Review

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From Plastids to Lipid Droplets: Molecular Transporters Orchestrating Triacylglycerol Accumulation in Oilseed Crops

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Laboratório de Química e Função de Proteínas e Peptídeos, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ, Brazil.

* Correspondence: daykturquetti@gmail.com (D.K.T.-M.); tmvenancio@uenf.br (T.M.V.)

Abstract

Oilseed crops are essential for global agriculture, industry, and nutrition, relying on complex pathways governing fatty acid (FA) and triacylglycerol (TAG) synthesis. While the enzymatic steps of TAG formation are well established, mechanisms of lipid trafficking between organelles remain poorly understood. This review summarizes recent advances in identifying lipid transporters involved in intracellular lipid movement and lipid droplet (LD) formation in oilseeds. Key protein families include non-specific lipid transfer proteins (nsLTPs), lipid droplet-associated proteins (LDPs), acyltransferases, ABC transporters, and plant VPS13 paralogs. Manipulating LDPs can enhance oil accumulation, yet the regulatory networks coordinating lipid transport and storage remain unclear. Recent studies highlight membrane contact sites (MCSs), especially between the endoplasmic reticulum (ER) and plastids, as critical hubs for lipid exchange. However, key questions persist regarding transported lipid species, ER–plastid tethering mechanisms, and redundancy among MCS proteins. Future research should prioritize identifying ER-to-LD transporters and expanding characterization of MCS-associated proteins. Integrating multi-omics, advanced imaging, and genome editing will be essential to uncover protein–lipid interactions regulating TAG trafficking and storage, enabling metabolic engineering strategies to improve oil yield and composition in oilseed crops.

Keywords: lipid transporters; lipid droplets; triacylglycerol trafficking; agricultural biotechnology; *Glycine max*; *Brassica napus*; *Arachis hypogaea*; *Sesamum indicum*; *Helianthus annuus*

1. Introduction

The increasing global demand for plant-based oils has driven significant advances in the study of oilseed crops such as soybean (*Glycine max*), rapeseed (*Brassica napus*), peanuts (*Arachis hypogaea L.*), sesame (*Sesamum indicum*), and sunflower (*Helianthus annuus*) [1–3]. A significant increase in global vegetable oil production, reaching 217 million tons in 2023-2024 [4]. Oilseed crops are essential constituents in the realms of agriculture, industry, and human nutrition. They are primary sources of energy, critical components of biofuels, and fundamental raw materials for a wide range of industrial products, including cosmetics, pharmaceuticals, and food items [5,6].

Triacylglycerol (TAG) is a lipophilic neutral lipid composed of three fatty acid (FA) chains esterified to a glycerol backbone. It serves as the primary form of energy storage in plants, accumulating mainly in seeds to support germination and early seedling growth. The carbon skeletons required for FA chain elongation are derived from carbohydrates metabolized through pathways like glycolysis and the subsequent conversion of pyruvate to acetyl-CoA in plastids [7]. Prior to glycolysis, a fundamental step occurs: the transport of sugars. Sugar transporters play a critical upstream role by mediating the allocation of sugars to developing seeds. These transporters facilitate the import and distribution of sucrose, where it can be metabolized into acetyl-CoA, the

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precursor for FA synthesis. Numerous reviews have extensively covered the biochemical pathways involved in TAG biosynthesis [7–11]. In summary, TAG synthesis in plants primarily proceeds through two interconnected pathways: the prokaryotic (plastidial) pathway and the eukaryotic/Kennedy pathway in the endoplasmic reticulum (ER). TAG biosynthesis begins with the *de novo* synthesis of FA in plastids, where acetyl-CoA serves as the precursor. In oilseed crops, the first committed step in FA biosynthesis is the conversion of acetyl-CoA, derived from sucrose-driven glycolysis, into malonyl-CoA catalyzed by acetyl-CoA carboxylase (ACCase) [12–14]. Malonyl-CoA then serves as the two-carbon donor in successive condensation reactions mediated by the FA synthase, producing long-chain FAs [15]. These long-chain FAs, mainly 16- and 18-carbon species synthesized in the plastid, are then transported to the ER, where they undergo additional elongation and desaturation to generate polyunsaturated FAs. Both the plastid-derived and ER-modified acyl chains provide substrates for TAG assembly, in which acyl groups are sequentially esterified onto a glycerol backbone via the Kennedy pathway [7,10]. In several species, including soybean, an alternative acyl-editing pathway operates in parallel [16]. In this route, phosphatidylcholine (PC) functions as both a site for acyl modification and as a carrier that mediates the exchange and trafficking of acyl groups between organelles and membrane subdomains [7–9]. The newly synthesized TAGs are ultimately stored in lipid droplets (LDs) within the cytoplasm [7].

Because lipophilic molecules such as FAs and TAGs have limited solubility in the aqueous cellular environment, they require specialized and directional transport mechanisms [17]. Plants have evolved a variety of lipid trafficking systems to coordinate lipid synthesis, modification, and storage. Transport proteins involved in lipid metabolism perform distinct specific functions, for instance, FA translocation across cellular membranes and the regulation of LD formation [18]. These include lipid-binding proteins, such as acyl-CoA-binding proteins (ACBPs), lipid transfer proteins (LTPs), and LD-associated proteins (LDPs), as well as vesicle-associated transport proteins, notably soluble N-Ethylmaleimide-Sensitive Factor Attachment Protein Receptors (SNAREs), each contributing uniquely yet cooperatively to lipid trafficking [17–21]. Despite exhibiting low amino acid sequence similarity, LTPs and ACBPs share a conserved fold that forms an internal hydrophobic cavity, enabling them to bind and potentially facilitate the transfer of diverse lipid species [22]. Some studies have revealed the complexity of lipid transport systems and their impact on oil accumulation, highlighting the importance of these transporters in seed oil metabolism [18,23,24]. Consequently, advancing our understanding of lipid transporters, particularly those mediating the movement of TAGs and their precursors, remains crucial for improving oil yield and composition in crops.

For decades, the export of FAs from plastids to the ER was considered one of the least understood aspects of plant lipid metabolism [25,26]. Before the discovery of any membrane transporters, the prevailing view was that free FAs (FFAs) might diffuse passively across the plastid envelope, aided by their amphipathic nature and by the action of acyl-ACP thioesterases, which release them from acyl-carrier proteins (ACPs) [27]. Alternative hypotheses invoked ATP-binding cassette (ABC) transporters or other energy-dependent mechanisms, but none could be conclusively linked to direct FA export [28]. This conceptual uncertainty persisted until 2015, when the identification of FAX1 (Fatty Acid Export 1), localized to the inner envelope (IE) of plastids, provided the first strong molecular evidence for a dedicated FA exporter [29]. Functional analyses in *Arabidopsis thaliana* demonstrated that FAX1 mediates plastidial FA export, and that its disruption affects biomass, fertility, and lipid composition, firmly establishing the existence of a protein-mediated pathway bridging plastidial FA synthesis with ER-based acyl editing and TAG assembly [17]. The discovery of FAX1 transformed the understanding of intracellular lipid trafficking, revealing that plastid–ER communication is far more dynamic and regulated than previously assumed [19,30].

