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Regulation of storage product formation during embryo development in oilseeds

D J Murphy, C Batchelder, I Cummins, H Whitfield, J Keddie, Y Knight, J H E Ross

Embryo development in oilseeds is a complex process involving regulation of gene expression and metabolism on many different levels. Newly formed embryos characteristically undergo a cell division stage during which little or no storage product synthesis is apparent. During this stage, which lasts for about two weeks in oilseed rape, embryo pattern formation occurs. After about two weeks, cell division ceases and the second phase of embryo development begins. This involves cell enlargement and an accumulation of storage products, which continues over the next four weeks until embryo dehydration commences. During the dehydration phase, which lasts a further four weeks, some continued synthesis of storage products occurs. Following dehiscence, the mature seed enters a quiescent phase until imbibition and seed germination allow for the mobilization of seed storage products and the subsequent development of a new seedling. This process and the changes in the activity of the various classes of genes which accompany it are summarized in Fig. 4.

Mature embryos of oilseed rape contain approximately 50% storage oil and 25% storage protein. A further 5% of the embryo mass is accounted for by the newly discovered class of protein, the oleosins. All three types of seed storage products are accumulated specifically in the cotyledon cells of oilseed rape embryos. While they share a common spatial pattern of accumulation and mobilization, however, their temporal regulation is quite different, as shown in Fig. 5. During embryo development, storage oils are accumulated at least one week before the seed storage proteins, napin and cruciferin. Following germination, napin and cruciferin are mobilized within 2-3 days, while oil mobilization occurs more gradually from approximately 3-7 days. Oleosin accumulation only occurs during the dehydration phase of embryo development and its mobilization largely mirrors that of the storage oil, following germination.

We have speculated that the mechanism responsible for the differential temporal regulation of such storage product accumulation and mobilization is a differential expression of the genes involved in these respective processes. We have tested this hypothesis by examining the study state mRNA levels of storage proteins versus oleosins during embryo development. The results, shown in Fig. 6, suggest that mRNA levels are correlated with the rate of accumulation of these proteins, thus suggesting that this accumulation is regulated at the transcriptional level. In order to examine the differential timing of the expression of genes involved in the accumulation of different storage products, we are now examining the role of *cis*-acting elements of these genes and their possible interactions with *trans*-acting factors. The most abundant seed storage protein in oilseed rape is the 11S globulin cruciferin. Our analysis of the promoter region of the cruciferin genes that we and others have sequenced has allowed for the identification of a number of putative *cis*-acting elements. Oligonucleotide probes were constructed based on some of these *cis*-acting elements and were used in gel retardation assays to identify possible *trans*-acting factors, as shown in Fig. 7. In this figure, it can be seen that the nature of the interaction between the oligonucleotide probe and the putative *trans*-acting factor changes during the course of embryo development. Using such methods, we hope to be able to elucidate the promoter elements in the various storage product genes, which are responsible for their differential, temporal and spatial specifities of expression.

A further aim of this work is to manipulate the amount and type of storage products which accumulate in seeds. There are three major economically important categories of seed storage product, i.e. protein, starch and oil. Developing seeds import most of the carbon for the biosynthesis of their storage products as sucrose, which is translocated from source tissues, such as leaves. Sucrose can then be converted quite readily to starch or it can be metabolized to glycolytic intermediates from which storage proteins and oils are derived, as shown in Fig. 8. We have observed a transient accumulation of starch during the early stages of embryo development in oilseed rape. Later on in embryo development, it is apparent that carbon is channelled away from starch formation and towards oil synthesis. Later still, the sucrose-derived carbon is channelled more towards protein accumulation. We have identified stable genetic lines of oilseed rape where the ratio of carbon allocation towards storage oil versus storage protein is considerably different from that given above. Interestingly, this difference in storage product accumulation may be due to a combination of pre- and post-transcriptional events. A third approach is to use antisense methods to down-regulate the accumulation of selected seed storage products. At present, the only available genes are those encoding the seed storage proteins and the oleosins. Antisense constructs to the genes encoding these proteins have been, or are being made for insertion into transgenic *Arabidopsis* and oilseed rape plants, as shown in Fig. 9. The aim is to observe the effect of reducing a specific class of seed protein on the allocation of carbon to other seed storage products. A future aim is to down-regulate oil accumulation in high-oil seeds with the same view in mind. Using a combination of molecular, genetic and biochemical approaches, therefore, we hope to be able to study the mechanisms that underlie carbon allocation to the different classes of storage products in seeds.

