Immunological Characterization of a Tapetal Protein in Developing Anthers of *Lilium longiflorum*

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ABSTRACT

Antiserum was raised in rabbits against a lily (*Lilium longiflorum*) anther-specific protein (LLA-15). Monospecific anti-LLA-15 antibodies were prepared to investigate the distribution of LLA-15 during anther development in a variety of flowering plants. Immunoblot analyses of total protein from floral and vegetative organs confirmed that LLA-15 or LLA-15-like proteins accumulated to detectable levels only in a discrete stage of anther development. In situ localization using anti-rabbit immunoglobulin G conjugated with gold particles confirmed that LLA-15 was specifically localized in the tapetal tissue of lily anthers. The maximal level of LLA-15 was strictly coincident with the peak of tapetal secretory functions. Immunoblots of two-dimensional polyacrylamide gels of lily anther proteins indicated that the seven LLA-15 isoforms ranged from isoelectric point 5.6 to 6.1. In vitro translation of lily anther mRNAs showed that four of these isoforms were primary products, the additional three being a result of posttranslational processing of the primary translation products.

The premeiotic anther is composed primarily of sporogenous tissue surrounded by a number of wall layers. The sporogenous cells undergo meiosis and give rise to microspores that, in turn, divide mitotically and differentiate into pollen grains. In addition to the specialized sporogenous cells, the diverse cell types in the anther-wall layers have distinct features. For instance, lignification occurs on the wall of the endothecium (10). Both the endothecial and middle cells are enlarged and the breakdown products of starch in the cells serve as a nutritive source for the microspores and pollen grains (26). In the anther wall, the tapetum is the innermost layer that surrounds the sporogenous tissue. The tapetum has drawn much attention because of its apparently nutritive physiological role during microspore and pollen development. The tapetum is also involved in the production of proteins and enzymes (such as callase), the formation of the pollen wall, the production and release of locular fluid, and of pollenkitt, tryphine, and recognition substances all deposited in the pollen wall (5, 14, 26).

Given the hypothesis that each cell type in the anther performs specific functions, each should exhibit a unique pattern of proteins reflecting these various physiological roles. Support for this opinion comes from recent studies on anther-specific genes (11, 18, 20, 23) and on the distribution of mRNAs in lily, tobacco, and rice pollen (25, 30). There is also a similar literature on organ- and tissue-specific proteins in Japanese morning glory (2, 22), *Tulipa* (1, 3), *Gladiolus* pollen and stigma (7), *Lycopersicon* flowers (28), *Petunia* anthers (24), carrot stamen (17), maize and tobacco pollen (8, 35), and, most recently, of developing lily (*Lilium longiflorum*) anthers (33).

A number of studies on the stamen of lily have documented the histological changes during anther growth (12, 14, 26, 34). Only a few reports have dealt with anther proteins and mRNAs from which the proteins were translated. Hotta and Stern (15) and Hotta et al. (16) have examined a number of mitotic and meiotic-associated proteins. Callase in lily anthers has also been characterized by Stiegli and Stern (32). Schrauwen et al. (30) have investigated the mRNA distribution in lily pollen. In a previous report (33), we have described the changes in protein patterns in developing lily anthers, shown them to be closely correlated with histological events, and identified several anther-specific proteins. Here, we continue our examination by using immunological methods to characterize one of these anther-specific proteins, designated LLA-15, for *L. longiflorum* anther protein with a molecular mass of 15.0 kD.

MATERIALS AND METHODS

Plant Materials

Plants of Easter lily (*Lilium longiflorum* L. cv Nellie White) and tobacco (*Nicotiana tabacum* L. cv Xanthi) were grown in a greenhouse under ambient conditions. Plants of cape honeysuckle (*Tecoma capensis*) and red trumpet vine (*Phaedranthus buckinators*) family Bignoniaceae were grown in the field. Plant material was collected, dissected, and frozen as previously described (33).