Despite these foundational insights, major questions remain unresolved regarding the specificity, regulation, and coordination of FA and TAG transport across organelles, as well as how these processes integrate with LD biogenesis. Building upon these foundations, this review consolidates recent advances in the identification and functional characterization of lipid transporters that shape oil accumulation in plants (Supplementary Table 1). We highlight both established and

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emerging transport systems—spanning plastid, ER, and LD interfaces—and delineate the critical knowledge gaps that must be addressed to harness these molecular transporters for metabolic engineering of high-oil-yielding crops.

2. Carbon Supply and Sugar Transporters Feeding Lipid Biosynthesis

Sucrose is especially critical for supporting the biosynthetic and energetic demands of developing seeds, where it serves as a precursor for starch, protein, and lipid synthesis [31,32]. The allocation of carbon from photosynthetic source tissues to non-photosynthetic sink organs is a tightly coordinated process mediated by a network of sugar transporters [33]. Among these, sucrose transporters/carriers (SUTs/SUCs), Sugars Will Eventually Be Exported Transporters (SWEETs), and monosaccharide transporters (MSTs) coordinate sugar movement and compartmentalization across cellular and tissue interfaces. SWEET transporters mediate bidirectional sucrose and monosaccharide transport via facilitated diffusion, with flux direction determined by local concentration gradients, whereas most SUT and MST family members function as proton-coupled symporters driven by the proton gradient established by H^+-ATPase activity [31,34]. Although MSTs play important roles in intracellular hexose transport and metabolism, their contribution to oil biosynthesis is generally more indirect. Therefore, this review focuses primarily on SUTs/SUCs and SWEET transporters, which are key regulators of long-distance carbon allocation and sugar flux into developing seeds. Members of the SUT/SUC family mediate sucrose uptake into phloem sieve elements and companion cells, underscoring their central role in phloem loading and directional sugar transport [35] (Figure 1A). Notably, members of the SWEET family play a pivotal role in apoplastic sucrose efflux, exporting sugars from mesophyll cells or phloem parenchyma into the apoplast to facilitate phloem loading or delivery to sink tissues such as developing seeds [36] (Figure 1B). Within developing embryos, sucrose is subsequently imported via plasma membrane SUT transporters [37]. These transporters have been targets of selection during crop domestication, contributing to improvements in grain nutritional value, yield, and overall crop quality [38].

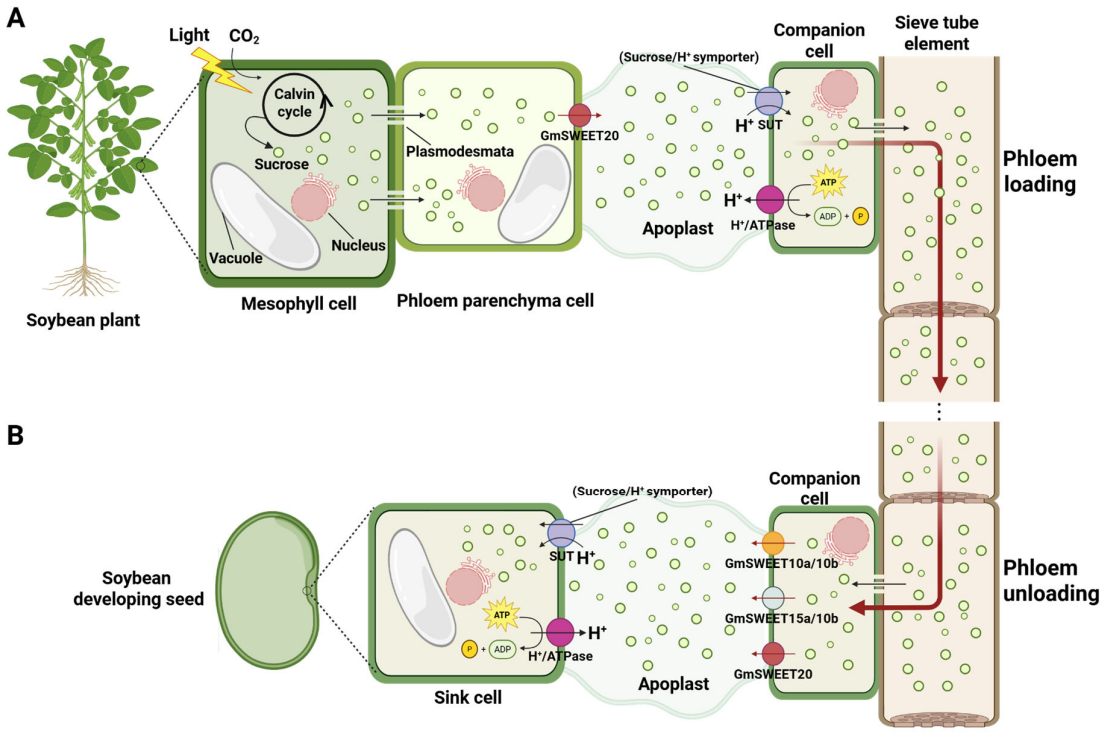

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Figure 1. Overview of sugar transport, including phloem loading and apoplastic transport, along the pathway from mesophyll cells to sink cells in the developing soybean embryo. **A.** Phloem sugar loading process. Sucrose produced in mesophyll cells is transported to phloem parenchyma cells through plasmodesmata-mediated symplastic transport. From the parenchyma cell, sucrose is exported to the apoplast via GmSWEET20 and subsequently taken up into the phloem companion cell by SUT (sucrose/H^+ symporter) transporters. The sucrose is then translocated through the sieve elements toward sink tissues. **B.** Phloem unloading process. Sucrose is released from the phloem into the apoplast by SWEET transporters, including GmSWEET10a/10b, GmSWEET15a/15b, and GmSWEET20. The sugar is subsequently imported into embryo sink cells by SUT transporters, driven by the proton gradient across the plasma membrane. Created in https://BioRender.com.

The importance of intracellular sugar compartmentalization is underscored by studies in *Arabidopsis* showing that redirecting vacuolar sugars to the cytosol enhances FA and TAG accumulation [39]. This effect appears to involve both metabolic and signaling pathways mediated by sugar-responsive regulators such as SnRK1 and WRI1. Although demonstrated in leaves, these findings provide a conceptual framework for manipulating sugar flux to boost TAG production in seeds as well.

Functional studies have established the central role of sugar transporters in seed development and storage reserve accumulation. In *A. thaliana*, *AtSWEET11*, *AtSWEET12*, and *AtSWEET15* cooperate in maternal-filial sucrose transfer, and the *sweet11;12;15* triple mutant exhibits impaired embryogenesis and reduced seed weight, starch, and lipid content [40]. Similar conservation of SWEET function is observed across oilseed crops. In *B. napus*, 68 *SWEET* and 22 *SUC* genes have been identified, showing tissue-specific expression in leaves, siliques, and developing seeds [41]. High-oil genotypes showed consistently higher expression of SUT and SWEET genes, suggesting that enhanced source-to-sink carbon flux underlies greater oil accumulation [42]. In *G. max*, *GmSWEET15a/b* knockout lines displayed impaired embryo development and reduced seed sugar levels, effects partially rescued by increased light intensity [43]. Although phylogenetically related to *Arabidopsis AtSWEET15*, *GmSWEET15* plays a more specific and critical role in seed development, highlighting the importance of assimilate partitioning for seed yield and quality. Moreover, allelic variation in *GmSWEET10a/b* influences sucrose supply and oil accumulation, and CRISPR–Cas9-engineered haplotypes have improved oil and protein content in field-grown lines without yield penalties [44,45]. Consistent with this, AlphaFold-based modeling revealed structural variation among *GmSWEET10a/b* alleles, enabling structure-guided genome editing strategies to optimize transporter activity and carbon allocation, thereby increasing seed oil and protein content [45]. In addition, *GmSWEET20* also plays an important role in sugar transport. This gene is expressed in both leaves and developing seeds, and its overexpression has been associated with an increased number of seeds per plant and higher overall yield [46].

