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STATE-OF-THE-ART REVIEW

Regulation and function of the palmitoyl-acyltransferase ZDHHC5

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Keywords
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Protein palmitoylation (S-acylation) has emerged as an important player in a range of cellular processes, and as a result, the palmitoyl-acyltransferase (PAT) enzymes which mediate this modification have entered into the spotlight. Palmitoyltransferase ZDHHC5 (ZDHHC5) is among the more unique members of the PAT family as it is mainly localised to the plasma membrane and contains an extended cytoplasmic domain with several regulatory features. ZDHHC5 plays a vital role in a wide range of processes in different cell types. In this review, we offer a summary of the functions of ZDHHC5 in synaptic plasticity, cardiac function, cell adhesion and fatty acid uptake, among other processes. We also explore recent work has revealed several mechanisms to control the activity, localisation and function of ZDHHC5.

Introduction

Post-translational modifications (PTMs) of proteins are used by organisms to regulate almost every cellular process. They can fundamentally alter the physiochemical properties of individual amino acid residues within proteins to regulate protein structure and function. The addition of lipids to proteins is a particularly potent class of PTMs as they make regions of proteins more hydrophobic and can act as anchors to attach soluble proteins to cellular membranes as well as pinning intracellular loops of integral membrane proteins to the membrane to regulate local protein conformations.

There are several types of fatty acid modifications, including myristoylation, prenylation and S-acylation (palmitoylation). These three types of modification vary in the types of lipids that are utilised, the residues they are attached to and, therefore, the types of bond used to attach them. As a result, each type confers different properties and are often used as an essential step in protein processing to target proteins to particular membranes within the cell to ensure correct protein localisation which is usually very important for proper protein function. Protein S-acylation is the addition of a long-chain fatty acid to the side chain of a cysteine residue via a thioester bond, thus increasing the hydrophobicity of the protein. S-acylation is often referred to as protein palmitoylation; as the saturated

Abbreviations
AP-MS, affinity purification-mass spectrometry; APT1, Acyl-protein thioesterase 1; ARVC, arrhythmogenic right ventricular cardiomyopathy; DHHC9, Palmitoyltransferase zDHHC9; DSG2, desmoglein-2; MEND, massive endocytosis; NCX1, Na(+)/Ca(2+)-exchange protein 1; NOD1, Nucleotide oligomerisation domain (NOD)-like receptor 1; NOD2, Nucleotide oligomerisation domain (NOD)-like receptor 2; PAT, palmitoyl-acyltransferase; PC7, Proprotein convertase subtilisin/kexin type 7; PKP3, plakophilin-3; PTMs, Post-translational modifications; RAS, GTPase HRas; zDHHC5, Palmitoyltransferase ZDHHC5.

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fatty acid, palmitic acid is the most common lipid added, although proteins can also be modified with stearic acid and oleic acid [1,2]. Furthermore, palmitic acid is not only attached to cysteine residues, but also be attached to proteins through N-palmitoylation [3] and O-palmitoylation [4]. As with the addition of other fatty acids to proteins, palmitoylation is primarily used for membrane anchoring [5], although it has also been shown to promote protein–protein interactions [6]. It is also important for the modulation of the activity of certain enzymes through the palmitoylation of active site cysteine residues [7]. Palmitoylation is fully reversible, like phosphorylation or acetylation, and only occurs post-translationally, unlike other fatty acid modifications to proteins, such as N-myristoylation, which generally occur co-translationally and are usually irreversible [8]. The reversible nature of this modification is important, as cycles of protein palmitoylation and depalmitoylation have roles in protein activation and inactivation in response to external stimuli or signalling pathways.

Palmitoylation is used by cells to alter protein localisation and regulate membrane trafficking, with the palmitoylation-mediated cycling of specific GTPase HRas (RAS) G-proteins between the plasma membrane, the Golgi and the other intracellular membranes in response to external triggers being a classic example [9]. All RAS isoforms are farnesylated on the C terminus [10]; however, H-RAS and N-RAS are also palmitoylated [9]. In this system, depalmitoylation of the Ras by ABHD17 proteins [11] occurs at the plasma membrane, leading to the protein being distributed amongst other intracellular membranes, before being palmitoylated at the Golgi by the Palmitoyltransferase zDHHC9 (DHHC9)-GCP16 palmitoyl-acyltransferase (PAT) complex [12] and then trafficked back to the plasma membrane. This palmitoylation-controlled cycling of RAS regulates the activity and correct subcellular localisation of this critical signalling protein; if it is disrupted, it can lead to diseases such as RAS-driven cancers. This example also highlights the importance of the reversibility of palmitoylation and how it can be leveraged by the cells to fine-tune signalling in specific contexts.

Dysregulated palmitoylation has been implicated in a range of diseases including various cancers, as mentioned above with RAS, [13,14] and diabetes [15]. It is particularly implicated in neurological disorders such as Huntington's disease [16], schizophrenia [17] and Alzheimer's disease [18]. As palmitic acid is a saturated fatty acid and can occur in combination with other lipid modifications, it is also involved in the partitioning of proteins into lipid raft subdomains [19,20] which can be used to concentrate or cluster proteins involved in a signalling pathway [21]. Stearic acid is also saturated so will have a similar effect, but oleic acid is unsaturated, so not all S-acylated proteins will necessarily preferentially partition into lipid raft domains [22]. Although palmitoylation was discovered over 40 years ago on viral proteins [2], it has only been within the past decade that the extent of protein palmitoylation has been uncovered with the development of proteomic approaches to study the palmitoylated proteome. This has led to the realisation that palmitoylation is much more widespread than first thought, with at least 10% of human genes having a protein product that is palmitoylated [23].

