if at all; and that Pd will apparently not superconduct, as has been predicted theoretically by Doniach (*5*). Apparently the disappearance of the superconductivity is intimately connected with the appearance of the high susceptibility. That such a relationship should exist was first pointed out by Clogston (*6*), and later by Doniach (*5*), and is a consequence of the fact that the interactions which enhance the susceptibility also affect the superconductivity.

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Comparison of Messenger RNA in Photoperiodically Induced and Noninduced Xanthium Buds

Abstract. *In response to the floral stimulus,* Xanthium *buds synthesize relatively more messenger RNA than do vegetative buds. This is demonstrated by fractionation, on methylated albumin–kieselguhr columns, of a mixture of nucleic acids from vegetative and induced buds, one being labeled with uridine-H³ and the other with uridine-2-C¹⁴. While floral induction stimulates a small increase in messenger RNA synthesis as revealed by labeling intact plants, this difference can be magnified by labeling excised buds in solution. From experiments with excised buds from* Xanthium *plants, it is concluded that buds from photoperiodically induced plants contain more messenger RNA than buds from noninduced ones do.*

Messenger RNA (mRNA) serves as the intermediate in the translation of the informational strand of DNA into protein molecules (*1*). Thus, the biological activities of a cell are a reflection of its enzymes, and its enzymes in turn are reflected by various RNA messengers. Therefore, the regulation of biological activities by environmental influences might be expected to result in an altered frequency of enzymes produced as well as RNA messengers. Spiegleman and Hayashi (*1*), using both bacteria and bacterial virus, have demonstrated qualitative differences in the synthesis of RNA during different times of growth of bacteria or different stages of infection by virus. They demonstrated differences in the labeling of mRNA's from two populations of bacteria or virus by labeling the RNA with either uridine-H^3 or uridine-C^{14} at different times and then fractionating a mixture of both RNA's on a methylated albumin–kieselguhr (MAK) column. There is little or no other information on differences in the types of RNA messengers in biological tissue except for changes in mRNA during seed germination (*2*) and variation in chromosomally directed protein synthesis from different parts of the pea plant (*3*). For other higher plants, there is no information available on this subject even though there are many instances in which changes in the environment specifically lead to the induction of enzymes or changes in cellular differentiation. The photoperiodic induction of *Xanthium* plants, for development of floral primordia by a single long night (16 hours of darkness), is an example of such an environmental control. Exposure of a *Xanthium* plant to a 16-hour period of darkness initiates the production of the flowering hormone in the mature leaf, and when it has been transported to the apex it causes the vegetative bud to grow into a reproductive bud (*4*). These changes are probably hormonal controls acting as gene corepressors or coactivators. At the onset of floral induction there may be changes in the species of mRNA produced or perhaps an increase in the relative frequency of some new mRNA's over others.

Changes in RNA synthesis in the *Xanthium* plant were investigated with a specific aim of determining whether floral induction causes the synthesis of different amounts or types of mRNA's. By means of double-labeling technique and fractionation of the nucleic acids on MAK columns, it was found that buds of photoperiodically induced plants contain more mRNA.

Seeds of *Xanthium pensylvanicum* Wall. (cocklebur) were washed in running tap water for 3 days and then germinated in vermiculite. The seedlings were transplanted and grown in a controlled-environment greenhouse. The day length was maintained at 18 hours with supplementary light, and the temperature was maintained at approximately 23°C during the day and 17°C at night. The plants were grown in this environment for approximately 6 weeks. At the beginning of each experiment, the plants were defoliated except for a single leaf, which was usually the third leaf from the apex (approximately 7 cm long). The plants were randomly selected and placed in either of two growth chambers. One growth chamber was regulated for 8 hours of light and 16 hours of darkness (short day, SD) while the second was set for 16 hours of light and 8 hours of darkness (long day, LD). In both chambers, the temperature was kept at 27°C during the light period and at 18°C during the dark period. These conditions were satisfactory to control photoperiodic induction; a single inductive period (16 hours of darkness) would result in the development of floral primordia (*5*).

