Differential expression of four members of the H\(^+\)-ATPase gene family during dormancy of vegetative buds of peach trees

Frédéric Gévaudant, Gilles Pétel, Agnès Guilliot

Laboratoire de Physiologie Intégrée de l’arbre Fruittier, Unité Associée I.N.R.A., Université Blaise Pascal, 24 avenue des Landais, 63177 Aubiere Cedex, France

Received: 15 May 2000 / Accepted: 19 August 2000

Abstract. Vegetative-bud dormancy in peach (Prunus persica L. Batsch) trees is known to be correlated, at least partially, with properties of the underlying bud tissues during winter. Variations in the activity and amount of plasma-membrane H\(^+\)-ATPase were observed. A full-length cDNA, PPA2 (Prunus persica H\(^+\)-ATPase 2) and three partial cDNAs (PPA1, PPA3 and PPA4) for the plasma-membrane H\(^+\)-ATPase from peach trees were isolated by reverse transcription (RT)-coupled rapid amplification of cDNA ends (RACE) polymerase chain reaction (PCR). The accumulation of plasma membrane H\(^+\)-ATPase transcripts was then studied in vegetative buds during dormancy and breaking of dormancy. Competitive RT-PCR analysis revealed that, during dormancy, the plasma membrane H\(^+\)-ATPase transcripts were higher in the tissues underlying the buds than in the buds themselves. After dormancy release, the level of PPA1, 2, 3 mRNA increased, whereas the level of PPA4 decreased in the buds. When trees were kept in a greenhouse (i.e. sheltered from chilling), no accumulation of PPA mRNA could be detected. These results suggest that there is a differential accumulation of H\(^+\)-ATPase mRNA between the bud and the underlying bud tissues during dormancy, and that chilling could act as a decisive factor.

Key words: Bud dormancy – Chilling (bud dormancy) – Gene expression (bud dormancy) – H\(^+\)-ATPase – Prunus (bud dormancy) – Tree dormancy

Introduction

Woody perennial plants of temperate areas are subjected to seasonal variations. Dormancy processes allow plants (and plant organs) to survive under adverse winter conditions. Dormant organs can resume their growth when environmental and endogenous conditions become propitious again. This developmental plasticity is critical for the survival of plants and it is therefore essential to determine the mechanism of growth inhibition during dormancy. The dormancy of vegetative buds could have three different origins (Lang et al. 1987): (i) unsuitable environmental factors (ecdormancy); (ii) endogenous factors within the affected structure (endodormancy); and (iii) physiological factors outside the affected structure (paradormancy or correlative inhibition). In order to study paradormancy in peach tree buds, we investigated the short-distance relationships between the bud and the underlying bud tissues.

Previously it was shown that bud dormancy in Jerusalem artichoke was partially correlated with the properties of the underlying tissues. The underlying parenchyma of dormant buds showed higher sucrose absorption (Gendraud and Lafleuriel 1983) and had a higher cytoplasmic pH (Gendraud 1981) than non-dormant structures. The higher cytoplasmic pH of underlying tissue cells was related to a higher plasma membrane (PM) H\(^+\)-ATPase activity in vitro (Pétel et al. 1992). It appears that buds will grow only when the nutrient-absorption capacities of the underlying tissues are low.

Similarly, variation in the active sucrose absorption of buds or their underlying tissues was observed in peach trees (Prunus persica L. Batsch cv. Redhaven) during winter dormancy. From November to December, the underlying bud tissues accumulated more nutrients than the bud (Marquat et al. 1996). The active absorption of sucrose in buds was lowest in December, while later in January an important increase was observed. The active sucrose absorption could be explained by an increase in H\(^+\)/sucrose co-transport. In fact, the PM H\(^+\)-ATPase generates a chemiosmotic H\(^+\) gradient across the plant.
PM. This gradient is used to drive solute uptake through H\(^+\)-coupled symporters (Delrot 1989; Bush 1993). Recently, PM H\(^+\)-ATPase activity was measured in PM-enriched fractions isolated from peach tree buds or their underlying tissues (Aue et al. 1999). Plasma membrane H\(^+\)-ATPase activity was higher in the tissues underlying the buds than in buds from October to November and was correlated, as shown by western blotting, with a lower amount of PM H\(^+\)-ATPase in buds than in their underlying tissues.

It is well known that plant H\(^+\)-ATPases are encoded by a multigene family (Ewing and Bennett 1994; Sussman 1994; Michelet and Boutrly 1995) and the differential transcriptional regulation of the H\(^+\)-ATPase genes among various organs has been demonstrated (DeWitt et al. 1991; Perez et al. 1992; Ewing and Bennett 1994; Harper 1994; Houlnè and Boutrly 1994; Michelet et al. 1994; Moriau et al. 1999; Oufaittole et al. 2000).

In order to understand the molecular mechanisms of bud growth inhibition during dormancy, we looked for a relationship between H\(^+\)-ATPase transcript accumulation and the morphogenetic potentialities of peach tree buds. We report the first isolation and characterization of cDNA clones encoding for a PM H\(^+\)-ATPase in a woody plant (i.e. *P. persica*). We analyzed the transcriptional expression of four H\(^+\)-ATPase genes in buds and their underlying tissues, both harvested from trees maintained in natural conditions or in a greenhouse (deprived of chilling).

