The PAQR-2 and HIF-1 pathways are physiologically essential for unsaturated fatty acid homeostasis in *C. elegans*

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To my family,

ABSTRACT

Since the 1930s, scientists have known that certain unsaturated fatty acids are essential in the human diet and act as precursors for further fatty acid synthesis. Unlike humans, the nematode *C. elegans* can *de novo* synthesize omega-3 and omega-6 polyunsaturated fatty acids, making it a useful species for studying fatty acid function. Fatty acids form the tails of phospholipids, the main component of cellular membranes, and modulating the identity of these tail fatty acids has important implications for membrane homeostasis. Saturated fatty acids have membrane rigidifying effects, while unsaturated fatty acids fluidize membranes; thus, a proper balance between the two is crucial for several membrane properties. One way by which cells achieve membrane homeostasis is through the PAQR-2 membrane fluidity regulator that responds to membrane rigidification by increasing fatty acid desaturation and incorporation of unsaturated fatty acids into phospholipids.

In the first part of this thesis, the effect of excessively rigid and excessively fluid membranes on several cellular and physiological traits were studied in *C. elegans* and revealed that deviation from optimal membrane composition in either direction is deleterious. Next, we further characterized the molecular basis of PAQR-2 activity, revealing that PAQR-2 recruits a complex containing enzymes important for fatty acid elongation and for channeling of unsaturated fatty acids into phospholipids. In the final part of this thesis, a forward genetics screen led to the discovery that the HIF-1 pathway can potentiate desaturase activity in a *C. elegans* mutant that is almost wholly devoid of polyunsaturated fatty acids. We conclude that the PAQR-2 and HIF-1 pathways are regulators of unsaturated fatty acid homeostasis essential for the physiological health of *C. elegans.*

Keywords: PAQR-2, HIF-1, FAT-2, fatty acid synthesis, membrane homeostasis, unsaturated fatty acid, *C. elegans*

SAMMANFATTNING PÅ SVENSKA

Sedan 1930-talet har forskare känt till att vissa omättade fettsyror är nödvändiga i människans kost och att de fungerar som prekursorer för vidare fettsyrasyntes. Till skillnad från människan kan nematoden *C. elegans* syntetisera fleromättade omega-3- och omega-6-fettsyror *de novo*, vilket gör den till en användbar art för att studera fettsyrornas funktion. Fettsyror bildar svansarna på fosfolipider, huvudkomponenten i cellmembran, och modulering av identiteten hos dessa fettsyrasvansar har viktiga konsekvenser för membranhomeostasen. Mättade fettsyror har en membranförstelnande effekt, medan omättade fettsyror gör membranen mer flytande; en korrekt balans mellan de två är därför avgörande för flera membranegenskaper. Ett sätt för celler att upprätthålla korrekt membranhomeostas är genom PAQR-2, en membranfluiditetsregulator som svarar på utmaningar i membranhomeostasen genom att öka fettsyradesaturationen och inkorporeringen av omättade fettsyror i fosfolipider.

I den första delen av denna avhandling försökte vi karakterisera effekten av alltför stela och alltför flytande membran på flera cellulära och fysiologiska egenskaper, och fastställde att avvikelser från optimal membransammansättning är skadligt i båda riktningarna. Därefter karakteriserade vi ytterligare mekanismerna nedströms från membranfluiditetsregulatorn PAQR-2 och fann att PAQR-2 rekryterar ett komplex innehållande enzymer som är viktiga för fettsyraförlängning och kanalisering av omättade fettsyror. I den sista delen av denna avhandling använde vi oss av framåtgenetik för att klassificera HIF-1-reaktionsvägen som en viktig regulator av omättade fettsyrors desaturering i *C. elegans*-mutanter som nästan helt saknar fleromättade fettsyror. Vi drar slutsatsen att PAQR-2 och HIF-1 är viktiga regulatorer för homeostasen av omättade fettsyror i *C. elegans.*

PUBLICATIONS

This thesis is based on the follow publications (* indicates shared first authorship)

I. **A genetic titration of membrane composition in *Caenorhabditis elegans* reveals its importance for multiple cellular and physiological traits**
Devkota R., Kaper D., Bodhicharla R., Henricsson M., Borén J., & Pilon M.
Genetics 2021

II. **AdipoR2 recruits protein interactors to promote fatty acid elongation and membrane fluidity**
Ruiz M.*, Devkota R.*, Kaper D.*, Ruhanen H., Busayavalasa K., Radović U., Henricsson M., Käkelä R., Borén J., & Pilon M.
Journal of Biological Chemistry 2023

III. **A *fat-2(wa17)* suppressor screen in *C. elegans* reveals genetic adaptations to polyunsaturated fatty acid deficiency**
Kaper D., Radović U., Bergh P-O., Qvist A., Henricsson M., Borén J., Pilon M.
eLife 2024 (Peer-reviewed preprint; https://doi.org/10.7554/eLife.104181.1.sa3)

Other publications not included in this thesis:

I. **Sphingosine 1-phosphate mediates adiponectin receptor signaling essential for lipid homeostasis and embryogenesis**
Ruiz M., Devkota R., Panagaki D., Bergh P.-O., Kaper D., Henricsson M., Nik A., Petkevicius K., Höög J. L., Bohlooly-Y M., Carlsson P., Borén J., & Pilon M.
Nature Communications 2022

II. **TLCD1 and TLCD2 regulate cellular phosphatidyl-ethanolamine composition and promote the progression of non-alcoholic steatohepatitis**
Petkevicius K., Palmgren H., Glover M., Ahnmark A., Andréasson A-C., MadeyskiBengtson K., Kawana H., Allman E., Kaper D., Uhrbom M., Andersson L., Aasehaug L., Forsström J., Wallin S., Ahlstedt I., Leke R., Karlsson D., Löfgren L., Nilsson R., Pellegrini G., Aoki J., Sienski G., Pilon M., Bohlooly-Y M., Maresca M., Peng XR.
Nature Communications 2022

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ABBREVIATIONS

AA	**A**rachidonic **a**cid
ACS	**A**cetyl-**C**oA **s**ynthetase
AdipoR	**Adipo**nectin **r**eceptor 2
ALA	**A**lpha-**l**inolenic **a**cid
DHA	**D**ocosa**h**exaenoic **a**cid
EMS	**E**thyl **m**ethane**s**ulfonate
EPA	**E**icosa**p**entaenoic **a**cid
FA	**F**atty **a**cid
FASN	**F**atty **a**cid **sy**n**n**thase
FRAP	**F**luorescence **r**ecovery **a**fter **p**hotobleaching
HIF	**H**ypoxia **i**nducible **f**actor
LA	**L**inoleic **a**cid
MDT	**Me**d**i**a**t**or
mmBCFA	**M**ono**m**ethyl **b**ranched-**c**hain **f**atty **a**cids
MUFA	**M**ono**u**nsaturated **f**atty **a**cid
NHR	**N**uclear **h**ormone **r**eceptor
OA	**O**leic **a**cid
PA	**P**almitic **a**cid
PAQR	**P**rogestin and **a**dipo**Q** **r**eceptor
PC	**P**hosphatidyl**c**holine
PE	**P**hosphatidyl**e**thanolamine
PUFA	**P**oly**u**nsaturated **f**atty **a**cid
SCD	**S**tearoyl-**C**oA **d**esaturase
SFA	**S**aturated **f**atty **a**cid
TAG	**T**ri**a**cyl**g**lyceride
UFA	**U**nsaturated **f**atty **a**cid
VLCFA	**V**ery **l**ong **c**hain **f**atty **a**cid

INTRODUCTORY WORDS

This thesis begins with a brief overview of cellular membrane composition and homeostasis. This is followed by a description of lipid metabolism and fatty acid synthesis in the model organism *Caenorhabditis elegans, C. elegans*. The PAQR-2 and HIF-1 pathways that have been in focus throughout much of the work will then be described in some details. The results section is then divided into three main parts: 1) effects of membrane composition on physiology; 2) membrane homeostasis pathways; and 3) mechanisms that compensate for low fatty acid desaturase activity.

DISCOVERY OF ESSENTIAL FATTY ACIDS

In 1929, George Burr discovered that excluding fat from the diet of rats led to severe side-effects and eventually death. When fed a fat-free diet, the rats developed what Burr deemed to be a deficiency disease characterized by scaly skin and sores, inflamed tails and paws, fur loss, and finally kidney degeneration that led to premature death. However, these severe effects of a fat-free diet could be reversed when small quantities of lard were added back to the diet, though not when only vitamins (some of which are fat-soluble) were added (Burr & Burr, 1929). Burr thus discovered that some dietary fats must be essential. In 1930, Burr published a follow-up paper detailing that certain polyunsaturated fatty acids (PUFAs) are the essential components missing from the fat-free diet. When attempting to rescue fat-free diet rats with different fats, it was found that providing the animals with butter or coconut oil, which contain no unsaturated fatty acids (UFAs), did not restore the rats to health. Instead, the addition of olive oil, linseed oil, and corn oil, all of which are high in linoleic acid, an 18:2 omega-6 (ω-6) fatty acid, rescued the deficiency disease phenotypes (Burr & Burr, 1930). Thus, linoleic acid was deemed to be an essential fatty acid in the diet. Subsequently in 1932, George Burr showed that alpha-linolenic acid, an 18:3 ω-3 fatty acid, is also an essential fatty acid (Burr et al., 1932). While mammals do have fatty acid desaturation and elongation mechanisms in place, they cannot produce PUFAs from *de novo* lipogenesis nor from

monounsaturated fatty acid (MUFA) precursors; linoleic and alpha-linolenic acid are therefore the precursors for all other PUFAs and without them no other PUFAs can be synthesized. During Burr's studies of fat-free diet rats, he remarked that "lack of dietary fat has so injured the tissues that they are no longer the normal membranes separating the interior of the animal from its relatively dry air environment" (Burr & Burr, 1930). What George Burr described above reflects an essential function of fatty acids, namely, to form membranes that separate the interior from the exterior of cells, and that disruptions to this membrane can cause a multitude of problems, which I will describe further in this thesis.

CELLULAR MEMBRANES

Cellular membranes are essential for numerous cellular properties and processes such as forming a boundary between extracellular and intracellular space, regulating signaling and transport into and out of the cell, and contributing to cell-cell interactions. The plasma membrane encapsulates the cell and separates the external environment from the cellular components, while internal cellular membranes define different organelles within the cell. The plasma membrane controls the transport of nutrients into the cell and waste out of the cell, maintains ion gradients and hosts numerous proteins with varied functions including transporters, structural proteins and signaling protein; internal membranes define organelles and often mediate communication and transport between them via vesicles or more direct membrane-to-membrane contacts (Singer & Nicolson, 1972; Stillwell, 2013).

The composition of cellular membranes determines their properties such as fluidity, compressibility, permeability, charge, etc. The main component of cellular membranes are phospholipids, each with a hydrophilic head group and two hydrophobic fatty acid tails (Fig. 1A); phospholipids form bilayers with the head groups in contact with the aqueous milieu and the fatty acid tails of each leaflet pointing towards those of the other (Singer & Nicolson, 1972). There are several types of phospholipids, and here only a

few will be mentioned. Phospholipids that contain a glycerol backbone are called glycerophospholipids and, depending on their head groups, include phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylserine (PS), and phosphatidic acid (PA) (Fig. 1B) (Van Meer et al., 2008). Glycerophospholipids have three "sn" positions; sn, or stereospecific numbering, refers to the carbon atoms of glycerol, with the sn3 esterified to a phosphate group that is then attached to a short-chain alcohol, for example choline, ethanolamine, etc (Kelly & Jacobs, 2011; Manni et al., 2018). The headgroup of the phospholipid can have effects on physical membrane properties such as curvature and packing. Of the two most common phospholipids in eukaryotic membranes, PCs, for example, have a larger head group than PEs and have an overall cylindrical shape that tends to form a flat bilayer membrane as opposed to the smaller headgroup in PEs that create a conical shape that tends to form a curved membrane (McMahon & Boucrot, 2015; Satouchi et al., 1993). Additionally, the ratio of PCs to PEs can affect additional membrane properties such as membrane thickness and permeability (McMahon & Boucrot, 2015).

The sn1 and sn2 positions of the glycerol are esterified with fatty acids; sn1 typically holds a saturated fatty acid while the sn2 fatty acid can be saturated or unsaturated (Manni et al., 2018). Fatty acids consist of a chain of carbon atoms attached to a carboxyl group and differ by their degree of saturation (or number of carbon-carbon double bonds in the acyl chain): saturated fatty acids (SFAs) have zero double bonds, monounsaturated fatty acids (MUFAs) have one double bond, and polyunsaturated fatty acids (PUFAs) have two or more double bonds in their carbon chain (Fig. 1C) (Stillwell, 2013). Fatty acids are commonly referred to by the number of carbons in the acyl chain and the number of double bonds. Oleic acid, for example, has an 18-carbon chain with one double bond and thus can be written as 18:1, though this system does not specify the position of the double bond (Köfeler, 2016). PUFAs are further classed as ω-3 or ω-6 depending on whether the first double bond is found either at the third or sixth carbon from the terminal methyl group (it is important to note that the ω position of FAs can also be written as "n", for example in 18:2n6, and

will be used as such throughout this thesis) (Köfeler, 2016). Membranes rich in SFAs tend to be more rigid and tightly packed, while the kinks caused by the double bonds in unsaturated fatty acids allow membranes rich in UFAs to be more fluid and loosely packed (Fig. 1D) (A. G. Lee, 1991).

While the majority of membrane lipids are phospholipids, other lipid classes found in biological membranes include sphingolipids, glycolipids and sterols. Instead of the phosphate group found in phospholipids, glycolipids contain a sugar molecule, such as glucose, that is attached to the glycerol, or if the sugar is attached to a sphingosine molecule the lipid is then called a glycosphingolipid. Sterols on the other hand, consist of a single hydroxyl group that is attached to a four- or five-ring steroid structure and a short hydrocarbon side chain (Fahy et al., 2005; Watson, 2015). There is great variation in the use of sterols as membrane components. While a small amount of dietary sterols are essential in the nematode *C. elegans,* they do not appear to serve a structural role in membranes (Kurzchalia & Ward, 2003). In contrast, mammals can synthesize sterols *de novo* and the sterol cholesterol accounts for ~45 mole% of total lipids in their plasma membranes (Das et al., 2013; Lange et al., 1989).

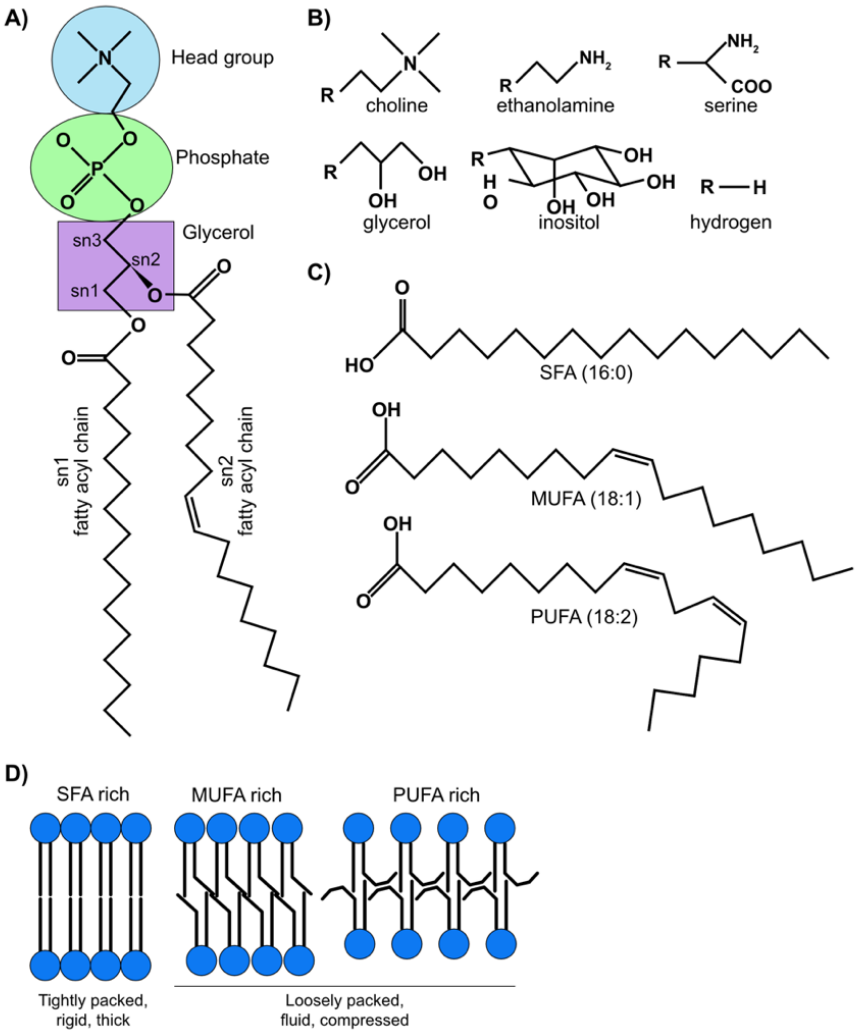

Figure 1. Overview of cellular membranes. A) Example of phospholipid structure. This phospholipid has a choline headgroup and fatty acyl chains at the sn1 and sn2 positions of the glycerol. B) Types of phospholipid head groups. C) Examples of fatty acid types. 16:0 (palmitic acid; PA) is a SFA, 18:1 (oleic acid; OA) is a MUFA, and 18:2 (linoleic acid; LA) is a PUFA. D) Phospholipid bilayers rich in SFAs are tightly packed and rigid while membranes rich in MUFAs or PUFAs are more loosely packed, thinner and fluid.

MEMBRANE HOMEOSTASIS

Membrane homeostasis is the process by which cells maintain the proper structure, composition, and function of their membranes despite eventual changes in the environment (like temperature, pH, lipid availability, etc.). The first study describing adaptive regulation of membrane composition concerned "homeoviscous adaptation" in *E. coli*, whereby the cell adapts to increasing temperature, which could lead to excessive membrane fluidity, by incorporating increasing amounts of long-chain and saturated FAs into its phospholipids (Sinensky, 1974). Cellular membranes, like other fatty substances, tend to become more rigid at cold temperatures and more fluid at warmer temperatures (Los & Murata, 2004), and regulating the type of FA that is incorporated into membranes is a way to maintain optimal membrane properties as temperatures vary. Homeoviscous adaptation not only occurs in *E. coli* but has also been well documented in several poikilotherms, i.e. animals whose body temperatures vary with environmental temperature: fish, insects, and nematodes, among others, boost incorporation of PUFAs into cellular membranes in cold conditions and incorporation of SFAs in warm conditions (Hazel, 1984, 1995; Overgaard et al., 2008; Watts & Ristow, 2017).

The molecular mechanisms through which organisms maintain their optimal membrane composition via homeoviscous adaptation differs between species; this thesis will focus mainly on membrane homeostasis in *C. elegans*. The nematode *C. elegans* is known for its ease of cultivation at temperatures between 15°C and 25°C, though 20°C is the standard temperature for laboratory maintenance. In colder conditions, membranes become more rigid, and the Δ9 desaturase FAT-7 is activated to boost the production and incorporation of UFAs into phospholipids (Murray et al., 2007). The FAT-7 desaturase creates the first double bond in the SFA stearic acid (SA, 18:0), producing the MUFA oleic acid (OA, 18:1) (Watts & Browse, 2000). Oleic acid is then able to be further desaturated by the Δ12 desaturase FAT-2 into linoleic acid (LA, 18:2) which is used as a substrate to produce longer PUFAs (Wallis et al., 2002). Δ9 desaturases insert a double bond at the 9^{th} carbon in the chain (starting from the carboxyl end), while

Δ12 desaturases insert a double bond at the 12th carbon. The FAT-7-dependent increase in UFAs is then responsible for restoring fluid membranes when worms encounter colder conditions (Murray et al., 2007). *C. elegans* is one of the few eukaryotes that can *de novo* synthesize PUFAs thanks to the FAT-2 desaturase that allows it to produce LA. In contrast, mammals and most other animals must obtain LA and ALA from the diet as starting ω-3 and -6 PUFAs to synthesize other PUFAs (Burr et al., 1932; Perez & Van Gilst, 2008; Rappleye et al., 2003; Wallis et al., 2002). How FAT-7 is regulated during temperature shifts is dependent in part on the evolutionarily conserved acyl-CoA-dehydrogenase ACDH-11 and PAQR-2 (Progestin and AdipoQ Receptor-2). During cold conditions, the membrane fluidity sensor PAQR-2 acts through the transcription factor NHR-49 to activate FAT-7 expression to boost desaturation and fluidize membranes (Svensk et al., 2013); more information about PAQR-2 will be presented later in this thesis. At the other end of the spectrum, one study has suggested that ACDH-11 is upregulated in high temperature conditions and sequesters C11/C12 fatty acids which in turn prevents NHR-49 activation and FAT-7 dependent desaturation, leading to increased SFA levels in membranes (Ma et al., 2015).

DIET AND MEMBRANE HOMEOSTASIS

While *C. elegans* can *de novo* synthesize most of its fatty acids, it can also obtain them from the diet (Perez & Van Gilst, 2008; Watts, 2009). Mammals, on the other hand, must obtain certain fatty acids from their diet and subsequently the nature of these ingested fatty acids can influence membrane composition. Over the years dietitians and other health professionals have claimed that the Mediterranean diet, which emphasizes an abundance of ω-3 rather than ω-6 PUFAs, is a diet of choice for a long and healthy life. When people eat ω-3 rich foods or supplements, the ω-3 FAs partially replace ω-6 FAs in the cell membranes with beneficial effects (Simopoulos, 1991). Several studies have suggested that adhering to the Mediterranean diet can reduce the risk of cardiovascular disease (Estruch et al., 2018; Kouli et al., 2019), diabetes (Martínez-González et al., 2008), inflammation (Sureda et al., 2018), and certain types of cancers (Bamia et

al., 2013; Pasanisi et al., 2018; Schneider et al., 2019; Schwingshackl et al., 2017). One study has found that the Mediterranean diet, which promotes the use of OA-rich olive oil and ALA-rich nuts, reduces the cholesterol/phospholipid ratio in erythrocyte cell membranes, which has a beneficial effect on membrane fluidity and, moreover, changes the composition of phospholipids by increasing levels of PEs. Additionally, the levels in membranes of ω-3 fatty acids increased while the concentrations of ω-6 fatty acids decreased (Barceló et al., 2009). A different study saw that while ω-3 fatty acid levels in erythrocytes did not change in subjects adopting the Mediterranean diet, levels of MUFAs did increase compared to the control group and compared to the test group's starting levels (Davis et al., 2017). Another paper noted that after 12 months on the Mediterranean diet, ω-3 levels in erythrocyte membranes did not increase significantly but that there was a decrease in ω-6 fatty acid levels (Seethaler et al., 2020). Thus, there does not seem to be a consensus on the effects of the Mediterranean diet on membrane composition, which could be due to variation in study designs. The age, sex, health and fitness levels, and study length were different for each of the three studies mentioned, and although they did not see the same changes in fatty acid profiles, they all did agree that the Mediterranean diet does have an impact on fatty acid composition in erythrocytes.

In controlled animal studies, when mouse diets are supplemented with either long-chain ω-3 PUFA-rich fish oil or ω-6 LA-rich corn oil, a robust incorporation of ω-3 PUFAs into cardiac membrane lipids of the fish oil fed mice relative to the corn oil fed mice was recorded (Levental et al., 2020). It seems, however, that it is mainly the ratio of ω-3 to ω-6 dietary PUFAs that influences membrane composition, but that overall SFA and UFA levels are homeostatically regulated regardless of dietary variation. In fact, in a study feeding rats diets varying in FA composition, membrane phospholipids maintained a constant membrane SFA content regardless of dietary SFA concentration, while MUFA and PUFA content varied only slightly with dietary variation. When examining triglycerides though, FA concentrations were heavily influenced by diet (Abbott et al., 2012).

Mechanisms must therefore exist that effectively guard membrane composition, but less so triglyceride composition, against the potential impacts of diets that can vary greatly in their fatty acid composition and content.