Palmitoyl-acyltransferase enzymes are a large family of integral membrane proteins (Fig. 1) with 23 proteins containing the signature DHHC motif in a cysteine-rich domain, found in the human genome [24,25]. After the discovery of palmitoylation, it took more than 20 years to discover and describe the PAT responsible for the palmitoylation of RAS in *Saccharomyces cerevisiae*, the Palmitoyltransferase erf2/Ras modification protein ERF4 complex [26], as before this it was thought that palmitoylation could be a non-enzymatic process. It was definitively shown that palmitoylation was mainly an enzymatic process by knockout of most of the PATs present in *S. cerevisiae* which led to the loss of the majority of cellular palmitoylation [27]. Each PAT has a distinct subcellular distribution, with the majority of PATs resident at the Golgi [24]. Due to this over-representation of palmitoylating enzymes at the Golgi, it has been suggested that the Golgi is the major centre of protein palmitoylation within the cell [28]. However, it is important to point out that the endoplasmic reticulum and the plasma membrane also have resident PATs. Palmitoyltransferase ZDHHC5 (ZDHHC5) is somewhat unusual as a PAT as it is mainly present at the plasma membrane, although some is present in the endosomal system. It also has an extended C-terminal tail (Fig. 1), which contains many PTMs including phosphorylation and palmitoylation [29,30], with a PDZ-binding ligand at the extreme C terminus of the protein, through which it could interact with PDZ domain-containing substrates and regulators [31].

Regulation of cardiac function by ZDHHC5

ZDHHC5 has several different roles in many specialised cell types, including the heart where it is the most abundant PAT [32]. The phenomenon of massive endocytosis (MEND), where up to 70% of the cell

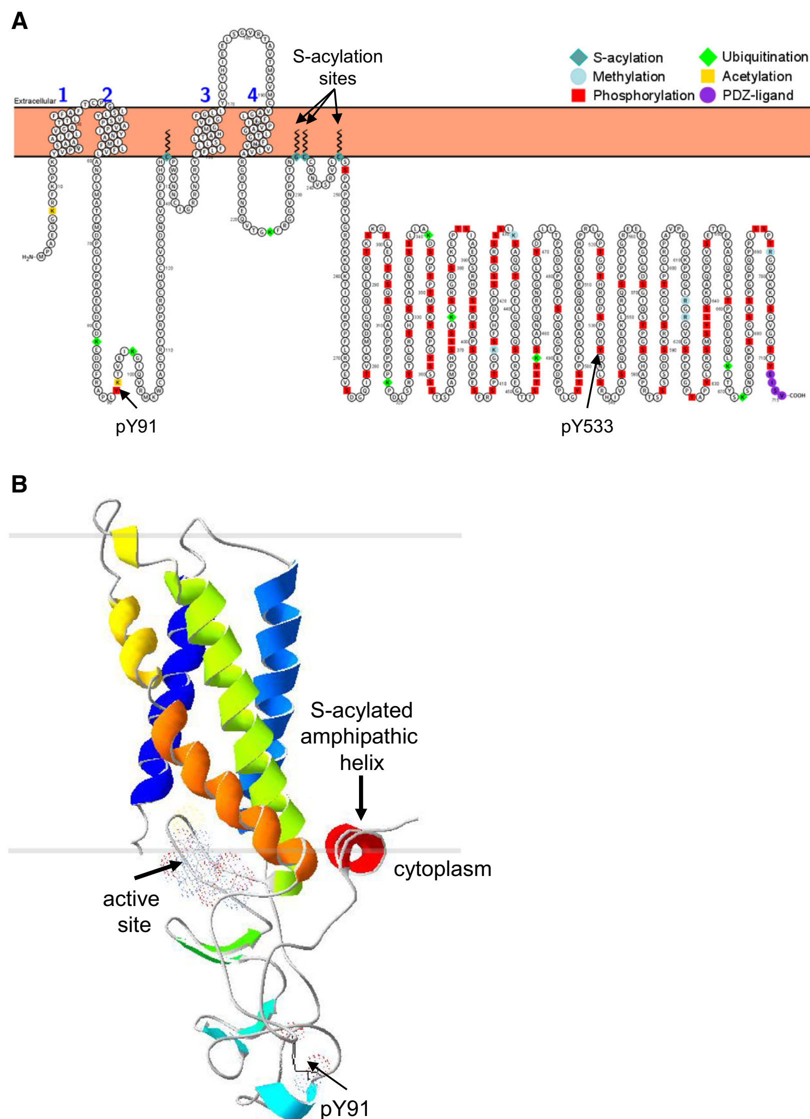

Fig. 1. (A) Membrane topology and known PTMs of ZDHHC5. PTM data is taken from the Phosphosite database [71]. (B) Predicted structure of ZDHHC5 based on the crystal structure of human DHHC17 created using the SWISS-MODEL platform [72]. The DHHC characteristic catalytic tetrad and activating phosphorylation site at Y91 are labelled. The S-acylated amphipathic helix just C-terminal to the fourth transmembrane helix is indicated. This model is missing most of the C-terminal tail (present in panel A) which is predicted to be unstructured.

