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Incorporation of Label from Radioactive Uridine into the Stylar Nucleic Acids of *Lilium longiflorum* Thunb. as Affected by Heat, 6-methylpurine and Actinomycin D*

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Summary. 5-^{3}H-uridine injected into the stylar canal of detached lily stigma-styles was taken up initially into the rapidly-labeled-RNA of the nucleic acid profile of a methylated albumin kieselguhr (MAK) column but with increasing time was found in all portions of the RNA profile, but not in the DNA. Heat treatment of the style before injection of 5-^{3}H-uridine greatly reduced the rate of incorporation of label into and the ultimate amount of label found in the RNA species of the lily style. Translocation of 5-^{3}H-uridine through the ovary into heat-treated pistils and the injection of 5-^{3}H-uridine into styles which had been incubated for 1 or 2 days after heat treatment resulted in stylar nucleic acids more highly labeled than nucleic acids in control styles, with an incorporation pattern different than control styles. Heat treatment of lily pistils resulted in detectable changes in the proportion of stylar RNA species as separated on MAK columns and measured as absorbance units. Actinomycin D and 6-methylpurine treated styles incorporated label from a stylar injection of radioactive uridine in patterns different than each other, different than heat-treated styles and different than non-treated styles. 6-methylpurine and heat treatment of styles only slightly reduced the rate at which 5-^{3}H-uridine was removed from the stylar canal into the stylar tissue.

Introduction

Lilium longiflorum Thunb., the Easter lily, possesses a self-incompatibility reaction which determines that pollen tubes will grow halfway down the 100 mm or longer style in 48 hr if derived from a self- or intraclonal-pollination, but will traverse most of the style if derived from a cross- or interclonal-pollination (Ascher and Peloquin 1966). The short, incompatible pollen tube growth can be converted into the longer, compatible-type pollen tube growth if the style is altered before pollination by plunging the styles into 50°C water for 6 min or by injecting the RNA synthesis inhibitors, 6-methylpurine and actinomycin D, into the hollow styles 6 hr before pollination (Hopper *et al.* 1967, Ascher 1971). Heat treatment affects styles differently than does inhibitor treatment for, when heat-treated styles are flushed with water and then pollinated, incompatible and compatible pollen tubes grow to incompatible lengths only (Ascher 1975). Flushing of inhibitor-treated styles has no effect on subsequent pollen tube growth, compatible and incompatible pollen tubes all growing to compatible lengths (Ascher 1971).

The primary purpose of this research was to ascertain what effect heat treatment had on the incorporation of label from 5-^{3}H-uridine into the stylar nucleic acids of *Lilium longiflorum* Thunb. A secondary purpose was to determine the effect of 6-methylpurine and actinomycin D on the incorporation of label from radioactive uridine into stylar nucleic acids.

Materials and Methods

Flowers of *Lilium longiflorum* 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. Stigma-styles were harvested from flowers the day after anthesis (unless specified otherwise) by cutting through the ovary with a triangular needle. Except where noted, stylar heat treatment involved submerging styles with ovary piece attached in distilled water at 50°C for 6 min. After heat treatment, the styles were blotted dry. For injection of a solution into the stylar canal, the ovary was snapped off and the solution injected through the stigma with a needle and syringe until a drop appeared at the ovarian end of the style. Incubation of styles was on moistened filter paper in petri plates at 23°C.

Experiment 1. To establish that radioactivity from 5-^{3}H-uridine injected into the lily stylar canal would be incorporated into stylar nucleic acids, 9 'NW' styles/incubation time were injected with 5-^{3}H-uridine[1] in 1×10^{-4}M uridine[2], incubated for time pe-

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[1] New England Nuclear, Specific Activity (SA) 29.3 curies (c)/mM, 1 mc/ml.

[2] Sigma Chemical Co.

riods of 1.5, 3, 6, 12, 24 and 48 hr, and the stylar nucleic acids extracted and separated on MAK columns. Extractions at 11 and 45 min after injection of label were done in much the same way except that 4 styles/treatment were used, radioactivity was diluted with water and 5 non-radioactive styles were added to each treatment prior to nucleic acid extraction.

Experiment 2. To determine the effect of heat treatment on the incorporation into stylar nucleic acids of label from 5-^{3}H-uridine previously injected into the stylar canal, 'Ace' styles were submerged in 49°C water for 6 min. Immediately, 50 ul of undiluted 5-^{3}H-uridine was injected into each heat-treated style and non-treated style and the styles incubated for 6, 24 and 48 hr. Similarly, 'NW' styles were heat treated at 50°C for 6 min, and the styles incubated for 11 min, 35 min and 5 days after injection of label. Two heat-treated and 2 control styles were each combined with 8 non-radioactive styles at the end of each incubation period for nucleic acid extraction.

Experiment 3. In the preceding experiment, label was injected into the style immediately after heat treatment. To determine whether a time interval between heat treatment and labeling might affect the incorporation of label into stylar nucleic acids, 'NW' styles were heat treated or left non-treated, incubated 24 or 48 hr and 50 ul of 5-^{3}H-uridine injected into each style. One hr later, 2 heat-treated and 2 control styles were added separately to enough 'NW' styles to make a total of 6.0 gr fresh weight for nucleic acid extraction. Two heat-treated and 2 control styles were pulsechased 24 hr after heat treatment, with 30 min between injection of 5-^{3}H-uridine and flushing with 10 drops of 1×10^{-4}M uridine per style. The 2 batches of styles were incubated for another 60 min before nucleic acid extraction.

Experiment 4. During the course of this study, Ascher (1975) discovered that a water flush of heat-treated styles caused both compatible and incompatible pollen tubes to grow as incompatible, as if the flushing washed out a substance stimulatory to pollen tube growth which had been released into the stylar canal by the heat treatment. In effect, injection of 5-^{3}H-uridine into heat-treated styles was the same as a stylar flush and would negate the heat treatment from the standpoint of subsequent pollen tube growth. Therefore, to determine the incorporation pattern of label from 5-^{3}H-uridine into the nucleic acids of heat-treated styles without injection of the label into the stylar canal, 20 'Ace' pistils were harvested 1 and 2 days after anthesis with 5 mm of ovary attached and 10 were heat treated. The 20 styles were inserted singly into small test tubes containing 100 ul redistilled water and 10 ul 5-^{3}H-uridine. Forty hr later, 0.5 ml water was added to each test tube. The styles wilted and recovered turgidity during the 40 hr, but the heat-treated styles wilted more rapidly. The ovary was snapped off after 48 hr and each style flushed with 2 drops of water which was collected on a 2.4 cm Whatman GF/A fiberglass filter disk. The disks were dried, placed in scintillation vials, 10 ml toluene scintillator[3] added and the vial counted twice for 10 min or until 20,000 counts accumulated. The 10 heat-treated and 10 non-treated styles were divided into 2 lots of 5 styles each, each lot added to enough 'Ace' style to give a final total fresh weight of 6gr and the nucleic acids extracted for MAK column chromatography.

