Gibberellic Acid-induced Phase Change in *Hedera helix* as Studied by Deoxyribonucleic Acid-Ribonucleic Acid Hybridization

Received for publication December 7, 1973 and in revised form February 12, 1974

CHARLES E. ROGLER and MICHAEL E. DAHMUS
Department of Biochemistry and Biophysics, University of California, Davis, California 95616

ABSTRACT

Applications of gibberellic acid to the mature form of *Hedera helix* induce morphological reversions to the juvenile form of growth. The juvenile forms produced are stable with time and differ dramatically from the mature in phenotype. DNA-RNA hybridization techniques have been used to study the RNA populations of juvenile, mature and gibberellic acid-treated mature apices. Hybridization competition experiments using RNA extracted by a hot phenol technique and uniformly labeled in *vitro* with radioactivity from labeled guanine show no qualitative differences between the species of RNA present in juvenile and mature apices. However, differences are observed in the frequency distribution of RNA species using both uniformly labeled or pulse-labeled RNA as a reference. RNA extracted from gibberellic acid-treated mature buds was a less effective competitor than control mature RNA and the difference observed was comparable to that observed between mature and juvenile RNA. These results indicate that at least part of the molecular basis of phase change and gibberellic acid action may involve an alteration in the rate of transcription of certain genes in the apices of the mature form.

RNA extracted using the hot phenol procedure contained a fraction of rapidly labeled RNA which was not extractable with cold phenol. When RNA extracted only with cold phenol was used in competition experiments sequences unique to the juvenile were detected and sequences unique to the mature were not detected. Implications of these results in relation to possible post-transcriptional control mechanisms are discussed.

Plant hormones have been extensively used to study the mechanism by which gene activity is regulated during plant development. This is due to the fact that many specific developmental events can be initiated by the addition of a particular hormone. We have characterized and quantitated the hormonal response in which GA<sub>3</sub> induces the mature form of *Hedera helix* to revert to the juvenile form of growth (unpublished data). This hormonal response involves a dramatic phenotypic change which is initiated in the apices of the mature plant and results in the reprogramming of the apical cells for the production of the juvenile form of growth.

The ability of GA<sub>3</sub> to stimulate RNA synthesis has been well demonstrated in barley aleurone layers. Zwar and Jacobsen (25) have reported that the effects of GA<sub>3</sub> were very small for ribosomal RNA and transfer RNA and comparatively large for RNA sedimenting between 5S and 14S. They termed the preferentially synthesized RNA, which is probably less than 1% of the total tissue RNA, gibberellic acid RNA, and concluded that α-amylase synthesis does not occur without the synthesis of this RNA.

Thompson (24) has studied the RNA populations from GA<sub>3</sub>-treated and control dwarf pea seedlings using the technique of DNA-RNA hybridization. He was unable to detect changes in RNA species hybridizing to the redundant DNA sequences in response to GA<sub>3</sub> in that system. However, in the same system, changes in transcription due to the addition of light were detectable. Dark-grown pea seedlings could not compete out a significant proportion of the RNA present in light-grown seedlings, indicating that the addition of light resulted in the activation of certain genes. In systems primarily involving the hormonal regulation of extension growth by auxin, similar results were obtained (23). No changes in the RNA populations in response to auxin were detectable when hypocotyl segments were used. However, in intact plants, in which swelling and possibly auxin-induced differentiation occur, new RNA sequences were detected in response to 2,4-D.

These results support the idea that a greater degree of gene reprogramming is necessary during developmental processes such as those induced by light or cell differentiation than those in which cell elongation is the primary response.

The system involving phase change in *Hedera helix* allows one to regulate a dramatic developmental change and may, therefore, be especially useful for the study of the control of transcription during plant development. The first step in the development of such a system was to determine if the molecular basis of phase change lies on the transcriptional level. We have used the technique of DNA-RNA hybridization to directly study the RNA species present in juvenile, mature and GA<sub>3</sub>-treated apices.

MATERIALS AND METHODS

Solutions. SSC: 1 × SSC is 0.15 M NaCl, 0.015 M Na citrate, pH 7.0; buffer A: 0.1 M tris HCl (pH 7.5), 0.1 M NaCl, 0.01 M Na EDTA; buffer B: 0.05 M tris HCl (pH 7.4), 0.05 M NaCl; buffer C: 0.05 M tris HCl (pH 6.7), 0.025 M KCl, 0.002 M MgCl<sub>2</sub>; buffer D: 0.01 M tris HCl (pH 7.4), 0.5 M NaCl, 0.001

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1 This work was supported by the Department of Environmental Horticulture, University of California, Davis, and by Grant HD 04899 from the National Institutes of Health.