In order to compare the RNA synthesized in the apical buds of *Xanthium* plants, the apices of either intact or excised buds were labeled with uridine-H^3 or uridine-2-C^{14}. In experiments where the intact buds were labeled, a drop of either uridine-H^3 (20 μc) or uridine-2-C^{14} (1 μc) was applied to the apex of the plant 16 hours prior to harvesting. In other experiments, buds (2 to 3 mm in length)

were incubated in 5 ml of a solution containing $10^{-4}M$ citric acid, *p*H 6.0 with NH_4OH; 1 percent sucrose; 5 μg of streptomycin per milliliter; and either 0.75 mc of uridine-H^3 or 30 μc of uridine-2-C^{14} for 4 hours. In all experiments, 50 buds were used for each sample.

Nucleic acids were isolated from *Xanthium* buds by a method in which phenol in the presence of 2 percent sodium lauryl sulfate was used. This method has been described for use with *Xanthium* buds (*5*) and peanut cotyledons (*6*). Two samples of nucleic acids labeled with either H^3 or C^{14} were appropriately mixed (*7*) and fractionated on MAK columns (*8*) with a linear gradient of NaCl in 0.05*M* phosphate buffer, *p*H 6.7. Fractions containing 5 ml each were collected, and their ultraviolet absorbancy was determined. Either a 1-ml or a 4-ml portion of each fraction was precipitated in the presence of carrier DNA in 5 percent trichloroacetic acid, and the precipitate containing the labeled nucleic acid was collected on a Millipore filter and counted in a Tricarb liquid scintillation spectrometer.

First attempts to distinguish between the synthesis of mRNA in photoperiodically induced and noninduced *Xanthium* buds involved labeling of the excised buds in solution with P^{32} and subsequently fractionation of the P^{32}-labeled nucleic acids separately on MAK columns (*5*). The plants were given a single induction period, and the buds were excised and labeled with P^{32} immediately after the dark period. From these initial experiments no significant differences in mRNA could be observed as a result of floral induction. Later, buds of plants exposed to a single photoinductive cycle were labeled intact during the inductive cycle with either uridine-H^3 or uridine-2-C^{14}. When nucleic acids from photoperiodically induced and noninduced buds were mixed and then separated on a MAK column the elution profiles of the H^3-RNA and C^{14}-RNA were nearly identical.

Since a single dark period is the minimum required for photoperiodic induction, the flowering response after 16 hours of darkness was weak. Also, probably very few changes in enzyme production and cellular differentiation occur immediately after a single photoperiodic-inductive dark period. There-

