As a matter of fat: Emerging roles of lipid-sensitive E3 ubiquitin ligases

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Abstract

The dynamic structure and composition of lipid membranes need to be tightly regulated to control the vast array of cellular processes from cell and organelle morphology to protein-protein interactions and signal transduction pathways. To maintain membrane integrity, sense-and-response systems monitor and adjust membrane lipid composition in response to the ever-changing environment, but only a relatively small number of control systems have been described. Here, we explore the emerging role of the ubiquitin-proteasome system in monitoring and maintaining membrane lipid composition. We focus on the ER-resident RNF145 E3 ubiquitin ligase, its role in regulating adiponectin receptor 2 (ADIPOR2), its lipid hydrolase substrate and the broader implications for understanding the homeostatic processes that fine-tune cellular membrane composition.

Introduction

Lipid membranes are essential to life. Fundamentally, they define the cell's boundary with its environment. Rather than representing a passive diffusion barrier, they are highly dynamic structures that enable and regulate a complex array of vital cellular processes [1]. This capability is intimately connected to maintenance of their lipid composition, which affects critical physical properties such as membrane thickness, viscosity and fluidity. In mammalian cells, the lipids dominating membrane properties in most organelles are typically phospholipids together with sterols and sphingolipids (in the plasma membrane) and cardiolipin (in the inner mitochondrial membrane).

Phospho- and sphingolipids contain both saturated and unsaturated acyl chains derived from fatty acids (FAs) of varying length. A high content (or local concentration) of saturated acyl chains decreases membrane fluidity due to its relatively compact structure, allowing for tight lipid packing in the plane of the membrane. In contrast, the *cis* double bonds of unsaturated fatty acyl chains act as molecular spacers within complex lipids and promote membrane fluidity [2]. Virtually all biological membranes contain a mixture of saturated and unsaturated lipids which are tuned to the particular requirements of their cellular organelles. Any aberrant excess of highly saturated lipids can upset this delicate balance and impair membrane function and integrity [3–5].

The exact lipid composition of biological membranes is not immutable but responds to dietary and environmental cues in a highly dynamic manner. Thus, complex rapid- and late-response systems must regulate the detection, storage and metabolism of lipids, enabling cells to adapt to fluctuations in dietary lipid supply [6]. Dysregulation of these mechanisms is linked to genetic, metabolic and immune diseases [7–10], including type II diabetes mellitus, neurodegenerative disorders, metabolic syndrome, and cancer [11,12].

Rapid-response mechanisms adjust the activity of lipid metabolic enzymes through post-translational modification and/or regulated turnover, which may be accompanied by long-term, transcriptional changes to restore homeostasis [9,13,14]. These sense-and-response systems are particularly challenging to investigate as the substrates to be regulated, sterols, free FAs and lipids are themselves sequestered within the membrane and must be 'sensed' by membrane-embedded proteins. Among these rapid responders, E3 ubiquitin ligases represent important lipid-responsive homeostatic regulators of lipid metabolic pathways [9,15–18].

E3 ubiquitin ligases regulate cellular signalling, endocytosis, and protein degradation [19,20], and ubiquitination controls the cellular proteome and provides an essential post-translational pathway to regulate protein abundance and stability [19]. E3 ubiquitin ligases covalently transfer ubiquitin to their target proteins. The human genome encodes ~ 600 E3 ligases typically containing either RING (really interesting new gene) domains, HECT (homologous to the E6AP carboxyl terminus) domains, RING-between-RING (RBR), or U-box domains, all of which are essential for ubiquitin transfer to E3 substrates [21,22]. E3 ubiquitin ligases do not function in isolation, but cooperate with E1 and E2 enzymes to achieve ubiquitin activation, conjugation and ligation to transfer ubiquitin onto the target protein [23,24].

Here we focus on the emerging role of E3 ubiquitin ligases as central players in homeostatic lipid 'sense-and-response' pathways and discuss how their activity is modulated by changing cellular membrane lipid content with special attention given to the underlying early upstream, pre-translational mechanisms.

E3 ubiquitin ligases, sensing and responding to changes in membrane lipid composition

An increasing number of membrane-resident and cytoplasmic E3 ubiquitin ligases either regulate or are regulated by cellular lipid content or membrane lipid composition (**Table 1**). Understanding how these ligases and their clients operate will provide a potential means of targeting these lipid-modulated pathways to influence diseases such as diet-induced diabetes. Here, we highlight a selection of E3 ubiquitin ligases involved in lipid signalling or lipid homeostasis.

Cytoplasmic ubiquitin ligases such as RNF213/Mysterin, TRAF2, IDOL, SMURF1, and Rsp5 (discussed below) are recruited to membranes via domains that recognise changes in membrane properties such as membrane charge or lipid composition (Figure 1 (i)). This is useful to many signalling and metabolic pathways as their activity can determine downstream cellular responses, for instance *via* the internalisation of cell surface receptors through K63 ubiquitination or K48 polyubiquitination and proteasomal degradation.

RNF213/Mysterin

RNF213/Mysterin is a cytoplasmic RING-E3 ubiquitin ligase; mutations in this protein are associated with Moyamoya disease (MMD), which causes strokes and haemorrhages due to a narrowing of arteries in the brain [25]. As the first known example of non-protein ubiquitination, RNF213 ubiquitinates lipopolysaccharide (LPS), a major component of many bacterial outer membranes, to stimulate xenophagy (macroautophagy of the invading bacterium), as part of an innate immune response upon bacterial entry into the cytosol [26]. RNF213-mediated ubiquitination also regulates triglyceride storage and its activities are contingent on fatty acid availability [27]. RNF213 associates with lipid droplets where it regulates the abundance of adipose triglyceride lipase (ATGL), a lipid droplet modulator, due to its ability to break down triglycerides into fatty acids and diglycerides [28]. RNF213-mediated degradation of ATGL stabilises lipid droplets and MMD-related mutations within its RING domain drastically reduce the cellular lipid droplet pool [27]. Oleic acid (OA; an unsaturated C18 fatty acid) stimulates RNF213's association with lipid droplets, implicating RNF213 as a fatty acid-responsive E3 ubiquitin ligase, although, rather than sensing OA directly, it may, more likely, be responding to an increase in lipid droplets and bind via an accessory protein(s) [27]. Furthermore, RNF213 depletion protects cells from the lipotoxic effects of palmitic acid (PA; a saturated C16 fatty acid), potentially by blocking RNF213's ability to suppress the activity of the SCD1 lipid desaturase [29].