2 Present address: Department of Plant Pathology, University of California, Davis, Calif. 95616.
m MgCl₂. RNase-free DNase (Worthington DPF) was dissolved in buffer C. DNase-free pancreatic RNase (preheated 10 min at 85°C) was dissolved in 1 × SSC.

Plant Materials. Actively growing buds from a clone of the juvenile and mature forms of Hedera helix were used as the source of juvenile and mature RNA in these studies. Juvenile vines were grown in a greenhouse bench under natural day-length and the 0.5 cm apical portion of the actively growing shoots were harvested. Mature apices were obtained by removing the terminal bud of mature shoots and allowing the lateral buds directly below the cut to become activated for 7 to 10 days. These buds were harvested at the bud break stage of development by snapping them off even with the stem.

Mature plants with a single stem, five leaves, and one lateral bud were treated with 18.2 μg of GA₃ using a tygon tubing infusion method (unpublished data). The primary shoots produced were cut off at the 10th node, and the lateral buds below the cut were activated and harvested as usual. The shoots produced from these lateral buds showed characteristics of an 85% reversion to the juvenile form. These methods of harvest provided a uniform source of plant material from juvenile, mature, and GA₃-treated mature plants.

Root tissue from mature plants, grown in hydroponic culture using one-half strength Hoagland’s solution, was used as a source of root RNA. Barley RNA was obtained from the shoots of 7-day-old barley seedlings (var. numar) which were grown in the dark for 6.5 days and moved to the light 12 hr before harvest.

All plant tissues were submerged in ice water immediately after harvest and then surface-sterilized in 5% Clorox containing 0.1% Tween 80 for 5 min. They were then rinsed in sterile-deionized water and stored at −80°C.

Preparation of RNA. In the experiments reported here two RNA extraction procedures were used. These will be designated as the cold phenol method, which is a modification of the method of Thompson (23), and the hot phenol method which is a modification of the methods of Penman (19) and Georgiev et al. (9).

Cold Phenol Method. All procedures were carried out at 4°C unless otherwise stated. Frozen bud tissue was homogenized in 5 volumes of buffer A containing 1% sodium dodecyl sulfate and 1 mg/ml Bentonite, and 10 volumes of water-saturated phenol containing 0.1% 8-hydroxyquinoline in a Waring Blender for 2 min at 60 v and then for 15 min at 20 v. The phases were separated by centrifugation at 10,000g for 5 min, and the aqueous phase was removed and re-extracted twice with an equal volume of phenol. The RNA was then precipitated by the addition of 2 volumes of cold ethanol at −20°C. The precipitate was dissolved in buffer B and precipitated with ethanol. The precipitate was then dissolved in buffer C and treated with 25 μg/ml DNase for 1 hr at room temperature. Sodium dodecyl sulfate and NaCl were then added to 1% and 1 M, respectively, and the aqueous phase was extracted three times, first with an equal volume of chloroform-isooctylalcohol (24:1, v/v), then with 0.5 volume of chloroform-isooctylalcohol, 0.5 volume of H₂O-saturated phenol and finally with an equal volume of phenol. The RNA was precipitated with ethanol and dissolved in SSC. The RNA was then treated with methoxyethanol and cetyltrimethylammonium bromide according to the method of Ralph and Bellamy (20). This step removes polysaccharides and also basic materials capable of interfering with specific hybrid formation (23). The RNA was then precipitated with ethanol, dissolved in buffer C and the DNase step repeated. After precipitation the RNA was dissolved in 1 × SSC and further purified by chromatography in Bio-Gel P-30 at room temperature. The excluded material was precipitated with ethanol, dissolved in H₂O, and stored at −20°C.