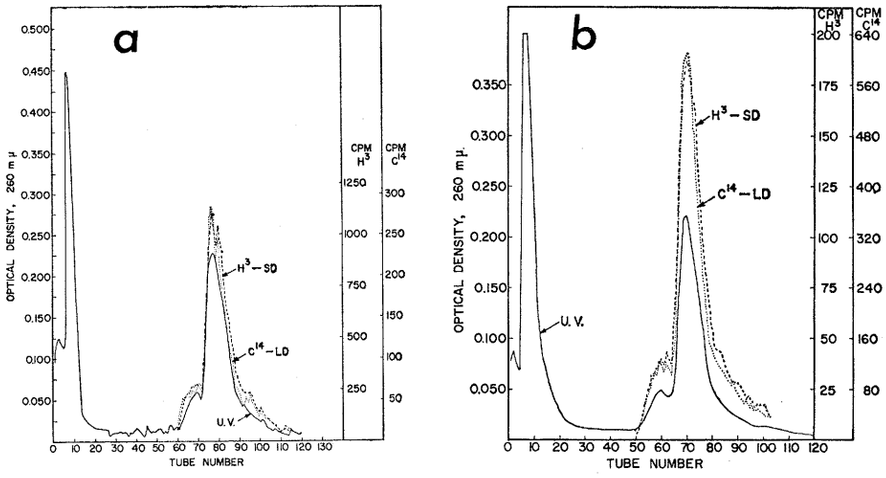

Fig. 1. Separation of double-labeled RNA from *Xanthium* buds. (*a*) Uridine-2-C^{14} was applied to the apex of *Xanthium* plants grown under continuous long days (LD). Uridine-H^3 was applied to the apex of *Xanthium* plants during the last 16 hours in which they were exposed to three cycles of short days (SD). After the plants were exposed to labeled uridine for 16 hours, the buds were removed and the nucleic acids were extracted. The H^3-RNA and C^{14}-RNA were mixed and fractionated on a MAK column with a linear gradient of NaCl from 0.65 to 1.10*M*. The small peak (tube 70) is light ribosomal RNA while the large peak (tube 80) is heavy ribosomal RNA. The RNA between tubes 90 to 110 is considered mRNA. (*b*) *Xanthium* plants (SD) were labeled with uridine-H^3 11 days after having been given three floral inductive cycles. The apices of noninduced plants (LD) were labeled with uridine-2-C^{14}. Sixteen hours after applying labeled uridine to the apices the nucleic acids were isolated from each set of plants and then mixed and fractionated on a MAK column. Messenger RNA is considered to be between tubes 80 to 100.

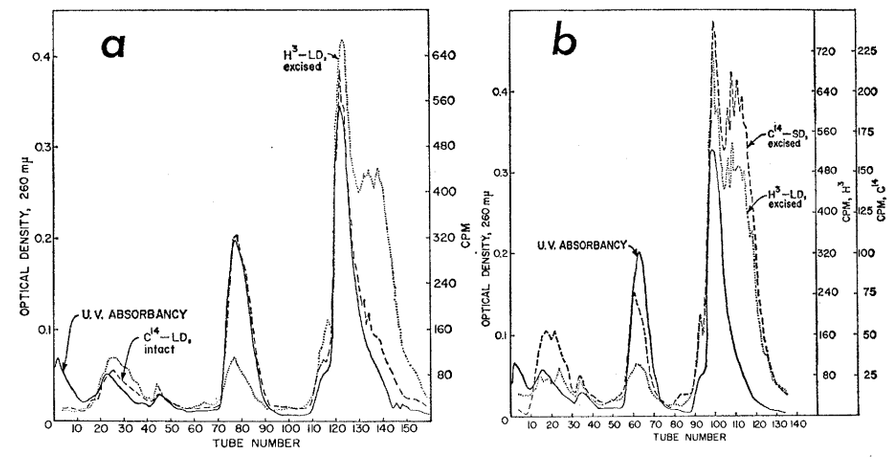

Fig. 2. Fractionation of double-labeled nucleic acids from *Xanthium* buds. (*a*) Noninduced plants (LD) were labeled with either uridine-H^3 or uridine-2-C^{14} in two ways. Uridine-2-C^{14} was applied to the apices of plants (intact) approximately 16 hours prior to harvesting the buds. Excised buds were incubated for 4 hours in 5 ml of a solution containing $10^{-4}M$ citric acid, *p*H 6.0 with NH_4OH; 1 percent sucrose; 5 μg of streptomycin per milliliter; and 0.75 mc of uridine-H^3. The nucleic acids were extracted from the intact labeled (C^{14}) and excised labeled (H^3) buds, mixed, and then fractionated on a MAK column. (*b*) Buds were excised from noninduced plants (LD) and plants 9 days after having been given three inductive cycles (SD) and each incubated in a 5-ml solution [see (*a*)] containing 0.75 mc uridine-H^3 or 30 μc of uridine-2-C^{14}, respectively, for 4 hours. The nucleic acids were extracted from each set of buds separately, mixed, and then fractionated on a MAK column, a linear gradient of NaCl from 0.35*M* to 1.10*M* being used. The first two ultraviolet-absorbing peaks are soluble RNA, and the third peak is DNA. The large difference between the amount of uridine-H^3 and uridine-2-C^{14} incorporated into the DNA fraction is explained by the fact that at least two-thirds of the H^3 of the uridine (purchased from Schwarz BioResearch, Inc.) is located at the 5-position of the uridine. Thus, when uridine is converted to thymidine by methylation at the 5-position two-thirds of the H^3 is removed, and this results in the large difference in labeling of the DNA noted in Figs. 2*a* and 2*b*.