Production and Purification of Antiserum

The extraction of total protein was performed according to Wang et al. (33). Approximately 20 μg of 2D-PAGE-purified lily anther LLA-15 (c, d, and e polypeptides in Fig. 1) was suspended in 0.5 mL of PBS (10 mM sodium phosphate, pH 7.4, 150 mM NaCl) and emulsified with 0.5 mL of Freund’s complete adjuvant (Difco). The emulsion was injected sub-

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cutaneously into a New Zealand white rabbit. The second injection with approximately 20 μg protein in PBS/Freund’s incomplete adjuvant was administered 4 weeks after the first injection. A booster injection with the same amount of protein in PBS/Freund’s incomplete adjuvant was then administered 1 week after the second injection. Blood was withdrawn from an ear vein every week for 30 d. The blood was allowed to clot for 2 h at room temperature and then stored at 4°C overnight. The clot was removed by centrifugation (15 min at 8000g) and the serum was aliquotted and stored at −20°C. Prior to antigen injection, a blood sample was taken to serve as the preimmune control.

Monospecific antibodies were purified according to the method of Smith and Fisher (31). The preparative blot to which LLA-15 was bound was excised and soaked in blocking solution containing 5% gelatin in TBST for 1 h. The blot was incubated with 5 mL of rabbit antiserum for 2 h. After two 10-min washes in TBST, antibodies were eluted with three 1-min washes with elution buffer (5 mM glycine-HCl, pH 2.3, 500 mM NaCl, 0.5% [v/v] Tween 20, and 100 μg/mL BSA). The eluates were immediately neutralized by the addition of 1 M Na-phosphate (pH 7.7) to a final concentration of 50 mM. Elution volume was 700 μL for each wash.

Immunoblot Analyses

2D-PAGE and SDS-PAGE were carried out according to previously published procedures (33). Protein samples were loaded at the basic end of the isoelectric focusing gels. The second dimension of 2D-PAGE was the same as performing SDS-PAGE. After electrophoresis, the gels were either stained with 0.25% Coomassie blue R-250, 10% acetic acid, and 5% ethanol for 4 h, and destained with 10% acetic acid and 20% ethanol, or electroblotted onto nitrocellulose (0.45 μm, Schleicher and Schuell BA85) in 20 mM Tris base, 150 mM glycine, and 20% methanol (4), at 0.8 A for 90 min using a Transphor TE apparatus (Hoefer Scientific Instruments, San Francisco, CA). The LLA-15 polypeptides were detected using an alkaline phosphatase assay according to the manufacturer (Bio-Rad). The blot was soaked for 1 h in blocking solution containing 3% gelatin in TBST buffer. The blot was then incubated for 2 h with monospecific anti-LLA-15 antibodies (1:50 dilution of eluate in TBST). After three 10-min washes in TBST, the blot was incubated for 1 h with affinity-purified goat anti-rabbit IgG conjugated to alkaline phosphatase diluted 1:3000 in TBST. After three 10-min washes in TBST followed by two 10-min washes in TBS (no Tween 20), the locations of antigen-antibody complexes were visualized by color development catalyzed by alkaline phosphatase with 5-bromo-4-chloro-3-indolyl phosphate substrate, and nitro blue tetrazolium, in a carbonate buffer (100 mM NaHCO₃, 1 mM MgCl₂, pH 9.8).

Isolation of Total RNA

Total RNA was prepared from 15 to 20 mm lily anthers by a guanidinium/cesium chloride method (6) with some modifications. One gram of powdered frozen anthers was thawed into 4 M guanidinium thiocyanate, 10 mM EDTA, 50 mM Tris-HCl (pH 7.5), and 5% β-mercaptoethanol. The mixture was centrifuged for 30 min, 4°C at 12,000g. The supernatant was heated at 60°C for 2 min in the presence of 2% N-lauroyl-sarcosine, then cesium chloride was added to a concentration of 0.1 g/mL. RNA was separated by ultracentrifugation through a dense cushion of 5.7 M cesium chloride buffered with 0.1 M EDTA (pH 7.0). After centrifugation at 131,000g for 16 h at room temperature, the RNA pellet was dissolved by heating at 60°C for 4 h in a convenient volume of 0.5% N-lauroylsarcosine, 5 mM EDTA, 5% β-mercaptoethanol. The resultant solution was extracted twice with an equal volume of phenol/chloroform (1:1, v/v) followed by a single chloroform/isoamyl alcohol (24:1, v/v) extraction. After ammonium acetate was added to a 2.0 M concentration, total RNA was precipitated with 2.5 volumes of 100% ethanol at −20°C. The pellet was dried, dissolved in 10 mM Tris-HCl (pH 7.5) and 0.1 mM EDTA, and stored at −80°C.