Experiment 5. To determine what qualitative effects heat treatment might have on the MAK column profile of stylar nucleic acids, 20 'Ace' and 20 'NW' styles were harvested 1 and 2 days after anthesis and 10 of each cultivar were heat treated. The 'NW' styles were incubated 24 hr and the 'Ace' styles, 48 hr. Stylar nucleic acids from the treated and control styles were extracted and the nucleic acid species separated on MAK columns. The absorbancy profiles of heat-treated styles were compared to the absorbancy profiles of control styles in the same manner as is described later in the Materials and Methods comparing absorbancy profiles and radioactivity patterns for experiments in which labeling was done.

Experiment 6. Two separate studies partially determined the effect of RNA synthesis inhibitors on the incorporation of label from radioactive uridine into stylar nucleic acids. The inhibitor and label were injected at the same time. Part A. Thirteen 'Ace' styles harvested 2 and 3 days after anthesis were injected with 1×10^{-3}M 6-methylpurine[4] containing 2-^{14}C-uridine[5] or with water containing 2-^{14}C-uridine, incubated 48 hr and the nucleic acids extracted from the 2 set of 13 styles. Part B. Forty 'Ace' styles were harvested the day of anthesis, 20 were injected with 1×10^{-3}M 6-methylpurine containing 5-^{3}H-uridine or 2×10^{-5} gr/ml actinomycin D[6] containing 5-^{3}H-uridine, incubated 3 or 6 hr and the stylar nucleic acids extracted. There were no controls.

Experiment 7. To test whether heat treatment or 6-methylpurine might be affecting the ability of the stylar canal cells to remove 5-^{3}H-uridine from the stylar canal, 'NW' and 'Ace' styles were heat treated or injected with 1×10^{-3}M 6-methylpurine 12 hr before labeling, and 40 ul of a 10 uc/ml 5-^{3}H-uridine solution injected into each style. Check styles for the 6-methylpurine treatment were injected with water 12 hr before labeling. At 11 min to 48 hr after injection of label, 0.4 ml of water was flushed through each of 2 styles/treatment/incubation time and collected in a scintillation vial, 10 ml of a 30% triton X scintillator[7] added to each vial, and the vials capped and vigorously shaken to develop a clear phase. Counts per min (CPM) data were obtained for each vial, twice, by counting for 5 min or until 900,000 counts accumulated.

For nucleic acid extraction, the styles were cooled on an ice bath, added to 30 ml ice-cold buffer[8]: 80 mg bentonite: 30 ml buffer-saturated phenol: 1% sodium lauryl sulfate, and ground in a Virtis Macro "45" ho-

[3] 4 gr PPO, 0.1 gr dimethyl-POPOP, 1000 ml toluene.

[4] K & K Laboratories, Inc.

[5] Schwarz BioResearch, SA 49.6 mc/mM, 10 uc/ml.

[6] Lot 940051, Calbiochem.

[7] 300 ml triton X-100 (Research Products International Corp.): 700 ml toluene scintillator.

[8] Experiments 1, 2, 5 and 6: 0.01M Tris-HCl, pH 7.6 containing 0.01M magnesium chloride and 0.06M potassium chloride. Experiments 3 and 4: 1xSSC, pH 7.6 = 0.01M tris (hydroxymethyl) aminomethane, 0.15M sodium chloride, 0.015M sodium citrate and 0.01M 2-mercaptoethanol.

mogenizer at top speed (45,000 rpm) for 3 min, decanted into an extraction flask, shaken at room temperature for 15 min and then centrifuged in a Sorvall RC2B in an S-34 head at 15,000 rpm for 10 min at 0°C. The supernatant was re-extracted against 40 ml buffer-saturated phenol plus 80 mg bentonite at 0°C, recentrifuged and the supernatant combined with 2 volumes 2-ethoxyethanol overnight at 20°C. The precipitate was collected by centrifugation at 15,000 rpm for 20 min, washed twice with room temperature 95% ethanol and stored upside down to drain at -20°C until chromatographed.

Nucleic acids in the samples were chromatographically separated on MAK columns, made in 3 layers of 1 gr cellulose powder, 12 gr MAK and, finally, 1 gr kieselguhr as the sample layer. Each layer was individually suspended in 0.1 M NaCl-$PO_4^{=}$ buffer, pH 7.6[9] and packed in turn at 4 lbs/in^2 air pressure into a 2 × 40 cm glass column. Before packing, the kieselguhr suspensions were boiled to remove the air. Then, 4 ml methylated albumin (MA) was stirred into the 12 gr kieselguhr suspension to produce the MAK. Each column was washed with 100 ml 0.3 NaCl-$PO_4^{=}$ buffer, pH 7.6.

The nucleic acid sample was dissolved in 20 ml 0.1M NaCl - $PO_4^{=}$ at 0°C, loaded onto the column and washed with 100 ml 0.3M NaCl - $PO_4^{=}$. A 600 ml gradient containing 300 ml 0.4M NaCl - $PO_4^{=}$ in the mixing chamber and 300 ml 1.2M NaCl - $PO_4^{=}$ in the reservoir was attached to the column and 3 lb/in^2 air pressure applied to the gradient. After the salt gradient, the column was washed with 100 ml of cold 0.74M NH_4OH. Fractions of 7.6 ml were collected.

The absorbance at 260 nm (A_{260}) for each of the approximately 100 fractions was obtained, the test tubes and their contents cooled to 0°C and 1 ml of a 1% albumin[10] solution added to each test tube. Enough 40% trichloroacetic acid (TCA) was added to each test tube to make the solutions 5% in TCA, and the test tube contents vigorously mixed. Six hr later, the precipitate was collected by filtering the solutions through 2.4 cm fiberglass filter disks, rinsing the test tubes twice with 5 ml cold 5% TCA. The disks were dried in scintillation vials and 10 ml of the toluene scintillator solution added. CPM data was obtained for each vial, several times, by counting in a Beckman Liquid Scintillation Spectrophotometer or a Packard 3375 Automatic TriCarb Liquid Scintillation Spectrophotometer at 8°C, with the gain set to give an automatic external standard (AES) ratio of 1.000 for the unquenched background standard found in each machine.

Quench curves were obtained for each machine with the formula of $y = ax^b$, plotting efficiency of counting the isotope versus AES ratio. These quench 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 replicates of counting. The efficiency of counting ^3H on the 2 spectrophotometers was approximately 45%.