C. ELEGANS

C. elegans is a small transparent nematode found in soil and that feeds mostly on the bacteria of rotting vegetation. *C. elegans* has a three-day-long life cycle in which it develops from embryo through four larval stages, L1-L4, before becoming an adult (Brenner, 1974). Once an adult, a single worm can live for approximately three weeks in optimal conditions, i.e. temperatures between 15°C and 25°C, although worms cultivated at 15°C live longer than those cultivated at 20°C or 25°C (Johnson & Hutchinson, 1993). There are two sexes in *C. elegans*: hermaphrodites, which produce both sperm and eggs allowing for self-fertilization, and males, which produce only sperm and can fertilize hermaphrodites (Nigon & Dougherty, 1949). Both sexes have five pair of autosomal chromosomes, while hermaphrodites have two X chromosomes (XX) and males have a single X chromosome (X0). A single hermaphrodite can self-fertilize to produce approximately 300 progeny, but if mated with a male can produce around 1,000 progeny (Nigon & Dougherty, 1949). An adult hermaphrodite has 959 somatic cells, of which 302 are neurons, while an adult male has 1,031 somatic cells including 381 neurons (Sulston et al., 1983). Adult hermaphrodites are ~1 mm in length when fully developed, and length is sometimes used as a measure of overall health because sick worms usually grow more slowly and are shorter (Brenner, 1974).

In the 1970s, Sydney Brenner began using *C. elegans* as a model organism due to its ease of cultivation and rapid life cycle, as well as for the ease with which it can be used for forward and reverse genetic studies, and *C. elegans* is today an established model supported by a worldwide research community (Kutscher & Shaham, 2014). In 1998, *C. elegans* became the first multicellular species to have its entire genome sequenced (Consortium*, 1998). The *C. elegans* haploid genome consists of approximately 100 million

base pairs and codes for around 20,000 proteins. Importantly, around 60-80% of human genes have *C. elegans* orthologs (Kaletta & Hengartner, 2006). Additionally, of the over 400 lipid metabolism-related genes in *C. elegans,* over 70% are homologous to mammalian lipid metabolism genes (Zhang et al., 2013).

C. ELEGANS LIPID METABOLISM

Much of what we know about fatty acid metabolism in *C. elegans* is thanks to forward genetic screens looking for mutants with abnormal fatty acid composition. The most comprehensive study on PUFA synthesis in *C. elegans* relied on ethyl methanesulfonate (EMS)-mutagenizing worms and identifying mutants with altered fatty acid composition by mass spectroscopy (Watts & Browse, 2002). Mutants were discovered with defects in *fat-1*, *fat-2*, *fat-3*, *fat-4*, and *elo-1,* together representing every step of the PUFA synthesis pathway, and following the conversion of 18:1n9 to 20:5n3. The study further revealed that each desaturation step is carried out exclusively by one enzyme while PUFA elongation is carried out by two or more enzymes (Watts & Browse, 2002).

C. elegans stores much of its fat as triacylglycerides (TAGs)-containing lipid droplets within the intestine, with the rest found mostly found in hypodermis or in the yolk of oocytes (Mullaney & Ashrafi, 2009). While *C. elegans* can *de novo* synthesize fatty acids, laboratory-grown worms obtain the majority of their fatty acids from the standard *E. coli* OP50 diet. In fact, although *C. elegans* can synthesize the SFA palmitic acid (16:0) from malonyl-CoA and acetyl-CoA, around 90% of the PA in a lab-grown worm comes from the diet (Perez & Van Gilst, 2008). The levels of MUFAs and PUFAs derived from diet (rather than *de novo* synthesized) is lower, with ~80% of UFAs coming from the bacterial diet (Perez & Van Gilst, 2008). While a large fraction of SFAs and UFAs are taken up from bacteria, *C. elegans* synthesize almost all monomethyl branched chain fatty acids (mmBCFA) *de novo* (Kniazeva et al., 2004). As mentioned earlier in this thesis, whereas mammals lack the enzymes required to synthesize PUFAs from MUFAs or to convert ω-6 fatty acids to ω-3 fatty acids, *C. elegans* does

have such enzymes (Burr et al., 1932; Perez & Van Gilst, 2008; Rappleye et al., 2003; Rivers et al., 1975; Wallis et al., 2002). Specifically, *C. elegans* can convert OA (18:1) to LA (18:2) with the Δ12 FAT-2 desaturase while the ω-3 fatty acid desaturase FAT-1 is responsible for adding an additional carbon-carbon double bond three carbons away from the terminal methyl group to create ω-3 fatty acids (Peyou-Ndi et al., 2000; Spychalla et al., 1997; Watts & Browse, 2002). Starting at the beginning of fatty acid synthesis in *C. elegans*, acetyl-CoA is converted to malonyl-CoA by POD-2, followed by fatty acid synthase FASN-1 (homolog of human FASN) catalyzing the production of PA through a seven-step process (Rappleye et al., 2003; Smith, 1994). PA can then be elongated to 18:0 by the elongase ELO-2 or desaturated by one of three Δ9 desaturases, i.e. FAT-5, -6 and-7, to produce 16:1n7 that is elongated to 18:1n7 (vaccenic acid) which is the most common 18:1 fatty acid in *C. elegans* (Watts, 2009; Watts & Browse, 2000); note that while *C. elegans* can synthesize vaccenic acid from scratch, this fatty acid is actually mostly obtained directly from the dietary *E. coli*. Separately 18:0 can be desaturated by FAT-6/FAT-7 to produce 18:1n9 (oleic acid), which is the substrate for desaturation by the Δ12 desaturase FAT-2 to begin the synthesis of PUFAs by producing 18:2n6 (LA). LA and subsequent PUFAs are then further elongated by the elongase ELO-1 or ELO-2, and further desaturated by FAT-1, FAT-3 or FAT-4 to produce the various PUFAs found in *C. elegans*, including the longest and most unsaturated one, namely 20:5 (EPA) (Watts, 2009; Watts & Browse, 2002). In a separate pathway downstream of acetyl-CoA, *C. elegans* also produce mmBCFAs with elongases ELO-5/ELO-6 (Kniazeva et al., 2004). The entire *C. elegans* FA synthesis pathway is illustrated in (Fig. 2). As mentioned above, the majority of fatty acids in *C. elegans* are obtained from the diet, except for mmBCFAs, SA, OA, and LA which are low in abundance in bacteria and almost exclusively synthesized by the worm (Kniazeva et al., 2004; Perez & Van Gilst, 2008).

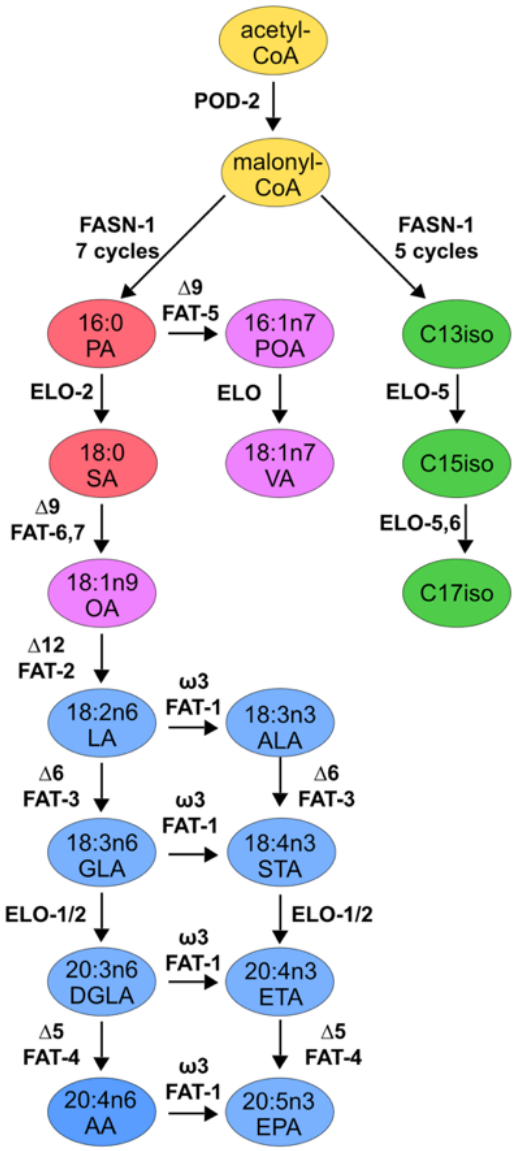

Figure 2. Fatty acid synthesis in *C. elegans*. Schematic of the fatty acid synthesis pathway. Ovals represent fatty acid products and the enzymes responsible are labeled next to arrows. Fatty acid abbreviations are as follows: palmitic acid (PA), palmitoleic acid (POA), vaccenic acid (VA), stearic acid (SA), oleic acid (OA), linoleic acid (LA), alpha-linolenic acid (ALA), gamma-linoleic acid (GLA), stearidonic acid (STA), dihomo-gamma-linolenic acid (DGLA), eicosatetraenoic acid (ETA), eicosapentaenoic acid (EPA). Adapted from (Watts & Ristow, 2017).

C. elegans can synthesize mmBCFAs composed of a saturated fatty acid with a single methyl group attached to the penultimate carbon (C13iso, C15iso, C17iso); their synthesis requires the elongases ELO-5 and ELO-6, and the 3-ketoacyl-CoA reductase LET-767 (Entchev et al., 2008; Kniazeva et al., 2004). *C. elegans* can also dietarily obtain ante-iso mmBCFAs (C15- and C17-ante-iso), where the methyl group is on the second to the terminal carbon, but do not *de novo* synthesize them (Diot et al., 2022; Kniazeva et al., 2004). mmBCFAs are essential in worms: mutants that are unable to synthesize them arrest as larvae (Entchev et al., 2008; Kniazeva et al., 2004; Vieira et al., 2022). While mmBCFAs can be found in TAGs, PCs, and PEs, all sphingolipids contain C17iso as the long chain acyl component in *C. elegans* (Chitwood et al., 1995; H. Zhu et al., 2013). Indeed, the larval arrest phenotype of *elo-5* mutants is dependent on the lack of C17iso in sphingolipids (H. Zhu et al., 2013).

Perhaps the best characterized fatty acid desaturases in *C. elegans* are FAT-5, FAT-6, and FAT-7. As mentioned above, FAT-7 is critical for temperature-dependent regulation of membrane saturation, but FAT-7 is not the only Δ9 desaturase in *C. elegans*. FAT-5 and FAT-6 are also Δ9 desaturases responsible for converting SFAs to MUFAs. FAT-6 and FAT-7 are stearoyl-CoA desaturases while FAT-5 is a palmitoyl-CoA desaturase (Brock et al., 2007). All three Δ9 desaturases convert SFAs to MUFAs but act on different starting substrates. FAT-5, for example, converts palmitic acid (16:0) to palmitoleic acid (16:1), while FAT-6 and FAT-7 convert stearic acid (18:0) to oleic acid (18:1Δ9). There is functional overlap between the three genes and single knockouts are viable because the expression of the other two is increased to compensate for the loss. The triple mutant, however, is lethal, showing that endogenous production of MUFAs is essential (Brock et al., 2006). When *fat-6;fat-7* are co-mutated, worms cannot synthesize oleic acid which is needed for production of PUFAs, and as a result have reduced growth and fertility, as well as reduced fat stores. However, when *fat-6* and *fat-7* are knocked out, FAT-5 can partially fill the role by promoting the synthesis of PUFAs from 16:1 instead of the typical 18:1 (Brock et al., 2007). When Δ9 desaturase activity is limited, *C. elegans* have reduced fat levels, showing that Δ9 desaturases are lipogenic enzymes (Brock et al., 2007; Ntambi et

al., 2002). In fact, Δ9 knockdown leads to increased expression of fatty acid oxidation genes which reduces lipotoxicity caused by excess SFAs (Flowers & Ntambi, 2008; Listenberger et al., 2003; Shi et al., 2013).

PAQR-2 PROTEIN AND PATHWAY

The first two papers in this thesis concern the function of the PAQR-2 protein. PAQR-2 belongs to the PAQR (Progestin and AdipoQ Receptor) protein family and is a regulator of membrane composition and fluidity. While this thesis will focus mostly on PAQR-2 in *C. elegans*, PAQR proteins are found in most species and even have distant homologs in bacteria. In *C. elegans* there are five PAQR proteins, while in humans there are eleven, in *E. coli* one, in yeast four, and in flies five (Y. T. Tang et al., 2005; Yamauchi et al., 2003). PAQR proteins have seven transmembrane domains but are unrelated to G-protein coupled receptors (GPCRs): in PAQR proteins, the C-terminus is extracellular while the N-terminus points into the cytosol. Beyond those two features found in all PAQR proteins, there is relatively low sequence homology (Y. T. Tang et al., 2005). Of the five PAQR proteins in *C. elegans*, only PAQR-1 and PAQR-2 are thought to act as membrane composition regulators (Svensson et al., 2011). Human AdipoR1 and AdipoR2, the closest homologs of the worm PAQR-1 and PAQR-2, were both originally identified as adiponectin receptors (Yamauchi et al., 2003) but mounting evidence suggests that adiponectin is not required for their function as membrane fluidity regulators (Palmgren et al., 2023; Ruiz, Ståhlman, et al., 2019).

As already mentioned, this thesis focuses on PAQR-2 in *C. elegans*. However, below is a short overview of what is known about PAQR-2 homologs in other species, and even more information can be found in recent reviews (Pilon, 2021; Pilon & Ruiz, 2023).

S. CEREVISIAE

The yeast species *S. cerevisiae* encodes four PAQR proteins, named IZH1-4 (Implicated in Zinc Homeostasis) which were originally found to be

regulated by zinc levels, but have since been found to be regulated by fatty acid levels as well (Karpichev et al., 2002; Lyons et al., 2004; Mattiazzi Ušaj et al., 2015). As such, expression of IZH genes is regulated by an oleate response element (ORE), and IZHs are inhibited by UFAs and induced by SFAs and glucose (Lyons et al., 2004). Interestingly, depletion of zinc in yeast causes an increase in PI and decrease in PE concentrations, which by itself has an effect of membrane properties (Carman & Han, 2007; Iwanyshyn et al., 2004). IZH2, the IZH gene with the highest homology to PAQR-2 and AdipoR2, is a ceramidase (it cleaves fatty acids from ceramides to produce sphingosine), validated by the overexpression of IZH2 causing an accumulation of sphingosine (Villa et al., 2009). In addition, IZH genes regulate zinc homeostasis by altering levels of membrane sterols (Lyons et al., 2004). Furthermore, in the IZH2 mutant several lipid metabolism genes are downregulated (Mattiazzi Ušaj et al., 2015). To summarize, yeast homologs of the PAQR proteins, IZH1-4, are regulated by zinc and fatty acid levels, have ceramidase activity, and regulate sterol homeostasis, and thus perform similar functions to PAQR proteins in different species, namely contributing to membrane homeostasis. This quote from one of the yeast studies helps motivate further research on these proteins: “IZH2 is a homolog of AdipoR, which plays an important role in type 2 diabetes. Lipid metabolism and zinc homeostasis are unbalanced in type 2 diabetes, and the role of IZH2 in yeast elucidated here could help better understand also the role of AdipoR in human health and disease.” (Mattiazzi Ušaj et al., 2015)

D. MELANOGASTER

There are five PAQR proteins in the fruit fly *D. melanogaster*, although only one, dAdipoR, is considered to be a homolog of PAQR-2 and also shows a 66% sequence similarity to human AdipoR1 (Kwak et al., 2013). dAdipoR is expressed in every developmental stage and in several tissues, including in insulin producing cells (IPCs), and null mutants are lethal, illustrating the essential function of the protein (Laws et al., 2015). When dAdipoR is inhibited specifically in IPCs, the flies exhibit impaired insulin secretion, reduced lifespan, increased sugar levels in hemolymph, and increased TAGs

(Kwak et al., 2013). Although dAdipoR is implicated in insulin production, a more recent study was not able to confirm the expression of dAdipoR in IPCs but saw that it was expressed in specific neurons where it contributes to insulin response (Arquier et al., 2021). Moreover, dAdipoR germ-line knockouts exhibited oogenesis defects that were insulin-dependent (Laws et al., 2015). Current knowledge about dAdipoR is limited and yields few clues that could connect it to membrane homeostasis. In the future it would be interesting to perform a lipidomics analysis on the fly dAdipoR mutant, and to test their tolerance to low temperature.

H. SAPIENS

There are eleven PAQR proteins in humans (PAQR1-11), although PAQR1 and PAQR2 are better known as AdipoR1 and AdipoR2 and are the two proteins closest to *C. elegans* PAQR-1 and PAQR-2 that have been implicated as having a role in membrane homeostasis (Svensson et al., 2011; Y. T. Tang et al., 2005; Yamauchi et al., 2003). Originally identified as adiponectin receptors (Yamauchi et al., 2003), this function of AdipoR1/R2 has since been questioned in newer studies (Palmgren et al., 2023; Ruiz, Ståhlman, et al., 2019). Adiponectin, a protein secreted by adipocytes, is primarily implicated in glucose regulation and low levels of adiponectin are associated with obesity as well as type-2 diabetes (Kubota et al., 2002; Maeda et al., 2002; Yamauchi et al., 2001). As such, AdipoR2 has been suggested to have an “antidiabetic” activity due to its presumed role as an adiponectin receptor (Yamauchi et al., 2003). However, more recent studies suggest that AdipoR2 is a membrane sensor that acts in an adiponectin-independent manner (Kita et al., 2019; Ruiz, Ståhlman, et al., 2019). Both AdipoR1 and AdipoR2 have been implicated in membrane homeostasis, with AdipoR2 playing a bigger role based on comparing the effects of silencing either gene on membrane fluidity and lipid composition (Devkota et al., 2017; Ruiz, Bodhicharla, et al., 2019; Ruiz et al., 2021, 2022; Ruiz, Ståhlman, et al., 2019; Volkmar et al., 2022). In fact, silencing AdipoR2 in several mammalian cell lines results in rigid membranes, especially when cells are challenged with membrane-rigidifying PA (Ruiz et al., 2022, 2023; Ruiz, Ståhlman, et al., 2019). Further, in several genome-wide CRISPR/Cas9

screens for genes involved in membrane regulation during SFA challenge, AdipoR2 emerged as one of the top hits (Jain et al., 2020; Unlu et al., 2022; X. G. Zhu et al., 2019). As with yeast IZH2, AdipoR2 has an intrinsic ceramidase activity, the output of which is a free fatty acid and a sphingosine (Holland et al., 2010). Through phosphorylation, the sphingosine can be converted into the signaling molecule sphingosine-1-phosphate (S1P) that may act as a ligand for PPARγ and promote fatty acid desaturation (Holland et al., 2010; Ruiz et al., 2022; Vasiliauskaité-Brooks et al., 2017).

C. ELEGANS

There are five PAQR proteins in *C. elegans*, with PAQR-1 and PAQR-2 being the most well characterized and closest homologs to mammalian AdipoR1 and AdipoR2 (Svensson et al., 2011; Y. T. Tang et al., 2005). Like AdipoR2 and IZH2, PAQR-2 also is a ceramidase that generates S1P that may be a ligand for NHR-49 (a distant homolog of PPARγ) to regulate desaturase expression (Ruiz et al., 2022). While *paqr-1* mutants have no visible phenotypes, the *paqr-2* mutant's most striking phenotypes are its deformed tail tip and intolerance to cold temperatures or dietary SFAs (Busayavalasa et al., 2020; Devkota et al., 2017; Devkota, et al., 2021; Svensson et al., 2011). The *paqr-1;paqr-2* double mutant has more severe mutant phenotypes than either single mutant, indicating functional redundancy between PAQR-1 and PAQR-2 (Busayavalasa et al., 2020; Svensson et al., 2011). Through lipidomic analysis, *paqr-2* mutants are revealed to have a high SFA to UFA ratio and these worms have severely rigid membranes, as evidenced by fluorescence recovery after photobleaching (FRAP) experiments (Bodhicharla et al., 2018; Devkota et al., 2017; Devkota & Pilon, 2018; Ruiz, Bodhicharla, et al., 2019; Ruiz et al., 2018; Svensk et al., 2016). Additionally, due to the excess of SFAs already present in the worm's phospholipids and their inability to regulate these levels, *paqr-2* mutant worms are extremely sensitive to exogenous SFAs (Devkota, Kaper, et al., 2021; Svensk et al., 2016). Many studies have been done to illuminate the function of PAQR-2 as well as the many interactors of PAQR-2 that act alongside or downstream of it to maintain membrane homeostasis. First, IGLR-2 was identified as an obligate

partner of PAQR-2, and both IGLR-2 and PAQR-2 must be functioning for membrane fluidity maintenance (Devkota, Henricsson, et al., 2021; Svensk et al., 2016). However, IGLR-2 is not required for PAQR-1 function, suggesting that PAQR-1 is constitutively active and/or acts together with some other protein partner (Busayavalasa et al., 2020).

Additionally, two pathway branches downstream of the PAQR-2/AdipoR2 protein complex that maintain membrane fluidity were revealed by forward genetic screens for suppressors of *paqr-2* mutant phenotypes (Devkota et al., 2017; Svensk et al., 2013). The first downstream branch promotes the transcription of fatty acid desaturases, revealed for example by the gain-of-function alleles of *nhr-49*, *mdt-15*, and *sbp-1* (Svensk et al., 2013). NHR-49 upregulates the expression of the fatty acid Δ9 desaturases *fat-5* and *fat-7*, and the gain-of-function allele can upregulate *fat-7* desaturase to suppress the *paqr-2* mutant phenotypes. MDT-15 (a Mediator subunit) acts together with NHR-49 and SBP-1 to promote fatty acid desaturase expression (Svensk et al., 2013). The second downstream branch of PAQR-2 promotes the incorporation of PUFAs into phospholipids and was revealed by loss-of-function alleles of *fld-1* and *acs-13* that can also partially suppress *paqr-2* mutant phenotype (Ruiz, Bodhicharla, et al., 2019). While the second branch downstream of PAQR-2 does promote PUFA incorporation into phospholipids, more recent research has showed that functional FLD-1 and ACS-13 actually promote the incorporation of SFAs into phospholipids; loss-of-function alleles of these genes therefore only indirectly result in increased PUFA levels in phospholipids (Sheokand et al., 2025). Thus, prior to this thesis, the actual molecular basis accounting for how PAQR-2 promotes PUFA incorporation into phospholipids was not resolved (Fig. 3). Note that combining *paqr-2* suppressors from both branch 1 and branch 2 is the only genetic way to fully suppress all *paqr-2* mutant phenotypes (Ruiz, Bodhicharla, et al., 2019; Ruiz et al., 2018).

It is important to note that PAQR-2-dependent rescue of rigid membranes is not confined to a single tissue: studies in genetically mosaic animals showed that once the PAQR-2 complex induces local production of UFAs,

these UFAs are then shared with tissues in the rest of the worm (Fig. 3) (Bodhicharla et al., 2018).

PAQR-2-ASSOCIATED PROTEINS

Below are short summaries of a few of the proteins that are important for the PAQR-2 membrane homeostasis pathway and are of relevance for this thesis.

NHR-49

The nuclear hormone receptor protein NHR-49 (homologous to human PPARα/PPARγ/HNF4α (Atherton et al., 2008; Bertrand et al., 2004; Ruiz et al., 2022; Van Gilst et al., 2005)) is a transcription factor that is important for the expression of the Δ9 desaturases *fat-5* and *fat-7*, as is the nuclear hormone receptor NHR-80 (Brock et al., 2006; Van Gilst et al., 2005). The expression levels of *fat-5* and *fat-7* are significantly decreased in *nhr-49* null worms, leading to an increased 18:0 to 18:1n9 ratio and subsequently a shorter lifespan (Van Gilst et al., 2005). While both NHR-49 and NHR-80 have similar effects on desaturases, NHR-49 is also a regulator of β-oxidation, that is the breakdown of fatty acids to acetyl-CoA that feeds to the tricarboxylic acid cycle to produce energy (Adeva-Andany et al., 2019; Watts & Ristow, 2017), and is also involved in the regulation of genes that are important for response to dietary response. Unsurprisingly, *nhr-49* mutants have more severe phenotypes than *nhr-80* mutants (Brock et al., 2006; Van Gilst et al., 2005). A loss-of-function mutation in *nhr-49* (but not of *nhr*-80) is synthetic lethal when combined with the *paqr-2* mutant; in contrast, *nhr-49* gain-of-function alleles suppress *paqr-2* mutant phenotypes by boosting *fat-7* desaturation and increasing UFA levels (Svensk et al., 2013; Svensson et al., 2011). While not studied in this thesis, the sterol regulatory binding protein SBP-1 (homolog of mammalian SREBP) is likewise activated by PAQR-2 during membrane rigidity challenges to promote Δ9 desaturation and is an important part of the PAQR-2 membrane fluidity regulation pathway (Svensk et al., 2013; F. Yang et al., 2006).