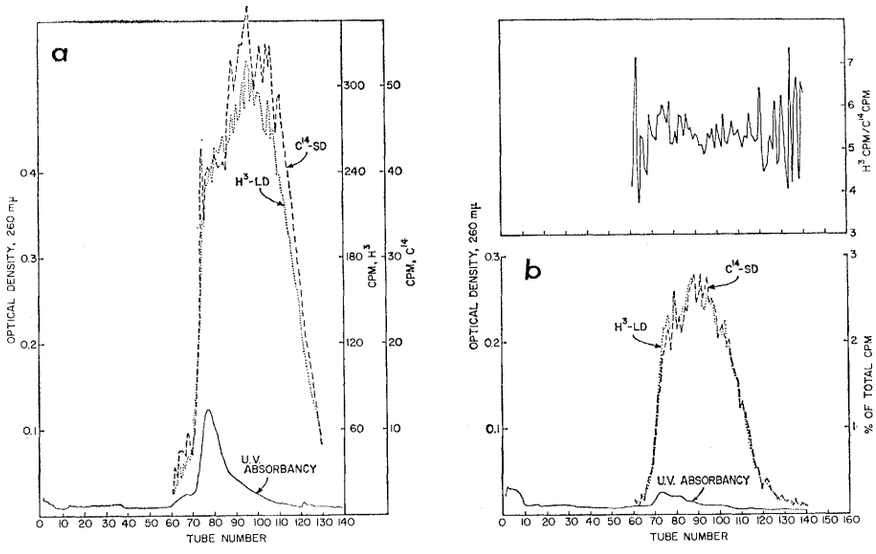

Fig. 3. Refractionation of double-labeled mRNA. (*a*) The mRNA fraction from a MAK column similar to the one presented in Fig. 2*b* was collected and mixed with an equal volume of 0.05*M* phosphate buffer, *p*H 6.7. This solution was added to a MAK column, and the RNA was eluted with a linear gradient of NaCl from 0.7*M* to 1.1*M*. Fractions containing 5 ml each were collected, and the ultraviolet absorbancy was estimated at 260 mμ. A 1-ml portion was removed from each fraction, mixed with 50 μg of carrier DNA, and then precipitated in 5 percent cold trichloroacetic acid. The precipitate containing the radioactive mRNA was collected on Millipore filters, and the radioactivity was determined in a scintillation spectrometer. (*b*) The mRNA from the fractionation shown in Fig. 3*a* (tubes 86 to 130) was collected, mixed with an equal volume of 0.05*M* phosphate buffer, *p*H 6.7, and again added to a MAK column. The RNA was eluted with a linear gradient of NaCl from 0.7*M* to 1.1*M*. A 4-ml portion from each successive 5-ml fraction was mixed with 0.2 mg of carrier DNA, and the nucleic acid was precipitated in 5 percent cold trichloroacetic acid. The precipitate was collected on a Millipore filter and the radioactivity was determined.

fore, *Xanthium* plants were given three photoperiodic-inductive cycles (16 hours darkness and 8 hours light per cycle) to allow development of the floral primordia and mRNA synthesis to occur. During the third dark period, the intact buds were labeled with uridine-H^3 and vegetative buds of noninduced plants were labeled with uridine-2-C^{14}. A sample of the nucleic acids obtained from these buds was fractionated on a MAK column (Fig. 1*a*). The major area of interest is the nucleic acids eluting from the MAK column on the back side of the ribosomal RNA fraction (tubes 85 to 110) where mRNA is fractionated (*1*, *2*, *5*–*7*). The elution profiles of the H^3-mRNA and C^{14}-mRNA are very similar. There appears to be slightly more newly formed mRNA from photoperiodically induced buds (SD) than from noninduced buds (LD). To further investigate this point, plants were given three photoperiodic-inductive cycles and returned to long days for 2 weeks to allow the floral primordia to develop still further. After 2 weeks, intact apices of photoperiodically induced and noninduced plants were labeled with either uridine-H^3 (SD) or uridine-2-C^{14} (LD), and the nucleic acids were extracted and fractionated on a MAK column (Fig. 1*b*). Again there was slightly more newly formed mRNA from buds of photoperiodically induced plants than from buds of noninduced plants.