surface is internalised [33] in fibroblasts, is inhibited by knockdown of ZDHHC5 [34]. In cardiac muscle, MEND occurs during reoxygenation following oxygen deprivation which would occur following a heart attack. The absence of ZDHHC5 reduces the occurrence of MEND in the heart and reduces the effects of reoxygenation injury on the heart [35]. Interestingly, when ZDHHC5-deficient cardiac cells undergo anoxia, it increases the plasma membrane localisation of the Na/Ca transporter Na(+)/Ca(2+)-exchange protein 1 (NCX1) and the sodium pump accessory subunit, phospholemman. This is an intriguing point as palmitoylation is often used to stabilise proteins at the plasma membrane. It may be that palmitoylation of these proteins is used as a way to trigger their endocytosis and that by removing the enzyme that palmitoylates them it leads to a stabilisation of NCX1 and phospholemman at the plasma membrane. Given that ZDHHC5 is required for MEND, and that MEND occurs after oxygen re-introduction following periods of anoxia and appears to be detrimental to the recovery of heart muscle cells, targeting ZDHHC5 specifically, or potentially palmitoylation more generally, could be a way to prevent or reduce the detrimental effects seen in cardiac muscle after heart attacks.

The triggering of MEND is a multi-step process, which originates in the mitochondria before a cascade reaches ZDHHC5 at the plasma membrane whereby the palmitoylation of plasma membrane proteins causes the process of MEND itself [34]. In cardiac

fibroblasts, a series of steps occurs that begins with the opening of mitochondrial pores, causing a large release of coenzyme A, which rapidly binds fatty acid chains to form acyl-CoA. The increase in acyl-CoA leads to palmitoylation of a large number of membrane proteins, presumably by ZDHHC5. The blockage of any step of this process, such as depleting fatty acids which will prevent acyl-CoA synthesis or preventing the opening of mitochondrial pores, inhibits the whole process which demonstrates that this is a specific cascade and that disruption at any point of it stops MEND [34].

DHHC5 plamitoylates phospholemman, which interacts with and regulates the cardiac sodium pump [36]. This palmitoylation event inhibits sodium pump but only when one of the two cytoplasmic cysteine residues of phospholemman is palmitoylated. Although ZDHHC5 palmitoylates phospholemman, they do not form a stable complex [37]. Instead, ZDHHC5 is brought into proximity of phospholemman through binding the third intracellular loop of the sodium pump α-subunit [37]. The interaction between the sodium pump a-subunit and ZDHHC5 is independent of the PDZ-binding domain of ZDHHC5 and has recently been mapped to a region consisting of around 40 amino acids (residues 223–267) which encompasses an amphipathic helix on the C-terminal side of the fourth and final transmembrane domain (Fig. 1) [37]. This amphipathic helix contains three cysteine residues which are palmitoylated [29,30] and forms a binding site for GOLGA7B, which regulates cell surface expression of ZDHHC5 [38]. Recently, using a proximity labelling approach, DHHC20 was found to interact with ZDHHC5 and to palmitoylate this amphipathic helix [37] which regulates the recruitment of the sodium pump and phospholemman palmitoylation [37].

Interestingly, treatment of cells with a cell-penetration peptide containing the sequence from the central portion of the sodium pump a-subunit binding site on ZDHHC5 inhibited the endogenous interaction, and therefore, ZDHHC5 mediated palmitoylation of phospholemman. This represents a novel and exciting pharmacological route to inhibit a PAT [37]. This mechanism of substrate recruitment explains to some extent the presence of an extended disordered cytoplasmic tail in ZDHHC5 and might be a mechanism shared by other PATs with similar C termini.

A role for ZDHHC5 in controlling the force of heart muscle contractions has been demonstrated by regulation of β-adrenergic signalling [39]. ZDHHC5 palmitoylates G-proteins associated with the β2-adrenergic receptor rapidly after receptor activation. The kinetics of palmitoylation correlated with the timing of downstream signalling events which lead to the contraction of cardiac muscle cells. Furthermore, receptor stimulation leads to a rapid increase (< 1 min) in the palmitoylation of the C-terminal tail of ZDHHC5, a region known to be important in controlling its localisation in the cells [38] and its lateral motility in the plasma membrane [39]. These data further establish ZDHHC5 as a vital regulator of crucial processes in the heart and highlight how palmitoylation can occur dynamically in response to receptor stimulation.

Activity-dependent synaptic palmitoylation by ZDHHC5

ZDHHC5 is also highly expressed in the brain, particularly in the hippocampus, where it localises to the postsynaptic density [40]. The generation of a ZDHHC5-GT (gene trap) mouse revealed some interesting roles for ZDHHC5 in the brain [40]. This mutation had a rate of embryonic lethality of around 50%, and behavioural testing of these mice showed that they had defects in fear conditioning, suggesting problems in their learning and memory. This implicates ZDHHC5 in various processes at the postsynaptic density involved with the formation and storage of memories. ZDHHC5 forms a complex with an important postsynaptic scaffolding protein PSD-95 [41,42] through an interaction between one of the PDZ domains of PSD-95 and the PDZ-binding ligand which is present at the very C terminus of ZDHHC5 (Fig. 2). Levels of PSD-95 palmitoylation are not altered in ZDHHC5 mutant mice. In fact, PSD-95 is palmitoylated by several other PATs; DHHC3, a Golgi-resident PAT, constitutively palmitoylates PSD-95 [43], DHHC2 palmitoylates PSD-95 in response to synaptic activity [44] and DHHC17 mediated palmitoylation of PSD-95 regulates its clustering in neurons [45]. Although ZDHHC5 does not palmitoylate PSD-95, several binding partners of PSD-95 are palmitoylated and may be recruited to ZDHHC5 by PSD-95 as substrates.

