Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 3674–3678, May 1989
Developmental Biology

Heat shock protein hsp70 cognate gene expression in vegetative and reproductive organs of *Lycopersicon esculentum*

(tomato/development/hsc70)

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Communicated by J. E. Varner, February 21, 1989

ABSTRACT **We have detected hsc70 gene expression (heat shock protein hsp70 cognate) during vegetative growth and reproductive development in tomato (*Lycopersicon esculentum*). Using RNA from a tomato hsc70 cDNA as a probe in *in situ* hybridizations, we have determined expression patterns of hsc70 in nonstressed tomato roots, stems, leaves, flowers, and developing fruits. We have localized high levels of hsc70 transcript to the vascular system of the ovary, dividing cells of the lateral root tips, and the inner integument of developing seeds. We also see expression in the transmitting tissue, in immature anthers, and in embryos. We cannot detect expression in mature pollen, xylem, or ovules. These data indicate that the expression of at least some tomato hsp70 family members is developmentally regulated.**

The heat shock response was first described in *Drosphila* in 1962 (1). Further analysis has shown that all organisms produce heat shock proteins (hsps) in response to heat, heavy metals, and a variety of other stresses (2); hsps range in size from 16 to 110 kDa, with the exact protein profile varying between organisms (3).

The sequence of the 70-kDa hsp (hsp70) is evolutionarily highly conserved, and in some organisms this is the most abundantly expressed of the hsps (2). In all organisms investigated except chicken (4), hsp70s are encoded by multigene families. The multigene family members are differentially regulated (5), and members that are expressed in the absence of heat stress are referred to as cognate hsp70s (hsc70s), or "cognates" (6). Some hsc70s have been associated with specific functions such as clathrin uncoating (7, 8), glycosylation-related reactions (9), and transferrin activities (10); other hsc70s seem to be developmentally regulated (11). hsc70s that are expressed during development have been detected during yeast sporulation (12), *Drosophila* oogenesis (13), in preimplantation mouse embryos (14), and during mouse, rat, and human spermatogenesis (15).

Recent evidence indicates that some of the yeast hsc70s are involved in the transport of proteins across membranes (16, 17). There is speculation (16) that the hsc70 unfolding activities used during transport may also be used in some of the other cognate activities. The localization studies presented in this paper indicate that there is nonstress, tissue-specific regulation of hsp70 family members in tomato. This work is the foundation for further analyses aimed at determining hsc70 functions during development and stress. Additionally, the promoters of such family members may be useful in chimeric gene constructions aimed at localizing foreign gene products in some of these tissues.

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MATERIALS AND METHODS

hsc-1 cDNA Isolation and Characterization. A cDNA library (in phage λGT10) made from tomato cv. VF36 mature pistil poly(A)$^+$ RNA was screened with a petunia hsp70 cDNA (18) under conditions of reduced stringency [40% formamide/5× SSC (1× = 0.15 M NaCl/0.015 M sodium citrate, pH 7)/1× Denhardt's solution (0.02% bovine serum albumin/0.02% polyvinylpyrrolidone/0.2% Ficoll)/300 μg of tRNA per ml/1% sodium dodecyl sulfate (SDS) at 42°C; washes were with 2× SSC/0.1% SDS at 50°C]. The *Eco*RI fragments of one of the positive plaques were subcloned into phage M13 and sequenced by using ^{35}S-substituted deoxyadenosine 5′-[thio]triphosphate and Amersham sequencing reagents. The >80% sequence homology between this cDNA, which we call hsc-1, and the well-characterized petunia hsp70 (20) verified that hsc-1 is an hsp70 family member.

Tissue Preparation. The VF36 tomato plants used in all of these experiments were maintained in growth chambers at 18°C on a 9-hr day/15-hr night light/dark cycle. Buds were collected and measured from the zone of abcission to bud tip, and the correlation between size and developmental stage was determined to be similar to that described by McCormick *et al.* (19). RNA was extracted for blot hybridization (Northern blots) from leaf tissue (3–5 cm) taken from mature plants or from ovaries of mature flowers. Pollen was vibrated free from dehiscing anthers and extracted for RNA. Measurements for organs used in *in situ* hybridizations are sited in figure legends or text.

