HORMONAL REGULATION OF TRANSLATION INHIBITION REQUIRING RNA SYNTHESIS

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Received January 22, 1970

Summary The control of the translation inhibition of germination mRNAs during cotton embryogenesis has been studied by determining the events necessary to remove the translation inhibition during precocious germination. The results indicate that the plant hormone abscisic acid may control translation inhibition by a mechanism involving RNA synthesis.

Introduction

During germination of cotton seeds a great deal of the protein synthesis that occurs in the cotyledons can occur in the presence of sufficient actinomycin D to inhibit RNA synthesis (Waters and Dure, 1966). This apparent example of "stored" mRNA is analogous to a number of developmental systems including germination in wheat (Chen et. al., 1968), fertilization in sea urchins (Gross and Cousineau, 1963) and fertilization in amphibians (Brown and Littna, 1966). In sea urchins the transcription of the "stored" mRNA may take place at least a month prior to maturation of the eggs (Piatagorsky and Tyler, 1967). In amphibians the "stored" mRNA appears to be synthesized during the last hours of oogenesis, following the hormonal stimulation which starts ovulation (Brown and Littna, 1966). In cotton the mRNA for a protease which appears normally during germination as a result of de novo synthesis is presumably transcribed by approximately 60% completion of embryogenesis (Ihle and Dure, 1969). The above evidence, therefore, suggests that a temporal separation of transcription and translation is extremely important in regulating developmental events. The ability to precociously germinate cotton embryos has afforded a useful system for investigating the controls of translation inhibition during development. The results reported here concern the possible mechanisms and factors

involved in maintaining translation inhibition during embryogenesis in cotton.

Methods

To study the phenomenon of translation inhibition, the following general procedures were used. Embryos of various stages of maturity were dissected from ovules and precociously germinated in petri dishes lined with miracloth which were saturated with various test solutions. The test solutions employed were, actinomycin D (20 ugms/ml), an aqueous extract of ovules (less their embryos), abscisic acid (10 ugms/ml) and combinations thereof. Precocious germination was followed by quantitative assay of a protease enzyme shown to be synthesized de novo during germination (Ihle and Dure, 1969). Rinsing the embryos prior to precocious germination was found to greatly stimulate the appearance of enzyme activity and visible germination. Consequently some of the embryos treated as above were washed prior to transfer to the petri dishes while others were transfered directly from the ovule.

Actinomycin D was obtained from Calbiochem. D,l abscisic acid was generously provided by Dr. Robert Schieferstein of Shell Development Company.

Results and Discussion

The time course of embryogenesis in cotton with respect to wet weight of the embryo is shown in figure 1. Twenty days after pollination the wet weight of the embryo increases rapidly until it reaches 80 to 90 mgs at 30 days. Following the initially rapid increase in wet weight, the embryo continues to increase in weight at a slower rate until it reaches 125 mgs at approximately 50 days following pollination. At approximately 50 days, dessication of the embryo and the sclerification (followed by death) of the ovule wall begins which ultimately results in formation of the mature seed. In the present study embryogenesis and seed formation have been divided into three phases based upon the apparent regulation of the appearance of the germination protease during precocious germination. The divisions are: 1.) stage I embryos, embryos smaller than 85 mgs wet weight, 2.) stage II embryos, embryos between 85 to 125 mgs wet weight, and 3.) stage III embryos, embryos older than 50 days which are undergoing seed formation.

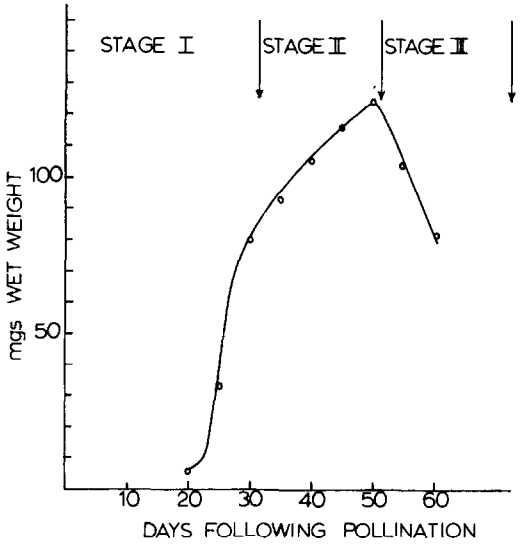

Figure 1. The wet weight of cotton embryos during embryogenesis.

