as conventional breeding and induced mutation have been used to generate new crop variance with different fatty acid profiles in their seed oils. The scope for introducing such variation into a crop has been widened considerably in the last few years by the availability of molecular genetic techniques. It is now possible, in principle, to transfer genes of interest from different crop species, or even from bacteria and animals, into a crop such as oilseed rape in order to produce the desired fatty acid profile in the seed oil. While these aims are straightforward and the concepts underlying them are easily understood this research is of necessity long term. This is due to several factors. Firstly, it will be necessary to elucidate in much greater detail the pathways and regulatory mechanisms responsible for the synthesis of storage products such as oil in developing seeds. Progress in this area has been slow but it is encouraging to note that several recent initiatives by Research Councils have resulted in an increase in research funds in the area of plant metabolism. Secondly, the vast majority of gene functions of interest involve singularly recalcitrant membrane-bound proteins whose purification has been bedeviled by many technical difficulties. It has been possible in some cases to use an oblique approach whereby analogous genes are cloned from more easily manipulated systems such as prokaryotes using techniques such as differential screening and complementation, but such an approach has not always been possible. Again while progress has been slow it is now accelerating in this area as well. These and other difficulties mean that it will probably be the turn of the century before we see our first transgenic oilseed crops in the field.

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Oleosins

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Oleosins are hydrophobic proteins which are specific to oleogenic tissues in plants. Oleosins were first characterized in oil seeds such as maize, soybean and sunflower. Oleosins have only been recognized as a new class of plant protein in the last 2-3 years. These proteins are uniquely associated with the storage oil bodies in plants and are not present in any other tissue or subcellular fraction. Oleosin like proteins have been found in all plant lipid storage tissues that we have so far investigated. These include avocado mesocarp, castorbean endosperm, maize scutellum and oilseed rape cotyledons to name but a few. In oilseeds, such as oilseed rape and sunflower, oleosins constitute in excess of 20% total seed protein. Despite their abundance and frequent location in seed, oleosins should not be considered as seed storage proteins. Oleosins are not found in seed protein bodies, but they are not nitrogen-rich, they are insoluble in aqueous media, regardless of salt concentration pH and, finally, they are synthesized and mobilized at different times to seed storage proteins. We have also found that the expression of oilseed rape oleosin genes, as determined by steady state mRNA levels, occurs later and shows different kinetics to oilseed rape storage protein genes.

The amount of oleosins in oleogenic plant tissues appears to be correlated with the oil content. On the other hand, it has been shown that oleosins are unlikely to play a role in the biosynthesis of triacylglycerols and their release as nascent oil droplets. That oleosins play an important role in the plant tissues in which they occur is evident from their great abundance. We suggest that the function of oleosins is to provide a

continuous monomolecular boundary layer around oil storage bodies in plants. Hence the size of the oil bodies would be determined by the quantity of oleosins synthesized. The oleosin boundary layer may serve to render oil bodies inert and to preserve them during the large changes in moisture content that accompany seed maturation and subsequent germination. The oleosins may also ensure that oil bodies present a large surface area of lipase-mediated oil mobilization after germination. It has also been suggested that oleosins may act as receptors for lipase binding in a manner analogous to apolipoprotein C-II binding of lipoprotein lipase in mammalian transport lipid bodies [1].

The term oleosin was originally an operational definition of the protein fraction that co-purified with the plant oil bodies. Recent progress in the sequencing and gene cloning of oleosins has allowed us to extend this and to recognize that oleosins are a structurally related family of proteins. First indications that oleosins from different plant species may be structurally related came from peptide mapping and immunological studies which showed that the oilseed rape 19 kDa oleosin shared immunological determinants and proteolytic cleaved sites with oleosins purified from more than 15 different species of the Cruciferae [2]. More recently we have begun to characterize the oleosin gene family in oilseed rape in detail. At least four different oleosin genes are expressed at high level during embryo development in oilseed rape. Southern blotting studies indicate that there may be as many as 8 genes in the oleosin gene family in rapeseed (Edwards, E W, Shaw, C H, Murphy, D J, unpublished results).

