Brassica and Oilseeds Research in Norwich

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Mechanisms of Storage Oil Deposition in Plants

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Virtually all plants produce oils in one or more tissues, including seed cotyledons and endosperm, the mesocarp of many fruits and in pollen grains. These oils are normally found in spheroidal triacylglycerol (TAG)-rich organelles, termed oil bodies, which can range from less than 0.1 μm in diameter to over 25 μm. In some species, mature seeds may contain in excess of 75% of their weight as storage oil. In most temperate seeds, storage oil bodies are enclosed by a monomolecular layer of unusual amphipathic proteins termed "oleosins". However, we have shown that in oil-storing fruit tissues, such as olive and oil palm mesocarp[1], and in pantropical oilseeds which do not normally undergo desiccation, these oleosins are either absent or very much reduced in quantity. It is thought that the oleosins prevent coalescence of oil bodies so that, following germination, a large surface area/volume ratio is maintained giving rapid access for lipases to mobilise the oil to support growth of the seedling. Our most recent findings suggest a new role for oleosins in preventing oil-body coalescence at the onset of seed imbibition. This is supported by low temperature scanning electron microscope observations which show coalescence of oil bodies at the onset of imbibition of dried oilseeds, such as cocoa, which contain few oleosins. In contrast, we observed that oil bodies maintained their structural integrity during imbibition in desiccation-tolerant oilseeds which invariably contained a large complement of oleosins.

We are also attempting to elucidate the mechanism of oil-body biogenesis in plants and the role, if any, of oleosins in this process. It has been proposed that oil bodies arise by budding-off from the endoplasmic reticulum (ER), although the mechanism for this process remains unknown. We have addressed this problem by fractionating the ER into distinct populations, using centrifugation of relatively shallow sucrose density gradients. This has allowed us to characterise two distinct ER-derived membrane fractions, one of which was enriched in TAG and TAG biosynthetic enzymes, while the other was more closely related in composition to the bulk ER[2]. It is possible that the low density TAG-enriched fraction is derived from a specialised region of the ER, in which enzymes of TAG biosynthesis are concentrated. Such regions could act as metabolons, channelling carbon from acyl-CoAs, directly into the formation of TAG. Such concentrated regions of TAG production may then give rise to droplets of TAG in the intra- bilayer of space. These droplets would eventually bud-off from the membrane to form nascent oil bodies, as shown in Figure 2.

In previous studies, we showed that oleosins are co- or post-translationally inserted into microsomes *in vitro*[3]. In order to elucidate the synthesis and targeting of oleosins *in vivo*, we followed the fate of endogenous 19 kDa rapeseed oleosins, and introduced 24 kDa soybean oleosins in transgenic rapeseed, using both EM immunocytochemical and subcellular fractionation methods[4]. The results showed, that oleosins are inserted into ER membranes, and that the soybean oleosins may undergo a post-translational modification during seed development. In view of these and other findings, we now propose a mechanism for oil-body biogenesis and maturation as outlined in Figure 3. In many non-desiccating tissues, TAG accumulation and oil-body formation proceeds in the absence of oleosin synthesis. In such cases, the nascent oil bodies continue to fuse in order to minimise the unfavourable free-energy associated with a high surface area:volume ratio. The final size of such large oil bodies can range from 10-25μm and is determined by factors such as the composition and viscosity of the surrounding cytoplasmic matrix (Figure 2A). If oleosin synthesis occurs concomitantly with TAG synthesis, the ratio of oleosin:TAG deposition will determine the final oil-body size. The oil droplets will continue to fuse until they are completely coated with oleosins which then act via steric hinderance and electrostatic repulsion to prevent further fusions. Therefore, under conditions of slow oleosin synthesis, each nascent oil body would undergo fewer fusion events before reaching a limiting size than in the absence of oleosin synthesis. This would lead to the accumulation of intermediate sized oil bodies of 1-5 μm diameter (Figure 2B). With rapid oleosin synthesis, very few fusions would occur before a final diameter of 0.1-1.0 μm is reached (Figure 2C). This may account for the observations made over many years that oil bodies made early in seed development, when the rate of oleosin synthesis is relatively low, are much larger than those made later in development when oleosins are made at high rates. We therefore propose that oleosins do not play a role in oil-body biogenesis *per se*, but are a major determinant of the final oil-body size and stability in plant tissues. Furthermore, oleosins appear to be an essential component in oil-rich seeds that undergo maturation drying.