The difference in elution profiles of the H^3-mRNA (SD) and C^{14}-mRNA (LD) is not as large as that found with bacteria or virus (*1*). However, in the bacterial or viral system there is a relatively larger amount of mRNA synthesis as judged by the amount of radioactivity, indicative of mRNA, in the chromatogram. It has been shown by labeling excised buds in solution that the relative amount of mRNA synthesized in *Xanthium* buds can be increased (*5*). Figure 2*a* illustrates the large increase in the relative amount of mRNA (tube 130 to 150) synthesized in excised buds as compared to buds labeled intact. The relative increase in mRNA may reflect a deficiency in some nutrient or other substances in the excised bud. This transition in the pattern of nucleic acids synthesized is analogous to the "step-down" cultures of bacteria (*9*). When bacteria are transferred from a complete to a minimum medium there is a much greater decrease in the synthesis of ribosomal RNA as compared to information RNA (mRNA). Apparently, the same phenomenon may occur in higher plants when the tissue from the intact plant is removed.

Since the relative amount of mRNA can be enhanced by labeling excised buds in solution, it was felt that this technique might be useful to magnify the small difference in mRNA synthesis as a result of floral induction. Therefore, excised buds from photoperiodically induced (three cycles, buds used 2 weeks after induction) and noninduced plants were labeled in solution with uridine-2-C^{14} or uridine-H^3, respectively. The elution profiles of the H^3-RNA and C^{14}-RNA are shown in Fig. 2*b*. The buds from photoperiodically induced plants contain considerably more mRNA than buds from noninduced plants. In order to determine whether the mRNA in photoperiodically induced buds is qualitatively different from that in noninduced buds the mRNA fraction (tubes 105 to 130) was collected from a MAK column similar to the one shown in Fig. 2*b*. This fraction of mRNA, which contains both C^{14}-mRNA from induced plants (SD) and H^3-mRNA noninduced plants (LD), was diluted with an equal volume of 0.05*M* phosphate buffer, *p*H 6.7. This solution was then added to a MAK column to absorb the mRNA which was then eluted with a linear gradient of NaCl (Fig. 3*a*). This mRNA fraction contained a small amount of light ribosomal (16*S*) and heavy ribosomal (23*S*) RNA's.

An examination of the radioactivity profiles indicates a relatively larger amount of mRNA from the induced plant (SD). However, since the shape of the H^3 and C^{14} profiles are similar but not identical, it seemed desirable to collect again the mRNA fraction (tubes 86 to 130) and refractionate on another MAK column. The second refractionated mRNA is presented in Fig. 3*b*. It should be noted that very little ultraviolet-absorbing material (ribosomal RNA) is present. The radioactivity profiles for H^3-mRNA and C^{14}-mRNA are now nearly identical as indicated by the small change in the ratio of H^3 to C^{14}. Thus the mRNA's from induced and noninduced *Xanthium* buds are not detectably dif-

ferent in quality as judged by the double-labeling method. The data presented show that buds of photoperiodically induced *Xanthium* plants synthesize a relatively larger amount of mRNA than do buds of noninduced plants. This suggests that the flowering hormone produced in the leaf acts in the apex either as a gene corepressor or coactivator mediating the production of more mRNA's.

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23 September 1965

Retina: Pathology of Neodymium and Ruby Laser Burns

Abstract. *Chorioretinal lesions have been produced in monkeys during experiments with ruby and neodymium lasers. Most of the energy from the ruby laser (wavelength 6943 angstroms) is absorbed by the pigment epithelium, where the greatest damage appears. With the neodymium laser (10,600 angstroms) the neural portions of the retina absorb more of the energy than the pigment layer does; consequently these portions exhibit more damage than the pigment epithelium and adjacent tissues.*

The absorption of high-intensity energy in the visible and near-visible portions of the spectrum, with a subsequent degradation into thermal energy, has long been known to be a source of ocular pathology (*1*). The optical properties of the eye make the retina and its surrounding structures particularly vulnerable, for the energy is focused in this region. The concentration of energy at the retinal region may be 1000 to 10,000 times greater per unit of area than that of energy incident upon the eye at the cornea (*2*). Retinal burns resulting from sungazing during an eclipse, from viewing an atomic fireball (*3*), from clinical photocoagulation (*4*), or, more recently, from exposure to radiation from a ruby laser (*5*) are well known. But because cornea, lens, and other parts of the ocular media have strong absorption bands for wavelengths below 4000 Å and above 12,000 Å (*6*), only energy in the visible and near-infrared region reaches the retina and choroid in sufficient amounts to be dangerous, except in unusual cases.