Hot Phenol Method. Tissues were ground to a fine powder in a mortar at −60°C and then homogenized at 4°C in a Waring Blender in buffer D (15 ml/g tissue) containing 1% sodium dodecyl sulfate and 1 mg/ml Bentonite and H₂O-saturated phenol (16 ml/g tissue) for 2 min at 60 v. After 15 min of vigorous shaking at 4°C, 0.5 volume of chloroform was added to stabilize the interphase, and the phases were separated by centrifugation at 12,000g for 5 min. The upper aqueous phase was removed, extracted at 4°C with 0.5 volume of water-saturated phenol and then with 0.5 volume of chloroform. The RNA was then precipitated with 2 volumes of ethanol. This fraction is referred to as the 4°C crude RNA fraction.

The interphase from the above 4°C extraction was suspended in buffer D (7.5 ml/g initial tissue) plus an equal volume of phenol and homogenized in a blender for 2 min at 60 v at room temperature. The mixture was then incubated at 85°C until the solution reached 78 to 80°C, and extracted for 3 min at this temperature. The extract was then cooled, diluted with 0.5 volume of chloroform, and centrifuged at 10,000g for 5 min. The organic phase was discarded, and the aqueous phase plus interphase were extracted with fresh phenol at 80°C as before. The aqueous phase was separated and re-extracted at 4°C with 0.5 volume of chloroform and then precipitated with 2 volumes of ethanol. This fraction is referred to as the 80°C crude RNA fraction.

The 4°C and 80°C crude RNA fractions were combined, dissolved in buffer C, treated with DNase (25 μg/ml for 1 hr at room temperature), and extracted as in the cold phenol procedure. If a large interphase formed, it was re-extracted with fresh buffer C at 80°C. The RNA was precipitated with ethanol, dissolved in 0.1 M Na acetate (pH 5.4), and precipitated by the addition of 1% CTAB in 0.1 M Na acetate (pH 5.4) to a final ratio of 2:1 CTAB-RNA (w/w). The precipitate was washed with ethanol-acetate mixture (70% ethanol and 0.1 M Na acetate) to remove CTAB and convert the RNA to the Na salt. The pellet was then dissolved in 0.01 M Na acetate, pH 5.4. The RNA solution was treated with methoxyethanol and CTAB according to the procedure of Ralph and Bellamy (20). The initial CTAB precipitation was found to be necessary to prevent loss of RNA in the interphase during the methoxyethanol step. This was not a problem with the cold phenol procedure. After the second CTAB precipitation and washing the RNA was dissolved in 1 × SSC and chromatographed on Bio-Gel P-30. The excluded RNA was precipitated with ethanol, dissolved in water, and stored at −20°C.

Labeling of RNA. Total RNA was uniformly labeled in vitro using 1H dimethyl sulfate according to a modification of the method of Smith et al. (21). This labeling procedure does not affect the hybridization properties of the RNA (21). The 1H dimethyl sulfate (1.7 mg) was dissolved in 25 μl of ether before the addition of RNA to the ampoule. In a typical labeling reaction 1.19 mg of RNA in 0.18 ml of 0.3 M Na phosphate buffer, pH 7.5, were incubated with 1.7 mg of carrier-free 1H dimethyl sulfate (2990 μc/mg) at room temperature for 3 hr. Following the reaction the RNA was precipitated three times with ethanol and chromatographed on Bio-Gel P-30. The specific radioactivity of the RNA under these conditions was 19,950 cpm/μg.

RNA was pulse-labeled in vivo according to the method of Ingle et al. (11). As an added precaution against bacterial con-

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*Abbreviation: CTAB: cetyltrimethylammonium bromide.
tamination, all operations were carried out under aseptic conditions. Samples of 3 to 10 g of buds (surface-sterilized 5 min in 5% Clorox and rinsed in sterile-deionized H2O) were incubated at 30 °C with continuous shaking in 50-ml Erlenmeyer flasks with from 5 to 15 ml of sterile incubation medium containing 1% sucrose, 0.5 mM citric acid neutralized to pH 6.0 with NH4OH and 20 µg/ml streptomycin.

The buds were preincubated for 2 hr before the addition of fresh medium containing neutralized carrier-free 32P. The final concentration of 32P was from 0.5 to 3.5 mc/ml. At the end of the 1 hr 32P incubation, the buds were rinsed with cold-deionized water and frozen in liquid N2 before grinding and RNA extraction.

