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A Meristem-Related Gene from Tomato Encodes a dUTPase: Analysis of Expression in Vegetative and Floral Meristems

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A meristem-specific gene coding for deoxyuridine triphosphatase (EC 3.6.1.23) (dUTPase) in tomato was isolated, and its developmental expression in vegetative and floral apices was monitored. An 18-kD polypeptide, P18, was isolated as a consequence of its accumulation in arrested floral meristems of *anantha* mutant plants. The corresponding cDNA isolated from an expression library exhibited a 40 to 60% similarity with the pseudoprotease sequences of poxviruses, genes that have been suggested to encode dUTPases. Enzymatic tests and conservation of peptide motifs common to bacterial and viral genes verified that the P18 cDNA clone indeed represents a eukaryotic dUTPase. Immunogold localization and in situ hybridization experiments showed that polypeptides and transcripts of dUTPase are maintained at high levels in apical meristematic cells of vegetative and floral meristems. dUTPase gene activity is also high in the potentially meristematic cells of the provascular and vascular system. Its expression is lower in the immediate parenchymal derivatives of the apical meristematic cells, and this downregulation marks, perhaps, the transition from totipotency to the first differentiated state.

INTRODUCTION

Primary tissues of the plant shoot and root arise continuously from apical meristems. Apical meristems differentiate during embryogeny and are developmentally autonomous. They retain their meristematic activity throughout the life cycle while generating primary tissues and new meristematic centers that form lateral organs (Walbot, 1985; Goldberg, 1988; Sussex, 1989; Poethig, 1990). Unlike vegetative meristems, floral meristems are not embryonic in origin. Transmissible physiological signals in the vegetative apex initiate floral evocation, which results in transformation to a floral apex (Bernier, 1988). Floral meristems, moreover, are considered to be determinate because in most cases they form only inflorescences. Understanding meristems is, therefore, a prerequisite for the understanding of plant development (Sussex, 1989). An impressive body of descriptive and classical experimental studies on this subject is discussed comprehensively by Esau (1977), Cutter (1980), and Steeves and Sussex (1989).

In an attempt to dissect the developmental processes in tomato meristems, we undertook the isolation of gene markers common to all plant meristems and others that are more specific to floral meristems. The repertoire of soluble and insoluble proteins of growing leaves and mature flowers was compared with that of *anantha* floral meristems. Floral meristems of *anantha* mutant plants are arrested at an early preorganogenesis stage and then duplicated repeatedly (Helm, 1951; Paddock and Alexander, 1952), thus providing an excellent source of meristematic tissue (Lifschitz, 1988). Selected polypeptides that appeared more abundant in *anantha* meristems were purified, antibodies were prepared, and cDNA clones were isolated. The tomato deoxyuridine triphosphatase (dUTPase) gene reported here was cloned as a consequence of its upregulation in *anantha* floral meristems.

dUTPase catalyses the hydrolysis of dUTP to dUMP, and dUMP is the precursor of thymidine diphosphate (dTDP) (Kornberg, 1980). In eukaryotes, one important pathway for dTDP requires the two-step production of dUMP from dCDP and a two-step conversion of dUMP to dTDP. Consequently, dUMP affects the pools of both dCDP and dTDP with many ramifications. dUMP is also produced by dUTPase, which, in this way, may intervene in the synthesis of both desoxythymidine triphosphate and the other three diethyl-*p*-nitrophenyl monothiophosphates. The biosynthesis of the other three deoxynucleotide diphosphates is accomplished by a one-step reduction of the corresponding ribonucleoside diphosphates (reviewed by Reichard, 1988). An additional crucial role of dUTPase in all organisms is to eliminate dUTP, thus preventing the damage that is inflicted on the DNA by the excision of misincorporated deoxyuridine (Shlomai and Kornberg, 1978; Kornberg, 1980). dUTPase is therefore an important enzyme in DNA metabolism.

In this report, we describe the isolation of the 18-kD polypeptide P18 and the corresponding cDNA clone and their

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conclusive identification as dUTPase and its coding sequence. The spatial and temporal expression patterns of dUTPase in meristematic zones of vegetative and floral apices of tomato are analyzed and the possible developmental implications evaluated.

RESULTS

Isolation of the P18 Protein and Its Coding Sequence

The *anantha* mutation was first characterized by Helm (1951). Paddock and Alexander (1952) discovered a similar, regular recessive mutation and appropriately named it *cauliflower.* Floral meristems of the *anantha* mutant inflorescences are arrested at the same early preorganogenesis stage, but the arrested primordia continue to duplicate and branch repeatedly to form a huge, loose, cauliflower-like, yellow-green inflorescence, as shown in Figure 1. All primordia are usually arrested at the same stage, as is normally found in the course of flower development (Chandra-Sekhar and Sawhney, 1984). Furthermore, the floral homeotic genes of the MADS box family (Schwarz-Sommer et al., 1990) are already expressed in *anantha* meristems (Pnueli et al., 1991), and under certain extreme conditions the meristematic buds develop gynoecial structures. As discussed in this report and in the accompanying report by Shahar et al. (1992), polyphenoloxidase (PPO) and dUTPase are expressed in *anantha* meristems in patterns characteristic of floral buds.

Analysis of protein profiles was the first step in the identification of presumptive meristematic genes. The relative abundancies of more than 1500 bands representing soluble proteins from *anantha* meristems, growing leaves, and normal mature flowers were evaluated. Six major polypeptides were identified as "specific" to meristems by Coomassie blue staining of one-dimensional denaturing gels (see Methods). The analysis of the gene coding for the protein P18 is presented here. The P18 polypeptide was eluted from the DEAE cellulose column with 0.15 M salt. After chromatography of this fraction on a hydrophobic (reversed phase) column, an abundant 18-kD polypeptide was identified in extracts from *anantha* meristems, as shown in Figure 2. A polyclonal antibody for P18 was prepared and subsequently used to probe a phage expression library constructed from poly(A) RNA of *anantha* floral meristems. One positive λgt11 clone was identified from among 1.25×10^5 screened, and the EcoRI insert, 764-bp long, was recloned into the EcoRI site of pTZ18u. DNA gel blot analysis of genomic DNA (data not shown) suggested that the gene coding for P18 is most likely unique in the genome.