At the postsynaptic density, ZDHHC5 also palmitoylates δ-catenin, an important scaffolding protein, in response to synaptic activity [46]. At the postsynaptic density, palmitoylated δ-catenin associates with the adhesion molecule N-cadherin, which regulates activity-dependent spike remodelling. Also, this palmitoylation event appears to increase the levels of AMPA receptor subunits at the postsynaptic density, which may sensitise the neuron to these signals. Context-dependent fear conditioning in mice increased the levels of δ-catenin palmitoylation, which links both δ-catenin

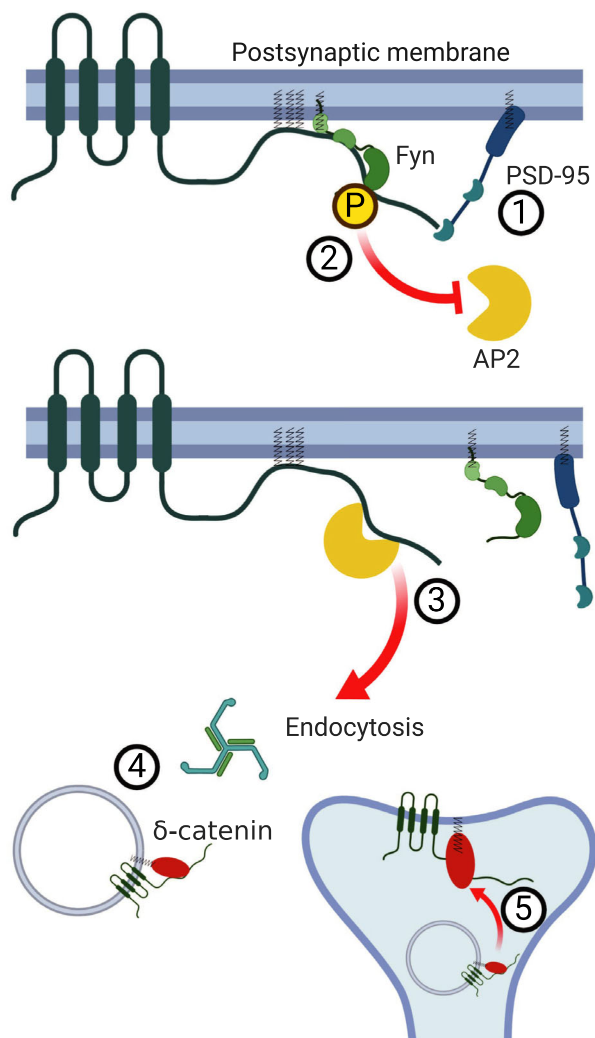

Fig. 2. Schematic of ZDHHC5 localisation and movement at the postsynaptic density in neurons. Adapted from [47]. (1) In basal conditions ZDHHC5 is localised to the postsynaptic membrane in complex with Fyn kinase and PSD-95. (2) Binding of AP2, and internalisation of ZDHHC5 is prevented by phosphorylation of ZDHHC5 by Fyn kinase. (3) Increased synaptic activity reduces Fyn kinase activity and ZDHHC5 phosphorylation. Reduced binding to Fyn and PSD-95 leads to internalisation of ZDHHC5 by AP2 mediated clathrin endocytosis. (4) After internalisation, ZDHHC5 traffics to dendritic shafts on recycling endosomes where it palmitoylates δ-catenin. (5) ZDHHC5 and δ-catenin are then trafficked together back to the postsynaptic membrane, leading to structural changes of the synapse.

to learning and memory and reveals a potential mechanism underlying the phenotypes of the ZDHHC5-GT mice [40].

Subsequent work has shed further light on the mechanism of ZDHHC5-mediated palmitoylation of δ-catenin and the nature of its interaction with PSD-95 [47]. At basal conditions, ZDHHC5 is at the plasma membrane at the postsynaptic density, while δ-catenin is usually present in the dendritic shafts of neurons [48]. Therefore, in order for ZDHHC5 to palmitoylate δ-catenin, one or both proteins must move to a shared location. This is accomplished as follows: ZDHHC5 is held at the plasma membrane through (a) an interaction with PSD-95, which is mediated by the PDZ-binding ligand on ZDHHC5, and (b) phosphorylation by the Src family kinase Fyn. In response to synaptic activity, ZDHHC5 is de-phosphorylated by STEP61 (Fig. 2) and the loss of phosphorylation causes ZDHHC5 to be endocytosed and trafficked to the dendritic shaft, where it then palmitoylates δ-catenin before both traffic back to the postsynaptic density allowing the recruitment and stabilisation of AMPA receptors. ZDHHC5 is endocytosed by the clathrin endocytosis system and requires the AP2 adaptor complex to bind at a canonical AP2-binding motif in the C terminus of ZDHHC5. As this AP2-binding motif contains the Fyn phosphorylation site, phosphorylation acts either to directly block the interaction with the AP2 complex or prevents the recognition of the motif on ZDHHC5 by the AP2 complex. The interaction with PSD-95 is unlikely to be dependent on the phosphorylation event given that the site of phosphorylation is not near the PDZ-binding domain of ZDHHC5, and the interaction is likely disrupted by the endocytosis of ZDHHC5. However, it is entirely possible that the phosphorylation event helps to stabilise the interaction between PSD-95 and ZDHHC5 and does not solely function to prevent association with the AP2 complex and the clathrin endocytosis machinery. The discovery that ZDHHC5 is trafficked away from the plasma membrane to palmitoylate δ-catenin opens up the possibility that ZDHHC5 has other substrates which are not present at the plasma membrane but are instead found in the dendritic shaft. It also demonstrates how important PTMs of the ZDHHC5 C-terminal tail is to the regulation of ZDHHC5, and how these PTMs could be significant in regulating the localisation of ZDHHC5.