Consistently, in *A. hypogaea*, *AhSWEET24* and other seed-expressed homologs show high expression in embryos and positive correlation with oil content [47]. Comprehensive genomic analyses have further expanded our understanding of the evolution of SWEET genes in peanuts. Li et al. (2024) identified 94 SWEET genes across three *Arachis* species [47]. Despite variations in exon-intron structure, most genes remained highly conserved, reflecting strong purifying selection during evolution. Whole-genome and tandem duplications were identified as key drivers of gene expansion, while promoter analyses revealed abundant upstream cis-elements in SWEET genes, including 31 development-related elements, 12 associated with phytohormone responses, and 7 involved in stress tolerance. Additionally, expression profiling indicated that 12 SWEET genes are highly expressed during seed development in multiple cultivars, supporting their role in regulating carbon allocation and oil accumulation [47].

Beyond SWEETs and SUCs, legume-specific sucrose facilitators (SUFs) have been identified in the seed coats of developing pea (*Pisum sativum*) and common bean (*Phaseolus vulgaris*). These uniporters, which have lost proton coupling, mediate sucrose efflux from maternal to filial tissues [48]. Phylogenetic evidence indicates that SUFs originated from recent gene duplications and

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represent a legume-specific adaptation associated with large seed development [48,49]. Notably, SUF homologs are absent in non-legume species such as *A. thaliana* or maize (*Zea mays*) [48], highlighting the evolutionary diversification of sucrose transport mechanisms among seed plants.

In parallel with studies focusing on sugar transporters, metabolic engineering efforts have explored strategies to enhance carbon flux toward oil synthesis. These approaches provide complementary evidence that increasing the availability of transport-derived carbon can be effectively coupled to enhanced lipid biosynthesis. Morley et al. (2023) demonstrated that transgenic soybean plants overexpressing *Arabidopsis* malic enzyme orthologs with distinct cofactor specificities and subcellular localizations, namely the NAD^+-dependent malic enzyme *AtME2* (extraplastidial/mitochondrial) and the $NADP^+$-dependent malic enzyme *AtME4* (plastidial), exhibited distinct metabolic outcomes. Malic enzymes catalyze the oxidative decarboxylation of malate into pyruvate, generating reducing equivalents in the form of NADH or NADPH, thereby linking carbon partitioning with redox balance and biosynthetic capacity. Subcellular targeting strongly influenced seed metabolism: *AtME4* transgenic lines increased NADPH and pyruvate pools that supported enhanced FA synthesis and a 0.5-2% increase in oil content, along with elevated oleic acid levels. In contrast, *AtME2* transgenic lines showed altered amino acid partitioning without increased oil. These findings indicate that plastidial generation of NADPH, rather than overall reducing power, is critical for enhancing FA synthesis, highlighting the importance of subcellular compartmentalization in determining the metabolic fate of transported carbon. Overall, current evidence indicates that sugar transporters not only determine carbon delivery to developing seeds but also set the quantitative framework that constrains downstream metabolic conversion into storage lipids, while highlighting plastidial NADP-ME flux as a target for increasing seed oil content [50].

3. Cytosolic Metabolism and Transport into Plastids

Plastids in land plants are surrounded by two concentric envelope membranes, designated as the outer (OE) and inner envelope (IE) membranes (Figure 2A). Far from being a passive barrier, the OE functions as a dynamic interface between plastids and the cytosol, equipped with transport proteins essential for metabolite fluxes. The OE mediates molecular exchange through β-barrel proteins that generate pores selective for cations or anions, whereas transport across the IE is mediated by a large repertoire of α-helical transporters with high substrate specificity [51]. Phylogenetic analyses reveal that the plastid phosphate translocator family, which includes glucose-6-phosphate translocator (GPT) and phosphoenolpyruvate/phosphate translocator (PPT), localized to the IE, originated in algae and diversified through gene duplications and losses during streptophyte evolution (Figure 2A) [52]. Within the OE, the β-barrel proteins OEP21, OEP24, OEP37, and OEP40 enable the movement of triose phosphate, dicarboxylic acids, amino acids, sugars, and phosphorylated glucose derivatives, with OEP21 being ATP-regulated (i.e., its transport activity is modulated by ATP binding, reflecting the cellular energy state) and OEP40 required for glucose export during starch breakdown. Additional OEPs, such as OEP16 and JASSY, facilitate the exchange of amino acids and 12-oxophytodieonic acid, respectively, while OEP23 has been implicated in stress responses, although its substrates remain to be fully defined [53].

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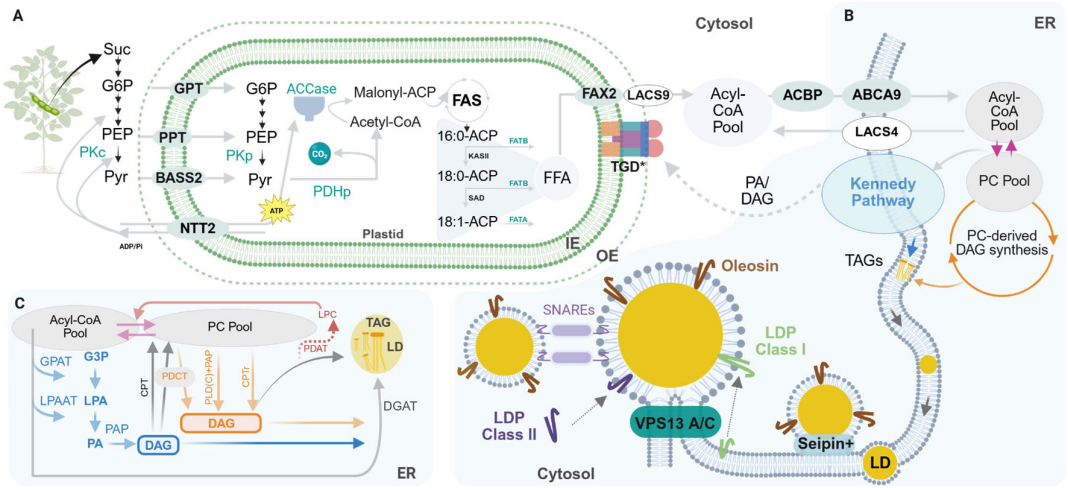