Preparation of DNA. Actively growing shoot tissue from either the juvenile or mature form of Hedera helix was surface-sterilized and rinsed before extraction. DNA extraction was conducted according to the method of Bendich and Bolton (2) to and including the initial precipitation of crude DNA. Good recoveries of DNA during subsequent phenol extractions require the removal of polysaccharides at this point. The DNA was dissolved in 1/10 SSC to approximately 2 mg/ml (A260 used as criterion to estimate DNA concentration) and NaCl was added to a final concentration of 1 M. An equal volume of 1% CTAB in water was then slowly added and the DNA wound out. The precipitate was washed twice with ethanol-acetone mixture and then dissolved in 1 M NaCl. According to Jones (13) polysaccharides remain as insoluble CTAB salts in 1 M NaCl and DNA is solubilized. The insoluble material was spun at 16,000g for 10 min. The supernatant was collected, and the DNA was precipitated. At this point the DNA is gelatinous in appearance.

The DNA was dissolved in 1/10 SSC, and contaminating RNA was removed by digestion with 20 µg/ml pancreatic RNase at room temperature for 2 hr. Pronase (autodigested for 90 min at 37 °C) was added to 20 µg/ml, and the incubation was continued for 3 hr. NaClO, was added to 1 M, and the solution was extracted twice with an equal volume of chloroform-isooamylic alcohol and then twice with 0.5 volume of chloroform-isooamylic alcohol and 0.5 volume of phenol. The DNA was precipitated and redissolved in 1 M Na phosphate, pH 6.8. The Na phosphate concentration was then increased to 0.2 M, and the DNA solution was passed over hydroxyapatite. The column was washed with 0.2 M Na phosphate, and the DNA was eluted with 0.5 M Na phosphate and dialyzed against 1/100 SSC. NaCl was added to 0.15 M, and the DNA was precipitated with 2 volumes of ethanol and dissolved in 1/100 SSC. DNA purified in this manner had an A280/260 = 0.55 and a 230/260 = 0.43. About 7.5 mg of purified DNA was obtained from 120 g of fresh tissue.

DNA-RNA Hybridization. DNA was immobilized on nitrocellulose filters (Millipore, size 0.22 µm) following the procedure of Gillespie and Spiegelman (10), except that 20 mM MgCl2 were added before the salt concentration was brought to 5 × SSC to maximize DNA retention (23). The filters were then washed twice with 50 mM 5 × SSC, air-dried overnight, and finally dried in a vacuum oven at 80 °C for 2 hr. With the aid of a paper punch, 6-mm discs were punched from the large filters. DNA retention at the end of the hybridization was determined by assaying the amount of diphenylamine-positive material according to the method of Burton (5).

The standard hybridization conditions were 5 × SSC and 50% formamide at 50 °C for 24 hr in a final volume of 0.2 ml. Each vial contained two DNA and two blank filters. Paraffin oil (0.1 ml) was added to the top of each reaction to prevent evaporation. After incubation, the filters were removed and washed at 50 °C twice with 5 × SSC, 50% formamide and twice with 5 × SSC. The filters were then treated with 20 µg/ml pancreatic RNase in 2 × SSC at room temperature for 30 min to remove nonspecifically bound RNA. They were then washed twice in 5 × SSC at 50 °C, dried, and counted. To obtain actual counts hybridized, the counts bound per blank filter were subtracted directly from those bound to the DNA filters. Counts bound to blank filters never constituted more than 10% of those bound to DNA filters.

The hybrids formed with 3H RNA under these conditions have a Tm of 75 C in SSC. The Tm of native Hedera helix DNA under these conditions is 86 C. Direct experiments to determine the per cent of the total hybridization due to ribosomal RNA in this system have not been conducted. However, from the competition by barley RNA in 32P and 3H hybridization competition experiments, it is unlikely that rRNA hybridization accounts for more than 30% of the hybrids formed in these experiments (3, 11).

RESULTS

The series of experiments described here were designed to characterize the RNA populations produced in shoot apices of juvenile and mature forms of Hedera helix. The initial work was conducted using total cell RNA extracted by the cold phenol method and labeled in vitro with 3H dimethyl sulfate. When increasing amounts of labeled juvenile or mature RNA were added to a series of reaction vials containing a fixed amount of denatured DNA the following DNA saturation curves were obtained (Fig. 1). The saturation curves for both RNA species are similar. When increasing amounts of mature RNA were added in the presence of a relatively high concentration of juvenile RNA, no large increase in the extent of hybridization was observed (upper frame). Similar results were obtained when labeled juvenile RNA was added in the presence of a high concentration of mature RNA (lower frame). In both cases when one RNA was substituted for another, midway in the saturation curve, the resulting curves did not differ significantly from those obtained in the presence of only one RNA type. These results indicate that, within the limitations of our assay, the portion of DNA hybridized by each RNA population is similar and that neither population contains a high percentage of sequences not contained in the other.