A role for ZDHHC5 in neuronal development has also been established [49]. When neuronal stem cells are starved of growth factors, a treatment which is often used to differentiate these cells, ZDHHC5 levels are rapidly (< 5 min) reduced [49]. This reduction mirrored the reduction of a stem cell marker during differentiation and could be reversed over shorter time points by addition of growth factors. Inhibition of the ubiquitin-proteasome system was able to reduce the degradation of ZDHHC5 and stabilisation of FGF signalling (through EGF or FGF2) prevented

ZDHHC5 turnover [49]. These data suggest that ZDHHC5 is rapidly turned over and that its levels are tightly regulated in response to extracellular signals. ZDHHC5 may be especially important to maintain stem cell pluripotency, and it would be interesting to track the fate of relevant substrates during differentiation to determine whether the reduction in ZDHHC5 leads to a reduction in either their abundance or cellular localisation.

ZDHHC5 regulates cell adhesion

A large number of adhesion proteins are palmitoylated [50,51], in particular, the desmosomal complex, with six palmitoylated components [52,53]. However, until recently, the identity of the PAT(s) responsible for desmosomal protein palmitoylation was unknown. Several threads of evidence pointed to a regulatory role of ZDHHC5 for one or more of these proteins. As ZDHHC5 is present at the plasma membrane, it would be in the same cellular compartment as desmosomal proteins to modify them. Recently, an affinity purification-mass spectrometry (AP-MS) study revealed that ZDHHC5-GOLGA7B interacts with the desmosomal cadherin desmoglein-2 (DSG2), a known palmitoylated desmosomal protein [38]. ZDHHC5 is the PAT responsible for palmitoylation of DSG2 [38], and depletion of ZDHHC5 leads to a mislocalisation of DSG2 in A431 cells. Depletion of ZDHHC5 also causes a significant inhibition in the trafficking of DSG2 back to the plasma membrane after disruption of desmosomes by calcium switch (calcium depletion and re-introduction). The localisation of ZDHHC5 is an important factor in mediating the palmitoylation of DSG2 as it is less palmitoylated when either ZDHHC5 or DSG2 was not localised to the plasma membrane. This did contradict the hypothesis put forward in previous work which suggested that DSG2 is palmitoylated at the Golgi [53]. However, it was subsequently shown that the majority of DSG2 palmitoylation likely takes place at the plasma membrane as when DSG2 is internalised by low calcium conditions and is thus separated from ZDHHC5 which is still present at the plasma membrane, palmitoylation of DSG2 is significantly reduced [38].

Knockdown of ZDHHC5 resulted in a mislocalisation of DSG2 [38] which was more severe than has been seen previously with palmitoylation-deficient mutants of DSG2 [53] but did more closely match the phenotype observed when palmitoylation of another desmosomal protein, plakophilin-3 (PKP3), is prevented [52]. In fact, siRNA-mediated knockdown of ZDHHC5 causes palmitoylation of PKP3 to be abolished. This points to the effects seen on the localisation of DSG2 being a combination of the loss of palmitoylation of both PKP3 and DSG2, and positions ZDHHC5 as the PAT for several desmosomal proteins.

As desmosomes are essential for proper cell adhesion and other cell adhesion components were found as interactors of ZDHHC5 in an AP-MS study, it was hypothesised that loss of ZDHHC5 could affect more types of cell adhesion [38]. This was tested by adhesion assays which showed that loss of ZDHHC5 led to a reduction in overall cell:cell adhesion, implicating ZDHHC5 not only in desmosomal protein localisation but in the wider process of cell adhesion [38]. This could be due to ZDHHC5 having roles in other cell adhesion processes as ZDHHC5 interacts with other cell adhesion proteins, such as the tight junction protein ZO-1. It would be interesting to investigate further the potential regulation of other adhesion processes by ZDHHC5, as it could position ZDHHC5 as a regulator of all of these adhesion complexes.

The reduction in adhesion that is driven by the loss of ZDHHC5 could have implications in several different diseases. Desmosomes and DSG2 and plakophilin, in particular, have been implicated in the serious heart condition arrhythmogenic right ventricular cardiomyopathy (ARVC) [54]. Mutations in these proteins have been shown to impair the adhesion of the cardiac muscle, which leads to damage of the cardiac muscle. To our knowledge, palmitoylation of desmosomal components has not been implicated in ARVC to date, but these recent findings open this up as a possibility and ZDHHC5, which is enriched in the cardiac muscle and regulates other relevant processes there [34,35] may play a role.