TRAF2

Tumour necrosis factor receptor-associated factor 2 (TRAF2) is a RING-type E3 ubiquitin ligase that regulates signalling downstream of the tumour necrosis factor (TNF) α receptor (TNFR) and also plays a role in IRE-1 activation during the unfolded protein response [30–32]. Binding of the pro-inflammatory cytokine TNF to TNFR1/2 recruits TRAF1/2 or TRAF5, which add K63-linked ubiquitin to RIPK1 and initiate NF-κB and JNK signalling [32]. Interestingly, TRAF2 is recruited to the plasma membrane through its C-terminus, where it interacts with membrane microdomains rich in GM1 gangliosides [33]. Therefore, TRAF2 membrane binding, or recruitment to activate TNFR, is contingent on the local lipid environment and receptor activation (Figure 1 (i)) [34]. Although lipid rafts allow recruitment of TRAF2, the activity of its ligase domain also depends on lipid signalling. The metabolic products of ceramide breakdown and sphingosine kinase 1 (Sphk1) activation,

sphingosine-1-phosphate (S1P) is required for TRAF2 ubiquitination of RIPK1 *in vitro* [34]. Membrane lipid regulation and subsequent production of S1P are therefore important for TRAF2 recruitment and activity. This evidence implies that TRAF2 activity is regulated not only by lipid content of the membrane but also lipid metabolites indicating it is a lipid responsive E3 ubiquitin ligase.

IDOL

The RING-type E3 ubiquitin ligase IDOL (Inducible degrader of low-density lipoprotein receptor; also known as MYLIP), an effector of the liver X receptor (LXR) transcription factor, is activated in cholesterol regulation and plays an important role in lipid metabolism and neuronal development [35–37]. Together with the E2 conjugases UBC13 and UBE2D, IDOL targets the low- and very-low-density-lipoprotein receptors (LDLR, ApoER2 and VLDLR, respectively) for lysosomal degradation [37–39].

Cell-surface LDLR binds to lipoprotein-complexed cholesterol to facilitate its import into cells. Excess cellular sterols trigger LDLR's IDOL-mediated polyubiquitination, stimulating internalisation and lysosomal degradation of the receptor, thereby staunching an unrestrained influx of lipoprotein-bound cholesterol [43,44]. IDOL's N-terminal 4.1/ezrin/radixin/moesin (FERM) domain enables its recruitment to the plasma membrane *via* interaction with negatively charged membrane phospholipids and is critical for the recognition, binding and subsequent ubiquitination of LDLR's cytoplasmic tail (Figure 1 (i)) [45]. The crystal structure of IDOL's FERM domain implies it has an auto-inhibited state in the absence of lipids, in this state the substrate binding site is obscured by FERM's F3c subdomain and IDOL has high inhibitory auto-ubiquitination activity [46]. Auto-ubiquitination of lysine residues in the F3c subdomain controls IDOL's stability in HEK293 cells, suggesting that auto-ubiquitination mediated turnover, membrane association and target engagement are tightly coupled [45]. Lipid rafts are essential for IDOL recruitment to LDLR, supported by the observation that IDOL seems to target an LDLR population restricted to lipid rafts in the plasma membrane [47]. Under limiting cellular cholesterol, LXR-drived IDOL expression is reduced and its F3c subdomain adopts an autoinhibitory state, promoting auto-ubiquitination, thus maintaining LDLR stabilisation and increased LDLR-assisted cholesterol uptake. Regulated auto-inhibition therefore tunes IDOL activity to cellular sterol levels and ensures regulated cholesterol uptake [45].

As mentioned, IDOL expression is regulated by the oxysterol-responsive LXR transcription factor family [40]. Enhanced LXR-dependent transcription of the IDOL gene triggered by LDLR-mediated cholesterol uptake provides a longer-term negative feedback loop, where de novo synthesis of IDOL further potentiates LDLR internalisation and degradation, and this reduces further LDLR-dependent cholesterol uptake. In comparative studies in mouse, Cynomolgus monkey and human hepatocytes LXR activation led to differing levels of LDL uptake while LDLR protein levels depleted in human and Cynomolgus monkey and increased in mouse. However, in peripheral tissue mouse responses mirrored human and monkey. This suggest LXR-dependent tissue- and species-specific roles of IDOL in LDLR stability and that mouse and human responses to are not always comparable [41,42].

Importantly, IDOL and TRAF2 rely on membrane association (through membrane raft or negative charge detection) as well as distal metabolic processes like LXR driven expression or ceramide and sphingolipid metabolism.

SMURF1

SMAD-specific E3 ubiquitin protein ligase 1 (SMURF1) is a lipid-responsive ligase involved in signalling pathways at the plasma membrane and may also change metabolic responses through lipid signalling [48]. It is a peripheral membrane protein and member of the NEDD4 subfamily of HECT E3 ligases that responds to changes in plasma membrane lipids [49,50]. SMURF1 regulates bone morphogenetic protein (BMP) activity by targeting BMP receptors type I (BMPR) and TGF-β receptor type I (TβRI) for proteasome-mediated degradation [50,51]. Phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) and phosphatidylinositol 3,4,5-triphosphate (PI(3,4,5)P3) regulate the plasma membrane localisation of SMURF1 through its C2 domain (a phospholipid and calcium binding domain first described in Protein Kinase C) [48,52]. Changes in the charge and lipid head group availability in the plasma membrane significantly affect the localisation and activity of this E3 ubiquitin ligase (Figure 1 (i)) [53].

