# Molecular cloning of the mRNA sequences for the storage proteins of *Brassica napus*

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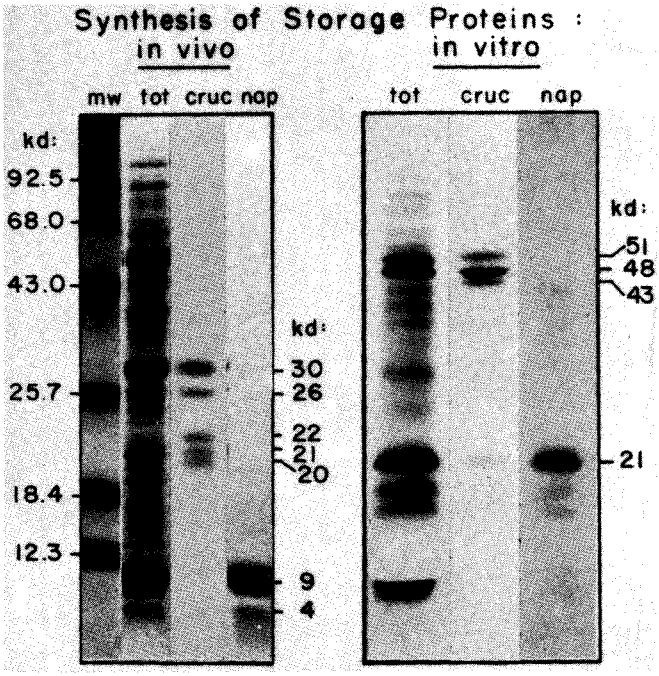
## Introduction

Two major storage proteins are synthesized in the cells of the cotyledons and axis of embryos of *Brassica napus* during seed maturation. Approximately 60% of seed protein is a 12S glycoprotein (1, 2), which we will call cruciferin to distinguish it from 12S storage proteins from other species. Another 20% of seed protein is a family of 1.7S basic proteins (3, 4), which we call napins. Both proteins are broken down during germination, and presumably not synthesized again during the life of the plant until the next cycle of seed development (2).

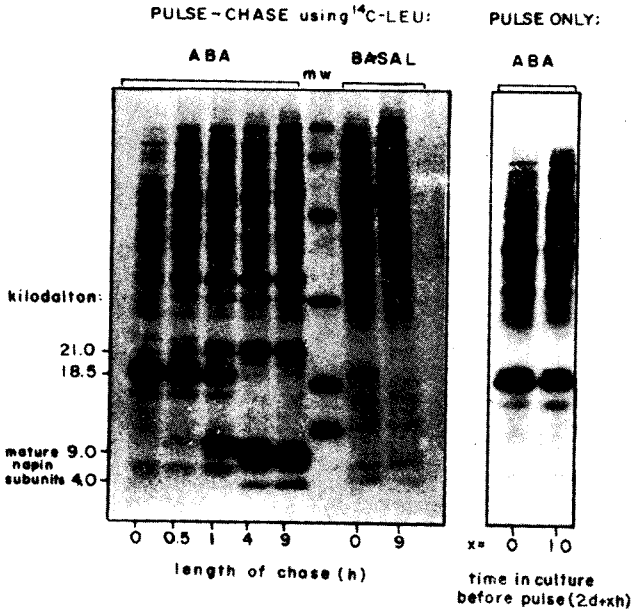
We have been studying the regulation of storage protein synthesis in *B. napus* embryos during development in the seed and in culture (2, 5). If embryos are removed from the seed when storage protein synthesis is just beginning (25d post anthesis) and cultured on a basal medium containing low sucrose and no hormones, storage protein accumulation will not exhibit the dramatic rise seen *in situ*. However, if the embryos are cultured on the same medium with $10^{-6}$ or $10^{-5}$M abscisic acid, storage proteins will accumulate at the highest rate observed in seeds. We are interested in the molecular events involved in ABA regulation of storage protein synthesis. Here we report that both napin and cruciferin are synthesized as precursor polypeptides. Using cloned cDNA probe for cruciferin and napin precursor mRNAs, we show that at least one of the effects of exogenous ABA is on the level of mRNA accumulation in cultured embryos.