MDT-15

The mediator subunit MDT-15, a homolog of mammalian MED15, is a transcriptional mediator that binds to and facilitates the activity of *nhr-49* and *sbp-1* and subsequently desaturase expression (Blazek et al., 2005; Taubert et al., 2006; F. Yang et al., 2006). A *mdt-15* loss-of-function mutation results in reduced *fat-7* expression and fat levels, but supplementation with oleic acid rescues these defects indicating that Δ9 desaturases are MDT-15 targets (F. Yang et al., 2006). Indeed, there is a partial overlap in the expression patterns of *mdt-15* and *nhr-49* suggesting that these genes are part of the same regulatory network (Taubert et al., 2006). In addition to regulating expression of *nhr-49* and *sbp-1*, which induce Δ9 desaturase expression, MDT-15 also regulates several fatty acid metabolism genes, including short-chain acyl-CoA-dehydrogenase ACDH-2, lipid binding protein LPB-1, and Δ12 desaturase FAT-2, among others (Taubert et al., 2006). Like gain-of-function mutations of *nhr-49* and *sbp-1*, a gain-of-function allele of *mdt-15* also suppresses the mutant phenotypes of *paqr-2* mutant worms (Svensk et al., 2013).

ACS-13

There are 23 acyl-CoA synthetase (ACS) genes in *C. elegans*, homologs to human ACSL genes, which catalyze the initial reaction in fatty acid metabolism and activate fatty acids for use in several processes (Watkins et al., 2007). ACS-13, a homolog of human ACSL1, was first proposed to promote incorporation of PUFAs into phospholipids by decreasing their utilization in mitochondria (Ruiz, Bodhicharla, et al., 2019); however, a new study suggests that it actually acts by activating SFAs and channeling them for incorporation into phospholipids, specifically PEs (Sheokand et al., 2025). A forward genetic screen identified a loss-of-function allele of *acs-13* as a suppressor of *paqr-2* mutant phenotypes; it was found to enhance the suppressing effects of the *mdt-15* gain-of-function mutant on *paqr-2* and was a partial *paqr-2* suppressor on its own (Ruiz, Bodhicharla, et al., 2019). The synergy with the *mdt-15* gain-of-function allele to fully suppress the *paqr-2* mutant suggest that *acs-13* requires elevated UFA levels (a result

of the *mdt-15* mutation) to efficiently act as a *paqr-2* suppressor. Additionally, ACS-13 was determined to localize to the mitochondria in intestine and hypodermis and this expression is influenced by MDT-15 (Ruiz, Bodhicharla, et al., 2019). Human ACLS1 can activate LCFAs prior to their import into the mitochondria, which led to the hypothesis that the *acs-13* mutant channels fewer LCFAs/PUFAs into mitochondria, freeing up FAs for incorporation into phospholipids and fluidizing membranes. However, as mentioned above, this hypothesis has been revised and it is now believed that functional ACS-13 promotes incorporation of SFAs into phospholipids and that its loss only indirectly leads to increased UFA levels in phospholipids (Ruiz, Bodhicharla, et al., 2019; Sheokand et al., 2025). Downstream of ACS-13, the membrane fluidity homeostasis protein FLD-1 (homolog of human TLCD1) is responsible for incorporating SFAs into lysophospholipids and, again, its loss only indirectly leads to increased UFAs in phospholipids (Sheokand et al., 2025).

ACS-4

Acyl-CoA synthetase ACS-4 (homolog of human ACSL4), a paralog of ACS-13, activates UFAs and channels them for incorporation into phospholipids. Loss of ACSL4 in human cells increases sensitivity to SFAs, particularly when AdipoR2 is knocked out (Ruiz et al., 2021; X. G. Zhu et al., 2019). In fact, in a CRISPR-Cas9 screen ACSL4 has been identified as crucial for protecting cells from SFA toxicity (X. G. Zhu et al., 2019). ACSL4 preferentially acts on PUFAs and loss of ACSL4 decreases levels of PUFA-containing phospholipids (Doll et al., 2017; Kang et al., 1997; Killion et al., 2018; Shimbara-Matsubayashi et al., 2019). *C. elegans acs-4* mutants develop similarly to wild-type during larval stages yet become sterile adults that produce neither sperm nor oocytes, showing that *acs-4* activity is essential for germline development (H. Tang & Han, 2017). ACS-4 is localized to lipid droplets in the intestine and hypodermis, and studies suggest that ACS-4 also acts in the germline to promote the spermatogenesis-to-oogenesis switch with free FAs produced in the soma acting as substrates of germline-expressed ACS-4 for this germ cell fate decision (H. Tang & Han, 2017; Vrablik et al., 2015).

HPO-8

Very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase HPO-8 (homolog of human HACD3) is an ER-bound enzyme that catalyzes the third of four reactions in the long-chain fatty acid elongation cycle that adds two carbons to the end of long- and very long-chain fatty acids (Haslam & Kunst, 2013; Ikeda et al., 2008). There are 4 HACD proteins in mammals that interact with ELOVL proteins to perform the condensation, reduction, dehydration, and reduction steps necessary for fatty acid elongation. In *C. elegans*, the elongation reactions are performed by LET-767, ELO-2, ART-1, and HPO-8 (Denic & Weissman, 2007; Leonard et al., 2004; Sassa & Kihara, 2014; Sawai et al., 2017). In a study of the four HACD human proteins, expression of HACD3 showed a slight activity toward the elongation of SFAs and MUFAs but had little effect on the elongation of PUFAs, while HACD1 and HACD2 had more broad activity (Sawai et al., 2017). HPO-8 is essential in *C. elegans* since the mutant arrests at larval stages, revealing the importance of long-chain fatty acids (Ruiz et al., 2023).

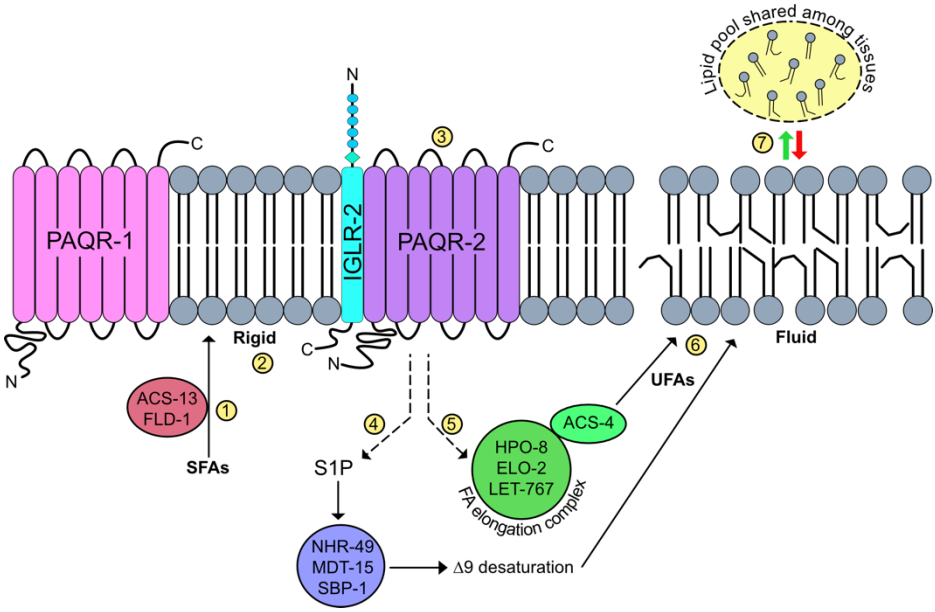

Figure 3. Model of the PAQR-2 pathway. 1) Dietary SFAs or the action of enzymes such as ACS-13 and FLD-1 leads to the incorporation of SFAs into membrane phospholipids resulting in 2) rigid membranes that 3) activate the PAQR-2/IGLR-2 complex whereby 4) PAQR-2 produces S1P that activates NHR-49/MDT-15/SBP-1 to promote Δ9 desaturation and 5) PAQR-2 recruits a fatty acid elongation complex that lengthens UFAs. 6) The newly produced UFAs are channeled for incorporation into phospholipids to fluidize membranes. 7) Lipid pools, including the UFAs produced downstream of PAQR-2 activity, are shared systemically. Pathway adapted from (Ruiz et al., 2023).

FATTY ACID DESATURATION

One of the papers produced in this thesis work revolves around the fatty acid desaturase FAT-2 and genetic suppressors of FAT-2 deficiency. The following sections summarize the role of FAT-2 in *C. elegans* as well as the HIF-1 pathway that was found to be relevant for FAT-2 activity in Paper III (discussed in Results).

FAT-2, A Δ12 DESATURASE

As mentioned above, FAT-2 is a Δ12 desaturase in *C. elegans* responsible for converting OA (18:1) into LA (18:2) by adding a second double bond to a fatty acid at the 12^{th} carbon from the carboxyl group (Peyou-Ndi et al., 2000; Watts & Browse, 2002; Zhou et al., 2011). While *C. elegans* are rare among animals for having Δ12 desaturase activity, a few other animal species can also synthesize OA to LA, including American cockroaches, crickets, slugs, and snails (Borgeson et al., 1990; Cai et al., 2020; Weinert et al., 1993). Through a screen looking for fatty acid desaturase mutants in *C. elegans*, the f*at-2(wa17*) allele (caused by a serine to phenylalanine substitution at position 101 in FAT-2, (Fig. 4A)) was identified as a near-null allele of *fat-2* with approximately 5% enzymatic activity compared to wild-type (Watts & Browse, 2002). *fat-2(wa17*) worms were found to contain only a small fraction of total PUFAs compared to wild-type worms (9.9 vs 39.6 mol %) and although they did have small amounts of 18:2 fatty acids, linoleic acid (18:2n6) was undetectable and instead small levels of unusual 18:2n9 and 18:2n7 were measured. Because *fat-2(wa17*) mutant worms have limited desaturase activity, MUFAs accumulate and, in fact, *fat-2(wa17)* worms have almost 10-fold more 18:1n9 compared to wild-type worms. This abnormal fatty acid profile in *fat-2(wa17)* worms is accompanied by many defects including sluggish movements, reduced brood size, and defective sperm motility (Kubagawa et al., 2006; Watts & Browse, 2002). Of particular importance for this thesis, while it takes wild-type worms approximately three days to develop into pregnant adults, *fat-2(wa17)* worms require almost seven days to develop into adults, and in many cases do not reach adulthood or do not produce viable offspring (Watts & Browse, 2002). Given

that the *fat-2* near-null mutation causes severe defects, we can assume that PUFAs are essential for growth and while there are several known functions of PUFAs, the third paper in this thesis will focus on identifying suppressors of PUFA deficiency phenotypes.

FAT-2, along with the other fatty acid desaturases in *C. elegans*, are diiron-oxo integral membrane proteins that require molecular oxygen, cytochrome $b_{5,}$ and NADH-cytochrome b_5 reductase as co-factors (Fig. 4A-B) (Paton & Ntambi, 2009; Strittmatter et al., 1974; Watts & Browse, 2002). Desaturase proteins each contain three conserved histidine-rich sequences (His-boxes) that coordinate the diiron-oxo structure at the active sites and each of these His-boxes is essential for enzyme function (Fig. 4A-B) (Shanklin et al., 1994; Shanklin & Cahoon, 1998). Desaturase reactions require two Fe^{2+} ions that bind to and activate an oxygen molecule to remove two hydrogens from an acyl chain which results in the formation of a double bond (Fig. 4C) (Cai et al., 2020; Shanklin & Cahoon, 1998; Shen et al., 2020). A study on mammalian stearoyl-CoA desaturase (SCD1) found that it loses its activity and becomes inactive on average after nine enzymatic cycles due to the loss of one Fe^{2+} ion in the diiron center, but that SCD1 can sustain its enzymatic activity if free ferrous ions are exogenously supplied (Shen et al., 2023). While no studies thus far have been published testing if exogenous Fe^{2+} can sustain the enzymatic activity of FAT-2, presumably all desaturases may benefit from an ample supply of Fe^{2+} given their structural and functional similarity.

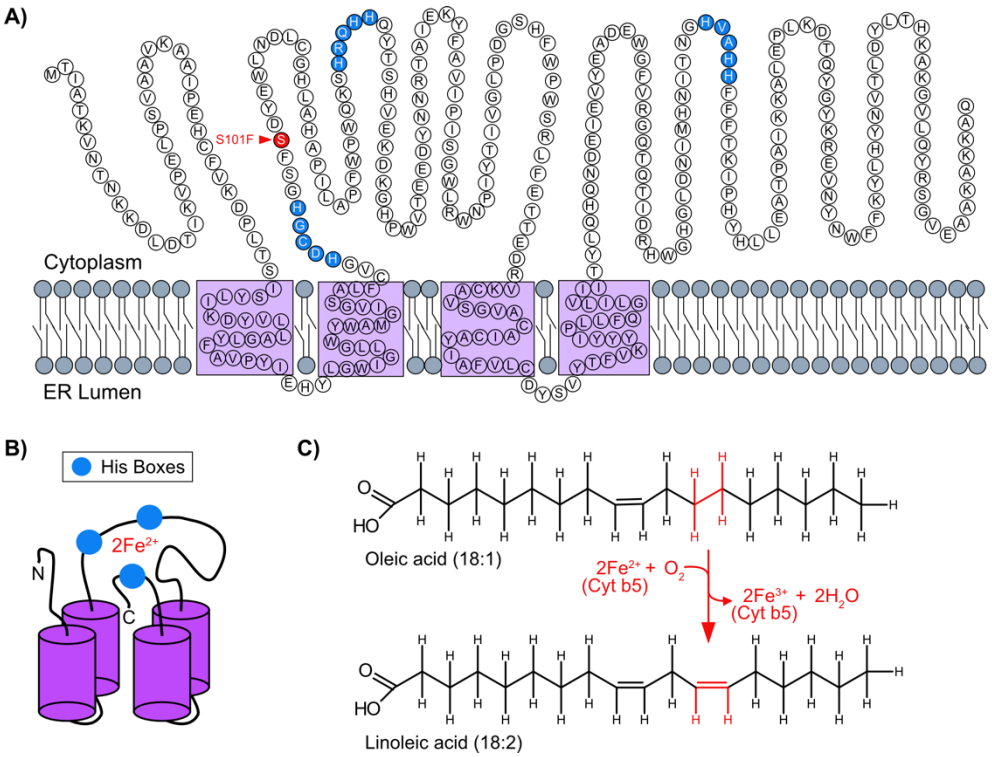

Figure 4. FAT-2 structure and activity. A) Structure of FAT-2 extrapolated from the structure of SCD (Paton & Ntambi, 2009). Purple boxes represent transmembrane domains and amino acids in blue are conserved histidine rich sequences (His Boxes). Red arrow indicates location of the S101F mutation that results in the *wa17* allele. B) Cartoon representing histidine boxes surrounding a diiron center. C) FAT-2 desaturase reaction converting 18:1 to 18:2. The reaction requires two ferrous ions and an oxygen molecule.

FUNCTION OF PUFAS

PUFAs are more than just potent membrane fluidizers: they affect many cellular processes, including mitochondrial function, oocyte development, insulin regulation, and autophagy, and act as precursors for signaling lipids (Calder, 2012; Harayama & Shimizu, 2020a; O'Rourke et al., 2013; Vrablik & Watts, 2013). As mentioned earlier in this thesis, PUFAs are essential nutrients that must be obtained from the diet in mammals; in particular ω-

3 PUFAs are believed to have powerful health benefits. Studies on knock-out mice lacking elongases or desaturases, or being fed a PUFA-deficient diet, revealed that PUFAs have important functions in several tissues throughout the body, including brain, gonads, bloods cells, and metabolic tissues (Harayama & Shimizu, 2020b; Roqueta-Rivera et al., 2010; Stoffel et al., 2008, 2013). For example, the lack of PUFA-derived eicosanoids, which are lipid-based signaling molecules, in AA-deprived mice resulted in reduced viability and reduced immune function (Fan et al., 2012). In another study of Δ6 desaturase knockout mice, the KO mice had significantly lower body weight than wild-type as well as poor learning and memory performance (Harauma et al., 2017). Additionally, in ELOVL5 knockout mice, the lack of AA and DHA resulted in the activation of genes involved in FA and triglyceride synthesis, which ultimately led to the development of hepatic steatosis in the KO mice (Moon et al., 2009). Aside from these examples, improper ratios of SFAs versus PUFAs is associated with diseases such as coronary heart disease, diabetes, and kidney disease (Simopoulos, 1999).

As mentioned, PUFAs are the precursors of the signaling molecules eicosanoids, which can have both pro- and anti-inflammatory effects (James et al., 2000). ω-6 and ω-3 PUFAs are precursors for different eicosanoids, with opposing metabolic effects, and a high ratio of ω-6 derived eicosanoids to ω-3 derived eicosanoids is associated with an increased risk of blood clots and atheroma (fatty buildup in arteries), as well as increased risk of developing allergic and inflammatory disorders (Brox et al., 1983; Lewis et al., 1986; Weber et al., 1986). Unsurprisingly, when the ω-6 to ω-3 ratio is high, the prevalence of type 2 diabetes increases as well (RAHEJA et al., 1993).

In *C. elegans*, PUFAs are required for proper development, as evidenced by the mutant phenotypes, such as slow development and low brood sizes, visible in desaturase and elongase mutants that can be rescued with exogenous PUFAs (Watts et al., 2003; Watts & Browse, 2002). PUFAs are also required for efficient neurotransmission in *C. elegans,* demonstrated

by Δ6 desaturase mutants that have reduced synaptic vesicles and release abnormally low levels of neurotransmitters (Lesa et al., 2003). This provides a link between *C. elegans* and human health as PUFAs are essential in humans for brain function as well (Bazinet & Layé, 2014). From these and numerous other studies, we can conclude that PUFAs are important not just for membrane function at the cellular level but are also essential for organismal health.

HIF-1 PATHWAY

Hypoxia-inducible factor (HIF) is an oxygen- and iron-dependent transcription factor that plays an essential role in cellular response to low oxygen levels (hypoxia). *C. elegans* possesses two HIF proteins, HIF-1 and AHA-1, which are homologs of the human HIF-1α and HIF-1β respectively. In humans, the HIF-1β subunit, also referred to as ARNT (aryl hydrocarbon receptor nuclear translocator), is constitutively expressed while HIF-1α is regulated by oxygen levels (J. W. Lee et al., 2004).

C. elegans HIF-1 regulates the expression of genes involved in numerous physiological processes including metabolism, growth, and development, especially when oxygen levels are low. HIF-1 activity varies with changing oxygen environments in *C. elegans*. During normoxia, HIF-1 is hydroxylated by the EGL-9/PHD prolyl hydroxylase, a Fe(II)- and 2-oxoglutarate-dependent dioxygenase. The ubiquitin E3 ligase VHL-1 (von Hippel-Lindau tumor suppressor homolog-1) then binds to HIF-1 and marks it for proteasomal degradation (Fig. 5A) (L. Fan et al., 2014; Semenza, 1999; C. Shen et al., 2005). During hypoxia, however, the lack of oxygen inhibits hydroxylation, causing HIF-1 to translocate to the nucleus where it dimerizes with AHA-1, activates the expression of target genes, and increases oxygen delivery or allows adaptation to hypoxia (Semenza, 1999).

The function of HIF-1 is not limited to oxygen sensing and hypoxia response but extends to other stress responses such as in oxidative stress and aging. In fact, HIF-1α in humans has been implicated in several neurogenerative diseases, including ALS, Alzheimer's, and Parkinson's disease (S. M. Kim et

al., 2013; Lall et al., 2019; Pinilla et al., 2021). Six HIF-1α SNPs have also been associated with increased risk of several cancer types including breast, lung, colorectal, renal cell carcinoma, and oral cancers, among others (M. K. Chen et al., 2009; Fransén et al., 2006; Gladek et al., 2017; Konac et al., 2007; J. Y. Lee et al., 2008; Naidu et al., 2009; Ollerenshaw et al., 2004). During hypoxic conditions, there is a reduction in cell proliferation rate in non-cancerous cells, meaning that there is not enough oxygen for new oxygen-consuming cells. Tumor microenvironments are often hypoxic, and while tumor cells thrive by activating HIF-1 and reprogramming metabolism and protein synthesis (Z. Chen, Han, et al., 2023), non-cancerous cells have to compete for the little available oxygen, allowing tumor cells to proliferate at a faster rate than healthy cells (Mbugua, 2022). HIF-1 is upregulated in cancer cells and as such plays a critical role in controlling the expression of genes associated with cancer development and metabolic changes (Karami Fath et al., 2023; Mylonis et al., 2019).

C. elegans null mutants of *hif-1* are hypersensitive to hypoxia but are viable during normoxia and, interestingly, have increased lifespans compared to wild-type worms as deleting HIF-1 activates the pro-longevity DAF-16 stress response (Bellier et al., 2009; Jiang et al., 2001; Zhang et al., 2009). *hif-1* null mutant worms also have lower levels of total iron and manganese but are more resistant to oxidative stress (Bellier et al., 2009; Romney et al., 2011). As stated above, HIF-1 is hydroxylated by EGL-9 during normoxia, and this reaction requires both oxygen and iron. Once HIF-1 is hydroxylated, it is targeted by VHL-1 for degradation (Fig. 5A). However, if iron or oxygen are deficient, EGL-9 is inactivated thus allowing HIF-1 to modulate target gene expression (Ivan et al., 2001). For instance, during iron deficiency HIF-1 regulates iron uptake and storage by causing increased expression of the intestinal Fe^{2+} transporter SMF-3 and inhibiting expression of the ferritin proteins FTN-1 and FTN-2 (Ackerman & Gems, 2012; Romney et al., 2008, 2011).

Iron metabolism is relatively well conserved between mammals and *C. elegans*, with many of the genes involved in mammalian iron metabolism having *C. elegans* homologs. There are two mammalian ferritins, ferritin

heavy subunit (FTH) and ferritin light subunit (FTL), that store iron in a stable mineralized form to prevent free radicals (Theil, 2013). FTH has an intrinsic ferroxidase activity that converts ferrous (Fe^{2+}) to ferric (Fe^{3+}) ions, while FTL does not. The two *C. elegans* ferritins are more homologous to FTH than FTL as both have ferroxidase activity, with *ftn-1* being expressed primarily in intestine while *ftn-2* is expressed in the intestine and other tissues such as the pharynx, body-wall muscles, and hypodermis (Gourley et al., 2003; Y. Il Kim et al., 2004). Both *ftn-1* and *ftn-2* are induced by high iron exposure, although *ftn-1* is induced to a higher extent, and only *ftn-1* mutants are iron sensitive (Y. Il Kim et al., 2004; Valentini et al., 2012). During iron deficiency, *ftn-1* and *ftn-2* are repressed as HIF-1 binds to hypoxia response elements located in the iron-dependent enhancer (IDE) upstream of both *ftn-1* and *ftn-2*. To sum up, during iron deficiency or hypoxia, EGL-9 is downregulated which prevents HIF-1 degradation, HIF-1 being active then inhibits *ftn-1* and *ftn-2* which increases ferrous ion pools (Fig. 5B) (Ackerman & Gems, 2012; Y. Il Kim et al., 2004; Romney et al., 2008, 2011). The increased iron levels inhibit hypoxia-induced cell death, reduce mitochondrial damage, and restore mitochondrial function to increase cell survival rate (Hu et al., 2022). HIF-1 therefore regulates iron homeostasis by up- or down-regulating genes involved in iron uptake and storage.