The primary sequence data for oleosins have been used to derive predictions concerning their possible secondary structures [3]. The most striking structural motif shared by all oleosins sequenced to date is a highly conserved central region of about 70 uninterrupted hydrophobic/non-polar residues. Such an extensive hydrophobic domain with no predicted turns is virtually without precedent and is quite different from any other class of integral membrane protein that has been studied. Another anomaly exhibited by the oleosins is that the hydrophobic region is strongly predicted by all the algorithms that we have used, to be a ß-strand structure as shown in Fig. 1. In this regard, it is interesting that a similar type of ß-strand structure is predicted to exist in the hydrophobic, lipid-embedded domains of human apolipoprotein B-100, which is similar to oleosins in being located at a neutral lipid-water interface[1].

One must, of course, be cautious in the use of algorithms based almost exclusively on the structures of soluble globular proteins for structure predictions of insoluble, interfacial and membrane-associated proteins. Such algorithms can be useful in generating experimentally verifiable predictions and have been used with some success in predicting the amphipathic α-helical domains of mammalian apolipoproteins and the membrane-spanning ß-sheet domains of bacterial porins. Nevertheless, the potential pitfalls of such predictions are exemplified when they are compared with experimental data using direct physical techniques such as circular dichroism (CD) or Fourier-transfer infra-red (FR-IT) spectroscopy. The prediction from Fig. 1 and elsewhere is that the oilseed rape oleosins should contain about 38% ß-strand and only 19% α-helix. However, our data from CD spectra (Fig. 2) indicate that native oilseed rape oleosins containing a molar ratio of fatty acid:protein of 14:1 had 56% α-helix and only 21% ß-strand. Another preparation of rapeseed oleosins with a lower ratio of fatty acid:protein (5:1) had less α-helix (38%) and and the same amount of ß-strand structure (22%). This indicates that oleosins may indeed adopt a predominantly ß-strand structure when they are completely delipidated in an aqueous medium but that the more biologically relevant structure is the more α-helical form exhibited when they are able to bind lipid, i.e. on the surface of an oil body.

In addition to their central hydrophobic domain, oleosins have rather polar C- and N-terminal domains (Fig. 2). In contrast to the hydrophobic domain, the polar regions of oleosins from different plant species show relatively low levels of sequence homology, particularly at the N-terminus. This may indicate that the function of these hydrophilic terminal domains is merely to give oleosins their amphipathic character. Nevertheless, there are some conserved features in these domains which exhibit interesting similarities with animal serum apolipoproteins. For example, there are putative complex amphipathic α-helical domains found near the C-terminals of the maize,oilseed rape and carrot oleosins. These domains resemble the proline-initiated, amphipathic α-helical, 11-residue tandem repeats that are the major structural motif of all human and animal apolipoproteins A, C and E. These tandem repeats are believed to form amphipathic α-helices with a hydrophobic face flanked by basic residues and an exposed polar face containing acidic residues. An Edmunson-wheel diagram of the 22-residue consensus sequences of animal apolipoproteins A,C, and E shows remarkable similarities with similar diagrams of the C-terminal amphipathic domains of the oilseed rape and maize oleosins (Fig. 3). While generalized amphipathic α-helices are not uncommon structural motifs in proteins, the specific organization of the basic and acidic residues on the polar face of the α-helix as shown in Fig. 3 is quite rare. The structural similarities of the putative amphipathic α-helical domains of these two quite diverse classes of triacylglycerol-associated protein from animals and plants may imply that similar mechanisms of protein-lipid binding are used in all organisms.

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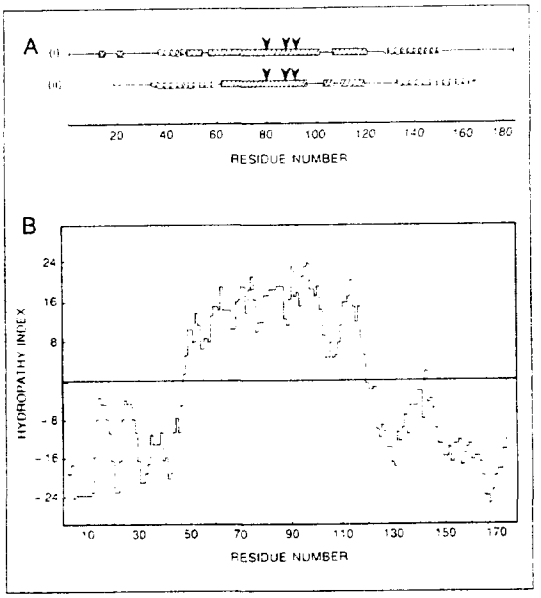

Fig. 1 Hydropathy plot and predicted secondary structure of oleosins. (A) secondary structure prediction for oleosins from (i) oilseed rape and (ii) maize. Coils indicate α-helix and hatched boxes indicate β-strand. Conserved proline residues are arrowed (B) hydropathy plot of the amino acid sequence of the rapeseed oleosin nap-II, using the Kyte and Doolittle method. Note the central hydrophobic domain from residues 48-119.

