Incorporation of Radioactive Label into Nucleic Acids of Compatible and Incompatible Pollen Tubes of *Lilium longiflorum* Thunb.*

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Summary. Stylar canal cells of *Lilium longiflorum* were labeled before pollination with 5-3H-uridine or 5-3H-orotic acid dissolved in water, or the stylar canal was filled 6 hr after pollination with stigmatic exudate supplemented with 5-3H-uridine, 2-14C-uridine or H32PO4. Nucleic acids extracted from compatible and incompatible pollen tubes grown in these styles were separated by methylated albumin kieselguhr column chromatography. Label occurred in all portions of the RNA profile, but no label appeared in the DNA peak. Using 5-3H-uridine, pollen tubes of different genotype had different labeling patterns.

Introduction

Pollen tubes grown in artificial media synthesize RNA (Dashek and Rosen 1966; Linskens et al. 1971; Mascarenhas 1971b; Mascarenhas and Bell 1970; Mascarenhas and Goralnick 1971; Rosen 1968; Steffensen 1966; Tano and Takahashi 1964). Part of this synthesis occurs both in the generative cell and vegetative nucleus (LaFontaine and Mascarenhas 1972; Mascarenhas 1966; Rosen 1968; Stanley and Young 1962). This new RNA probably is not needed for germination or early pollen tube growth but is thought to be required for later pollen tube elongation (Mascarenhas 1968). Most likely, this RNA is not t-RNA or r-RNA, but is m-RNA (Mascarenhas 1971a; Mascarenhas and Bell 1970; Mascarenhas and Goralnick 1971; Steffensen 1966, 1971; Tano and Takahashi 1964).

Injection of the RNA synthesis inhibitor, 6-methylpurine, dissolved in stigmatic exudate into the stylar canal of *Lilium longiflorum* resulted in compatible pollen tube lengths typical of incompatible pollen tubes, but did not affect incompatible pollen tubes (Ascher and Drewlow 1970). Self incompatibility, a common mechanism enforcing outbreeding in angiosperms, is expressed in the Easter lily as a restriction of incompatible pollen tube length to half that of compatible pollen tubes in the amount of time it takes compatible tubes to traverse the 100 mm long lily style. Apparently, new RNA is needed for compatible pollen tube growth. However, even without this RNA synthesis, lily pollen tubes grow half the length of the style, at least 5 times farther than they grow in vitro.

Germinating and growing pollen in artificial media is the usual method for studying pollen tube metabolism (Linskens 1964; Rosen 1968). This procedure has been justified under the assumption that pollen tube metabolism in vitro is wholly that of the pollen tube, free of stigma and stylar influences. Perhaps, because of the greater ease of procedure and the relative certainty that in vitro results would not be different, pollen germination and initial pollen tube growth is best studied in vitro. But, biochemical studies of later stages of in vitro pollen tube growth are studied of a greatly impaired metabolism since the length of pollen tubes in vitro is usually only a fraction of normal pollen tube length.

Another justification for using in vitro systems is that the pollen tubes grown in vivo often can not be easily isolated from stylar tissue. Many plant species have loosely packed parenchymatous cells in the center of the style among which pollen tubes grow. This transmitting tissue with embedded pollen tubes has been isolated and analyzed biochemically (Linskens 1958, 1959). While this study pointed out differences in pollen tube-stylar metabolisms between compatible and incompatible pollinations in *Petunia*, it did not separate pollen tube metabolism from stylar metabolism. However, other plant species such as those in the Liliaceae have large, hollow styles lined with specialized cells on which pollen tubes grow. These pollen tubes can be easily removed from the style by bisecting the style and lifting the pollen tubes out of the canal.

The purpose of this research was to study the incorporation of radioactivity into nucleic acids of in vivo grown pollen tubes of *Lilium longiflorum*, the radioactivity coming from stylar canal cells previously labeled.

with radioactive nucleic acid precursors or from stigmatic exudate supplemented with label and injected into the stylar canal 6 hr after pollination. A secondary purpose was to see whether unique incorporation patterns were associated with pollen tubes of different genotype or with compatible and incompatible pollen tubes.

