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Review

Human DHHC proteins: A spotlight on the hidden player of palmitoylation

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ABSTRACT

Palmitoylation is one of the most common posttranslational lipid modifications of proteins and we now know quite a lot about it. However, the state of knowledge about the enzymes that catalyze this process is clearly insufficient. This review is focused on 23 human *DHHC* genes and their products – protein palmitoyltransferases. Here we describe mainly the structure and function of these proteins, but also, to a lesser degree, what the substrates of the enzymes are and whether they are related to various diseases. The main aim of this review was to catalogue existing information concerning the human DHHC family of genes/proteins, making them and their functions easier to understand.

Palmitoylation at a glance

Palmitoylation was first described about 40 years ago and it is one of the most frequent posttranslational modifications of proteins, defined as the addition of saturated 16-carbon palmitic acid to specific cysteine residues. Depending on the type of palmitoylation, it occurs through an N-amide bond (N-palmitoylation) or through the formation of a labile thioester bond (S-palmitoylation). This review is focused only on S-palmitoylation. S-palmitoylation is a unique, reversible modification with a potential to regulate the function of proteins through cycles of palmitoylation and depalmitoylation catalyzed by protein palmitoyltransferases and protein thioesterases. Palmitoyltransferases are responsible for catalyzing the addition of palmitate to the protein substrate, and thioesterases catalyze the removal of palmitate (Fang et al., 2006; Greaves et al., 2011; Linder and Deschenes, 2003; Mitchell et al., 2006; Planey et al., 2009; Raymond et al., 2007; Resh, 2006a). In this context, palmitoylation can be considered as the lipid version of protein phosphorylation (Ducker et al., 2006; Jennings et al., 2009; Varner et al., 2003; Yang et al., 2010). There is also evidence that palmitoylation is a spontaneous modification, in which the cysteine SH group, which is a strong nucleophile, attacks the thioester bond between CoA and palmitate (Bijlmakers and Marsh, 2003; Dietrich and Ungermann, 2004). This non-enzymatic autoacylation occurs in the case of several proteins, for example the G protein α subunit (Gα) (Duncan and Gilman, 1996) and the SNAP-25 protein (Veit, 2000). It was also found that this process, when it occurs on PAT (protein acyltransferase) itself, may be involved in the regulation of PAT specificity and/or activity (Yang et al., 2010). This possibility is supported by evidence that DHHC5, which appears to be S-acylated on three cysteine residues within a novel $CCX_{7-13}C(S/T)$ motif downstream of a conserved DHHC domain forming a short loop upon palmitoylation, functions as a scaffold. This scaffold may recruit either specific substrates to DHHC5 and in this way influence its specificity or specific regulators that block interaction between DHHC5 and the substrate, which, in consequence, inhibits the palmitoylation of the substrates. Depalmitoylation of DHHC5 may change the structure of this enzyme and regulate its specificity and/or activity (Yang et al., 2010). To date, three thioesterases, named APT1 (acyl-protein thioesterase-1), APT2 (acyl-protein thioesterase-2) and PPT1 (palmitoyl-protein thioesterase-1), catalyzing the depalmitoylation process, have been discovered. Interestingly, only APT1 appears to be engaged in regulation of the reversible palmitoylation of cytoplasmic proteins such as Ras, Gα subunits, endothelial nitric oxide synthase (eNOS) and SNAP-23 (Dekker et al., 2010; Tomatis et al., 2010; Yang et al., 2010; Zeidman et al., 2009). It is still unclear how this enzyme efficiently accesses its membrane-associated substrates, but one possibility is that palmitoylation of APT1 may tether this protein to the membrane and in consequence facilitate the depalmitoylation of its substrates (Yang et al., 2010). APT2 is a thioesterase which catalyzes the depalmitoylation of peripheral

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membrane-associated GAP-43 (Tomatis et al., 2010). PPT1, on the other hand, is a lysosomal enzyme involved in the degradation of palmitoylated proteins (Tomatis et al., 2010; Verkruyse and Hofmann, 1996). It was found that PPT1 deficiency causes neuronal ceroid lipofuscinosis of infants (Zeidman et al., 2009).

It is important to add that there are proteins, named acyl-CoA-binding proteins (ACBPs), which may effectively protect membrane proteins against spontaneous palmitoylation, despite the fact that these proteins do not supply palmitate to PATs. However, the mechanism of this process is still unclear (Dunphy et al., 2000; Leventis et al., 1997).

The roles of palmitoylation are diverse. The most commonly described function of this process is increasing the affinity of proteins for membranes, which can thereby affect the target protein localization and function. Palmitoylation may also affect protein–protein interactions, modulate protein stability and regulate protein degradation (Baekkeskov and Kanaani, 2009; Bijlmakers and Marsh, 2003; Conibear and Davis, 2010; Draper and Smith, 2009; Fang et al., 2006; Greaves and Chamberlain, 2011; Greaves et al., 2011; Linder and Deschenes, 2003). Moreover, it was found that palmitoylated proteins are highly enriched in lipid rafts, caveolae and tetraspanin-enriched microdomains, which suggests an essential role of the reversible palmitoylation process in regulating the dynamics of the multiprotein assemblies associated with these structures (Levental et al., 2010; Yang et al., 2010). Palmitoylation of proteins engaged in intracellular signaling is a subject of intense research and some of those proteins are mentioned below as substrates while others have been the subject of excellent reviews (Planey and Zacharias, 2009; Smotrys and Linder, 2004).

