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Fatty acyl donor selectivity in membrane bound O-acyltransferases and communal cell fate decision-making

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Abstract

The post-translational modification of proteins with lipid moieties confers spatial and temporal control of protein function by restricting their subcellular distribution or movement in the extracellular milieu. Yet, little is known about the significance of lipid selectivity to the activity of proteins targeted for such modifications. Membrane bound O-acyl transferases (MBOATs) are a superfamily of multipass enzymes that transfer fatty acids on to lipid or protein substrates. Three MBOATs constitute a subfamily with secreted signalling molecules for substrates, the Wnt, Hedgehog (Hh) and Ghrelin proteins. Given their important roles in adult tissue homeostasis, all three molecules and their respective associated acyltransferases provide a framework for interrogating the role of extracellular acylation events in cell-to-cell communication. Here, we discuss how the preference for a fatty acyl donor in the Wnt acyltransferase porcupine (Porcn) and possibly in other protein lipidation enzymes may provide a means for coupling metabolic health at the single cell level to communal cell fate decision-making in complex multicellular organisms.

Keywords

intercellular signalling; lipid metabolism; membrane bound O-acyltransferases; protein fatty acylation; Wnt; Hedgehog

Background

The cell membrane represents the primary point of information exchange between cells and their surroundings. Proteins that inhabit this nexus are frequently charged with the task of transducing extracellular cues across the cell membrane or converting intrinsic cues into cellular responses to the environment such as changes in cell morphology or movement. Unlike transmembrane proteins that are embedded into the lipid bilayer with one or more stretches of hydrophobic protein sequence, lipidated proteins are more nomadic and typically exhibit malleability with respect to membrane association.

Much of our understanding of lipid adduct contribution to target protein function is derived from studies focused on intracellular proteins such as the Ras proto-oncogenes, a gene

family with activating mutations in 30% of all cancers [1]. A collection of enzymes that affix or remove isoprenoid or palmitoyl adducts on Ras proteins reversibly controls protein association with the membranes of subcellular compartments such as the endoplasmic reticulum (ER), Golgi apparatus or plasma membrane [2]. This subcellular distribution in turn dictates Ras protein access to a number of effector molecules that regulate diverse cellular responses [1].

Unlike the palmitoyl transferases belonging to the zinc finger, Asp-His-His-Cys-type containing (ZDHHC) protein family that fatty acylate intracellular proteins such as Ras [3], the membrane bound O-acyl transferases (MBOATs) immobilize a diverse set of lipids on to protein and lipid substrates (Table 1). The understanding that Hedgehog (Hh), Wnt and Ghrelin are modified with unique fatty acyl moieties suggests that a particular lipid adduct may influence the function of the targeted protein that extends beyond merely membrane immobilization. In this review, we discuss recent observations from studies focused on porcupine (Porcn) and the Hh acyltransferase (Hhat) that expand the canonical roles of protein lipidation to include the support of signalling molecule capacitation, receptor/ligand interaction and possibly cellular metabolism coupling to cell fate outcomes.

Divergent roles of fatty acylation in Hh and Wnt signalling

The Wnt and Hh proteins embody a nearly universal strategy for coordinating cell fate decision-making during development and tissue homeostasis in metazoans. The lipid adducts found on these proteins have largely been attributed roles as molecular brakes that prevent protein diffusion and instead promote their graded distribution from ligand producing cells. The mature Hh protein is dually lipidated with a palmitate and cholesterol molecule (Figure 1A). Whereas Hhat affixes the palmitoyl adduct on to the N-terminal cysteine residue, the regulatory domain of the Hh precursor protein facilitates a cholesterol-mediated intramolecular attack to release an N-terminal signalling domain [4] (Figure 1A). As a result, the cholesterol molecule is covalently attached to the C-terminus of the newly generated signalling domain. Substituting the cysteine residue targeted for acylation with an alanine modestly reduces Hh protein ability to elicit cellular responses *in vivo* [5] and does not decrease Hh affinity for the Hh receptor Patched (Ptc) *in vitro* [6] suggesting acylation is not essential to its engagement of Ptc. Loss of Hhat then may compromise the ability of cholesterol-modified Hh to be lifted from the cell membrane by an enzyme called Dispatched (Disp) or to multimerize thus crippling the long range movement of Hh protein [7–9] (Figure 1A).