Figure 2. Overview of FA, TAG, and LD biosynthesis, as well as lipid transport involved in TAG accumulation. **A**. Transport of carbon precursors and FA biosynthesis. FFAs are exported from the plastid via FAX2 and subsequently esterified to acyl-CoAs by LACS9 at the outer envelope, feeding the cytosolic acyl-CoA pool. **B**. Acyl-CoAs are trafficked to the ER via ACBP and ABCA9. In the ER, FAs undergo acyl editing/elongation, and there is complex crosstalk between the acyl-CoA pool and the PC pool, increasing the diversity of FAs incorporated into TAG or other lipids such as phospholipids. Some lipid intermediates can return to the plastid through the TGD complex. TAG synthesized in the ER is subsequently packaged into LDs, a process coordinated by LDPs, such as seipin+, which organizes ER-LD biogenesis sites; SNARE proteins, which mediate membrane fusion events involving LDs; and oleosins, which stabilize LDs and prevent uncontrolled droplet fusion. **C**. Detailed steps and enzymes involved in TAG biosynthesis, including the Kennedy pathway (blue) and an alternative pathway (orange). TAG synthesis is predominantly mediated by DGAT, while PDAT provides an acyl-CoA-independent route, indicated by the red dashed arrow, contributing to TAG assembly under specific physiological conditions. Further details on the processes occurring at each step can be found in the main text or in the following references. Adapted from. [7,54–56] Created in https://BioRender.com. **Abbreviations**: **Suc**, sucrose; **G6P**, Glucose-6-phosphate; **PEP**, phosphoenolpyruvate; **PKc**, cytosolic pyruvate kinase; **PKp**, plastidic pyruvate kinase; **Pyr**, pyruvate; **ATP**, adenosine triphosphate; **ADP**, adenosine diphosphate; **Pi**, inorganic phosphate; **GPT**, Glucose-6-phosphate translocator; **PPT**, phosphoenolpyruvate/phosphate translocator; **BASS2**, bile-acid sodium symporter 2; **NTT2**, nucleotide triphosphate transporter 2; **ACCase**, acetyl-CoA carboxylase; **PDHp**, plastidial pyruvate dehydrogenase complex; **CO_2**, carbon dioxide; **CoA**, coenzyme A; **ACP**, acyl carrier protein; **FA**, fatty acid; **FAS**, FA synthase; **16:0-ACP**, palmitoyl-ACP; **18:0-ACP**, stearoyl-ACP; **18:1-ACP**, oleoyl-ACP; **FAT**, FA thioesterase; **KAS**, ketoacyl-ACP synthase; **SAD**, stearoyl-ACP desaturase; **FFA**, free FA; **IE**, plastid inner envelope; **OE**, plastid outer envelope; **FAX**, FA export 2; **LACS**, long-chain acyl-CoA synthetases; **ACBP**, acyl-CoA-binding protein; **ABCA9**, ATP-binding cassette A subfamily member 9; **PC**, phosphatidylcholine; **DAG**, diacylglycerol; **TAG**, triacylglycerol; **PA**, phosphatidic acid; **TGD***, trigalactosyldiacylglycerol protein complex comprising TGD1 (IE-localized ABC transporter; purple), TGD2 (lipid-binding protein for PA/DAG transfer; green), TGD3 (ATPase driving transport via ATP hydrolysis; orange), and TGD4 (red) and TGD5 (blue), which associate with the OE and are proposed to mediate lipid capture from the ER at plastid–ER contact sites; **LD**, lipid droplet; **Seipin+**, Seipin-containing protein complexes; **LDP**, LD-associated protein; **VPS13 A/C**, vacuolar protein sorting-associated protein 13 A/C; **SNARE**, soluble N-ethylmaleimide-sensitive factor attachment protein receptors; **G3P**, glycerol-3-phosphate; **GPAT**, acyl-CoA:G3P; **LPA**, lysophosphatidic acid; **LPAAT**, acyl-CoA LPA acyltransferase; **PAP**, phosphatidic acid phosphatase; **CPT**, cytidine diphosphate-choline DAG cholinephosphotransferase; **CPTr**, CPT reverse; **PDCT**, phosphatidylcholine DAG cholinephosphotransferase; **PLC**, phospholipase C; **PLD**, phospholipase D; **DGAT**, diacylglycerol acyltransferase; **PDAT**, phospholipid: DAG acyltransferase; **LPC**, lysophosphatidylcholine; **ER**, endoplasmic reticulum.

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3.1. Transport of Carbon Precursors for Fatty Acid Biosynthesis

Within the plastid stroma, *de novo* FA synthesis occurs as acyl chains elongate on the ACP, yielding mainly C16:0-ACP and C18:1-ACP for subsequent lipid assembly [10,57]. To sustain this biosynthetic process, plastids require a continuous supply of carbon precursors and energy. Organic acids and phosphorylated sugars derived from glycolysis must cross the IE through specific α-helical transporters such as bile acid:sodium symporter family protein 2 (BASS2), PPT, and GPT (Figure 2A). These systems provide complementary routes for carbon skeletons and reducing equivalents required for FA biosynthesis [52,58].

Recent advances have highlighted the importance of these transporters in oilseed crops. Functional studies in *B. napus* demonstrated that *BnaBASS2*, a sodium-dependent pyruvate transporter, directly controls seed oil accumulation: overexpression increased plastidial pyruvate availability, boosting FA synthesis and oil yield, whereas knockout mutants showed reduced oil content and altered FA profiles [59]. Similarly, phosphoenolpyruvate (PEP)/phosphate translocator in *B. napus* (*BnaPPT1*), responsible for PEP import, has emerged as a key regulator of both primary metabolism and lipid biosynthesis. Overexpression of *BnaPPT1* enhanced photosynthetic efficiency, chlorophyll accumulation, and seed oil content, while loss-of-function mutants displayed impaired growth, reduced seed yield, and decreased lipid levels [60]. These results indicate that efficient plastidial import of PEP is essential not only for FA biosynthesis but also for maintaining the metabolic network that sustains plant productivity. Collectively, these findings confirm that pyruvate and PEP supply through *BnaBASS2* and *BnaPPT1* constitute critical bottlenecks for oil accumulation in *B. napus*.

3.2. Energy Transport and ATP/ADP Exchange Across Plastid Membranes

In addition to carbon precursors, ATP-dependent steps of lipid metabolism, including the ACCase-catalyzed production of malonyl-CoA and the export of FA to the ER, require an efficient plastidial ATP supply. Since nucleotides cannot freely diffuse across the IE, this energetic demand is fulfilled by nucleotide triphosphate transporters (NTTs), particularly NTT2, which exchanges cytosolic ATP for plastidial ADP (Figure 2A) [61–64]. In *Arabidopsis*, two related genes, *AtNTT1* and *AtNTT2*, have been identified: *AtNTT2* is broadly expressed across tissues, while *AtNTT1* is a sugar-inducible gene, mainly active in stems and roots [65]. Functional studies revealed that disruption of NTT genes impairs chlorophyll accumulation, root growth, and overall plant development, whereas co-expression of *AtNTT1* with a GPT enhanced starch and tuber yield in potato [66]. In *B. napus*, *BnaNTT2* was recently characterized as a key ATP/ADP antiporter mediating ATP import from the cytosol into plastids. Overexpression of *BnaNTT2* increased chloroplast ATP levels, maintained enhanced glycolytic flux, and led to elevated starch, leaf FA content, and seed oil yield, whereas CRISPR/Cas9-mediated knockout of *BnaNTT2* reduced ATP availability, disrupted thylakoid architecture, and decreased oil accumulation [67]. These findings highlight that NTTs regulate plastidial ATP/ADP exchange and thereby sustain lipid metabolism. Supporting this, several studies have demonstrated that exogenous ATP can stimulate FA synthesis in isolated plastids [62,65].

Collectively, the coordination of metabolite and energy transport across plastid membranes underscores a finely tuned system, integrating β-barrel channels in the OE with substrate-specific α-helical transporters in the IE. Recent evidence from *B. napus* confirms that *BnaBASS2*, *BnaPPT1*, and *BnaNTT2* are central players in lipid biosynthesis and seed oil accumulation. This orchestration ensures the allocation of sugars, organic acids, and ATP required to sustain FA biosynthesis, directly impacting plant growth, stress resilience, and agronomic productivity.