To standardize A_{260} profiles and the radioactivity profiles 1 experiment to the other (see Fig. 2), the RNA portions of the MAK profile were replotted with each test tube's A_{260} and DPM designated as a % of the total A_{260} units or DPMs present in the total RNA of the MAK chromatographic profile for that experiment (see Fig. 2). According to standard methods of describing the profile of plant nucleic acids separated on MAK columns by salt gradient, the 2 major RNA portions of the MAK profile were divided into 5 separate areas (Fig. 1): the light-RNA was divided into 4S-RNA and 5S-RNA, and the heavy-RNA was divided into 18S-RNA and 28S-RNA. The 28S-RNA was subdivided into peak-RNA (from 18S-RNA to 2 fractions past the highest A_{260} reading in the 28S portion) and rapidly-labeled-RNA (the shoulder of the 28S-RNA portion). The A_{260} units or radioactivity within the 5 portions of the RNA profile were summed and the 5 sectional totals expressed as a % of the total A_{260} units or radioactivity present in RNA. Subsequently, the ratio $R_{D/A}$ was calculated using this formula,

$$R_{D/A} = \frac{\%DPM_X}{\%A_X}$$

where X was 1 of the 5 portions of the RNA profile or was any summed part of the profile. $R_{D/A}$ values were calculated also for the 2 major areas of the RNA profile, the light-RNA and heavy-RNA (see Fig. 2).

Results

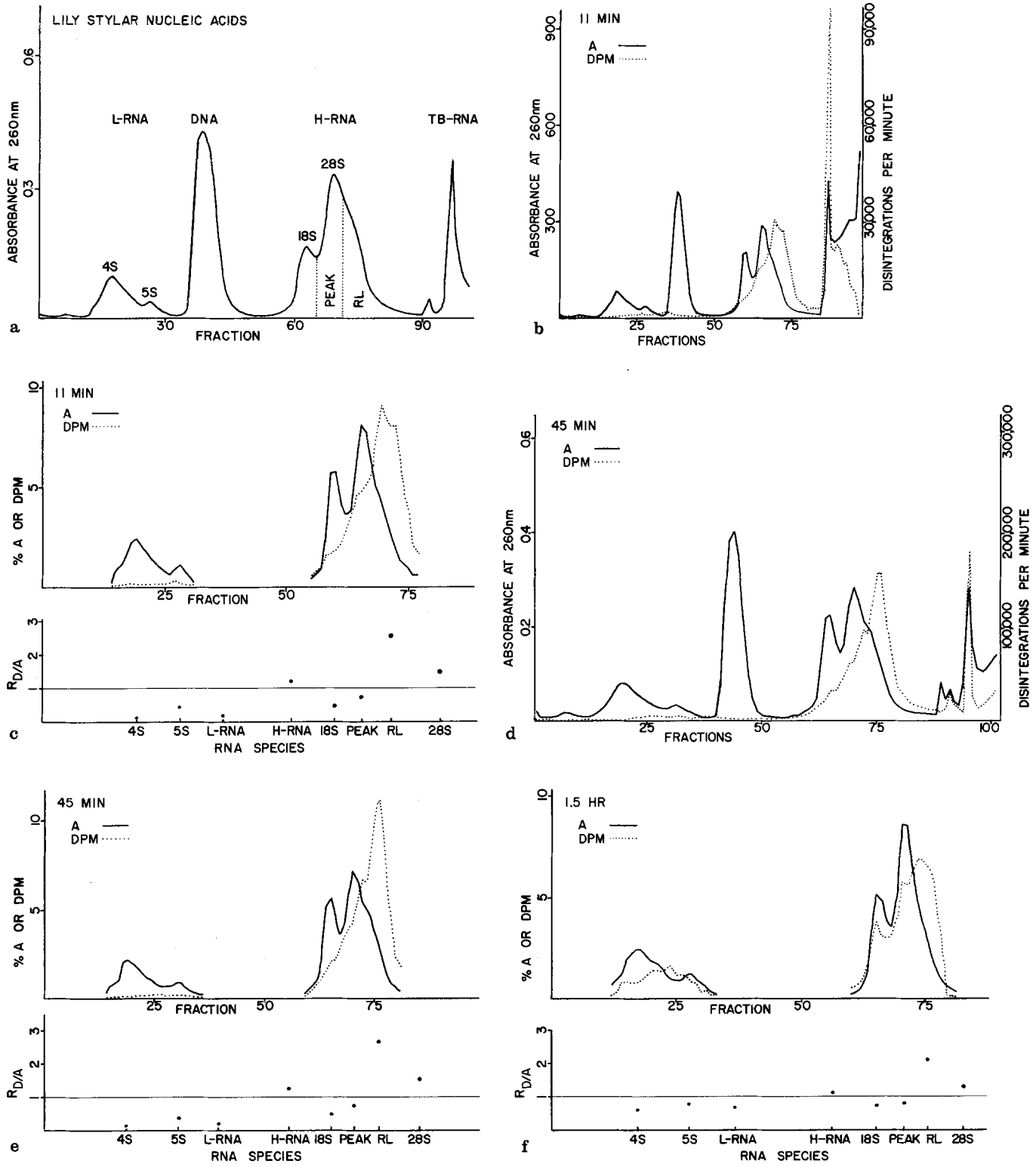
Experiment 1. Label derived from 5-^3H-uridine injected into non-treated, day-after-anthesis styles first appeared as a tall slender peak in the rapidly-labeled-RNA region of the A_{260} profile and as a broad, low peak between the 4S- and 5S-RNA (Fig. 2a-d). As the time of labeling increased, the labeling pattern de-emphasized the rapidly-labeled-RNA and by 48 hr closely fit the A_{260} profile of the 18S- and 28S-RNA peaks (Fig. 1e,g). The fit improved after 5 days labeling. The same occurred for the 4S- and 5S-RNA region, with the label following closely the A_{260} profile for the light-RNA region by 48 hr (Fig. 1e, g). The proportion of label in light-RNA increased steadily from 11 min to 5 days (Fig. 1c, e, g). Tenaciously-bound-RNA acquired label while DNA did not acquire label in this and all other experiments. Non-labeled DNA was expected since 5-^3H-uridine is a specific precursor of RNA synthesis (Hayhoe and Quaglino 1965).

The results of the 3, 6, 12 and 24 hr incubation times were marred by drastically reduced amounts of heavy-RNA caused by poor extraction or some other factor. However, the qualitative picture of how the

[9] 0.1, 0.3, 0.4 and 1.2 M sodium chloride in 0.05 M sodium phosphate buffer, pH 7.6.

[10] Bovine serum albumin, Fraction V, Sigma Chemical Co.

label became distributed over time in the stylar nucleic acids is sound. Quantitative data involving percentage recovery of injected radioactivity in stylar RNA species was derived from control treatments in Experiment 2 (Fig. 2a). The percentage of injected label incorporated into the total stylar RNA and the heavy-RNA species increased until 24 hr of labeling and in light-RNA increased until 48 hr of labeling.



