terminal domain lacking two of the conserved Cys; this subgroup may be altered in their ability to bind metal ions or in their specificity of ions that they bind.

There have been no previous reports of metallothionein-like proteins being expressed during fruit development and it is not understood what role they are performing in the fruit, either early in fruit development (pKIWI504) or late in fruit development and during fruit ripening (pKIWI503). pKIWI504-like clones were abundant in the cDNA library, and the pKIWI503 probe gave intense bands on northern blots using RNA isolated late in fruit maturation and during ripening. This high abundance suggests that the metallothionein-like proteins are of some importance for the growth and development of the fruit. However, there is no physiological evidence that they play a role in metal ion homeostasis. Although the first peak of pKIWI504 expression in early fruit development coincides with the period when copper concentrations (and probably those of other metal ions) appear to be highest in kiwi-

fruit [7], the concentrations of copper and other metals did not undergo the biphasic change found in pKIWI504 transcript levels. Apart from potassium, metal concentrations in ripe fruit are generally lower than in other parts of the plant [33]. Orchard records of the vines used in this study indicated that during the fruit growth period there were no applications of sprays which contained heavy metals. The role of these metallothionein-like proteins in fruit has yet to be determined.

The β -subunit of ATP synthase is the catalytic region of the enzyme which is involved in the final step of ATP production. The partial clone pKIWI505 is homologous to a large number of plant and animal ATP synthase β -subunit genes. The high levels of expression in root tips and young fruit may reflect a high demand for ATP in these tissues to fuel cell division. However, only low levels of pKIWI505 were found in young leaves, when cells should also be undergoing high levels of division.

This paper describes the characterization of five differential clones isolated from fruit harvested 8–10 days after anthesis, and represents an initial molecular description of early fruit development in kiwifruit. These genes will be used to isolate promoters specific to certain stages of fruit development.

Acknowledgements

We thank Jonathan Narita for the tomato HMGR clone, Kevin Davies for the pTIP6 clone and Dave Whittaker for the northern membrane of the ripening series of *A. chinensis* S.L. was in receipt of a Ph.D. scholarship from the New Zealand Institute for Crop & Food Research Limited.

References

1. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ: Basic local alignment search tool. *J Mol Biol* 215: 403–410 (1990).
2. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K: *Current Protocols in Molecular Biology*. John Wiley, New York (1989).
3. Boedtker H, Frischauf A, Lehrach H: Isolation and translation of calvaria procollagen messenger ribonucleic acids. *Biochemistry* 15: 4765–4770 (1976).
4. Boutry M, Chua N: A nuclear gene encoding the β -subunit of the mitochondrial ATP synthase in *Nicotiana glauca*. *EMBO J* 4: 2159–2165 (1985).
5. Callahan AM, Morgens PH, Cohen RA: Isolation and initial characterization of cDNAs for mRNAs regulated during peach fruit development. *J Am Soc Hort Sci* 118: 531–537 (1993).
6. Chye M, Tan C: Isolation and nucleotide sequence of a cDNA clone encoding the β -subunit of mitochondrial ATP synthase from *Hevea brasiliensis*. *Plant Mol Biol* 18: 611–612 (1992).
7. Clark CJ, Smith GS: Seasonal accumulation of mineral nutrients by kiwifruit. 2. Fruit. *New Phytol* 108: 399–409 (1988).
8. de Framond AJ: A metallothionein-like gene from maize (*Zea mays*): cloning and characterization. *FEBS Lett* 290: 103–106 (1991).
9. de Miranda JR, Thomas MA, Thurman DA, Tomsett AB: Metallothionein genes from the flowering plant *Mimulus guttatus*. *FEBS Lett* 260: 277–280 (1990).
10. Devereux J, Haeblerli P, Smithies O: A comprehensive set of sequence analysis programs for the VAX. *Nucl Acids Res* 12: 387–395 (1984).
11. Evans IM, Gatehouse LN, Gatehouse JA, Robinson NJ, Croy RR: A gene from pea (*Pisum sativum* L.) with homology to metallothionein genes. *FEBS Lett* 262: 29–32 (1990).
12. Evans KM, Gatehouse JA, Lindsay WP, Shi J, Tommey AM, Robinson NJ: Expression of the pea metallothionein-like gene *PsmTA* in *Escherichia coli* and *Arabidopsis thaliana* and analysis of trace metal ion accumulation; implications for *PsmTA* function. *Plant Mol Biol* 20: 1019–1028 (1992).
13. Fournier RM, Miyakoshi J, Day RS, Paterson MC: Northern blotting: efficient RNA staining and transfer. *Focus* 10: 5–7 (1988).
14. Garboczi DN, Fox AH, Gerring SL, Pedersen PL: β -subunit of rat liver mitochondrial ATP synthase: cDNA cloning, amino acid sequence, expression in *Escherichia coli*, and structural relationship to adenylate kinase. *Biochemistry* 27: 553–560 (1988).
15. Gray J, Picton S, Shabbeer J, Schuch W, Grierson D: Molecular biology of fruit ripening and its manipulation with antisense genes. *Plant Mol Biol* 19: 69–87 (1992).
16. Harvey CF, Fraser LG, Pavis SE, Considine JA: Floral biology of two species of *Actinidia* (Actinidiaceae). I. The stigma, pollination, and fertilization. *Bot Gaz* 148: 426–432 (1987).
17. Hopping ME: Structure and development of fruit and seeds in Chinese gooseberry (*Actinidia chinensis* Planch.). *NZ J Bot* 14: 63–68 (1976).
18. Kawashima I, Inokuchi Y, Chino M, Kimura M, Shimizu N: Isolation of a gene for a metallothionein-like pro-