The P18 Gene Is Coding for the Tomato dUTPase

The 764-bp sequence of the P18 cDNA clone, along with the deduced amino acid sequence of the P18 polypeptide (M_r 17.931) are shown in Figure 3. In a search of the protein data bank (Swiss Prot 13), we found a similarity to the pseudoprotease genes of *vaccinia* and *orf* poxviruses, and to a lesser degree to other pseudoproteases. Pseudoproteases were named because of their modest similarity to retroviral proteases (McClure et al., 1987; Mercer et al., 1989; Slabaugh and Roseman, 1989), but their actual function has never been

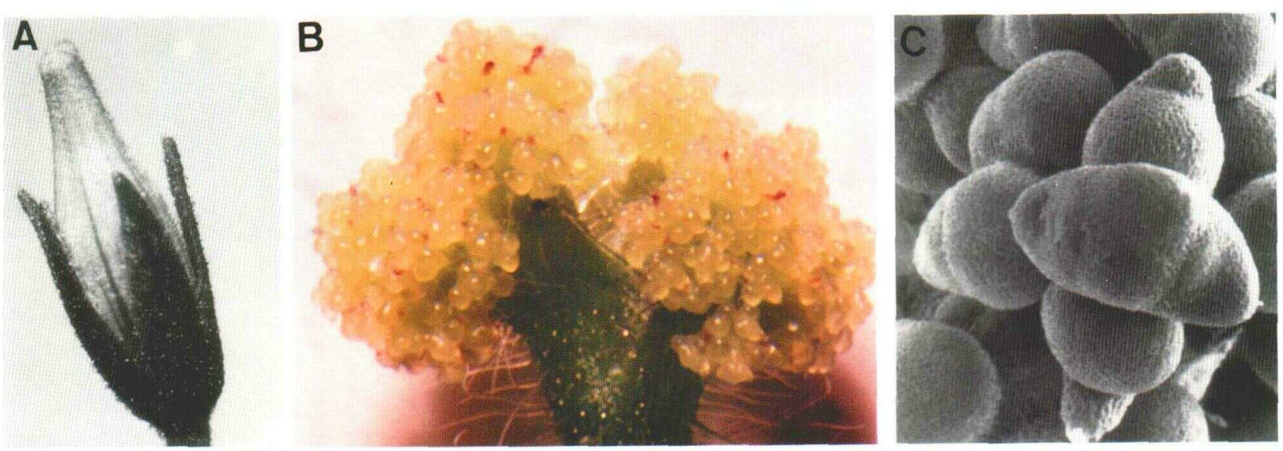

Figure 1. Normal Tomato Flower and the *Anantha* Floral Meristems.

(A) Normal tomato flower, 2 days before anthesis.
(B) A branch of the *anantha* inflorescence.
(C) Scanning electron micrograph of an *anantha* floral primordia. See Chandra-Sekhar and Sawhney (1984) for comparison with normal floral primordia.

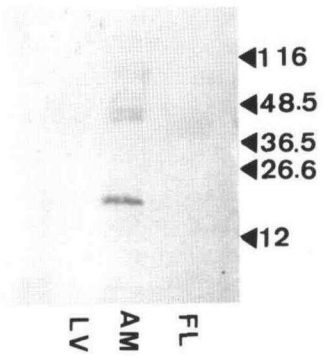

Figure 2. Protein Gel Blot Analysis of P18 with Anti-P18 Polyclonal Antibody.

Total soluble proteins were fractionated on 15% SDS-PAGE gel, blotted onto nitrocellulose filters, and reacted with anti-P18 antibody in 10^{-4} dilution of the original antiserum. LV, total soluble proteins of leaves; AM, *anantha* floral meristems; FL, normal flowers.

elucidated by direct experimentation. Recently, McGeoch (1990) presented suggestive evidence, based on the conservation of five key motif sequences, that pseudoprotease sequences are related to genes coding for dUTPase from herpes viruses and also from *Escherichia coli.*

The comparison of the amino acid sequence of P18 with those of *vaccinia* pseudoprotease and *E. coli* dUTPase genes is given in Figure 4. The five peptide motifs that, according to McGeoch, characterize the dUTPase gene family are also conserved in the P18 gene (shaded sequences in Figure 4). Consequently, the dUTPase activity of the protein fraction that contains the P18 polypeptide was tested. Results presented in Table 1 indicate that dUTPase activity and P18 are coeluted from the DEAE cellulose column in 0.15 M salt (Table 1, part A), that the hydrolytic activity of the 0.15 M salt fraction is absolutely specific to dUTP (Table 1, part B), and that hydrolysis of dUTP by this column fraction is almost completely blocked by the addition of antibody for P18 but not by the preimmune serum (Table 1, part C).

The predicted molecular weight for P18 is 17.931 kD. The monomer is thus somewhat larger than that of *E. coli* (152 residues) or of *vaccinia* virus. Estimated molecular weights for enzymes from other eukaryotes vary between 36 and 68 kD, depending, presumably, on subunit composition. Polypeptides of 12, 24, and 36 kD were reported in *Allium cepa* (Pardo and Gutiérrez, 1990), and the figure for the larger protein agrees well with a dimer form of the tomato polypeptide.

A unique feature of the plant polypeptide primary structure is the proportion and distribution of prolines in its amino terminal half. Prolines comprise 13 to 14% of the first 84 amino acid residues of P18 and are regularly separated by five to seven other residues. Interestingly, when the gap between prolines is duplicated or triplicated, the codons found in the "missing links" can be derived with only one base substitution from proline codons.