RNA Isolation and Analysis. The tissue used for RNA analysis was collected and immediately frozen in liquid nitrogen. The RNA extraction, blotting, and wash procedure are described by Rochester *et al.* (20). High specific activity nick-translated or random-oligo-labeled hsc-1 (1×10^6 cpm/ml) was added to each hybridization mix. Blots were hybridized at 48°C for 24 hr. After strigent washing (0.3× SSC/0.1% SDS at 68°C), the blots were exposed to XAR-5 film with one intensifying screen.

***In Situ* Hybridization Procedures.** Tissue was fixed, embedded in paraffin, and hybridized *in situ* with ^{35}S-labeled RNA probes (21–23). Glutaraldehyde only (1%) or 1% glutaraldehyde/0.05 M phosphate buffer, pH 7.0, was used as a fixative in order to avoid fixative agents that could induce stress before fixation was complete. The rapid fix time under these conditions and the agreement between Northern blots and *in situ* data give us confidence that there is no glutaraldehyde induction of hsp70 in these tissues. The template for the RNA probe used in *in situ* hybridizations was the most 3′ 1000 base-pair (bp) *Eco*RI fragment of the tomato hsc-1 cDNA subcloned into pGEM4 (Promega). ^{35}S-substituted uridine 5′-[thio]triphosphate was incorporated into the hsc-1 RNA (RNA opposite in polarity to the hsc70 mRNA of

Abbreviations: hsp, heat shock protein; hsc, cognate hsp.

tomato), and the final product was alkaline-digested to yield 150- to 200-base probes. The hybridizations were done in a 50% formamide/0.3 M NaCl buffer (22) at 42°C overnight. After hybridization the slides were treated with RNase and washed in 0.1× SSC/1 mM dithiothreitol at 57°C. Slides were then dehydrated and, after drying, dipped in liquid emulsion (Kodak) at 45°C. Slides were incubated in darkness at 4°C for 3–7 days and developed in D-19 (Kodak) developer.

Control *in situ* experiments were performed in an identical fashion except that an RNA probe that does not hybridize to tomato RNA (made from the transcription template supplied with the Promega phage SP6 *in vitro* transcription kit) was used in the hybridization. Sections were stained with toluidine blue and visualized by dark-field microscopy. Bright-field photographs represent sections that did not undergo hybridization treatments, so that the integrity of the tissue would be maintained for histological analysis.

RESULTS

RNA Blot-Hybridization (Northern) Analyses. A brief survey of endogenous hsc70 mRNA levels in tomato tissues was initially performed by Northern blot analysis. The endogenous level of hsc70 in ovary tissues was ≈10-fold higher than that of 3- to 5-cm leaves (Fig. 1*A*). Since we were unable to detect any hsc70 transcript in the toal RNA from mature pollen, poly(A)$^+$ RNA was made and probed with both the tomato hsc-1 cDNA (Fig. 1*B*) and a pollen-expressed cDNA probe, 9656. 9656 was used to verify that the pollen poly(A)$^+$ RNA was intact.

Although a weak hsc70 RNA signal was detected in mature anthers, even using longer exposures did not show a signal in the pollen lane. To determine within these and other organs where hsc70 gene expression is highest, we used *in situ* hybridizations.

***In Situ* Hybridization Analyses.** We have performed *in situ* hybridizations on root, stem, leaf, floral, and fruit sections. Because of space limitations, we are showing only select *in situ* photographs and describing the others.

Buds and Fruits. We have examined buds from the 2-mm size to pollination. hsc70 hybridization to a longitudinal section of a mature ovary supports the Northern blot data and indicates that there is an hsc70 mRNA signal associated with the vascular system and the transmitting tissue (Fig. 2). We have seen this pattern throughout ovary development. Cross sections from the receptacle of a 3.5-mm bud best demonstrate the vascular system hybridization patterns (Fig. 3). The receptacle is the swollen part of the pedicel that supports the rest of the bud and from which the vascular system branches out into traces that will supply specific floral organs with their own vascular bundles (24). The hybridization to vascular tissue throughout the bud is very strong and also is seen in cross sections of sepal, petal, and anthers (and is responsible for the mature anther signal seen in Fig. 1*B*). Although there is a signal associated with the phloem of tomato stem, leaf, and root, it is weak compared with that of the bud (data not shown). A cross section of a style (13-mm bud) best demonstrates the hybridization to the transmitting tissue (Fig. 4). In tomato, several channels of transmitting tissue extend from the stigma to the ovary. The hsc70 signal in Fig. 4 is associated with these channels.