4. Fatty Acid Export from Plastids to the Endoplasmic Reticulum

In oilseeds, the bulk of FAs produced by *de novo* synthesis in the plastid stroma are exported to the ER, where they are elongated, desaturated, and incorporated into TAGs [7,68]. Consequently, the existence of a plastid exporter for FAs and an ER importer for acyl-CoAs is required. This process

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depends on a highly coordinated network of FA exporters, activating enzymes, binding proteins, and ER transporters, which collectively sustain the high metabolic flux required for seed filling [11]. In addition, ER–plastid membrane contact sites (MCSs) play an essential role in facilitating lipid exchange and maintaining the balance between plastidial FA production and extraplastidial TAG assembly [69,70]. MCSs are specialized regions where organelle membranes are in close proximity, enabling lipid transfer while preserving membrane identity [55]. Interactions between the ER and plastids are increasingly recognized as important, yet remain insufficiently characterized in plant cells. Recent advances, especially in lipid metabolism, have clarified aspects of these MCSs and suggest roles in plant development and stress adaptation, as reviewed by Huercano et al. (2025) [55].

4.1. A Decades-Old Question: How Do Fatty Acids Leave the Plastid?

Although it was widely recognized that FFAs are exported from plastids, the precise mechanism underlying this process remained elusive for decades [10,71]. Early models proposed that FAs released by thioesterases could simply diffuse across the plastid envelopes,[25,72] whereas alternative hypotheses suggested the involvement of protein-mediated transfer [71]. However, kinetic studies indicated that the pool of FFAs at the plastid boundary was extremely transient, raising doubts about passive diffusion as the major mechanism [25]. Thus, a central unanswered question was whether plastid FA export required a dedicated transporter. This longstanding gap was bridged in 2015 with the identification of a protein anchored in the IE of the plastid—FAX1, the first membrane-intrinsic protein shown to directly mediate FA export from the plastid, supplying substrates for extraplastidial lipid metabolism [29]. Functional characterization of FAX1 in *Arabidopsis* demonstrated that its loss disrupts biomass accumulation, pollen development, and lipid homeostasis, leading to reduced TAG content in leaves and flowers, but not in seeds, and whereas its overexpression promotes TAG accumulation in vegetative tissues [18,29,73,74]. Studies in *B. napus* demonstrated that FAX1 function is conserved in oilseed crops: knockdown lines displayed reduced seed size, lower oil content, and altered FA composition, whereas overexpression of FAX1 significantly increased seed oil accumulation. In addition to its role in lipid metabolism, changes in FAX1 expression also affected vegetative growth, biomass, and fertility [75,76].

While FAX1 is broadly expressed, FAX2 and FAX4 have emerged as the key paralogs controlling oilseed metabolism (Figure 2A) [77,78]. Both, FAX2 and FAX4, are highly expressed during the early stages of seed development, and the double mutant (*fax2 fax4*) shows a 30% reduction in TAG accumulation, shrunken seeds, and accumulation of plastid lipids compared with wild-type seeds [77]. Radiolabeling assays confirmed that FAX2/FAX4 are especially important for C18 FA export, which fuels the eukaryotic pathway in the ER. Overexpression of FAX2 or FAX4 under seed-specific promoters boosts oil accumulation by more than 120%, highlighting their direct role in determining seed oil yield [77]. Conversely, the relatively minor shifts in the molar composition of TAG molecular species observed between the mutants and wild-type indicate that other transporters may also contribute to sustaining TAG biosynthesis during seed development [77].

Following their release from the plastid IE, FFAs are thought to cross the OE via vectorial acylation, a process mediated by long-chain acyl-CoA synthetases (LACSs). These enzymes activate FFAs into acyl-CoAs, which are then available for downstream lipid assembly in extraplastidial compartments such as the ER (Figure 2A) [7,18]. In *A. thaliana,* nine members of the LACS family have been described, exhibiting both overlapping and specialized functions [79]. LACS9, LACS1, and LACS4 are especially important for plastidial FA export. The *lacs1 lacs9* and *lacs4 lacs9* double mutants show 11% and 27% reductions in seed TAG content, respectively, highlighting their synergistic roles in TAG biosynthesis [80,81].

Comparative genomics has revealed a strong conservation of LACS genes across oilseed species. In *B. napus*, genome-wide survey identified 34 LACS genes classified into four phylogenetic clades, of which 18 are strongly expressed in developing seeds [82]. In addition, comparative expression analyses between high- and low-oil cultivars of *B. napus* suggested that *BnaLACS6-4, BnaLACS9-3*, and *BnaLACS9-4* may be important for plastidial FA synthesis, whereas *BnaLACS1-10* and *BnaLACS4-*

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1 are more directly associated with downstream lipid biosynthesis [82]. Notably, *AtLACS9* has two close homologs in *B. napus*, consistent with its allopolyploidy, and these copies are proposed to function in plastidial FA synthesis and lipid trafficking. Parallel studies in *H. annuus* demonstrated a similar conservation: *HaLACS1* and *HaLACS2*, orthologs to *AtLACS9* and *AtLACS8*, are highly expressed during seed development and are implicated in plastidial FA activation and ER lipid remodeling [83].

The functional output of *BnLACS9* extends beyond FA activation. Recent evidence indicates that *BnLACS9* promotes chloroplast glycolipid synthesis, particularly monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG), which may contribute to increased chlorophyll content and improved photosynthetic efficiency. Overexpression of *BnLACS9* increased plant biomass, delayed silique senescence, and significantly increased seed oil content, reaching up to 45.6% in some lines compared with 39.7% in the wild type [84]. This dual role in photosynthesis and storage-lipid biosynthesis suggests that *BnLACS9* functions as a central node linking chloroplast activity with seed oil production. Functional divergence within the LACS family is also evident. *BnLACS2*, an ortholog of *AtLACS2*, localizes to the ER and exerts a strong influence on seed oil accumulation in *B. napus*. Overexpression of *BnLACS2* increased seed oil content by 6–8%, accompanied by elevated levels of long-chain FAs such as C18:2, C20:0, C20:1, and C22:0, whereas RNAi suppression reduced oil content by 3-6%. Beyond directly supplying acyl-CoAs for TAG synthesis, *BnLACS2* positively regulates the expression of genes involved in glycolysis and lipid biosynthesis, indicating a broader regulatory role in carbon partitioning during seed development [85].

Beyond transporter- and activation-based mechanisms, plastid lipases have also emerged as contributors to FA export. One such case is PLASTID LIPASE1 (PLIP1), a thylakoid-associated phospholipase A1 in *Arabidopsis*. Through a combination of in vitro enzyme assays, transgenic overexpression lines, and pulse–chase radiolabeling experiments, Wang et al. (2017) demonstrated that PLIP1 specifically hydrolyzes polyunsaturated acyl groups from the unusual chloroplast phosphatidylglycerol species 16:1Δ^3trans-PG. The released acyl chains are exported from plastids, incorporated into PC, and subsequently funneled into seed TAGs. Consistent with this role, *plip1* mutants show ~10% reductions in seed oil content and delayed germination, whereas overexpression lines accumulate 40–50% more TAG despite growth and fertility penalties. Supporting evidence from *fad4* mutants, which lack the 16:1Δ^3trans modification required for PLIP1 activity, further confirmed this functional coupling. Although PLIP1 contributes only a minor fraction of total seed oil, it illustrates a lipid remodeling-based route for plastidial FA export that complements the canonical FAX/LACS pathway [86].

