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Seed Storage Protein Transcription and mRNA Levels in *Brassica napus* during Development and in Response to Exogenous Abscisic Acid[1]

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ABSTRACT

Transcription rates and mRNA levels for *Brassica napus* seed storage protein families, cruciferin and napin, have been determined in embryos developing in the seed, as well as in embryos cultured with and without abscisic acid. Cruciferin and napin mRNAs are high during the cell expansion phase of embryo development, representing as much as 11 and 8%, respectively, of the total embryo mRNA. During the same time cruciferin and napin gene transcription rates, as measured in isolated nuclei, are also high. The data indicate that cruciferin mRNA is more stable than napin mRNA because while the napin transcription rate is higher than the cruciferin transcription rate, the cruciferin mRNA accumulates to higher levels. However, late in embryo development, both cruciferin and napin mRNAs seem to be less stable than earlier because comparable transcription rates result in lower mRNA levels. When embryos are cultured in the presence of abscisic acid, the levels of cruciferin and napin mRNAs are two- to threefold higher than in embryos cultured on basal medium. The transcription rates show a similar increase in the presence of abscisic acid, suggesting that abscisic acid is responsible for the increased mRNA level at least in part through an increase in the transcription rate of the two genes.

Few proteins from plants have been studied as extensively as the seed storage proteins. By definition, seed storage proteins accumulate during the development of the seed, are stable during the period of developmental arrest that separates embryogeny from germination, and then are specifically degraded during germination and seedling growth to provide a source of carbon and nitrogen. Thus, storage proteins are abundant and stage-specific, making them good subjects for studies of developmental control of gene expression in plants.

We have been studying the regulation of expression of the two major seed storage protein families in *Brassica napus L.* (rapeseed), cruciferin, and napin. Cruciferin is a legumin-like protein, soluble in salt solutions, with a mol wt of 300,000. The holoprotein is composed of six subunits, and each subunit consists of a 30 kD A polypeptide disulfide-bonded to a 20 kD B polypeptide. Each subunit is processed from a single 50 kD precursor polypeptide. The subunits that constitute cruciferin are actually a family of polypeptides, and whether the holoprotein consists of one family member or several is not known. Napin is a family of small, water-soluble proteins, each composed of a disulfide-bonded 13 and 4 kD polypeptide, processed from a larger precursor. Cruciferin and napin are synthesized during the last half of embryo development in cells of the cotyledons and the embryo axis and are presumably sequestered in membrane-bound protein bodies. At seed maturity, cruciferin makes up 60% and napin 20 to 30% of total protein (6).

The pattern of expression of the storage proteins in *B. napus* has been followed by measuring protein accumulation (6) and mRNA levels (8, 15) in embryos of different developmental stages. The major period of accumulation for both proteins begins during the cell expansion phase, which occurs about half way through seed development (25 dpa[3]) after tissues and organs have formed and cell division has ceased, and during which storage reserves increase. Napin accumulation stops about 20 d later (45 dpa), when the seeds are drying down (desiccation phase), while cruciferin accumulation continues until just before developmental arrest. Using cDNA clones, the mRNAs for napin and cruciferin can be detected quite early; napin mRNA is detected at 18 dpa just past the 'heart stage' of development and cruciferin mRNA can be detected 5 d later. Napin mRNA levels increase to their maximal level by 33 dpa while cruciferin mRNA levels do not peak until 38 dpa (8, 15). Both mRNAs decline during late embryogeny and are barely detectable in dry seeds. Thus, although both napin and cruciferin mRNAs are detectable early in embryogeny, are modulated up during embryo maturation, and decline to low levels in the dry seed, napin mRNA is detectable several days before cruciferin mRNA. We are particularly interested in understanding how these two gene families are regulated, considering the fact that they show the same developmental stage specificity but different temporal regulation.

The endogenous factors that are responsible for regulating the pattern of cruciferin and napin gene expression are unknown. However, the growth regulator ABA has been shown to modulate the levels of storage proteins and mRNAs in cultured embryos (8, 15). For example, embryos taken from the seed at the beginning of seed storage protein accumulation (25–27 dpa) will accumulate both seed storage protein and

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[3] Abbreviations: dpa, days postanthesis.

seed storage protein mRNAs at rates comparable to development in the seed if 1 to 10 μM ABA is included in the culture medium. In the absence of ABA, seed storage protein and seed storage protein mRNAs decrease to low levels.