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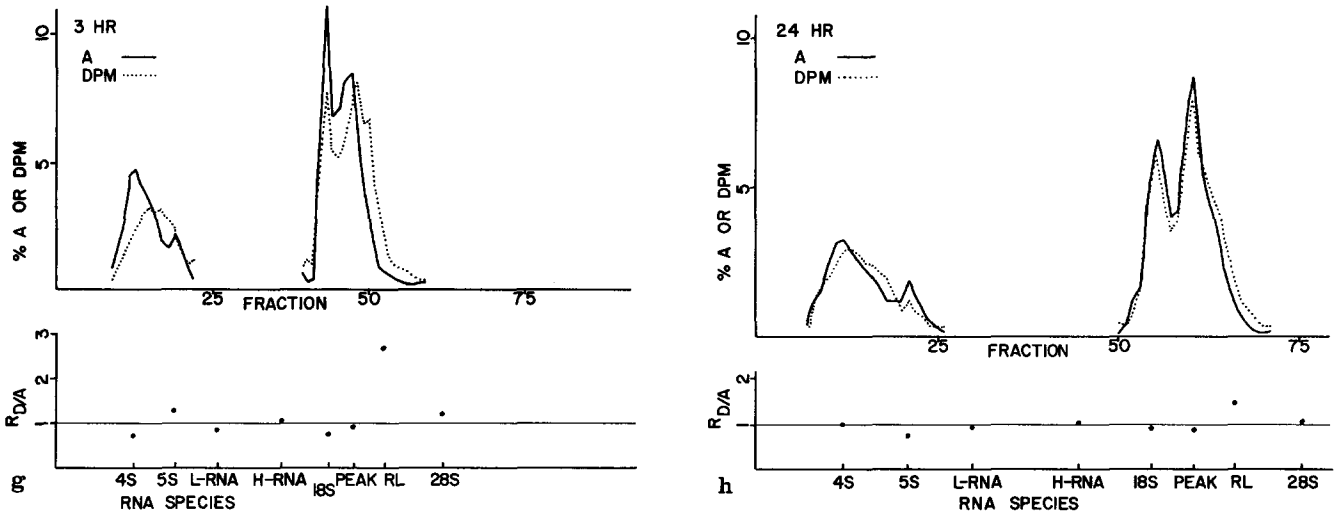


Fig. 1a-h. Absorbancy profile of *Lilium longiflorum* stylar nucleic acids chromatographed on a MAK column. a) Typical nucleic acid profile: light-RNA (1-RNA) + 4S + 5S-RNA; heavy-RNA (h-RNA) = 18S- + 28S-RNA; peak- + rapidly-labeled-(rl) RNA = 28S-RNA; tenaciously-bound-(tb) RNA is removed by NH_4OH. b- h) Nucleic acid profile with superimposed radio-activity profile: label in the stylar RNA from 5-^{3}H-uridine injected into the stylar canal of non-treated (b,c,e,g) or heat-treated (d,f,h) stigma-styles. Stigma-styles were incubated 11 min (b,c,d), 6 hr (e,f) or 48 hr (g,h) after injection of label. b) Entire A_{260} and radioactivity profile. c-h) Adjusted absorbancy and radioactivity profiles (see text)

Experiment 2. In heat-treated styles, the incorporation pattern over time remained the same as in non-treated styles, but the heat treatment caused a slowdown in the initial phases of that pattern and an overall lowering of the amount of label incorporated (Fig. 1d, f, h, 2a). For instance, the pattern and level of labeling after 11 min in non-treated styles was not reached in heat-treated styles until after approximately 6 hr of labeling (Fig. 1c, f, 2a). The labeling pattern after 24 hr for both heat-treated and check styles showed a DPM curve that followed closely the A_{260} peaks of 4S-, 5S-, 18S- and 28S-RNA indicating that qualitative differences in labeling were disappearing. The amount of the label originally injected found in stylar RNA species decreased after 48 hr in heat-treated styles rather than after 24 hr as in non-treated styles (Fig. 2a). After 11 min, 2 days and 5 days of labeling, the RNA from the heat-treated styles contained 2%, 54% and 27% of the label incorporated into the RNA of the control styles.

Experiment 3. When injection of 5-^{3}H-uridine occurred 24 and 48 hr after heat treatment, heat-treated lily styles incorporated slightly more label into stylar RNA but less label into light-RNA than did control styles with a pattern of incorporation different than control styles (Fig. 3a, b). The level of incorporation in heat-treated and control styles incubated 24 hr (Fig. 2b) and the pattern of incorporation for heat-treated and control styles incubated 24 hr (Fig. 3a, b) and 48 hr was the same as expected for non-treated styles after 60 or 90 min of labeling (Fig. 2a). In other words, the major peak of radioactivity was centered in the rapidly-labeled-RNA portion of the A_{260} profile, it showed indications of an 18S-RNA shoulder and possibly a 28S-RNA shoulder, and the peak of label in the light-RNA portions was associated with 5S-RNA. According to $R_{D/A}$ values, heat-treated styles had less highly labeled 5S- and 4S-RNA and more highly labeled rapidly-labeled- and 18S-RNA. The level of labeling for heat-treated and check styles incubated 48 hr before labeling was approximately 50% of the level in the styles incubated 24 hr before labeling and was so low as to be less than the incorporation level of non-treated styles incubated 11 min after injection of label (Fig. 2a, b). This may have been the result of stylar ageing.

Experiment 4. Label from 5-^{3}H-uridine absorbed into lily pistils through the ovary was incorporated in 48 hr into stylar nucleic acids of both heat-treated and check styles with the labeling profile closely following the nucleic acid profile (Fig. 3c, d). Like the heat-treated styles compared to their controls in Ex-