4.2. The Cytosolic Journey: Guiding Fatty Acids Toward the Endoplasmic Reticulum

FFAs, once activated to acyl-CoAs by LACSs, are subsequently delivered to the ER through the action of ACBPs (Figure 2A). ACBPs comprise a conserved protein family with high affinity for C12–C22 acyl-CoA esters [87,88]. In addition to acting as intracellular carriers, ACBPs stabilize the acyl-CoA pool, preventing the detergent-like toxicity of free acyl-CoAs and channeling substrates into defined metabolic pathways [89,90]. Their activity ensures continuity between plastidial FA synthesis and extraplastidial lipid metabolism, making them central regulators of seed oil accumulation.

Plant ACBPs are organized into four major classes: (I) small ACBPs, (II) ankyrin-repeat ACBPs, (III) large ACBPs, and (IV) kelch-motif ACBPs. Despite their conserved four-helix bundle structure that forms a hydrophobic cavity for acyl-CoA binding, the addition of ankyrin repeats or kelch motifs confers distinct subcellular targeting and protein–protein interaction capacities [91]. This structural diversification underlies the functional specialization of ACBPs across tissues and developmental contexts. Small cytosolic isoforms such as *AtACBP6* mainly act as acyl-CoA buffers that stabilize intracellular lipid pools, whereas membrane-associated ankyrin-repeat isoforms (*AtACBP1* and *AtACBP2*) engage in signaling and stress-response pathways. This modular organization indicates

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that, even when highly conserved at the sequence level, orthologous ACBPs in different plant species are unlikely to be functionally redundant [89].

Comparative genomics confirms that ACBPs are ubiquitous in higher plants, including legumes, cereals, and oilseed crops. In legumes such as *G. max* and *P. vulgaris*, genome-wide surveys revealed conservation of acyl-CoA binding domains but divergence in subcellular localization and cis-regulatory elements, suggesting evolutionary adaptation of ACBPs to lineage-specific lipid requirements [92]. Such diversity has also been noted in major oil crops like sunflower and rapeseed, where ACBPs contribute to both seed oil biosynthesis and broader cellular processes such as stress tolerance and membrane remodeling [89].

Mutants of *Arabidopsis AtACBP6* show ~50% reductions in TAG and altered lysophosphatidylcholine/PC ratios, whereas *acbp4 acbp5* double mutants reveal their complementary roles in LPC metabolism and acyl editing [93]. In *B. napus*, transcriptomic profiling further highlights the functional divergence of ACBP isoforms across tissues. Notably, ankyrin-repeat *BnACBP2* is up-regulated during embryo oil accumulation, while *BnACBP6* expression declines in embryos but increases in seed coats, indicating differential partitioning of acyl fluxes across tissues [94]. Such spatial differentiation likely reflects coordinated regulation of storage-lipid biosynthesis in embryos and protective lipid metabolism in seed coats.

From an applied perspective, manipulation of ACBP expression holds promise for metabolic engineering. Overexpression studies in model plants have already shown that altering ACBP levels can modify both seed oil content and composition,[93,95] while evolutionary studies in oil crops suggest that particular homologs may be amenable to selective breeding or biotechnological optimization [89]. In the context of rising global demand for plant-derived oils, ACBPs thus emerge not only as key regulators of lipid homeostasis but also as strategic targets for improving oil yield and quality, particularly under fluctuating environmental conditions.

4.3. ATP-Binding Cassette Transporters and Fatty Acid Transfer to the Endoplasmic Reticulum

ABC transporters represent one of the largest and most versatile protein superfamilies in plants, mediating the transport of a wide variety of substrates, including hormones, secondary metabolites, and lipids [96]. In *Arabidopsis*, *AtABCA9* was the first member to be functionally characterized as a FA/acyl-CoA transporter localized to the ER (Figure 2B). Loss-of-function mutants displayed smaller seeds with reduced TAG content, whereas overexpression enhanced TAG accumulation by up to 40%, providing compelling evidence that *AtABCA9* supplies acyl substrates for TAG biosynthesis during the seed-filling stage [97].

Recent studies have expanded our understanding of ABCA transporters. In *Arabidopsis*, *AtABCA10* was shown to play an essential role during early seed development. Unlike *AtABCA9*, which is strongly expressed during seed filling, *AtABCA10* is selectively expressed in female gametophytes and young developing seeds. Loss of function of *AtABCA10* resulted in defective endosperm development, delayed embryogenesis, and shrunken seeds with markedly reduced TAG levels, while overexpression stimulated TAG overaccumulation without altering carbohydrate or protein reserves [98]. These findings suggest that ABCA9 and ABCA10 function in distinct temporal windows of seed development: ABCA10 supports early endosperm expansion, whereas ABCA9 ensures lipid supply during the subsequent phase of storage oil accumulation [97,98].

At a broader scale, diversity analyses reveal that land plants harbor an unusually high abundance of ABC transporters. Oilseed plants, in particular, encode more ABCA genes than non-oilseed species, reinforcing the hypothesis that ABCA expansion correlates with lipid metabolism and storage capacity [99]. Moreover, emerging work demonstrates that engineering strategies targeting lipid transporters may hold promise for crop improvement. For instance, simultaneous overexpression of *AtFAX1* and *AtABCA9* synergistically enhanced seed oil accumulation in *Camelina sativa*, surpassing the effects of single-gene overexpression [100]. Taken together, these findings demonstrate that ABCA transporters play multifaceted roles in plant lipid metabolism, ranging from early endosperm development (*AtABCA10*) to storage lipid accumulation (*AtABCA9*), and

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potentially lipid turnover in oilseed crops. Their functional diversity and evolutionary expansion position them as compelling targets for biotechnological interventions aimed at boosting seed oil yield and quality.

4.4. The Trigalactosyldiacylglycerol Complex: Mediating Lipid Feedback from the Endoplasmic Reticulum to Plastids

FAs exported from plastids to the ER are incorporated into phospholipids through a series of acyl editing reactions, particularly involving PC. Some of these newly formed phospholipids can subsequently return to plastids, where they serve as precursors for the synthesis of plastid-specific lipids, such as MGDG and DGDG. This intricate bidirectional lipid exchange between the ER and plastids depends on the trigalactosyldiacylglycerol (TGD) protein complex, a non-canonical ABC transporter system spanning both plastid envelope membranes (Figure 2A and Figure 2B) [18,30]. According to a recent review, the TGD system remains the best-defined machinery for directional import of ER-derived phospholipid precursors into plastids [55].

The TGD complex comprises multiple subunits: TGD1 (Figure 2A, TGD* purple structure), an ABC transporter embedded in the IE membrane; TGD2 (Figure 2A, TGD* green structure), a lipid-binding protein that recognizes and transfers PA or DAG; TGD3 (Figure 2A, TGD* orange structure), an ATPase that drives transport through ATP hydrolysis; and TGD4 (Figure 2A, TGD* red structure) and TGD5 (Figure 2A, TGD* blue structure), which associate with the OE membrane and are proposed to mediate lipid capture from the ER at plastid–ER contact sites [18,30]. Together, these components form a trans-envelope lipid transfer system that facilitates the import of ER-derived lipid precursors into plastids, thereby supporting galactolipid assembly and maintaining plastid membrane homeostasis.

