which causes the fraction of pentameric ion channels to increase on the same slow timescale as is observed when pore dilation occurs.

The authors also used cryo-electron microscopy (cryo-EM) to obtain a structure of pentameric TRPV3 with a resolution of 4.4 ångströms. Not all of the details can be resolved, but the structure clearly shows that the pore of the ion channel at its narrowest constriction point is more than 8 Å across — much larger than the pore in the open tetramer, which is about 3.5 Å in diameter at its narrowest point. Taken together, the authors' findings show that TRPV3 pentamers meet both the temporal and architectural criteria associated with pore dilation of these channels.

So why have pentameric TRP channels not been observed previously? The answer could be that expectations often drive observations: that is, the overwhelming success story of the structural biology of TRP channels might have shaped scientists' expectations of what these channels look like.

Furthermore, the protocols used to prepare proteins for structural-biology experiments typically include a final step in which the proteins are separated by their size and shape. The expectation that proteins will form a complex from a particular number of subunits could therefore have resulted in protein species with higher molecular weights being discarded as possible contaminants or non-functional aggregates; removing such material is generally regarded as good biochemical practice. In contrast, Lansky and co-workers were specifically looking for DPBA-enriched pentamers as a result of their HS-AFM experiments, highlighting the need for structural biologists to use complementary methods that account for the highly dynamic nature of TRP channels[2,3,9].

It remains to be seen whether the ability of TRPV3 to exchange subunits is universal among TRP channels and whether researchers can now retroactively identify pentamers in their cryo-EM data sets — possibly even for non-TRP channel types that undergo pore dilation[11]. Perhaps subunit exchange will add another layer of complexity to the already complicated array of processes known to regulate ion-channel activity.

Further studies are now needed to verify the existence of pentameric TRP channels in cells, and to determine the role of naturally occurring molecules and lipids in promoting pentamer formation. The energetics and structural changes associated with subunit release from, and re-incorporation into, protein assemblies also warrant investigation. In the meantime, Lansky and colleagues' study shows that there is still much more to learn about the proteins that spice up our lives.

Ute A. Hellmich is in the Faculty of Chemistry and Earth Sciences, Institute of Organic Chemistry and Macromolecular Chemistry, Friedrich Schiller University Jena, 07743 Jena, Germany; the Cluster of Excellence Balance of the Microverse, Friedrich Schiller University Jena; and at the Center for Biomolecular Magnetic Resonance (BMRZ), Goethe University, Frankfurt, Germany.
e-mail: ute.hellmich@uni-jena.de

1. Liao, M., Cao, E., Julius, D. & Cheng, Y. *Nature* **504**, 107–112 (2013).
2. Goretzki, B., Guhl, C., Tebbe, F., Harder, J.-M. & Hellmich, U. A. *J. Mol. Biol.* **433**, 166931 (2021).
3. Lansky, S. *et al. Nature* **621**, 206–214 (2023).
4. Svedberg, T. & Fåhraeus, R. *J. Am. Chem. Soc.* **48**, 430–438 (1926).
5. Monod, J., Wyman, J. & Changeux, J.-P. *J. Mol. Biol.***12**, 88–118 (1965).
6. Marianayagam, N. J., Sunde, M. & Matthews, J. M. *Trends Biochem. Sci.* **29**, 618–625 (2004).
7. Klotz, I. M., Langebman, N. R. & Dahnall, D. W. *Annu. Rev. Biochem.* **39**, 25–62 (1970).
8. Yang, P. & Zhu, M. X. *Handb. Exp. Pharmacol.* **222**, 273–291 (2014).
9. Heath, G. R. & Scheuring, S. *Curr. Opin. Struct. Biol.* **57**, 93–102 (2019).
10. Yue, L. & Xu, H. *J. Cell Sci.* **134**, jcs258372 (2021).
11. Ferreira, L. G. B. & Faria, R. X. *J. Bioenerg. Biomembr.* **48**, 1–12 (2016).
12. Chung, M.-K., Güler, A. D. & Caterina, M. J. *J. Biol. Chem.* **280**, 15928–15941 (2005).

The author declares no competing interests.
This article was published online on 30 August 2023.

Metabolism

A road less travelled for lipid synthesis

Jean E. Schaffer

The pathway used by mammalian cells to make triglyceride lipids when supplies of fat molecules are high has long been known. A route that works when fat supplies are low has now been discovered. **See p.171**

Triglycerides are key lipids used in the transport of fatty acids through the bloodstream in animals, and serve as the most efficient medium for energy storage in both animal and plant cells. In humans, accumulation of triglycerides in lipid droplets in non-fat tissues is commonly observed in lipid-overloaded states, such as obesity-related metabolic syndrome and type 2 diabetes. It can also be associated with cardiovascular disease[1] and non-alcoholic steatohepatitis[2] (a disease of the liver). Thus, the characterization of molecular pathways for triglyceride synthesis and their regulation is crucial for understanding human physiology and disease. On page 171, McLelland *et al*.[3] uncover a previously unknown pathway for triglyceride synthesis in mammalian cells.