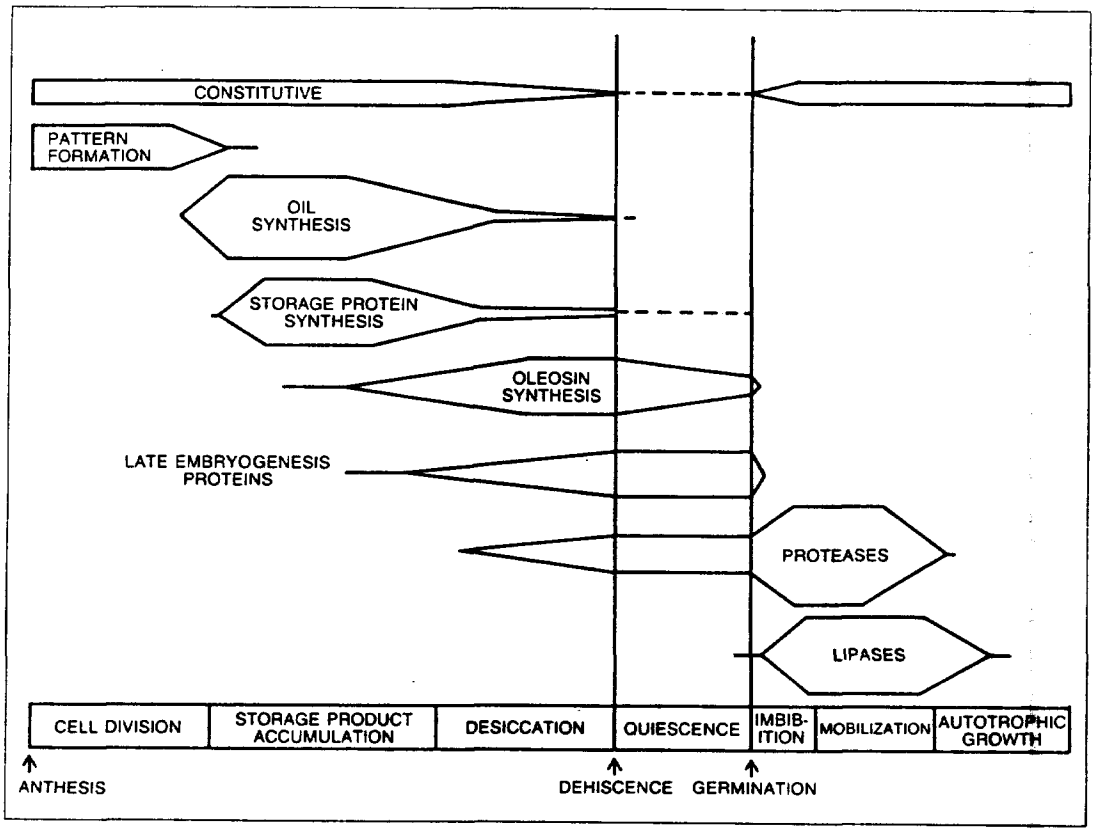


Fig. 4. Patterns of gene regulation during embryo development in oilseed rape.

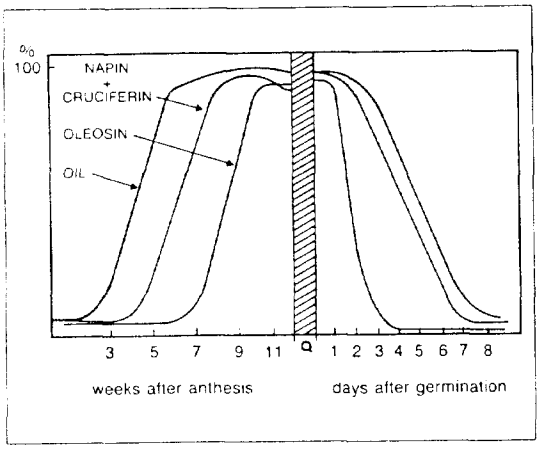


Fig. 5 Timing of the accumulation and mobilization of the major seed storage products in cotyledons of oilseed rape.