To compare the RNA sequences present in the two populations more completely, competition hybridization experiments were conducted. When increasing amounts of unlabeled juvenile or mature RNA were competed against a constant amount of reference juvenile RNA the results presented in Figure 2 were obtained. The fact that the competition curve obtained in the presence of mature RNA plateaued at a value above that of the homologous juvenile RNA competition curve indicates that the juvenile RNA contains sequences not represented in the mature RNA (Fig. 2A). This experiment was repeated three times, and in each case the results indicated 13 to 20% of the sequences contained in juvenile RNA were absent in the mature RNA population. Sequences unique to the mature RNA population were not detected (Fig. 2B).

The technique of in vitro labeling allows one to compare the total RNA populations including the more stable RNA species which would not be labeled following a short pulse. To further extend these studies, it was of interest to study the rapidly labeled RNA produced in juvenile and mature apices. Rapidly labeled RNA accounts for only a small proportion of the total cellular RNA and in short term labeling experiments is primarily localized in the nucleus (1, 7, 8). Furthermore, Georgiev et al. (9) and Armelin and Marques (1) have determined that phenol extractions at elevated temperatures greatly en-
Fig. 1. Similarity of saturation curves using various combinations of $^3$H-labeled juvenile or mature RNA. Standard hybridization conditions were used. Each DNA filter contained 4.5 μg of DNA. Upper: Mature $^3$H RNA (6250 cpm/μg) substituted for juvenile $^3$H RNA (6050 cpm/μg). ●: juvenile RNA only; ○: 131 μg of juvenile RNA plus designated amount of mature RNA substituted for juvenile RNA. Lower: Juvenile $^3$H RNA substituted for mature $^3$H RNA. —●— mature RNA only; —○— 145 μg mature RNA plus designated amount of juvenile RNA substituted for mature RNA.

Fig. 2. DNA-RNA competition hybridization experiments to compare the RNA species present in juvenile and mature RNA extracted by the hot phenol method. A: 20 μg of uniformly labeled $^3$H juvenile RNA (9290 cpm/μg) used as reference in each vial in the presence of increasing amounts of unlabeled juvenile or mature competitor RNA; B: 20 μg of $^3$H mature RNA (9594 cpm/μg) used as reference in each vial in combination with competitor RNA as in A. Each DNA filter contained 4 μg of DNA. In the absence of competitor, 190 cpm above background were hybridized in A and 310 cpm above background in B.

Table 1. Extraction of Pulse-labeled RNA at Various Temperatures

Juvenile RNA was pulse labeled with $^{32}$P for 1 hr and the RNA was purified by the hot phenol method outlined under "Materials and Methods." The interphase from the 55 C extraction was saved and successively re-extracted for 5 min with fresh buffer and phenol at the desired temperature. Each RNA fraction was further purified by the standard hot phenol technique.

<table>
<thead>
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<th>RNA Fraction</th>
<th>Amount (μg)</th>
<th>Specific Radioactivity (cpm/μg)</th>
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<td>2.48</td>
<td>350</td>
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<td>65</td>
<td>0.18</td>
<td>1050</td>
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<td>80</td>
<td>0.02</td>
<td>3060</td>
<td>5</td>
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Each DNA method was used as reference against competitor RNA from either mature plants or GA3-treated mature plants. The RNA from the lateral buds on GA3-treated plants appears slightly less efficient in competition experiments against mature RNA (Fig. 5). Extension of these curves beyond 20% hybrid remaining is essential to determine if this difference represents a qualitative or quantitative change in RNA populations.

The results obtained from the experiments involving uniformly labeled RNA (3H) indicated the presence of sequences in juvenile RNA absent from mature populations whereas the results with pulse-labeled RNA suggest the presence of additional sequences in mature RNA populations. These results are not inconsistent due to the different RNA labeling and extraction procedures used in each case. The question arises as to whether the different results were due to the different labeling techniques or whether the RNA extraction procedure played a significant role. The latter was a strong possibility due to the fact that the hot phenol extraction procedure results in a greater yield of rapidly labeled RNA. Therefore, it was necessary to repeat the initial experiments using in vitro labeled RNA extracted by the hot phenol procedure.

