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# New Insights into the Mechanisms of Protein Palmitoylation†

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ABSTRACT: Since its discovery more than 30 years ago, protein palmitoylation has been shown to have a role in protein−membrane interactions, protein trafficking, and enzyme activity. Until recently, however, the molecular machinery that carries out reversible palmitoylation of proteins has been elusive. In fact, both enzymatic and nonenzymatic S-acylation reaction mechanisms have been proposed. Recent reports of protein palmitoyltransferases in *Saccharomyces cerevisiae* and *Drosophila* provide the first glimpse of enzymes that carry out protein palmitoylation. Equally important is the mechanism of depalmitoylation. Two major classes of protein palmitoylthioesterases have been described. One family is lysosomal and is involved in protein degradation. The second is cytosolic and removes palmitoyl moieties preferentially from proteins associated with membranes. This review discusses recent advances in the understanding of mechanisms of addition of palmitate to proteins and removal of palmitate from proteins.

The covalent attachment of fatty acids to eukaryotic proteins was first described ~30 years ago (*1*−*3*), and since that time, the number of acylated proteins that have been identified has continued to grow. There are three classes of protein fatty acylation in eukaryotic cells:[1] N-myristoylation, S-palmitoylation, and N-palmitoylation. Protein N-myristoylation refers to the covalent attachment of myristate, a 14-carbon saturated fatty acid, to the N-terminal glycine of proteins. In most cases, N-myristoylation occurs cotranslationally and is a stable modification. S-Palmitoylation is the reversible addition of palmitate or other long chain fatty acids to proteins at cysteine residues via a thioester linkage. N-Palmitoylation was first described for sonic hedgehog, a secreted signaling protein, and recently has been reported as a modification of the G-protein $G_{s\alpha}$. The N-terminal cysteine residue of hedgehog is modified with amide-linked palmitate. In $G_{s\alpha}$, N-palmitoylation occurs at the N-terminal glycine. Whereas the biology and enzymology of protein N-myristoylation have been extensively characterized (see ref *5* for a recent review), these processes are less well understood for protein palmitoylation and are under active investigation.

The types of proteins that undergo palmitoylation are quite diverse and include intrinsic and peripherally associated membrane proteins, as well as mitochondrial proteins (Table 1). The function of palmitoylation will depend on the protein that is being considered. Palmitoylation increases the hydrophobicity of proteins or protein domains and contributes to their membrane association. Palmitoylation also appears

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[1] In the bacteria *Bordatella pertussis*, adenylate cyclase is modified with amide-linked palmitate at an internal lysine residue (*4*).

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Table 1: Palmitoylation Motifs