Rsp5

Rsp5, a *S. cerevisiae* NEDD4 E3 ubiquitin ligase related to SMURF1, also contains a C2 domain and is regulated by lipid metabolism. Rsp5 has an established role in modulating endocytosis and multivesicular body (MVB) formation and is recruited to these membranes via its C2 domain [54,55]. Rsp5 alleviates saturated lipid-mediated ER stress through regulation of the Ole1 lipid desaturase [54,55]. Survival of Rsp5-deficient yeast mutants is dependent on their supplementation with the unsaturated fatty acid OA, the end product of Ole1's catalytic activity [56]. Rsp5 controls Ole1 by ubiquitinating the fungi-specific membrane-bound transcription factor Mga2, which senses temperature-change induced alterations in lipid crowding in the ER [57]. Rsp5-mediated Mga2 ubiquitination and subsequent degradation releases transcriptionally-active elements of Mga2 from the ER membrane, followed by their nuclear translocation and induction of Ole1 gene expression [58]. Spt23, a fungal transcription factor homologous to Mga2, also monitors ER membrane properties and is subject to similar regulation by Rsp5 [59].

A role for membrane-resident E3 ubiquitin ligases regulating lipid metabolism

While some cytoplasmic E3 ubiquitin ligases directly recognise and interact with membrane lipids electrostatically (via lipid-binding domains such as the C2 domain, see above), lipids which affect bulk membrane properties are usually contained within the lipid bilayer. Sensing these changes is challenging and requires capabilities that extend beyond the recognition of lipid head groups. To access and continually probe their immediate membrane lipid microenvironment requires polytopic proteins which detect lipid property changes, such as increased fatty acid chain length or saturation or increased lipid or sterol concentrations, through specialised transmembrane domains. Many of these lipid-sensing proteins are also E3 ubiquitin ligases or their substrates. Polytopic E3 ubiquitin ligases are restricted to specific membrane compartments, allowing them to target distinct membrane-resident or -associated lipid-metabolic enzymes. Membrane-embedded E3 ubiquitin ligases can therefore be modulated by specific lipid classes or by the properties of the membrane environment itself (Figure 1 (ii)).

Here, we discuss how membrane lipids influence membrane-resident E3 ubiquitin ligases and how these interactions can regulate lipid homeostasis.

MARCHF5

Membrane-associated RING-CH finger 5 (MARCHF5), an integral E3 ubiquitin ligase of the outer mitochondrial membrane (OMM), helps remove unfolded mitochondrial membrane proteins. MARCHF5 interacts with the lipids cardiolipin (CL) and phosphatidic acid via a positively charged region formed from transmembrane domain 2 and 4 [60] altering MARCHF5's ability to ubiquitinate itself or its substrates. Nano-differential scanning fluorimetry showed that MARCHF5 auto-ubiquitination and dimerization was enhanced in the presence of CL, correlating with its decreased stability[60] , while phosphatidic acid had the opposite effect. The strong impact of CL on MARCHF5 is surprising as CL makes up only ~ 6% of the OMM. This likely increases the sensitivity of MARCHF5 activity when CL relocates from the IMM to the OMM, where it signals loss of membrane integrity and mitochondrial damage [61,62]. MARCHF5-deficient cells lose the interconnected mitochondrial network in favour of mitochondria trapped in their fissile state, highlighting MARCHF5's role in regulating mitochondrial morphology [63]. Importantly, as MARCHF5 stability is regulated by different lipid content in the OMM, it points towards MARCHF5 as a lipid-specific E3 ubiquitin ligase which regulates mitochondrial fission and fusion events.

Putative sterol-sensing domains in membrane-resident E3 ubiquitin ligases

Sensing of cellular sterol levels is often dependent on 'sterol-sensing domains' (SSD), an evolutionarily conserved, but poorly understood motif in the membrane-spanning domains of polytopic proteins and best characterised in regulators of cholesterol homeostasis [64]. SSD-containing proteins include the enzymatic target of statins, HMG-CoA reductase (HMGCR), the chaperone SREBP cleavage activating protein (SCAP) and the cholesterol transporter Niemann-Pick type C1 (NPC1)). The stability of HMGCR, and the transport of SCAP from the ER to the Golgi apparatus, are critically dependent on their SSDs, which respond to sterol-dependent conformational changes by differential binding to the regulatory proteins INSIG1/2. As a case in point, 25-hydroxycholesterol, a naturally occurring lipid metabolite, stabilises the interaction of INSIG1/2 with SCAP and HMGCR. While sterols are high, SCAP's interaction with INSIG1/2 masks a COPII binding sequence, which retains the SCAP-SREBP2-INSIG complex in the ER [65]. As sterol levels decrease, SCAP is released from its INSIG1/2 anchor and traffics with SREBP2 to the Golgi apparatus, where regulated proteolytic cleavage of SREBP2 results in its activation and

transcriptional upregulation of multiple genes involved in *de novo* cholesterol biosynthesis [65]. In contrast to SCAP, sterol-induced binding of INSIGs to HMGCR recruits the membrane-resident E3 ubiquitin ligases RNF145 and gp78 resulting in the ubiquitination and degradation of HMGCR, the rate-limiting enzyme of *de novo* cholesterol biosynthesis. Importantly, the SSD of HMGCR is engaged by INSIG1/2 and adopts a dramatically different conformation than free HMGCR, allowing the SSD-distal TMD7 to directly bind INSIG1/2 [66]. These structural rearrangements within HMGCR's SSD suggest that SSDs act as lipid-sensitive conformational switches that gate the interaction with accessory proteins (Figure 1 (ii)) [66].

Putative SSDs have been described in several ER resident E3 ubiquitin ligases (MARCHF6, TRC8/RNF139 and RNF145), but their SSD amino acid sequence diverges from those in HMGCR and SCAP family members, which themselves differ from NPC1 and Patched SSD proteins [64]. The function of these putative SSDs remains poorly defined, and no experimentally obtained structures are available. Sequence differences in (putative) SSDs may point to an evolutionary adaptation of the original SSD function in sterol metabolism to different modes of lipid-mediated regulation [18,67,68]. In the following, we discuss the function of these structural motifs in the context of their E3 ubiquitin ligases.