In Vitro Translation and Immunoprecipitation

Total RNA was translated in the rabbit reticulocyte lysate (Promega). Each translation mixture (25 μL) consists of 70% (v/v) reticulocyte lysate, 70 mM potassium acetate, 0.5 mM magnesium acetate, 20 units of RNAsin (Promega), 30 μCi [35S]-methionine (Amersham, specific activity 1031 Ci/mmol), and 5 μg of total RNA from 15 to 20 mm lily anthers. Total RNA was denatured by heating at 68°C for 10 min prior to translation. The in vitro translations were incubated at 30°C for 1 h; the reaction was terminated by freezing (−20°C) the reaction mixture.

Immunoprecipitation of translated proteins was according to the method of Kessler (19). The translation mixture was diluted to 1.0 mL with 20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% SDS, 0.02% NaN₃, and 1 mg/mL unlabeled l-methionine. The mixture was precleared by adding 25 μL of preimmune serum and a 25-μL slurry of immunoprecipitin (Bethesda Research Laboratories). After incubating for 10 min at 4°C, the mixture was centrifuged at 14,000g for 2 min. The supernatant was then incubated with 80 μL of anti-LLA-15 antiserum for 1 h at 4°C. Antigen-antibody complexes were precipitated with 25 μL immunoprecipitin as indicated above. The pellet was resuspended and washed with five 1.0-mL changes of 1% Triton X-100 in PBS buffer followed by one wash with PBS (without 1% Triton X-100). The final protein pellet was boiled in 2% SDS and 6 μL urea for 2 min. After removal of the immunoprecipitin by centrifugation, sample buffer was added into the supernatant to give a final concentration of 2.3% SDS, 5% β-mercaptoethanol, 10% glycerol, 0.05% bromphenol blue, and 62.3 mM Tris-HCl (pH 6.75). The mixture was analyzed by SDS-PAGE. For 2D-PAGE, the protein pellet was heated in the solubilization buffer (9.5 μL urea, 2% [v/v] Triton X-100, 5 mM K₂CO₃, 0.5% DTT, 500 μg/mL L-lysine, 0.4% ampholines, pH 3–10 [Serva], and 1.6% ampholines, pH 5–7 [Serva]) at 45°C for 5 min (33). Gels were stained, destained, impregnated with Amplify (Amersham) for 30 min, and dried before fluorography.

Immunogold Labeling

Lily anthers were fixed in 2.5% glutaraldehyde in 25 mM potassium phosphate buffer (pH 6.8), dehydrated in a graded
ethanol series, and embedded in glycol methacrylate (Polysciences, Warrington, PA). Sections were cut 3-μm thick on a Bright 5030 microtome and mounted on glass slides. Treatments of slides were performed in moisture chambers using small drops of reagents placed directly on the sections. The procedure was according to the method of Faye et al. (9) except that sections were washed in TBST-2 containing 0.1% BSA, and 1% gelatin was used instead of 0.5% in the reaction of silver enhancement. The primary antibodies were anti-LLA-15 monospecific antibodies with a 1:5 dilution of eluate in TBST containing 1% gelatin. The second antibodies were goat anti-rabbit IgG conjugated with 10 nm colloidal gold particles (Sigma) diluted 1:5 in TBST containing 1% gelatin. After air drying, slides were dipped in Histoclear (National Diagnostics, Sommerville, NJ) and coverslips were mounted with Permoun, a synthetic mounting medium. Sections were examined on a Zeiss microscope (Carl Zeiss, Oberkochen, Germany) using phase contrast and darkfield illumination and photographed using Kodak Technical Pan 2415 film.

RESULTS

LLA-15 Protein Is Abundant, Heterogenous, and Anther-Specific

A group of proteins in lily anthers have been reported to accumulate coordinately during anther development (33). These polypeptides, designated here as LLA-15, were abundant in the 15-mm anther (indicated by arrowheads in Fig. 1) and 2D-PAGE resolved these proteins into three classes according to their pI: 6.1 (a and b), 5.9 (c, d, e, and f), and 5.7 (f) (Fig. 1). It was not possible to separate the LLA-15c, d, and e polypeptides; therefore, an antisem was raised against a mixture of the three. Antibodies to LAA-15c-e were purified from antisem and the specificity of the antibodies was determined by immunoblot analyses of lily anther proteins (Fig. 2 and Fig. 3, lane 5). The antibodies reacted only with LLA-15 proteins, indicating a high specificity. No proteins were detected when identical blots were incubated with antibodies purified from preimmune serum (data not shown).