| protein class / examples | location of palmitoylated cysteines: sequence[a] | ref |
|---|---|---|
| (I) transmembrane proteins | | |
| G-protein-coupled receptors | cytoplasmic domain following the seventh TM span | |
| rhodopsin | $^{309}$M/NKQFRNCMVTTL**C**$\underline{\mathbf{C}}$GKNP−COOH | *92* |
| $\beta$2-adrenergic receptor | $^{325}$I/YCRSPDFRIAFQEL$\underline{\mathbf{L}}$**C**$\underline{\mathbf{L}}$RRSS$^{346}$- - - - | *93* |
| tetraspanins | N- and C-terminal cytoplasmic domains | |
| CD151 | $^{1}$MGEFNEKKTT**C**GTV**C**LK/YLL$^{20}$<br>$^{236}$FGMIFT/$\underline{\mathbf{CC}}$L$\overline{\mathrm{Y}}$RSLK$\overline{\mathrm{L}}$EHY-COOH | *94* |
| viral glycoproteins | cytoplasmic domain near membrane interface | |
| VSV G-protein | $^{481}$VL/RVGIHL**C**IKLK$^{493}$ | *95* |
| Semliki forest virus E1 | $^{429}$VVVT$\underline{\mathbf{C}}$IGL/$\overline{\mathrm{R}}$R-COOH | *95* |
| (II) dually lipidated proteins | | |
| (A) farnesylation and S-palmitoylation | | |
| Ras GTPases | cysteines upstream of farnesylated and methylated C-terminus | |
| H-Ras | G**C**MS**C**KCfarn | *80* |
| N-Ras | G$\overline{\mathbf{C}}$MG$\overline{\mathrm{L}}$PCfarn | *80* |
| Ras2p (*S. cerevisiae*) | K$\overline{\mathrm{S}}$GSGG$\underline{\mathbf{C}}$Cfarn | *41* |
| (B) N-myristoylation and S-palmitoylation | | |
| | cysteines following myristoylated N-terminus | |
| Fyn | myr-N-G**C**VQ$\underline{\mathbf{C}}$KDKE | *96* |
| $G_{i\alpha1}$ | myr-N-G$\overline{\mathbf{C}}$TL$\overline{\mathrm{S}}$AEDK | *77* |
| eNOS | myr-N-G$\overline{\mathrm{N}}$LKSVAQEPGPP$\underline{\mathbf{C}}$GLGLGLGLGL$\underline{\mathbf{C}}$G | *97* |
| (C) N-palmitoylation and C-terminal cholesterol modification | | |
| hedgehog (*Drosophila*) | palm-N-$^{85}$CGPGP- - -G$^{257}$-O-cholesterol | *55* |
| | fully processed N-terminal signaling fragment | *54* |
| (D) N-palmitoylation and S-palmitoylation | | |
| $G_{s\alpha}$ | palm-N−G$\underline{\mathbf{C}}$LGNSKTE | *57, 85* |
| (III) exclusively palmitoylated cytoplasmic proteins | | |
| (A) N-terminal motifs | | |
| GAP-43 | $^{1}$ML$\underline{\mathbf{CC}}$M | *98* |
| PSD-95 | $^{1}$MD$\overline{\mathbf{C}}$L$\overline{\mathbf{C}}$ITT | *99* |
| $G_{q\alpha}$ | $^{1}$MTL$\overline{\mathrm{E}}$S$\overline{\mathrm{I}}$MA$\underline{\mathbf{CC}}$ | *100* |
| RGS4 | $^{1}$M$\underline{\mathbf{C}}$KGLAGL$\overline{\mathrm{P}}$AS$\underline{\mathbf{C}}$LR | *78* |
| (B) C-terminal motif | | |
| Yck2p (*S. cerevisiae*) | $^{534}$KSSKGFFSKLG$\underline{\mathbf{CC}}$-COOH | *16* |
| (C) cysteine string motifs | | |
| SNAP-25b | $^{83}$KF$\underline{\mathbf{C}}$GL$\underline{\mathbf{C}}$V$\underline{\mathbf{C}}$P$\underline{\mathbf{C}}$NKL$^{95}$ | *101* |
| cysteine string protein 1 | $^{116}$LT$\overline{\mathbf{CC}}$Y$\overline{\mathbf{C}}\overline{\mathbf{C}}\overline{\mathbf{C}}$L$\underline{\mathbf{CC}}$CFN$\underline{\mathbf{CC}}$GK$\underline{\mathbf{C}}$KPK$^{139}$ | *102* |
| GAIP | $^{36}$RNP$\underline{\mathbf{CC}}$L$\underline{\mathbf{C}}$W$\underline{\mathbf{CCCC}}$S$\underline{\mathbf{C}}$SW$^{51}$ | *103* |
| (IV) mitochondrial proteins, palmitoylated at active site cysteines | | |
| methylmalonate semialdehyde dehydrogenase (MMSDH) | | *25* |
| carbamoyl-phosphate synthase (CPSI) | | *29* |

[a] Thioacylated cysteines are underlined and bold. The superscript numbers refer to the amino acid position in the protein sequence. Amino acid sequences are human unless indicated otherwise. A slant signifies the border between the transmembrane and cytoplasmic domains of the polypeptide.

