

Rapid communication

Identification and distribution of profilin in tomato (*Lycopersicon esculentum* Mill.)

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Abstract. Profilin is a G-actin monomer-binding protein which has been shown to participate in actin-based tip-growth of animal cells. The abundance of profilin in pollen and its occurrence in several vegetable foods raises the question of the role of profilin in plants. First, its distribution throughout various parts of the plant needs to be determined. This paper describes observations on the presence of profilin in the tomato plant (*Lycopersicon esculentum* Mill.). The distribution of profilin in flower buds, stems, leaves, roots, and fruits of tomato was determined by immunoblotting and by tissue printing, showing that profilin is present in most if not all parts of the tomato plant.

Key words: Actin – Allergen – Cytoskeleton – *Lycopersicon* – Profilin – Tissue printing

Pollen contains an abundance of the eukaryotic actin-binding protein profilin, a major pollen allergen (Valenta et al. 1991a; Valenta et al. 1992) that is also present in many vegetable foods (Vallier et al. 1992; van Ree et al. 1992). These findings have stimulated interest in this important regulatory actin-binding protein in plants, and particularly in pollen.

Information on the biochemistry and molecular biology of profilin is increasing rapidly in both plant and animal systems. Profilins in plants and animals have a relative molecular mass (M_r) of 12–15 kDa, and profilin has been shown to be a G-actin (globular=monomeric) binding-protein in both animals (Carlsson et al. 1976) and plants (Valenta et al. 1993). Coding sequences have been cloned from several species including yeast (Magdolen et al. 1988), *Drosophila* (Cooley et al. 1992), and amoebae (Binette et al. 1990; Haugwitz et al. 1991) and from plant species including birch (Valenta et al. 1991b), tobacco (Mittermann et al. 1995), common bean (Vidali et al. 1995), maize (Staiger et al. 1993), timothy grass (Valenta et al. 1994), and wheat (Rihs et al. 1994).

Profilin plays an important role in modulating the actin cytoskeleton in many cell types. For example *Drosophila* oocytes require profilin in nurse cells to control changes in actin architecture which are vital for normal egg formation (Cooley et al. 1992). *Drosophila* bristles also require profilin expression for normal structure formation (Verheyen and Cooley 1994).

Profilin probably performs several functions besides binding to G-actin, including binding to 4,5-bisphosphoinositides and thus inhibiting phospholipase C-mediated cell-signalling in both plants and animals (Goldschmidt-Clermont et al. 1990; Drøbak et al. 1994); and promotion of the exchange of ADP for ATP on actin (Goldschmidt-Clermont et al. 1991).

Though the action of profilin is often associated with tip-growing cells such as fibroblasts (Giuliano and Taylor 1994), in plants such cells are limited to pollen tubes, root hairs, leaf hairs, and stigmatic papillary cells. Here we survey the occurrence of profilin in various parts of the plant by tissue printing and immunoblotting.

Freshly harvested material from *Lycopersicon esculentum* Mill. cv. MoneyMaker was frozen in liquid nitrogen and ground rapidly to a fine powder with a precooled mortar and pestle. This powder was immediately thawed at 100 mg tissue/ml in a standard SDS-containing sample buffer. Protein concentrations were determined by the method of Larson et al. (1986), and 5 µg of total protein per lane (10 µg for tomato pollen) was loaded onto each of a pair of replicate SDS polyacrylamide minigels (5% stacking, 20% resolving) along with wide-molecular-weight-range Rainbow Standards (Sigma, St. Louis, Mo., USA). After electrophoretic separation the proteins in one gel were electroblotted onto 0.22-µm nitrocellulose (NitroME; MSI, Westboro, Mass., USA). The other gel was stained for protein using Coomassie Blue. As positive controls, each blot included a lane containing 50 ng of recombinantly produced birch profilin (Valenta et al. 1991b) and a lane containing 5 µg of maize pollen total protein.