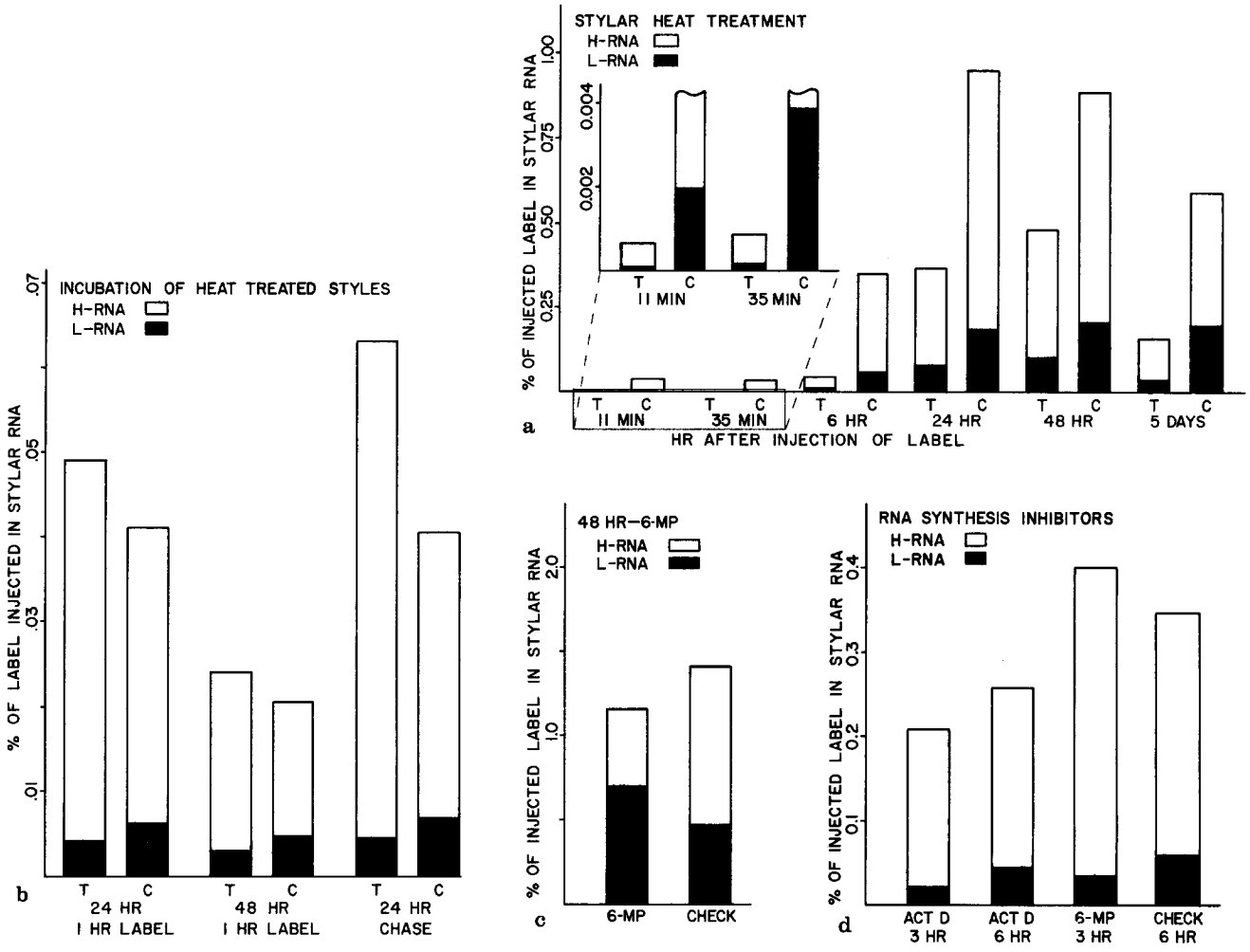


Fig.2a-d. Percentage of injected label incorporated into light-RNA and heavy-RNA of lily styles: a,b) Heat-treated (T) and non-treated (C) styles: a) Styles were treated, immediately injected with 5-^{3}H-uridine, and incubated for various times. b) Styles were treated, and either incubated 24 or 48 hr, injected with 5-^{3}H-uridine, and incubated 1 hr more (1 hr label) or incubated 24 hr, injected with 5-^{3}H-uridine, incubated 30 min, the label flushed out of the style, and the styles incubated 1 hr more (chase). c) Styles injected with 2-^{14}C-uridine either in water (check) or in 1×10^{-3} M 6-methylpurine (6-MP) and incubated 48 hr. d) Styles injected with 5-^{3}H-uridine in either 2×10^{-5} gr/ml actinomycin D or 1×10^{-3} M 6-methylpurine and incubated 3 or 6 hr. The 6 hr check is from Fig.2a

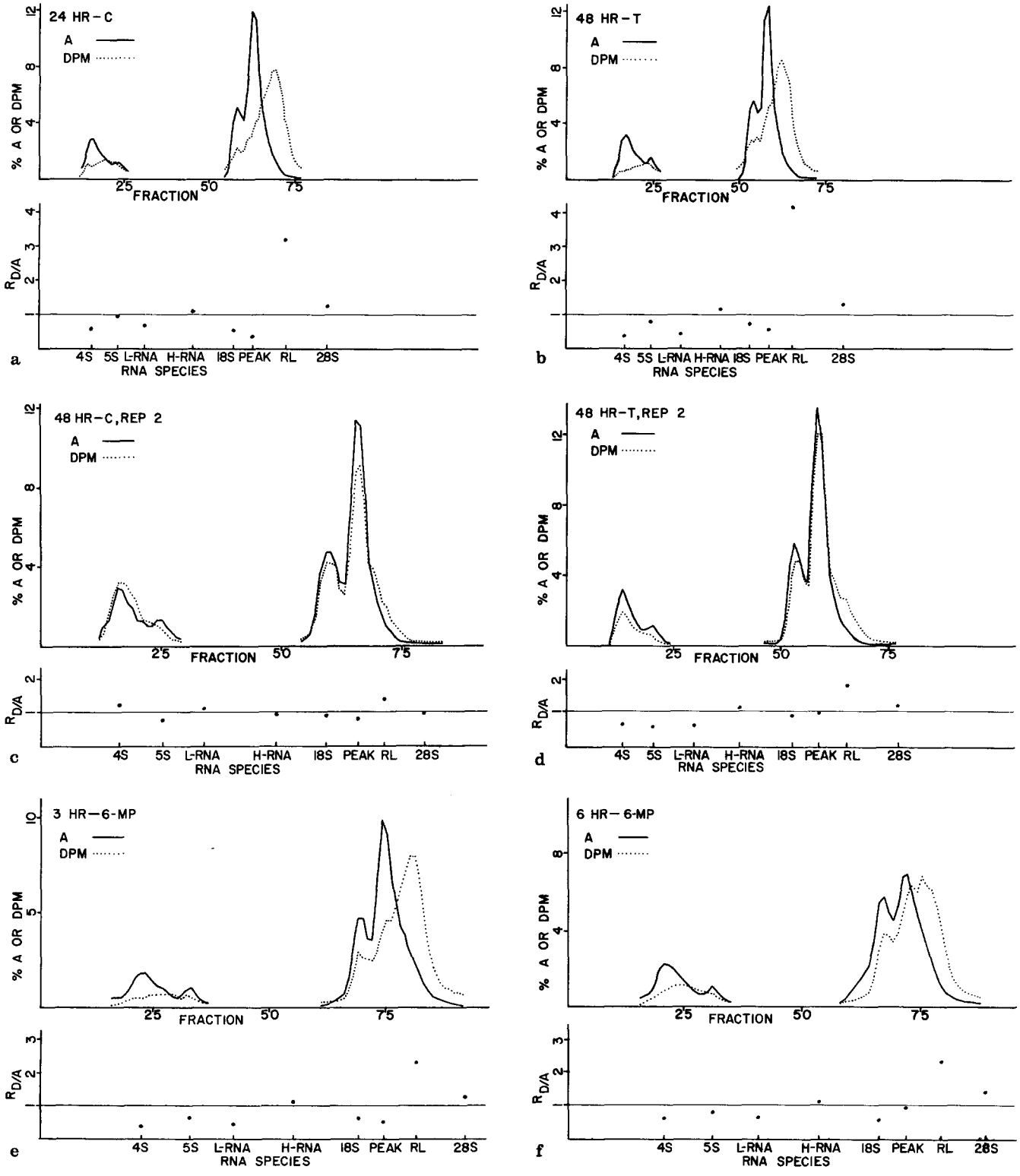
periment 3 (Fig.2b) and like the 24 hr control styles compared to the 48 hr control styles in Experiment 2 (Table 1), the light-RNA species of the heat-treated styles in this experiment had a lower $R_{D/A}$ value and also contained a smaller % of the absorbed label than the light-RNA of the controls while the total RNA of the heat-treated styles contained a greater % of absorbed label than did the RNA of the control styles (Table 1).