The results of competition experiments utilizing juvenile or mature RNA labeled in vitro with dimethyl sulfate and extracted at elevated temperatures are shown in Figure 6. Sequences unique to the juvenile RNA were not detected (Fig. 6A). With the exception of the RNA extraction procedure this experiment is directly analogous to that presented in Figure 2A. Therefore, it appears that when one accomplishes a more quantitative extraction of RNA, including the fraction of RNA extracted with hot phenol, the differences are no longer evident.

In the reverse competition, using mature RNA as the reference, the results again indicate no qualitative differences in the hybridizable RNA sequences of juvenile and mature apices (Fig. 6B). However, a small difference in the slopes of the curves, juvenile and GA3-treated buds in comparison to mature control, indicates there may be small frequency differences in the RNA species present in the two forms. Absolute differences in the RNA species, if they exist, are too small to be detected using uniformly labeled RNA.

![Graph 3](image3.png)

**Fig. 3.** DNA saturation curve with pulse labeled juvenile RNA (152 cpm/µg). RNA was labeled for 1 hr with 32P (500 µc/ml). Each DNA filter contained 3.4 µg of DNA. Counts bound to blank filters ranged from 2 to 15.

![Graph 4](image4.png)

**Fig. 4.** Comparison of the RNA species from juvenile and mature buds using 32P pulse labeled RNA extracted by the hot phenol method. A: 180 µg of juvenile 32P pulse labeled RNA (345 cpm/µg) was used as reference; B: 200 µg of mature 32P pulse labeled RNA (380 cpm/µg) was used. Competitor RNA was mixed with the appropriate reference RNA and incubated under standard conditions. Each DNA filter contained 3.4 µg of DNA. In the absence of competitor 292 cpm above background were hybridized in A and 303 cpm above background in B.

![Graph 5](image5.png)

**Fig. 5.** Comparison of the RNA species from mature and GA3-treated buds. 180 µg of 32P pulse labeled mature RNA (1500 cpm/µg) was used as reference per vial. All RNA was extracted by the hot phenol method and each DNA filter contained 1.5 µg of DNA. Competitor RNA was obtained from mature control buds or from buds on a mature plant previously treated with 18 µg of GA3 which exhibited an average of 85% reversion to the juvenile form of growth. In the absence of competitor 210 cpm above.
DISCUSSION

The juvenile and mature forms of *Hedera* differ not only in morphology but also in many physiological characteristics. The juvenile form has a much more rapid growth rate than the mature, forms adventitious roots more readily, produces an abundance of anthocyanins, and has a plagiotropic growth habit compared to the mature which has an orthotropic growth habit. The mature form, however, has the ability to flower whereas the juvenile does not. In tissue culture, callus of the juvenile form also maintains a much higher growth rate than mature callus on similar defined media (23). The morphological and physiological differences between the two forms indicate there may be a significant difference in gene activity between them.

Evidence has been obtained to show that the RNA sequences present in different organs of the plant vary significantly (23). We have also found that mature *Hedera* root RNA is a fairly poor competitor with mature *Hedera* bud RNA (Fig. 4B). However, an absolute qualitative difference in sequences has not been established in our system. In the case of phase change, we are investigating the possible role of RNA in the differentiation of the shoot apical cells for the production of one defined phenotype (mature) or another (juvenile). The cells comprising the leaf primordia of juvenile and mature apices may contain similar RNA sequences and would, therefore, be expected to compete with each other for binding sites on the DNA. Thus, common sequences from analogous differentiated parts of the bud may contribute significantly to make differences present in the apical dome more difficult to detect. Therefore, any differences which are observed will be a minimal estimate of the differences which exist in a localized portion of the apex.

The hybridization conditions employed in these experiments would be expected to result in hybridization primarily to the repeated DNA sequences (6, 15, 17). Unique sequences, due to their lower effective concentration, will not contribute measurably to the observed hybridization (4, 6, 16). Furthermore, the fact that closely related sequences can cross react also leads to a minimum estimate of the differences between RNA populations. A more complete discussion of this subject has recently been presented by several investigators (6, 24).