In all fruits examined we have seen hsc70 transcript in the vascular bundles. In young fruits we see signal associated with the placenta, but the most dramatic signal is associated with the inner integument of the developing seed (Fig. 5). The inner integument is sometimes referred to as the "seed tapetum" (25) because it is believed to transfer nutrients to the developing embryo (in fact, we see a weak but repeatable signal associated with the developing tapetum of the immature anther). The hsc70 signal in the developing seed persists until seed desiccation. After desiccation there is a signal associated with the embryo only (the embryo signal is already apparent in Fig. 5*C*).

Vegetative Tissue. Other than very immature (4-mm) leaves, the highest level of hsc70 gene expression that we

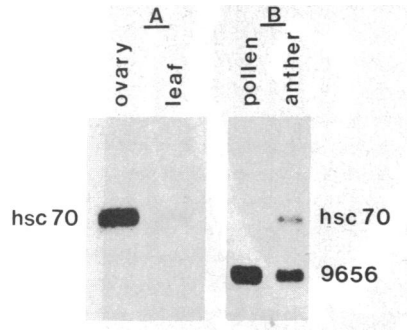

FIG. 1. Northern blots of RNA from nonstressed tomato ovary, leaf, pollen, and mature anther. (*A*) Total RNA (50 μg) from ovaries of mature tomato flowers or from 3- to 5-cm tomato leaves transferred to GeneScreen and hybridized to the tomato hsc-1 cDNA probe. (*B*) Poly(A)$^+$ RNA (2 μg) from pollen of mature tomato flowers or from mature anthers, probed first with hsc-1 and then with cDNA 9656 (an anther-specific cDNA characterized in ref. 19).

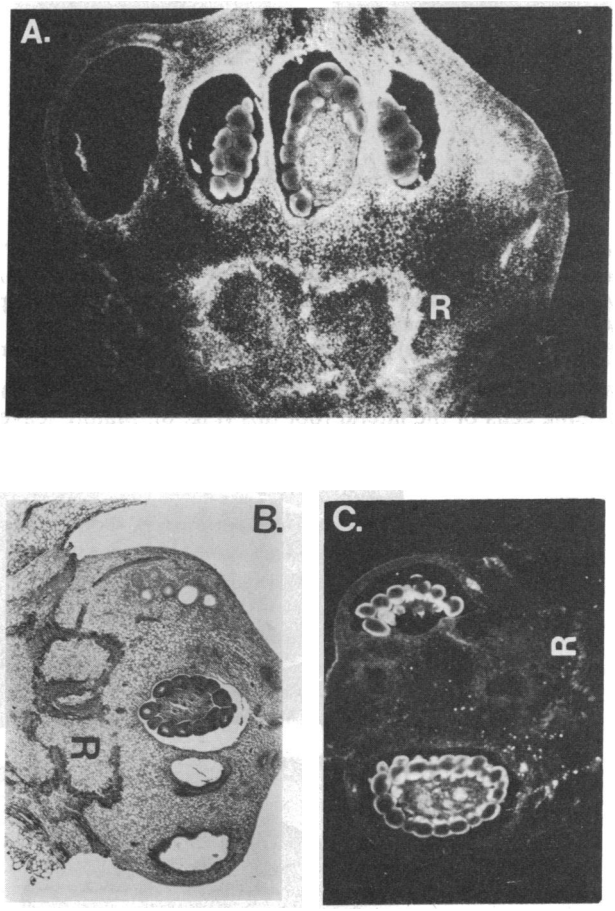

FIG. 2. Localization of hsc70 mRNA in a longitudinal section of tomato ovary dissected from a mature flower. (*A*) Hybridization of an ovary section to a ^{35}S-labeled hsc-1 RNA probe. RNA·RNA hybrids are indicated by white grains. Note that there is no hsc70 gene expression in the ovules. Regardless of the developmental stage of the ovary, ovules do not appear to produce hsc70 mRNA. (*B*) Bright-field photograph of a section from the same ovary as in *A* and *C*. (*C*) Hybridization of an ovary section to the control probe defined in *Materials and Methods*. R, receptacle area of flower.