### Materials and methods

#### Plant material

Four-year-old peach trees (*Prunus persica* L. Batsch cv. Redhaven) grafted on cultivar GF 305 were grown outdoors, in natural conditions (seasonal climatic variations of France). Some trees, cultivated in 200-l containers, were transferred to a greenhouse and maintained at temperatures higher than 15 °C from October 1997 to April 1998, in order to deprive the trees of winter chilling. Organs were harvested from both tree samples and frozen immediately in liquid nitrogen.

#### Extraction of RNA

Total RNA was extracted from several organs (roots, stems, tissues underlying the buds, vegetative buds, leaves) according to the method recently described (Gévauuant et al. 1999). The RNA was quantified spectrophotometrically and its quality and integrity was checked by gel electrophoresis. Aliquots of RNA were treated with 2 units per µg RNA of RNase-free DNase I (Promega), to remove template DNA, and diluted to 10 ng µl\(^{-1}\) for use in competitive reverse transcription-polymerase chain reaction (RT-PCR). Messenger RNA was purified using the PolyATtract mRNA-isolation system (Promega).

#### Isolation of an H\(^+\)-ATPase cDNA clone

The highly conserved ATP-binding domain was chosen in known PM H\(^+\)-ATPase sequences to design two degenerate oligonucleotides for PCR (Ewing and Bennett 1994). A 23-mer, designated as P1 [5' TT(T,T,A) CCA(T,A) GAG(A) CAC AAA(G) TAT(C) GAG(A) AT 3'], and an antisense 23-mer, designated as P2 [5' GC A(G)TA T(G)AT AC(T,T)GT A(G)TT C(T,T)T CTAT 3'], corresponding to amino acids (FPFHKYE) and (MKNYTIIY), respectively, were synthesized.

The first-strand cDNAs were produced with 2 µg of total RNA using the avian myeloblastosis virus (AMV) reverse transcriptase from the First Strand Synthesis kit (Amersham), following the manufacturer's instructions. Amplification by PCR was carried out in 25 µl total volume, using 0.5 units of Taq polymerase (Appli-gen), 200 µM deoxyribonucleotide triphosphates and 0.8 µM of primers (P1 and P2). Diluted cDNA was used as the template, and PCR amplification was performed during 40 cycles at 94 °C (30 s), 45 °C (30 s), and 72 °C (1 min). The resulting 254-bp DNA fragments were cloned into the pCR II T-vector (Invitrogen).

**Isolation of the full-length H\(^+\)-ATPase cDNA (PPA2)**

The 5' and 3' end of a cDNA were isolated using the RACE system (Marathon cDNA amplification Kit; Clontech Laboratories, Palo Alto, Calif., USA), following the manufacturer's instructions. The PCR amplification was carried out using a *PPA2*-specific primer (R5') [5' GCC AGA AGA AGA CAA CTA CTA TTA GC 3'] for the 5' end and (R3') [5' GCT ACA GGA GAG GAA GTA CAT CTG 3'] for the 3' end.

Finally, the full-length cDNA clone was obtained by hot-start PCR using two cDNA-specific primers based on the sequences of the 5' and 3' ends: A250 primer [5' CGA TTC GCC GTA CTA CCT CAT CCT CAT CC 3'] and an antisense primer A230 [5' GCC TAC AAA AGA GAC ATC CCC TGC C 3'].

**Identification and characterization of cDNA clones**

The cDNA clones were partially sequenced using the deoxy chain termination method with the T7-polymerase (Pharmacia). The complete sequencing was performed with an automated sequencer (Genome Express, Grenoble, France).

Sequence alignment was performed and similarity scores were calculated using the CLUSTAL-W 1.8 program (Gibson et al. 1994).

**Preparation of standard RNA**

Competitive PCR for the quantification of H\(^+\)-ATPase mRNA was conducted according to the method described by Siebert and Larrick (1992). Standard RNA was prepared as follows. The cDNA clone APR3 was modified by insertion of a 518-bp heterologous DNA fragment (an *Alul*-HindII fragment from nucleotides 331 to 849 of the cDNA for the adenine nucleotide translocator of *Solanum tuberosum*; EMBL accession number X57557) within the region amplified by the primer pair.

The RNA standard was obtained by in vitro transcription with T7 RNA polymerase of the linearized plasmid. After treatment with RNase-free DNase I (Promega), the mixture was extracted with phenol/chloroform and precipitated with ethanol. The transcript was resuspended in diethylpyrocarbonate (DEPC)-treated water, quantified spectrometrically and its integrity was determined by gel electrophoresis.

**Quantification of H\(^+\)-ATPase mRNA by competitive RT-PCR**

The RT-PCR was performed using the Titan one Tube RT-PCR System (Boehringer Mannheim) according to the manufacturer’s specifications, with the following modifications: 25 ng of standard transcript was added to 10 ng of plant RNA, and 25 µM downstream and upstream primers (P1, P2 described above).