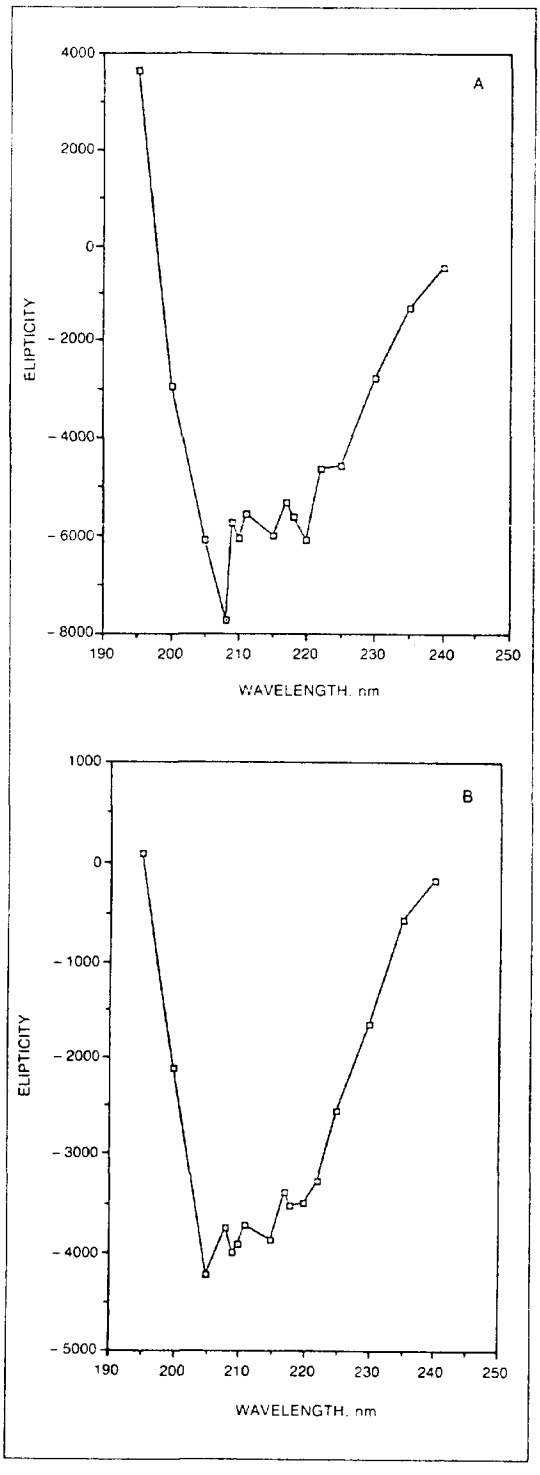

Fig. 2 Circular dichroism spectra of rapeseed oleosins. (A) native oleosin purified by gel-filtration and ion-exchange FPLC. (B) partially delipidated oleosin purified by preparative SDS-PAGE.

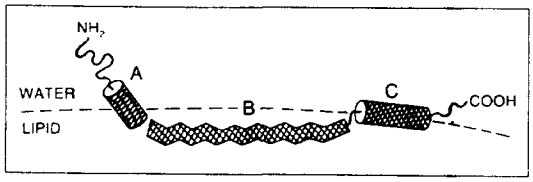

Fig. 3 Proposed model of oleosins at the lipid:water interface of a storage oil body in plants.
(A) N-terminal proximal polar α-helical domain. In the case of one of the rapeseed oleosins, this domain contains a sequence bearing some similarity to the lipoprotein lipase binding domain of human apolipoprotein CII. This domain may therefore, be the site of attachment of the lipase responsible for oil body mobilization.
(B) central 70-residue proline-rich hydrophobic domain. Due to its hydrophobic nature, this domain is probably immersed in the lipid phase of the storage oil body.
(C) C-terminal proximal, amphipathic α-helical domain. This contains a possible 4 x 11 residue tandem repeat, which has some similarities with the 11 residue tandem repeats found in all human and animal serum apolipoproteins sequenced to date.