Does the maternal genome influence seedling protein biochemistry during plant embryogenesis?

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Abstract: Much data has been published on the importance of maternal RNA transcripts on early embryogenesis in animals. However, parallel studies on plants have been lacking. We previously compared protein banding in seed and seedlings of two plant families (Brassicaceae and Fabaceae). Thus, the purpose of the study is to determine whether proteins expressed during seedling embryogenesis in these families were the product of maternal transcripts. As in animals, the cytoplasm of the megagametocyte (female gamete) in plants could be expected to provide RNA transcripts needed to synthesize proteins that could facilitate early embryogenesis; this has been suggested in Brassica napa (Wu et al., 2005) and Oryza sativa (Zhu et al.,1980). Thus, plant seedlings will be treated with the transcription inhibitor actinomycin-D to determine whether there might exist long-lived templates critical to early embryogenesis in plants; a protein synthesis inhibitor, cycloheximide will also be used to complement our studies. It is our hope that such studies will encourage undergraduate and college professors to consider using plants models as biochemical tools to investigate developing systems. This exercise provides some insights into the lingering influence of maternal genome on early embryogenesis.

INTRODUCTION

Biochemical studies of plant seedlings may provide insights into embryogenesis. The focus of the present paper deals with a phenomenon which was first discovered in sea urchin embryos (Gross et al. 1964). It was proposed that maternal m-RNA templates might be directing development during early embryogenesis. These investigators demonstrated that a transcriptional inhibitor (actinomycin D) did not interfere with protein synthesis during the early phase of development, since the embryo used maternal transcripts which were preformed before fertilization. However, after 10 hours or so (blastula stage) , the embryo began to transcribe embryonic templates de novo to translate a new genre of protein synthesis. This switch over, known as the maternal to zygotic transition (MZT) has also been demonstrated in other animal models (Baroux et al, 2008).

However less is known about its MZT in plant systems. Early studies on rice (Zhiping et al, 1980) and more recent studies in rapeseed plant (Wu et al, 2005) have suggested that there is maternal mediation during early development.

Thus the purpose of this study is to encourage students to explore whether MZT occurs in the plant kingdom. In order to facilitate the study, we have used seeds of members of the Brassicaceae (cabbage and broccoli) as well as one member of the Fabaceae (snow pea). The seed is an example of a quiescent embryo which has the potential of germination under hydrated conditions. The study will employ the use of electrophoresis to determine whether protein bands in germinated seeds are expressed in the presence of transcriptional and translational inhibitors (cycloheximide). It should also be possible to measure protein expression by measuring the expression of enzymes such as peroxidase activity (Zhiping et al 1980).

METHODOLOGY

1.Seed Growth and development: To disinfect, seeds were exposed to 10% Bleach for 10 minutes, after which they were rinsed in distilled water three times. To initiate germination, seeds were then submersed in distilled water in a 50 ml beaker overnight. Seeds were divided into three groups: Group "C" (Control) was then placed in sterile distilled water (one hour); Group "A" (Experimental- Act D) in a solution of 10 mg % actinomycin D (Enzo) solution (one hour) and Group "B" (Experimental – Hex) was placed in a solution of 10 mg % cycloheximide (Enzo) solution (one hour). (Warnings: consult MSDS sheets for these chemicals). Afterwards, these seeds were placed on sterile filter paper in separate sterile plastic Petri dishes and covered with more sterilized filter paper. Paper was soaked in sterile distilled water, covered with lid, and placed in a drawer at 25-30 °C. Seeds were checked for moisture at least once every day.

2.Sample preparation: The seedlings are harvested each week for a three week period. Testae and cotyledons were removed with scissors and forceps; the remaining whole seedlings are weighed at 0.16-0.17g and placed in microfuge tubes. Tubes are frozen at -80 C, minimally overnight, to disrupt cell membranes. For pea seedlings, the seedling were selectively cut into root, stem and leaf that would be weight equivalent.

3.Preparation of the seedling homogenate: After thawing, distilled water is added in proportion to the weight of pooled sample (e.g. 160ul for a 0.16g sample). Bio-Rad mini-pulverizing tool is used to make a paste of samples on ice. One might increase the total volume of water to create a visible slurry. This is then briefly vortexed and centrifuged at 10,000 g (4˚C, 2 X 10 min). Supernatant aliquots are delivered into separate microfuge tubes by decanting and/or using a loading-pipet tip. This point can be considered a "stop point" and the samples may be frozen for later analysis.

4.Protein Calibration Assay: Total protein of each sample is determined using a standard curve derived by using different concentrations of bovine serum albumin, Bio-Rad protein assay procedure (http://www.bio-rad.com/webroot/web/pdf/lsr/literature/LIT33.pdf).