Many investigators have shown that hormone treatment of plant tissues can affect the rate of RNA synthesis (14) and that the template activity of isolated chromatin can be increased as a result of hormone treatment of intact plants (12). It has recently been reported that purified protein factors have been isolated, which in conjunction with plant hormones and native plant RNA polymerase appear to regulate RNA transcription *in vitro* (18). In order to unambiguously relate effects on RNA synthesis obtained *in vitro* to a physiological response one must show that the RNA products produced both *in vivo* and *in vitro* in response to the hormone are similar. Before such comparisons can be made it is essential to establish the relationship between RNA populations present in control and hormone-treated plants.

In the case of *Hedera*, the juvenile and mature forms represent two natural reference points. Therefore, our original work was carried out to study the RNA populations produced in juvenile and mature buds and was later extended to include buds from GA$_3$-treated plants. When experiments were conducted using rapidly labeled RNA from juvenile or mature buds as reference, sequences unique to the juvenile form could not be detected. Reverse competition experiments suggested that the mature RNA contained a small proportion of sequences which were either not present in the juvenile RNA or present in only very low concentrations. The results indicate that some DNA sequences transcribed in the mature form are not active in the juvenile form.

RNA extracted from GA$_3$-treated mature buds was a less effective competitor than mature control RNA at low levels of competitor RNA. The difference is small and comparable to that observed between mature and juvenile RNA. These differences may be due to either additional RNA sequences present in the GA$_3$ mature RNA population, which dilute out the normal mature RNA, or the reduction in the rate of transcription from certain genes active in the mature which may have lowered their relative frequency in relation to the total RNA population. Therefore, the significance of these small quantitative differences in relation to phase change or GA$_3$ induction of juvenile characteristics is not directly apparent from this experiment.

In order to extend our results to include the more stable RNAs a similar set of experiments was conducted with RNA extracted by the same hot phenol extraction procedure and labeled uniformly *in vitro* with $^3$H dimethyl sulfate. In these experiments, as before, no RNA sequences unique to the juvenile or GA$_3$-treated mature buds could be detected (Fig. 6A). A small difference was apparent in the slope of the competition curves for juvenile RNA and RNA from GA$_3$-treated mature buds when compared to mature RNA (Fig. 6B). The slope of the curve for GA$_3$ mature RNA was more similar to that of the juvenile RNA than the mature RNA. Therefore, these small proportional differences (Figs. 5 and 6B), which represent a minimum estimate of differences in the total RNA populations,
action may lie in alteration of the rate of transcription of different RNA sequences.

In all the experiments involving the use of RNA isolated by the hot phenol extraction procedure sequences unique to the juvenile form could not be detected. However, when a cold phenol method was used, a unique fraction was reproducibly found. An unequivocal explanation for these results is not possible but several considerations should be mentioned. It is apparent from Table I and the work of numerous laboratories (1, 9, 19) that the type of RNA recovered is highly dependent on the conditions of the phenol extraction. The RNA recovered by extraction of 80 C should contain the greatest RNA sequence diversity and most of the sequences transcribed within that particular tissue. The RNA extracted by the cold phenol procedure is devoid of a fraction of nuclear RNA extractable only at elevated temperatures and is probably composed primarily of ribosomal, transfer, and cytoplasmic messenger RNA. The differences observed with the cold phenol-extracted RNA may therefore, reflect changes in processing or transport of nuclear RNA transcribed from repeated DNA sequences.

The results presented in this paper indicate total phase change does not involve large alterations in the species of RNA transcribed from the redundant DNA sequences in mature and juvenile apices. RNA sequences represented only in the juvenile bud RNA were not detectable using either pulse-labeled or uniformly labeled RNA extracted using a hot phenol extraction. A small fraction of mature RNA was not competed out by juvenile RNA in experiments using pulse-labeled RNA, indicating that some genes may be active in the mature form which are not active in the juvenile form. However, the RNA transcribed from these genes must represent a small proportion of the total RNA due to the fact that these qualitative differences are not detectable using uniformly labeled mature RNA. The small differences observed in the frequency distribution of RNA species, as judged by differences in the slopes of competition curves, indicate that at least part of the molecular basis of phase change and GA action may involve an alteration in the rate of transcription of certain RNA sequences in the buds of the mature form.

Acknowledgment—We thank Dr. Wesley P. Hackett for his helpful advice on the physiological aspects of the problem.

LITERATURE CITED