The 20 drops of water flushed through the 10 heat-treated styles contained a total of 331,493.4 DPM or 39.5% of the 839,708.8 DPM flushed out of the 10 control styles. Analysis of variance indicated that the difference was very highly significant. The amount flushed out was 1.1% of the total fed to the styles.

Translocation of label through the ovary was less efficient in labeling stylar RNA than injection of label into the stylar canal. The % of label translocated through the ovary and incorporated into RNA of heat-treated styles was at about the same level as in heat-treated styles incubated 24 hr after a stylar injection of 5-^{3}H-uridine (Fig.2a) and in the control styles was a little lower than the incorporation level in non-treated styles incubated 6 hr after label injection (Fig.2a). Quicker wilting of heat-treated styles and the secretion of less free label into the stylar canal argues that

heat treatment disrupted the normal flow of solutes within the stylar tissue.

Experiment 5. Stylar heat treatment changed the relative amounts of stylar RNA species resolved by MAK column chromatography. The proportionality ratios derived by comparing the corresponding parts of the RNA profiles from the heat-treated and control styles for both the 24 hr and 48 hr incubation times



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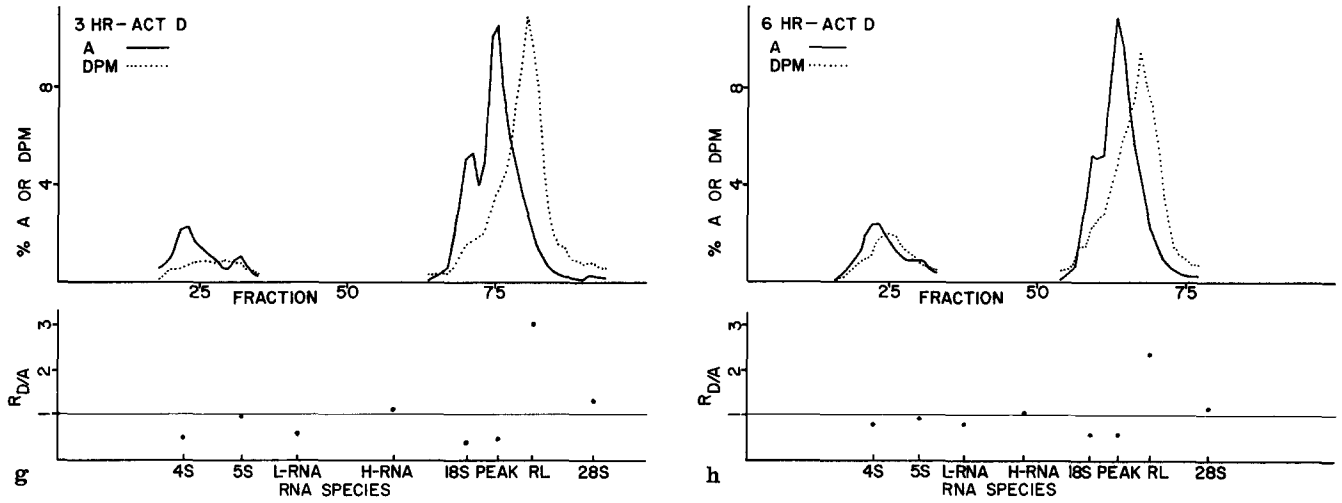

Fig.3a-h. Adjusted absorbancy and radioactivity profiles of lily styles: a,b) Heat-treated or non-treated styles incubated 24 hr before 5-^{3}H-uridine was injected and labeled 1 hr. c,d) Heat-treated or non-treated styles absorbed 5-^{3}H-uridine through the ovary for 48 hr. Data is the second replicate. e-h) RNA-synthesis-inhibitor treated styles incubated 3 or 6 hr with 5-^{3}H-uridine (see Fig.2d)

Table 1. Percentage label incorporated into stylar nucleic acids of *Lilium longiflorum* Thunb. comparing label injected into the stylar canal (Exp.2) to label translocated into the pistil through the ovary (Exp.4)

Stylar canal injection				Translocation through ovary			
Treatment	L-RNA	H-RNA	RNA	Treatment	L-RNA	H-RNA	RNA
24 hr control	0.183	0.768	0.950	48 hr heated			
				Run 1	0.0648	0.295	0.360
				Run 2	0.0427	0.326	0.369
48 hr control	0.205	0.673	0.879	48 hr control			
				Run 1	0.0848	0.242	0.327
				Run 2	0.0870	0.252	0.339

after heat treatment were not equal to 1.0 (Fig.4). The heat-treated 'NW' styles incubated 24 hr (Fig.4a) and heat-treated 'Ace' styles incubated 48 hr (Fig.4b) had a greater proportion of nucleic acids in the light-RNA than the controls, the 5S-RNA portions of the light-RNA being especially higher. Both sets of heat-treated styles exhibited increased proportions of nucleic acids in the rapidly-labeled-RNA and to a lesser extent in the 18S-RNA, apparently at the expense of nucleic acids in the peak-RNA portion of the profile. Comparisons between treated and control styles over time is confounded with differences in cultivars and should not be made.

Experiment 6. Part A. The addition of 1×10^{-3}M 6-methylpurine to the 2-^{14}C-uridine injected into the lily stylar canal reduced the amount of label incorporated into the heavy-RNA of the style by 41% and increased the label in the light-RNA by 25% (Fig.2c). There was a loss of label in the 3 portions of the heavy-RNA, a 53% reduction in peak-RNA, a 29% reduction in rapidly-labeled-RNA and a 26% reduction in 18S-RNA.