to play an important role in subcellular trafficking of proteins between membrane organelles and within microdomains of individual membrane compartments, as well as modulating certain protein−protein interactions (*6*−*11*). While the identification of palmitoylated proteins has proceeded at a brisk pace, the understanding of the molecular mechanisms that underlie modification with palmitate has advanced more slowly. There are several reasons for this, one being that no single mechanism is likely to account for the diverse nature of palmitoylated proteins (Table 1). Two distinct views of the mechanism of protein palmitoylation have emerged. One view is that palmitoylation occurs spontaneously, while the other invokes a more traditional enzymatic mechanism by a protein acyltransferase (PAT).[2] As described below, both may be correct.

The reversibility of palmitoylation speaks to the possibility of a regulated modification much like protein phosphorylation. Two protein palmitoylthioesterases, one a lysosomal hydrolase (PPT1) and the other a cytoplasmic enzyme (APT1), have been identified and characterized. In both cases, crystal structures have been determined, providing insight into the mechanism of the thioesterase reaction. These studies and the appearance of several recent reports describing novel palmitoyltransferases make this a good time to review the status of the field (*12*−*16*). This review will focus on the mechanisms that underlie addition and removal of palmitate from proteins and will not venture into the extensive body of work on the functional consequences of palmitoylation. Several excellent reviews have appeared recently addressing the regulatory significance of protein palmitoylation (*6*−*11*).

## *Mechanisms of Palmitate Addition*

*Evidence for Spontaneous S-Acylation*. In the absence of cellular factors, palmitoyl-CoA is capable of spontaneously

[2] Abbreviations: ACBP, acyl-CoA binding protein; APT, acylprotein thioesterase 1; CoA, coenzyme A; C-terminus, carboxyl terminus; CRD, cysteine rich domain; DHHC, Asp-His-His-Cys; DFP, diisopropylfluorophosphate; eNOS, endothelial nitric oxide synthase; HDSF, hexadecylsulfonylfluoride; Hh, hedgehog; INCL, infantile neuronal ceroid lipofuscinosis; MMSDH, methylmalonyl semialdehyde dehydrogenase; PAT, protein palmitoyl acyl transferase; PMSF, phenylmethanesulfonyl fluoride; PPT, protein palmitoylthioesterase; Shh, sonic hedgehog; Ski, skinny hedgehog; Sit, sightless.

S-acylating cysteinyl thiols. This can occur in the context of short peptides as well as folded proteins. For example, peptides derived from palmitoylated proteins such as myristoyl-GCG, myristoyl-GCV, and IRYCWLRR undergo spontaneous S-acylation in the presence of palmitoyl-CoA and large unilamellar vesicles (*17*, *18*). Under similar conditions, rhodopsin undergoes spontaneous S-acylation with a $K_m$ of approximately 40 $\mu$M (*19*). Similar results have been seen with G-protein α subunits (*20*, *21*). Interestingly, the efficiency of spontaneous palmitoylation varies. A peptide derived from myelin P0 glycoprotein (RYCWLRR) is efficiently acylated using palmitoyl-CoA, whereas other Cys-containing peptides were not substrates (*18*). Similarly, attempts to autoacylate other known palmitoylated proteins *in vitro*, including SNAP-25, GAP-43, and Fyn kinase, have not met with success (*20*).

It is difficult to assess the role of spontaneous palmitoylation *in vivo*. The lack of an identifiable consensus sequence for palmitoylation appears to argue for a nonenzymatic mechanism. However, mutagenesis studies have shown that palmitoylation is influenced by the position of cysteine or neighboring residues, arguing for an enzymatic mechanism. This also may not be a warranted assumption. Autoacylation of $G_{i\alpha1}$ has been shown to be influenced by the position of basic amino acids which may create a favorable environment for thiol anion formation (*20*, *22*). Autoacylation can also be influenced by the presence of an associated subunit as is seen in the case of the $G_{\beta\gamma}$ subunit enhancing the spontaneous palmitoylation of $G_{i\alpha1}$ (*20*).