Regulation of storage protein expression during development and in culture is likely to be complex. For example, early embryos (27 dpa) cultured on ABA accumulate cruciferin mRNA and protein to levels comparable to that in the seed. However, in older embryos (40 dpa) cultured on ABA, the cruciferin mRNA level is much lower than that in embryos in the seed, but the protein accumulates to a similar level in both. At either stage, embryos cultured on basal medium show low levels of both cruciferin mRNA and protein (15). Therefore, ABA may modulate both the levels and the translation of cruciferin mRNA in older embryos.

To begin to dissect the levels of regulation and to compare the regulation of cruciferin and napin gene expression, we have looked at the contribution of transcription rates to the pattern of seed storage protein mRNA levels both during development in the seed, and in response to ABA in culture, by measuring *in vitro* transcription rates in isolated nuclei. Although mRNA levels for cruciferin and napin had been determined previously (8, 15), they were repeated here for direct comparison to transcription rates.

MATERIALS AND METHODS

Plant Growth

Plants (*Brassica napus*) were grown as described previously (15). On the day of anthesis, flowers were pollinated with bee sticks (29) and tagged with the date. The date of pollination was considered to be d 0 of embryo development. Embryos were staged based on dpa.

Embryo Culture

Embryos were dissected aseptically using tungsten knives (9) and placed immediately either in liquid nitrogen or in a 60 × 30 mm Petri dish containing a modification of Monnier's embryo culture medium (22) (described below), at a density of 15 to 20 embryos per plate. Each sample consisted of 40 embryos, 30 of which were used for isolation of nuclei, and 10 of which were used for RNA isolation. Tissue for nuclear isolation or RNA isolation was frozen in liquid nitrogen, weighed, and stored at −70°C.

Monnier's culture medium contains inorganic salts, reduced nitrogen, and 0.35 M sucrose (12%) and is hormone free. For the basal medium, the sucrose concentration was lowered to 0.06 M (2%). For the medium containing ABA, 10 mM ABA (mixed isomers, grade IV, Sigma Chemical Co.) in DMSO (50% v/v) was added to give the appropriate concentration. In all cases, the media ingredients were mixed, pH was adjusted to 5.5 with 1.0 N KOH, and powdered agar (Difco-Bacto; Difco Laboratories) was added to 0.7%. The medium was autoclaved and dispensed 10 mL/dish. The dishes were sealed with Parafilm (American Can Co.) and cultured at 28°C in continuous light from cool-white fluorescent bulbs (General Electric).

RNA Isolation

RNA was extracted by a modification of the procedure of Galau *et al.* (16). Tissue was ground in 2 mL of homogenization buffer (0.1 M Tris-HCl [pH 8.5], 0.1 M NaCl, 1 mM EDTA, 0.5% SDS) in a sintered glass homogenizer, on ice. Pronase (Boehringer Mannheim Biochemicals) that had been self-digested at 37°C for 60 min was added to a final concentration of 0.5 mg/mL and incubated at 37°C for 60 min. The pH was lowered by adding one-tenth volume of 2 M sodium acetate (pH 5), and the homogenate was extracted with an equal volume of water saturated phenol:chloroform:isoamyl alcohol (50:24:1) at 55°C (20). The homogenate was reextracted several times with an equal volume of water saturated phenol:chloroform:isoamyl alcohol (25:24:1) at room temperature, followed by an extraction with chloroform:isoamyl alcohol (24:1). This was followed by an ethanol precipitation, a LiCl precipitation, and a final ethanol precipitation. The RNA was stored in 10 mM Tris, 1 mM EDTA (pH 7.6) at −20°C.

RNA Quantitation

RNA was quantitated by dot blots as described (15), except that the standard curve was made in the following way. Napin or cruciferin RNA (mRNA strand) was transcribed *in vitro* from the corresponding cDNAs subcloned in Bluescribe (Stratagene), a vector which contains the phage T3 and T7 promoters. RNAs were transcribed and purified according to the manufacturer's protocol and were quantitated by optical density. Serial dilutions were made using yeast RNA as a carrier so that there was a total of 2 μg of total RNA per dot. Standard curves were linear from less than 25 pg to 2 ng cruciferin or napin mRNA, and from less than 10 pg to 1 ng actin mRNA, per μg total RNA.