Part B. Compared to the 6-methylpurine-treated styles, the actinomycin D-treated had proportionally more label in the light-RNA species after 3 and 6 hr of labeling and lacked on the slender rapidly-labeled-RNA radioactivity peak the definite peaks in the 18S- and 28S-RNA regions of the A_{260} profile (Fig.3e-h). Label profiles from the 2 sets of the actinomycin D-treated styles most closely resembled the 11 min labeling profile of non-treated styles (Fig.1b) although the % of injected label incorporated was 5 to 6 times higher (Fig.2a, d). The labeling profile after 3 hr in 6-methylpurine-treated styles resembled most closely the labeling occurring after 11 to 35 min in control styles (Fig.1b, 3e) although the % of injected label

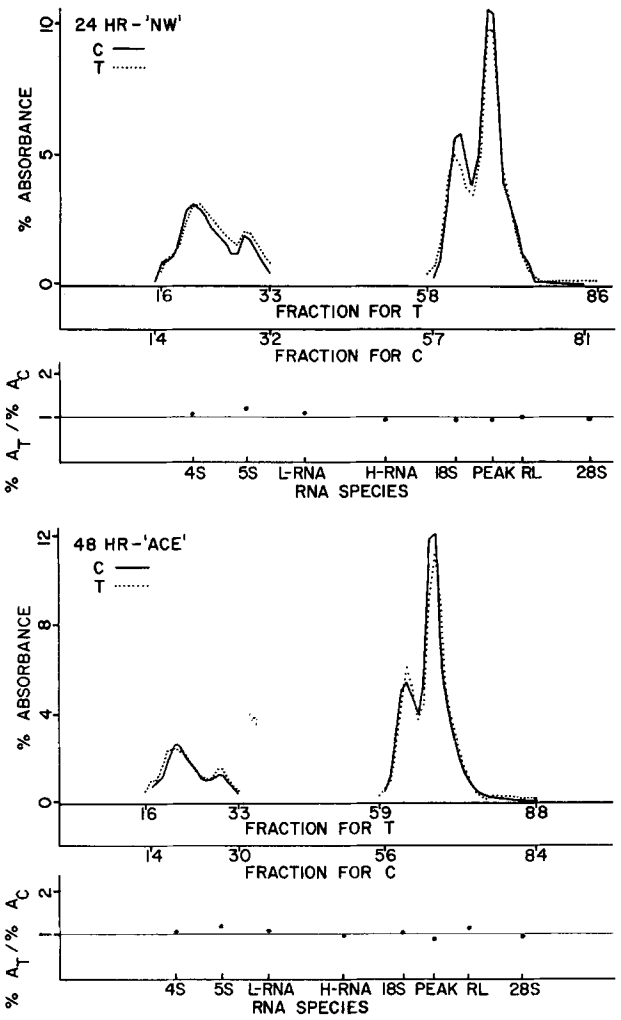

Fig.4a,b. Adjusted absorbancy profiles of nucleic acids extracted from heat-treated and non-heat-treated 'NW' styles incubated 24 hr after treatment (a) and 'Ace' styles incubated 48 hr after treatment (b). The ratio was calculated as was $R_{D/A}$ except that $\%A_T/\%A_C$ is a ratio of the percentage A_{260} units of heat-treated stylar RNA present in any one portion of the profile divided by the A_{260} units present in the corresponding portion of the profile of non-heat-treated stylar RNA

incorporated was 10 times higher (Fig.2a, d). The labeling profile after 6 hr most closely resembled the 1.5 hr labeling profile in control styles.

Little can be said about the possible effects of the inhibitors on the % of injected label incorporated into stylar nucleic acids because of the lack of adequate controls, except that the actinomycin D-treated styles during 6 hr incorporated 25% less label into stylar RNA than did the control styles in Experiment 2 (Fig. 2a, d).

Experiment 7. Both heat treatment and 6-methylpurine injection 12 hr before pollination slightly reduced the stylar ability to remove label from the canal. The reduction was at its greatest for both heat- and inhibitor-treated styles between 6 and 24 hr (Fig.5). This reduced ability in heat-treated styles, which was 13% of the control level at its maximum, could not be the cause of the reduction in incorporation of label from a stylar injection of 5-^{3}H-uridine into stylar nucleic acids of heat-treated styles, which at 11 min labeling was 99.4% of the control styles and at 48 hr was 46% of the labeling level in control styles.

Discussion

Label from radioactive uridine was incorporated into lily stylar nucleic acids in the pattern usually attributed to processes of RNA synthesis in plant tissue. As reviewed by Loening (1968), short bursts of labeled RNA synthesis precursors applied to various plant systems initially appear on the MAK column as 2 peaks, one just after the 28S-ribosomal RNA peak and inseparable from it, and the other as a much smaller, broad peak between 4S- and 5S-RNA. The rapid labeling on the edge of the ribosomal-RNA area is thought to result from synthesis of a nuclear precursor to either cytoplasmic messenger-RNA or to cytoplasmic ribosomal-RNA synthesis. Progression of label into all portions of the 18S- and 28S-ribosomal RNA peaks usually results from ribosomal-RNA synthesis. The rapidly-labeled intermediate in the 4S- and 5S-RNA area gradually disappears and soon develops 2 peaks of label corresponding to the 4S- and 5S-RNA peaks. Label in the 4S-RNA area comes from transfer-RNA synthesis (or from addition of label to the 3' end of transfer-RNA as reported by Mascarenhas and Goralnick (1971)) while label in the 5S-RNA area comes from the synthesis of the 5S-ribosomal molecule. Transfer-RNA, 5S-ribosomal RNA, 18S- and 28S-ribosomal RNA labeling occur at about the same rate. Therefore, the incorporation pattern of label into lily stylar nucleic acids seems to indicate the synthesis of various RNA species in the lily styles, except for the label in the 4S-RNA peak which may be addition to the 3' end of transfer-RNA. Base analysis of the labeled RNA from lily styles and separation of stylar RNA species by other biochemical methods would determine in fact

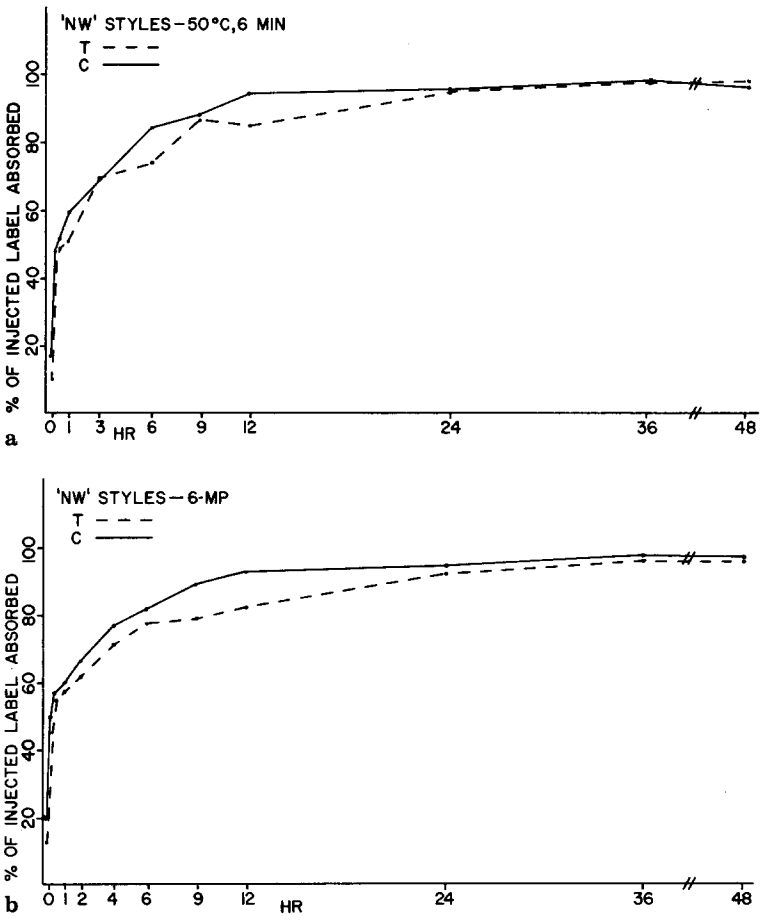

Fig. 5a, b. Percent injected label removed over time from the stylar canal by stylar tissue, comparing heat-treated (a) and 6-methylpurine-treated (b) 'NW' styles to controls

whether each of the separable species contained label and had undergone synthesis to acquire that label.