Fig. 1. Cruciferin and napin proteins are synthesized as precursor polypeptides in vitro.




In order to identify cDNA probes to study RNA synthesis, we needed to determine the approximate sizes of the storage protein mRNAs and their in vitro translation products. We extracted total RNA (6) from embryos (25d post anthesis) cultured on $10^{-5}$M ABA for 3d (2), and then translated the RNA in vitro in a rabbit reticulocyte system. The polypeptide pattern of total RNA translated in vitro is shown in Fig. 1, Second panel (tot). Antibodies made against mature cruciferin or mature napin were used to immunoprecipitate (7) the storage protein precursors from the in vitro translation products (Second panel; cruc, nap). Cruciferin antibodies precipitate a group of polypeptides between 43-51 kd. Napin antibodies precipitate a 21 kd polypeptide. For comparison, the protein synthesized in vivo in embryos from which the RNA was isolated is shown in Fig. 1, first panel (tot) with the immunoprecipitated cruciferin polypeptides between 20-30 kd (cruc). Purified napin has two polypeptides between 9 and 4 kd (nap). Therefore, it appears that both storage proteins are synthesized as precursor polypeptides that are large enough to contain more than one of the mature polypeptides.

Fig. 2, 3: Storage protein precursors are also found in vivo.




We were interested in determining whether we could observe precursor forms of storage proteins in vivo, and so performed a pulse/chase experiment. We cultured embryos (26d post anthesis) for 2d on $10^{-5}$M ABA, or on basal medium, and then pulsed the embryos for 15 min in $^{14}$C-leucine (2). The embryos were then transferred to medium without label for a chase of 0, 0.5, 1, 4, or 9 hours. Proteins were extracted and electrophoresed on SDS-polyacrylamide gels, and the fluorograph is shown in Fig. 2. After a 15 min pulse, the most prominant polypeptide in a fluorograph of extracts from embryos grown on ABA is a polypeptide with an apparant mol. wt. of 18.5 kd, but after a chase of 4 h this polypeptide is not detectable. Conversely, the mature subunits of napin at 9 and 4 kd are not visible until 9 h after labelling. It appears that the 18.5 kd polypeptide is chased into polypeptides a little larger than 9 and 4 kd over a period of a few hours, and then into polypeptides that comigrate with purified mature napin subunits. To observe cruciferin precursors we immunoprecipitated the extracts in Fig. 2 with cruciferin antibodies (7). The results (Fig. 3) indicate that the in vivo cruciferin precursors are 43 and 48 kd, and are processed over a period of hours to the mature polypeptides. Note that both the napin and cruciferin in vivo precursors are a few thousand daltons smaller than the in vitro translation products, which suggests that a signal peptide is removed cotranslationally and that the proteins are synthesized on rough endoplasmic reticulum.

Fig. 3:
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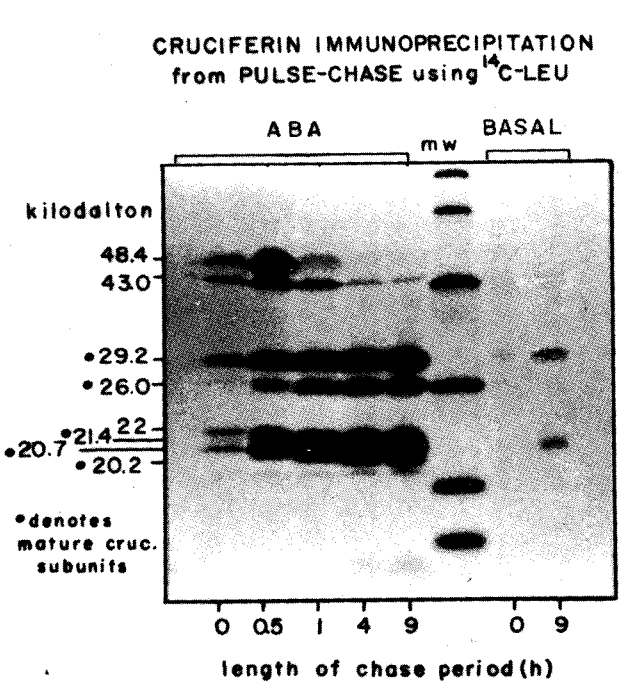



Fig. 4:
Construction and identification of cDNA clones for the napin and cruciferin mRNAs.