Isolation of Nuclei, Transcription, and RNA Isolation

Nuclei were isolated and transcribed *in vitro* by modifications of published procedures (20, 28; L Walling, R Goldberg, personal communication). All steps were carried out at 0 to 4°C unless stated otherwise. Briefly, tissue which had been frozen in liquid nitrogen was ground with a mortar and pestle in liquid nitrogen. The frozen powder was transferred to a sintered glass homogenizer with 3 to 5 mL Honda buffer (25 mM Tris-HCl [pH 8.5], 0.44 M sucrose, 2.5% Ficoll, 5% dextran-T40, 5 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 0.5% Triton X-100, 2 mM spermine, 0.05% diethylpyrocarbonate) (18), and homogenized. The homogenate was filtered through three layers of 74 μm mesh polyester sieve cloth, and the filtrate was centrifuged at 4000*g* in a Sorval HB-4 rotor to pellet the nuclei. At this point, one of two procedures was followed. In the first, the pellet was resuspended in 4.5 mL Honda buffer minus spermine and diethylpyrocarbonate, loaded on a Percoll step gradient which was 4.5 mL each 40, 60, and 80% Percoll over 2 M sucrose in gradient buffer (25 mM Tris-HCl [pH 8.5], 10 mM $MgCl_2$). Alternatively, the pellet was resuspended in 1.5 mL Honda buffer minus spermine and diethylpyrocarbonate, diluted with 3 mL 2 M sucrose in gradient buffer, and layered over 4.5 mL 2 M sucrose in

gradient buffer. The gradients were centrifuged in a Sorval HB-4 rotor at 4000*g* for 30 min. With all tissues used, the nuclei pelleted through either type of gradient. The nuclei were washed in a small volume of nuclei resuspension buffer (50 mM Tris-HCl [pH 8.5], 5 mM $MgCl_2$, 10 mM 2-mercaptoethanol) containing 20% glycerol, resuspended in the same buffer containing 50% glycerol, frozen in liquid nitrogen, and stored at −70°C.

For the transcription reaction, nuclei (approximately 10^8 per reaction) were diluted 1:5 to a final concentration of 100 mM $(NH_4)_2SO_4$, 4 mM $MgCl_2$, 0.3 μM phosphocreatin, 500 μM ATP, 500 μM UTP, 500 μM CTP, 30 μM GTP, 0.025 μg/mL creatin phosphokinase, 80 to 100 μCi [^{32}P]GTP (>600 Ci/mmol, New England Nuclear), and 100 to 500 units RNasin (Promega Biotec), in a total volume of 75 μL. The reaction was routinely incubated for 10 min at 30°C. The reaction was stopped and RNA was isolated according to Marzluff and Huang (21).

Control experiments showed that the transcription reaction was linear only for the first few minutes (Fig. 1A). However, using 500 units of RNasin in a pulse-chase experiment showed that RNA which was labeled for 5 min is stable during a 25 min chase (Fig. 1B). Using 100 units of RNasin showed considerable degradation of RNA during the chase (data not shown). However, the relative amounts of cruciferin, napin, actin, and rRNA were the same before and after the chase, suggesting that there is no specific degradation of any of the RNAs in the nuclei. Alpha-amanitin (10 μg/mL) inhibits the incorporation of [^{32}P]GTP into total RNA by 40 to 50% (Fig. 1A) and into cruciferin, napin, and actin RNA by 80 to 90% (data not shown), indicating that RNA polymerase II is responsible for this fraction of the transcription. Incorporation of label is dependent on the presence of all four nucleotides, since if any one of them is left out of the reaction, there is no detectable incorporation of label (Fig. 1A). Using single strand probes, it was determined that there is detectable transcription from only the expected strand of DNA (data not shown).