In addition to iron metabolism, HIF-1 plays a role in lipid metabolism as well. Fatty acid binding proteins FABP3 and FABP7, which are involved in FA uptake, are both induced by hypoxia in a HIF-1α-dependent manner and lead to significant lipid droplet accumulation in mammalian cells. This lipid droplet accumulation is due to FABP3/7-dependent fatty acid uptake, as FA synthesis is repressed during hypoxia (Bensaad et al., 2014). In a separate study, silencing of HIF-1α promoted lipid accumulation in non-alcoholic fatty liver disease (NAFLD) cells and increased the levels of TAGs. Additionally treating HIF-1-silenced cells with OA and/or PA resulted in the upregulation of lipid metabolism-related genes and further increased lipid accumulation in NAFLD cells. Interestingly, HIF-1α was found to bind to PPARα, and this relationship was enriched in NAFLD cells, but when HIF-1α was silenced the PPARα signaling pathway was inhibited. By itself, PPARα

can improve steatosis, inflammation, and fibrosis in NAFLD, however when inhibited due to HIF-1α silencing these beneficial effects go away (He et al., 2021). Several papers have found that HIF-1 upregulates the expression of genes which facilitate fatty acid synthesis and lipid storage, which in turn are metabolic responses to hypoxia (Furuta et al., 2008; Menendez & Lupu, 2007; Mylonis et al., 2012).

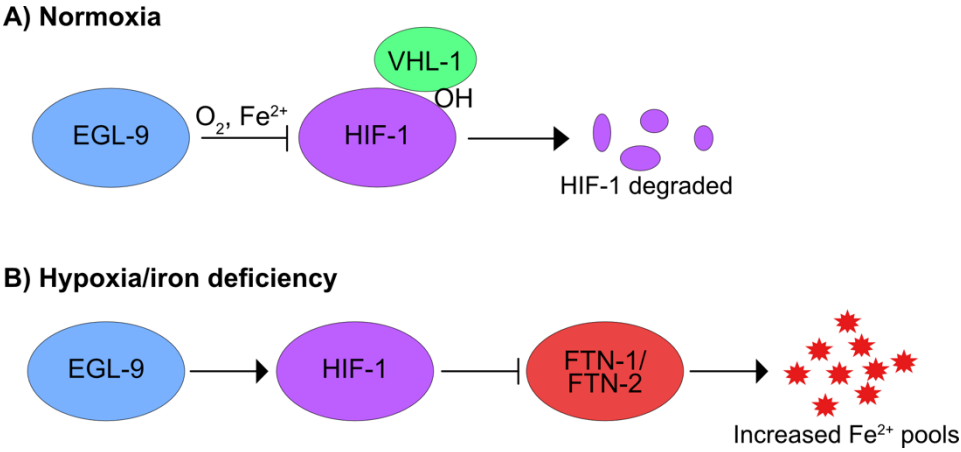

Figure 5. HIF-1 pathway during normoxia and hypoxia. A) HIF-1 signaling during normoxia where HIF-1 is degraded by EGL-9 and VHL-1. B) HIF-1 signaling during hypoxia and/or iron deficiency where HIF-1 upregulation inhibits ferritin to increase ferrous ion pools.

DISEASE RELEVANCE

As established in this thesis, the PAQR-2 and HIF-1 pathways are both important for unsaturated fatty acid synthesis in *C. elegans.* The research touched on is not only relevant for studies in worms, but it also has connections to human diseases because both AdipoR2 and HIF-1α have been implicated in disease progression. As previously discussed, the fatty acid composition within membranes is influenced by diet, and improper fatty acid homeostasis can increase risk for developing diseases such as cardiovascular disease, diabetes, and cancers (Estruch et al., 2018; Martínez-González et al., 2008; Schwingshackl et al., 2017). The AdipoR2

pathway is essential for responding to membrane fluidity challenges, and as such mutations in AdipoR2 and its downstream interactors can result in increased adiposity/obesity and shift lipidomic profiles, as well as increase insulin resistance. Such changes put individuals at increased risk for developing diabetes, certain cancers, heart disease, and neurodegenerative disorders such as Alzheimer's and Parkinson's (Z. Chen, Yang, et al., 2023; Kubota et al., 2002; Mociño-Rodríguez et al., 2017; Tiwari et al., 2015; Yamauchi et al., 2003).

We found that the HIF-1 pathway can contribute to unsaturated fatty acid homeostasis by boosting residual desaturase activity in a *C. elegans* mutant, as discussed further in Paper III results. This too could have implications for human health since imbalances in PUFA levels are already implicated in progression of several diseases. Improper PUFA levels can, for example, increase risk of blood clots and atheroma, lead to heart disease, type 2 diabetes, NAFLD, kidney disease, and autoimmune disorders (Brox et al., 1983; Lewis et al., 1986; Moon et al., 2009; RAHEJA et al., 1993; Simopoulos, 1991, 1999; Weber et al., 1986). Additionally, several mutations in the HIF-1 pathway have connections to the development of cancers and neurodegenerative diseases (specifics have been discussed above under the HIF-1 pathway) (M. K. Chen et al., 2009; Fransén et al., 2006; Gladek et al., 2017; S. M. Kim et al., 2013; Konac et al., 2007; Lall et al., 2019; Pinilla et al., 2021).

Mutations within the AdipoR2 and HIF-1 pathways therefore both have effects on fatty acid homeostasis and additionally confer increased risk for developing several of the same diseases (diabetes, cancers, heart disease, to name a few) (Fig. 6). Although much of the work detailed in this thesis has been done in *C. elegans*, it would be interesting to know if the research presented here could be used to eventually develop treatments to human diseases.

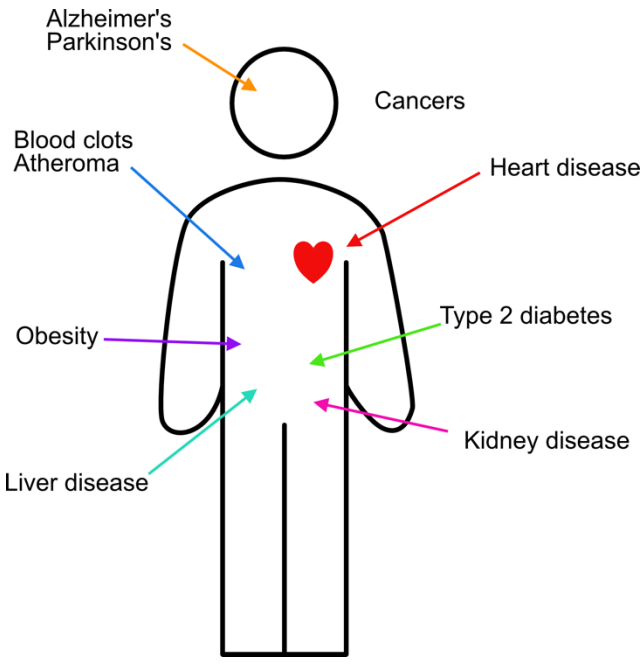

Figure 6. Diseases associated with UFA homeostasis defects. Cartoon showing some of the health issues resulting from or associated with a UFA imbalance and for which the AdipoR2 and HIF-1 pathways may be of relevance in humans.

AIM

The aim of this thesis is to better understand the molecular genetics basis and physiological roles of fatty acid homeostasis using *C. elegans* as a model organism. **PAPER I** focuses on detailing the effect of impaired membrane homeostasis on worm physiology. Worm mutants with a range of membrane composition profiles were created to study the deleterious effects of suboptimal membrane fluidity on numerous cellular and physiological traits. **PAPER II** further defines the PAQR-2 membrane homeostasis pathway by identifying protein interactors of PAQR-2 that likely provide a molecular basis for what had hitherto been called the "second branch" of the PAQR-2 pathway, i.e. the promotion of PUFA insertion in phospholipids. **PAPER III** shifts the focus to identifying mutations that compensate for polyunsaturated fatty acid deficiency in worms that are nearly deficient in FAT-2 desaturase activity. This paper uncovers a novel role of the HIF-1 pathway in fatty acid homeostasis.

RESULTS AND DISCUSSION

PAPER I: A genetic titration of membrane composition in *Caenorhabditis elegans* reveals its importance for multiple cellular and physiological traits

This paper follows previous research from the Pilon lab where they identified the partially redundant roles of the membrane fluidity sensors/regulators PAQR-1 and PAQR-2 and determined that a double *paqr-1;paqr-2* mutant worsens the mutant phenotypes of the *paqr-2* mutant (Busayavalasa et al., 2020; Svensk et al., 2016; Svensson et al., 2011). The Pilon lab also identified mutations that increase UFA content in phospholipids and fall on the other side of the membrane fluidity spectrum from *paqr-1;paqr-2.* Such mutations include gain-of-function mutations of *mdt-15* and *nhr-49* that promote desaturase expression and loss-of-function of *acs-13* that indirectly promotes channeling of PUFAs into membrane phospholipids (Ruiz, Bodhicharla, et al., 2019; Sheokand et al., 2025; Svensk et al., 2013) (Fig. 7A). The aim of this paper, therefore, was to characterize the cellular and physiological effects caused by a range of too fluid to too rigid membraned mutants.

RESULTS

Membrane composition directly impacts membrane fluidity

We generated five *C. elegans* strains with varying degrees of membrane fluidity. On the rigid side, a *paqr-1(tm3262);paqr-2(tm3410*) double mutant (referred to as *p1p2)* as well as a *paqr-2(tm3410)* single mutant (*p2*). With wild-type N2 worms acting as the middle of the fluidity spectrum, the excess fluidity strains consisted of a double *mdt-15(et14);nhr-49(et8)* mutant (*mn*) and an *mdt-15(et14);acs-13(et54);nhr-49(et8)* triple mutant (*man*) (Fig. 7B). After confirming the fluidity gradient with fluorescence recovery after photobleaching (FRAP), we determined that membrane fluidity was heavily influenced by fatty acid composition. While N2 worms

change their membrane composition to adapt to temperature changes, i.e. becoming rich in UFAs at cold temperatures and rich in SFAs at warm temperatures, the composition of the five strains varied substantially when all grown in identical conditions. The rigid *p1p2* strain had an abundance of rigidifying SFAs, while the fluid *man* strain was rich in fluidizing PUFAs, seen at all three temperatures tested (15°, 20°, 25°C) as well as in both PEs and PCs. Therefore, the membrane fluidity correlates directly with membrane composition.

Multiple physiological traits correlate with membrane fluidity

Characterization of several physiological traits revealed that both excessively rigid and excessively fluid membranes can have deleterious effects on worm health (Fig. 7C). Plasma membrane leakiness, measured by Hoechst 34580 staining, was seen in all strains except for N2, confirming that while excess UFA levels can cause gaps and leakiness in membranes, excess SFAs can also lead to membrane leakiness due to loss of membrane integrity.

Tolerance to different temperatures and diets also varied among the strains, with the rigid strains unable to grow at membrane-rigidifying cold temperatures, and fluid strains growing less than N2 at membrane-fluidizing warm temperatures. Unsurprisingly, *p1p2* and *p2* failed to grow in the presence of SFA-rich diets, and although *mn* and *man* were not particularly affected by any diet, they did experience a slight reduction of growth on fluidizing NP40 treatment, confirming that fluidizing already fluid strains is detrimental while rigidifying rigid strains is detrimental. Both the fluid and rigid strains also exhibited reduced lifespans and reduced brood sizes relative to N2, underlining the importance of proper membrane composition for health. To further investigate the cause of the reduced brood sizes in each strain, we visualized vitellogenin (lipoproteins that transport lipids and nutrients from the intestine to gonads) trafficking. In N2 worms, vitellogenin accumulates within developing oocytes, however this trait was impaired in the other strains. *p1p2* and *p2* worms showed low levels of vitellogenin overall, and it accumulated into the pseudocoelomic

cavity rather than in oocytes. In contrast, the excessively fluid strains exhibited an excess accumulation of vitellogenin in oocytes relative to N2. While further investigating reproductive defects, we saw that the rigid strains had disorganized membranes within the gonad as well as disorganized oocytes and embryos. Although the excess fluidity strains had relatively well-organized gonad membranes, they did accumulate late-stage embryos in the uterus, especially at warmer temperatures.

Cellular defects correlate with membrane fluidity

In addition to physiological defects associated with changes from optimal membrane composition, several cellular processes are affected as well. When examining lipid peroxidation levels in the fluid and rigid strains, we saw that those strains low in PUFAs, *p1p2* and *p2*, had low levels of lipid peroxidation while strains with an excess of PUFAs, *mn* and *man*, had high levels of lipid peroxidation. This falls in line with previously accepted knowledge that lipid peroxidation is propagated by PUFAs. Similarly, we showed contrasting effects on membrane composition of autophagosome formation, with rigid strains having almost no autophagosomes and *man* having an excess of autophagosomes compared to N2, most likely due to both increased autophagosome formation and impaired degradation of autophagolysosomes. Finally, shifting the worms to colder temperatures induced autophagy, showing that increased rigidity correlates with higher autophagy and increased fluidity correlates with lower autophagy.

DISCUSSION

In this paper, we established a panel of *C. elegans* strains that genetically titrate membrane fluidity, ranging from those with overly rigid membranes to those with overly fluid membranes, and additionally detailed the effects of suboptimal membrane composition on several traits. We determined that both the rigid and the fluid strains have permeable membranes, reduced lifespans, and reduced brood sizes, but that the rigid and fluid strains differ in their susceptibility to lipid peroxidation and autophagy as well as having vastly different lipidomic profiles consistent with their

membrane fluidity phenotypes. We concluded that proper membrane composition is crucial for several processes, not just those related to membrane fluidity, and that most cellular processes are likely dependent on membrane homeostasis.

This paper is consistent with several previously hypothesized ideas about how membrane fluidity impacts different processes and phenotypes (Fanning et al., 2019; Guschina & Harwood, 2006; Manni et al., 2018; W. S. Yang et al., 2016), both in worm strains that have excessively fluid membranes and excessively rigid membranes, and interestingly that excess fluidity/rigidity can have the same effects on organisms. Both the rigid and fluid strains had similar defects in oogenesis, membrane permeability, and lifespan, even though they had opposite phospholipid compositions. It makes sense that having excess fluidity will not improve oogenesis or lifespan compared to the wild-type or the rigid strains, as the worms are still not entirely healthy, but it was surprising to note that the rigid strains also had leaky membranes. One can imagine that fluid membranes will be leaky due to gaps between phospholipids, however it is not as obvious that rigid membranes would be leaky as well.

It is also important to note that the mutant alleles in this paper are known to have functions outside of membrane homeostasis regulation, meaning that the results seen cannot be due entirely to faulty membranes. ACS-13, MDT-15, and NHR-49 are known to have roles as regulators of PUFA utilization, stress response, and β-oxidation (Goh et al., 2013; Moreno-Arriola et al., 2016; Ruiz, Bodhicharla, et al., 2019; Taubert et al., 2006). Even with the possibility that other factors are influencing the phenotypes studied in this paper, the strains created should still serve as a guide for the effects membrane fluidity can have on an organism.

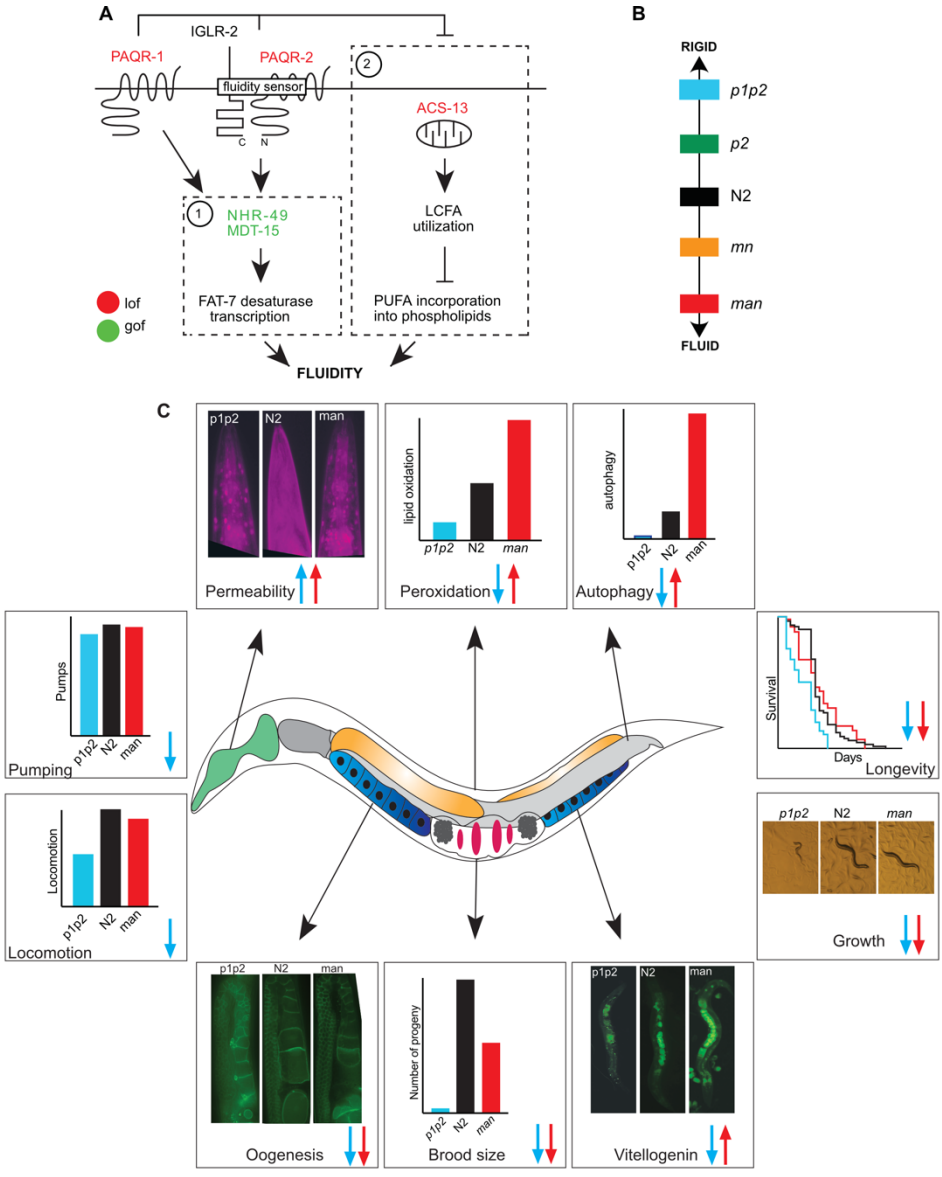

Figure 7. Summary of Paper I results. A) The PAQR-2 pathway as understood at the start of Paper I, showing the role of mutant alleles used in the study. B) Overview of the five strains used in the paper showing their place along the rigid/fluid scale. C) Summary of defects resulting from impaired membrane homeostasis. Blue and red arrows show the direction of change in rigid strains and fluid strains, respectively, relative to the wild type. Adapted from (Devkota, Kaper, et al., 2021).

Own contributions to Paper I:

1. Growth experiments: I performed growth experiments on NGM, 25°C, EPA, and NP40.
2. Lifespan assays: I did one of the replicates of the lifespan assay for all five strains at 15°C, 20°C, and 25°C.
3. Oogenesis assays: I crossed the *pie-1::gfp* reporter into strains and imaged strains at three temperatures. Additionally, I Hoechst-stained worms to visualize germline defects at three temperatures.

PAPER II: AdipoR2 recruits protein interactors to promote fatty acid elongation and membrane fluidity

RESULTS

AdipoR2 is required for elongation and desaturation of linoleic acid

We began this paper by confirming that AdipoR2 is important for fatty acid elongation and desaturation and PUFA incorporation into phospholipids. By incubating AdipoR2 siRNA-silenced HEK293 cells with ^{13}C-labeled OA or LA, we found that AdipoR2 silencing limits uptake and incorporation of exogenous OA into phospholipids, as evidenced by a reduced total level of ^{13}C-labeled OA and its derivatives in PCs and PEs. AdipoR2 silencing had no effect on total levels of ^{13}C-labeled LA or its derivatives in PCs and PEs, but did significantly increase the levels of short, more saturated PCs and PEs while decreasing the levels of longer, desaturated phospholipids. The reduction of long, desaturated PUFAs indicates that AdipoR2 is required for elongation and desaturation of LA and perhaps also the incorporation of long-chain PUFAs into phospholipids.

PAQR-2 and AdipoR2 interact with fatty acid metabolism proteins

To determine how AdipoR2/PAQR-2 influence fatty acid elongation and desaturation, we performed immunoprecipitations to identify protein interactors of AdipoR2 and PAQR-2 in both human HEK293 cells and *C. elegans*. After filtering for proteins that were only present in PAQR-2::HA and AdipoR2::HA samples and not their respective controls, we identified several proteins that are involved in fatty acid metabolism (Fig. 8A). Several proteins associated with the life cycle of transmembrane proteins were also pulled down in the immunoprecipitations, but for this paper we chose to focus on proteins that could explain PAQR-2/AdipoR2's involvement in fatty acid processing. Among the fatty acid metabolism proteins, we identified FASN-1/FASN (fatty acid synthetase), LBP-5,6/FABP4 (fatty acid binding protein), as well as three of the four enzymes required for fatty acid elongation (ELO-2/ELOVL3/6, LET-767/HSD17B12, HPO-8/HACD-3) and the

fatty acid CoA synthetase ACS-4/ACSL4. We chose to continue the paper by confirming the interaction of PAQR-2/AdipoR2 with HPO-8/HACD-3 and ACS-4/ACSL4. HPO-8/HACD3 was chosen because it was the protein in the fatty acid elongation complex that came down in both worm and human IPs, and although ACS-4 was only identified in the worm IPs, previous work has shown that it is important for AdipoR2s function in mammals as well and was therefore also chosen for further studies.

PAQR-2/AdipoR2 interacts with HPO-8/HACD3 and ACS-4/ACSL4

As PAQR-2/AdipoR2 interact with three of the four ER-bound enzymes required for fatty acid elongation, we chose to focus on and confirm the interaction of PAQR-2 and AdipoR2 with HPO-8 and HACD3 respectively. HPO-8/HACD-3 is a 3-hydroxyacyl-CoA dehydratase that catalyzes the third step in the long-chain fatty acid elongation cycle and adds two carbons to the chain of long- and very-long FAs. First, we confirmed the interaction of PAQR-2 with HPO-8 by co-immunoprecipitations and colocalization in *C. elegans* samples and followed this with confirming the interaction of AdipoR2 and HACD3 by bimolecular fluorescence complementation (BiFC) and colocalization experiments.

Next, we confirmed the interaction of PAQR-2/AdipoR2 with the FA CoA synthetase ACS-4/ACSL4. Although in the original immunoprecipitations the two proteins only interacted in the worm samples, prior research indicated that the proteins should also interact in human samples (Ruiz et al., 2021; X. G. Zhu et al., 2019). Indeed, after confirming via co-immunoprecipitations and colocalizations experiments that PAQR-2 and ACS-4 interact in worms, BiFC and colocalization experiments in human cells also confirmed that ACSL4 interacts with AdipoR2, indicating that the original IP method was not as sensitive as the further tests and that some protein interactors might be missed.

Interestingly, we next showed by co-IPs that PAQR-2, HPO-8, and ACS-4 are all part of one complex (Fig. 8B) indicating that PAQR-2 may recruit HPO-8 and ACS-4, along with other fatty acid metabolism proteins not studied in

this paper, together to elongate fatty acids and channel their incorporation into phospholipids to regulate membrane fluidity.

HPO-8 and ACS-4 act downstream of PAQR-2 and *hpo-8* mutants display membrane defects

HPO-8 and ACS-4 are essential proteins and as such *hpo-8* mutants arrest at larval stages and *acs-4* mutants are sterile. Like the *paqr-2* mutant, FRAP revealed that *hpo-8* worms have rigid intestinal membranes but *acs-4* mutants do not have rigid membranes (Fig. 8C). The *hpo-8* mutant likewise arrests at 15°C but benefits from exogenous OA, PA, OA and PA, and surprisingly glucose. While PA and glucose provide the worms with a high concentration of SFAs that are harmful to *paqr-2* mutant worms, we believe that *hpo-8* mutants benefit from the addition of any longer fatty acids that they are lacking. Unlike *hpo-8*, no treatments benefitted the *acs-4* mutant which grew to like wild-type lengths but was sterile in every condition tested. This most likely is the result of there being 22 other fatty acid CoA synthetases with partially redundant functions that can channel UFAs into tissues other than the gonads when *acs-4* is absent.

HACD3 and ACSL4 contribute to fluid membranes in human cells

Finally, we Laurdan-stained HEK293 cells in which HACD3 or ACLS4 had been silenced and determined that silencing either protein results in tightly packed (i.e. rigid) membranes, although not to the degree exhibited in AdipoR2-silenced cells (Fig. 8D). HACD3-silenced cells became depleted of LCFAs and a previous paper revealed that ACSL4 silencing results in an excess of SFAs in phospholipids (Ruiz et al., 2021). These results are consistent with the theory that ACSL4 and HACD3 are acting downstream of AdipoR2 to influence and regulate membrane composition.