6-methylpurine and actinomycin D appeared to affect the incorporation of label into lily stylar RNA in much the same way as they do in other plants. 6-methylpurine depresses adenine incorporation into RNA and the conversion of adenylic acid (adenosine monophosphate) to guanylic acid (guanosine monophosphate)(Miller and Kempner 1963). In lily styles, 6-methylpurine depressed but did not block the incorporation of label from uridine into RNA or, in other words, 6-methylpurine depressed RNA synthesis in lily styles. This depressed RNA synthesis was somehow responsible for the slight loss of stylar ability to remove 5-^{3}H-uridine from the stylar canal (Fig. 5b). It also must have increased, through non-competition, the amount of free tritiated nucleosides and nucleotides in the pools since light-RNA acquired more label than controls, possibly through addition to the 3' end of the transfer-RNA (Fig. 2c).

Actinomycin D binds to DNA, inhibiting DNA-directed RNA synthesis in both eukaryotes and prokaryotes by prohibiting chain elongation of the RNA. At concentrations that do not suppress all RNA synthesis in a cell, actinomycin D selectively suppresses synthesis of certain RNA species, ribosomal-RNA synthesis being the most sensitive (Goldberg and Friedman 1971). Since synthesis in lily styles apparently occurred, actinomycin D must have been acting at concentrations that did not inhibit all RNA synthesis. Therefore, the removal of the 18S- and 28S-RNA shoulders on the rapidly-labeled-RNA peak of radioactivity in the heavy-RNA portion of the profile seemed to confirm that these shoulders represented ribosomal RNA and that ribosomal synthesis occurs in lily styles.

An explanation for the effect of heat treatment must incorporate these phenomena: 1) The lagging but otherwise unchanged pattern of stylar RNA labeling; 2) the reduction in the ability of the stylar tissue to remove 5-^{3}H-uridine from the stylar canal; 3) the loss of heavy-RNA which was still apparent after 48 hr in heat-treated styles (Fig. 4), even though the style appeared to be replenishing the heavy-RNA content with large amounts of synthesis (Fig. 3a, b and 4) the

dependence of the stylar part of the self-incompatibility reaction in Easter lily on protein synthesis only until the flower opens, but its dependence on a continued RNA synthesis from before the flower opens to some days after anthesis (Ascher 1974). This last point requires elaboration. Pre-pollination injection of puromycin, an inhibitor of protein synthesis, blocks the self-incompatibility reaction allowing incompatible pollen tubes to reach compatible lengths only when the styles are injected before anthesis. However, 6-methylpurine produces this effect whether styles are treated in the bud (about 12hr pre-anthesis), at anthesis or 1 or 2 days post-anthesis.

Whether injected into the stylar canal or absorbed into the vascular system through the ovary, the tritiated nucleoside uridine would have passed cell walls and membranes and moved through the cytoplasm into nucleoside pools in the stylar cells. Such movement could require energy. Subsequently, the nucleoside would be metabolized with the aid of various enzymes to uridine monophosphate and then uridine diphosphate which is incorporated into stylar RNA with the aid of RNA polymerase. The tritiated uridine might also have been metabolized into the other pyrimidines losing tritiated hydrogen on carbon 5 if metabolized into thymidine (Hayhoe and Quaglino 1965), or the uridine might have labeled transfer-RNA by addition. Heat treatment could have attacked 3 vulnerable spots in the movement of the labeled nucleoside, by 1) destroying the energy sources for uridine movement from the outside of the cell to nucleoside pools; 2) impairing the ability of enzymes to a) transform uridine to uridine diphosphate, b) synthesize RNA by affecting an effector equivalent to the sigma factor involved with RNA polymerase, or c) carry on any vital cell function; or 3) destroying the stylar RNA. Presumably, metabolic processes would work to reverse the effect of any of these events.

A heat effect at any of these 3 metabolic points could explain the reduced ability of the style to remove 5-^{3}H-uridine from the stylar canal. Heat treatment might impair enzymes besides those involved in RNA synthesis and conversion of nucleoside to nucleotide and some of these other enzymes might affect the energetics of nucleoside movement into the cell. Also, destruction of RNA might simulate the 6-methylpurine impairment of stylar ability to remove 5-^{3}H-uridine from the canal. However, stylar RNA destruction can not explain the lag in label incorporation. In fact, the loss of RNA likely would have forced new RNA synthesis so that heat-treated styles should have incorporated label as fast or faster than control styles. Also, an attack on the movement of labeled nucleoside from outside the cell to the pools would not have caused reductions in the amount of stylar RNA. Therefore, the effect of heat must involve an impairment of enzyme function.

This theory for the effect of heat treatment can be experimentally tested. If heat treatment destroyed the enzymatic functioning of proteins, among them the long-lived protein postulated by Ascher (1974) to be necessary for the RNA synthesis that conditions the stylar portion of the self-incompatibility reactions, RNA synthesis dependent on the affected proteins would not occur and the amount of stylar RNA would decrease. The protein would have to be re-synthesized to recover normal RNA levels. Therefore, heat-treated styles should exhibit these characteristics: Immediate reduced amounts of enzymatically active proteins possibly RNA polymerase, the enzymes that metabolize uridine to uridine diphosphate, or other stylar enzymes; rapidly decreasing amounts of RNA; and subsequent protein synthesis giving increased enzymatic activity of heat-impaired enzymes followed by increased RNA synthesis.

This model explaining the effects of heat treatment on the style provides an explanation of the labeling pattern exhibited by light-RNA species in heat-treated styles. In styles labeled immediately after heat treatment, the low labeling level of the 4S- and 5S-RNA (Fig.1, 2a) argues that 4S- and 5S-RNA synthesis was hindered or that the functioning of enzymes responsible for addition to the 3' end of transfer-RNA was impaired. However, in heat-treated styles incubated 1 or 2 days before labeling, functional enzymes again would be present helping to re-synthesize needed RNA (Fig.5) and the tritiated nucleoside available for 3' addition would be limited because of competition from RNA synthesis. Therefore, the light-RNA in these styles would accumulate as they did a smaller % of injected label than controls (Fig.2b).

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