2D-PAGE immunoblot analysis (Fig. 2) showed that, in addition to c, d, and e polypeptides, a, b, f, and g polypeptides were recognized by the monospecific anti-LLA-15 antibodies. These results showed the immunological relatedness of the LLA-15a–f polypeptides that were initially identified by silver staining (33). In addition, the monospecific antibodies detected a polypeptide LLA-15g with a pI of 5.6, which was below the detection level of Coomassie blue staining (Fig. 1) or silver staining (33).

Earlier, we reported (33) that LLA-15 polypeptides were anther specific. To confirm the organ specificity of these polypeptides, monospecific anti-LLA-15 antibodies were used in immunoblot studies of total protein extracted from vegetative organs (foliar leaves, roots, stems, and bulb...
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**Figure 3.** Immunoblot analysis of the distribution of LLA-15 protein in L. longiflorum organs. Total protein was extracted from various vegetative and floral organs of 25-mm buds. Approximately 12 μg of total protein from each organ was fractionated by SDS-PAGE, electroblotted onto nitrocellulose, and immunochimically detected using monospecific anti-LLA-15 antibodies. Foliage leaves (lane 1), roots (lane 2), stems (lane 3), bulb leaves (lane 4), anthers (lane 5), filaments (lane 6), tepals (lane 7), ovaries (lane 8), styles (lane 9), and stigmas (lane 10). Positions of molecular mass markers are indicated.

**Figure 4.** Temporal accumulation of LLA-15 proteins during anther development in L. longiflorum. Total protein was extracted from 14 size classes of anthers: 1 = 2 mm; 2 = 3 mm; 3 = 4 mm; 4 = 5 mm; 5 = 6 mm; 6 = 9 mm; 7 = 15 mm (25-mm bud); 8 = 20 mm; 9 = 24 mm; 10 = 26 mm; 11 = 27 mm (65-mm bud); 12 = 27 mm (85-mm bud); 13 = 27 mm (105-mm bud); 14 = 28 mm (125-mm bud). Approximately 12 μg of total protein from each anther size class was fractionated by SDS-PAGE, electroblotted onto nitrocellulose, and immunochimically detected using monospecific anti-LLA-15 antibodies. Three anther developmental phases (I–III) are indicated above the size classes (33). Phase I corresponds to the premeiotic anthers. Phase II corresponds to a period of microспорon development and major cytological changes in the wall layers. Phase III corresponds to pollen maturation. Positions of molecular mass markers are indicated.

**Figure 5.** Temporal accumulation of LLA-15-like proteins during anther development in cape honeysuckle (panel A), red trumpet vine (panel B), and tobacco (panel C). Total protein was extracted from anthers of three flower bud size classes of the cape honeysuckle: 4 to 7 mm (A1), 7 to 10 mm (A2), and 10 to 13 mm (A3); of five bud size classes of red trumpet vine buds: <10 mm (B1), 10 to 20 mm (B2), 20 to 30 mm (B3), 30 to 40 mm (B4), and 40 to 50 mm (B5); of five bud size classes of tobacco: <7 mm (C1), 7 to 14 mm (C2), 14 to 24 mm (C3), 24 to 35 mm (C4), and 35 to 45 mm (C5), and of 25-mm lily buds (D). Approximately 12 μg of anther total protein from each size class was fractionated by SDS-PAGE, electroblotted onto nitrocellulose, and immunochimically detected using monospecific anti-LLA-15 antibodies. M indicates lanes containing marker proteins (97.4, 66.2, 42.7, 31.0, 21.5, and 14.4 kD). leaves), and from floral organs (anthers, filaments, tepals, stigmas, styles, and ovaries) from 25-mm lily buds (Fig. 3). In 25-mm lily buds, anthers are 15 mm in length and the level of LLA-15 proteins was close to the maximum (33). LLA-15 proteins were detected only in the anthers.