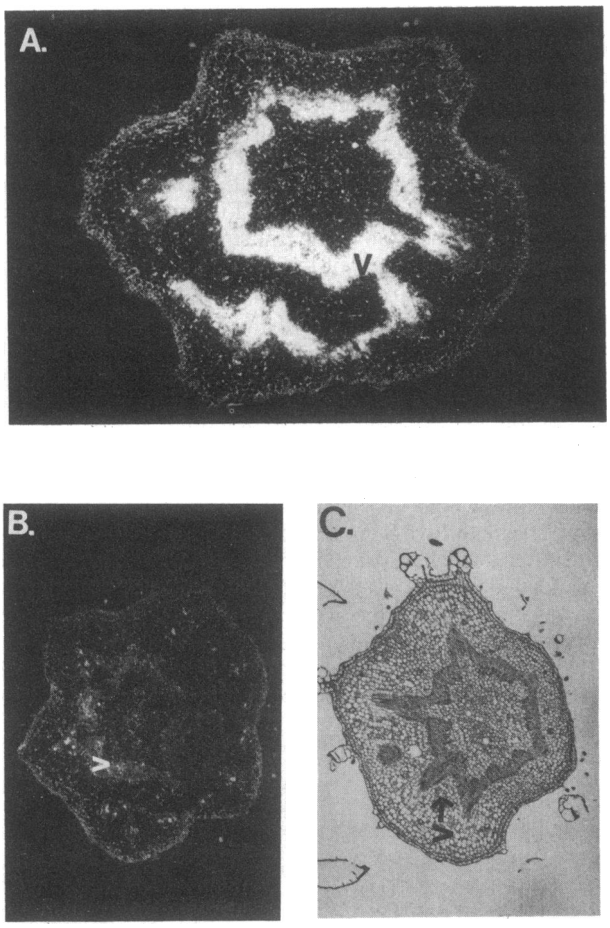

FIG. 3. Localization of hsc70 mRNA in the cross section of a receptacle from a 3.5-mm tomato flower bud. (*A*) Hybridization of a receptacle section to a ^{35}S-labeled hsc-1 RNA probe. RNA·RNA hybrids are indicated by white grains. (*B*) Hybridization of a receptacle section to the control probe. (*C*) Bright-field photograph of a section from the same ovary as used in *A* and *B*. v, Vascular system.

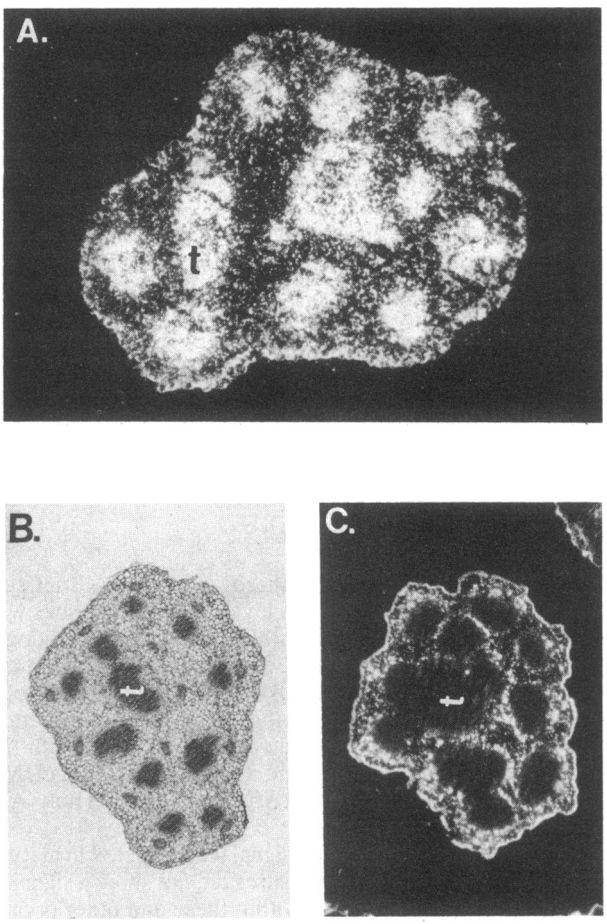

FIG. 4. Localization of hsc70 mRNA in a cross section of the style from a 13-mm tomato bud. (*A*) Hybridization of a style section to a ^{35}S-labeled hsc-1 RNA probe. RNA·RNA hybrids are indicated by white grains. (*B*) Bright-field photograph. (*C*) Hybridization of a style section to the control probe. t, Transmitting tissue.

have detected in vegetative tissue is associated with the dividing cells of the lateral root tips (Fig. 6). Mature leaves and stem have a lower level of hsc70 that seems to be associated entirely with the vascular bundles.