Given the above results, we proposed that PAQR-2/AdipoR2 recruit a complex that locally promote fatty acid elongation and channels the resulting long-chain UFAs for incorporation, via ACS-4/ACSL4, into phospholipids to maintain fluidity (Fig. 8E).

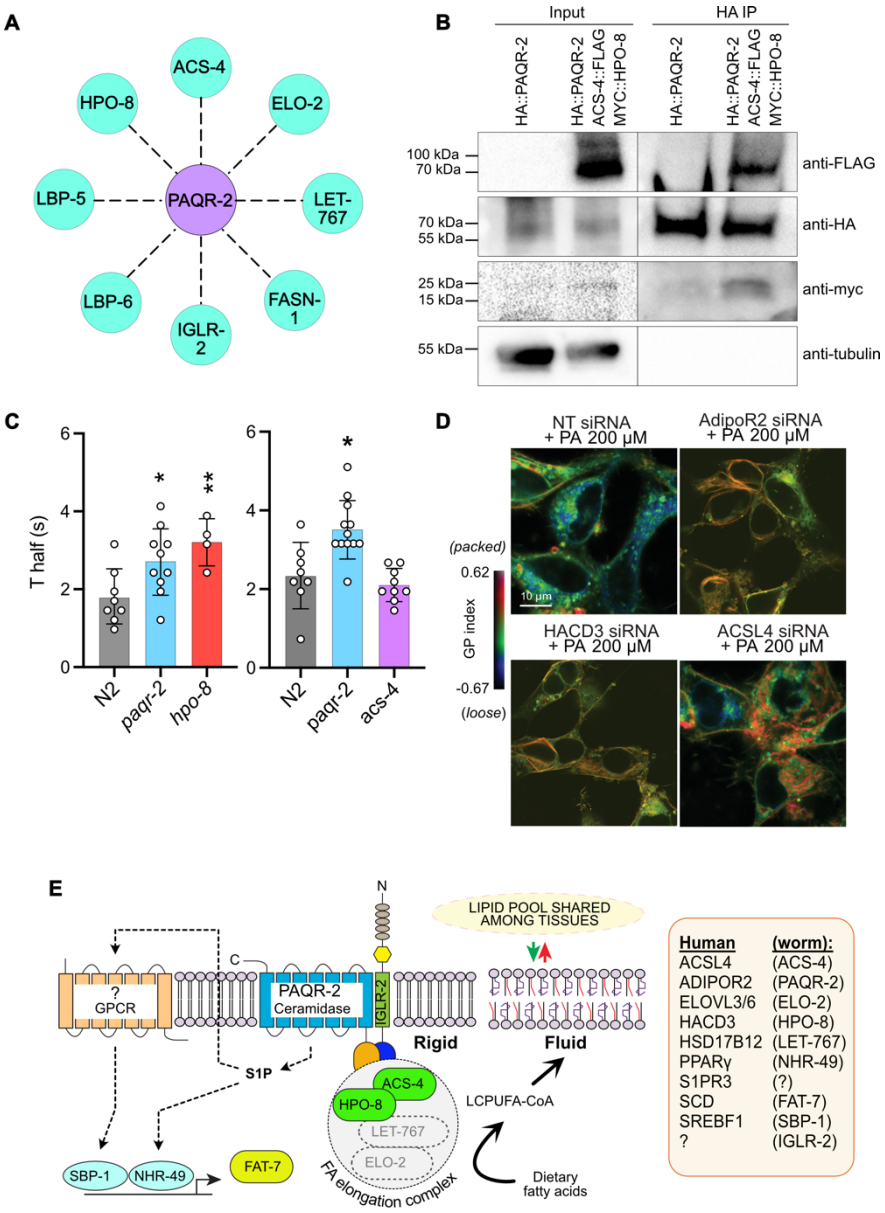

Figure 8. Summary of Paper II results. A) Summary of proteomic results showing interactors of interest with PAQR-2/AdipoR2 (worm protein names are listed). B) IP/western blot showing interaction of PAQR-2/ACS-4/HPO-8 in one complex. Silencing *hpo-8*/HACD3 or *acs-4*/ACSL4 results in rigid membranes in C) worms (measured by FRAP) and D) human cells (measured by Laurdan staining). E) PAQR-2/AdipoR2 pathway as understood after Paper II. Adapted from (Ruiz et al., 2023).

DISCUSSION

In this paper we searched for protein interactors of PAQR-2 in worms and AdipoR2 in human cells to clarify the mechanisms by which they can sustain membrane fluidity. Through immunoprecipitation and proteomics analysis, we identified several proteins that interact with PAQR-2 and/or AdipoR2, and we focused on proteins associated with fatty acid metabolism, in particular HPO-8/HACD3 and ACS-4/ACSL4. Given that *C. elegans* and humans are separated by over 700 million years of independent evolution (Nido et al., 2011), the odds of PAQR-2 and AdipoR2 evolving independently to interact with HPO-8/HACD3 and ACS-4/ACSL4 are very low, and, more importantly, is an indication that these two proteins are vital in the pathway to maintain membrane homeostasis.

Previous work within the Pilon group has suggested that there are two separate pathway branches downstream of PAQR-2/AdipoR2 that help restore membrane fluidity: the first "branch" acts through S1P to promote desaturase transcription via SREBF1 and PPARγ (human homologs of worm SBP-1 and NHR-49, respectively) (Ruiz et al., 2022), and the second to promote UFA incorporation into phospholipids, as evidenced by the *fld-1* and *acs-13* mutant alleles that act as *paqr-2* mutant suppressors (TLCD1 and ACSL1 are the human homologs of FLD-1 and ACS-13) (Ruiz, Bodhicharla, et al., 2019; Ruiz et al., 2018). Prior to Paper II, the second branch of PAQR-2/AdipoR2 had not been conclusively defined, and the proteomics approach in this paper aimed to resolve this.

Although ACSL4 was only identified in the initial immunoprecipitations in the worm samples, previous work suggested that it should be involved in AdipoR2-dependent membrane regulation (Ruiz et al., 2021; X. G. Zhu et al., 2019), and in fact further experiments revealed that ACSL4 does indeed interact with AdipoR2, and it must have been the sensitivity of the initial proteomics screen that missed some interactors. HPO-8 and HACD3 were pulled down in both species during the initial immunoprecipitations and later confirmed with further experiments, however the other two proteins within the four-protein fatty acid elongation complex that HPO-8/HACD3

are a part of were only seen in the worm samples. This could again be the case of lack of sensitivity in the initial experiment, or perhaps that in mammals the elongation step initiated by HACD3 is the most crucial for membrane fluidity regulation. Interestingly, within the same category of interactors, fatty acid synthetase (worm FASN-1 and human FASN) only interacted with PAQR-2 and not AdipoR2. FASN-1/FASN is an enzyme that catalyzes the synthesis of palmitic acid from acetyl-CoA and malonyl-CoA and it is especially important for fatty acid synthesis as it produces the substrate (PA) that is required by the fatty acid elongation complex (Smith, 1994). Again, this could be a case of the immunoprecipitations/mass spectroscopy not being sensitive enough to detect FASN in the human samples, or it could be that FASN interacts with PAQR-2 in worms but not with AdipoR2 in HEK293 human cells.

While PAQR-2 and AdipoR2 by no means act alone to promote membrane fluidity, in this paper we proposed that PAQR-2/AdipoR2 recruits an ER-bound fatty acid elongation complex, including HPO-8/HACD3, to synthesize UFAs and then utilizes ACS-4/ACSL4 to channel the UFAs into phospholipids, thus restoring membrane homeostasis. Several other proteins involved in fatty acid synthesis/metabolism were identified as PAQR-2/AdipoR2 interactors and these too may have important roles connected to PAQR-2/AdipoR2's function. For example, according to the proteomics results, PAQR-2 and AdipoR2 interact with the fatty acid binding proteins LBP-5/6 (worm) and FABP4 (human). LBP-5/6 and FABP4 regulate fat accumulation and storage and act as lipid chaperones. LBPs and FABPs can facilitate the transport of lipids to specific compartments for storage (Furuhashi & Hotamisligil, 2008; Xu et al., 2011) and as interactors of PAQR-2/AdipoR2 could help with transporting the necessary FAs into membranes to protect cells from membrane rigidification.

The findings in this paper confirm the presence of a previously uncharacterized second "branch" of PAQR-2 and AdipoR2 that promotes membrane fluidity. Prior to this paper, the first branch of PAQR-2/AdipoR2 was defined as promoting desaturase transcription via NHR-49 and MDT-15, among others (Ruiz et al., 2022; Svensk et al., 2013), while the second

branch had been genetically revealed by the changes in PUFA incorporation into phospholipids seen in the *fld-1* and *acs-13* mutants (Ruiz, Bodhicharla, et al., 2019; Ruiz et al., 2018). More recently it has been suggested that FLD-1 and ACS-13 act together to promote membrane rigidity by channeling SFAs into phospholipids, and that PAQR-2 can boost desaturase expression to counter this; the two pathways thus provide an accelerator and a brake required for membrane homeostasis (Sheokand et al., 2025). By using proteomics to identify PAQR-2/AdipoR2 interactors, this paper clarified how PAQR-2/AdipoR2 mechanistically promote the incorporation of PUFAs into membrane phospholipids.

Own contributions to Paper II:

1. Confirmation of PAQR-2 interaction with HPO-8 and ACS-4: I performed the coimmunoprecipitations and colocalization experiments confirming the interaction between the proteins in worms.
2. FRAP experiments: I performed FRAP on the *hpo-8* and *acs-4* mutants.
3. Mutant characterization: I performed the growth experiments on *acs-4* and *hpo-8* mutants.
4. Confocal microscopy: Crossed the HPO-8::mCherry and ACS-4::GFP reporters into *paqr-2* mutants worms for imaging of protein expression patterns.

PAPER III: A *fat-2(wa17)* suppressor screen in *C. elegans* reveals genetic adaptation to polyunsaturated fatty acid deficiency

RESULTS

The *fat-2(wa17)* mutant is deficient in PUFAs and benefits from exogenous PUFAs

The *fat-2(wa17)* mutant is a near-null allele of the Δ12 desaturase FAT-2 and as such the mutant has a limited capacity to convert OA to LA and subsequently limits further PUFA synthesis. As a result, *fat-2(wa17)* worms have severely limited growth, reduced brood size and locomotion, among other defects. We first confirmed that the defects seen in *fat-2(wa17)* are due to the mutation in *fat-2* by rescuing *fat-2(wa17*) with wild-type *fat-2(+)* on an extrachromosomal array, then showed that *fat-2(wa17)* has rigid membranes, similar to the *paqr*-2 mutant, most likely due to the *fat-2(wa17*)'s lack of fluidizing PUFAs. Unsurprisingly*, fat-2(wa17*) is rescued by exogenously provided PUFAs, both LA and EPA which are normally produced by wild-type worms, and DHA which worms do not produce themselves (Fig. 9D). This rescue is transient, however, and when worms grown on LA are transferred back to nematode growth media (NGM; the control media for culturing worms) their progeny do not receive any growth benefit. *fat-2(wa17)* additionally is temperature sensitive, with worms grown at 15°C having further limited growth compared to 20°C, and worms grown at 25°C developing better. Interestingly, although *fat-2(wa17)* has rigid membranes, the addition of fluidizing diets, such as NP-40 and OA, did not rescue *fat-2(wa17)* growth in any way. However, while fluidizing treatments did not rescue the slow growth of *fat-2(wa17),* NP-40 diet did rescue the rigid membranes of *fat-2(wa17)* (unpublished). Additionally, exogenous PA and glucose, both sources of high concentrations of SFAs, did not further hinder *fat-2(wa17)* growth.

Lipidomic analysis confirmed what has previously been published: *fat-2(wa17)* has an excess of 18:1 fatty acids both in PCs and PEs, although the method we used could not distinguish between 18:1n7 (vaccenic acid) and

18:1n9 (OA). Furthermore, *fat-2(wa17)* has a significant reduction in total PUFAs, in particular of 20:5, which is the endpoint fatty acid in *C. elegans* fatty acid synthesis/elongation and likely suffers from the lack of LA being synthesized. Exogenous LA decreased the MUFA levels relative to control *fat-2(wa17)* worms and increased the PUFA levels, and transferring LA-grown worms back to NGM 6 hours before analysis caused the lipidomics profile to trend back towards *fat-2(wa17)* control worms.

Mutations within the HIF-1 pathway suppress *fat-2(wa17)* mutant phenotypes

In a first genetics approach, we sought to determine if mutations known to suppress *paqr-2* mutant phenotypes could also suppress *fat-2(wa17)* phenotypes; these experiments were prompted by the similarities between the two mutant strains. We found that *mdt-15(et14), nhr-49(et8), fld-1(et46), paqr-1(et52),* and *acs-13(et54)* provide only partial suppression of *fat-2(wa17)* mutant phenotypes, further confirming that fluidizing *fat-2(wa17)*'s membranes (either with fluidizing diets or with *paqr-2* suppressors) does not provide much benefit and that membrane rigidity is likely not the only or main cause of *fat-2(wa17)*'s defects.

Next, we performed a forward genetic screen for suppressors of *fat-2(wa17)*'s slow growth, i.e. mutations that allowed *fat-2(wa17)* to develop into adults within 72 hours as opposed to the 120 hours commonly seen in the mutant (Fig. 9A). After EMS-mutagenesis and screening of approximately 40,000 haploid genomes, we identified ten novel mutations that suppressed the *fat-2(wa17)* growth defect. Four of the alleles found (*et63-et66*) were within the *fat-2* gene itself and may work by stabilizing the FAT-2 protein and allowing for improved desaturase activity. The other six suppressors identified were within the HIF-1 pathway: 3 alleles of *egl-9 (et60-et62)*, 2 alleles of *ftn-2 (et67-et68)*, and one allele of *hif-1(et69*) (Fig. 9B). EGL-9 is a proline hydroxylase that regulates the response to iron depletion and hypoxia, and can hydroxylate HIF-1 leading to its degradation. Strikingly, the 3 alleles found all affected the same amino acid, an arginine at position 557, leading to a hypomorph with reduced EGL-9

function. EGL-9 additionally interacts through its R557 amino acid with Fe^{2+}/2-oxoglutarate, a required cofactor for its oxygenase activity. We hypothesize that the mutated version of EGL-9 is not able to hydroxylate HIF-1 but retains other functions.

FTN-2 is a ferritin protein that is constitutively expressed in the intestine, muscles, and neurons and has ferroxidase activity to oxidize reactive Fe^{2+} ferrous ions to stable Fe^{3+} ferric ions. As FTN-2 is a major iron binder, we expect that the two loss-of-function *ftn-2* mutants have less iron than wild-type worms, but a higher Fe^{2+}/Fe^{3+} ratio. As ferrous ions are required for desaturase activity, we believe that loss of FTN-2 function allows for more sustained desaturase activity. Indeed, as *ftn-2(et68)* suppresses both *fat-2(wa17*) and *fat-2* RNAi silenced worms, but not the *fat-2* null mutant, it appears that this increase of ferrous ions allows for the little amount of desaturase activity left to be used more efficiently but that it cannot boost desaturation if the enzyme is fully missing.

Lastly, HIF-1 is a hypoxia inducible factor that is involved in sensing and responding to low oxygen conditions. The *hif-1(et69)* allele is the result of a splice acceptor mutation in the 5^{th} intron of HIF-1, that prevents EGL-9 from hydroxylating HIF-1 and leads to a gain-of-function mutation where HIF-1 is constitutively active. HIF-1 is known to suppress *ftn-2* expression so a gain-of-function mutation should suppress *ftn-2*, leading to an increase of ferrous ions and eventually increased FAT-2 desaturase activity.

Using *ftn-2(et68)* as a proxy for the HIF-1 pathways suppressors as all of them converge on FTN-2 inhibition, we saw that in addition to suppressing *fat-2(wa17)* mutant's slow growth, *ftn-2(et68)* also suppressed several stress responses that were activated in *fat-2(wa17),* namely ER UPR, mitochondrial UPR, and DAF-16 stress responses. *ftn-2(et68)* also restored membrane fluidity to *fat-2(wa17).*

fat-2(wa17) suppressors influence protein expression and lipidomic profile

After determining that all suppressors (outside of the intragenic *fat-2* alleles) fall within the HIF-1 pathway, we wanted to verify this via western blot. After tagging HIF-1 with a 3XFLAG tag and confirming that the protein was properly tagged by seeing an increase in HIF-1 levels after incubating wild-type worms in hypoxic conditions for 6 or 20 hours, we revealed that levels of HIF-1 within *fat-2(wa17)* are reduced, but that the addition of one of our novel *egl-9* mutant alleles leads to a sharp increase in HIF-1 levels, further confirming that the *egl-9* hypomorphs upregulate HIF-1 (Fig. 9F). Further, the levels of HIF-1 in the *ftn-2(et68);fat-2(wa17)* strain were very weak, indicating that HIF-1 is functioning upstream of FTN-2 and that mutations in *ftn-2* acts after HIF-1.

Subsequently we tested the levels of FAT-2 within the suppressor strains by western blot with an HA-tagged FAT-2. First, we confirmed that the levels of FAT-2 protein in *fat-2(wa17)* are significantly diminished, and that the suppressors can increase this signal, although there was quite a large variation in signal between experiments (Fig. 9G; unpublished at the time of writing).

Lastly, the *hif-1(et69)* mutant was found to have reduced FTN-2 levels, as evidenced by qPCR, verifying that *hif-1* gain-of-function inhibits FTN-2 (Fig. 9H; unpublished at the time of writing). We did not test the levels of FTN-2 in the *egl-9* mutant as it is already well established that EGL-9 loss of function upregulates HIF-1.

After measuring protein and mRNA levels of the genes of interest, we performed lipidomics analysis on the suppressors to confirm that each of the *fat-2(wa17)* suppressors boost PUFA levels, particularly levels of 20:5, validating the protein expression patterns seen from the western blots (Fig. 9C). Thus, we revealed that the only way to rescue worms with severely reduced FAT-2 desaturase activity is to boost desaturase activity and not through other mechanisms that can bypass the need for PUFAs.

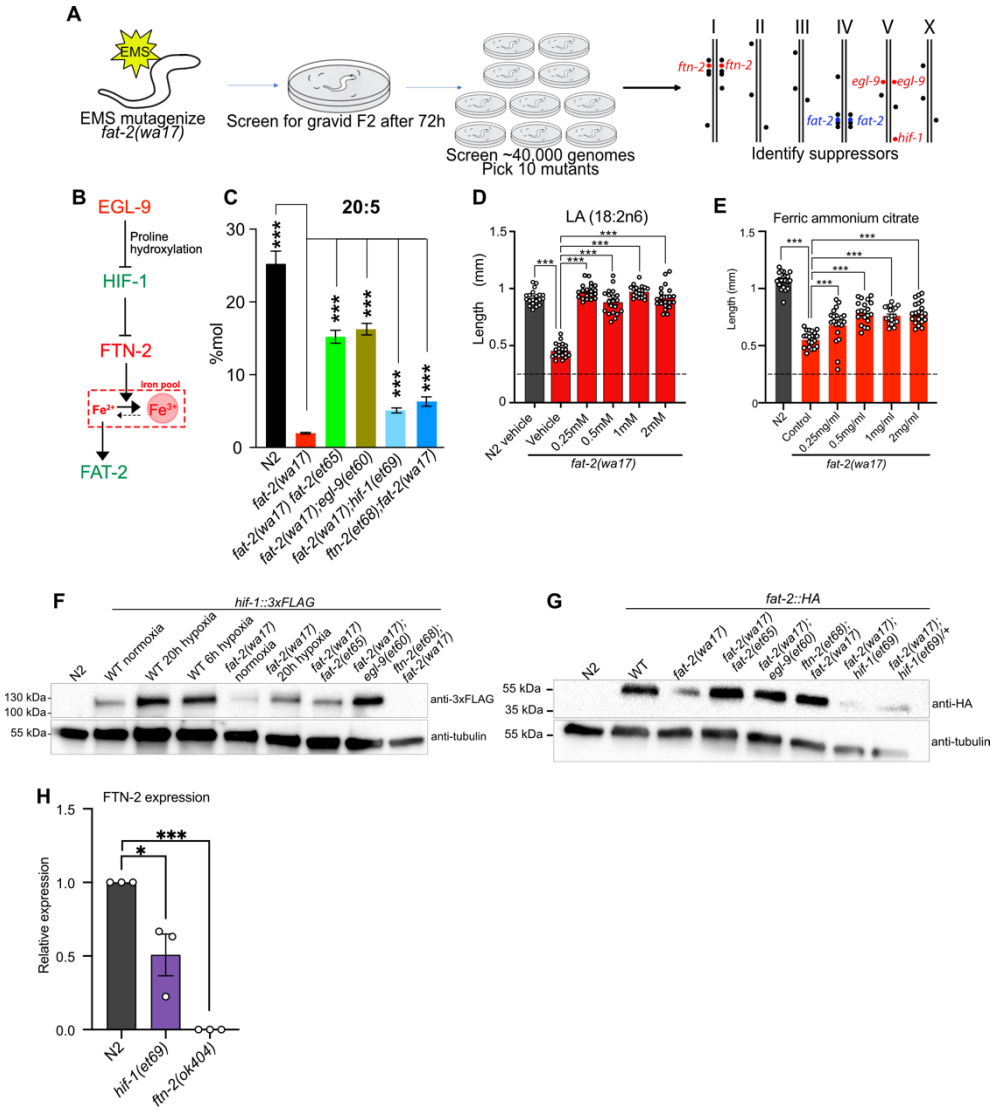

Figure 9. Summary of Paper III results. A) Schematic of the forward genetic screen that identified *ftn-2, egl-9,* and *hif-1* as suppressors of *fat-2(wa17).* B) *fat-2(wa17)* suppressors belong in the HIF-1 pathway. Red names indicate loss- or reduction- of function mutations, green indicates gain-of-function mutations. C) Lipidomics shows that *fat-2(wa17)* suppressors increase levels of 20:5 FAs. *fat-2(wa17)* is rescued by D) exogenous PUFAs (LA) and E) treatments that mimic FTN-2 mutations (ferric ammonium citrate). F) HIF-1 protein expression is upregulated in *fat-2(wa17)* suppressors and downregulated in *fat-2(wa17).* G) FAT-2 proteins levels are upregulated in suppressors. H) Gain-of-function *hif-1* reduces FTN-2 mRNA levels as measured by qPCR. Adapted from (Kaper et al., 2024).

fat-2(wa17) is partially rescued by treatments that mimic suppressor mutants

To better understand the mechanisms behind how mutations within the HIF-1 pathway rescue *fat-2(wa17)*, we attempted to rescue *fat-2(wa17)* with treatments that mimic the suppressors. Exogenously providing iron in the form of ferric ammonium citrate (FAC) led to a slight rescue of *fat-2(wa17)* (Fig. 9E), likewise HIF-1-activating paraquat (PQ) slightly rescued the growth of the mutant worms, but a combination of FAC and PQ did not yield any further rescue. Hydrogen peroxide, another HIF-1 activator, yielded a slight rescue, while hypoxia mimetics cobalt chloride and sodium sulfite did not rescue *fat-2(wa17)*. Short incubation under hypoxia itself, however, did lead to a mild rescue but longer hypoxia treatments were not beneficial. These results show that increasing iron levels and activating HIF-1 are beneficial to the *fat-2(wa17)* worms that are PUFA-deficient, although finding the correct dose and delivering it to the right tissues may be difficult.

DISCUSSION

In this paper we isolated ten novel alleles that suppress the slow growth phenotype of *fat-2(wa17)* worms that almost entirely lack PUFAs. The suppressors fall into two categories; (i) internal mutations within *fat-2* itself; or (ii) mutations in genes that belong in the HIF-1 pathway. As there were no other, unrelated, suppressors identified, we believe that the screen was at or near saturation. From the screen and identity of the suppressors, we hypothesize that the only way to rescue worms that are almost wholly devoid of PUFAs is to boost the small amount of desaturase activity left to create more PUFAs. Importantly, the suppressors only function as suppressors of *fat-2(wa17)* or *fat-2* RNAi-treated worms: they are not able to suppress the lethal *fat-2* null mutant. This shows that there must be some desaturase activity for the suppressors to enhance and that they cannot create PUFAs via some other means.