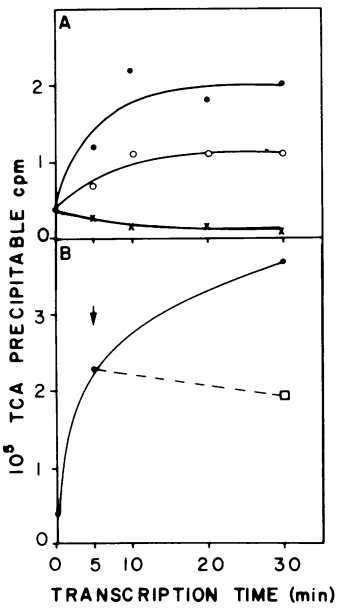

Figure 1. Transcription controls. A, Effect of α-amanitin on transcription rate and requirement for exogenous nucleotides for transcription. In parallel transcription reactions, nuclei were incubated for the indicated times in the normal transcription mix containing [^{32}P]GTP (●), or in the same mix plus 10 μg/mL α-amanitin (○), or minus CTP (×). At the indicated times, an aliquot of the reaction was precipitated with ice-cold 10% TCA, 1% pyrophosphate, and the amount of ^{32}P incorporated was determined. B, Pulse-chase labeling of transcription reaction. In duplicate reactions, nuclei were incubated in transcription mix containing [^{32}P]GTP (○). After 5 min (arrow), nuclei from one reaction were pelleted and resuspended in the same reaction mix minus [^{32}P]GTP, containing 500 μM unlabeled GTP, and incubated for 25 min (□). At the indicated times, aliquots of the reactions were precipitated as described in A. Data shown are from a representative experiment.

DNA Excess Filter Hybridization

Relative amounts of incorporation of label into specific RNAs was determined by DNA excess filter hybridization (17). The cDNA probes pC1 (26) and pN2 (7) were used to detect cruciferin and napin RNAs, respectively. For controls, a soybean actin genomic clone, pSAC3, supplied by Richard Meagher (25), and a radish rRNA clone, pRE12, supplied by Michel Delseny (11), were also used. A control filter containing either pUC8 or no DNA was included in hybridizations to determine the background. There was no detectable difference between background hybridization to either.

RESULTS

Cruciferin and Napin Transcription Rate and mRNA Levels during Development

It has been shown previously that cruciferin and napin mRNAs are present only in the embryo, and that the steady state levels of these messages are high during the expansion phase of embryo development (8, 15). However, mRNA levels were expressed in arbitrary units because the standards used in the dot blots contained unknown concentrations of the respective mRNAs. In order to compare cruciferin and napin gene expression more accurately, absolute amounts of their corresponding mRNAs and gene transcription rates were determined.

RNA standards used in dot blot determinations of cruciferin and napin mRNA levels were known amounts of RNAs produced from cDNA clones in vectors with T3 and T7 promoter/RNA polymerase systems. At their highest levels, cruciferin and napin represent about 0.11 and 0.08%, respectively, of total embryo RNA by mass (Fig. 2). In *Brassica napus* embryos, mRNA is 1% of total RNA (John Harada, personal communication). Therefore, cruciferin and napin represent 11 and 8%, respectively, of embryo mRNA.

To compare cruciferin and napin mRNA levels and gene transcription rates, both parameters were determined in parallel experiments. Previously, it was shown that napin mRNA is first detectable at 18 dpa, and cruciferin mRNA is first detectable 5 d later (8, 15). In this study, the earliest day examined was 24 dpa because of the difficulty in obtaining enough tissue for transcription reactions from earlier stages. However, both napin mRNA level and transcription rate are

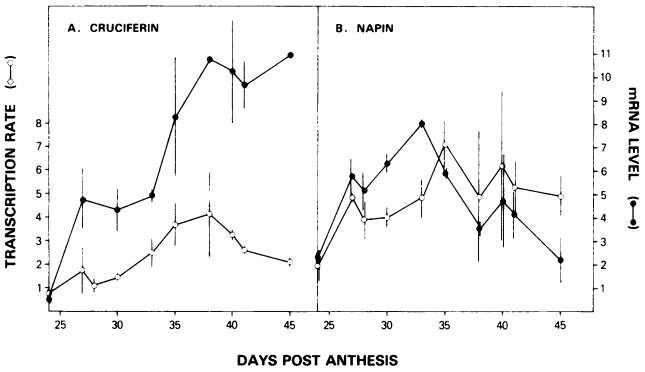

Figure 2. Seed storage protein mRNA levels and transcription rates during embryo development. In parallel experiments, mRNA levels and transcription rates were determined for cruciferin (A) and napin (B). Total RNA and nuclei were isolated from embryos at the indicated times during development. The mRNA levels relative to total RNA (●) were determined using dot blots. The relative transcription rates (○) were determined by incubating the nuclei in an *in vitro* transcription mix with [^{32}P]GTP, isolating the transcription products, and quantitating the transcription rates of cruciferin and napin genes by DNA excess filter hybridization. Units for transcription rate are 10^{-4} cpm hybridized/total, and units for mRNA level are 10^{-4} μg/μg total RNA. Error bars indicate ±1 SD.