The appearance of enzyme activity in embryos of stages I, II and III, precociously germinated under a variety of conditions, is shown in figure 2. In all cases, the appearance of enzyme activity is concomitant with the visible germination of the cotyledons. As can be seen and as has been previously reported (Ihle and Dure, 1969) stage II and III embryos can accumulate the protease enzyme in the presence of sufficient actinomycin D to stop detectable RNA synthesis (values from actinomycin D treated embryos are indicated by the dashed lines). Embryos below approximately 85 mgs do not accumulate the enzyme in the presence of actinomycin D. This observation has been interpreted to indicate that the mRNA for the protease is transcribed when the embryos are approximately 85 mgs wet weight. During normal embryogenesis, the mRNA is presumably conserved and not translated until germination has begun. This suggests that its translation is inhibited in some regulated fashion until the embryo is induced to commence germination.

The regulation of translation inhibition for the protease mRNA during embryogenesis and the factors involved in the release of this inhibition during

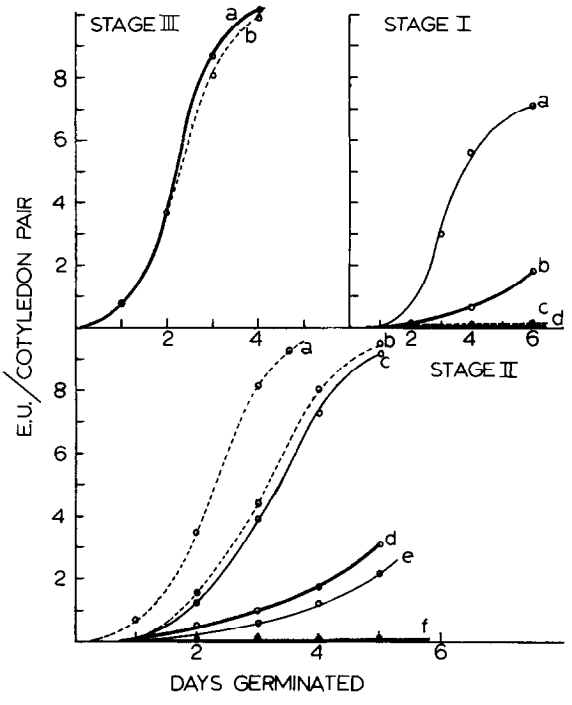

Figure 2. The development of protease activity in cotton embryos during precocious germination.
Stage I embryos: line a, washed embryos; line b, unwashed control embryos; line c, embryos treated with the ovule extract or abscisic acid; line d, embryos precociously germinated under any of the experimental conditions in the presence of actinomycin D.
Stage II embryos: line a, unwashed, actinomycin D treated embryos or washed embryos treated with the ovule extract and actinomycin D; line b, washed embryos treated with actinomycin D or actinomycin D and abscisic acid; line c, washed embryos; line d, unwashed, control embryos; line e, washed embryos treated with the ovule extract; line f, washed embryos treated with abscisic acid.
Stage III embryos: line a is obtained with abscisic acid treated, ovule extract treated and unwashed, control embryos; line b is obtained with washed, unwashed, abscisic acid treated or ovule extract treated embryos precociously germinated in the presence of actinomycin D.

precocious germination are demonstrated by the responses of stage II embryos to various treatments during precocious germination. These responses are shown in figure 2, lower graph. The stimulatory effect on enzyme appearance and precocious germination of simply washing the dissected embryos in distilled water prior to placing them on miracloth is demonstrated in figure 2 (compare line c with d). If stage II embryos are washed with a solution of actinomycin D and are precociously germinated in the presence of actinomycin D,

the stimulation is still observed (compare line b with d). If unwashed embryos are precociously germinated in the presence of actinomycin D, the appearance of enzyme activity is stimulated approximately four fold above the unwashed controls (compare line a with d).