Blots were blocked 1 h in 1×PBS (phosphate-buffered saline=0.137 M NaCl, 1.47 mM KH_2PO_4 , 8.10 mM Na_2HPO_4 , 2.70 mM KCl, pH 7.1, 10% nonfat Carnation dry milk (Carnation Company, Los Angeles, Calif., USA), 1% Tween 20 (Sigma); and then incubated 1 h with the primary antibody RP1 (Valenta et al. 1993) diluted 1:1000 in the blocking buffer. Blots were washed 4×5 min in 1×PBS, 1% Tween 20, incubated 1 h with 1:1000-dilut-

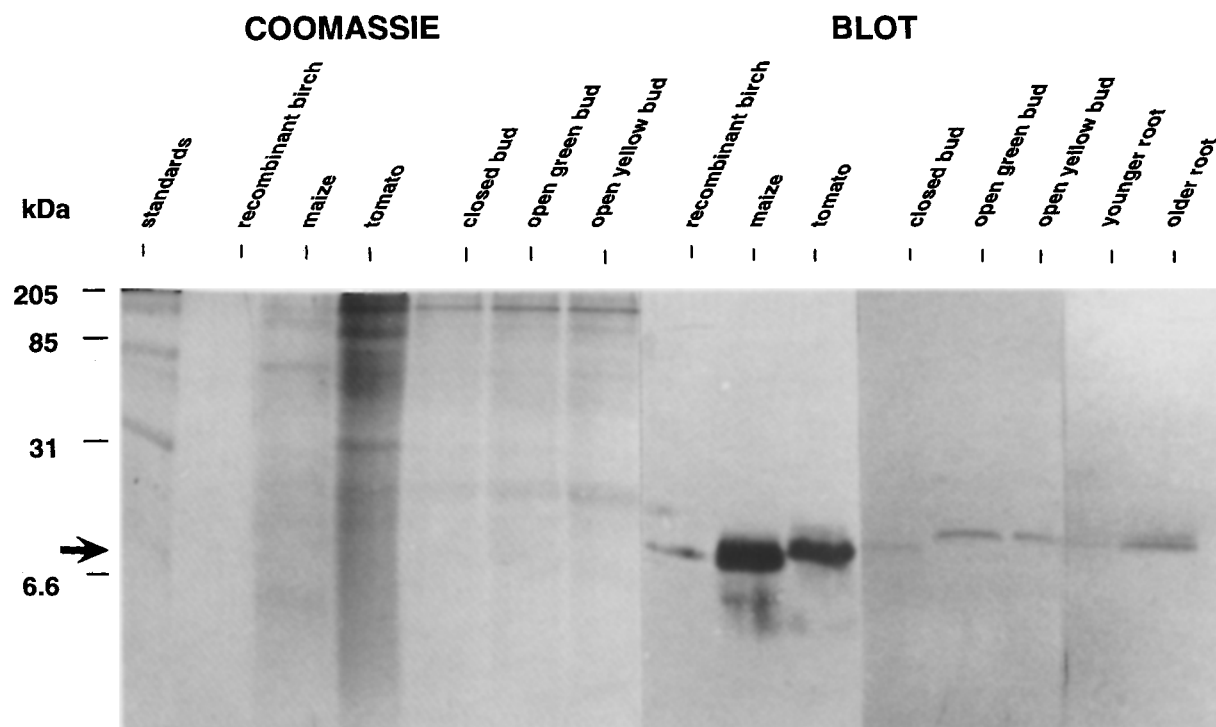


Fig. 1. Immunoblots of various tomato tissues. Immunoblots of total protein extracts of reproductive materials. Lanes labeled as *maize* and *tomato* refer to pollen. *Recombinant birch* refers to recombinant birch pollen profilin. Approximate bud development: *closed green bud*, a mix from pollen mother cells to early micro-

spores; *open green bud*, microspores up to and including pollen grains; *open yellow bud*, mature pollen grains, anthers dehiscing. Arrow indicates approximately 14 kDa, the molecular weight of profilin. 5 µg protein/lane except 10 µg for tomato pollen

ed goat anti-rabbit IgG alkaline-phosphatase-conjugated secondary antibodies (whole molecule; USB, Cleveland, Ohio, USA), and washed again for 4×5 min. Antibody detection used NBT (nitro blue tetrazolium)/BCIP (5-bromo-4-chloro-3-indolyl phosphate; Harlow and Lane 1988).