Spatial and Temporal Expression of the P18 Gene in Plant Meristems

Results shown in Figure 5A verify that the P18 cDNA clone recognizes a poly(A) RNA of ~750 bp. The level of this RNA is ~20-fold higher in extracts of *anantha* meristems than in extracts of young expanding leaves or normal mature flowers. The expression of the P18 gene in the major organs of the tomato plants, as recorded in blots of total RNA, is depicted in Figure 5B.

P18 was isolated under the assumption that accumulation of a particular protein in *anantha* floral primordia reflects a possible role in meristems in general. Plant meristems are composed of several types of cells, however, and their borders are not accurately defined. Mere quantitation of a particular mRNA or protein in extracts of whole organs is not satisfactory and can sometimes lead to erroneous conclusions. To determine in detail the tissue and cell specificity of gene P18, we used an indirect immunolabeling detection technique to localize gene products to the single cell level.

The distribution of the P18 antigen in cells and tissues of floral and vegetative meristems is illustrated in Figure 6. The most dense signals for P18 in *anantha* meristems are located in the central zones of the apices of the arrested floral primordia and along the provascular strands. Note, in particular, that

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      taaacaatggct
        || ||| ||
gaattcccaaaaatggcagaaaatcagatcaactctcctgagatcaca  48
              M  A  E  N  Q  I  N  S  P  E  I  T
gaaccttctcccaaggttcagaaactggaccaccctgaaaacggcaat  96
 E  P  S  P  K  V  Q  K  L  D  H  P  E  N  G  N
gtccccttttccgagtgaagaagctctctgaaaacgctgttttgccc 144
 V  P  F  F  R  V  K  K  L  S  E  N  A  V  L  P
tcaagagcctcttctcttgctgctggttatgatctatcaagtgctgca 192
 S  R  A  S  S  L  A  A  G  Y  D  L  S  S  A  A
gagactaaagttcccgccagaggcaaggctcttgtacccacagatctc 240
 E  T  K  V  P  A  R  G  K  A  L  V  P  T  D  L
agtattgctgttcctcaaggaacctatgctcgtattgcacctcgttct 288
 S  I  A  V  P  Q  G  T  Y  A  R  I  A  P  R  S
ggtttggcatggaagtattctatagatgttggagctggagtcatagat 336
 G  L  A  W  K  Y  S  I  D  V  G  A  G  V  I  D
gctgattatagagggcctgttggggtagtattgttcaaccactctgaa 384
 A  D  Y  R  G  P  V  G  V  V  L  F  N  H  S  E
gttgattttgaagtcaaggttggtgataggatagctcagcttattgtt 342
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taagtacttgatgaatatattgttcgaaaaaaaaaaaaaaaaaaa    764
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Figure 3. Nucleotide and Deduced Amino Acid Sequence of the P18 cDNA Clone.

Translation initiation site was inferred from the comparison with the plant consensus sequence (Joshi, 1987; Lütke et al., 1987) (shown above the 5′ end of the P18 sequence).

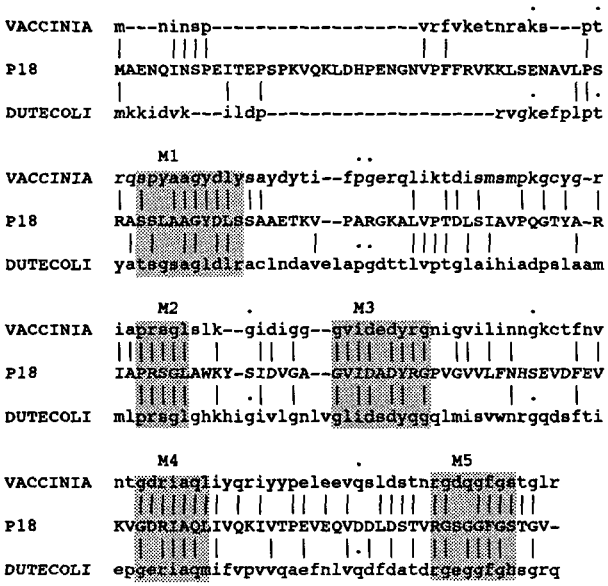

Figure 4. Primary Amino Acid Sequences of P18, *Vaccinia* Pseudoprotease, and *E. coli* dUTPase.

The five boxes assigned by McGeoch (1990) as characterizing dUTPases are shaded and numbered. Homologies of P18 with any of the two genes are indicated by vertical lines, and homologies between *E. coli* and *vaccinia* genes only are indicated by dots. For more extensive comparisons of pseudoprotease sequences with known viral dUTPases, the reader is referred to McGeoch (1990).

labeling is diminished as distance from the apical dome increases, except in the provascular strands where signal strength remains high (Figure 6A).

Similar patterns of tissue specificity are found for P18 in longitudinal sections of normal floral buds at a stage parallel to that of *anantha* (Figure 6C). The antigen is localized in the apical cells and provascular tissues of the normal floral primordia. This pattern is exactly complementary to that of PPO in the very same organs (Shahar et al., 1992).

Because expression of the P18 gene is not restricted to floral meristems (Figure 5), a comparison of tissue specificities in vegetative and floral meristems was conducted. As in floral apices, P18 antigen is most abundant in the central zone of the apical vegetative meristem and in provascular strands, marking probable meristematic activity (Figure 6B). We noticed, however, that unlike floral meristems, the peripheral cell layer of the shoot apex, the tunica, is consistently devoid of P18. In agreement with the RNA studies (Figure 5), the level of P18 antigen in the root apex is low, but the meristematic tissues and provascular tissues are still differentially labeled (Figure 6D).

For controls, these spatial patterns were contrasted with the distribution of the light-harvesting (LH) protein of photosystem II (Figure 6E), or with the preimmune serum that was substituted for the antibody P18 (Figure 6F). The chloroplast-bound LH protein (Figure 6E) is found mostly in parenchyma cells of the ground tissue, just below the epidermal layer.