MARCHF6

The MARCHF6 (RNF176) ER-resident RING E3 ubiquitin ligase has 14 transmembrane domains and acts in multiple pathways including the unfolded protein response, cholesterol biosynthesis and ferroptosis [18,69–71]. MARCHF6 binding to NADPH, in its C-terminal regulatory region, adjusts MARCHF6-mediated degradation of the ferroptosis effectors p53, squalene monooxygenase (SM) and ACSL4 in response to ferroptosis-inducing stress [71]. MARCHF6 directly binds cholesterol and regulates the turnover of multiple lipid metabolic enzymes (e.g. the lipid droplet protein PLIN2, ACSL4, SM), the latter being an important enzyme in sterol *de novo* biosynthesis [18,72,73]. MARCHF6 is stabilised by high cholesterol levels, facilitating the ubiquitination and degradation of SM [74]. Stabilisation is lost in MARCHF6 mutants which cannot autoregulate, suggesting that cholesterol stabilises MARCHF6 by attenuating its autoubiquitination activity. However, MARCHF6's putative SSD may be dispensable for cholesterol-mediated stabilisation as mutating conserved residues in MARCHF6, known to affect cholesterol responsiveness in

SCAP, did not affect MARCHF6's stabilisation in cholesterol [74]. Moreover, depletion of INSIGs does not affect the sterol-induced stabilisation of MARCHF6, suggesting that MARCHF6's regulation is fundamentally different from that of HMGCR and SCAP, in which INSIG1/2 binds a tetrapeptide motif within their SSDs. Nevertheless, MARCHF6's putative SSD plays an important role in its basal stability as deletion mutants are unstable [74]. Therefore, despite the evidence that MARCHF6 plays an important role in cholesterol detection and regulation, how this is achieved remains unclear as it does not need INSIGs for its function but does require its SSD for membrane stability. It will be important to determine how MARCHF6 detects changes in cholesterol and degrades SM.

RNF145

RNF145 is a ubiquitous ER-resident RING E3 ubiquitin ligase with essential roles in sterol and phospholipid homeostasis. Single nucleotide polymorphisms within RNF145's coding region in humans are associated with obesity and dyslipidemia while its differential expression in hepatocellular carcinoma, affects disease progression [75–78].

RNF145's lipid-modulating effects are intimately linked to its ability to target lipid metabolic enzymes for degradation [75,79–82]. Accordingly, RNF145's substrates include the ER-resident lipid hydrolase adiponectin receptor 2 (ADIPOR2) and the cholesterol biosynthetic enzyme HMGCR [75,79–81]. By tailoring the abundance and/or localisation of these central lipid modulators, RNF145 indirectly adjusts lipid membrane composition to support shifting cellular demands.

Genetic deletion of RNF145 in mice increases the expression of multiple cholesterol biosynthetic genes, likely in an SREBP-dependent fashion, and leads to increased liver and plasma cholesterol levels [75]. These findings indicate that this E3 ligase is intimately linked to organismal lipid homeostasis. Indeed, expression of RNF145 is reportedly dependent on LXR signalling for expression [68,75]. However, the impact of the LXR pathway on the regulation of RNF145 protein levels remains contested [80]. Importantly, early point RNF145 protein stability is exquisitely sensitive to membrane saturated lipid content which has not been reported to act on the LXR axis.

Like MARCHF6, RNF145 contains 14 predicted TMDs, 5 of which (TMD 1-5) have been recognised as a putative SSD by sequence similarity with the SSDs of SCAP and HMGCR. This potential SSD contains the INSIG-binding tetrapeptide motif YLYF, modified versions of which are also present in the RNF145 homologue TRC8 (YIID), HMGCR (YIYF) and SCAP (YIYF) [79]. RNF145 binding to INSIG proteins is sterol-dependent and, together with the GP78 E3 ubiquitin ligase, required for HMGCR turnover (Figure 1 (ii)). While INSIGs are indispensable for targeting RNF145 and GP78 to HMGCR, this is not the case for RNF145 targeting of ADIPOR2, its other known substrate (Figure 1 (iii)) [81]. Whether RNF145's putative SSD acts as a lipid-responsive switch and is required for the lipid-enhanced interaction between RNF145 and ADIPOR2 is not known.

Mammalian cells express a distant homologue of RNF145, named TRC8/RNF139 (27% amino acid identity). Both E3 ubiquitin ligases localise to the ER membrane and contain a putative SSD, but they perform distinct cellular functions. TRC8 induces degradation of tail-anchored proteins like heme oxygenase-1 after cleavage by SPP in the ER, the misfolded hERG protein[70,83] and MHC-I molecules in the presence of herpesviral (CMV) infection [84].

TRC8 also plays a role in lipid homeostasis by antagonising the trafficking of the sterol-regulatory proteins SREBP2 and SCAP in a ubiquitination-independent manner [85,86]. Overexpressed TRC8 binds to INSIG1/2 via an SSD-localised YIID motif, resulting in INSIG1/2 ubiquitination and degradation [85]. While endogenous TRC8 protein accumulates in HEK293 cells upon 12-24h of sterol depletion, but TRC8 mRNA levels remained unchanged [85]. These findings indicate that TRC8 might tune the activity of sterol-regulatory proteins (SREBP2, INSIGs, SCAP), how TRC8 responds to sterols is not known and warrants further investigation.

In summary, multiple membrane-resident or -associated E3 ubiquitin ligases respond to changes in the lipid composition of membranes, likely involving specialised domains such as putative SSDs and auto-regulatory domains. The lipid-responsiveness of these ligases is typically accompanied by altered auto-ubiquitination activity and therefore ligase stability and/or altered substrate ubiquitination.

Homeoviscous adaptation - the cellular response to a changing membrane lipid environment

‘Homeoviscous adaptation’ is the dynamic adjustment of cellular lipid membrane properties to environmental changes [2]. Lipid sense-and-response systems facilitate the cell’s adaptation to the lipid composition of its membranes. Sensing membrane lipid imbalances can occur by direct ligand binding, as seen in the control of cellular cholesterol levels, or the detection of changes in biophysical membrane properties (e.g. thickness, viscosity). The sensor might itself restore homeostasis or initiate corrective signalling cascades via modulation of the activity of downstream responders (e.g. enzymes or transcription factors). After resolution, the sensors and responders return to steady state or are degraded to prevent overcorrection.