A modification of standard procedures (Reid and Pont-Leizca 1992) was used. Fresh hand-sections were pressed for 15 s onto 0.22 µm NitroME. At least three replicate prints were made with the same-age materials. Results were consistent between experiments and among the duplicates in a given experiment. Prints were treated as immunoblots except that (i) blocking buffer contained 1×PBS, 0.1% Tween 20, 2% each of teleostean (cold water) fish skin gelatin (Sigma) and bovine serum albumin (Fraction V; Sigma); (ii) washes after the secondary antibody were for 2×5 min. and then 1×3 h in 1×PBS, 1% Tween 20, 1% SDS (Sigma). Controls included no antibody (blocking buffer substituted for primary and secondary antibodies) and secondary antibody only (blocking buffer substituted for primary antibody). One set of tissue prints was silver-stained for protein by incubating for 5 min in 0.004% AgNO₃ and then developing for 15 min with 0.04% formalin in 2.5% Na₂CO₃.

In general, immunolocalization in tissue prints was visible after a longer incubation in NBT/BCIP than was needed for the corresponding immunoblot. Some immunoblots shown were overdeveloped to improve visualization. The results obtained were generally similar to those obtained with RP2 (Valenta et al. 1993). For a given material, antibody-treated tissue prints were handled identically throughout antibody incubation, alkaline-phosphatase development, and photographic processing.

Tissue prints were photographed with Kodak Plus-X Pan film (Eastman Kodak Company, Rochester, N.Y., USA) using an SHZ ILLD (Olympus, Tokyo, Japan) dissecting microscope with an orange filter. Prints were made using AGFA Multigrade paper (Gevaert AG, Germany) and Multicontrast filters (Ilford, Moberley, Cheshire, UK).

Figures 1 and 2 illustrate that profilin occurs in most if not all organs of the tomato plant, including pollen, buds, and roots. It is also present in mature leaves (blade and petiole), stems, and fruit (data not shown). Both immunoblots and tissue prints indicated a greater abundance of profilin in older or mature organs compared to younger or immature organs of the same type, with the possible exception of floral buds at some stages. For example, Fig. 1 illustrates immunoblotted bud proteins in which profilin appears to be more abundant in open floral buds than in closed green buds. This is also the case for younger roots compared to older roots (Fig. 1).

From tissue-printing evidence, profilin appears to be expressed in most tissues. In tomato fruit profilin is found in pericarp, locule, and seed (Fig. 2a), and in both stem and root, profilin is present throughout the organ (Fig. 2e, g). Profilin localizes most strongly in the vascular cylinder (Fig. 2e, g). Whether or not profilin is expressed in the cambial region is unclear since heavy browning of that region leads to the dark cambial staining present in control tissue prints (Fig. 2f). Consistent with immunoblotting of total protein extracts, profilin is most strongly expressed in the most mature or oldest stage of development examined for a given organ such as stem or fruit. The stem and fruit sections shown are the most mature stages examined for each of those organs.