about twofold higher at this point than cruciferin mRNA level and transcription rate (Fig. 2). During the next several days of embryo development, an increase in both cruciferin and napin mRNA levels is accompanied by an increase in the respective gene transcription rate. The cruciferin mRNA level and transcription rate both increase until about 38 dpa (Fig. 2A). After 38 dpa, the cruciferin mRNA level remains the same while the transcription rate begins to decline. The napin mRNA level and transcription rate are both high until 33 dpa, after which the transcription rate remains high and the mRNA level falls. mRNA levels are a function of both mRNA synthesis (transcription) rate, and the rate of mRNA degradation. Based on the data presented here, several conclusions can be made. Cruciferin mRNA is more stable than napin mRNA because although napin gene transcription rate is higher than cruciferin gene transcription rate, napin mRNA accumulates to lower levels than cruciferin mRNA. In addition, later in embryo development, mRNA degradation has a greater influence on both cruciferin and napin mRNA levels. For example, cruciferin transcription rates are very similar at 35 and 40 dpa, but the mRNA level is increasing at 35 dpa and declining at 40 dpa (Fig. 2A). The results of a similar analysis are even more striking for napin, comparing for example, 33 and 38 dpa (Fig. 2B) when mRNA levels are falling, but transcription rates remain the same.

In Figure 3, A and B, the open bars show mRNA levels and transcription rates respectively, for actin at four stages of embryo development. The patterns are very different from those of the seed storage proteins. Except at the earliest stage, actin mRNA levels and transcription rate are both at least 10-fold lower than what is observed for the seed storage proteins. Also, unlike the seed storage proteins, the actin mRNA levels are relatively constant, with less than 2-fold variation between the four stages. However, the actin transcription rate varies as much as 3-fold, suggesting that posttranscriptional control is also important in determining actin mRNA levels.

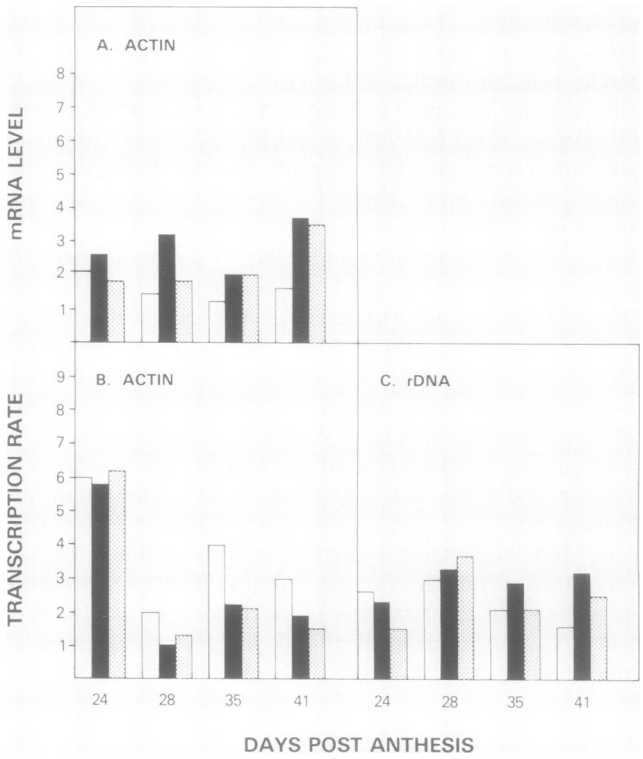

Figure 3. Actin mRNA levels and actin and rDNA transcription rates for different stage embryos cultured with and without ABA. Embryos were excised from the seed at the indicated days postanthesis and placed in culture for 5 d on medium containing 1 μM ABA (solid bars) or basal medium (stippled bars). Open bars represent mRNA levels or transcription rates at the time embryos were excised from the seed. Actin mRNA levels (A) were determined as in Figure 4, and relative transcription rates for actin (B) and rDNA (C) were determined as in Figure 2. Units for mRNA level are 10^{-5} μg/μg total RNA. Units for actin transcription rate are 10^{-5} cpm hybridized/total, and 10^{-1} cpm hybridized/total for rDNA transcription rate.

While actin transcription can be measured in leaves, neither seed storage protein mRNA nor gene transcription (Table I) are detectable in leaves, which is consistent with developmental stage specific expression of these genes.

