Short sequence-paper

A *rab11*-like gene is developmentally regulated in ripening mango (*Mangifera indica* L.) fruit

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Abstract

A full-length cDNA clone from mango (*Mangifera indica* L.) fruit has homology to the *rab11/YPT3* class of small GTPases. The corresponding mRNA is expressed in fruit, only during ripening. The likely involvement of this RabX protein in trafficking cell-wall modifying enzymes through the trans-Golgi network is discussed.

Keywords: Fruit ripening; Secretion; *rab11/YPT3*; GTPase; *Mangifera indica* L.

Secretory mechanisms, including the control of trafficking, are of great importance, but have been poorly characterised at the molecular level in plants. A large number of genes involved in membrane trafficking have been identified and characterised in animal and yeast systems, including the *YPT/Rab* class of small GTP-binding proteins (see [1]). These appear to be involved in controlling membrane fusion, either by a proofreading mechanism [2] or by directly promoting membrane fusion in the GTP-bound state [3]. Some of these GTP-binding proteins have homologues in plants, though their role has usually not been well characterised (see [4]).

One important area in which secretory mechanisms would be expected to play a key role is in the synthesis and turnover of the plant cell wall. The ripening of most fruits involves cell wall degradation. In tomato, this has been shown to involve the secretion of cell wall degrading enzymes such as polygalacturonase that are synthesised specifically during ripening and result in degradation of the pectic components of the wall (see [5]).

The situation in mango, is less well understood though changes in the structure of cell wall polymers in general and pectins in particular have been convincingly demonstrated [6–10]. Several cell wall degrading enzymes have been assayed in mango fruit [6,11,12] and at least one, β-galactanase, has been shown to be expressed in a ripening-specific manner (Los and Tucker, unpublished). Fruit ripening has been shown to occur in conjunction with the appearance of several ripening-specific gene products. Several of these have been identified in tomato and other fruit but, as yet, none of these ripening-specific genes appear to be involved in secretion. In a differential screen of a mango fruit cDNA library for ripening related genes, we have identified a *YPT/Rab* homologue, which may be involved in the secretion of material to the cell wall.
Fig. 1. Sequence of mango RabX and its predicted translation product. The putative polyadenylation signal is underlined.

Mango (Mangifera indica L. cv. Tommy Atkins) green fruit were treated with 10 \( \mu l \) l\(^{-1} \) ethene for 24 h at 20\(^\circ\)C and then left to ripen in air at 20\(^\circ\)C. Poly(A)-rich RNA was prepared as described [13] after 0, 2, 4, and 6 days and a cDNA library of half a million clones was constructed from the pooled samples in a lambda-ZAP vector. Screening of the library with cDNA probes from untreated fruit and fruit two days after the onset of ethene treatment revealed several clones that were expressed only in ripening fruit. One of these clones, which was unique, was converted to plasmid form by in vivo excision and was named pNY602.

The complete DNA sequence of the pNY602 cDNA insert is shown in Fig. 1. The longest open reading frame begins at a sequence similar to the consensus for plants (AACAATGGGC) [14] and encodes a 217 amino acid polypeptide with a molecular weight of 24 kDa and a predicted pI of 5.8. The 5' untranslated region (5'UTR) contains nine repeats of the trinucleotide TTC and seven additional repeats of the dinucleotide TC. The 3' untranslated region (3'UTR) also contains two and nine occurrences, respectively. The sequence TTTT also occurs five times in the 5'UTR and seven times in the 3'UTR, sometimes overlapping the TC sequences. The TC repeats are similar to those found in an Arabidopsis thaliana small GTP-binding protein gene [15].

Comparison of the predicted polypeptide sequence with the SWISSPROT protein sequence database by means of the FASTA program revealed homology to small GTP binding proteins of the Rab/YPT type. The best four scores in decreasing order of similarity were rice Ric2 [16], tobacco Np-Ypt3 [17], Arabidopsis Ara2 [18] and canine, human and rat Rab11 [19–21].