Localization of P18 in Tissues of the Differentiated Floral Organs

Developing floral primordia sequentially form new tissues and organs. The localization of P18 in the developing organs of tomato flowers is documented in Figure 7. An oblique section of a floral bud, at a stage where only sepal primordia have been formed, is shown in Figure 7A (compare with Figures 6A and 6C). A longitudinal tangential section in a floral bud,

Table 1. dUTPase Activity Assays

A		B		C	
Column Fraction	Units (SE)*	Competitive Nucleotides	Activity Units (SE)*	Serum Added	Activity Units (SE)*
SP	5.37 (1.27)	None	13.71 (0.28)	None	14.46 (0.13)
0.1 M	0.58 (0.03)	dUTP × 10^3	7.21 (0.45)	AP18 1:500	9.27 (2.67)
0.15 M	12.05 (0.93)	dUTP × 3 × 10^3	4.20 (0.30)	AP18 1:166	5.78 (1.60)
0.2 M	4.81 (0.89)	dUTP × 10^4	1.51 (0.35)	AP18 1:100	3.30 (1.12)
0.25 M	2.73 (0.71)	dUTP × 3.3 × 10^4	0.55 (0.01)	AP18 1:50	0.86 (0.17)
0.3 M	1.51 (0.21)	dA, dG, dC, dT, rU	12.62 (0.77)	Preimmune	14.01 (0.66)
VV	2.12 (0.21)				

(A) Hydrolysis of dUTP by DEAE cellulose salt fractions of total soluble proteins from *anantha* meristems. SP, total soluble proteins; 0.1 to 0.3 M, NaCl step gradient fractions; VV, column void volume fraction.

(B) Specificity of dUTPase activity. Competition assays were conducted under standard reaction conditions with the addition of dUTP in the multiplication factors indicated in the table. Similar tests were conducted for each of the four dNTPs and UTP, and the results were pooled, as no inhibition was detected.

(C) Inhibition of dUTP hydrolysis by anti-P18 antiserum. P18 antibody in 1:50 to 1:500 dilutions was used to immunoprecipitate 0.1 μg protein of the peak column fraction (see column **A**), and supernatants were assayed for dUTP hydrolysis. Preimmune serum was used with identical dilutions, but results of all four tests were pooled, as no inhibition was observed.

* One unit = production of 1 pmol dUMP/min under standard assay conditions (Methods). All values are given as a mean and SE of at least three tests.

following emergence of all organs, is shown in Figure 7B. The two pictures verify that P18 antigen is most abundant in apical cells of growing zones. It diminishes basipetally or when extensive cell proliferation subsides but is always found in cells of the vascular bundles.

A more advanced stage, in which major tissues of all organs are already differentiated but proliferation of sporogenic cells in the anther has just begun, is shown in Figure 7C. Only vascular bundles of all organs and the region of the anther where sporogenic tissue and stomium differentiate (arrowheads in Figure 7C) are labeled. After formation of pollen mother cells (Figure 7D), however, only the stomium, its flanking epidermal cells, and a few cells in the connective tissue are labeled. At the same stage of development, signals are found in the integuments of the ovules, in the endocarp cell layer lining the locule wall, and in an additional separated cell layer in the ovary wall (Figure 7E). As in earlier developmental stages, P18 antigen is always detected in cells of the vascular bundles of the floral organs long after it has disappeared from the rest of the organ's cells. The most noteworthy demonstration of this phenomenon is observed in the central vascular system of the flower receptacle (Figure 7F).

The distribution of dUTPase antigen suggested that gene expression may be correlated with the meristematic potential of cells and not only with the proliferative activity of the particular tissue. It is essential, however, to investigate the relation between gene expression and presence of the protein. The results of the in situ hybridization experiments shown in Figure 8 led to the conclusion that the P18 antigen molecules found in cells of the mature vascular system are not the remnants of those present in the progenitor meristematic cells but rather are the consequence of de novo gene expression.

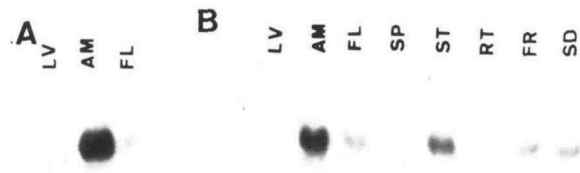

Figure 5. RNA Gel Blot Analysis of P18 Gene Expression in Tomato Organs.

(A) Analysis of poly(A) RNA. 0.3 μg of each RNA was fractionated by 1.25% denaturing agarose gels, transferred onto nylon filters, and probed with a gel-purified ^{32}P-labeled P18 cDNA fragment.
(B) Analysis of P18 transcripts in total RNA samples from tomato plant organs. 20 μg of total RNA was loaded per lane. RNA was also calibrated by hybridization to labeled rDNA probe.
LV, young growing leaves; AM, *anantha* floral meristems: FL, sepalless flowers; SP, sepals; ST, shoots (5 cm long, six leaves stage); RT, roots; FR, fruits, 5 mm diameter; SD, seeds.

DISCUSSION

The P18 Gene Codes for the Tomato dUTPase

The tomato P18 gene was isolated as a consequence of its abundance in floral primordia of the *anantha* mutant inflorescences. The *anantha* inflorescences, like those of cauliflower curds, are a preferred source for the isolation of meristem-specific genes in general and floral meristem genes in particular (Lifschitz, 1988). The compelling evidence for the P18 gene being dUTPase depended on isolation of the protein and an antibody for testing. A flower-specific gene was positively identified as the plant threonine deaminase using similar methods (Samach et al., 1991).

The P18 gene is moderately similar to dUTPase genes from *E. coli* and herpes simplex virus and is highly similar to the so-called pseudoprotease sequences of two poxviruses. McGeoch (1990) has recently argued that sequences defined as pseudoproteases in oncoviruses, lentiviruses, and poxviruses are, in fact, related to dUTPase rather than to retroviral proteases. This suggestion was based primarily on the identification of five short peptide motifs (Figure 4) common to pseudoproteases and to known dUTPase genes from *E. coli* and herpes viruses. The P18 protein coelutes with bona fide dUTPase activity. Such activity is blocked by anti-P18 antibody. Furthermore, the cloned sequence contains five motifs common to bacterial and viral dUTPases and exhibits a 40 to 60% overall similarity to poxvirus pseudoprotease genes. These findings clearly support McGeoch's conclusion. We do not know why tomato dUTPase is more closely related to dUTPase genes (pseudoprotease) of the poxviruses that replicate in the cytoplasm rather than to herpes viruses that replicate in the nucleus. The fact is that for all five motifs (~30 residues), homology between P18 and poxviruses genes exceeds 90%.