**LLA-15 Protein Is Developmentally Regulated in Anthers of Lily and Other Plants**

To determine when LLA-15 protein could be first detected during anther development in L. longiflorum, total protein from 14 size classes of lily anther was fractionated by SDS-PAGE. An immunoblot was incubated with monospecific anti-LLA-15 antibodies to show that the LLA-15 proteins were detected during phase II of anther growth when meiosis occurs in the pollen mother cells and major cytological changes occur in the wall layers (Fig. 4). LLA-15 proteins were first detected in 9-mm anthers (lane 6), and accumulated to the maximal level around 20-mm anthers (lane 8). The temporal distribution of LLA-15 during lily anther development is consistent with the earlier report of its detection in phase II anthers by silver staining (33).

To determine if LLA-15-related proteins were present in other plants, total protein was extracted from anthers of various species and immunoblots were probed using monospecific anti-LLA-15 antibodies (Fig. 5). These immunoblots revealed that anthers of cape honeysuckle, red trumpet vine, and tobacco had cross-reactive bands ranging from 16.0 to 23.0 kD. No protein was detected when an identical blot incubated with monospecific antibodies purified from preimmune serum was used as a control (data not shown). As in lily anthers, the accumulation of the LLA-15-like proteins appears to be developmentally regulated during anther growth in these three species.
LLA-15 Protein Is Encoded by a Number of mRNAs

Total RNA isolated from 15- to 20-mm lily anthers was translated in vitro in the presence of [35S]L-methionine in a rabbit reticulocyte lysate system. A single endogenous translation product of 26.0 kD corresponding to globin was detected (Fig. 6, lane 2), although no LLA-15 proteins were observed. When lily anther RNAs were translated, an array of proteins was synthesized (lane 3). A translation product with a molecular mass of approximately 15.0 kD was immunoprecipitated by anti-LLA-15 antiserum (lane 4), whereas no translation product was detected after immunoprecipitation with preimmune serum (lane 5). To determine the number of LLA-15 primary translation products, the immunoprecipitable polypeptides were fractionated by 2D-PAGE (Fig. 7). LLA-15 proteins were resolved into four polypeptides. These data suggest that four mRNAs encode the LLA-15 proteins. The positions of the four translation products on the 2D gel corresponded to the a, d, f, and g LLA-15 isoforms that were detected by immunoblot analysis (Fig. 2).

Subcellular Localization of LLA-15 Protein

The LLA-15 protein was localized in anther sections by light microscopy with anti-LLA-15 monospecific antibodies and goat anti-rabbit IgG conjugated with gold particles. A survey of sections of developing lily anthers (Fig. 8, A, B, and D) showed that LLA-15 proteins were detectable only in the tapetal cells of 15-mm (Fig. 8, B and F) and 20-mm anthers (Fig. 8D) when the tapetum became polarized, highly secretory, and showed loss of cell walls. No LLA-15 proteins were detected in the 5-mm anther (Fig. 8A). A control, reacted with antibodies purified from preimmune serum from the same rabbit, showed low background (Fig. 8, C and E). The accumulation of LLA-15 proteins in tapetal cells correlated with what was observed on the immunoblots (Fig. 4). However, LLA-15 protein was detectable in 24-mm anthers on the immunoblot, whereas the sections of 22-mm anthers showed no tapetal tissue present and no detectable LLA-15 protein in the anther (data not shown).

DISCUSSION

Our previous report on the proteins of the lily stamen suggested that a 15.0-kD protein was anther specific (33). Because Coomassie blue staining of the total proteins from 15-mm lily anthers showed the 15.0-kD polypeptides (designated here as LLA-15) to be abundant, we isolated LLA-15c-e polypeptides and prepared monospecific antibodies to them. Both the polyclonal antiserum and monospecific antibodies to LLA-15 were used to determine the organ- and tissue-specific accumulation of the LLA-15 protein family and to demonstrate the size and pI of the primary translation products.

There are at least seven isoforms of the LLA-15 protein. The pI difference of these polypeptides may be due to changes in amino acid residues and/or modifications of amino acid residues upon translation. Of the seven different LLA-15 polypeptides identified by immunoblot analysis, four are evident among immunoprecipitated in vitro translation products. The mRNAs of the remaining three LLA-15 isoforms may not be translated in vitro or their presence may be below the limits of detection. Alternatively, they may arise by modifications of the primary translation products. The presence of several primary translation products of the LLA-15 protein may reflect a mRNA population that is encoded by a gene family. The translation products have the same apparent mol wt as the in vivo polypeptides. However, it is possible that the LLA-15 protein contains a signal peptide because the protein has been identified as a glycoprotein (our unpublished data); cleavage of a signal peptide and subsequent glycosylation may result in LLA-15 proteins that migrate to the same position as the primary translation products on the SDS gel.