Figure 1

Oil-body biogenesis on endoplasmic reticulum (ER) membranes. Triacylglcyerol (TAG) accumulates in discrete regions of the ER that are enriched in enzymes of TAG biosynthesis. Eventually the TAG buds off from the ER as a nascent oil-body droplet.

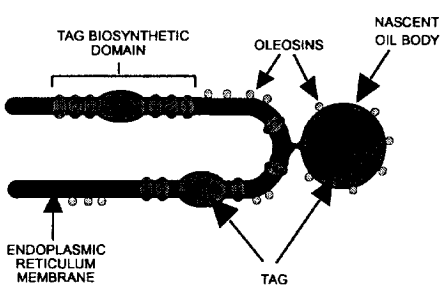
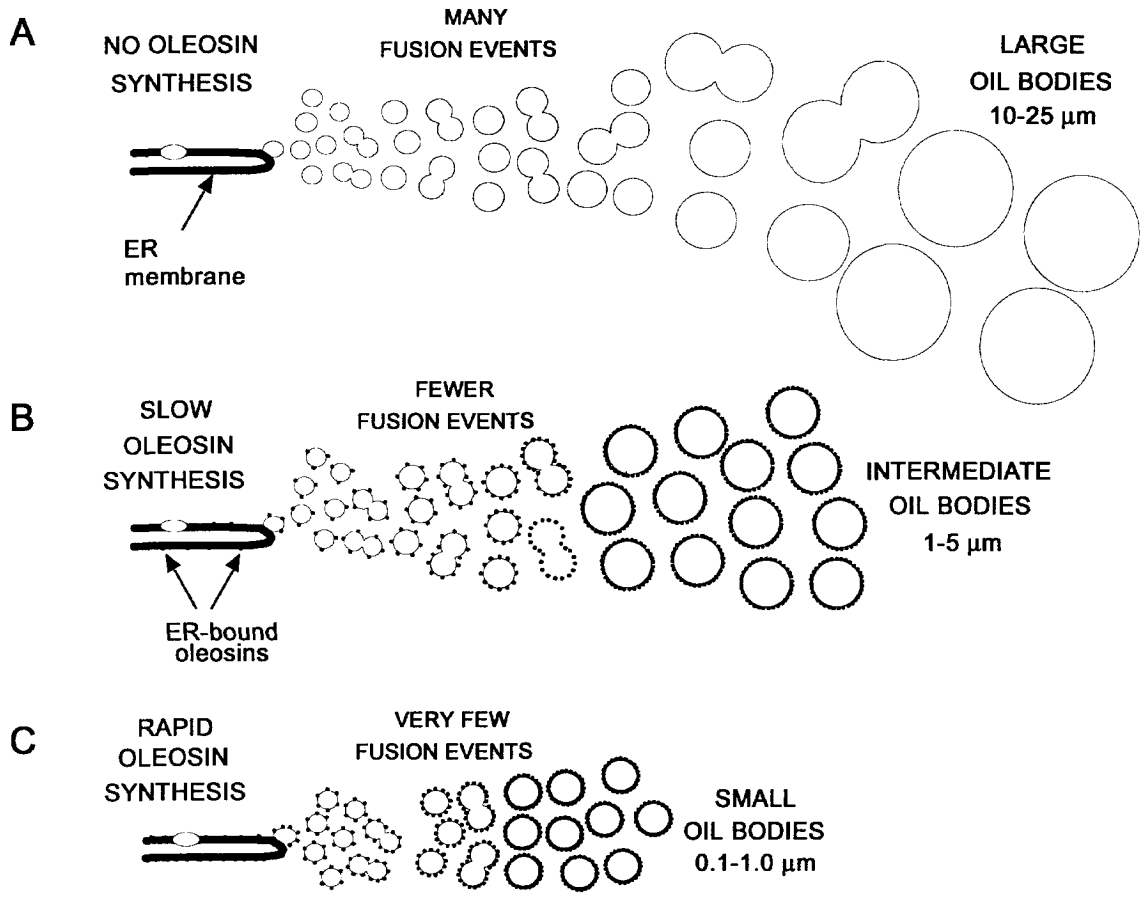


Figure 2
Oil-body maturation and the role of oleosins. In the absence of oleosins, oil bodies are free to fuse until they reach a size determined by the nature of the external medium, i.e. the cytosol. In the presence of oleosins, oil-body size will be limited by the oleosin:TAG ratio.



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