Our observations suggest that all the non-intragenic *fat-2(wa17)* suppressors converge on FTN-2 inhibition, first through reduction of *egl-9*

function that results in constitutively active HIF-1. HIF-1 being permanently activated (both through the *egl-9* mutations and the *hif-1* gain-of-function allele) leads to the inhibition of FTN-2. FTN-2 is responsible for the stable storage of mineralized iron by converting reactive Fe^{2+} ions to stable Fe^{3+} ions, and as such inhibition of *ftn-2* should result in increased levels of Fe^{2+} (Romney et al., 2011). Because Fe^{2+} and oxygen are both substrates required for desaturase reactions (J. Shen et al., 2023), the increase in Fe^{2+} likely increases and sustains the activity of the defective FAT-2 Δ12 desaturase.

Interestingly, the biggest change seen in the lipidomic profiles of the suppressors versus *fat-2(wa17*) was not in the levels of LA, the fatty acid that *fat-2(wa17)* is defective in synthesizing, but in the levels of 20:5, which is most likely EPA. As EPA is the last fatty acid in the *C. elegans* fatty acid synthesis pathway (Watts & Ristow, 2017), it is likely that the additional LA that is synthesized in the suppressor strains is immediately further elongated and desaturated instead of accumulating, and as a result accumulates as EPA. And indeed, in wild-type worms EPA is the most abundant fatty acid (Henry et al., 2016; Watts & Browse, 2002), so the increase in EPA levels is likely a big reason the suppressor worms are healthier than *fat-2(wa17).* It appears that the increase in EPA levels do not need to reach the same concentration as in N2 worms, since with as little as an ~5% mol increase in EPA compared to *fat-2(wa17)* (Fig. 9C), the *fat-2(wa17);hif-1(et69)* worms are clearly healthier. It is worth noting however, that *hif-1(et69)* suppresses *fat-2(wa17)* best when it is heterozygous, but lipidomics was performed on *hif-1(et69)* homozygous worms, so it is likely that in the heterozygous state the levels of EPA in the *hif-1* suppressor might have been further increased. Phospholipids, particularly PCs, with two EPA chains are abundant in *C. elegans,* particularly in worms cultivated in colder temperatures, suggesting that EPA is important to fluidize membranes (Kosel et al., 2011). However, *fat-2(wa17)* mutant development did not benefit from other membrane fluidizing treatments (e.g. NP40 or OA) indicating in this case that fluidizing membranes is not the most critical function of PUFAs. EPA is precursor for eicosanoids, and EPA-derived eicosanoids are important for cell signaling and migration in *C. elegans*

(Hoang et al., 2013; Horikawa & Sakamoto, 2010; Kosel et al., 2011), but interestingly *fat-2(wa17)* did not benefit from an exogenously supplied eicosanoid mix, suggesting it is not the lack of eicosanoids that confers the mutant phenotypes of *fat-2(wa17).*

Although mammals do not have a FAT-2 homolog and thus require LA to be obtained dietarily, the findings in this paper can be translated into research on mammal desaturases and can provide insight into the genetic mechanisms that can compensate for PUFA-deficiency. For instance, at the time of writing this thesis, we are investigating the effects of ferritin mutations on desaturase expression in SCD-silenced human cells (unpublished). Because humans lack a Δ12-desaturase, we are using SCD as the closest alternative as SCD-silenced cells lack MUFAs and as a result have excessively rigid membranes and do not react well to exogenous SFAs (Ruiz et al., 2021, 2022). Preliminarily, it appears that knocking-down SCD results in rigid membranes as visualized by Laurdan staining and this rigidity worsens when cells are treated with PA (as previously published) (Fig. 10A); additionally knocking down ferritin heavy chain (FTH) reverses some of the membrane rigidity phenotype (Fig. 10B). Further work will be done to determine if additional mutations in the HIF-1 pathway, or treatments such as iron supplementation or hypoxia, can boost desaturase activity in human cells as it does in *C. elegans*.

Own contributions to Paper III:

1. EMS screen for *fat-2(wa17)* suppressors: Performed the suppressor screen and isolated ten alleles. Screen was performed with help from another lab member.
2. Characterization of *fat-2(wa17)*: I cloned and microinjected *fat-2(+)* into *fat-2(wa17)* and performed all the growth experiments of *fat-2(wa17)* on different treatments.
3. FRAP experiments: I performed all FRAP experiments to test whether *fat-2(wa17)* has rigid membranes, and if fluidizing treatments and novel suppressors rescue this phenotype.

4. Lipidomics sample preparation: I prepared all samples for lipidomics. The actual lipidomics analysis is performed by collaborators.
5. Confirmation of *fat-2(wa17)* suppressors identities and function: I tested if null alleles of *hif-1, ftn-2,* and *egl-9* rescue *fat-2(wa17*) to determine if the suppressor alleles are loss-of-function. I performed *fat-2(RNAi)* on the *ftn-2(et68)* strain. I confirmed the identity of *ftn-2(et68*) as a suppressor and that *hif-1(et69)* works best as a suppressor when heterozygous.
6. Westen blots and qPCR: I performed all western blots and qPCR to test levels of HIF-1, FAT-2, FTN-2 protein/mRNA levels in suppressor strains.
7. Stress response assay: I imaged and analyzed *fat-2(wa17*)'s mitochondrial stress response and its rescue by *ftn-2(et68).*
8. Rescuing *fat-2(wa17*) with suppressor mimics: Apart from hypoxia and sodium sulfite treatments, I performed all rescue experiment of *fat-2(wa17).*

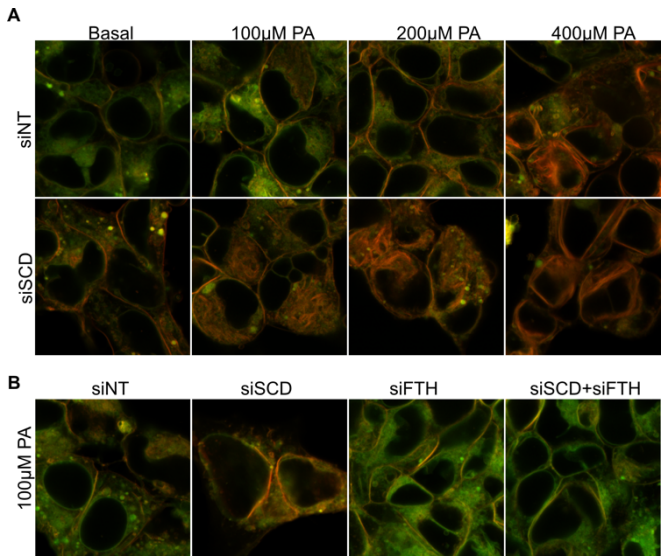

Figure 10. Translating Paper III findings into human cells. A) Laurdan staining of siSCD cells having rigid membranes, which is worsened with PA treatment. B) Co-silencing of FTH rescues SCD membrane rigidity defect. In these images, the green-to-red color range correlates with loosely packed/fluid-to-tightly packed/rigid membranes.

CONCLUSIONS AND FUTURE PERSPECTIVES

Throughout this thesis I have detailed the role of PAQR-2 on membrane fluidity homeostasis and the role of HIF-1 on polyunsaturated fatty acid synthesis.

With regard to the PAQR-2 pathway, we have shown that *C. elegans* are phenotypically best when they have ideal membrane homeostasis. Any changes to membrane composition, and thus membrane homeostasis, results in deleterious changes to several traits, both cellular and physiological. While worms with excessively fluid membranes fare better than worms with excessively rigid membranes, deviation towards either side of the membrane homeostasis scale results in less-than-ideal phenotypes. Additionally, we confirmed that PAQR-2 interacts with several proteins involved with fatty acid elongation and recruits an ER-bound complex made up of these proteins as well as with an acyl-coA synthetase to promote the elongation of unsaturated fatty acids and channel UFA incorporation into phospholipids to fluidize membranes during rigidity challenges. PAQR-2 is localized to the plasma membrane (Yamauchi et al., 2003), yet is able to recruit ER-localized proteins presumably to the plasma membrane to promote FA synthesis, yet we have never confirmed where this interaction occurs. It would be interesting to use MAPPER, an ER-PM junction marker (Y. J. Chen et al., 2019), to visualize if PAQR-2 is interacting with HPO-8/ACS-4 at this junction point or if the interaction is entirely ER or plasma membrane based. If PAQR-2/HPO-8/ACS-4 are interacting at these junctions it would provide proof that UFAs are being shared directly between the ER and plasma membrane to locally and more quickly influence FA levels as opposed relying on vesicular trafficking for FA transport.

The HIF-1 pathway having relevance to this thesis was surprising initially, as we did not expect mutations within the pathway to be the only mutations that can compensate for fatty acid deficiency. And in a way, mutations within the HIF-1 pathway are not really compensating for fatty acid deficiency by finding other mechanisms for worms to survive without

PUFAs. Instead, the suppressors are simply allowing the residual *fat-2(wa17)* desaturase activity to be more efficient. Indeed, one of the limiting factors of a fatty acid deficiency reaction is the availability of ferrous ions, and through our genetic screen we identified that each of the suppressors converges on inhibition of the ferritin FTN-2. Inhibiting FTN-2 should increase the availability of ferrous ions and therefore allow the mutated FAT-2 desaturase to more efficiently function and produce more PUFAs. As discussed under Paper III, I am currently attempting to translate these findings into mammalian cells, and it will be interesting to see if mutations in the HIF-1 pathway reliably improve desaturase efficiency in a conserved manner in other species. Humans with mutations in FADS1 or FADS2, FA desaturase genes that catalyze the several desaturation steps starting with conversion of 18:2n6 to 18:3n6, have irregular serum lipid levels and increased risk for conditions such as inflammation, cardiovascular disease, diabetes, liver disease and ischemic stroke (Ameur et al., 2012; Malerba et al., 2008; Voruganti et al., 2012; Q. Yang et al., 2015). As the western diet shifts more heavily toward ω-6 PUFA-rich as opposed to ω-3-rich, it would be interesting to identify if treatments that mimic mutations in the HIF-1 pathway could help reduce the risk of such health problems caused by FA deficiencies or imbalances, especially as risk of these diseases grows.

To conclude, the papers in this thesis provided further characterization of the previously well-studied PAQR-2 membrane fluidity regulation pathway and introduced a new pathway involved in fatty acid homeostasis in *C. elegans*. By studying both PAQR-2 and HIF-1 (more specifically FTN-2 within the HIF-1 pathway) we gain further insight into the importance of membrane and lipid homeostasis. Perhaps in the future, further studies can be performed to more directly translate our findings into mammalian models to provide a basis for disease treatments.

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REFERENCES

Ackerman, D., & Gems, D. (2012). Insulin/IGF-1 and Hypoxia Signaling Act in Concert to Regulate Iron Homeostasis in Caenorhabditis elegans. *PLOS Genetics*, *8*(3), e1002498.

Adeva-Andany, M. M., Carneiro-Freire, N., Seco-Filgueira, M., Fernández-Fernández, C., & Mouriño-Bayolo, D. (2019). Mitochondrial β-oxidation of saturated fatty acids in humans. *Mitochondrion*, *46*, 73–90.

Ameur, A., Enroth, S., Johansson, Å., Zaboli, G., Igl, W., Johansson, A. C. V., Rivas, M. A., Daly, M. J., Schmitz, G., Hicks, A. A., Meitinger, T., Feuk, L., Van Duijn, C., Oostra, B., Pramstaller, P. P., Rudan, I., Wright, A. F., Wilson, J. F., Campbell, H., & Gyllensten, U. (2012). Genetic Adaptation of Fatty-Acid Metabolism: A Human-Specific Haplotype Increasing the Biosynthesis of Long-Chain Omega-3 and Omega-6 Fatty Acids. *American Journal of Human Genetics*, *90*(5), 809.

Arquier, N., Bjordal, M., Hammann, P., Kuhn, L., & Léopold, P. (2021). Brain adiponectin signaling controls peripheral insulin response in Drosophila. *Nature Communications*, *12*(1).

Atherton, H. J., Jones, O. A. H., Malik, S., Miska, E. A., & Griffin, J. L. (2008). A comparative metabolomic study of NHR-49 in Caenorhabditis elegans and PPAR-α in the mouse. *FEBS Letters*, *582*(12), 1661.

Bamia, C., Lagiou, P., Buckland, G., Grioni, S., Agnoli, C., Taylor, A. J., Dahm, C. C., Overvad, K., Olsen, A., Tjønneland, A., Cottet, V., Boutron-Ruault, M. C., Morois, S., Grote, V., Teucher, B., Boeing, H., Buijsse, B., Trichopoulos, D., Adarakis, G., … Trichopoulou, A. (2013). Mediterranean diet and colorectal cancer risk: Results from a European cohort. *European Journal of Epidemiology*, *28*(4), 317–328.

Barceló, F., Perona, J. S., Prades, J., Funari, S. S., Gomez-Gracia, E., Conde, M., Estruch, R., & Ruiz-Gutiérrez, V. (2009). Mediterranean-style diet effect on the structural properties of the erythrocyte cell membrane of hypertensive patients: The prevencion con dieta mediterranea study. *Hypertension*, *54*(5), 1143–1150.

Bazinet, R. P., & Layé, S. (2014). Polyunsaturated fatty acids and their metabolites in brain function and disease. *Nature Reviews Neuroscience 2014 15:12*, *15*(12), 771–785.

Bellier, A., Chen, C. S., Kao, C. Y., Cinar, H. N., & Aroian, R. V. (2009). Hypoxia and the Hypoxic Response Pathway Protect against Pore-Forming Toxins in C. elegans. *PLOS Pathogens*, *5*(12), e1000689.

Bensaad, K., Favaro, E., Lewis, C. A., Peck, B., Lord, S., Collins, J. M., Pinnick, K. E., Wigfield, S., Buffa, F. M., Li, J. L., Zhang, Q., Wakelam, M. J. O., Karpe, F., Schulze, A., & Harris, A. L. (2014). Fatty Acid Uptake and Lipid Storage

Induced by HIF-1α Contribute to Cell Growth and Survival after Hypoxia-Reoxygenation. *Cell Reports*, *9*(1), 349–365.

Bertrand, S., Brunet, F. G., Escriva, H., Parmentier, G., Laudet, V., & Robinson-Rechavi, M. (2004). Evolutionary genomics of nuclear receptors: from twenty-five ancestral genes to derived endocrine systems. *Molecular Biology and Evolution*, *21*(10), 1923–1937.

Blazek, E., Mittler, G., & Meisterernst, M. (2005). The mediator of RNA polymerase II. *Chromosoma*, *113*(8), 399–408.

Bodhicharla, R., Devkota, R., Ruiz, M., & Pilon, M. (2018). Membrane Fluidity Is Regulated Cell Nonautonomously by Caenorhabditis elegans PAQR-2 and Its Mammalian Homolog AdipoR2. *Genetics*, *210*(1), 189–201.

Borgeson, C. E., Renobales, M. de, & Blomquist, G. J. (1990). Characterization of the delta 12 desaturase in the American cockroach, Periplaneta americana: the nature of the substrate. *Biochimica et Biophysica Acta*, *1047*(2), 135–140.

Brenner, S. (1974). THE GENETICS OF CAENORHABDITIS ELEGANS. *Genetics*, *77*(1), 71–94.

Brock, T. J., Browse, J., & Watts, J. L. (2006). Genetic Regulation of Unsaturated Fatty Acid Composition in C. elegans. *PLOS Genetics*, *2*(7), e108.

Brock, T. J., Browse, J., & Watts, J. L. (2007). Fatty Acid Desaturation and the Regulation of Adiposity in Caenorhabditis elegans. *Genetics*, *176*(2), 865.

Brox, J. H., Killie, J. E., Østerud, B., Holme, S., & Nordøy, A. (1983). Effects of Cod Liver Oil on Platelets and Coagulation in Familial Hypercholesterolemia (Type IIa). *Acta Medica Scandinavica*, *213*(2), 137–144.

Burr, G. O., & Burr, M. M. (1929). A NEW DEFICIENCY DISEASE PRODUCED BY THE RIGID EXCLUSION OF FAT FROM THE DIET. *Journal of Biological Chemistry*, *82*(2), 345–367.

Burr, G. O., & Burr, M. M. (1930). ON THE NATURE AND RÔLE OF THE FATTY ACIDS ESSENTIAL IN NUTRITION. *Journal of Biological Chemistry*, *86*(2), 587–621.

Burr, G. O., Burr, M. M., & Miller, E. S. (1932). ON THE FATTY ACIDS ESSENTIAL IN NUTRITION. III. *Journal of Biological Chemistry*, *97*(1), 1–9.

Busayavalasa, K., Ruiz, M., Devkota, R., Ståhlman, M., Bodhicharla, R., Svensk, E., Hermansson, N.-O., Borén, J., & Pilon, M. (2020). Leveraging a gain-of-function allele of Caenorhabditis elegans paqr-1 to elucidate membrane homeostasis by PAQR proteins. *PLOS Genetics*, *16*(8), e1008975.

Cai, Y., Yu, X. H., Chai, J., Liu, C. J., & Shanklin, J. (2020). A conserved evolutionary mechanism permits Δ9 desaturation of very-long-chain fatty acyl lipids. *The Journal of Biological Chemistry*, *295*(32), 11337.

Calder, P. C. (2012). Mechanisms of action of (n-3) fatty acids. *The Journal of Nutrition*, *142*(3).

Carman, G. M., & Han, G. S. (2007). Regulation of phospholipid synthesis in Saccharomyces cerevisiae by zinc depletion. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1771*(3), 322–330.

Chen, M. K., Chiou, H. L., Su, S. C., Chung, T. Te, Tseng, H. C., Tsai, H. T., & Yang, S. F. (2009). The association between hypoxia inducible factor-1α gene polymorphisms and increased susceptibility to oral cancer. *Oral Oncology*, *45*(12), e222–e226.

Chen, Y. J., Quintanilla, C. G., & Liou, J. (2019). Recent Insights into Mammalian ER-PM Junctions. *Current Opinion in Cell Biology*, *57*, 99.

Chen, Z., Han, F., Du, Y., Shi, H., & Zhou, W. (2023). Hypoxic microenvironment in cancer: molecular mechanisms and therapeutic interventions. *Signal Transduction and Targeted Therapy 2023 8:1*, *8*(1), 1–23.

Chen, Z., Yang, H., Ren, Y., Yang, Z., Huang, J., Li, C., Xiong, Y., & Yu, B. (2023). Distinct roles of ADIPOR1 and ADIPOR2: A pan-cancer analysis. *Frontiers in Endocrinology*, *14*, 1119534.

Chitwood, D. J., Lusby, W. R., Thompson, M. J., Kochansky, J. P., & Howarth, O. W. (1995). The glycosylceramides of the nematodeCaenorhabditis elegans contain an unusual, branched-chain sphingoid base. *Lipids*, *30*(6), 567–573.

Consortium*, T. C. elegans S. (1998). Genome sequence of the nematode C. elegans: A platform for investigating biology. *Science*, *282*(5396), 2012–2018.

Das, A., Goldstein, J. L., Anderson, D. D., Brown, M. S., & Radhakrishnan, A. (2013). Use of mutant 125I-Perfringolysin O to probe transport and organization of cholesterol in membranes of animal cells. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(26), 10580–10585.

Davis, C., Hodgson, J., Bryan, J., Garg, M., Woodman, R., & Murphy, K. (2017). Older Australians Can Achieve High Adherence to the Mediterranean Diet during a 6 Month Randomised Intervention; Results from the Medley Study. *Nutrients 2017, Vol. 9, Page 534*, *9*(6), 534.

Denic, V., & Weissman, J. S. (2007). A Molecular Caliper Mechanism for Determining Very Long-Chain Fatty Acid Length. *Cell*, *130*(4), 663–677.

Devkota, R., Henricsson, M., Borén, J., & Pilon, M. (2021). The C. elegans PAQR-2 and IGLR-2 membrane homeostasis proteins are uniquely essential for tolerating dietary saturated fats. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1866*(4), 158883.

Devkota, R., Kaper, D., Bodhicharla, R., Henricsson, M., Borén, J., & Pilon, M. (2021). A genetic titration of membrane composition in Caenorhabditis elegans reveals its importance for multiple cellular and physiological traits. *Genetics*, *219*(1).

Devkota, R., & Pilon, M. (2018). FRAP: A Powerful Method to Evaluate Membrane Fluidity in Caenorhabditis elegans. *BIO-PROTOCOL*, *8*(13).

Devkota, R., Svensk, E., Ruiz, M., Ståhlman, M., Borén, J., & Pilon, M. (2017). The adiponectin receptor AdipoR2 and its Caenorhabditis elegans homolog PAQR-2 prevent membrane rigidification by exogenous saturated fatty acids. *PLOS Genetics*, *13*(9), e1007004.

Diot, C., García-González, A. P., Vieira, A. F., Walker, M., Honeywell, M., Doyle, H., Ponomarova, O., Rivera, Y., Na, H., Zhang, H., Lee, M., Olsen, C. P., & Walhout, A. J. M. (2022). Bacterial diet modulates tamoxifen-induced death via host fatty acid metabolism. *Nature Communications 2022 13:1*, *13*(1), 1–15.

Doll, S., Proneth, B., Tyurina, Y. Y., Panzilius, E., Kobayashi, S., Ingold, I., Irmler, M., Beckers, J., Aichler, M., Walch, A., Prokisch, H., Trümbach, D., Mao, G., Qu, F., Bayir, H., Füllekrug, J., Scheel, C. H., Wurst, W., Schick, J. A., … Conrad, M. (2017). ACSL4 dictates ferroptosis sensitivity by shaping cellular lipid composition. *Nature Chemical Biology*, *13*(1), 91–98.

Entchev, E. V., Schwudke, D., Zagoriy, V., Matyash, V., Bogdanova, A., Habermann, B., Zhu, L., Shevchenko, A., & Kurzchalia, T. V. (2008). LET-767 is required for the production of branched chain and long chain fatty acids in Caenorhabditis elegans. *The Journal of Biological Chemistry*, *283*(25), 17550–17560. https://doi.org/10.1074/JBC.M800965200

Estruch, R., Ros, E., Salas-Salvadó, J., Covas, M.-I., Corella, D., Arós, F., Gómez-Gracia, E., Ruiz-Gutiérrez, V., Fiol, M., Lapetra, J., Lamuela-Raventos, R. M., Serra-Majem, L., Pintó, X., Basora, J., Muñoz, M. A., Sorlí, J. V., Martínez, J. A., Fitó, M., Gea, A., … Martínez-González, M. A. (2018). Primary Prevention of Cardiovascular Disease with a Mediterranean Diet Supplemented with Extra-Virgin Olive Oil or Nuts. *New England Journal of Medicine*, *378*(25).

Fahy, E., Subramaniam, S., Brown, H. A., Glass, C. K., Merrill, A. H., Murphy, R. C., Raetz, C. R. H., Russell, D. W., Seyama, Y., Shaw, W., Shimizu, T., Spener, F., Van Meer, G., VanNieuwenhze, M. S., White, S. H., Witztum, J. L., & Dennis, E. A. (2005). A comprehensive classification system for lipids. *Journal of Lipid Research*, *46*(5), 839–861.

Fan, L., Li, J., Yu, Z., Dang, X., & Wang, K. (2014). The Hypoxia-Inducible Factor Pathway, Prolyl Hydroxylase Domain Protein Inhibitors, and Their Roles in Bone Repair and Regeneration. *BioMed Research International*, *2014*, 239356.

Fan, Y. Y., Monk, J. M., Hou, T. Y., Callway, E., Vincent, L., Weeks, B., Yang, P., & Chapkin, R. S. (2012). Characterization of an arachidonic acid-deficient (Fads1 knockout) mouse model. *Journal of Lipid Research*, *53*(7), 1287.

Fanning, S., Haque, A., Imberdis, T., Baru, V., Barrasa, M. I., Nuber, S., Termine, D., Ramalingam, N., Ho, G. P. H., Noble, T., Sandoe, J., Lou, Y., Landgraf, D.,

Freyzon, Y., Newby, G., Soldner, F., Terry-Kantor, E., Kim, T. E., Hofbauer, H. F., … Selkoe, D. (2019). Lipidomic Analysis of α-Synuclein Neurotoxicity Identifies Stearoyl CoA Desaturase as a Target for Parkinson Treatment. *Molecular Cell*, *73*(5), 1001-1014.e8.