A diverse range of protein-based lipid sensing systems detect changes in the lipid bilayer in bacteria, yeast and metazoa (reviewed in [87]). These systems likely co-evolved with the increasing structural complexity of membrane lipids and membrane lipid composition of subcellular membrane compartments [88].

In bacteria, the bifunctional thermosensor DesK behaves as a phosphatase at elevated temperatures, while its kinase activity dominates at low ambient temperature [89–91]. This remarkable catalytic switch is initiated by an elongation of the DesK transmembrane domain due to increased lipid bilayer thickness at low temperatures. Under these conditions, DesK phosphorylates the DesR transcription factor, which translocates to the nucleus to increase expression of the Des Δ5 fatty acid desaturase. Des desaturates phospholipid acyl chains, increasing lipid disorder and reducing membrane thickness and viscosity [89–91], as discussed [87,92].

Budding yeast use a more complex homeoviscous sense-and-response system regulated by ER lipid packing density [58]. The system’s principal components consist of two sensors and transcription factors, a cytoplasmic E3 ubiquitin ligase and a lipid desaturase. The Mga2 and Spt23 sensors detect local changes in lipid crowding through tyrosine residues in their transmembrane regions. Their rotation under high membrane lipid packing induces a conformational change in the cytoplasmic domain, exposing lysine residues for ubiquitination by Rsp5, signalling Mga2 cleavage. The released, transcriptionally active Mga2 N-terminus enters the nucleus to initiate transcription of Ole1 desaturase, which, analogous to Des (**Δ**5 desaturase), desaturates lipids, directly reducing lipid packing (Figure 1 (iv)) [58,93].

In C.*elegans*, the paqr-2 lipid hydrolase is essential to prevent membrane rigidification under cold conditions or upon ingestion of excess dietary fats such as PA [94–97]. Paqr-2 is an orthologue of ADIPOR2, a mammalian anti-diabetic protein. Importantly, both ADIPOR2 and paqr-2 regulate membrane fluidity following PA supplementation [98]. Paqr-2 activity is regulated by binding the integral membrane protein iglr-2, which has no direct orthologue in mammals [5,99,100].

ADIPOR2 - an ER-resident lipid hydrolase regulating membrane fluidity and homeoviscous adaptation

The mammalian lipid hydrolase ADIPOR2 is essential for homeoviscous adaptation [5]. Initially described as a metabotropic receptor and plasma-membrane resident protein for the adipokine adiponectin [101], ADIPOR2 was recently shown to be an ER-resident protein which initiates a homeoviscous response to restore membrane fluidity in the presence of excess saturated fatty acids [81,102]. This activity is independent of adiponectin [97]. ADIPOR2 has an inverted 7-TMD G-protein coupled receptor topology and is structurally and catalytically similar to ceramidases of the CREST superfamily [103–105]. Its ceramidase activity may partially explain its function, inducing sphingosine-1-phosphate-mediated activation of PPAR**γ** or SREBP1 through sphingosine-1-phosphate receptors and transcriptional upregulation of lipid metabolic enzymes including the lipid desaturase SCD1 [82,106]. However, many aspects relating to the molecular function and regulation of ADIPOR2 remain unclear such as how ADIPOR2 senses and responds to PA-induced stress in the absence of an ilgr-2 orthologue [81]. An important lipid-dependent binding partner of ADIPOR2 is the RNF145 E3 ligase, which maintains steady-state levels of ADIPOR2 as well as regulating ADIPOR2 abundance in response to dietary fatty acids (discussed in sections below) [81,107].

ADIPOR2's catalytic activity is required for the processing of dipalmitoyl phospholipids, as deletion of endogenous ADIPOR2 leads to the accumulation of dipalmitoyl phospholipids that was only reversed by expressing catalytically active ADIPOR2 [81,82]. As well as potential ceramidase activity, ADIPOR2 is therefore likely to have a more general lipid hydrolase activity. SCD1, FADS1/2, ADIPOR1 and SREBF1/2 mRNA levels were suppressed when ADIPOR2-depleted cells were treated with PA, implying that ADIPOR2 depletion may have

a more widespread effect on the entire lipid modifying pathway as part of the cellular saturated fatty acid response [108].

How is the activity of ADIPOR2 regulated?

RNF145 levels and its interactions with ADIPOR2 are highly lipid sensitive. As concentrations of OA increase, RNF145 is stabilised and binds and ubiquitinates ADIPOR2, which is rapidly degraded. In contrast, as saturated fatty acids increase, two key events occur: (i) RNF145 dissociates from ADIPOR2 which is then stabilised resulting in increased lipid hydrolase activity. (ii) RNF145 protein levels drop due in part to decreased stability, autoubiquitination and degradation. This stabilisation of ADIPOR2 and loss of RNF145 provides an important rheostat which regulates saturated fatty acid incorporation into phospholipids and cellular membranes and most likely regulates lipid storage and fat metabolism in many cell types (Figure 1 (iii)). Therefore, in high unsaturated fatty acids, ADIPOR2 is rapidly turned over in a 'futile cycle' of continuous production and degradation, thus limiting its activity. While maintaining ADIPOR2 translation appears somewhat wasteful, such 'futile cycles' are common, as seen with both HMGCR in sterol regulation and HIF in the regulation of oxygen levels. These cycles have the advantage of anticipating and reacting to conditions requiring a rapid corrective response (e.g. saturated FA influx and incorporation into cellular membranes). Since many E3 ubiquitin ligases play an integral role in sterol and lipid regulation, the rapid and effective response offered by the ubiquitin-proteasome degradation pathway appears to be the preferred rapid-response control system.

This continuous post-translational turnover of ADIPOR2, rather than transcriptional regulation, is the favoured cellular control system and enhances the cell's ability to detect and respond to membrane saturation or lipid saturation in a timely manner. This implies that enhanced lipid saturation (in the presence of dietary saturated fatty acids) is detrimental to cells and indeed the continuous supply of PA is lipotoxic [81].