A comparison of the mango protein (which we have called RabX) with several similar proteins (Fig. 2) shows that several regions known to contain functionally significant motifs (see [22]) are well conserved. These motifs include regions I, III, V and VI that participate in GTP binding and hydrolysis and the paired cysteines of region VII that are essential for membrane attachment and the effector domain (region II). In addition, region IV has been shown to be conserved in all Rab/YPT proteins [22].

RNA from green fruit (Fig. 3) and from the same four stages of ripening that were used to construct the library were subjected to northern blot analysis with the mango RabX cDNA as probe. Expression of a 1.2 kb transcript is seen in ripe fruit (days 4 and 6) but not in green fruit or fruit in the early stages of ripening.

The northern blot analysis clearly supports the hypothesis that the mango RabX gene is expressed in a developmentally regulated manner during ethene induced ripening of mango fruit and in addition, the general sequence homology suggests that RabX belongs to the rab11/YPT3 family. This is supported by the good match to a Rab11-specific consensus [23]. The products of these genes are involved in protein targeting and are thought to be located in the trans-Golgi network (see [4]), however, the role of the RabX gene in ripening fruit is not clear. It is, perhaps, surprising that no cDNAs encoding components of the secretory pathway have been among the large numbers of ripening specific cDNAs so far identified by differential screens in several types of fruit. It, however, clear that at least some components of the secretory system, including the RabX protein and two recently discovered rab1/YPT1 homologues in tomato [28], are specifically induced during this hormonally-regulated ripening process in fruit. This suggests that they are associated with a regulated secretion pathway. Fruit may be expected to alter the components secreted to the wall during ripening. For instance, ripening fruit do synthesise, in
a ripening specific manner, a number of enzymes such as polygalacturonase [24] which act specifically upon the cell wall and would need, therefore, to be

Fig. 3. Northern hybridisation. Samples of fruit RNA were separated and probed with pNY602 cDNA insert. Samples were from: untreated green fruit (A), fruit treated with ethene for 24 h (B), or fruit treated with ethene for 24 h and left to ripen in air for an additional 24 h (C), 48 h (D) or 96 h (E).

specifically secreted and actively targeted to the apoplast. Polygalacturonase is synthesised with a long N-terminal presequence (77 amino acids) that is removed during secretion [25]. In addition, novel cell wall polysaccharides or other components may be secreted to the wall during ripening. The RabX protein may have a role in trafficking these enzymes or altered cell wall components during ripening, perhaps by acting as a specific docking protein within the Golgi or by facilitating fusion between elements of the trans-Golgi network and the plasmalemma. Recently, Rab11 was shown to be associated with zymogen granules and the plasma membrane in pancreatic acini [26]. However, by expressing hybrid Rab proteins in BHK cells, Chavrier et al. [27] showed that it was the naturally variable C-terminal domain, consisting of the last 34 amino acids, that conferred targeting specificity upon the protein. The homology to the other proteins is particularly poor in this region, though a database search employing only the last 35 amino acids still identifies Ric2, Ara2 and

Fig. 2. Comparison of mango RabX protein with similar proteins. The boxed regions are conserved sequences discussed in the text. The sequences listed are: mrabx, mango RabX (this study); ric2, rice Ric2 (77.4%; P40393) [16]; nypt3, tobacco Np-Ypt3 (70.5%; Q01111) [17]; pra5 and pra6, pea pra5 (70.5%) and pra6 (76.5%) [29]; ara2, Arabidopsis Ara2 (69.4%; P28185) [18]; sypt3, Schizosaccharomyces pombe YPT3 (57.5%; P17610) [30,31]; rao3, Discopyge ommata Ora3 (59.9%; P22129) [32]; mbr1, mouse Rab11b (59.9%; P46638) [33]; hbr1, canine, human and rat Rab11 (61.6%; P24410) [19–21]. Figures in parentheses indicate the percentage similarities to the mango sequence and the Swissprot database accession numbers, where applicable. Sequence alignments were by the PRETTY program of the University of Wisconsin Genetics Computer Group (GCG) package [34].
Np-Ypt3 as the three best matches. However, it is possible that RabX, and perhaps Ric2 belong to a new class of Rab protein not previously identified in other kingdoms and possibly playing a hitherto unidentified role.

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References