The monospecific antibodies against this monocot LLA-15 protein react with proteins having molecular masses ranging...
Figure 8. Immunocytochemical detection of LLA-15 protein in developing anthers of *L. longiflorum*. A through E are phase contrast, F is the same sample as B viewed under darkfield illumination. A, B, and D sections were treated with rabbit anti-LLA-15 monospecific antibodies followed by goat anti-rabbit IgG conjugated with gold particles. A, No signal apparent in the 5-mm anther; B and D, the detection of silver-enhanced immunogold conjugates in the tapetum of the 15-mm (B) and 20-mm (D) anthers. C and E are control sections of 15-mm (B) and 20-mm (D) anthers, respectively, treated with monospecific antibodies from rabbit preimmune serum. Bar = 50 μm. E, Epidermis; En, endothecium; M, middle layers; T, tapetum; S, sporogenous tissue; P, pollen.
from 16.0 to 23.0 kD in anthers of three widely divergent dicot species. The various isoforms of LLA-15 protein, the different mol wt of LLA-15-like proteins, and the striking difference in the strength of the signal between lily and three dicots in different families suggest that an ancestral LLA-15 gene diverged into a complex pattern in the past.

In situ localization confirmed that LLA-15 is a tapetal protein. The relatively high density of immunogold particles staining the region of tapetal cells (Fig. 8) also suggests that LLA-15 is an abundant protein. The accumulation of LLA-15 protein was first detected when the tapetal cells became vacuolated. The protein continued to accumulate thereafter, and when it reached its maximal level in 20-mm anthers, the tapetal cells were polarized and highly secretory. The LLA-15 protein may be secreted subsequently into the anther lobe, where it would be lost from the sections during the washing steps necessary for immunogold labeling. This would account for the absence of detectable immunogold particles in the sections of 22-mm anthers when the immunoblots demonstrate the protein’s presence in 24-mm anthers. The period of LLA-15 protein accumulation in the lily anther encompasses the phase I/II transition when meiosis occurs, phase II when microspores develop, and the phase II/III transition when microspore mitosis occurs to form pollen (33).

Regarding the parallel of the LLA-15 protein production with tapetal secretory function, we can suggest a number of possibilities. The tapetum is known for its nutritive role during microspore and pollen development (5). In addition to this role, LLA-15 may be involved in one or more of the following developmental events in lily anthers. First, esterases in the tapetum are known to be active during pollen wall formation and tapetal degeneration (24, 29). Second, peroxidases and other proteins are associated with the synthesis of sporopollenin or its precursor in the tapetum of lily (26). Third, proteins are related to the deposition of sporopollenin on the microspore to form the exine (21). Rowley (27) has detected a proteinaceous component embedded within the exine. Finally, proteins are involved in the synthesis of carotenoids and flavonoids that, in turn, form the pollenkitt, a substance deposited on the pollen wall with tapetal debris (3, 14).

It is worth noting that caspase is also produced by tapetal cells in lily. This enzyme dissolves the caspase that encases the tetrads during microsporogenesis. Because the enzymatic activity of caspase decreases sharply before microspore formation (32), it is unlikely that the LLA-15 protein is caspase. Male sterility is due to the dysfunction of the tapetum during or after meiosis because relations between tapetal and sporogenous cells are assumed to be intricate and precisely ordered. The best source of evidence supporting such a role is from the genetic work on male sterile mutants (13), but the mechanism of this interplay is still unknown.

Here we report a tapetal-specific protein, LLA-15, characterized by using immunological methods. The LLA-15 protein accumulates primarily in phase II of anther development in Lilium longiflorum and consists of a group of seven isoforms that are encoded by a number of mRNAs. In addition, posttranslational modifications occur during the maturation process of this protein. The detection of LLA-15 proteins in lily (a monocot) and LLA-15-like proteins in three widely divergent dicot species suggests that LLA-15 may play an essential role in anther development, but its function is unknown as yet.

LITERATURE CITED

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