What is the molecular mechanism for sensing PA-induced altered cellular states (i.e. decreased membrane fluidity)? In bacterial membranes, an increase in saturated fatty acyl tails of membrane lipids results in a separation of membrane lipids into at least 2 domains – highly saturated, rigid microdomains and less saturated, more fluid microdomains, in

turn triggering membrane protein partitioning [109]. It will be interesting to determine whether a similar phenomenon might occur in the ER of mammalian cells and lead to partitioning of RNF145 away from ADIPOR2.

Alternatively, changes to the biophysical properties of the ER membrane, induced by different FAs, may induce conformational changes in the transmembrane region of RNF145 and ADIPOR2, affecting their interaction. Similar changes occur in bacterial (DesK), yeast (Mga2) and mammalian systems (SCAP/HMGCR/INSIGs) [64–66,110].

ADIPOR2 does not respond to changes in its lipid microenvironment in the absence of RNF145 and is therefore unlikely to be a lipid sensor. Although ADIPOR2 can bind OA in its hydrophobic channel [105], there is no evidence this plays a role in ADIPOR2 biology. Crystallographic evidence showing how ADIPOR1 can cycle from a closed to open conformation was not seen in ADIPOR2 with a similar mutation that revealed the change in ADIPOR1 [105] . It is therefore unlikely that ADIPOR2 harbours a conformational TMD switch that directly responds to an altered membrane lipid composition.

Recent structural insights into the regulation of SCAP and HMGCR highlight their ability to switch conformation and orientations of their transmembrane domains in a sterol-sensitive manner [64–66,110]. RNF145, with its 14 predicted transmembrane domains, 5 of which make up an unconventional SSD, is well-positioned to respond to intramembrane substrates, enhancing its interaction with ADIPOR2. It therefore seems likely that RNF145 senses changes in membrane lipid saturation, and responds by modulating both its own degradation and the ubiquitination and degradation of ADIPOR2. Since ADIPOR2 abundance is strictly dependent on RNF145 (but not *vice versa*), this membrane-resident E3 ubiquitin ligase directly adjusts ADIPOR2 levels to fine-tune membrane lipid hydrolysis in response to cellular demand. These changes therefore maintain cellular membrane homeostasis despite continual fluctuations in dietary FA supply.

We therefore propose a sequence of events in which RNF145 is the main sensor of changes in membrane FA saturation, and in turn regulates ADIPOR2 to respond to these changes in membrane lipid saturation.

Future Challenges

How membrane-resident E3 ubiquitin ligases sense changes in membrane properties and/or lipid composition and how this contributes to membrane lipid homeostasis remains challenging.

Investigation of lipid changes in cells and the use of *in vitro* reconstitution systems have demonstrated clear correlations between the activity and stability of a number of E3 ubiquitin ligases in different membrane lipid compositions. However, due to the technical difficulty of investigating membrane-embedded proteins, there is still a lack of detailed structural information. This has stymied investigations of dynamic protein-lipid interactions and the molecular changes that relate lipid microenvironment, protein structure and activity.

Changes in membrane properties (rigidity, crowding, fluidity, viscosity) have drastically different consequences for protein dynamics in different subcellular compartments, each of which show distinct steady-state membrane lipid compositions. This helps explain why a variety of membrane property sensors in various organelles have been discovered.

How the micro- and nanoscale organisation of membrane-resident E3 ubiquitin ligases affects their activity and ability to bind cognate substrates is unclear. Indeed, the identification of cognate substrates for many E3 ubiquitin ligases remains fragmentary. We know little about how acute changes in membrane lipid composition alter the dynamics within and between different membrane proteins. To address these fundamental questions, requires more sophisticated tools to investigate the nanoscale organisation of membrane protein complexes.

Furthermore, understanding the specific structural features which enable membrane proteins to sense changes in their membrane microenvironment is essential. The recent improvements in *in silico* structure prediction by deep learning algorithms such as AlphaFold 2 and RoseTTAFold signal new opportunities in predicting such features, particularly as these tools are refined. Powerful as these algorithms are, modelling membrane protein conformations need to understand the restraints imposed by surrounding membranes and different membrane lipid compositions. New ways to image biophysical membrane properties at high spatial resolution *in vivo* (i.e. [112,113]), will significantly enhance our understanding of the role of E3 ubiquitin ligase in modulating lipid homeostasis.

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Data availability statement

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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Graphical abstract text

Lipids are important components of cellular membranes. E3 ubiquitin ligases are emerging as important regulators of lipid homeostasis of membranes in cells. E3s involved in responding to changes in sterols levels and lipid saturation detect and directly act to relieve these changes in membrane properties.

Figure 1. Mechanisms of E3 ligase recruitment mediated by changes in lipids. (i) E3 ligases (dark red), such as TRAF2 and IDOL remain inactive in the cytoplasm until their cognate receptor (blue) is activated by ligand binding (yellow) this often stimulates changes to the membrane properties or recruits the receptor to regions of the membrane concentrated in phospholipids (PIP3) or regions of the membrane known as rafts (pink). The E3 is recruited to the membrane and binds to the receptor to ubiquitinate (ubiquitination in green) the receptor where it is degraded (for IDOL, shown) or the E3 targets proteins recruited to the receptor complex for ubiquitination (TRAF2). **(ii)** In low sterol conditions the polytopic E3 ligase (dark red) is maintained at low levels in the membrane or the accessory proteins (purple) is degraded to prevent interactions between the E3 and the target protein (lipid metabolic enzyme; blue). In excess sterol conditions cholesterol (red) stabilises the ligase, accessory protein or both and this allows for the interaction between the E3, accessory protein and lipid metabolic enzyme resulting in the lipid metabolic enzyme's ubiquitination (green) and degradation. **(iii)** Membrane property sensing E3 ubiquitin ligases (red) act directly as the sensor for changing membrane properties such as membrane saturation. In steady state the ligase interacts with the lipid modifying enzyme (blue) and ubiquitinates (green) the enzyme leading to its degradation. Under conditions where excess dietary saturated fatty acids (FA) are increased the E3 is no longer able to interact with the lipid modifying enzyme and the E3 self immolates. **(iv)** In steady state the membrane sensor (light blue and grey) is in a closed conformation. The sensor becomes active when there is an altered state such as increased lipid spacing/crowding this is when the E3 ligase (dark re) is recruited to the sensor. This interaction results in the ubiquitination and release of an important transcriptional element (grey).