There could be more than one dUTPase gene in tomato, although it is clear that there is no other gene with close sequence homology to P18. It is possible that genes coding for organelle dUTPases are not sufficiently similar to P18 to be detected by DNA hybridization.

Speculation on the Role of dUTPase in Meristematic Zones

dUTPase may be expressed at high levels in keeping with a strictly metabolic role, thus making dUTP unavailable for DNA polymerase (Shlomai and Kornberg, 1978) and providing an alternative route for the biosynthesis of dUMP (Reichard, 1988). dUTPase can also modulate the incorporation of deoxyuridine into DNA for developmental purposes, as for the regulated degradation of chloroplast DNA in Chlamydomonas (Burton et al., 1979) or of metamorphosed cells in Drosophila (Giroir and Deutsch, 1987).

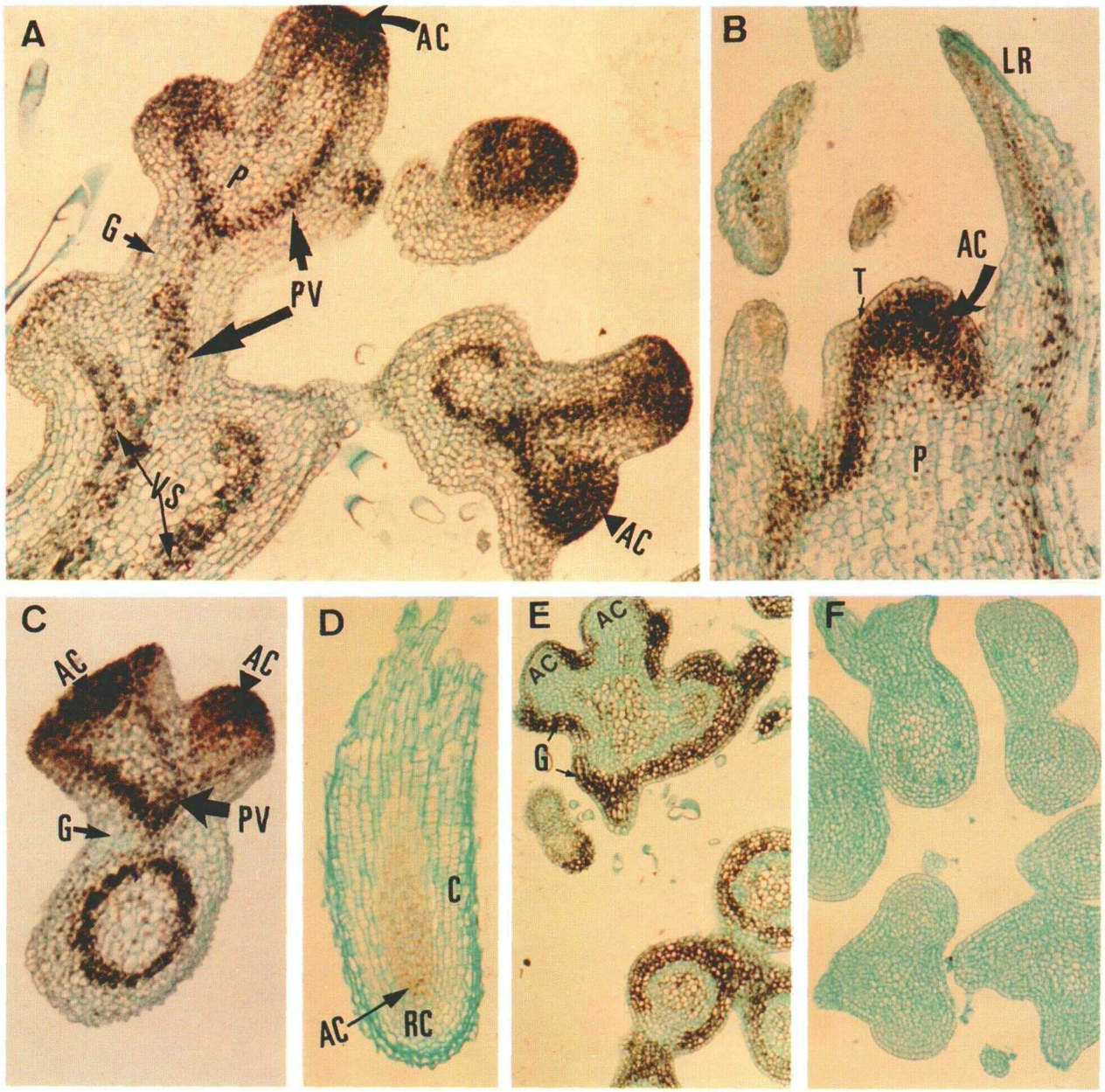

Figure 6. Localization of P18 Antigen and Expression of P18 Gene in Tomato Meristems.

(A) to **(F)** Immunogold detection of P18 antigen.

(A) *Anantha* floral primordia. Signals are restricted to cells of the apical dome and provascular tissue. Note the continuity between the two tissue systems and the lack of labeling in ground cells and pith cells.

(B) Vegetative shoot apex, four true leaves stage. Longitudinal section. Note the differential decoration of the tunica (first peripheral cell layer, T) and corpus (see text).

(C) Normal flower primordia. Note the similarity with *anantha* primordia. The labeled round vascular tissue actually represents another floral bud.

(D) Root apex, oblique section.

(E) Localization of the LH protein of photosystem II in floral primordia of *anantha* meristems.

(F) *Anantha* meristems probed with preimmune serum.