Effect of Different Concentrations of ABA on Cruciferin and Napin mRNA Levels

It has been shown that by culturing cell expansion phase *B. napus* embryos on medium containing ABA they will accumulate high levels of seed storage proteins and their corresponding mRNAs, while embryos cultured on basal medium accumulate lower levels of both (8, 15). As a prelude to further studies of the effects of ABA on cruciferin and napin gene expression, we determined the effect of different concentrations of ABA on the steady state levels of their mRNAs. The results are shown in Figure 4. Embryos were dissected from the seed at 27 dpa, and placed in culture for 3 d on medium containing no ABA, or from 0.1 to 50 μM ABA. Total RNA was isolated, and the amounts of cruciferin and napin mRNA

Table I. *DNA Excess Filter Hybridization Data from a Representative Nuclear Transcription Experiment*

The numbers indicate counts per minute in RNA bound to filters containing plasmids pC1 (cruciferin), pN2 (napin), pSAC3 (actin), and pRE12 (rDNA), and no plasmid (background); 35 dpa are nuclei from embryos at 35 dpa, +ABA are nuclei from embryos of the same age cultured for 5 d on ABA, −ABA are nuclei from embryos cultured for 5 d on basal medium, and LEAF are nuclei from leaves. Cruciferin, napin, actin, and background filters were included in a single hybridization mix with the total labeled RNA indicated in the following column. rDNA and background filters were hybridized in a separate mix with the total labeled RNA indicated in the following column.

Nuclei	Cruciferin	Napin	Actin	Background	Total	rDNA	Background	Total
35 dpa	322	762	87	34	8.4×10^5	2298	35	11367
5 + ABA	670	1450	129	67	5.5×10^6	4831	40	9039
5 − ABA	329	546	129	40	6.3×10^6	3835	42	9144
LEAF	53	58	113	71	1.4×10^6	94	25	7706

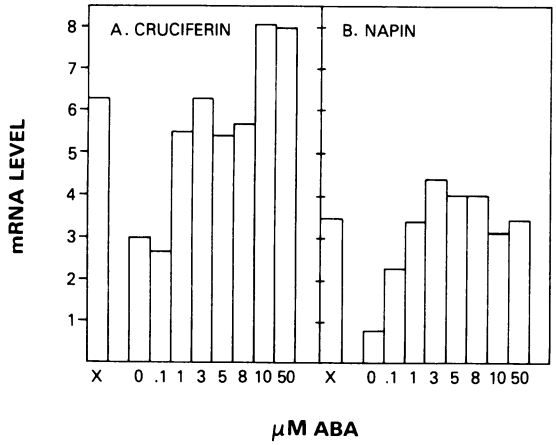

Figure 4. Effect of different concentrations of ABA on seed storage protein mRNA levels. Embryos were dissected from the seed at 27 dpa and placed in culture with the indicated concentrations of ABA for 3 d. Total RNA was isolated and the amounts of cruciferin mRNA (A) and napin mRNA (B) relative to total RNA were determined using dot blots. (×), Respective mRNA level at the time the embryos were excised from the seed. Units for mRNA level are 10^{-4} μg/μg total RNA.

relative to total RNA were determined using dot blots. Compared to embryos cultured on medium without ABA, there was a twofold and a fourfold increase in cruciferin and napin mRNA levels, respectively, when embryos were cultured on 1 μM ABA. ABA (1 μM) was chosen as the concentration to use in subsequent experiments for several reasons. Culturing embryos on increasing concentrations of ABA showed little change in napin mRNA levels, and 1 μM ABA resulted in approximately the same level of both cruciferin and napin mRNA as in embryos at the time when they were dissected from the seed. In addition, previous studies had shown that embryos younger than 37 dpa cultured on medium containing 1 μM ABA have ABA contents similar to embryos in the seed, and that higher levels of ABA showed toxic effects on older embryos (15).

mRNA Levels and Transcription of Cruciferin and Napin in Embryos Cultured on Medium With or Without ABA