The concentration of acyl-CoA must be considered when evaluating the likelihood of spontaneous acylation occurring *in vivo*. However, this has been difficult to ascertain. In tissues and cell lines, estimates of long chain acyl-CoA concentrations and their distribution in subcellular compartments vary widely (*23*). Most of the cytosolic acyl-CoA in the cell is bound to acyl-CoA binding proteins (ACBPs). This sequesters acyl-CoAs and provides a mechanism for minimizing otherwise uncontrolled effects of acyl-CoAs on cellular processes. The rate of spontaneous acylation of short cysteinyl peptides *in vitro* is very slow in the presence of ACBP, acyl-CoA, and membrane lipids, arguing against a nonenzymatic mechanism for palmitoylation of proteins exposed to the cytoplasm (*24*). However, as described below, mitochondrial proteins may be substrates for spontaneous acylation.

The best evidence to date for a regulatory role of palmitoylation is found in the S-palmitoylation of mitochondrial enzymes. Bovine methylmalonyl semialdehyde dehydrogenase (MMSDH) is acylated by an $^{125}$I-labeled analogue of myristoyl-CoA on an active site cysteine, resulting in enzyme inhibition (*25*). This observation together with evidence that palmitoyl-CoA inhibits the activity of several mitochondrial enzymes suggests a regulatory role of S-acylation in metabolism (*26*−*29*). In the case of carbamoyl-phosphate synthetase I, Corvi et al. (*29*) have presented evidence that active site S-palmitoylation occurs spontaneously at physiological concentrations of palmitoyl-CoA. Inhibition of CPSI by long chain fatty acyl-CoAs might serve to reduce the extent of amino acid degradation during starvation. This form of regulation may represent a general form of metabolic cross talk between amino acid and fatty acid catabolism.

*Biochemical Characterization of Protein Palmitoyltransferase Activities*. Protein acyl transferase (PAT) activity has been detected in membrane fractions derived from a variety of cell types, with the first reports dating back to the mid 1980s (*30*, *31*). Additional reports of PAT activity in membrane preparations enriched in ER, Golgi, or the plasma membrane have appeared (*32*−*38*). This could be interpreted as follows: one ubiquitously expressed enzyme or multiple PATs, each localized to distinct subcellular compartments. Given the diverse nature of palmitoylated proteins, it would not be surprising if there were multiple protein acyltransferases.

Identification of *bona fide* protein acyltransferases has been hampered on two fronts. First, PAT activity is displayed by enzymes that have a primary role in lipid metabolism. A Ras PAT activity purified to apparent homogeneity was subsequently identified as thiolase A, a peroxisomal enzyme that catalyzes the final step of fatty acid $\beta$-oxidation (*35*, *36*). The subcellular distribution of thiolase makes it an unlikely candidate for Ras PAT. A second example is the p260−p270 heterodimeric complex from *Bombyx mori* (p260−p270). The sequences of both p260 and p270 are significantly homologous to that of rat fatty acid synthase. Purified p260−p270 is a soluble enzyme that transfers palmitate to C-terminal Ras peptides but does not require prenylation. Given that expression of p260−p270 is restricted to early stages of embryogenesis, it seems unlikely to be a Ras PAT. The facile transfer of activated fatty acids from acyl-CoA donors to protein thiols by these and potentially other enzymes involved in acyl-CoA metabolism documents the promiscuity of protein acyltransferase reactions *in vitro*. The second issue that confounds molecular characterization of PAT activities is their inherent instability. A number of studies have found PAT activity that requires detergent (Triton X-100) for solubilization (*32*). Only limited purification of this activity has been achieved due to the rapid loss of PAT activity during chromatographic separation, precluding the molecular identification of the active species (*39*).