AC, apical cells; C, cortex; G, ground cells; LR, leaf primordium; P, pith cells; PV, provascular strands; RC, root cap; T, tunica; VS, vascular system.

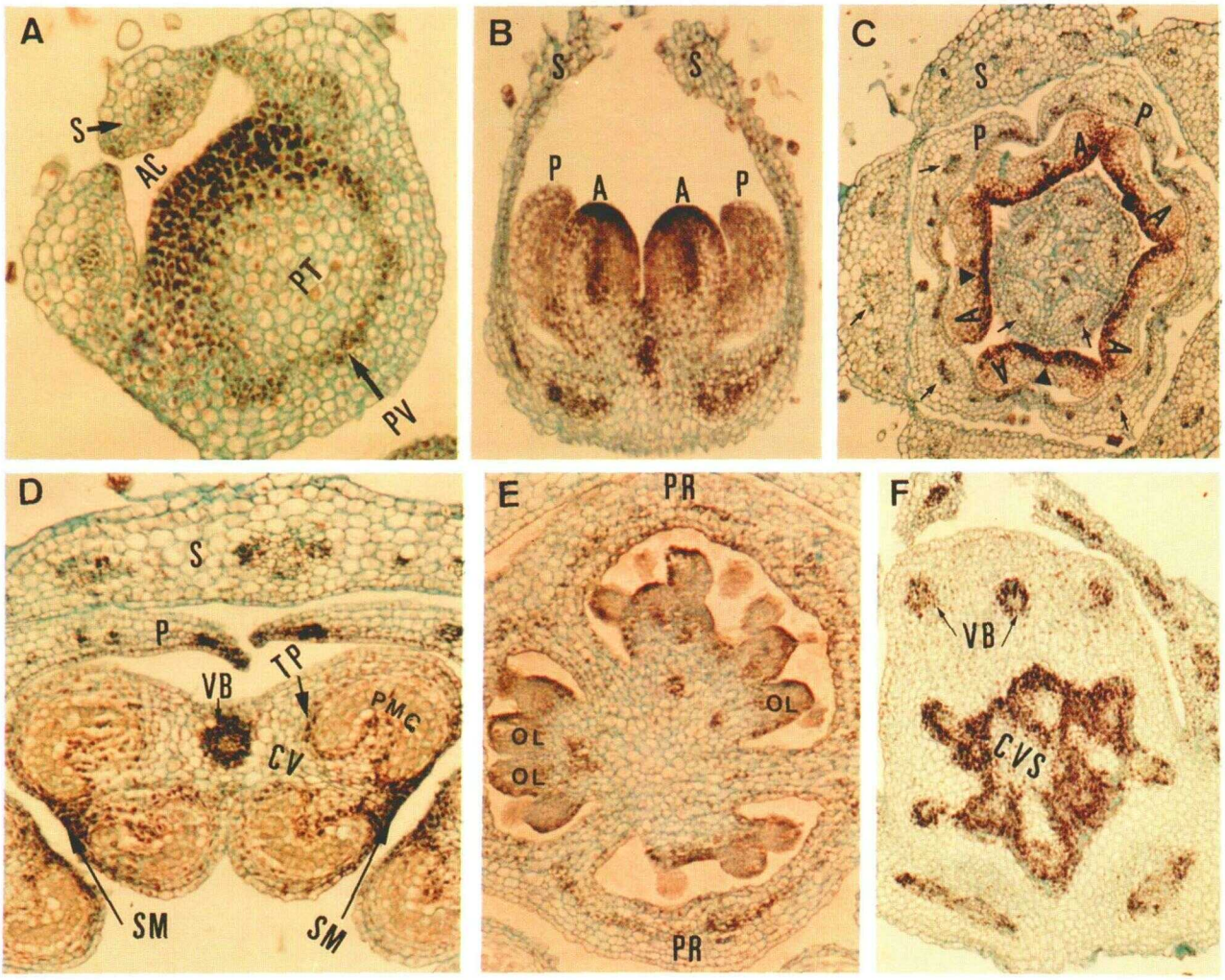

Figure 7. Localization of P18 Antigen and Expression of the P18 Gene in the Developing Tomato Flower.

(A) Floral primordia, oblique section.

(B) Differentiated floral bud. Longitudinal median section. Note that the most recently developed organ primordium (the stamen) is most intensely decorated.

(C) Early differentiated flower. Anthers and ovary are still in primordial stage and have not completed differentiation. Arrows mark vascular bundles.

(D) Cross-section of corolla and androecium of mature flower after differentiation of PMC.

(E) Cross-section of ovary of mature flower. The same flower as in **(C)**.

(F) Localization of P18 antigen in the vascular system of the flower receptacle.

A, anthers; AC, apical cells; CV, connective; CVS, central vascular system; PR, pericarp; OL, ovule; P, petals; PT, pith cells; PMC, pollen mother cells; PV, provascular strands; S, sepals; SM, stomium; TP, tapetum; VB, vascular bundles.

Alternatively, dUTPase could have a regulatory, rather than a strictly metabolic, role in meristematic cells. Cell cycles can be regulated at the level of the single cell, as in yeast (Hartwell and Weinert, 1989). In yeast, dUTPase, dCMP deaminase, and DHFR (McIntosh et al., 1986) are found throughout the cell cycle, whereas ribonucleotide reductase (Lowden and Vitols, 1973; Elledge and Davis, 1990; Lowndes et al., 1991), thymidilate synthase (Storms et al., 1984), and thymidilate kinase (White et al., 1987) fluctuate according to the cell cycle. In plants, resting and proliferating meristematic root cells of *A. cepa* have similar levels of dUTPase activity, provided that the cells have not been arrested with hydroxyurea (Pardo and Gutiérrez, 1990). The multicellular nature of meristematic zones and their function also requires regulation at the organ level so that cell division in various parts of the apex can be coordinated. This may be accomplished by