To compare the effect of ABA on cruciferin and napin gene expression, parallel determinations of mRNA level and transcription rate were determined in embryos cultured on basal medium and on medium containing 1 μM ABA. Because embryos of different stages respond differently to ABA, we chose to examine four different stages of embryos: late cell division phase (24 dpa) when cruciferin mRNA is relatively low and napin mRNA level is already increasing; early expansion phase (28 dpa) when cruciferin mRNA level is increasing, and napin mRNA level is high; middle expansion phase (35 dpa) when both cruciferin and napin mRNA levels are high; and desiccation phase (41 dpa) when cruciferin mRNA level is still high, and napin mRNA level is declining. The data from these experiments are shown in Figure 5. The bar graphs illustrate the cruciferin and napin mRNA levels and transcription rates when the embryos were excised from the seed, and after 5 d in culture with or without ABA. The results represent the average of two experiments. There were not enough replicates of each stage to determine a statistically significant effect of ABA on mRNA level or transcription rate for either cruciferin or napin which correlates with the stage of development. However, by combining the data for embryos cultured with and without ABA at all stages, it was possible to do an analysis of variance of the effect of ABA on cruciferin mRNA level, napin mRNA level, cruciferin transcription rate, and napin transcription rate. The analysis of variance showed that the effect of ABA is greater than chance for all of these dependent variables at the >95% confidence level. For all parameters examined, there was a 1.3- to 3-fold increase in those embryos which were cultured on ABA compared to no ABA. The higher mRNA level was accompanied by a higher transcription rate for both cruciferin and napin. However, the only stage when ABA causes an increase in transcription rate over that seen in the seed is the earliest stage examined (24 dpa), suggesting that the culture system does not precisely mimic development in the seed.

To determine whether ABA has a general effect on transcription rather than a specific effect on transcription of the seed storage protein genes, transcription rates of rDNA and actin were determined as examples of RNA polymerase I and II genes, respectively. Actin mRNA levels were also determined, and the results shown in Figure 3. rRNA levels were not determined because, in all cases examined, they were greater than 90% of total (data not shown). The data in Figure 3 represent the average of two experiments. Unlike the effect of ABA on cruciferin and napin, an analysis of variance showed that there is no significant overall effect of ABA on actin transcription, mRNA level, and rDNA transcription. However, there is some suggestion that the culture conditions themselves may have an effect on actin and rDNA gene

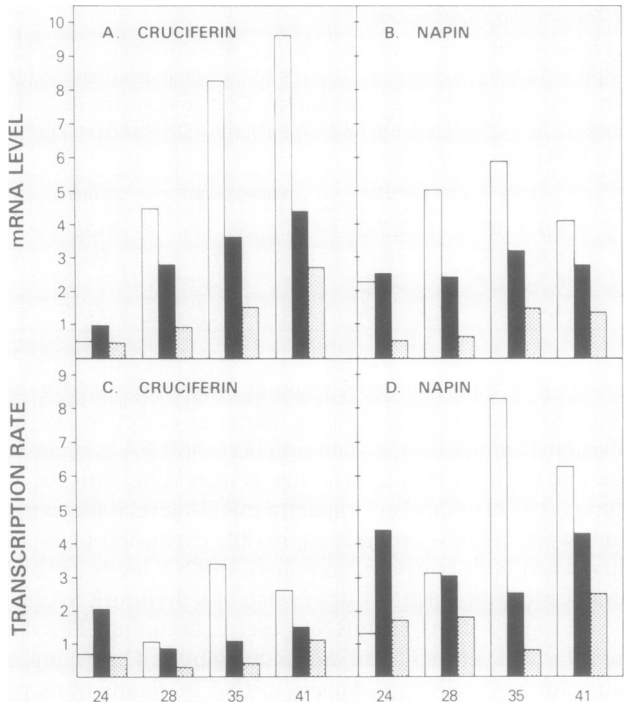

Figure 5. Seed storage protein mRNA levels and transcription rates for different stage embryos cultured with and without ABA. Embryos were excised from the seed at the indicated days postanthesis and placed in culture for 5 d on medium containing 1 μM ABA (solid bars), or basal medium (stippled bars). The open bars indicate mRNA levels and transcription rates at the time the embryos were excised from the seed. Total RNA or nuclei were isolated from identical samples. The amount of cruciferin mRNA (A) and napin mRNA (B) relative to total RNA was determined using dot blots. The transcription rates relative to total transcription for cruciferin (C) and napin (D) were determined as described in Figure 2. Units for transcription rate are 10^{-4} cpm hybridized/total, and units for mRNA level are 10^{-4} μg/μg total RNA.

expression, especially at later stages, although further experiments along this line are warranted.

DISCUSSION

Brassica napus seed storage protein mRNA levels and gene transcription rates have been examined during embryo development, and in response to exogenous ABA. During development in the seed, cruciferin and napin mRNAs constitute as much as 11 and 8%, respectively, of the mRNA in the embryo. This is comparable to what has been determined for soybean seed storage protein mRNAs. Messages for β-conglycinin and glycinin represent about 11 and 10%, respectively, of the mRNA in the embryo (28).