Although the proteins that are modified by palmitoylation have divergent functions and no general consensus sequence specifying palmitoylation has been found, a compelling factor that decides whether a cysteine residue is palmitoylated is undoubtedly its proximity to the membrane (Greaves et al., 2009). Similarities between the sites of S-palmitoylation were sorted by Resh (2006b) into 4 classes.

One group consists of transmembrane proteins that are *S*-acylated on cysteines at or near the transmembrane domain. A second group is made up of proteins in which palmitoylation occurs within the C-terminal region and is dependent on prior prenylation of the cysteine residue within the C-terminal "CAAX" box (RAS family). A third group consists of proteins that are palmitoylated at one or more cysteines near the N- or C-terminus. A fourth comprises the members of the Src family of tyrosine protein kinases and several α subunits of heterotrimeric G proteins contain a consensus sequence for dual acylation within their N-terminal SH4 domain: MGC. The glycine residue is *N*-myristoylated and this modification is required for subsequent palmitoylation at the C-3 residue, probably because the presence of a myristate alkyl chain enhances accessibility to a membrane-bound palmitoyl acyl transferase (Ducker et al., 2006; Fukata et al., 2006; Resh, 1996, 1999; Varner et al., 2003).

Human palmitoylating enzymes (PATs)

Palmitoylation is catalyzed by a family of enzymes that contain a DHHC (Asp-His-His-Cys) cysteine-rich domain which is directly involved in the palmitoyl transfer reaction (it is the apparent catalytic center of the enzyme) (Mitchell et al., 2006). The DHHC cysteine-rich domain is a highly conserved motif that can be found in multiple eukaryotic proteins (Ohno et al., 2006). This domain includes a region with the form of a Cys4 zinc-finger-like metal ion binding site followed by a complex Cys-His region and may be summarized as $CX_2CX_9HCX_2CX_2CH_4DHHCCX_5CX_4NX_3FX_4$ (Mitchell et al., 2006; Putilina et al., 1999). It was found that mutations in the DHHC box abolish the PAT activity of DHHC protein (Lobo et al., 2002; Roth et al., 2002; Yang et al., 2010).

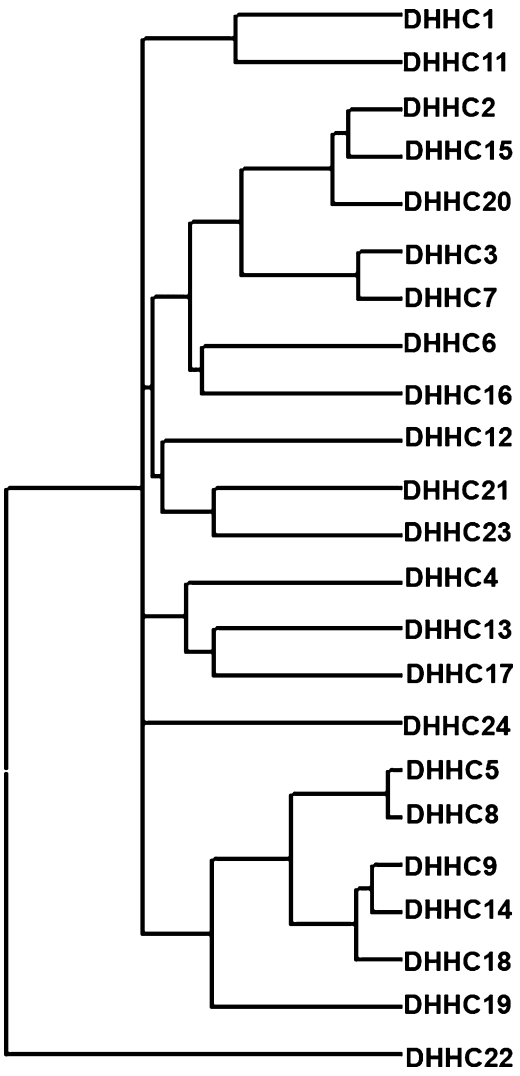

Fig. 1. The phylogenetic tree of the human DHHC family of proteins. This phylogenetic analysis was made based on alignment made using BioEdit program version 7.0.5 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html) and ClustalW accessory application of the DHHC domains, and exactly on its 51 amino acid residue sequences, using Mega5 program version 5.0 (http://www.megasoftware.net).

Closely related proteins containing this domain belong to the DHHC family of proteins (Fig. 1). There are 23 human DHHC proteins (DHHC1–DHHC24) encoded by 23 human Z*DHHC* genes, named *ZDHHC1–ZDHHC24* (*ZDHHC10* is omitted) (Fang et al., 2006; Linder and Deschenes, 2004; Mitchell et al., 2006). Besides the DHHC domain, these proteins also contain four or more transmembrane domains (the DHHC domain is usually located between TM2 and TM3). Additionally, DHHC13 and DHHC17 also contain an ankyrin repeat domain (Greaves et al., 2011; Mitchell et al., 2006; Planey et al., 2009) (Fig. 2).

Human *ZDHHC* genes and proteins

Summarized information about the size of each gene, its location in the human genome and transcription products and also intracellular localization of proteins is presented in Tables 1a and 1b. Most of the DHHC proteins are localized to the Golgi apparatus and to the endoplasmic reticulum (ER). However, some of them have been localized to the plasma