An engineered Wnt molecule with a transmembrane anchor can substitute for the wild-type protein in development suggesting that the mono-unsaturated fatty acyl adduct found on all 19 Wnt molecules (Table 1) restricts their signalling to adjacent cells and prevents their long-range signalling [10]. Additionally, distinguishing the role of fatty acylation in Wnt and Hh signalling is the affinity of the Wntless (Wls) for only lipidated Wnt molecules thus imposing a check-point on Wnt ligand biosynthesis that is absent from the case of Hh molecules [11–13] (Figure 1B). The Frizzled (Fzd) receptors constitute a large family of seven transmembrane proteins that transduce Wnt signals across the membrane [14]. In contrast with the Ptc receptor which appears agnostic to Hh acylation status, the Fzd

proteins harbour a pocket for a long chain fatty acid and contribute to Wnt binding [15]. Thus, the fatty acyl modification is essential at several stages in the Wnt protein life cycle.

The source of cellular palmitoleoyl-CoA (coenzyme A) in part stems from the desaturation of palmitoyl-CoA by the ER-resident stearoyl-CoA desaturase (SCD) enzymes [16]. The participation of SCDs in the production of Wnt proteins [17] may support a mechanism for coupling cell intrinsic fatty acid metabolism with the coordination of cell fate outcomes during development and tissue homeostasis. Indeed, stem cells and cancer initiating cells appear to rely more on *de novo* fatty acid desaturation than scavenging pathways for maintaining homeostatic levels of desaturated phospholipids that are essential for membrane fluidity [18,19]. Remarkably, chemical disruption of SCD provokes transcriptional up-regulation of many Wnt family members in induced pluripotent stem cells thus further providing evidence for the coupling of cellular metabolism and tissue homeostatic renewal [18].

MBOAT substrate and acyl donor specificity

Hhat and Porcn appear to occupy unique roles across multiple species thus signifying a selectivity of each enzyme for their cognate substrates. Indeed, no compensatory mechanisms have so far been identified that enable recovery of Wnt or Hh lipidation upon targeted disruption of either Porcn or Hhat respectively [5,20,21]. Clearly, contributing to enzyme-substrate recognition is the presence of protein-protein interaction determinants in both MBOATs and substrates [22,23] (Figure 2). At the same time, despite having access to both palmitoyl- and palmitoleoyl-CoA molecules, Wnt and Hh proteins are nevertheless predominantly modified with a single fatty acyl species [6,24]. Thus, mechanisms underpinning fatty acyl donor selectivity for MBOATs must also be in place. Whereas Porcn exhibits some predilection for fatty acids with a certain chain length (C10–16) [17,25], this alone cannot account for Porcn acyl donor selectivity given that Wnt proteins appear to be mostly modified with palmitoleate. Thus, the active site of Porcn must accommodate an acyl donor with a double-bond-induced bend that is nine carbon length distal to the CoA moiety. At the same time, the lack of fatty acyl donor selectivity observed *in vitro* [26] and *in vivo* (unpublished work; Rubina Tuladhar and Lawrence Lum) for Hhat when provided with excess fatty acid analogues suggests additional mechanisms enforcing selectivity exist. The relative cytoplasmic abundance of palmitoyl-CoA compared with other acyl-CoA species in addition to active site determinants that limit stearate may dictate Hhat selectivity *in vivo* [27]. Although the mechanistic basis underlying these selectivity issues clearly require further examination, these early studies focused on Porcn and Hhat have already revealed general strategies for achieving fatty acylation selectivity that are probably employed by the MBOAT family at large.