Cruciferin and napin both show high mRNA levels and gene transcription rates during the expansion phase of embryo development. However, it is clear that these two seed storage protein families are controlled differently at the transcriptional and posttranscriptional levels. There have been several reports of seed storage protein gene transcription rates and mRNA levels during embryo development in other plants. Transcription of legumin and vicilin from *Pisum sativum* were compared, but the relative transcription rates of the two genes depended on the length of the transcription reaction, making it difficult to draw conclusions from the data (14). Transcription rate and mRNA level of the *Phaseolus vulgaris* seed storage protein phaseolin was shown to be very similar to that of the lectin phytohemagglutinin. While phaseolin was not compared to another seed storage protein, both transcription and mRNA stability appear to be important in controlling gene expression because the transcription rate is high early in embryo development, then declines, while the mRNA continues to accumulate (5). On the other hand, when the soybean seed storage proteins glycinin and β-conglycinin were compared, differences were seen in timing and organ specificity. β-Conglycinin transcription and mRNA are both detectable several days earlier than glycinin. In addition, when embryo axis and cotyledons were compared separately, it was found that while the glycinin transcription rate in the axis is much less than that of β-conglycinin, the mRNA levels are similar. These data suggest that the glycinin mRNA is more stable than the β-conglycinin mRNA in the embryo axis (28). Therefore, different modes of control of the seed storage protein genes may be a general phenomenon.

Several groups have reported that exogenous ABA stimulates the accumulation of seed proteins (*B. napus*, 8, 15; *V. faba*, 3; rice, 27; soybean, 1, 4, 13; wheat, 23) and in those cases which have been examined, the corresponding mRNAs (*B. napus*, 8, 15; soybean, 4, 13; wheat, 24, 30). Cotton and pea are exceptions, in that accumulation of seed storage proteins does not occur in culture in the presence of exogenous ABA (10, 12). However, it is difficult to make comparisons between these systems because of variation in culture conditions, stage of embryos, and organs of the embryo which were used. This is the first report of the effects of ABA on the transcription rates of embryo specific genes. The data show that there is an increase in storage protein gene transcription rates in the presence of ABA. However, there is no evidence that ABA acts directly on the seed storage protein genes, and it is not possible to rule out posttranscriptional effects. In fact, as mentioned above, it is likely that there are other levels of control because it has been shown that the levels of cruciferin protein are as high in older embryos cultured with ABA as in embryos developing in the seed, even though the cruciferin mRNA levels are much lower (15). As controls, rDNA and actin transcription rates were shown to be unaffected by ABA. Measuring rDNA transcription in nuclei from barley aleurone protoplasts, Jacobsen and Beach (19) showed that gibberellin inhibits rDNA transcription and that ABA can reverse that effect. However, they did not report on the effects of ABA alone.

This report of a 2-fold increase in the levels of cruciferin and napin mRNAs in response to ABA is consistent with one report from our laboratory (15), but not with another (8) which showed a 10-fold increase in mRNA levels in 25 to 27 dpa embryos cultured on medium containing ABA compared to basal medium. All differences between the studies presented here and the previous report of a 10-fold increase have been ruled out except one. That difference is the way in which the plants were grown. The plants which were used for this study

and the study which showed results consistent with this report (15), were grown under controlled conditions, but the plants used in the report which showed a greater response to ABA (8) were grown in the field and in window sills where conditions of light intensity, temperature, and humidity can show considerable variation. A similar phenomenon has been observed in the response of barley aleurone to giberellic acid where the magnitude of the α-amylase response varies with seed batch, depending on the year in which the grain was harvested (2). It would be interesting to compare the effect of ABA on the seed storage protein genes of embryos from plants grown under varying conditions.

Results presented here show that cruciferin and napin gene families are controlled differently at the transcriptional and posttranscriptional levels. In the mature seed, cruciferin protein is 2 to 3 times as abundant as napin protein by mass, but in terms of numbers of molecules of each protein, they are very similar. In addition, both proteins are embryo specific. However, there are several differences which we have noted. There are approximately 5 cruciferin genes and 15 napin genes. While the cruciferin and napin families show different temporal and quantitative transcription rates and mRNA levels, it is not known if all of the family members are transcribed, or if the individual genes are differentially regulated. We are currently analyzing the individual cruciferin and napin genes to address these questions.

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