*Discovery of Yeast S-Palmitoyltransferases Containing DHHC-Cysteine Rich Domains*. Conservation of protein palmitoylation in genetic model systems such as *Saccharomyces cerevisiae* and *Drosophila* provided an opportunity to use a genetic approach to identify and characterize PATs. In yeast, the Ras oncogene homologues, Ras1p and Ras2p, undergo reversible palmitoylation on a Cys residue adjacent to the canonical C*aaX* box prenylation motif at the C-terminus of the protein (*40*, *41*). Mutation of the palmitoylated Cys to Ser abolishes palmitoylation and results in a mislocalization of Ras2p from the plasma membrane to endomembranes. This corresponds to a partial attenuation of the Ras-dependent glucose stimulation of intracellular cAMP levels (*42*). Using a genetic screen based on palmitoylation-dependent RAS2 alleles, mutations in two genes, *ERF2* and *ERF4*/*SHR5*, were isolated. Deletion of *ERF2* and/or *ERF4*/*SHR5* causes a decrease in the level of Ras palmitoylation and partial mislocalization of Ras2p from the PM to endomembrane compartments (*43*, *44*). *ERF2* encodes a 42 kDa protein with four predicted membrane-spanning domains and a cysteine rich domain (CRD) containing a conserved DHHC (Asp-His-His-Cys) motif. Orthologs of *ERF2* can be found in the genomes of every eukaryote examined to date. Figure 1 shows an alignment of repre-

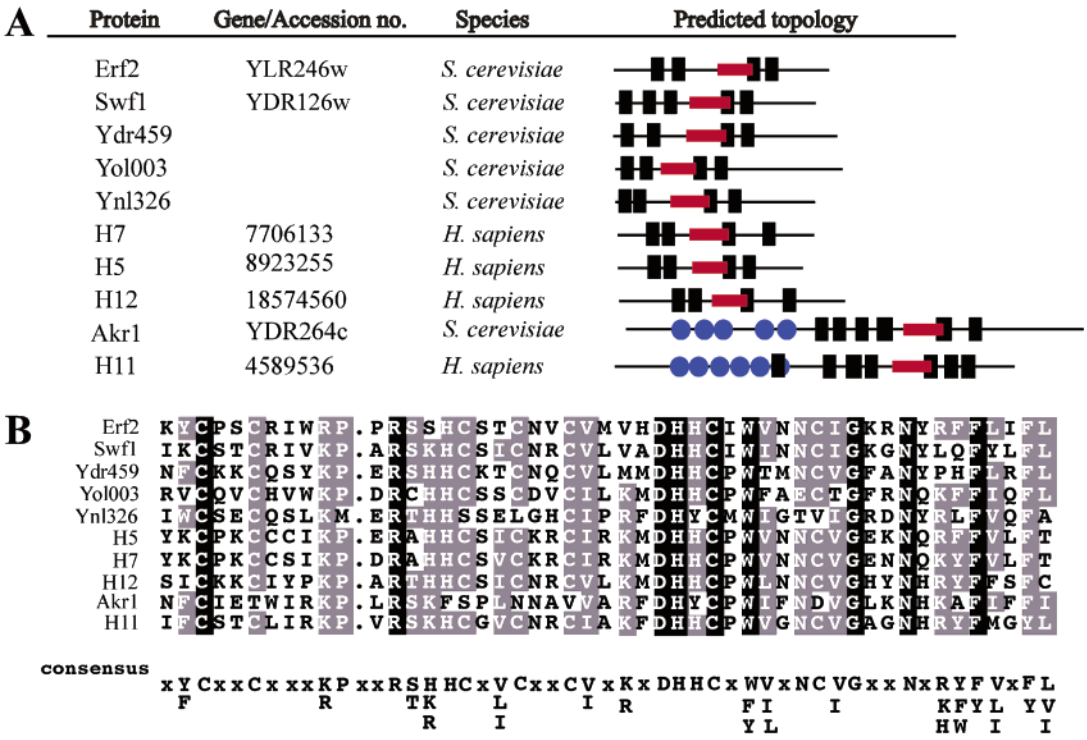


FIGURE 1: Representative examples of DHHC-CRD proteins from *S. cerevisiae* and *Homo sapiens*. (A) The predicted domain structure is shown for transmembrane domains (black boxes), DHHC-CRD (red boxes), and ankyrin repeats (blue circles). (B) Sequence alignments of selected DHHC-CRD motifs.