Genetic and biochemical studies in *A. thaliana* have revealed that disruption of the TGD1–3 complex severely impairs ER-to-plastid lipid trafficking. In the *tgd1* mutant, this disruption creates a metabolic bottleneck in lipid exchange between the ER and plastids, leading to the accumulation of aberrant lipid species and a marked increase in TAG levels [101]. Concomitantly, plastidic ACCase becomes post-translationally activated, stimulating *de novo* FA synthesis and further increasing acyl flux through the ER lipid network. The excess FAs are subsequently redirected into storage lipids, likely as a protective mechanism to prevent FFA toxicity. Consistent with this model, double-mutant analyses demonstrated that LPCAT1/2 and ROD1—key enzymes involved in PC acyl editing—are essential for viability in the *tgd1* background, highlighting the tight coordination between TGD-mediated lipid trafficking and ER phospholipid remodeling in maintaining lipid homeostasis [101].

A recent study by Renna et al. (2024) reveal that ER-plastid contact sites are increasingly understood as regulatory platforms controlling acyl flux, where multiple LTPs, including TGD4, the Sec14 proteins SFH5/SFH7, and VaP27-ORP2A complexes operate in parallel [102]. Within this network, the TGD complex remains the primary route for delivering ER-synthesized PA/DAG into the plastid, reinforcing its central role in sustaining galactolipid biosynthesis and preventing acyl overload in the ER. By maintaining this lipid-exchange equilibrium, the TGD machinery indirectly modulates storage-lipid formation: when ER-to-plastid lipid flow is interrupted, acyl flux is diverted toward TAG accumulation in the ER and associated LDs, a phenomenon observed not only in *Arabidopsis tgd* mutants but also in algal systems such as *Chlamydomonas*, where defects in TGD2 similarly enhance TAG formation from plastid-derived MGDG reservoirs [102].

In summary, the TGD complex represents a crucial conduit for inter-organelle lipid exchange, coupling ER phospholipid metabolism with plastid galactolipid synthesis. Its activity prevents excessive ER acyl accumulation and thereby constrains TAG formation under normal conditions. Conversely, TGD disruption leads to elevated FA synthesis, imbalanced acyl flux, and pronounced TAG accumulation. Acting in concert with other ER-plastid contact-site transport systems and ABCA-type acyl exporters, the TGD complex helps determine the metabolic balance between membrane-lipid assembly and storage-lipid deposition in plant cells.

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5. Lipid Droplet Formation and Seed Triacylglycerol Storage

Once synthesized, TAG molecules accumulate in cytosolic LDs—also known as oil bodies or oleosomes—which are specialized organelles surrounded by a phospholipid monolayer and associated proteins (Figure 2B)[103]. The formation of LDs originates at the ER through phase separation and budding of TAG lenses. A comprehensive overview of the molecular events underlying LD biogenesis was recently provided by Du et al., who described the nucleation, budding, and growth stages and highlighted key proteins, such as SEIPIN and SEIPIN-containing protein complexes [56].

Soybean seeds, for example, contain small, stable LDs that account for approximately 18–22% of its total mass. These LDs consist of structures of TAGs enriched with minor bioactive components and surrounded by a phospholipid monolayer embedded with multiple oleosins, caleosins, and steroleosins [104]. Such diversity in LDPs reflects functional specialization that extends beyond structural stabilization. Some studies indicate that these proteins exhibit distinct molecular architectures, enabling additional cellular roles such as stress adaptation and inter-organelle communication [105]. Key ER–LD bridging proteins, including SEIPINs, LDPs, and LD-Interacting Proteins, have been reported as essential regulators of LD nucleation, growth, and stability [105–107]. SEIPIN complexes, in association with their partners—LD assembly factor 1 (LDAF1), Nuclear Envelope Morphology 1 (NEM1), and Peroxin 30 (PEX30)—define specific ER subdomains where neutral lipids accumulate to initiate droplet formation (Figure 2B) [56].

Evolutionary analyses have shown that genes encoding oleosins, SEIPINs, and TAG lipases experienced positive selection in several oilseed species, including *G. max*, *B.napus*, *S. indicum*, and *A. hypogaea*, reflecting adaptive diversification of lipid storage and mobilization systems across these lineages [108]. The evolution of LDPs contributed to enhanced desiccation tolerance, efficient oil accumulation, and improved germination success. Despite these advances, the detailed molecular coordination of lipid flux between the ER and LDs, as well as the targeting and turnover of LD surface proteins, remains incompletely understood.

Kumar et al. (2018) indicated that Vacuolar Protein Sorting-Associated Protein 13 A/C (VPS13A/C) act as conserved lipid transporters that mediate neutral lipid flux between the ER, LDs, and mitochondria in mammals [109]. These proteins bridge membranes through extended hydrophobic channels capable of directly transferring TAGs and phospholipids across organelles without vesicle formation (Figure 2B) [56]. Comparative structural analyses have shown that VPS13 belongs to a broader superfamily of bridge-like LTPs characterized by repeating β-groove domains that form elongated conduits spanning 15–30nm between membranes [110,111]. This configuration enables the direct flow of lipids at MCSs, redefining our understanding of intracellular lipid transport. This family is evolutionarily conserved across eukaryotes, with six homologs identified in flowering plants [112]. Plant VPS13 genes cluster into four major clades (VPS13A-D), maintaining the domain organization typical of metazoan orthologs. Transcriptomic and co-expression analyses indicated that VPS13C and VPS13D homologs are preferentially expressed in tissues with active lipid metabolism, including developing seeds and young seedlings, consistent with a role in LD–ER interactions. In photosynthetic organisms, the diversification of VPS13 likely reflects adaptation to unique metabolic demands, such as coordinating lipid trafficking between the ER, plastids, and peroxisomes during storage lipid synthesis and mobilization. Hence, plant VPS13 proteins, particularly VPS13C-like members in oilseed species, may establish ER–plastid or ER–LD contact sites analogous to those described in mammalian cells, supporting lipid transfer required for TAG accumulation and membrane expansion [112].

Similar large-scale lipid transport mechanisms have also been proposed at ER–peroxisome interfaces, where peroxisomal β-oxidation relies on LD-derived substrates, suggesting a bidirectional lipid exchange network [20]. In parallel, sSNAREs contribute to LD biogenesis and expansion by mediating membrane fusion events between the ER and nascent droplets [113]. This process may facilitate transient inter-organelle bridges that coordinate the transfer of membrane lipids and proteins. Additionally, LTPs have been proposed to participate in extracellular or inter-organelle

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lipid exchange in seeds, possibly acting at LD–ER or LD–plastid contact sites to facilitate phospholipid redistribution and TAG remodeling [20].

LTPs facilitate the transfer of lipids between membranes, playing a role in LD formation and stabilization. Their small size and versatility make them attractive targets for metabolic engineering [114]. Although LTPs have been widely described in plants as multifunctional proteins implicated in defense responses, cuticle formation, and stress adaptation, their involvement in seed lipid metabolism has remained poorly characterized [115–118].