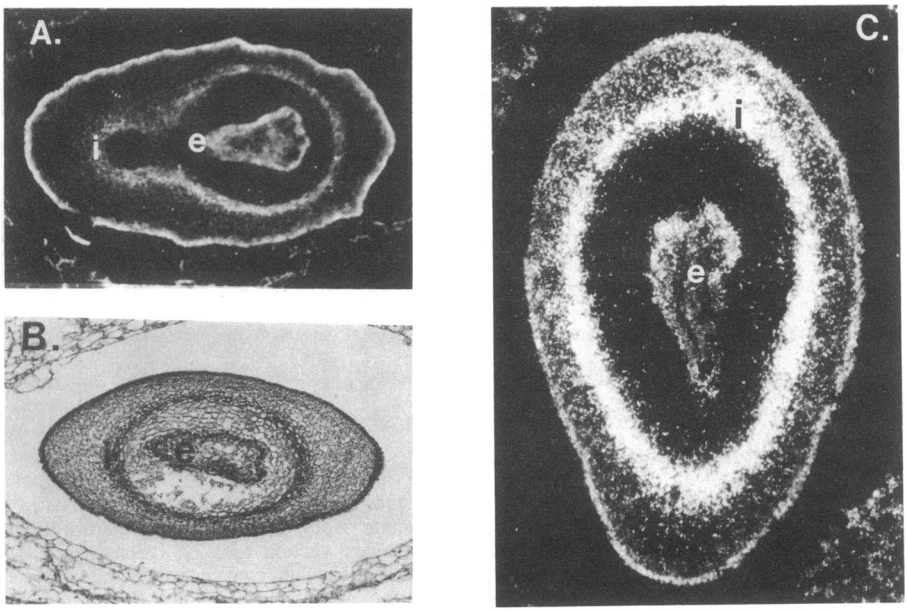

FIG. 5. Localization of hsc70 mRNA in a developing tomato seed from a 1.4-g fruit. (*A*) Enlargement to show a developing seed from a fruit cross section hybridized to the control probe. (*B*) Bright-field photograph. (*C*) Enlargement to show a developing seed from a fruit cross section hybridized to a ^{35}S-labeled hsc-1 RNA probe. White grains represent RNA·RNA hybrids. e, Embryo; i, integument.