ZDHHC5 in host–pathogen interactions

There are several examples of important roles for palmitoylation in regulating host–pathogen interactions, and ZDHHC5 is involved in both the protection of host cells from bacterial invasion and the pathogenesis of secreted pathogenic toxins. Many toxins require cleavage by host proteases, including the anthrax toxin and aerolysin, a toxin that forms a pore in the plasma membrane of host cells [55]. The cleavage of these proteins is mediated by the proteins Furin and Proprotein convertase subtilisin/kexin type 7 (PC7), both of which are ZDHHC5 substrates [55]. Palmitoylation of these proteins by ZDHHC5 is required for Furin/PC7 to partition into lipid microdomains where their proximity to the anthrax toxin and aerolysin facilitates their

cleavage [55]. Furthermore, ZDHHC5 also has a role in the recycling of these proteins to the plasma membrane [55]. This effect is likely to be indirect and linked to the function of ZDHHC5 in endosome to Golgi retrieval [56]. The palmitoylation of Furin and PC7 appears to control the precise localisation of these proteins to particular lipid microdomains at the plasma membrane [55]. Further investigation on the acyl chain length added by ZDHHC5 to both Furin and PC7 could be interesting as ZDHHC5 can use lipids of differing chain lengths [57] which would all confer different levels of hydrophobicity to these proteins and presumably affect their partitioning.

ZDHHC5 also has a highly significant role in sensing bacteria that invade the cell and the subsequent immune response. ZDHHC5 palmitoylates a pair of proteins Nucleotide oligomerisation domain (NOD)-like receptor 1 (NOD1) and NOD2, which are both essential for the sensing of bacteria present within the cell [58]. These proteins are present on endosomal membranes and the plasma membrane, and preventing their palmitoylation causes them to lose their membrane localisation. ZDHHC5 knockdown also causes a similar phenotype and also prevents NOD1/2 from localising to vacuoles containing pathogens [58]. ZDHHC5-mediated palmitoylation of NOD1/2 appears to be essential for the correct function of NOD1/2 in this respect. Diseases caused by mutations in NOD1/2, including Crohn's disease, involve the region of NOD1/2 that is palmitoylated, which could implicate ZDHHC5 in these diseases also.

To identify potential drugs to fight the recent COVID-19 pandemic, a large-scale AP-MS screen of SARS-CoV-2 host–virus interactions was performed [59]. Epitope-tagged versions of 26 of the 29 SARS-CoV-2 viral proteins were expressed in HEK293 cells, and host–protein interactors were identified using pull-downs and mass spectrometry-based proteomics. Out of 322 high confidence host–virus interactions, drugs targeting 66 human protein were identified. Interestingly, the viral Spike protein was found to interact with just two proteins, ZDHHC5 and GOLGA7 [59]. The Spike protein of other coronaviruses is S-acylated (by an unknown PAT) and that this modification is very likely essential for viral fusion. S-acylation was first discovered as a PTM in viruses over 40 years ago [1]. Since this initial discovery, protein S-acylation has emerged as an important regulatory mechanism for several proteins in many viruses [60]. S-acylation of the Spike protein in coronaviruses regulates host cell fusion to allow infection to spread directly from cell to cell [61] as well as regulating the major conformational changes that the spike protein must undergo during the process of fusion of cell and viral membranes [62]. To date, the enzymes responsible for the S-acylation of just one viral protein has been identified [63]. Clearly, the identification of the PAT or PATs responsible for Spike protein palmitoylation is urgently required to understand better how the virus manipulates the host cell to replicate and complete its life cycle. The subcellular location of spike protein palmitoylation is also of interest given that palmitoylation occurs in the ER just before the assembly of viral particles for other similar viruses. It is possible that the co-localisation and therefore interaction of the Spike protein and ZDHHC5 is an artefact of expression of the spike protein alone in the absence of the other viral proteins. Whether palmitoylation of the Spike protein at the plasma membrane or in endosomes by ZDHHC5 is possible or biologically relevant remains to be determined.

Regulation of ZDHHC5 by GOLGA7B

ZDHHC5 is a PAT which is generally localised to the plasma membrane [24]. However, it can be trafficked from the postsynaptic density to recycling endosomes, through the AP2-mediated clathrin endocytic pathway, [47]. However, until recently it was unknown if other factors contribute to ZDHHC5 maintenance at the plasma membrane in different cell types or if palmitoylation of ZDHHC5 has any impact on its subcellular localisation [29,30]. Woodley *et al.* [38] recently identified and characterised the function of a novel ZDHHC5 interactor and S-acylated protein GOLG7B. GOLGA7B is closely related to GOLGA7, which is a co-factor of the RAS PAT, DHHC9 [12] and acts to stabilise the DHHC9-acyl chain intermediate [64].

ZDHHC5 S-acylates GOLGA7B but the interaction between ZDHHC5 and GOLGA7B is not dependent on the palmitoylation of GOLGA7B as the mutation of these palmitoylation sites does not prevent the interaction [38]. However, preventing palmitoylation of the three palmitoylation sites on the C-terminal tail of ZDHHC5 abolishes the interaction between ZDHHC5 and GOLGA7B [38]. This could be due to a disruption of the local protein structure that is formed when the C-terminal tail is pinned to the membrane by palmitoylation.