Triglycerides are complex lipids composed of a glycerol molecule linked to three fatty acids. Given the many possible combinations of fatty acids of differing length and saturation and the three positions to which they can bind on glycerol, a vast number of triglyceride molecules can potentially be made.

Since the first description[4] of the enzymatic synthesis of triglycerides in liver extracts in 1960, two enzymes that catalyse the final step of the synthesis have been extensively characterized in mammalian cells, mice and humans[5] (Fig. 1a). Known as DGAT1 and DGAT2, these 'acyltransferase' enzymes add a fatty-acid derivative (a fatty acyl-CoA molecule) to a diacylglycerol — a glycerol molecule that already has two fatty-acid-derived groups (fatty acyl chains) attached to it. DGAT1 is embedded in the membrane of the endoplasmic reticulum, the cellular organelle that acts as a main site of lipid and protein synthesis, and also catalyses the reaction of acyl-CoA molecules with long-chain alcohols, vitamin A and monoacylglycerols (glycerol molecules with just one fatty acyl chain attached). DGAT2 localizes to both the endoplasmic reticulum and to growing lipid droplets and is involved mainly in triglyceride synthesis.

Mice that have been genetically engineered to have non-functional DGAT enzymes have striking traits (phenotypes), providing strong evidence that triglyceride-synthesis pathways involving DGATs are crucial for regulating lipid levels in mammalian tissues[6,7]. Other pathways for triglyceride synthesis that are independent of fatty acyl-CoAs, and which instead use phospholipid molecules as fatty acyl donors, have been described in yeast and plants[8]. But whether alternative routes for triglyceride synthesis exist in mammals has been largely unexplored — until now.

McLelland and colleagues used a method known as sequential CRISPR loss-of-function screening to identify one such alternative pathway in a human cell line (HAP1 cells). To search for pathways that are independent of known DGATs, they engineered HAP1 cells to be deficient in both DGAT1 and DGAT2. The

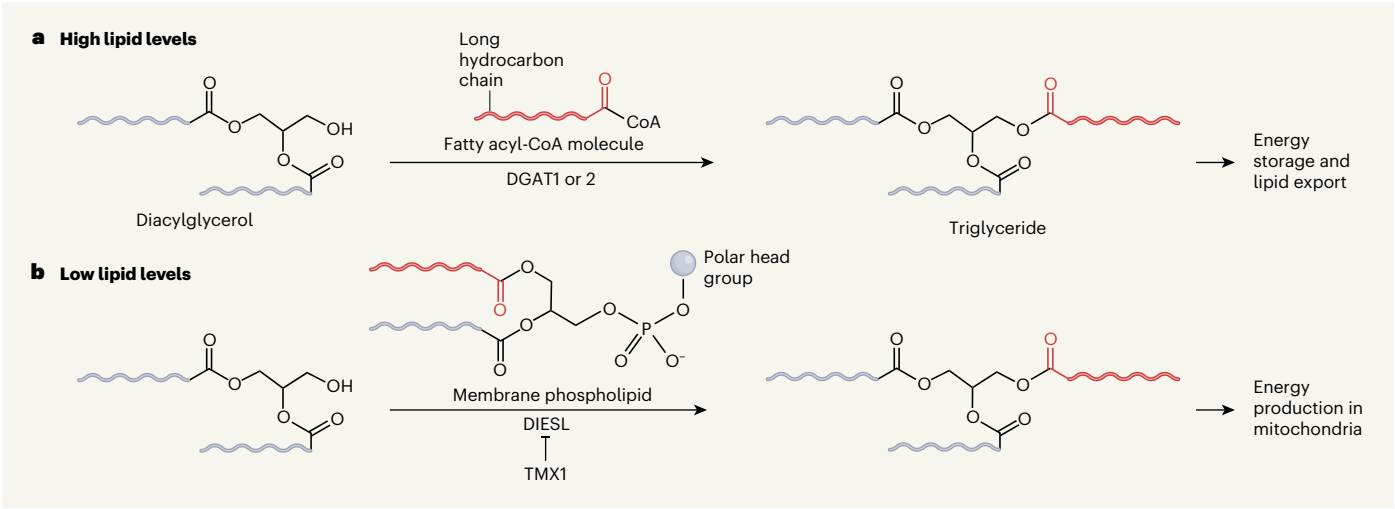

Figure 1 | Independent pathways for the biosynthesis of triglycerides. **a**, Lipids called triglycerides are the main constituents of fats stored in plants and animals. Triglyceride biosynthesis in mammals involves the reaction of diacylglycerol molecules with derivatives of fatty acids, known as fatty acyl-CoA molecules; CoA is an enzyme cofactor. An enzyme (DGAT1 or DGAT2) catalyses the transfer of a fatty acyl group (red) from the fatty acyl-CoA to the diacylglycerol, thereby forming a triglyceride. This pathway operates when lipid supplies to cells are high, and the products are used for energy storage or to export lipids from cells. **b**, McLelland *et al.*[3] report an independent pathway for mammalian triglyceride synthesis, which operates when lipid supplies are low. The enzyme DIESL catalyses the transfer of fatty acyl groups from membrane phospholipids to diacylglycerols, an activity that is regulated by the protein TMX1. Triglycerides formed in this way seem to support the functions of mitochondria, the power-generating organelles in cells.

authors then made further random gene disruptions in these cells, and discovered that loss of the protein transmembrane thioredoxin 1 (TMX1) increases triglyceride accumulation when no extra fatty acids were supplied to the cells. Because TMX1 is an enzyme that does not itself catalyse triglyceride synthesis, this finding suggested that TMX1 usually restricts triglyceride formation by an enzyme that is distinct from DGAT1 and DGAT2.