5.Electrophoresis: Samples are vortexed and centrifuged for 5 minutes in a Serofuge centrifuge (3400 rpm). After calculating the volume of the supernatant needed to deliver about 10 ug to each well(see 4.), an equal volume of modified 2X Laemmli buffer (5ul mercaptoethanol per 95ul of Laemmli) is added. Each of the wells of a precast 4-15% Tris-Glycine polyacrylamide-TGX gel is then loaded. At least one lane should contain a marker, with 10 pre-stained known proteins *(Bio-rad* Precision Plus Protein™ Dual Color Standards). Two PAGE protocols explain details of the process.(Racusen and Thompson, 1996; Frame, 2000). Electrophoresis should be run at 150V for 40 minutes.

6.Data analysis: After the electrophoretic run, gels are rinsed for 10 minutes (3X) in distilled water on a rocking platform, stained with Bio-Rad Coomassie Blue *Safe stain* and then destained in distilled water over 2 days. Gels can be scanned (Bio-Rad ChemiDoc™ XRS scanner) and the image will reveal the position and intensity of the protein bands in each of the lanes. The image can also be labelled. Other scanners will perform similarly but remember that all images (sometimes stored using a different extension) must be exported to the .jpg format before presentation. Scans of band intensity are often quantified using software from the scanner and this data can be exported to Microsoft Excel. Once exported, this data can be plotted with Excel's software.

Figure 1: Germinating sugar snap pea seedlings (Fabaceae family) after 2 weeks. "Exp" seed was previously soaked in 10 mg % actinomycin D for one hour. Note bleaching of the treated seedlings

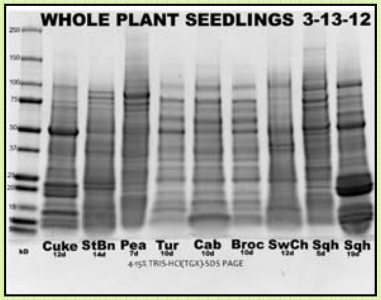

Figure 2: Protein banding profiles of germinating seedlings at different time periods. Note similar banding patterns among members of the Brassicaceae (lanes 5-turnip, 6-cabbage, 7-broccoli). (Blando-Hoegler and Hoegler, 2013)

Figure 3: Germinating broccoli seedlings (Brassicaceae family) after 2 weeks. "Exp" seed was previously soaked in 10 mg actinomycin D for one hour. Note that the treated seedling is stunted.

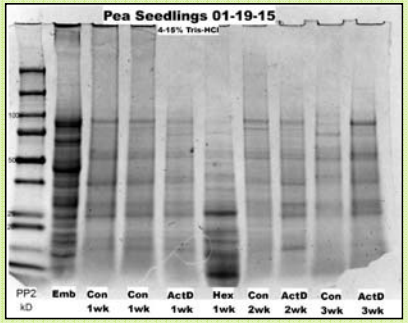

Figure 4: Protein banding profiles of germinating pea (*Fabaceae*) seedlings. The twenty-four hour pea embryo pattern (lane 2) contrasts with 1 week controls (lanes 3, 4). After one-(lane 5) and two-week (lane 8), the actinomycin-D (ActD) -treated seedlings exhibit similar banding to controls (lanes 4 and 7 resp.). The ActD-treated three week seedlings (lane 10) exhibit different banding than controls (lane 9). Cycloheximide-treated (lane 6) specimens exhibit different banding than controls (lane 4) after one week.

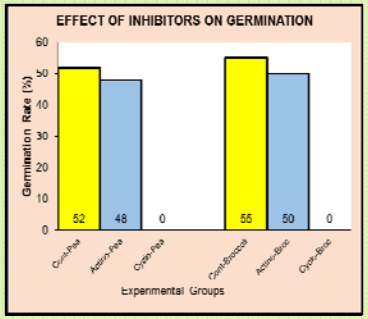

Figure 5: Graph showing the germination rates of pea and broccoli seeds two weeks after being exposed to a transcription inhibitor (actinomycin D) and translational inhibitor (cycloheximide) for one hour.

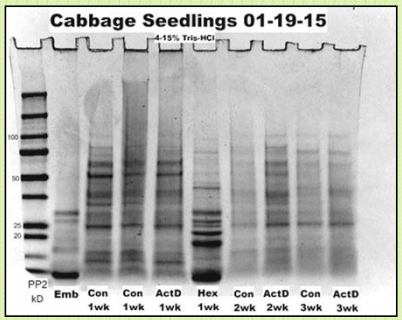

Figure 6: Protein banding profiles from cabbage (Brassicaceae) seedlings. The twenty-four hour embryonic pattern (lane 2) contrasts with 1 week controls (lanes 3, 4).After one-week (lane 5), two-week (lane 8) and three-weeks (lane 10), the actinomycin-D (ActD)-treated exhibit similar banding to controls (lanes 4 , 7 and 9 resp.). Cycloheximide-treated (lane 6) specimens exhibit different banding than controls (lane 4) after one week.