Over-expression of ZDHHC5 generally causes a mislocalisation of the protein leading to it being present in other compartments of the cell as well as the plasma membrane. This is a feature that has been observed by others previously [65], but the reasons for this were unknown. Recently, it was determined that over-expression induced mislocalisation of

ZDHHC5 could be reversed by co-expression of wild-type GOLGA7B, but not by co-expression of the palmitoylation-deficient mutant GOLGA7B [38]. Furthermore, siRNA-mediated depletion of GOLGA7B causes ZDHHC5 to be mislocalised similarly, and this is not rescued by the expression of the closely related protein GOLGA7 [38]. This suggests that when ZDHHC5 is over-expressed, it is the lack of GOLGA7B that causes the mislocalisation phenotype and that this effect on ZDHHC5 is likely to be specific to GOLGA7B and not a more general feature of the Golgin protein family [66]. The C-terminal S-acylation mutant ZDHHC5 is unaffected by either wild-type or mutant GOLGA7B expression and is also exclusively present at the plasma membrane even when over-expressed [38].

These results allow several conclusions to be drawn. Firstly, plasma membrane localisation of ZDHHC5 is stabilised by GOLGA7B, but only when GOLGA7B is palmitoylated. Secondly, if palmitoylation of GOLGA7B is prevented, then ZDHHC5 is internalised (Fig. 3) and appears to be present throughout the cell. It is also apparent that the level of GOLGA7B in the cell is important in ensuring that ZDHHC5 is present at the plasma membrane, as depletion of GOLGA7B causes endogenous ZDHHC5 to be mislocalised to internal membranes away from the plasma membrane and over-expression of ZDHHC5 without corresponding over-expression of wild-type GOLGA7B leads to a similar phenotype. This points to the presence of palmitoylated GOLGA7B being a protective factor from ZDHHC5 internalisation. Also, palmitoylation of the C-terminal tail of ZDHHC5 is an important factor in its interaction with GOLGA7B, but perhaps more interestingly, it also plays a role in maintaining the plasma membrane localisation of the protein. When the C terminus of ZDHHC5 cannot be palmitoylated, trafficking of ZDHHC5 appears to be unaffected as ZDHHC5 is at the plasma membrane. However, this palmitoylation is likely to be important in the endocytosis of ZDHHC5 as when the palmitoylation sites are mutated ZDHHC5 is present at the plasma membrane. This points to palmitoylation of the ZDHHC5 C terminus as being an important regulator or trigger for ZDHHC5 endocytosis.

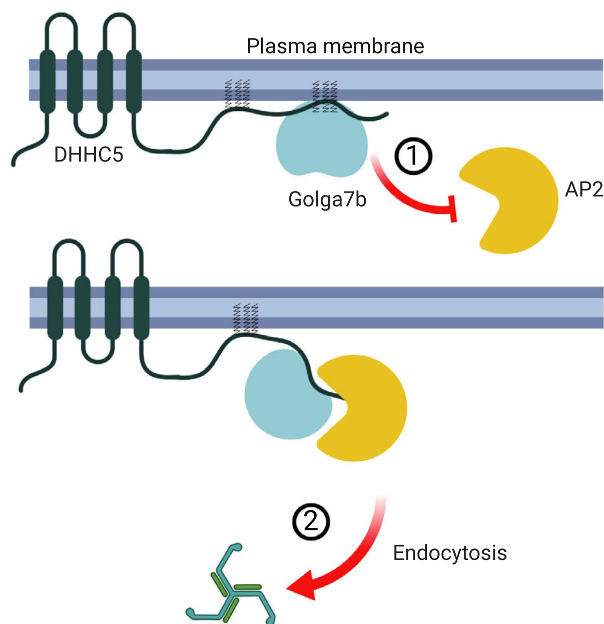

Fig. 3. Proposed model of how palmitoylation of Golga7b regulates plasma membrane localisation of ZDHHC5. (1) Binding to and S-acylation of Golga7b by ZDHHC5 stabilises the complex at the plasma membrane by preventing AP2 mediated endocytosis. (2) Loss of Golga7b S-acylation allows binding of AP2 to ZDHHC5 and its endocytosis.

Emerging mechanisms for ZDHHC5 regulation

A recent study has shown that ZDHHC5 has an important, and previously unrecognised, role in a novel form of cell death which is distinct from the classical cell death pathways [67]. This work showed that ZDHHC5 forms a complex with the DHHC9 co-factor GOLGA7 at the plasma membrane, which then leads to palmitoylation of unknown downstream proteins to trigger cell death caused by a synthetic small molecule CIL56 [67]. Cell death triggered by CIL56 is dependent on the formation of a ZDHHC5-GOLGA7 complex, the catalytic activity of ZDHHC5, C-terminal acylation of ZDHHC5 but not binding of clathrin to ZDHHC5. This opens up the possibility that ZDHHC5 and GOLGA7 form a functional PAT complex similar to that of DHHC9 and GOLGA7, although the work does not directly investigate this possibility. A potentially more intriguing possibility is that GOLGA7 is required to target ZDHHC5 to specific substrates, which require palmitoylation for CIL56-mediated cell death.