The authors then searched for this triglyceride-synthesis enzyme using a genetic approach known as a suppressor screen. In this case, they looked for genes that, when disrupted, limited triglyceride synthesis in HAP1 cells lacking TMX1. This led them to a previously uncharacterized protein — TMEM68, found in the endoplasmic reticulum — as the enzyme responsible for triglyceride accumulation when TMX1 is disrupted. The authors renamed this protein DGAT1/2-independent enzyme synthesizing storage lipids (DIESL).

Through a series of biochemical and genetic analyses, McLelland *et al.* demonstrated that DIESL is a diacylglycerol acyltransferase, the activity of which is decreased by interaction with TMX1 (Fig. 1b). When the authors introduced DIESL into cells lacking DGAT1, DGAT2, DIESL and TMX1, they observed increases in triglyceride levels, and a concomitant decrease in diacylglycerols and membrane phospholipids — suggesting that membrane phospholipids serve as donors of fatty acyl groups in the reaction catalysed by DIESL.

So what is the biological role of DIESL? McLelland and colleagues found that, in cultured cells, DIESL plays a key part in maintaining the function of mitochondria — the organelles that act as powerhouses for cells — under conditions in which the supply of lipids is limited. Furthermore, when the authors generated 'knockout' mice lacking the gene that encodes DIESL, the animals were found to gain weight more slowly than did wild-type mice around the time of weaning, a metabolically stressful period when the amount of lipid in the diet decreases markedly. Moreover, compared with wild-type mice, the adult knockout mice had lower body weight, less body fat and lower levels of triglycerides in the bloodstream.

What are the implications of this newly discovered pathway for triglyceride synthesis? In contrast to the DGAT enzymes, which support triglyceride synthesis when extracellular lipid concentrations are high, TMX1 and DIESL seem to promote triglyceride synthesis when lipid supply is low, consuming membrane phospholipids in the process. Whether triglycerides are synthesized through TMX1–DIESL pathways or through DGAT-mediated routes might affect the size and location of lipid droplets in cells, and this could be key in determining the ultimate fate of the fatty acids released when those triglycerides are hydrolysed. TMX1 and DIESL are widely expressed in human organs, suggesting that they could have a role in maintaining lipid levels in many tissues.

McLelland and colleagues' study sets the stage for work to establish the context in which TMX1 and DIESL contribute to lipid metabolism. An important next step will be the definitive identification of the source of the acyl chains that are incorporated by DIESL into triglycerides, and the determination of the ultimate fate of those triglycerides. This will require techniques in which labelled acyl chains are followed as they are incorporated into triglyceride molecules and subsequently released after their hydrolysis, as well as examination of many classes of lipid. An unanswered question is whether the interaction between TMX1 and DIESL is modulated by the availability of nutrients.

Mouse studies in which DIESL is disrupted in specific cell types, and in which animals are tested under several nutrient conditions, will help to clarify the physiological importance of this pathway. Investigations into the genetic variation and regulation of TMX1 and DIESL in human tissues will also shed light on the pathway's relevance in disease. Whether physiological triglyceride synthesis is mediated by DGAT proteins, or proceeds through the less-travelled TMX1–DIESL route, could make all the difference for metabolic homeostasis.

Jean E. Schaffer is at the Joslin Diabetes Center and in the Department of Medicine, Harvard Medical School, Boston, Massachusetts 02215, USA.
e-mail: jean.schaffer@joslin.harvard.edu

1. Goldberg, I. J. *et al. Circulation* **138**, 305–315 (2018).
2. Diehl, A. M. & Day, C. *N. Engl. J. Med.* **377**, 2063–2072 (2017).
3. McLelland, G.-L. *et al. Nature* **621**, 171–178 (2023).
4. Weiss, S. B., Kennedy, E. P. & Kiyasu, J. Y. *J. Biol. Chem.* **235**, 40–44 (1960).
5. Farese, R. V. Jr & Walther, T. C. *Cold Spring Harb. Perspect. Biol.* **15**, a041246 (2023).
6. Liu, L. *et al. J. Biol. Chem.* **289**, 29881–29891 (2014).
7. Gluchowski, N. *et al. Hepatology* **70**, 1972–1985 (2019).
8. Dahlqvist, A. *et al. Proc. Natl Acad. Sci. USA* **97**, 6487–6492 (2000).

The author declares no competing interests.
This article was published online on 30 August 2023.