Flowers, M. T., & Ntambi, J. M. (2008). Role of stearoyl-coenzyme A desaturase in regulating lipid metabolism. *Current Opinion in Lipidology*, *19*(3), 248–256.

Fransén, K., Fenech, M., Fredrikson, M., Dabrosin, C., & Söderkvist, P. (2006). Association between ulcerative growth and hypoxia inducible factor-1alpha polymorphisms in colorectal cancer patients. *Molecular Carcinogenesis*, *45*(11), 833–840.

Furuhashi, M., & Hotamisligil, G. S. (2008). Fatty acid-binding proteins: role in metabolic diseases and potential as drug targets. *Nature Reviews Drug Discovery 2008 7:6*, *7*(6), 489–503.

Furuta, E., Pai, S. K., Zhan, R., Bandyopadhyay, S., Watabe, M., Mo, Y. Y., Hirota, S., Hosobe, S., Tsukada, T., Miura, K., Kamada, S., Saito, K., Iiizumi, M., Liu, W., Ericsson, J., & Watabe, K. (2008). Fatty acid synthase gene is up-regulated by hypoxia via activation of Akt and sterol regulatory element binding protein-1. *Cancer Research*, *68*(4), 1003–1011.

Gladek, I., Ferdin, J., Horvat, S., Calin, G. A., & Kunej, T. (2017). HIF1A gene polymorphisms and human diseases: Graphical review of 97 association studies. *Genes, Chromosomes and Cancer*, *56*(6), 439–452.

Goh, G. Y. S., Martelli, K. L., Parhar, K. S., Kwong, A. W. L., Wong, M. A., Mah, A., Hou, N. S., & Taubert, S. (2013). The conserved Mediator subunit MDT-15 is required for oxidative stress responses in Caenorhabditis elegans. *Aging Cell*, *13*(1), 70.

Gourley, B. L., Parker, S. B., Jones, B. J., Zumbrennen, K. B., & Leibold, E. A. (2003). Cytosolic Aconitase and Ferritin Are Regulated by Iron in Caenorhabditis elegans. *Journal of Biological Chemistry*, *278*(5), 3227–3234.

Greer, E. R., Pérez, C. L., Van Gilst, M. R., Lee, B. H., & Ashrafi, K. (2008). Neural and Molecular Dissection of a C. elegans Sensory Circuit that Regulates Fat and Feeding. *Cell Metabolism*, *8*(2), 118–131.

Guschina, I. A., & Harwood, J. L. (2006). Mechanisms of temperature adaptation in poikilotherms. *FEBS Letters*, *580*(23), 5477–5483.

Harauma, A., Hatanaka, E., Yasuda, H., Nakamura, M. T., Salem, N., & Moriguchi, T. (2017). Effects of arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid on brain development using artificial rearing of delta-6-desaturase knockout mice. *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, *127*, 32–39.

Harayama, T., & Shimizu, T. (2020a). Roles of polyunsaturated fatty acids, from mediators to membranes. *Journal of Lipid Research*, *61*(8), 1150.

Harayama, T., & Shimizu, T. (2020b). Roles of polyunsaturated fatty acids, from mediators to membranes. *Journal of Lipid Research*, *61*(8), 1150.

Haslam, T. M., & Kunst, L. (2013). Extending the story of very-long-chain fatty acid elongation. *Plant Science*, *210*, 93–107.

Hazel, J. R. (1984). Effects of temperature on the structure and metabolism of cell membranes in fish. *The American Journal of Physiology*, *246*(4 Pt 2).

Hazel, J. R. (1995). Thermal adaptation in biological membranes: Is homeoviscous adaptation the explanation? *Annual Review of Physiology*, *57*(Volume 57,), 19–42.

He, Y., Yang, W., Gan, L., Liu, S., Ni, Q., Bi, Y., Han, T., Liu, Q., Chen, H., Hu, Y., Long, Y., & Yang, L. (2021). Silencing HIF-1α aggravates non-alcoholic fatty liver disease in vitro through inhibiting PPAR-α/ANGPTL4 singling pathway. *Gastroenterología y Hepatología (English Edition)*, *44*(5), 355–365.

Henry, P., Owopetu, O., Adisa, D., Nguyen, T., Anthony, K., Ijoni-Animadu, D., Jamadar, S., Abdel-Rahman, F., & Saleh, M. A. (2016). Fatty acids composition of Caenorhabditis elegans using accurate mass GCMS-QTOF. *Journal of Environmental Science and Health, Part B*, *51*(8), 546–552.

Hoang, H. D., Prasain, J. K., Dorand, D., & Miller, M. A. (2013). A Heterogeneous Mixture of F-Series Prostaglandins Promotes Sperm Guidance in the Caenorhabditis elegans Reproductive Tract. *PLOS Genetics*, *9*(1), e1003271. https://doi.org/10.1371/JOURNAL.PGEN.1003271

Holland, W. L., Miller, R. A., Wang, Z. V., Sun, K., Barth, B. M., Bui, H. H., Davis, K. E., Bikman, B. T., Halberg, N., Rutkowski, J. M., Wade, M. R., Tenorio, V. M., Kuo, M. S., Brozinick, J. T., Zhang, B. B., Birnbaum, M. J., Summers, S. A., & Scherer, P. E. (2010). Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. *Nature Medicine 2010 17:1*, *17*(1), 55–63.

Horikawa, M., & Sakamoto, K. (2010). Polyunsaturated fatty acids are involved in regulatory mechanism of fatty acid homeostasis via daf-2/insulin signaling in Caenorhabditis elegans. *Molecular and Cellular Endocrinology*, *323*(2), 183–192.

Hu, R., Li, G., Xu, Q., & Chen, L. (2022). Iron supplementation inhibits hypoxia-induced mitochondrial damage and protects zebrafish liver cells from death. *Frontiers in Physiology*, *13*, 925752.

Ikeda, M., Kanao, Y., Yamanaka, M., Sakuraba, H., Mizutani, Y., Igarashi, Y., & Kihara, A. (2008). Characterization of four mammalian 3-hydroxyacyl-CoA dehydratases involved in very long-chain fatty acid synthesis. *FEBS Letters*, *582*(16), 2435–2440.

Ivan, M., Kondo, K., Yang, H., Kim, W., Valiando, J., Ohh, M., Salic, A., Asara, J. M., Lane, W. S., & Kaelin, J. (2001). HIFalpha targeted for VHL-mediated

destruction by proline hydroxylation: implications for O2 sensing. *Science (New York, N.Y.)*, *292*(5516), 464–468.

Iwanyshyn, W. M., Han, G. S., & Carman, G. M. (2004). Regulation of Phospholipid Synthesis in Saccharomyces cerevisiae by Zinc. *Journal of Biological Chemistry*, *279*(21), 21976–21983.

Jain, I. H., Calvo, S. E., Markhard, A. L., Skinner, O. S., To, T. L., Ast, T., & Mootha, V. K. (2020). Genetic Screen for Cell Fitness in High or Low Oxygen Highlights Mitochondrial and Lipid Metabolism. *Cell*, *181*(3), 716-727.e11.

James, M. J., Gibson, R. A., & Cleland, L. G. (2000). Dietary polyunsaturated fatty acids and inflammatory mediator production. *The American Journal of Clinical Nutrition*, *71*(1), 343s–348s.

Jaramillo-Lambert, A., Fuchsman, A. S., Fabritius, A. S., Smith, H. E., & Golden, A. (2015). Rapid and efficient identification of caenorhabditis elegans legacy mutations using Hawaiian SNP-based mapping and whole-genome sequencing. *G3: Genes, Genomes, Genetics*, *5*(5), 1007–1019.

Jiang, H., Guo, R., & Powell-Coffman, J. A. (2001). The Caenorhabditis elegans hif-1 gene encodes a bHLH-PAS protein that is required for adaptation to hypoxia. *Proceedings of the National Academy of Sciences of the United States of America*, *98*(14), 7916.

Johnson, T. E., & Hutchinson, E. W. (1993). Absence of strong heterosis for life span and other life history traits in Caenorhabditis elegans. *Genetics*, *134*(2), 465–474.

Kaletta, T., & Hengartner, M. O. (2006). Finding function in novel targets: C. elegans as a model organism. *Nature Reviews Drug Discovery 2006 5:5*, *5*(5), 387–399.

Kang, M. J., Fujino, T., Sasano, H., Minekura, H., Yabuki, N., Nagura, H., Iijima, H., & Yamamoto, T. T. (1997). A novel arachidonate-preferring acyl-CoA synthetase is present in steroidogenic cells of the rat adrenal, ovary, and testis. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(7), 2880–2884.

Kaper, D., Radović, U., Bergh, P.-O., Qvist, A., Henricsson, M., Borén, J., & Pilon, M. (2024). A fat-2(wa17) suppressor screen in C. elegans reveals genetic adaptations to polyunsaturated fatty acid deficiency. *ELife*, *13*.

Karami Fath, M., Garousi, S., Mottahedi, M., Ghasemzadeh, N., Salmani, K., Olfati, F., Beit Saeed, M., Sotoudeh, S., & Barati, G. (2023). The role of hypoxia-inducible factors in breast cancer stem cell specification. *Pathology - Research and Practice*, *243*, 154349.

Karpichev, I. V., Cornivelli, L., & Small, G. M. (2002). Multiple Regulatory Roles of a Novel Saccharomyces cerevisiae Protein, Encoded by YOL002c, in Lipid and Phosphate Metabolism. *Journal of Biological Chemistry*, *277*(22), 19609–19617.

Kelly, K., & Jacobs, R. (2011). *Phospholipid Biosynthesis*.

Killion, E. A., Reeves, A. R., El Azzouny, M. A., Yan, Q. W., Surujon, D., Griffin, J. D., Bowman, T. A., Wang, C., Matthan, N. R., Klett, E. L., Kong, D., Newman, J. W., Han, X., Lee, M. J., Coleman, R. A., & Greenberg, A. S. (2018). A role for long-chain acyl-CoA synthetase-4 (ACSL4) in diet-induced phospholipid remodeling and obesity-associated adipocyte dysfunction. *Molecular Metabolism*, *9*, 43–56.

Kim, Y. Il, Cho, J. H., Yoo, O. J., & Ahnn, J. (2004). Transcriptional Regulation and Life-span Modulation of Cytosolic Aconitase and Ferritin Genes in C. elegans. *Journal of Molecular Biology*, *342*(2), 421–433.

Kim, S. M., Kim, H., Lee, J. S., Park, K. S., Jeon, G. S., Shon, J., Ahn, S. W., Kim, S. H., Lee, K. M., Sung, J. J., & Lee, K. W. (2013). Intermittent hypoxia can aggravate motor neuronal loss and cognitive dysfunction in ALS mice. *PloS One*, *8*(11).

Kita, S., Fukuda, S., Maeda, N., & Shimomura, I. (2019). Native adiponectin in serum binds to mammalian cells expressing t-cadherin, but not adipors or calreticulin. *ELife*, *8*.

Kniazeva, M., Crawford, Q. T., Seiber, M., Wang, C. Y., & Han, M. (2004). Monomethyl Branched-Chain Fatty Acids Play an Essential Role in Caenorhabditis elegans Development. *PLOS Biology*, *2*(9), e257.

Köfeler, H. C. (2016). Nomenclature of Fatty Acids. *Encyclopedia of Lipidomics*, 1–3.

Konac, E., Onen, H. I., Metindir, J., Alp, E., Biri, A. A., & Ekmekci, A. (2007). An investigation of relationships between hypoxia-inducible factor-1α gene polymorphisms and ovarian, cervical and endometrial cancers. *Cancer Detection and Prevention*, *31*(2), 102–109.

Kosel, M., Wild, W., Bell, A., Rothe, M., Lindschau, C., Steinberg, C. E. W., Schunck, W. H., & Menzel, R. (2011). Eicosanoid formation by a cytochrome P450 isoform expressed in the pharynx of Caenorhabditis elegans. *Biochemical Journal*, *435*(3), 689–700.

Kouli, G. M., Panagiotakos, D. B., Kyrou, I., Magriplis, E., Georgousopoulou, E. N., Chrysohoou, C., Tsigos, C., Tousoulis, D., & Pitsavos, C. (2019). Olive oil consumption and 10-year (2002–2012) cardiovascular disease incidence: the ATTICA study. *European Journal of Nutrition*, *58*(1), 131–138.

Kubagawa, H. M., Watts, J. L., Corrigan, C., Edmonds, J. W., Sztul, E., Browse, J., & Miller, M. A. (2006). Oocyte signals derived from polyunsaturated fatty acids control sperm recruitment in vivo. *Nature Cell Biology 2006 8:10*, *8*(10), 1143–1148.

Kubota, N., Terauchi, Y., Yamauchi, T., Kubota, T., Moroi, M., Matsui, J., Eto, K., Yamashita, T., Kamon, J., Satoh, H., Yano, W., Froguel, P., Nagai, R., Kimura, S., Kadowaki, T., & Noda, T. (2002). Disruption of adiponectin causes

insulin resistance and neointimal formation. *The Journal of Biological Chemistry*, *277*(29), 25863–25866.

Kurzchalia, T. V., & Ward, S. (2003). Why do worms need cholesterol? *Nature Cell Biology 2003 5:8*, *5*(8), 684–688.

Kutscher, L. M., & Shaham, S. (2014). Forward and reverse mutagenesis in C. elegans. *WormBook : The Online Review of C. Elegans Biology*, 1.

Kwak, S. J., Hong, S. H., Bajracharya, R., Yang, S. Y., Lee, K. S., & Yu, K. (2013). Drosophila Adiponectin Receptor in Insulin Producing Cells Regulates Glucose and Lipid Metabolism by Controlling Insulin Secretion. *PLOS ONE*, *8*(7), e68641.

Lall, R., Mohammed, R., & Ojha, U. (2019). What are the links between hypoxia and alzheimer's disease? *Neuropsychiatric Disease and Treatment*, *15*, 1343–1354.

Lange, Y., Swaisgood, M. H., Ramos, B. V., & Steck, T. L. (1989). Plasma Membranes Contain Half the Phospholipid and 90% of the Cholesterol and Sphingomyelin in Cultured Human Fibroblasts. *Journal of Biological Chemistry*, *264*(7), 3786–3793.

Laws, K. M., Sampson, L. L., & Drummond-Barbosa, D. (2015). Insulin-independent role of adiponectin receptor signaling in Drosophila germline stem cell maintenance. *Developmental Biology*, *399*(2), 226–236.

Lee, A. G. (1991). Lipids and their effects on membrane proteins: Evidence against a role for fluidity. *Progress in Lipid Research*, *30*(4), 323–348.

Lee, J. W., Bae, S. H., Jeong, J. W., Kim, S. H., & Kim, K. W. (2004). Hypoxia-inducible factor (HIF-1)α: its protein stability and biological functions. *Experimental & Molecular Medicine 2004 36:1*, *36*(1), 1–12.

Lee, J. Y., Choi, J. Y., Lee, K. M., Park, S. K., Han, S. H., Noh, D. Y., Ahn, S. H., Kim, D. H., Hong, Y. C., Ha, E., Yoo, K. Y., Ambrosone, C. B., & Kang, D. (2008). Rare variant of hypoxia-inducible factor-1α (HIF-1A) and breast cancer risk in Korean women. *Clinica Chimica Acta*, *389*(1–2), 167–170.

Leonard, A. E., Pereira, S. L., Sprecher, H., & Huang, Y. S. (2004). Elongation of long-chain fatty acids. *Progress in Lipid Research*, *43*(1), 36–54.

Lesa, G. M., Palfreyman, M., Hall, D. H., Clandinin, M. T., Rudolph, C., Jorgensen, E. M., & Schiavo, G. (2003). Long chain polyunsaturated fatty acids are required for efficient neurotransmission in C. elegans. *Journal of Cell Science*, *116*(24), 4965–4975.

Levental, K. R., Malmberg, E., Symons, J. L., Fan, Y. Y., Chapkin, R. S., Ernst, R., & Levental, I. (2020). Lipidomic and biophysical homeostasis of mammalian membranes counteracts dietary lipid perturbations to maintain cellular fitness. *Nature Communications 2020 11:1*, *11*(1), 1–13.

Lewis, R. A., Lee, T. H., & Austen, K. F. (1986). EFFECTS OF OMEGA–3 FATTY ACIDS ON THE GENERATION OF PRODUCTS OF THE 5-LIPOXYGENASE

PATHWAY. *Health Effects of Polyunsaturated Fatty Acids in Seafoods*, 227–238.

Listenberger, L. L., Han, X., Lewis, S. E., Cases, S., Farese, R. V., Ory, D. S., & Schaffer, J. E. (2003). Triglyceride accumulation protects against fatty acid-induced lipotoxicity. *Proceedings of the National Academy of Sciences*, *100*(6), 3077–3082.

Los, D. A., & Murata, N. (2004). Membrane fluidity and its roles in the perception of environmental signals. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1666*(1–2), 142–157.

Lyons, T. J., Villa, N. Y., Regalla, L. M., Kupchak, B. R., Vagstad, A., & Eide, D. J. (2004). Metalloregulation of yeast membrane steroid receptor homologs. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(15), 5506–5511.

Ma, D. K., Li, Z., Lu, A. Y., Sun, F., Chen, S., Rothe, M., Menzel, R., Sun, F., & Horvitz, H. R. (2015). Acyl-CoA dehydrogenase drives heat adaptation by sequestering fatty acids. *Cell*, *161*(5), 1152.

Maeda, N., Shimomura, I., Kishida, K., Nishizawa, H., Matsuda, M., Nagaretani, H., Furuyama, N., Kondo, H., Takahashi, M., Arita, Y., Komuro, R., Ouchi, N., Kihara, S., Tochino, Y., Okutomi, K., Horie, M., Takeda, S., Aoyamaa, T., Funahashi, T., & Matsuzawa, Y. (2002). Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nature Medicine*, *8*(7), 731–737.

Malerba, G., Schaeffer, L., Xumerle, L., Klopp, N., Trabetti, E., Biscuola, M., Cavallari, U., Galavotti, R., Martinelli, N., Guarini, P., Girelli, D., Olivieri, O., Corrocher, R., Heinrich, J., Pignatti, P. F., & Illig, T. (2008). SNPs of the FADS gene cluster are associated with polyunsaturated fatty acids in a cohort of patients with cardiovascular disease. *Lipids*, *43*(4), 289–299.

Manni, M. M., Tiberti, M. L., Pagnotta, S., Barelli, H., Gautier, R., & Antonny, B. (2018). Acyl chain asymmetry and polyunsaturation of brain phospholipids facilitate membrane vesiculation without leakage. *ELife*, *7*, e34394.

Martínez-González, M. Á., De La Fuente-Arrillaga, C., Nunez-Cordoba, J. M., Basterra-Gortari, F. J., Beunza, J. J., Vazquez, Z., Benito, S., Tortosa, A., & Bes-Rastrollo, M. (2008). Adherence to Mediterranean diet and risk of developing diabetes: prospective cohort study. *BMJ*, *336*(7657), 1348–1351.

Mattiazzi Ušaj, M., Prelec, M., Brložnik, M., Primo, C., Curk, T., Ščančar, J., Yenush, L., & Petrovič, U. (2015). Yeast Saccharomyces cerevisiae adiponectin receptor homolog Izh2 is involved in the regulation of zinc, phospholipid and pH homeostasis. *Metallomics*, *7*(9), 1338–1351.

Mbugua, S. N. (2022). Targeting Tumor Microenvironment by Metal Peroxide Nanoparticles in Cancer Therapy. *Bioinorganic Chemistry and Applications*, *2022*(1), 5041399.

McMahon, H. T., & Boucrot, E. (2015). Membrane curvature at a glance. *Journal of Cell Science*, *128*(6), 1065–1070.

Menendez, J. A., & Lupu, R. (2007). Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. *Nature Reviews. Cancer*, *7*(10), 763–777.

Mociño-Rodríguez, M. D., Santillán-Benítez, J. G., Dozal-Domínguez, D. S., Hernández-Navarro, M. D., Flores-Merino, M. V., Sandoval-Cabrera, A., & García Vázquez, F. J. (2017). Expression of AdipoR1 and AdipoR2 Receptors as Leptin-Breast Cancer Regulation Mechanisms. *Disease Markers*, *2017*(1), 4862016.

Moon, Y. A., Hammer, R. E., & Horton, J. D. (2009). Deletion of ELOVL5 leads to fatty liver through activation of SREBP-1c in mice. *Journal of Lipid Research*, *50*(3), 412.

Moreno-Arriola, E., El Hafidi, M., Ortega-Cuéllar, D., & Carvajal, K. (2016). AMP-Activated Protein Kinase Regulates Oxidative Metabolism in Caenorhabditis elegans through the NHR-49 and MDT-15 Transcriptional Regulators. *PloS One*, *11*(1).

Mullaney, B. C., & Ashrafi, K. (2009). C. elegans fat storage and metabolic regulation. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1791*(6), 474–478.

Murray, P., Hayward, S. A. L., Govan, G. G., Gracey, A. Y., & Cossins, A. R. (2007). An explicit test of the phospholipid saturation hypothesis of acquired cold tolerance in Caenorhabditis elegans. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(13), 5489–5494.

Mylonis, I., Sembongi, H., Befani, C., Liakos, P., Siniossoglou, S., & Simos, G. (2012). Hypoxia causes triglyceride accumulation by HIF-1-mediated stimulation of lipin 1 expression. *Journal of Cell Science*, *125*(14), 3485–3493.

Mylonis, I., Simos, G., & Paraskeva, E. (2019). Hypoxia-Inducible Factors and the Regulation of Lipid Metabolism. *Cells*, *8*(3).

Naidu, R., Har, Y. C., & Taib, N. A. (2009). Associations between hypoxia-inducible factor-1α (HIF-1α) gene polymorphisms and risk of developing breast cancer. *Neoplasma*, *56*(5), 441–447.

Nido, G. S., Méndez, R., Pascual-García, A., Abia, D., & Bastolla, U. (2011). Protein disorder in the centrosome correlates with complexity in cell types number. *Molecular BioSystems*, *8*(1), 353–367.

Nigon, V., & Dougherty, E. C. (1949). Reproductive patterns and attempts at reciprocal crossing of Rhabditis elegans maupas, 1900, and Rhabditis briggsae Dougherty and nigon, 1949 (Nematoda: Rhabditidae). *Journal of Experimental Zoology*, *112*(3), 485–503.

Ntambi, J. M., Miyazaki, M., Stoehr, J. P., Lan, H., Kendziorski, C. M., Yandell, B. S., Song, Y., Cohen, P., Friedman, J. M., & Attie, A. D. (2002). Loss of stearoyl–CoA desaturase-1 function protects mice against adiposity. *Proceedings of the National Academy of Sciences*, *99*(17), 11482–11486.

Ollerenshaw, M., Page, T., Hammonds, J., & Demaine, A. (2004). Polymorphisms in the hypoxia inducible factor-1α gene (HIF1A) are associated with the renal cell carcinoma phenotype. *Cancer Genetics and Cytogenetics*, *153*(2), 122–126.

O'Rourke, E. J., Kuballa, P., Xavier, R., & Ruvkun, G. (2013). ω-6 Polyunsaturated fatty acids extend life span through the activation of autophagy. *Genes & Development*, *27*(4), 429.

Overgaard, J., Tomčala, A., Sørensen, J. G., Holmstrup, M., Krogh, P. H., Šimek, P., & Koštál, V. (2008). Effects of acclimation temperature on thermal tolerance and membrane phospholipid composition in the fruit fly Drosophila melanogaster. *Journal of Insect Physiology*, *54*(3), 619–629.

Palmgren, H., Petkevicius, K., Bartesaghi, S., Ahnmark, A., Ruiz, M., Nilsson, R., Löfgren, L., Glover, M. S., Andreasson, A. C., Andersson, L., Becquart, C., Kurczy, M., Kull, B., Wallin, S., Karlsson, D., Hess, S., Maresca, M., Bohlooly-Y, M., Peng, X. R., & Pilon, M. (2023). Elevated Adipocyte Membrane Phospholipid Saturation Does Not Compromise Insulin Signaling. *Diabetes*, *72*(10), 1350–1363.