Table 1. Lipid-regulated E3 ubiquitin ligases and their features

aa, amino acid; ER, endoplasmic reticulum; OMM,outer mitochondrial membrane; CL, cardiolipin; OMM, outer mitochondrial membrane; LPS, lipopolysaccharide; PhA,

phosphatidic acid; PI, phosphoinositides; PI(4)P, phsophatidyl-inosito-4-phosphate; S(1)P, sphingosine-1-phosphate; SSD, sterol sensing domain; TMD, transmembrane domain

* Proteins shown to be regulated by respective E3 ubiquitin ligase not necessarily related to lipid modulation

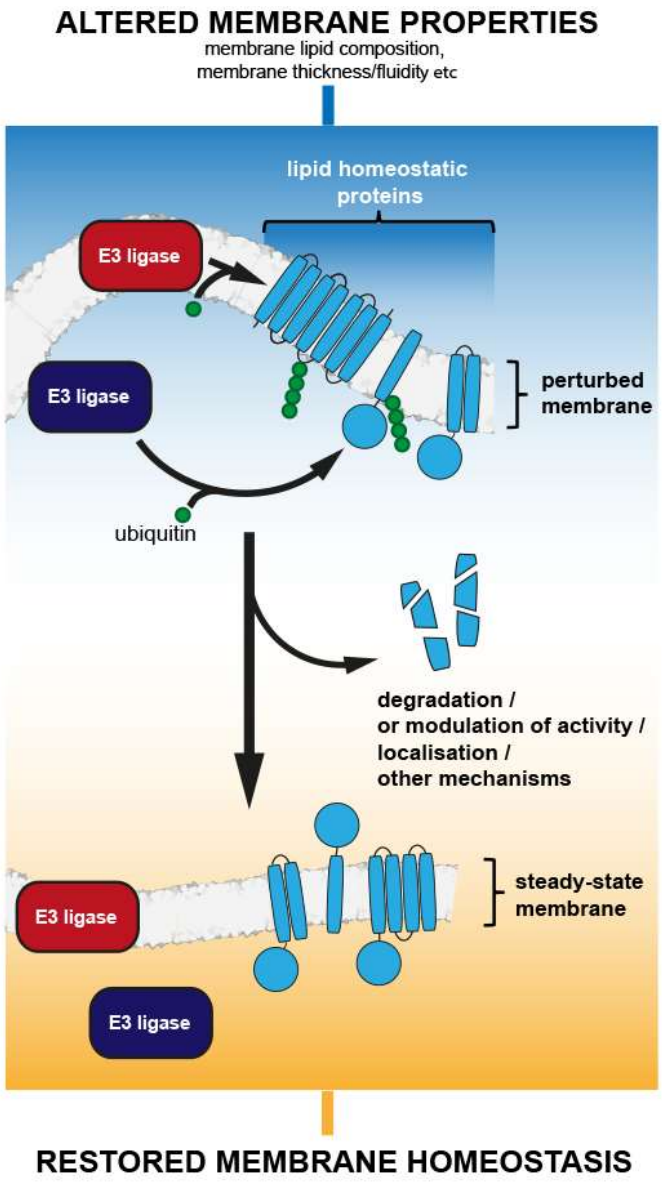
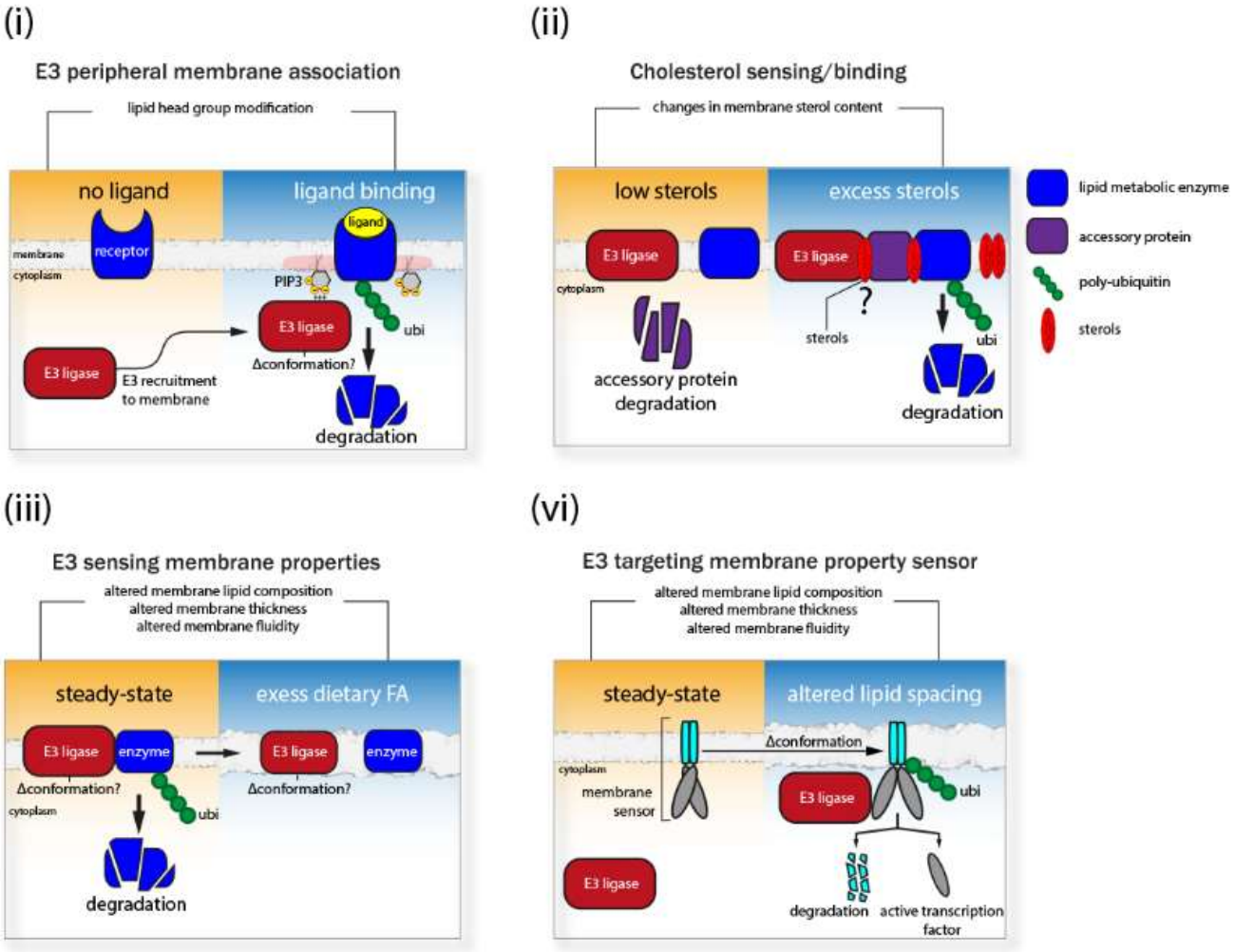