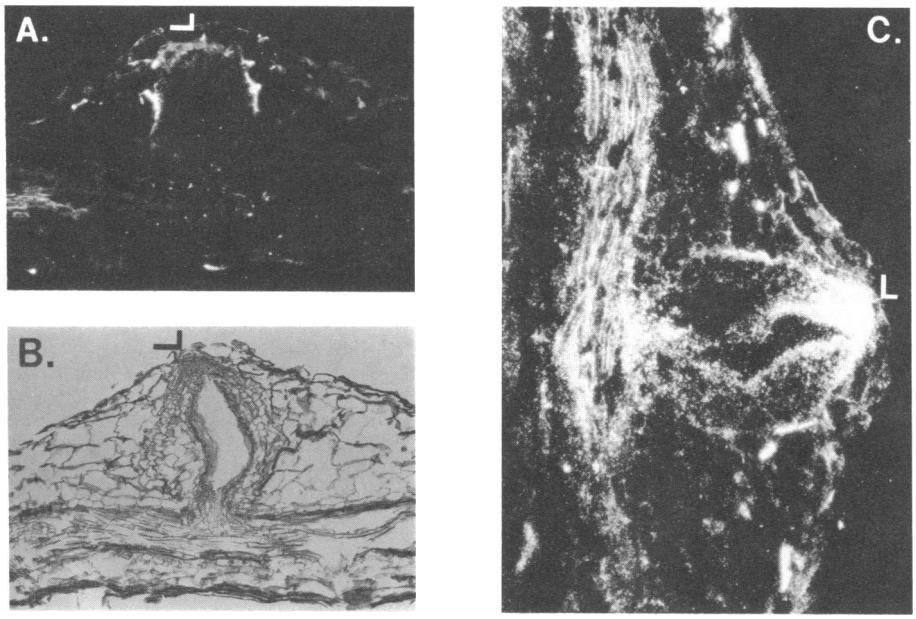

FIG. 6. Localization of hsc70 mRNA in the emerging lateral root of a 5-day-old seedling. (*A*) Hybridization of a longitudinal root section to the control probe. (*B*) Bright-field photograph. (*C*) Hybridization of a longitudinal root section to the ^{35}S-labeled hsc-1 RNA probe. White grains indicate RNA·RNA hybrids. L, lateral root.

DISCUSSION

The tomato hsp70 multigene family member that we have used to map hsc70 gene expression throughout the tomato life-cycle cross-hybridizes to other tomato family members on a Southern blot (data not shown). We hypothesize that the expression patterns that we have identified may represent the differential or simultaneous expression of several family members of this small multigene family. Further experimentation using monoclonal antibodies should clarify this point.

To date, the functions associated with hsc70 proteins fall into two broad categories: those with transport-related or glycosylation-related activities (16, 17) and those with DNA replication-related activities (26). Based on our localization studies and the previously defined hsc70 activities, we can speculate as to the functions of tomato hsc70s. It may not be a coincidence that many of the tomato tissues that express hsc70 transcripts are either highly secretory tissues or organs with zones of rapidly dividing cells. The developing tapetum of the immature anther, the inner integument of the developing seed, and the transmitting tissue are all tissues that make glycoproteins and secrete proteins. As mentioned in the introduction, there is evidence in yeast (16, 17) for hsc70 involvement in transport activities. However, proteins are also transported to the surface of pollen cells (27), and we see no indication of hsc70 gene expression (or transcript storage) in mature pollen.

Kao *et al.* (28) have shown that an hsp70 family member in HeLa cells is induced during adenovirus infection. Additionally, Milarski and Morimoto (29) have shown that hsp70 mRNA levels increase during the G_1/S interphase of the HeLa cell cycle. The DnaK protein of *Escherichia coli*, which is 49% homologous to eucaryotic hsp70s, is essential for phage λ replication (3). Thus, it is possible that the hsc70 gene expression in the rapidly dividing cells of the meristem of the lateral root is involved in DNA replication or cell division.

Until we unambiguously determine which cells within the vascular bundles are responsible for the expression of hsc70 mRNA throughout the plant, it is difficult to speculate upon its function. In stem cross sections, the hsc70 mRNA signal appears to be associated with both inner and outer phloem; however, we need greater resolution to identify the cell type responsible for this hsc70 gene expression. The hsc70 function could be related to the metabolite transfer function of some phloem cells. This could explain why the hsc70 signal is exaggerated in developing buds, since buds are considered to be "sink" tissues during development.

We cannot eliminate the possibility that hsp70 transcripts are stored in some tissues in preparation for stress. In *Xenopus* oocytes, hsp70 transcripts are stored and translated only during stress (30). Further analysis is necessary to determine whether this is happening in any of the tomato tissues.

In summary, we see expression of hsc70 transcripts to various degrees in all tomato tissues except ovules, mature pollen, and xylem. Palter *et al.* (13) have documented an almost ubiquitous presence of hsc70s in *Drosophila* tissues. They detect the highest levels of expression in ovaries and embryos with 5-fold less expression in larva, pupae, and adults. We see high levels of hsc70 transcripts in the vascular bundles of developing buds, the inner integument of developing seeds, and the tips of lateral roots and 4-mm leaves. We see a concentration of hsc70 transcripts in developing embryos and a lower level of hsc70 transcripts in the transmitting tissue of the style and in the developing tapetum of immature anthers. A comparatively weak but repeatable signal indicates the expression of hsc70 gene(s) in the vascular system that runs throughout the vegetative tissue.

We thank Bob Goldberg for sending us his *in situ* procedure before publishing it (21–23). We thank Al Smith for technical training and advice, and Kim Sachs for giving us the tomato pistil cDNA library. We thank Marco Wallroth for technical advice and photographic assistance. We thank Dawn Cleeton for patiently typing the manuscript. This research was supported by U.S. Department of Agriculture Grant 88-37261-3899.

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