There are some interesting similarities between the interactions that occur between ZDHHC5 and GOLGA7 [67] and GOLGA7B [38]. Both appear to require the palmitoylation of the C-terminal tail of ZDHHC5 to form stable complexes, as a C-terminal acylation mutant is unable to form stable complexes with either GOLGA7 or GOLGA7B, but neither require ZDHHC5 to be active. This implicates a possible conformational change in the region immediately

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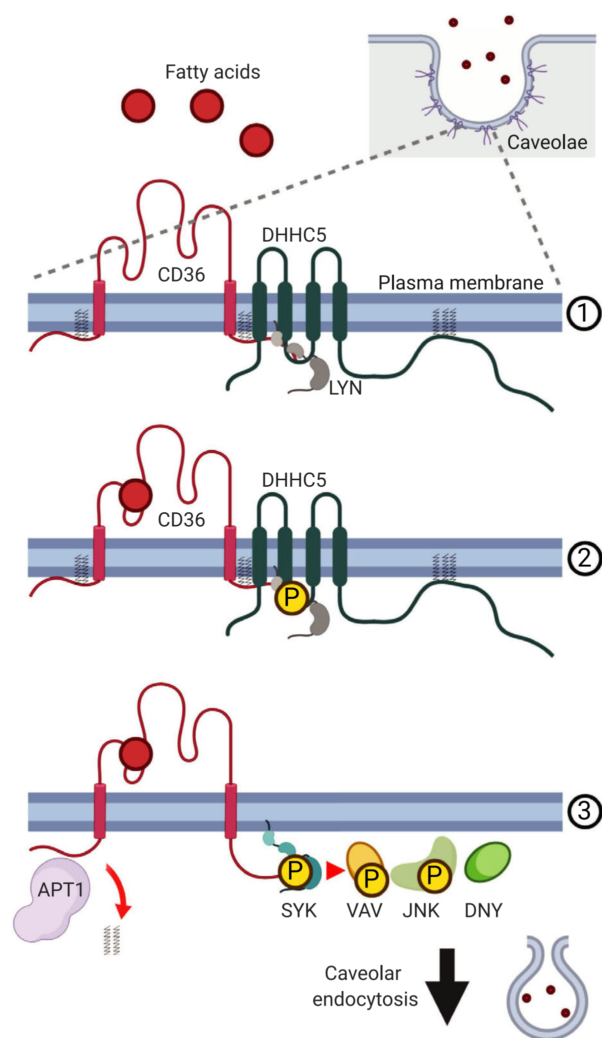

Fig. 4. Regulation of fatty acid uptake by ZDHHC5-mediated S-acylation of CD36. (1) In basal conditions CD36 is S-acylated by ZDHHC5 in caveolae. (2) Binding of fatty acids to CD36 leads to activation of LYN and phosphorylation of ZDHHC5 and its inactivation. (3) De-acylation of CD36 by APT1 leads to caveolar endocytosis and fatty acid uptake.

at the C-terminal of the 4th transmembrane domain of ZDHHC5 when it is palmitoylated and that this palmitoylation event allows this region of ZDHHC5 to become a critical interaction interface for both GOLGA7 and GOLGA7B.

These two studies reported differences in the importance of ZDHHC5 C-terminal palmitoylation for its localisation at the plasma membrane. One found ZDHHC5 to be localised to the plasma membrane exclusively when palmitoylation of the C-terminal tail was abolished [38] whereas the other found it to accumulate in cytosolic puncta [67]. Another recent study found that in neurons, the cell surface expression of ZDHHC5 C-terminal palmitoylation mutant was significantly increased compared with wild-type ZDHHC5 due to a decrease in ZDHHC5 turnover [68] supporting the former study [38]. A potential explanation of these conflicting results may be due to differences in the cell types used.

Recent work focusing on the elucidation of fatty acid uptake by CD36 revealed that CD36 is targeted to the plasma membrane by S-acylation mediated by DHHC4 and ZDHHC5 [69]. The activity of ZDHHC5 was found to be regulated by phosphorylation of tyrosine-91, which is located in the same intracellular loop as the DHHC motif [70]. This site is phosphorylated by the tyrosine kinase Lyn [70], a protein very highly enriched in ZDHHC5 pulldowns analysed by mass spectrometry [38]. Intriguingly, phosphorylation of ZDHHC5 by Lyn at Y91 is induced by fatty acid (oleate) treatment of cells, and this phosphorylation acts to inhibit ZDHHC5 activity [70]. This inhibition of ZDHHC5 allows the depalmitoylation of CD36 by Acyl-protein thioesterase 1 (APT1), which initiates CD36-mediated caveolar endocytosis of fatty acids [70]. In this way, palmitoylated CD36 captures fatty acids at the plasma membrane, and its depalmitoylation triggers its endocytosis and therefore the uptake of captured fatty acids (Fig. 4). It remains to be determined how exactly phosphorylation of ZDHHC5 at Y91 regulates its enzymatic activity, but phosphorylation may cause a conformational change in the cytosolic loop that contains the DHHC motif (Fig. 1). Detailed structural studies should resolve whether this is a likely mechanism or not.

Conclusions

A model of ZDHHC5 regulation is emerging that utilises phosphorylation by tyrosine kinases to regulate its activity and localisation; in the case of Y91 by directly regulating enzyme activity and for Y533 by regulating its cell surface expression by blocking its endocytosis. ZDHHC5 cell surface expression is also regulated by S-acylation of the C terminus of ZDHHC5 and by binding to GOLGA7 or GOLGA7B. Whilst some of these mechanisms of ZDHHC5 regulation have been shown to occur in different cell types, it is not yet clear which are employed universally or in a cell type-specific manner. More work is required to refine our current understanding of ZDHHC5 regulation in a wider variety of cell and tissue types, and the extensive PTM of the sizeable C-terminal domain remains relatively unexplored.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

KTW and MOC wrote the manuscript.

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