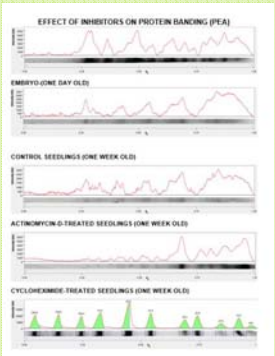

Figure 7: A graphic representation of protein banding in one-week old pea seedlings previously exposed to inhibitors. Note the similarity in pattern between controls and ActD-exposed; there is less similarity between controls and cycloheximide-exposed. The banding of the embryos is also uniquely different from seedlings.

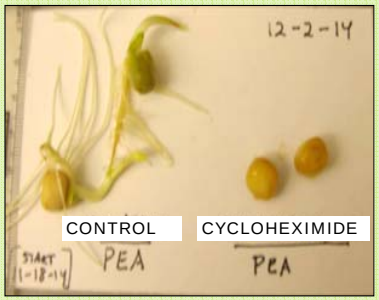

Figure 8: Germinating sugar snap pea seedlings (Fabaceae family) after 2 weeks. "Exp" seed was previously soaked in 10 mg % cycloheximide for one hour. Note the lack of germination in the cycloheximide-exposed seeds.

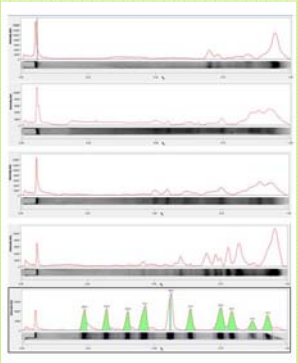

Figure 9: A graphic representation of protein banding in one-week old cabbage seedlings. Note the similarity in pattern between controls and ActD-exposed and the lack thereof between controls and cycloheximide specimens. Top to bottom panels: Embryo (1 d); Control (1 wk); Actinomycin (1 wk); Cycloheximide (1 wk) Molecular weight marker on bottom.

RESULTS

(1)Though actinomycin D (transcriptional inhibitor) does not prevent germination of pea, cabbage and broccoli seeds, it is evident that after two weeks, there is retardation of their physical development (Fig 1, 3). It appears that actinomycin-D treated pea seedlings are somewhat stunted and prone to infection.

(2)Protein banding pattern in actinomycin-treated seedlings is similar to the banding in control seedlings during the first two weeks of development in both peas(Fig 4,7) broccoli and cabbage.(Fig. 6, 9)

(3)Cycloheximide (translational inhibitor) prevents germination of all seeds (Fig 8) The seeds do not appear to sprout and show signs of infection.

(4)After one week of development, protein banding at high molecular weights (>25kD) in cycloheximide-treated seedlings is reduced in intensity and number in both peas (Fig 7) and cabbage (Fig 9), compared to controls. Yet, there are also increases in banding intensity in the range of MW less than 25kD. (Fig. 4, lane 6 and Fig. 6, lane 6).

CONCLUSION

A. Plant embryos exhibit protein bands of a wide range of molecular weights.

B. Depending on the plant, early seedling development may involve increases in the varieties of proteins (cabbage) or there may be a decrease in the protein variety (pea).

In peas, cabbage and broccoli seedlings, the range of expressed protein during the first three weeks of development remain stable for any one plant member. This may at first suggest that the RNA transcripts or RNAi species also show stability.

C. If one poisons the seedling with a transcriptional inhibitor (actinomycin D), it seems that the protein fingerprint during the first three weeks is identical to the controls, suggesting that the RNA transcripts were preformed maternally. In the third week, there is a loss of protein banding, suggesting that actinomycin D may inhibiting both embryonic transcription as well as the production of new proteins *de novo*. At the same time, extant preformed proteins or those made from maternal transcripts stored in the cytoplasm may be degraded. If this is the case, we suggest the existence of MZT occurring at third week of germination for certain plants, such as pea, but not for others in the *Brassicaceae*.

D. If one poisons the seedling with a translational inhibitor, very few of the seeds will germinate. In one week old poisoned embryos, the expressed proteins (25-75kD) resemble the day-old embryos as well as week-old seedlings, though less intense. In addition there is an increased supply of low molecular proteins (less than 25kD).

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