- tein from soybean. *Plant Cell Physiol* 32: 913-916 (1991).
19. King GA, Davies KM: Identification, cDNA cloning and analysis of mRNAs having altered expression in tips of harvested asparagus spears. *Plant Physiol* 100: 1661-1669 (1992).
 20. Klermsdal SS, Hughes W, Lonneborg A, Aalen RB, Olsen O: Primary structure of a novel barley gene differentially expressed in immature aleurone layers. *Mol Gen Genet* 228: 9-16 (1991).
 21. Koning AJ, Tanimoto EY, Kiehne K, Rost T, Comai L: Cell-specific expression of plant histone H2A genes. *Plant Cell* 3: 657-665 (1991).
 22. Martineau B, McBride KE, Houck CM: Regulation of metallopeptidase inhibitor gene expression in tomato. *Mol Gen Genet* 228: 281-286 (1991).
 23. Moore KB, Oishi KK: Characterization of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity during maize seed development, germination and seedling emergence. *Plant Physiol* 101: 485-491 (1993).
 24. Narita JO, Gruissem W: Tomato hydroxymethylglutaryl-CoA reductase is required early in fruit development but not during ripening. *Plant Cell* 1: 181-190 (1989).
 25. Ohta S, Kagawa Y: Human F₁-ATPase: molecular cloning of cDNA for the β -subunit. *J Biochem* 99: 135-141 (1986).
 26. Okumura N, Nishizawa N, Umehara Y, Mori S: An iron deficiency-specific cDNA from barley roots having two homologous cysteine-rich MT domains. *Plant Mol Biol* 17: 531-533 (1991).
 27. Pear JR, Ridge N, Rasmussen R, Rose RE, Houck CM: Isolation and characterization of a fruit-specific cDNA and the corresponding genomic clone from tomato. *Plant Mol Biol* 13: 639-651 (1989).
 28. Prackell UM, McKee RA, Smith H: Molecular analysis of actinidin, the cysteine proteinase of *Actinidia chinensis*. *Plant Mol Biol* 10: 193-202 (1988).
 29. Pratt HK, Reid MS: Chinese Gooseberry: Seasonal patterns in fruit growth and maturation, ripening, respiration and the role of ethylene. *J Sci Food Agric* 25: 747-757 (1974).
 30. Sakamoto M, Shimada H, Jugimura T: Nucleotide sequence of a cDNA encoding a β -subunit of the mitochondrial ATPase from rice (*Oryza sativa*). *Plant Mol Biol* 20: 171-174 (1992).
 31. Salts Y, Wachs R, Gruissem W, Barg R: Sequence coding for a novel proline-rich protein preferentially expressed in young tomato fruit. *Plant Mol Biol* 17: 149-150 (1991).
 32. Sanger F, Nicklen S, Coulson AR: DNA sequencing with chain terminating inhibitors. *Proc Natl Acad Sci USA* 74: 5463-5467 (1977).
 33. Smith GS, Buwalda JG, Clark CJ: Nutrient dynamics of a kiwifruit ecosystem. *Sci Hort* 37: 87-109 (1988).
 34. Snowden KC, Gardner RC: Five genes induced by aluminum in wheat (*Triticum aestivum* L.) roots. *Plant Physiol* 103: 855-861 (1993).
 35. Tommey AM, Shi J, Lindsay WP, Urwin PE, Robinson NJ: Expression of the pea gene *P5MTA* in *E. coli*: metal-binding properties of the expressed protein. *FEBS Lett* 292: 48-52 (1991).
 36. Van Haaren MJ, Houck CM: A functional map of the fruit-specific promoter of the tomato 2A11 gene. *Plant Mol Biol* 21: 625-640 (1993).
 37. Varga A, Bruinsma J: Tomato. In: Monselise SP (ed) *CRC Handbook of Fruit Set and Development*, pp. 461-480. CRC Press, Boca Raton, FL (1986).
 38. Walton EF, de Jong TM: Growth and compositional changes in kiwifruit berries from three Californian locations. *Ann Bot* 66: 285-298 (1990).
 39. Winning BM, Bathgate B, Purdue PE, Leaver CJ: Nucleotide sequence of a cDNA encoding the β -subunit of the mitochondrial ATP synthase from *Zea mays*. *Nucl Acids Res* 18: 5885 (1990).