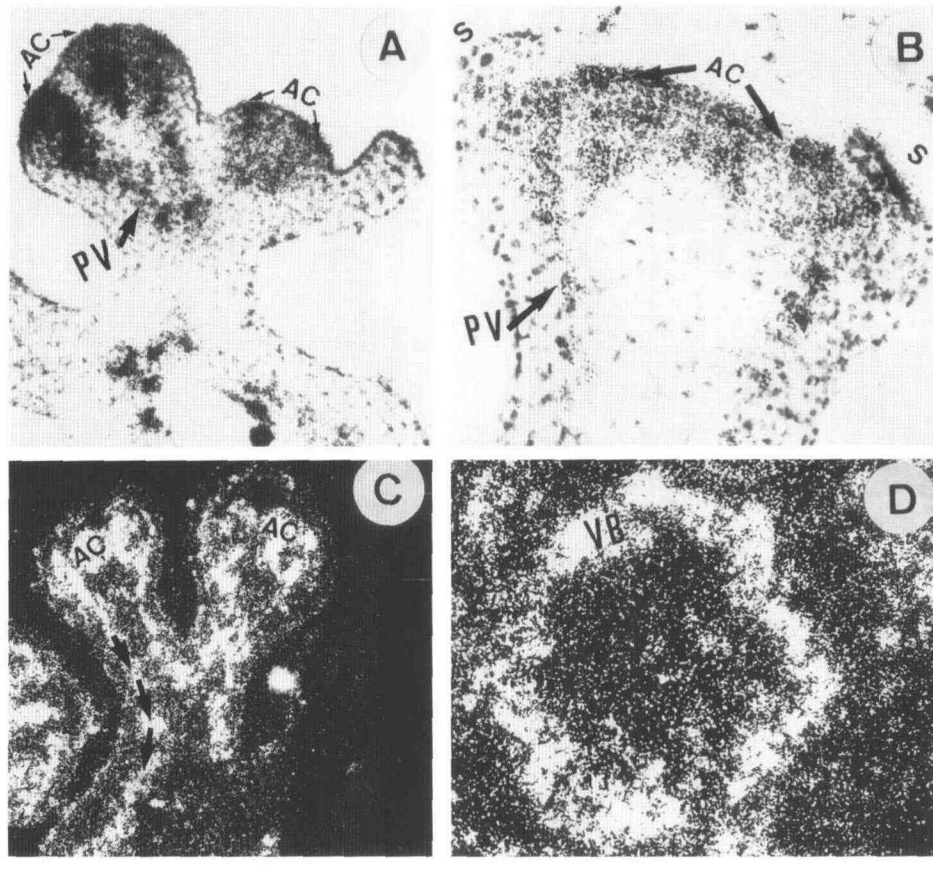

Figure 8. In situ Hybridization of P18 Antisense RNA with *Anantha* and Normal Floral Primordia.

(A) *Anantha* meristems (compare with Figure 6A). Bright-field microscopy.
(B) and **(C)** Normal floral buds (compare with Figures 6C, 7A, and 7B). Arrows in **(C)** delineate provascular strands. **(B)** Bright-field photography.
(C) Dark-field photography.
(D) Vascular system of the floral receptaculum (compare with Figure 7F). Dark-field photography.
Exposure time, 12 days. AC, apical cells; PV, provascular strands; S, sepal primordia; VB, vascular bundles. Hybridization with P18 sense RNA resulted in only low homogeneous background signal.

the modulation of one or several key enzymes of DNA metabolism. Like ribonucleotide reductase in yeast (Elledge and Davis, 1990), dUTPase may be regulated by the hypothetical meristematic program while at the same time participating in the regulation of this program.

In tomato, the dUTPase gene maintains a high level of activity in apical dome cells of shoot and floral apices. Within the boundaries of these expression domains, all cells, resting or dividing, in the quiescent center of flanking regions, exhibit a high level of gene activity. It is inferred, therefore, that expression of dUTPase in meristematic zones is independent of cell cycle. In addition, the expression patterns of dUTPase shown in Figures 6 and 7 indicate: (1) homogeneous labeling of meristematic cells in the main central region of apical domes, (2) downregulation of dUTPase gene activity in the immediate parenchymal derivatives of the apical meristem cells, again independent of their division rate, and (3) the retention of high activity of dUTPase in the immediate provascular derivatives of the apical dome cells.

This pattern deviates sharply from that of other genes whose expression was found to be associated with meristems. Histone H2A, the only other identified gene with high expression levels in shoot meristems, is expressed in single scattered cells of the shoot apex and derived tissues in a pattern that is consistent with that expected of cycling cells (Koning et al., 1991). Other, as yet unidentified, "apex-specific" genes in tobacco (Kelly et al., 1990) or cauliflower-curd–specific genes (Medford et al., 1991) are expressed only in subdomains of meristematic zones rather than in the homogeneous pattern exhibited by dUTPase. Likewise, genes expressed during transition from vegetative to floral stages in *Sinapis alba* (Melzer et al., 1990) or floral homeotic genes

(Schwarz-Sommer et al., 1990; Coen and Meyerowitz, 1991) are only expressed in specific developmental territories of floral meristems.

If there is indeed a developmental program that distinguishes a meristematic state of cells from other states, it is expected to be represented by genes that are active in all plant meristems, in all cells within a given meristematic apex and independent of superimposed developmental programs. dUTPase, we surmise, is thus far the best candidate for such a marker. This speculation is supported by the maintenance of high gene activity in cells associated with vascular tissues that may have a potential cambial activity even when expression in surrounding dividing tissues has subsided.

The inactivity of dUTPase observed in L1 cells of the vegetative shoot apex would seem to contradict such a view. This phenomenon, however, is in accord with the unique expression patterns described for PPO in the shoot apex (Shahar et al., 1992). PPO is upregulated specifically in the L1 cells where dUTPase is inactive, again distinguishing the epidermal progenitors from other meristematic cells. The alternative expression of dUTPase and PPO in L1 cells of shoot apices is by no means trivial. The two genes exhibit remarkable complementary patterns in floral apices as well, where dUTPase is expressed in all apical meristem cells and in their immediate provascular derivatives, whereas PPO is upregulated only in the differentiating parenchymal derivatives of the apical dome cells. As illustrated by Shahar et al. (1992), the two genes mark alternative expression domains in several developing organs as well.