ALTERED MEMBRANE PROPERTIES
membrane lipid composition,
membrane thickness/fluidity etc
lipid homeostatic proteins
E3 ligase
E3 ligase
perturbed membrane
ubiquitin
degradation /
or modulation of activity /
localisation /
other mechanisms
steady-state membrane
E3 ligase
E3 ligase
RESTORED MEMBRANE HOMEOSTASIS

FIGURE 1


(i)
E3 peripheral membrane association
lipid head group modification
no ligand
ligand binding
receptor
ligand
membrane
cytoplasm
PIP3
E3 ligase
ubi
E3 ligase
E3 recruitment to membrane
Δconformation?
degradation
(ii)
Cholesterol sensing/binding
changes in membrane sterol content
low sterols
excess sterols
E3 ligase
E3 ligase
cytoplasm
?
sterols
ubi
accessory protein degradation
degradation
lipid metabolic enzyme
accessory protein
poly-ubiquitin
sterols
(iii)
E3 sensing membrane properties
altered membrane lipid composition
altered membrane thickness
altered membrane fluidity
steady-state
exess dietary FA
E3 ligase
enzyme
E3 ligase
enzyme
Δconformation?
Δconformation?
cytoplasm
ubi
degradation
(vi)
E3 targeting membrane property sensor
altered membrane lipid composition
altered membrane thickness
altered membrane fluidity
steady-state
altered lipid spacing
Δconformation
cytoplasm
membrane sensor
E3 ligase
ubi
E3 ligase
degradation
active transcription factor

E3 ligase	Demonstrated role in	Structural features	Subcellular compartment	Responsive to	Substrates*	Mechanism	Evidence level	Notes	Selected references demonstrating involvement of lipids or links to lipid metabolism
RNF145	*H. sapiens*	14 TMDs putative SSD (aa 52-188) YLYF motif RING finger domain	ER	PA, OA, FAs of different chain lengths, sterols	ADIPOR2 HMGCR SCAP CYBA CYBB	transcription/expression levels and protein stability PA: reduces protein levels OA: increases stability & activity	in cellulo	Resident of the ER membrane.	[68,75,80,81]
TRAF2	*N/A*	RING finger domain TRAF1/TRAF2 Zinc finger domain BIRC2/3 binding region MATH domain	cytoplasmic	lipid raft microdomains, S(1)P	BIRC3 RIPK1 TICAM1	recruited to the membrane by changes in membrane charge after TNFα binding TNFR S(1)P binding to RING domain stimulates K63-specific ubiquitination of RIPK1	in vitro	Associates with lipid membranes and leads to their deformation	[33,114]
MARCHF5/ RNF153	*H. sapiens*	4 TMDs RING finger domain	OMM	CL, PhA	RMDM3/PIPIP51 Drp1 MiD49	CL increases auto-ubiquitination phosphatidic acid increases stability	in vitro	Transcriptionally regulated by PPARγ localised to the OMM	[60,115]
MARCHF6	*H. sapiens*	14 TMDs RING finger domain putative SSD (aa 330–543; TMD4–8) YILL motif (aa 340-343)	ER	sterols	SQLE SC4MOL	sterols inhibit MARCHF6 degradation likely by inhibiting auto-ubiquitination	in cellulo	Binds cholesterol directly	[72,74,116]
MYLIP/IDOL	*H. sapiens* *M. musculus*	FERM domain RING finger domain	cytosol	LXR agonists	LDLR VLDLR ApoER2	recruited to membranes by LDLR binding and membrane charge transcriptional induction by LXR agonist	in cellulo	FERM domain interacts with membrane lipid headgroups	[46,117,118]
Rsp5	*S. cerevisiae*	C-terminal HECT domain C2 domain	cytosol	Phospholipids PI	Spt5, Mga2 RMDN3	senses the availability of Mga2 lysines for ubiquitination after lipid crowding events in the ER membrane C2 domain-dependent binding to PI and ubiquitination of endosomal cargo	in vivo	required for the ubiquitination and processing of Mga2/Spt5 for transcriptional upregulation of Ole1	[55,56,119]
RNF213/Mysterin	*H. sapiens*	dynein core domain 4x inactive ATPase domains Rz type zinc finger domain	cytosol, lipid droplets	OA, TNFα, LPS, PA	LPS ATGL NFAT1	ubiquitinates LPS on bacterial membranes to stimulate xenophagy depletes ATGL to maintain lipid droplets	in cellulo	LPS as first non-protein substrate for an E3 ubiquitin ligase	[17,26,27,29]
SMURF1	*H. sapiens*	C2 domain	cytosol, membrane-associated	PIPs, Ca^{2+}	SMAD1 SMAD5	regulates bone morphogenetic protein signalling by degrading downstream signalling proteins from the BMP receptor	in cellulo In vitro	PIPs are required for SMURF1 recruitment to membranes	[48]