We constructed a cDNA library from total RNA of embryos cultured for 3d on $10^{-5}$M ABA, using the G-C tailing method, in the PstI site of plasmid pBR322. The methods will soon be published in detail (Crouch et al., submitted for publication). Three cDNA clones were chosen for further analysis (Fig. 4). Two clones represent napin mRNAs (pN1 and pN2), and pC1 is a cruciferin cDNA clone, as demonstrated by hybrid-release-translation experiments (8).

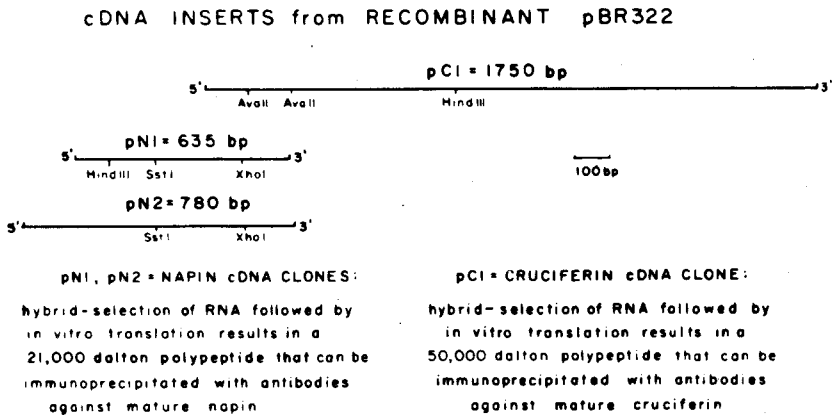
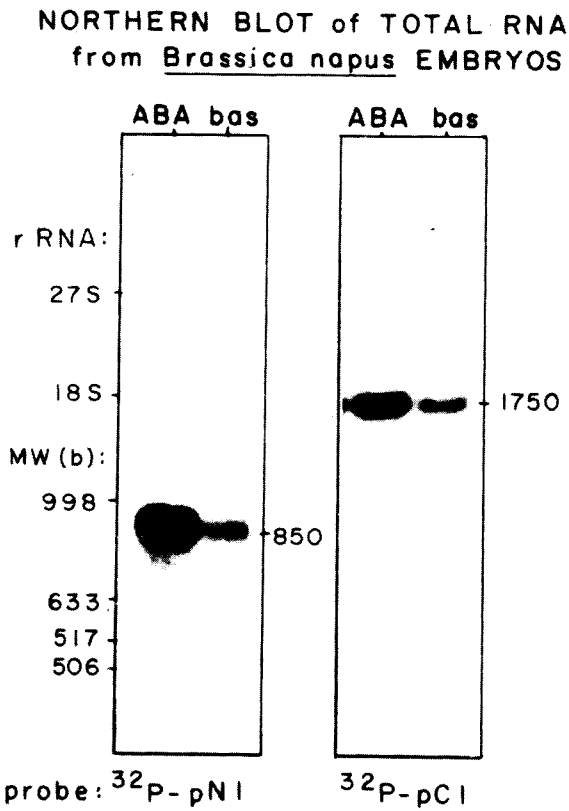


Fig. 5: Storage protein mRNAs are more abundant in embryos cultured on ABA-containing media.




The cDNA clones for napin and cruciferin mRNA sequences were labelled with $^{32}P$ by nick translation, and used to probe Northern blots of RNA extracted from embryos cultured with or without ABA (9). Each lane of the formaldehyde-agarose gel (1%) contains 5 g total RNA. The results show that napin mRNA is 850 bases (first panel), and cruciferin mRNA is 1750 bases (second panel). Both are more abundant relative to total RNA in embryos cultured with ABA, suggesting that a major effect of ABA on storage protein synthesis is at the level of specific mRNA accumulation. We are currently studying this phenomenon in more detail.

References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