Short Communication

Increased DNA Template and RNA Polymerase Associated With the Breaking of Seed Dormancy[1]

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Dormant seeds of Hazel (*Corylus avellana* L.) require a period of after-ripening at low temperature before they become capable of subsequent germination. Gibberellic acid (GA_3) can fully replace this requirement (2, 6). The identification of gibberellin-like substances in extracts of chilled (non-dormant) seeds led Frankland and Wareing (7) to suggest that gibberellin synthesis may be involved in the after-ripening process. GA_3 has also been shown to induce synthesis of hydrolytic enzymes in other seed tissues (13) and enhance RNA synthesis in isolated nuclei of pea seedlings (9).

Since derepression of genetic material has been shown to accompany the breaking of potato bud dormancy by ethylene chlorohydrin (12) the possibility arises that the breaking of dormancy by GA_3 may similarly result in gene derepression. To test this possibility we have studied the effect of GA_3 on chromatin-directed RNA synthesis in relation to the breaking of Hazel seed dormancy.

All seeds were sterilized for 5 min in 0.5 % sodium hypochlorite, sown on absorbant paper soaked with water or GA_3 (10 μg/ml) and subsequently maintained at 25° in the dark for the required period. Embryonic axes were then excised by severing the cotyledonary petioles. RNA synthesis was measured by $^{32}PO_4^{3-}$ incorporation. Twenty embryonic axes were incubated for 3 hr in 1 ml of medium (125 μc $^{32}PO_4^{3-}$, 1 % sucrose and 10^{-4} M ammonium acetate buffer, pH 6.0) at 25° and nucleic acids subsequently extracted by the phenol-dupanol method of Cherry *et al.* (5). Polyacrylamide gel electrophoresis was used to fractionate the RNA species (based on 1), although the data presented here represents total RNA. Gel electrophoresis also established, on the basis of molecular weight, that there was no significant incorporation of label into bacterial RNA in any samples (unpublished data). Chromatin was prepared from embryonic axes by general methods based on those of Huang and Bonner (8) and similar to those previously used in this laboratory (10). The activity of chromatin-bound RNA polymerase was assayed in a reaction mixture containing in μmoles the following; GTP, 0.2; CTP, 0.2; ATP, 0.2; $MgCl_2$, 1.0; $MnCl_2$, 0.25; Cleland's reagent, 5.25; tris-HCl, pH 8.0, 35.0 and 20 μc of ^{3}H-UTP (2.0 mc/μmole) and chromatin (equivalent to 4–6 μg DNA) in a final volume of 0.35 ml. The capacity of chromatin DNA to support RNA synthesis was determined in a similar assay mixture except that the chromatin was reduced to an equivalent of 0.4 to 0.6 μg DNA and *E. coli* RNA polymerase (purchased from Biopolymers Inc., Pinebrook, New Jersey) was included in excess (6 units according to Chamberlin and Berg, 4). The reaction was performed at 37° for 20 min and then terminated by the addition of 4 ml of ice-cold 10 % TCA. The precipitates were subsequently transferred to membrane filters (Schleicher and Schuell, type B-6) and washed with 40 ml cold 5 % TCA The membranes were dried and counted in a Tri-Carb liquid scintillation spectrometer.

DNA determinations were according to Burton (3) following hydrolysis in 0.5 M perchloric acid at 70° for 45 min.

Seeds treated with GA_3 begin to germinate on the fifth day and all viable seeds germinate within 11 days. Ten percent of the seeds sown in water do eventually germinate although no germination is observed until the seventh day. Growth of the embryonic axis precedes radicle protrusion, a GA_3-induced increase in fresh weight being apparent by the third day. The changes described below markedly precede changes in fresh and dry weight and in total RNA content (unpublished data).

The rate of $^{32}PO_4^{3-}$ incorporation into total RNA is markedly stimulated by gibberellic acid (fig 1). RNA synthesis in embryonic axes is detectable after 11 hr in GA_3 (8500 cpm/axis) with a progressive increase in the rate of synthesis up to the sixty-sixth hr. Embryonic axes of seeds sown in water, however, exhibit no RNA synthesis by 11 hr, limited RNA synthesis at 24 hr and only about one-seventh that of the GA_3-treated seeds at 66 hr. Such data are consistent with the hypothesis that the breaking of dormancy involves changes in the level of nucleic

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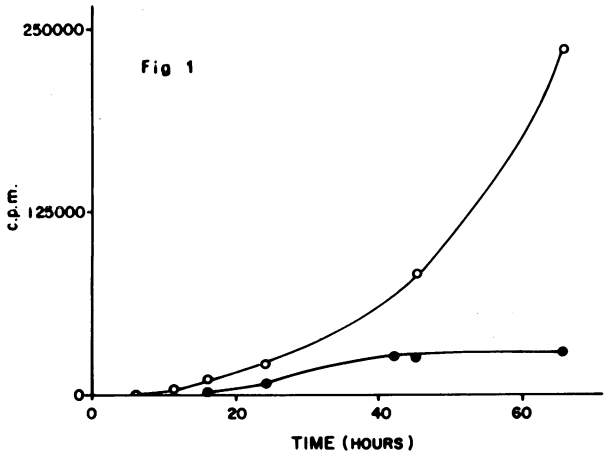