Pasanisi, P., Bruno, E., Venturelli, E., Morelli, D., Oliverio, A., Baldassari, I., Rovera, F., Iula, G., Taborelli, M., Peissel, B., Azzolini, J., & Manoukian, S. (2018). A Dietary Intervention to Lower Serum Levels of IGF-I in BRCA Mutation Carriers. *Cancers 2018, Vol. 10, Page 309*, *10*(9), 309.

Paton, C. M., & Ntambi, J. M. (2009). Biochemical and physiological function of stearoyl-CoA desaturase. *American Journal of Physiology - Endocrinology and Metabolism*, *297*(1), E28.

Perez, C. L., & Van Gilst, M. R. (2008). A 13C Isotope Labeling Strategy Reveals the Influence of Insulin Signaling on Lipogenesis in C. elegans. *Cell Metabolism*, *8*(3), 266–274.

Peyou-Ndi, M. M., Watts, J. L., & Browse, J. (2000). Identification and Characterization of an Animal Δ12 Fatty Acid Desaturase Gene by Heterologous Expression in Saccharomyces cerevisiae. *Archives of Biochemistry and Biophysics*, *376*(2), 399–408.

Pilon, M. (2021). Paradigm shift: the primary function of the "Adiponectin Receptors" is to regulate cell membrane composition. *Lipids in Health and Disease 2021 20:1*, *20*(1), 1–8.

Pilon, M., & Ruiz, M. (2023). PAQR proteins and the evolution of a superpower: Eating all kinds of fats. *BioEssays*, *45*(9), 2300079.

Pinilla, L. L., Ugun-Klusek, A., Rutella, S., & De Girolamo, L. A. (2021). Hypoxia Signaling in Parkinson's Disease: There Is Use in Asking "What HIF?" *Biology 2021, Vol. 10, Page 723*, *10*(8), 723.

RAHEJA, B. S., SADIKOT, S. M., PHATAK, R. B., & RAO, M. B. (1993). Significance of the N-6/N-3 Ratio for Insulin Action in Diabetes. *Annals of the New York Academy of Sciences*, *683*(1), 258–271.

Rappleye, C. A., Tagawa, A., Le Bot, N., Ahringer, J., & Aroian, R. V. (2003). Involvement of fatty acid pathways and cortical interaction of the pronuclear complex in Caenorhabditis elegans embryonic polarity. *BMC Developmental Biology*, *3*(1), 1–15.

Rivers, J. P. W., Sinclair, A. J., & Crawford, M. A. (1975). Inability of the cat to desaturate essential fatty acids. *Nature 1975 258:5531*, *258*(5531), 171–173.

Romney, S. J., Newman, B. S., Thacker, C., & Leibold, E. A. (2011). HIF-1 Regulates Iron Homeostasis in Caenorhabditis elegans by Activation and Inhibition of Genes Involved in Iron Uptake and Storage. *PLOS Genetics*, *7*(12), e1002394.

Romney, S. J., Thacker, C., & Leibold, E. A. (2008). An Iron Enhancer Element in the FTN-1 Gene Directs Iron-dependent Expression in Caenorhabditis elegans Intestine. *Journal of Biological Chemistry*, *283*(2), 716–725.

Roqueta-Rivera, M., Stroud, C. K., Haschek, W. M., Akare, S. J., Segre, M., Brush, R. S., Agbaga, M. P., Anderson, R. E., Hess, R. A., & Nakamura, M. T. (2010). Docosahexaenoic acid supplementation fully restores fertility and spermatogenesis in male delta-6 desaturase-null mice. *Journal of Lipid Research*, *51*(2), 360.

Ruiz, M., Bodhicharla, R., Ståhlman, M., Svensk, E., Busayavalasa, K., Palmgren, H., Ruhanen, H., Boren, J., & Pilon, M. (2019). Evolutionarily 1 conserved long-chain acyl-coa synthetases regulate membrane composition and fluidity. *ELife*, *8*.

Ruiz, M., Bodhicharla, R., Svensk, E., Devkota, R., Busayavalasa, K., Palmgren, H., Ståhlman, M., Boren, J., & Pilon, M. (2018). Membrane fluidity is regulated by the C. Elegans transmembrane protein FLD-1 and its human homologs TLCD1/2. *ELife*, *7*.

Ruiz, M., Devkota, R., Kaper, D., Ruhanen, H., Busayavalasa, K., Radović, U., Henricsson, M., Käkelä, R., Borén, J., & Pilon, M. (2023). AdipoR2 recruits protein interactors to promote fatty acid elongation and membrane fluidity. *Journal of Biological Chemistry*, *299*(6), 104799.

Ruiz, M., Devkota, R., Panagaki, D., Bergh, P.-O., Kaper, D., Henricsson, M., Nik, A., Petkevicius, K., Höög, J. L., Bohlooly-Y, M., Carlsson, P., Borén, J., & Pilon, M. (2022). Sphingosine 1-phosphate mediates adiponectin receptor signaling essential for lipid homeostasis and embryogenesis. *Nature Communications 2022 13:1*, *13*(1), 1–19.

Ruiz, M., Palmgren, H., Henricsson, M., Devkota, R., Jaiswal, H., Maresca, M., Bohlooly-Y, M., Peng, X. R., Borén, J., & Pilon, M. (2021). Extensive transcription mis-regulation and membrane defects in AdipoR2-deficient cells challenged with saturated fatty acids. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1866*(4), 158884.

Ruiz, M., Ståhlman, M., Borén, J., & Pilon, M. (2019). AdipoR1 and AdipoR2 maintain membrane fluidity in most human cell types and independently of adiponectin. *Journal of Lipid Research*, *60*(5), 995–1004.

Sassa, T., & Kihara, A. (2014). Metabolism of Very Long-Chain Fatty Acids: Genes and Pathophysiology. *Biomolecules & Therapeutics*, *22*(2), 83.

Satouchi, K., Hirano, K., Sakaguchi, M., Takehara, H., & Matsuura, F. (1993). Phospholipids from the free-living nematode Caenorhabditis elegans. *Lipids*, *28*(9), 837–840.

Sawai, M., Uchida, Y., Ohno, Y., Miyamoto, M., Nishioka, C., Itohara, S., Sassa, T., & Kihara, A. (2017). The 3-hydroxyacyl-CoA dehydratases HACD1 and HACD2 exhibit functional redundancy and are active in a wide range of fatty acid elongation pathways. *Journal of Biological Chemistry*, *292*(37), 15538–15551.

Schneider, L., Su, L. J., Arab, L., Bensen, J. T., Farnan, L., Fontham, E. T. H., Song, L., Hussey, J., Merchant, A. T., Mohler, J. L., & Steck, S. E. (2019). Dietary patterns based on the Mediterranean diet and DASH diet are inversely associated with high aggressive prostate cancer in PCaP. *Annals of Epidemiology*, *29*, 16-22.e1.

Schwingshackl, L., Schwedhelm, C., Galbete, C., & Hoffmann, G. (2017). Adherence to Mediterranean Diet and Risk of Cancer: An Updated Systematic Review and Meta-Analysis. *Nutrients 2017, Vol. 9, Page 1063*, *9*(10), 1063.

Seethaler, B., Basrai, M., Vetter, W., Lehnert, K., Engel, C., Siniatchkin, M., Halle, M., Kiechle, M., & Bischoff, S. C. (2020). Fatty acid profiles in erythrocyte membranes following the Mediterranean diet – data from a multicenter lifestyle intervention study in women with hereditary breast cancer (LIBRE). *Clinical Nutrition*, *39*(8), 2389–2398.

Semenza, G. L. (1999). Regulation of mammalian O2 homeostasis by hypoxia-inducible factor 1. *Annual Review of Cell and Developmental Biology*, *15*, 551–578.

Shanklin, J., & Cahoon, E. B. (1998). Desaturation and related modifications of fatty acids. *Annual Review of Plant Biology*, *49*(Volume 49, 1998), 611–641.

Shanklin, J., Whittle, E., & Fox, B. G. (1994). Eight Histidine Residues Are Catalytically Essential in a Membrane-Associated Iron Enzyme, Stearoyl-CoA Desaturase, and Are Conserved in Alkane Hydroxylase and Xylene Monooxygenase. *Biochemistry*, *33*(43), 12787–12794.

Shen, C., Nettleton, D., Jiang, M., Kim, S. K., & Powell-Coffman, J. A. (2005). Roles of the HIF-1 hypoxia-inducible factor during hypoxia response in Caenorhabditis elegans. *Journal of Biological Chemistry*, *280*(21), 20580–20588.

Shen, J., Wu, G., Pierce, B. S., Tsai, A. L., & Zhou, M. (2023). Free ferrous ions sustain activity of mammalian stearoyl-CoA desaturase-1. *Journal of Biological Chemistry*, *299*(7

Shen, J., Wu, G., Tsai, A. L., & Zhou, M. (2020). Structure and Mechanism of a Unique Diiron Center in Mammalian Stearoyl-CoA Desaturase. *Journal of Molecular Biology*, *432*(18), 5152–5161.

Sheokand, P. K., James, A. M., Jenkins, B., Lysyganicz, P. K., Lacabanne, D., King, M. S., Kunji, E. R. S., Siniossoglou, S., Koulman, A., Murphy, M. P., & Petkevicius, K. (2025). TRAM-LAG1-CLN8 family proteins are acyltransferases regulating phospholipid composition. *Science Advances*, *11*(8), eadr3723.

Shi, X., Li, J., Zou, X., Greggain, J., Rødkær, S. V., Færgeman, N. J., Liang, B., & Watts, J. L. (2013). Regulation of lipid droplet size and phospholipid composition by stearoyl-CoA desaturase. *Journal of Lipid Research*, *54*(9), 2504–2514.

Shimbara-Matsubayashi, S., Kuwata, H., Tanaka, N., Kato, M., & Hara, S. (2019). Analysis on the Substrate Specificity of Recombinant Human Acyl-CoA Synthetase ACSL4 Variants. *Biological and Pharmaceutical Bulletin*, *42*(5), 850–855.

Simopoulos, A. P. (1991). Omega-3 fatty acids in health and disease and in growth and development. *The American Journal of Clinical Nutrition*, *54*(3), 438–463.

Simopoulos, A. P. (1999). Essential fatty acids in health and chronic disease. *The American Journal of Clinical Nutrition*, *70*(3), 560s–569s.

Sinensky, M. (1974). Homeoviscous adaptation: a homeostatic process that regulates the viscosity of membrane lipids in Escherichia coli. *Proceedings of the National Academy of Sciences of the United States of America*, *71*(2), 522–525.

Singer, S. J., & Nicolson, G. L. (1972). The Fluid Mosaic Model of the Structure of Cell Membranes. *Science*, *175*(4023), 720–731.

Smith, S. (1994). The animal fatty acid synthase: one gene, one polypeptide, seven enzymes. *The FASEB Journal*, *8*(15), 1248–1259.

Spychalla, J. P., Kinney, A. J., & Browse, J. (1997). Identification of an animal ω-3 fatty acid desaturase by heterologous expression in Arabidopsis. *Proceedings of the National Academy of Sciences*, *94*(4), 1142–1147.

Stillwell, W. (2013). An Introduction to Biological Membranes: From Bilayers to Rafts. *An Introduction to Biological Membranes: From Bilayers to Rafts*, 1–367.

Stoffel, W., Hammels, I., Jenke, B., Binczek, E., Schmidt-Soltau, I., Brodesser, S., Odenthal, M., & Thevis, M. (2013). Obesity resistance and deregulation of lipogenesis in Δ6-fatty acid desaturase (FADS2) deficiency. *EMBO Reports*, *15*(1), 110.

Stoffel, W., Holz, B., Jenke, B., Binczek, E., Günter, R. H., Kiss, C., Karakesisoglou, I., Thevis, M., Weber, A. A., Arnhold, S., & Addicks, K. (2008). Δ6-Desaturase (FADS2) deficiency unveils the role of ω3- and ω6-polyunsaturated fatty acids. *The EMBO Journal*, *27*(17), 2281.

Strittmatter, P., Spatz, L., Corcoran, D., Rogers, M. J., Setlow, B., & Redline, R. (1974). Purification and properties of rat liver microsomal stearyl coenzyme A desaturase. *Proceedings of the National Academy of Sciences of the United States of America*, *71*(11), 4565–4569.

Sulston, J. E., Schierenberg, E., White, J. G., & Thomson, J. N. (1983). The embryonic cell lineage of the nematode Caenorhabditis elegans. *Developmental Biology*, *100*(1), 64–119.

Sureda, A., del Mar Bibiloni, M., Julibert, A., Bouzas, C., Argelich, E., Llompart, I., Pons, A., & Tur, J. A. (2018). Adherence to the Mediterranean Diet and Inflammatory Markers. *Nutrients 2018, Vol. 10, Page 62*, *10*(1), 62.

Svensk, E., Devkota, R., Ståhlman, M., Ranji, P., Rauthan, M., Magnusson, F., Hammarsten, S., Johansson, M., Borén, J., & Pilon, M. (2016). Caenorhabditis elegans PAQR-2 and IGLR-2 Protect against Glucose Toxicity by Modulating Membrane Lipid Composition. *PLOS Genetics*, *12*(4), e1005982.

Svensk, E., Ståhlman, M., Andersson, C. H., Johansson, M., Borén, J., & Pilon, M. (2013). PAQR-2 Regulates Fatty Acid Desaturation during Cold Adaptation in C. elegans. *PLOS Genetics*, *9*(9), e1003801.

Svensson, E., Olsen, L., Mörck, C., Brackmann, C., Enejder, A., Faergeman, N. J., & Pilon, M. (2011). The Adiponectin Receptor Homologs in C. elegans Promote Energy Utilization and Homeostasis. *PLOS ONE*, *6*(6), e21343.

Tang, H., & Han, M. (2017). Fatty Acids Regulate Germline Sex Determination through ACS-4-Dependent Myristoylation. *Cell*, *169*(3), 457-469.e13.

Tang, Y. T., Hu, T., Arterburn, M., Boyle, B., Bright, J. M., Emtage, P. C., & Funk, W. D. (2005). PAQR proteins: A novel membrane receptor family defined by an ancient 7-transmembrane pass motif. *Journal of Molecular Evolution*, *61*(3), 372–380.

Taubert, S., Van Gilst, M. R., Hansen, M., & Yamamoto, K. R. (2006). A Mediator subunit, MDT-15, integrates regulation of fatty acid metabolism by NHR-49-dependent and -independent pathways in C. elegans. *Genes & Development*, *20*(9), 1137–1149.

Theil, E. C. (2013). Ferritin: The protein nanocage and iron biomineral in health and in disease. *Inorganic Chemistry*, *52*(21), 12223–12233.

Tiwari, A., Ocon-Grove, O. M., Hadley, J. A., Giles, J. R., Johnson, P. A., & Ramachandran, R. (2015). Expression of Adiponectin and Its Receptors Is Altered in Epithelial Ovarian Tumors and Ascites-Derived Ovarian Cancer Cell Lines. *International Journal of Gynecological Cancer*, *25*(3), 399–406.

Unlu, G., Prizer, B., Erdal, R., Yeh, H. W., Bayraktar, E. C., & Birsoy, K. (2022). Metabolic-scale gene activation screens identify SLCO2B1 as a heme transporter that enhances cellular iron availability. *Molecular Cell*, *82*(15), 2832-2843.e7.

Valentini, S., Cabreiro, F., Ackerman, D., Alam, M. M., Kunze, M. B. A., Kay, C. W. M., & Gems, D. (2012). Manipulation of in vivo iron levels can alter resistance to oxidative stress without affecting ageing in the nematode C. elegans. *Mechanisms of Ageing and Development*, *133*(5), 282.

Van Gilst, M. R., Hadjivassiliou, H., Jolly, A., & Yamamoto, K. R. (2005). Nuclear Hormone Receptor NHR-49 Controls Fat Consumption and Fatty Acid Composition in C. elegans. *PLoS Biology*, *3*(2), e53.

Van Meer, G., Voelker, D. R., & Feigenson, G. W. (2008). Membrane lipids: where they are and how they behave. *Nature Reviews. Molecular Cell Biology*, *9*(2), 112.

Vasiliauskaité-Brooks, I., Sounier, R., Rochaix, P., Bellot, G., Fortier, M., Hoh, F., De Colibus, L., Bechara, C., Saied, E. M., Arenz, C., Leyrat, C., & Granier, S. (2017). Structural insights into adiponectin receptors suggest ceramidase activity. *Nature*, *544*(7648), 120.

Vieira, A. F. C., Xatse, M. A., Tifeki, H., Diot, C., Walhout, A. J. M., & Olsen, C. P. (2022). Monomethyl branched-chain fatty acids are critical for Caenorhabitis elegans survival in elevated glucose conditions. *The Journal of Biological Chemistry*, *298*(2).

Villa, N. Y., Kupchak, B. R., Garitaonandia, I., Smith, J. L., Alonso, E., Alford, C., Cowart, L. A., Hannun, Y. A., & Lyons, T. J. (2009). Sphingolipids function as downstream effectors of a fungal PAQF. *Molecular Pharmacology*, *75*(4), 866–875.

Volkmar, N., Gawden-Bone, C. M., Williamson, J. C., Nixon-Abell, J., West, J. A., St George-Hyslop, P. H., Kaser, A., & Lehner, P. J. (2022). Regulation of membrane fluidity by RNF145 -triggered degradation of the lipid hydrolase ADIPOR2 . *The EMBO Journal*, *41*(19).

Voruganti, V. S., Higgins, P. B., Ebbesson, S. O. E., Kennish, J., Göring, H. H. H., Haack, K., Laston, S., Drigalenko, E., Wenger, C. R., Harris, W. S., Fabsitz, R. R., Devereux, R. B., MacCluer, J. W., Curran, J. E., Carless, M. A., Johnson, M. P., Moses, E. K., Blangero, J., Umans, J. G., … Comuzzie, A. G. (2012). Variants in CPT1A, FADS1, and FADS2 are associated with higher levels of estimated plasma and erythrocyte delta-5 desaturases in Alaskan Eskimos. *Frontiers in Genetics*, *3*(JUN), 24918.

Vrablik, T. L., Petyuk, V. A., Larson, E. M., Smith, R. D., & Watts, J. L. (2015). Lipidomic and proteomic analysis of Caenorhabditis elegans lipid droplets and identification of ACS-4 as a lipid droplet-associated protein. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, *1851*(10), 1337–1345.

Vrablik, T. L., & Watts, J. L. (2013). Polyunsaturated fatty acid derived signaling in reproduction and development: Insights from Caenorhabditis elegans and Drosophila melanogaster. *Molecular Reproduction and Development*, *80*(4), 244–259.

Wallis, J. G., Watts, J. L., & Browse, J. (2002). Polyunsaturated fatty acid synthesis: what will they think of next? *Trends in Biochemical Sciences*, *27*(9), 467–473.

Watkins, P. A., Maiguel, D., Jia, Z., & Pevsner, J. (2007). Evidence for 26 distinct acyl-coenzyme A synthetase genes in the human genome. *Journal of Lipid Research*, *48*(12), 2736–2750.

Watson, H. (2015). Biological membranes. *Essays in Biochemistry*, *59*, 43.

Watts, J. L. (2009). Fat synthesis and adiposity regulation in Caenorhabditis elegans. *Trends in Endocrinology & Metabolism*, *20*(2), 58–65.

Watts, J. L., & Browse, J. (2000). A Palmitoyl-CoA-Specific Δ9 Fatty Acid Desaturase from Caenorhabditis elegans. *Biochemical and Biophysical Research Communications*, *272*(1), 263–269.

Watts, J. L., & Browse, J. (2002). Genetic dissection of polyunsaturated fatty acid synthesis in Caenorhabditis elegans. *Proceedings of the National Academy of Sciences of the United States of America*, *99*(9), 5854–5859.

Watts, J. L., Phillips, E., Griffing, K. R., & Browse, J. (2003). Deficiencies in C20 polyunsaturated fatty acids cause behavioral and developmental defects in Caenorhabditis elegans fat-3 mutants. *Genetics*, *163*(2), 581–589.

Watts, J. L., & Ristow, M. (2017). Lipid and carbohydrate metabolism in Caenorhabditis elegans. *Genetics*, *207*(2), 413–446.

Weber, P. C., Fischer, S., Schacky, C. von, Lorenz, R., & Strasser, T. (1986). DIETARY OMEGA–3 POLYUNSATURATED FATTY ACIDS AND EICOSANOID FORMATION IN MAN. *Health Effects of Polyunsaturated Fatty Acids in Seafoods*, 49–60.

Weinert, J., Blomquist, G. J., & Borgeson, C. E. (1993). De novo biosynthesis of linoleic acid in two non-insect invertebrates: The land slug and the garden snail. *Experientia*, *49*(10), 919–921.

Xu, M., Joo, H. J., & Paik, Y. K. (2011). Novel Functions of Lipid-binding Protein 5 in Caenorhabditis elegans Fat Metabolism. *Journal of Biological Chemistry*, *286*(32), 28111–28118.

Yamauchi, T., Kamon, J., Ito, Y., Tsuchida, A., Yokomizo, T., Kita, S., Sugiyama, T., Miyagishi, M., Hara, K., Tsunoda, M., Murakami, K., Ohteki, T., Uchida, S., Takekawa, S., Waki, H., Tsuno, N. H., Shibata, Y., Terauchi, Y., Froguel, P., …

Kadowaki, T. (2003). Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature 2003 423:6941*, *423*(6941), 762–769.

Yamauchi, T., Kamon, J., Waki, H., Terauchi, Y., Kubota, N., Hara, K., Mori, Y., Ide, T., Murakami, K., Tsuboyama-Kasaoka, N., Ezaki, O., Akanuma, Y., Gavrilova, O., Vinson, C., Reitman, M. L., Kagechika, H., Shudo, K., Yoda, M., Nakano, Y., … Kadowaki, T. (2001). The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nature Medicine*, *7*(8), 941–946.

Yang, F., Vought, B. W., Satterlee, J. S., Walker, A. K., Jim Sun, Z. Y., Watts, J. L., DeBeaumont, R., Mako Saito, R., Hyberts, S. G., Yang, S., Macol, C., Iyer, L., Tjian, R., Van Den Heuvel, S., Hart, A. C., Wagner, G., & Näär, A. M. (2006). An ARC/Mediator subunit required for SREBP control of cholesterol and lipid homeostasis. *Nature*, *442*(7103), 700–704.

Yang, Q., Yin, R. X., Cao, X. L., Wu, D. F., Chen, W. X., & Zhou, Y. J. (2015). Association of two polymorphisms in the FADS1/FADS2 gene cluster and the risk of coronary artery disease and ischemic stroke. *International Journal of Clinical and Experimental Pathology*, *8*(6), 7318.

Yang, W. S., Kim, K. J., Gaschler, M. M., Patel, M., Shchepinov, M. S., & Stockwell, B. R. (2016). Peroxidation of polyunsaturated fatty acids by lipoxygenases drives ferroptosis. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(34), E4966–E4975.

Zhang, Y., Shao, Z., Zhai, Z., Shen, C., & Powell-Coffman, J. A. (2009). The HIF-1 Hypoxia-Inducible Factor Modulates Lifespan in C. elegans. *PLOS ONE*, *4*(7), e6348.

Zhang, Y., Zou, X., Ding, Y., Wang, H., Wu, X., & Liang, B. (2013). Comparative genomics and functional study of lipid metabolic genes in Caenorhabditis elegans. *BMC Genomics*, *14*(1).

Zhou, X. R., Green, A. G., & Singh, S. P. (2011). Caenorhabditis elegans Δ12-Desaturase FAT-2 Is a Bifunctional Desaturase Able to Desaturate a Diverse Range of Fatty Acid Substrates at the Δ12 and Δ15 Positions. *Journal of Biological Chemistry*, *286*(51), 43644–43650.

Zhu, H., Shen, H., Sewell, A. K., Kniazeva, M., & Han, M. (2013). A novel sphingolipid-TORC1 pathway critically promotes postembryonic development in Caenorhabditis elegans. *ELife*, *2013*(2).

Zhu, X. G., Nicholson Puthenveedu, S., Shen, Y., La, K., Ozlu, C., Wang, T., Klompstra, D., Gultekin, Y., Chi, J., Fidelin, J., Peng, T., Molina, H., Hang, H. C., Min, W., & Birsoy, K. (2019). CHP1 Regulates Compartmentalized Glycerolipid Synthesis by Activating GPAT4. *Molecular Cell*, *74*(1), 45-58.e7.