The above data may be interpreted to indicate the presence of an inhibitor which in some manner controls translation inhibition of the mRNA for the protease enzyme. The inhibitor can be removed by simply washing the embryos, or its effect on translation inhibition can be nullified by stopping RNA synthesis with actinomycin D.

To further test the above hypothesis, an ovule extract was obtained from ovule walls. When washed embryos are precociously germinated in the presence of the ovule extract, the development of enzyme activity is somewhat inhibited as is germination in general (compare line e with d). If, however, embryos are washed in actinomycin D and subsequently germinated in the presence of the ovule extract containing actinomycin D, enzyme synthesis is restored to that of the actinomycin D treated, unwashed control embryos. These data suggest that a factor or factors are present in the ovule wall which diffuse into the embryo during embryogenesis and maintain translation inhibition of the protease mRNA and suppress germination in general, presumably by a mechanism involving RNA synthesis.

The presence of abscisic acid in developing cotton bolls has been well documented (Ohkuma et. al., 1963) and the ability of abscisic acid to inhibit germination has been demonstrated in a number of plant systems (Galston and Davies, 1969). Therefore, the effect of this plant hormone on precocious germination of stage II cotton embryos was determined. As is demonstrated in figure 2, when washed embryos are precociously germinated in the presence of abscisic acid, the development of enzyme activity is completely inhibited as is visible germination (compare line f with c). If, however, embryos are washed in actinomycin D and are precociously germinated in the presence of both abscisic acid and actinomycin D, the appearance of enzyme activity is

restored to that of washed embryos (compare line b with c).

Stage III embryos, however, are not sensitive to the ovule extract nor are they sensitive to abscisic acid (figure 2, upper left hand graph). In addition, the presence of actinomycin D under any of the experimental conditions does not inhibit or stimulate the appearance of enzyme activity during the precocious germination of these mature embryos. The factors involved in obtaining this independence from apparent abscisic acid inhibition during precocious germination are not known. However, by this time the ovule wall is dead and is highly sclerified, and consequently the germination of these embryos may be considered as normal seed germination, the early stages of which have been shown to be actinomycin D and abscisic acid insensitive.

The responses of stage I embryos to the various experimental treatments are shown in figure 2 (upper right hand graph). If stage I embryos are washed prior to culturing, precocious germination and the appearance of enzyme activity are stimulated (compare line a with b). However, the presence of abscisic acid, the ovule extract or actinomycin D inhibits precocious germination and the appearance of enzyme activity (compare lines c and d with b).

The response of stage II embryos to the various experimental treatments described above during precocious germination suggests a scheme of translation inhibition diagramatically presented in figure 3. At a point corresponding to approximately 85 mgs wet weight, the mRNA for the protease enzyme has been transcribed. The presence of abscisic acid diffusing into the embryos from the ovule wall precludes translation of this mRNA. If embryos in stage II are washed to remove the abscisic acid, translation of the mRNA and germination can occur more rapidly. The slow precocious germination of untreated embryos may reflect the gradual loss of endogeneous and adhering abscisic acid. The mode of action of abscisic acid may be envisioned as involving either a direct stimulation of RNA synthesis which ultimately results in translation inhibition or it may act as a corepressor functioning in concert with a subsequent product of RNA synthesis to effect

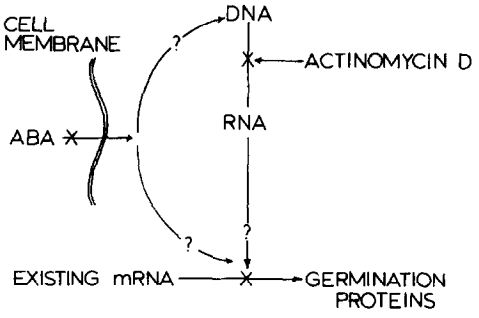

Figure 3. Possible mechanisms by which abscisic acid (ABA) could inhibit translation.

translation inhibition. In either case the inhibition of RNA synthesis by actinomycin D would allow translation of the protease mRNA (and presumably other mRNAs) and visible germination to commence.

This research was supported in part by an NSF grant and a USAEC contract.

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