### Materials and Methods

**Flowers of Lilium longiflorum** Thunb. cultivars Ace and Nellie White (NW) were cut early the day of anthesis and placed in jars of water on the laboratory bench at room temperature for 1 day. Stigma-styles were removed from the flowers by cutting through the ovary with a triangular needle, and the remaining portion of the ovary was snapped off.

Radioactively labeled nucleic acid precursors diluted with water or stigmatic exudate were injected through the stigma until a drop appeared at the ovarian end of the style. 5-2H-uridine* diluted with water to concentrations of 50 to 500 microcuries per experiment (uc/exp) was injected 12 hr before pollination into sets of 32 'Ace' or 'NW' styles, and incubated at 23 °C until pollination. 5-3H-uridine is a specific precursor of RNA synthesis and is not used in DNA synthesis (Hayhoe and Quaglini, 1965). At first, the styles were flushed with 10 drops of water just before pollination to remove any free label left in the stylar canal. Since flushing removed only 0.1% of the label injected suggesting that the stylar tissue had absorbed the rest, flushing was discontinued for the remaining experiments. For another set of experiments, 5-2H-uridine or 5-3H-ortic acid was diluted with 9 volumes of water and injected just before pollination into 12 'Ace' or 'NW' styles, 2-14C-uridine, H32P04, and 5-2H-uridine diluted with stigmatic exudate collected from 'Ace' flowers to concentrations of 1 to 5 uc/exp for 2-14C-uridine, 300 uc/exp for H32P04 and 100 uc/exp for 5-2H-uridine were injected 6 hr after pollination into 32 'Ace' or 'NW' styles. 32P was added to 400 ul ice-cold 1xSSC buffer; 30 ml buffer-saturated phenol at 4°C, and the average disintegrations per min (DPM) per fraction was calculated utilizing the raw activity versus AES ratio. These quench curves were erratic or there was signs of micro-organism contamination. Therefore, each individual experiments were discarded before critical analysis (including all the 'Ace' × 'NW') because the absorbancy profile had very low counts and poor nucleic acid extraction or a column problem, or because the isotope versus AES ratio did not fit the curve. These quench curves were also used to estimate the background standard found in each machine.

Quench curves were obtained for each machine with the formula of \( y = ax^2 \), plotting efficiency of counting the isotope versus Bq/µl. These curves were encoded in a computer program for a Wang 2000 desk computer, and the average disintegrations per min (DPM) per fraction was calculated utilizing the raw CPM data and the AES ratio from the 2 replicates of counting. Efficiency of counting on the 2 spectrophotometers was close to 100% for 14C and 32P, and approximately 45% for 2H.

The planned comparisons were 'Ace' × 'Ace', 'NW' × 'NW', 'Ace' × 'NW' and 'NW' × 'Ace' pollen tubes using a 5-2H-uridine stylar labeling. While the Easter lily cultivars are clones and are intraclonally (self) incompatible and interclonally (cross) compatible when pollinated. The detached styles were clonally (self) incompatible and interclonally (cross) incompatible when pollinated. The detached styles were used to discriminate between the isotope versus Bq/µl. These curves were encoded in a computer program for a Wang 2000 desk computer, and the average disintegrations per min (DPM) per fraction was calculated utilizing the raw activity versus AES ratio. These quench curves were erratic or there was signs of micro-organism contamination. Therefore, each individual experiments were discarded before critical analysis (including all the 'Ace' × 'NW') because the absorbancy profile had very low counts and was erratic or there was signs of micro-organism contamination.

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1. 5-2H-uridine, New England Nuclear (NEN), Specific Activity (SA) 29.3 curies (c)/mM, 1 mc/ml; 5-2H-ortic acid, NEN, SA 12.2 c/mM, 1 mc/ml; 2-14C-uridine, Schwarz BioResearch, SA 49.6 c/mM, 1 mc/ml; H32P04, carrier-free, NEN, 1 mc/ml.
2. 0.01 M tris (hydroxymethyl) aminomethane, 0.15 M sodium chloride, 0.015 M sodium citrate, 0.01 M 2-mercaptoethanol.
3. 0.1 M, 0.3 M, 0.4 M and 1.2 M NaCl in 0.05 M Na phosphate buffer, pH 7.6.
5. 4 gr 2,5-diphenyloxazole (PPO), 0.1 gr dimethyl POPOP, 1000 ml toluene.