FIG. 1. The amount of $^{32}PO_4^{3-}$ incorporated into the RNA of embryonic axes of hazel seeds sown in gibberellic acid (○) or water (●). Data given as cpm/embryonic axis.

acid synthesis. However, the rate of nucleic acid synthesis is controlled by available DNA template sites and RNA polymerase activity. Consequently, to test the relative importance of these 2 possibilities, changes in chromatin-directed RNA synthesis, at endogenous and non-limiting levels of RNA polymerase, were studied.

The RNA polymerase activity of chromatin prepared from embryonic axes of GA_3-treated seeds begins to increase several hr earlier than that of control seeds. Also the rate of increase is more rapid in the case of GA_3-treated seeds (fig 2). Thus, it appears that the *in vivo* stimulation of RNA synthesis by GA_3 can be attributed, at least in part, to increased RNA polymerase activity.

DNA template availability was assayed by saturating the chromatin system with *E. coli* RNA polymerase (fig 3). In all chromatin preparations the amount of endogenous polymerase is limiting RNA synthesis, as shown by the large stimulation with exogenous polymerase, 100-fold, for example, with chromatin from seeds treated with GA_3 for 36 hr. However, it is possible that RNA polymerase does not limit RNA synthesis *in vivo* since there may be additional soluble enzyme as in corn seedlings (11). No attempt has yet been made to isolate soluble RNA polymerase from hazel seeds. Chromatin from

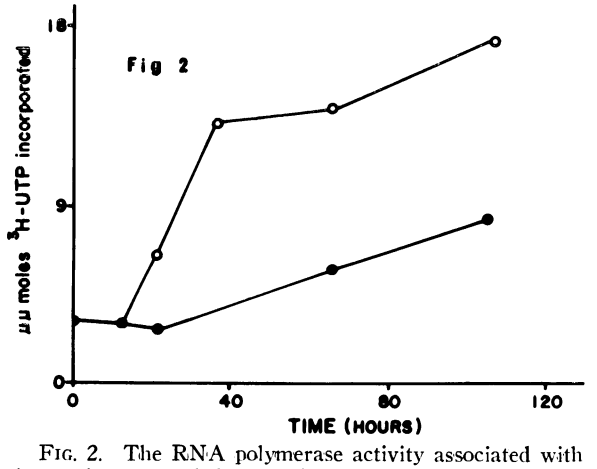

FIG. 2. The RNA polymerase activity associated with chromatin prepared from embryonic axes of seeds sown in gibberellic acid (○) or water (●). Results expressed as $\mu\mu$moles ^{3}H-UTP incorporated/100 μg DNA.

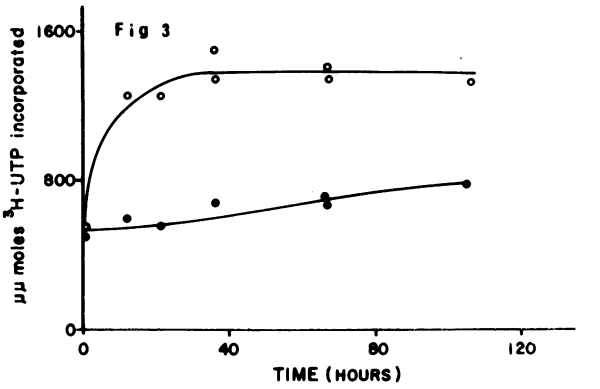

FIG. 3. DNA template availability of chromatin prepared from embryonic axes of seeds sown in gibberellic acid (○) or water (●). Results expressed as $\mu\mu$moles ^{3}H-UTP incorporated into RNA/100 μg DNA.

seeds in GA_3 possesses 2 to 3 times more available DNA template than that of control seeds, the GA_3-induced increase in available DNA template being virtually complete within 11 hr, with little further change prior to germination. The small increases in template availability, RNA polymerase activity and *in vivo* RNA synthesis observed in untreated seeds probably reflect the fact that 10% of these seeds would eventually germinate.

The earliest change detected in embryonic axes of GA_3-treated seeds is in the amount of DNA available for transcription. This is followed by increased chromatin RNA polymerase activity, increased RNA synthesis as measured *in vivo* and subsequently growth of the embryonic axis. Whether, or not, the change in DNA template availability is the initial step in the breaking of dormancy remains to be further examined, since these data do not necessarily indicate that GA_3 affects the transcriptional process directly, or even if this is the main or sole function of GA_3 in breaking dormancy.

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