Concluding remarks

The MBOATs constitute a novel family of highly druggable and therapeutically relevant targets in diseases of metabolism, cancer and tissue degeneration [28–31]. Challenges typically associated with the structural biology of highly hydrophobic proteins and recent reports that Porcn is itself fatty acylated [25] will probably limit the contribution of tools

such as crystallography to drug development agendas targeting these enzymes. To compensate for the absence of structural insights that typically guide small molecule development projects, new tools for probing the enzymology of these unique proteins are required. Indeed, the chemical basis of enzyme selectivity for substrate and fatty acyl donors will be essential for the development of agents targeting other MBOATs in addition to the Porcn inhibitor that has entered clinical testing [32]. Finally, clues as to how these enzymes accomplish their feat of bringing together intracellular lipids and extracellular proteins to generate hybrid signalling molecules will undoubtedly increase our understanding of the ancient mechanisms borne from protein and lipid co-existence that synchronize metabolic governance with cell fate determination.

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Abbreviations

CoA	coenzyme A
Disp	dispatched
ER	endoplasmic reticulum
Fzd	frizzled
Hh	Hedgehog
Hhat	Hh acyltransferase
MBOAT	membrane bound O-acyl transferase
Porcn	porcupine
Ptch	patched
SCD	stearoyl-CoA desaturase
Wls	Wntless.

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Figure 1. The role of fatty acylation in Wnt and Hh signalling

(A) Porcn utilizes a palmitoleoyl-CoA donor to modify Wnt molecules. Sources of palmitoleoyl-CoA include SCD-mediated desaturation of palmitoyl-CoA. Acylated Wnt protein is chaperoned by the Wntless (Wls) multipass receptor to the cell surface. Secreted Wnt proteins then bind to Fzd receptors with the acyl moiety inserting into a groove found in the extracellular cysteine-rich domain. (B) Hh molecules are dually lipidated with a cholesterol adduct on the C-terminal domain and a palmitate on the N-terminal domain. Palmitoylation of Hh is mediated by the Hhat. Acylated Hh proteins are released from the

membrane by the multipass protein Disp and form multimeric complexes which enable long-range signalling and support formation of graded Hh responses in cells surrounding the ligand producing cells. Hh binding to the Ptch receptor lifts the inhibitory action of Ptch on Smoothened (Smo) thus resulting in transcriptional activation of Hh target genes.

Figure 2. Mechanisms underlying MBOAT acyl donor selectivity
Wnt fatty acylation by the Porcn enzyme is modelled here.

Table 1

Diverse cellular functions of MBOATs dictated by fatty acyl donor and substrate selectivity

Function	Gene	Substrate	Acyl donor
Lipid biosynthesis	ACAT1, ACAT2	Cholesterol	Long chain acyl-CoA
Lipid biosynthesis	DGAT1	Diacylglycerol	Long chain acyl-CoA
Protein acylation	PORCN	Wnt	Palmitoleoyl-CoA
Protein acylation	HHAT	Hh	Palmitoyl-CoA
?	HHATL/MBOAT3	?	?
Protein acylation	GOAT/MBOAT4	Ghrelin	Octanoyl-CoA
Phospholipid remodelling pathway	LPEAT1/MBOAT1	LPS	Oleoyl-CoA
Phospholipid remodelling pathway	LPLAT5/MBOAT5	LPS and LPC	Polyunsaturated fatty acyl-CoA
Phospholipid remodelling pathway	LPLAT2/MBOAT2	LPE and LPA	Oleoyl-CoA
Phospholipid remodelling pathway	LPIAT1/MBOAT7	LPI	Arachidonoyl-CoA

Abbreviations: ACAT, acyl-CoA cholesterol acyltransferase; DGAT, diacylglycerol acyltransferase; GOAT, ghrelin O-acyl acyltransferase; HHATL, hedgehog acyltransferase-like; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPEAT, lysophosphatidylethanolamine acyltransferase; LPI, lysophosphatidylinositol; LPIAT, lysophosphatidylinositol acyltransferase; LPLAT, lysophospholipid acyltransferase; LPS, lysophosphatidylserine.