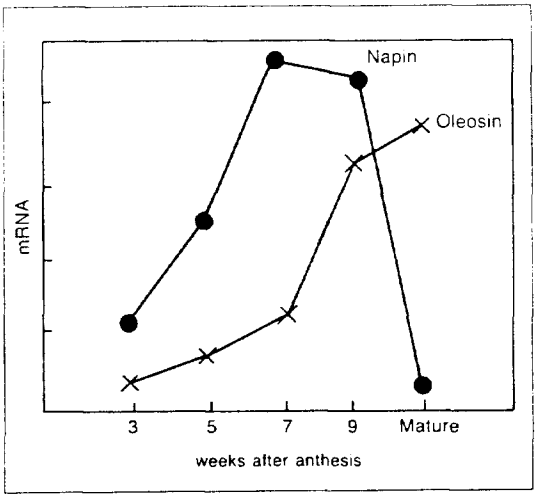


Fig. 6 Pattern of mRNA abundance levels of the embryo-specific proteins napin and oleosin, during embryo development in oilseed rape.

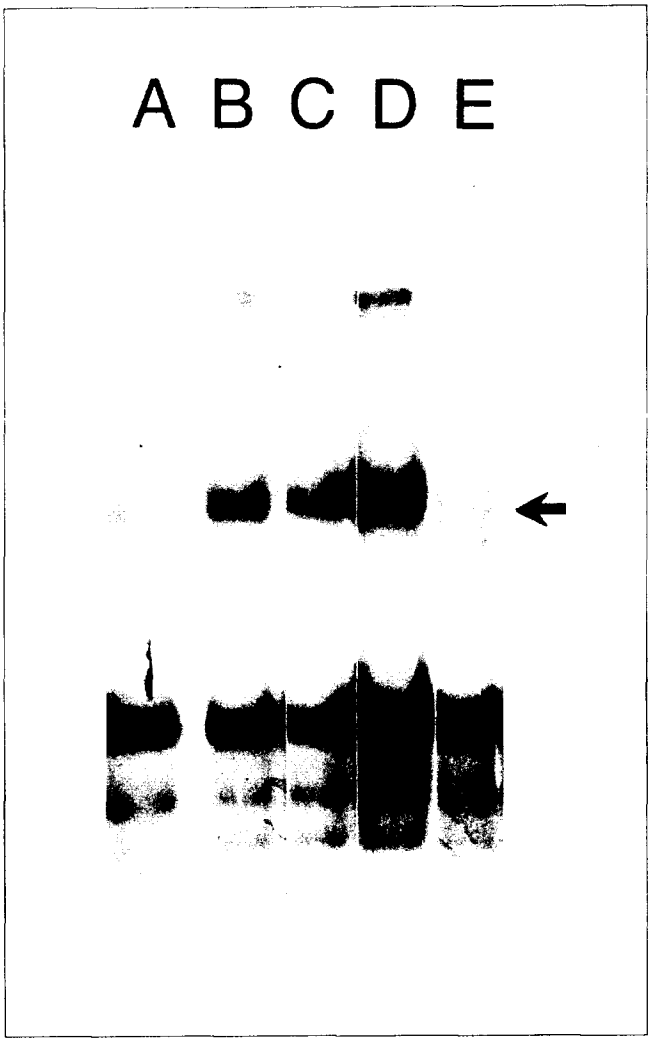
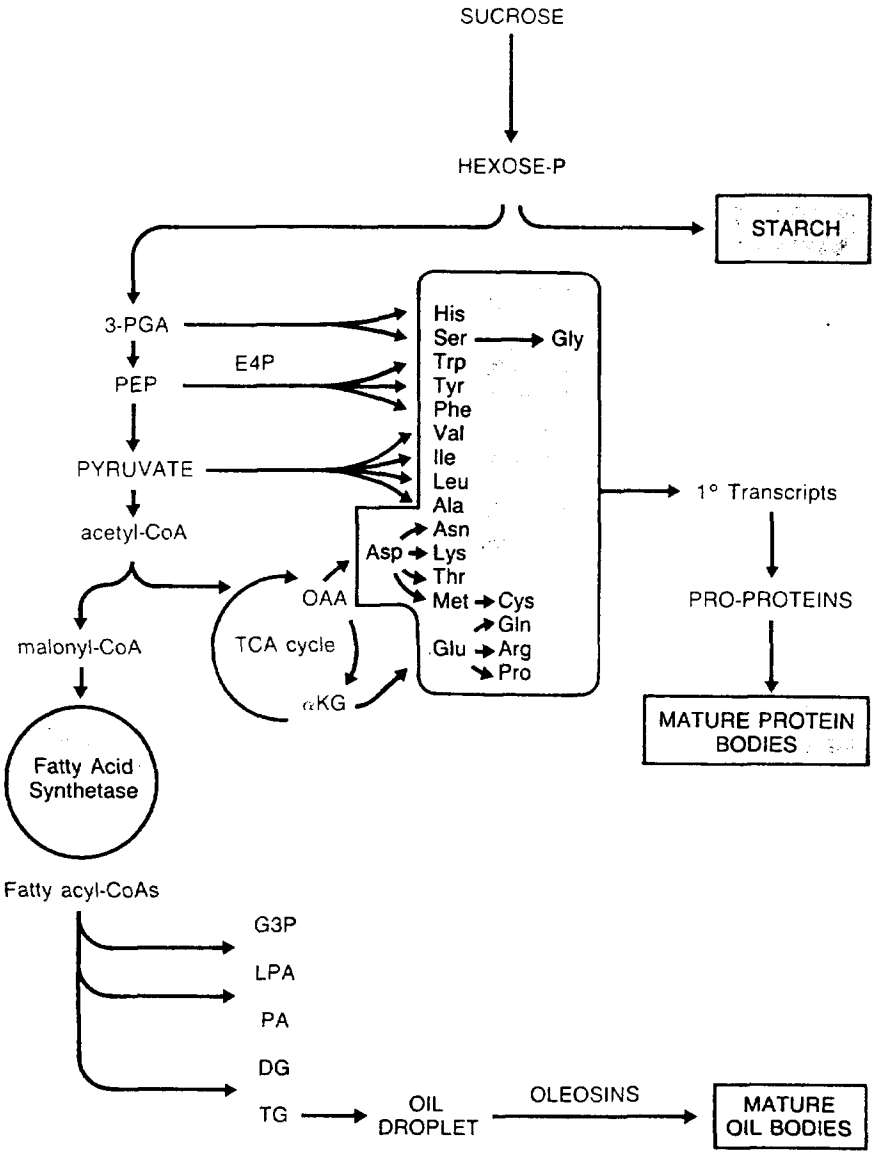