As expected from the presence of profilin in pollen from various mono- and dicotyledonous species, tomato pollen also contains profilin. The protein is also present in most of the tomato plant parts examined. Unexpected-

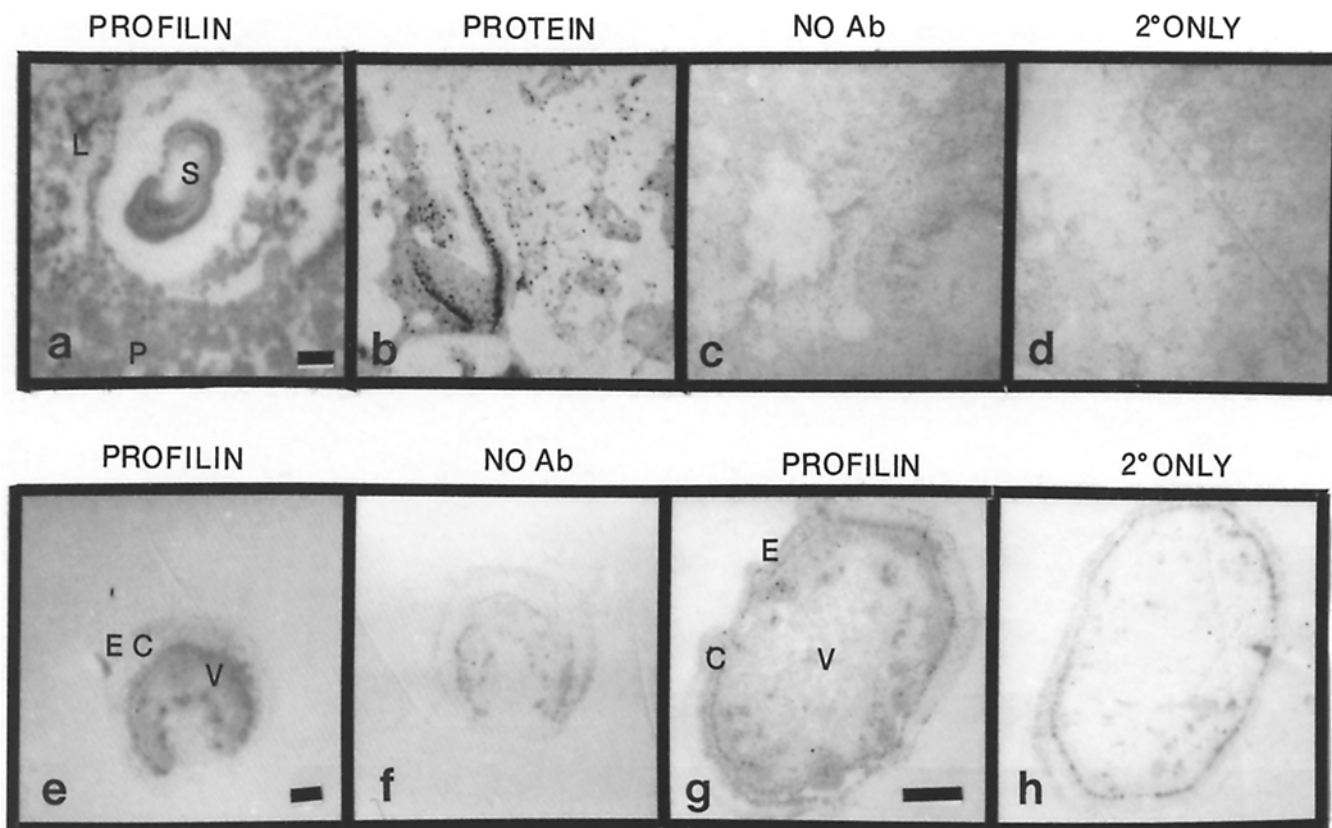


Fig. 2a-h. Tissue Prints. **a-d** Mature tomato fruit. $\times 40$. **e-f** Mature tomato petiole. $\times 40$. **g-h** Mature tomato stem. $\times 40$. All tissue prints of a given material represent the same stage of development. The areas represented were chosen for near transverse sectioning of cell walls. In fruits, seeds that were not stably positioned often made poor contact with the membrane. Thus they do