In *S. indicum*, Wang et al. demonstrated that LTPs play an active role in determining seed oil content, providing molecular evidence for their contribution to lipid accumulation [119]. By comparing transcriptomes of high (~59%) and low-oil (~48 to 50%) genotypes across seed developmental stages (10–30 days post-anthesis, DPA), the authors found that the LTP-encoding genes were consistently upregulated in the high-oil variety, particularly during the late oil biosynthesis phase at 30 DPA. Among these genes, *SIN_1019175* and *SIN_1019172* (LTP1-type), and *SIN_1010009* (LTP5-type) showed markedly higher expression in high-oil seeds, suggesting that enhanced lipid transport between organelles or membranes facilitates TAG accumulation. These LTPs were among a core set of 23 candidate genes identified as major contributors to oil content variation. Their expression coincided with the upregulation of key Kennedy pathway genes (e.g., *GPAT*, *DGAT*, *PDAT*), indicating that LTP-mediated lipid trafficking supports the efficiency of TAG biosynthesis (Figure 2C).

Chen et al. provided a comprehensive analysis of LTPs in *B. napus*, addressing a long-standing gap in understanding their role in oil accumulation [120]. By combining genome-wide identification, structural analysis, and transcriptomic profiling, the authors identified 66 *BnLTP* genes grouped into six phylogenetic clusters, revealing conserved motifs and gene structures suggestive of evolutionary conservation with functional divergence. Expression profiling showed that most *BnLTPs* were highly expressed at 25 days after flowering, coinciding with the phase of active oil biosynthesis. Notably, genes such as *BnaC01g26640D*, *BnaA08g00590D*, and *BnaC03g71570D* exhibited significantly elevated expression at this stage across all three cultivars analyzed, particularly in high-oil lines (~35–49% oil content). Furthermore, gene coexpression networks linked *BnLTPs* to biological processes such as oxidative phosphorylation, ATP metabolism, and defense response, suggesting that these proteins may mediate lipid transport while integrating metabolic energy demands and stress signaling. Collectively, this work represents one of the first systematic efforts to elucidate the contribution of LTPs to oil biosynthesis in *B. napus*, highlighting specific gene candidates and regulatory features that could be leveraged for metabolic engineering in oilseed crops [120].

6. Outstanding Questions and Future Perspectives

Despite substantial advances in clarifying TAG biosynthesis and LD biogenesis in oilseeds, lipid transport remains a major bottleneck in building predictive models of oil accumulation. Multi-compartmental flux from plastids to the ER and then into LDs is known to involve, at least in part, specific exporters and importers such as FAX2/FAX4 and ABCA9, whose perturbation markedly alters seed TAG content, yet only a handful of such transporters have been molecularly and functionally defined in embryos [77,78,97,121]. The situation is further complicated by the strong tissue and cell-type heterogeneity of lipid metabolism within a single seed, where distinct embryonic regions exhibit different acyl-CoA pools, PC composition, and TAG profiles [122,123]. Thus, a central open question is how transporter activities, local acyl flux, and LD biogenesis are coordinated spatiotemporally across seed tissues to determine final oil content and composition.

Emerging work on ER-plastid MCSs reveals that complexes such as TGD1-5, VAP27-ORP2A, and Sec14-like proteins mediate targeted transfer of PA, DAG, and sterols between compartments, playing essential roles in plastid lipid homeostasis and stress responses [24,55]. However, whether and how these ER-plastid MCS machineries channel acyl precursors into TAG biosynthetic pathways during seed filling, or supply regulatory lipids that tune the activity of DGAT, PDAT or TAG-remodeling lipases, remains largely unresolved [55,122,124,125]. In particular, disentangling direct

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contributions of MCS-mediated flux from bulk FA export via FAX and ABC transporters is a key challenge.

Functional annotation of lipid transporter and LDPs families in oilseeds is still fragmentary. Only a small subset of nsLTPs has experimentally validated lipid substrates or developmental roles, despite clear structural diversification and predicted ligand-binding specificities across the family [24,126]. Similarly, LD structural proteins such as oleosins and LDPs are known to stabilize LDs and can substantially increase TAG content when overexpressed, yet the rules by which LD surface proteomes modulate TAG remodeling, lipase access, and futile cycling are only beginning to emerge [127–129]. The molecular identity and regulation of TAG lipases that act in remodeling rather than degradation, as recently shown for TAGL1-mediated TAG remodeling in *Physaria*, is another important knowledge gap for engineering unusual or stress-responsive oils without compromising total TAG levels [125].

Addressing these questions will require integrative strategies that combine high-resolution lipidomics and spatial metabolite imaging with cell-type-resolved transcriptomics and proteomics of developing seeds [121–124]. Integrating these datasets with CRISPR/Cas-based reverse genetics, multiplex editing of lipid transporters, and heterologous testing of candidate nsLTPs, ABC transporters, FAX proteins, and TAG-remodeling enzymes should enable systematic identification of acyl flux control points [24,77,78,97,121,130]. Structure-guided protein engineering, supported by improved structural models of nsLTPs and lipid transporters, may then allow modification of substrate specificity or interactions with LDs. Ultimately, these strategies could enable the design of synthetic "push–pull–protect" modules that couple enhanced FA export, efficient TAG assembly, and stabilized LD storage [24,127,129,131,132].

Future work could therefore prioritize: (i) defining the full complement and kinetics of plastid-to-ER FA exporters and ER acyl importers in oilseed embryos, including crop orthologs of FAX2/FAX4 and ABCA9 [77,78,97,121]; (ii) resolving how ER–plastid MCS complexes partition PA, DAG, and regulatory lipids between membrane biogenesis and storage TAG synthesis during seed filling [24,55,122,124]; (iii) mapping LD surface proteomes and identifying lipid-selective TAG lipases and their DGAT partners that drive TAG remodeling versus net turnover [125,127,129]; and (iv) integrating transporter and transcriptional networks that coordinate carbon allocation between starch, protein and oil, leveraging population-scale transcriptomics and eQTL/TWAS approaches already successful in major oil crops [121,122,124,130]. Such mechanistic insights will underpin next-generation engineering strategies aimed at simultaneously maximizing oil yield, tailoring FA composition and maintaining physiological robustness under field conditions [24,121,124,128,130,131].

Conclusions

Lipid transport and storage now emerge as central, rather than peripheral, determinants of oil accumulation in seeds. Work in *Arabidopsis* and *Brassica* species demonstrate that manipulating plastid FA exporters (FAX2/FAX4), ER acyl importers (ABCA9), and key acyltransferases can substantially raise or lower TAG content, highlighting transporter-mediated flux as a major control point in oilseed metabolism. In parallel, studies on oleosins, LDPs, and engineered LD proteins show that strengthening the LD sink and limiting TAG turnover can boost TAG levels in both seeds and vegetative tissues, and that TAG stability is crucial to avoid futile cycles and growth penalties in high-oil lines. At the subcellular level, ER–plastid MCSs and their associated lipid-transfer complexes are now recognized as key hubs for glycerolipid exchange, but their specific roles in channeling precursors into storage TAG during seed filling remain poorly understood. Integrating transporter biology, MCS function and LD surface regulation with transcriptional control networks and spatially resolved lipidomics will be essential to move from pathway-centric views of TAG biosynthesis to a holistic understanding of how coordinated lipid flux shapes oil yield and composition in oilseed crops. In summary, future gains in oilseed productivity are likely to rely less on identifying new

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biosynthetic enzymes and more on deciphering and redirecting the lipid transport and storage networks that control acyl flux across cellular and subcellular compartments.

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