Fig. 7 Gel retardation analysis of nuclear protein extracts from oilseed rape. An oligo- nucleotide probe based upon the TATA box domain of the seed storage protein cruciferin was used to probe nuclear extracts from maturing rapeseed cotyledons of increasing ages as shown in lanes A,B,C, and D. Lane E was a control of leaf nuclear protein extracts.

Fig. 8 Channelling of carbon towards storage products in developing oilseeds.



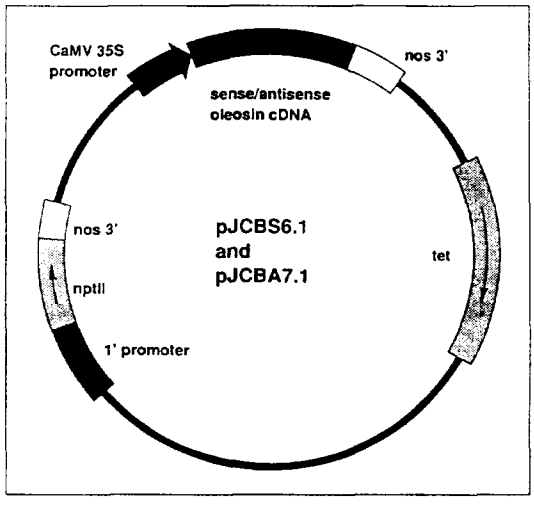


Fig. 9 Antisense oleosin construct. This construct is based on vectors made available by the Sainsbury Laboratory at Norwich.