not appear in all prints. *L*, Locule; *P*, pericarp; *S*, seed; *E*, epidermis; *C*, cortex; *V* vasculature. Bars 100 μ m. *Profilin*, stained for profilin using antibody RP1; *Protein*, silver stained for protein distribution; *No Ab*, no antibody control; *2° only*, secondary antibody only control

ly, mature or older organs appear to contain more profilin than immature or younger ones. One possible exception to this increase in profilin abundance with age or maturity occurs in floral buds. Once they contain older microspores or pollen grains, buds do not appear to accumulate more profilin. There may actually be a decrease in profilin abundance by the open yellow bud stage, though up to the open green bud stage there is a clear increase in profilin abundance. This increase in profilin abundance is true assuming that the antibody RP1 recognizes equally different profilin isoforms which may be expressed at different times during the development of an organ. This is an important assumption given that different profilin isoforms can be expressed at the RNA level in pollen (Staiger et al. 1993). The doublet observed in the older root blot (Fig. 1) may be due either to protein degradation or to the presence of different isoforms with molecular weights that fall within the range of known profilin molecular weights (12–15 kDa).

Profilin is known to be present in cells that lack tip-growth, including those in most of the plant organs examined. The amoeba *Physarum polycephalum* was found to express profilin in all cells of its life cycle, including plasmodia, amoebas, and spores (Binette et al. 1990). In *Drosophila*, profilin mutations affect not only oocytes, where the effects of these mutations were first

noted (Cooley et al. 1992), but also sperm cells and somatic cells (Verheyen and Cooley 1994). Profilin has been identified by an enzyme-linked immunosorbent assay technique in a number of vegetable foods, including tomato fruit and macadamia nuts (van Ree et al. 1992). Staiger et al. (1993) detected more profilin genes in the maize genome than were expressed in pollen. The finding of profilin in various organs of the tomato plant is in agreement with the finding of profilin in seeds, leaves, and pollen of tobacco by Mittermann et al. (1995).

The functions of profilin in plant cells which do not display tip-growth can only be speculated upon. In the cells of the tomato plant which do not undergo tip-growth, such as those of the developing fruit, profilin may be acting to maintain dynamically stable networks of actin filaments or to control the formation of important transient actin networks. *Drosophila* oogenesis depends on one such network, and its loss upon the deletion of profilin expression from the nurse cells of the developing egg chamber causes female sterility (Cooley et al. 1992). Based on the roles described for profilin in animal cells, profilin may control the polymerization state of actin in plant cells by G-actin binding, by controlling ADP/ATP exchange on actin, and/or by controlling 4,5-bis-phosphoinositides-based cell-signalling. This last role in signalling has already been suggested by in-vitro

experiments using plant profilin (Drøbak et al. 1994). The increased amounts of profilin present in mature tissues of tomato also suggest that the protein may play a direct or indirect role in the maturation process. The finding of strong profilin expression within the vascular cylinder agrees with a similar finding in the common bean by Vidali et al. (1995).

Precise localization of profilin and actin in pollen and buds will be needed to determine the possible association of profilin with dynamic actin filaments and/or regions important for cell-signalling in the tip-growing pollen tube. Important associations may also occur during the earlier stages of pollen development.

Antisense alteration of profilin expression has shown significant effects in the amoeba *Dictyostelium* (Haugwitz et al. 1994). An important further step in studying the role of profilin in tomato pollen is determining the effect of profilin antisense expression in developing and germinated pollen. However, the presence of profilin in most parts of the tomato plant poses an additional challenge for molecular genetic analysis of profilin function, particularly in pollen. In order to assess the role of profilin in a given cell type or organ, such as pollen, by antisense genetics, antisense constructs must be tightly regulated and specific to the tissue of interest. Improper alteration of the pattern of profilin expression in non-reproductive structures could indirectly and adversely affect pollen development. At the same time, similar studies could also shed important light on the role of profilin in fully developed, mature tissues, where it is strongly expressed.

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