The pathology of chorioretinal burns resulting from exposure to sunlight (*1*), atomic fireball (*3*), and ruby lasers (6943 Å) (*5*) has been described previously. In general, the injuries follow the course analyzed by Vos (*7*): most of the energy is absorbed in the melanin granules of the pigmented epithelium. These granules heat up and transfer heat by conduction, convection, and radiation to the proteins around them, which as a result of the

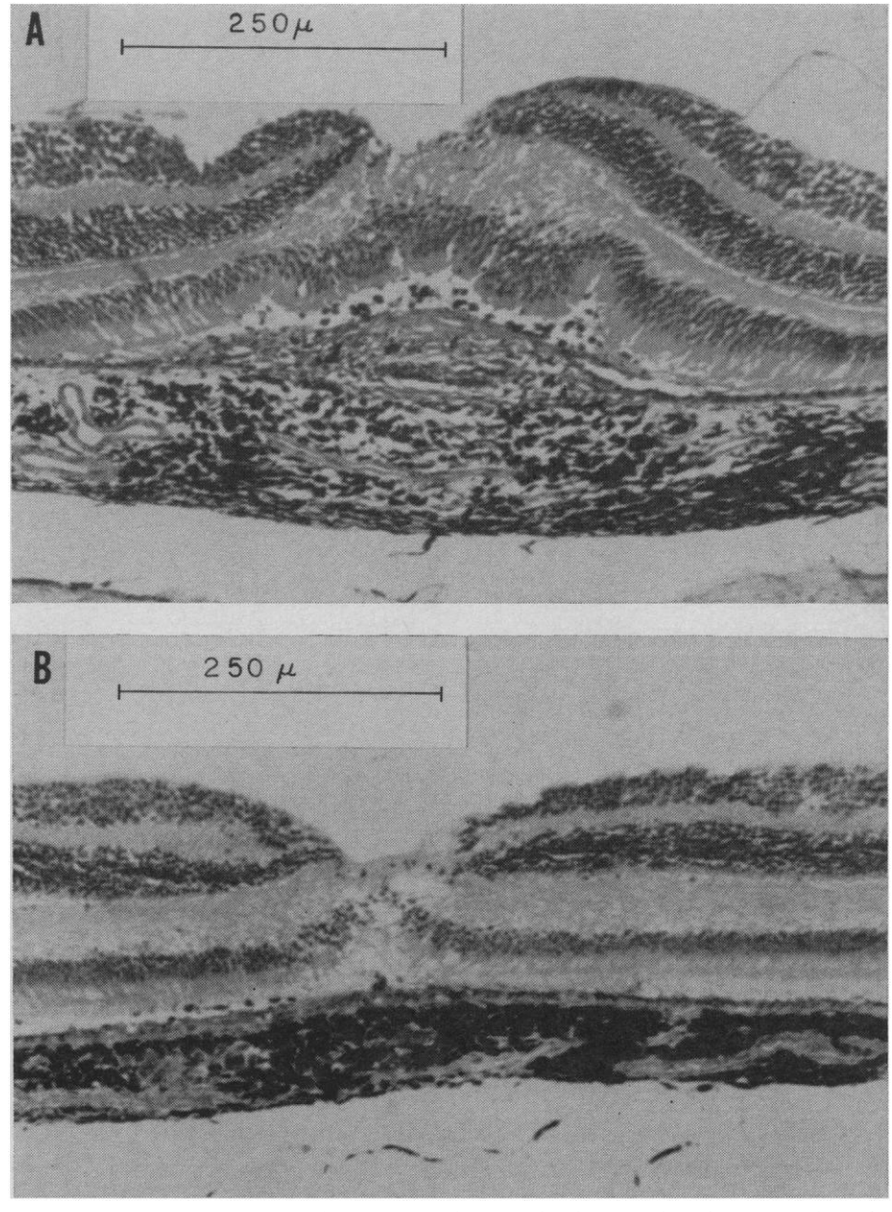

Fig. 1. Cross section of the retina of a monkey eye in the foveal region approximately 1 week after exposure to (*A*) ruby (6943 Å) laser pulse (non-Q switched) of approximately 60 millijoules and (*B*) neodymium (10,600 Å) laser pulse (non-Q switched) of approximately 200 millijoules. Both lesions were produced by an Optics Technology laser photocoagulator (model M-10, MK II) with the appropriate crystal mounted in it.