Thus, in addition to the different developmental fate of their progeny, L1 cells of the vegetative shoot apex of tomato are distinguished from the rest of the meristematic cells by at least two biochemical activities, e.g., dUTPase and PPO. As commitment and lineage are mostly irrelevant for developmental characterization of plant cells (Sussex, 1989), this observation calls for revised definitions of the meristematic nature of cells.

dUTPase genes or pseudoprotease sequences are found in genomes of large DNA viruses (McClure et al., 1987; Slabaugh and Roseman, 1989), along with other genes of DNA metabolism. Of general interest is the provoking observation that they are also conserved in the reduced genomes (~10 kb) of several retroviruses, like SRV-1 (Power et al., 1986), E1AV (Kawakami et al., 1987), or MMTV (Moore et al., 1987), where other genes of DNA or RNA metabolism have been eliminated. If, indeed, pseudoproteases are synonyms of dUTPases (McGeoch, 1990), this observation calls for a special, as yet unknown, regulatory role for dUTPase. Such a role may, in turn, be related to that played by dUTPase in meristematic cells.

To evaluate the possible developmental significance of the levels of dUTPase in meristematic zones, it is essential first to study the regulation of other key components of the replication machinery. We will then have to investigate how experimental modulation of these activities in the plant affects meristematic properties and potentials.

METHODS

Plant Material

The Tiny Tim cultivar (LA154; Stevens and Rick, 1986) served as a major source of normal plant organs. For the isolation of RNA and proteins, flowers were harvested 2 to 4 days before anthesis. Growing leaves were collected at ~10 to 15% of their final size. Early floral meristems were collected from plants homozygotous for the *anantha* mutation (LA536; Helm, 1951; Paddock and Alexander, 1952). Seeds were kindly provided by C. Rick (University of California at Davis, Davis, CA).

Nucleic Acid Procedures

RNA was extracted, as described by Ausubel et al. (1988), with modification (Samach et al., 1991). DNA was isolated as in Bernatzky and Tanksley (1986), but with the addition of 0.15% Triton X-100 to the extraction buffer. DNA and RNA gel blots were conducted as in Maniatis et al. (1982). A cDNA expression library was constructed in λgt11 (Huynh et al., 1985) from mRNA prepared from *anantha* meristems or from normal flowers, according to Gubler and Hoffman (1983).

Fractionation of Proteins and Comparison of Protein Profiles

Tissues were homogenized in 25 mM NaCl, 100 mM Tris-HCl, pH 7.4, and 1 mM phenylmethylsulfonyl fluoride (4 mL per 1 g fresh weight), five times for 20-sec cycles in a Waring (New Hartford, CT) blender. After low-speed centrifugation to remove crude debris, the homogenate was centrifuged at 100,000*g* for 30 min. The supernatant was diluted twofold with water and applied to a DEAE-cellulose column (DE52; Whatman). A void fraction, along with five fractions eluted by increasing concentration of an NaCl step gradient (0.1, 0.15, 0.2, 0.25, and 0.3 M NaCl), were collected. The void fraction was brought to 10 mM phosphate (pH 7.4), loaded onto a phosphocellulose column, and eluted with 1.2 M NaCl, 10 mM phosphate. Each of the final seven fractions was concentrated, dialyzed, and further chromatographed on a C3 reversed phase HPLC column (Protein plus, Zorbax series; Du Pont) using a 5 to 80% gradient of acetonitrile in 0.1% TFA. Twenty 1-min fractions of 1 mL were collected and subjected to one-dimensional SDS-PAGE (Laemmli, 1970) separation. Gels were stained with Coomassie blue, and protein profiles from growing leaves, *anantha* floral meristems, and normal flowers were compared. It is estimated that ~1500 major bands from each tissue were analyzed in this way.

Antibodies and in Situ Localization of P18 Antigen

P18 polypeptide was electroeluted from gel slices and rechromatographed, and the second eluate was transferred onto nitrocellulose filters. Strips of nitrocellulose loaded with ~15 μg protein were dissolved in DMSO and used to immunize rabbits, as described in Harlow and Lane (1988). A second immunization was carried out after 4 weeks, followed by two more booster immunizations at 10-day intervals.

For antigen localization, tissues were fixed in a 3:1 mixture of ethanol/acetic acid for 24 to 48 hr at 4°C. Primary antibody at a dilution

of 1:500 was applied to 8 μm paraffin in section for 18 hr. Detection was done with gold labeled (1 nm) goat anti-rabbit (Amersham International) second antibody according to the manufacturer's protocol. Slides were then counterstained with alcian-green and safranin (Yoel, 1983). IgG was purified using Avid AL columns. Affinity-purified, anti-P18 antibody was then eluted from nitrocellulose filters previously loaded with the fusion protein made by the λgt11 P18 clone and incubated with the IgG fraction of the primary antiserum.

In situ hybridization was carried out according to Cox and Goldberg (1988) with ^{35}S-labeled sense and antisense RNA as probes. Tissues were fixed in FAA (3.7% formaldehyde: 50% ethanol and 5% acetic acid) embedded in paraffin, and 8-μm sections were hybridized overnight with 8 × 10^5 cpm of fragmented probe. Exposure time was 12 days.

Enzyme Assays

dUTPase activity was assayed according to Shlomai and Kornberg (1978). Standard assay conditions were 0.1 μg protein, 15 μM ^{3}H-dUTP, 10 mM dithiothreitol, and 10 mM potassium phosphate buffer (pH 6.5) in 10 μL reaction mix. Incubation was at 30°C for 20 min. Protein concentration was estimated by the Coomassie blue method using bovine serum albumin as the standard. Immune precipitation of P18 protein was conducted according to Wilcox (1986).

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