sentative members of the DHHC-CRD family from yeast, flies, worms, plants, and humans. Note that the size and predicted topology of the proteins are also conserved, despite the relatively low level of sequence conservation outside of the DHHC-CRD domain. Erf4p is a 26 kDa protein with no recognizable motifs or apparent homologues except in closely related yeast with examples of more distant homologues in *Aspergillus fumagata* and *Neurospora crassa* (*43*, *45*). Erf2p and Erf4p associate in a complex on the ER membrane.

*In vivo* studies suggested that both Erf2p and Erf4p are involved in the palmitoylation of Ras2p (*44*). To determine if this effect was direct or indirect, the Erf2p−Erf4p complex (Erf2p−Erf4p) was isolated and incubated with farnesylated Ras2p and [$^3$H]palmitoyl-CoA. Erf2p−Erf4p-dependent palmitoyl transfer was observed with thioester bond formation occurring on the Cys residue modified *in vivo* (*15*). The Erf2p−Erf4p-dependent PAT activity was sensitive to heat as expected of a protein-mediated rather than a nonenzymatic event. Two lines of evidence link the DHHC-CRD domain to the PAT activity. First, mutations in the DHHC motif abolished PAT activity. Second, a palmitoylated intermediate of Erf2p is formed when the enzyme is incubated with palmitoyl-CoA in the absence of Ras substrate. This intermediate is not observed when the cysteine of the DHHC motif is mutated to alanine. Substrate selectivity was also observed. The yeast Erf2p−Erf4p Ras PAT worked best with yeast Ras2 protein and less well with mammalian myristoylated $G_{i\alpha}$ subunits or mammalian Ha-Ras. In addition, long chain acyl-CoA substrates (16 and 18 carbons) are preferred over shorter acyl chains (<14 carbons).

A second yeast DHHC-CRD protein, *AKR1*, was identified by genetic screens and by two-hybrid experiments to be involved in the pheromone response pathway (*46*−*48*). Akr1p and Erf2p both contain cysteine rich domains and predicted transmembrane domains; however, in Akr1p, the DHHC motif is replaced with a DHYC motif, and Akr1p also has ankyrin repeats that are not present in Erf2p. Strains lacking Akr1p exhibit reduced rates of pheromone receptor internalization (*47*). A very similar phenotype has been observed in mutants of the casein kinase genes, *YCK1* and *YCK2* (*49*). This led Davis and his colleagues to suggest that Yck1p and Yck2p may undergo Akr1p-dependent lipid modification. Yck2p terminates in a dicysteine motif which is generally assumed to undergo prenylation by a type II geranylgeranyl transferase system. Consistent with this, mutation of the CC pair to a SS pair abolishes the membrane localization of Yck2p. However, prenylation of the Yck2p dicysteine has never been directly demonstrated.

The molecular basis for Akr1p function in Yck2p subcellular localization was demonstrated by Davis and his colleagues, who showed that Akr1p is a palmitoyltransferase for Yck2p (*16*). Akr1p was purified from yeast to apparent homogeneity and shown to catalyze the transfer of palmitate from palmitoyl-CoA to a C-terminal Cys residue on Yck2p. Interestingly, an Akr1p-palmitoyl intermediate was also detected in the Akr1p-dependent PAT reactions. Both intermediate formation and acyl transfer to Yck2p were stimulated by ATP. Interestingly, palmitoylation appears to be the only lipid modification that occurs on Yck2p. This implies that Akr1p and the Erf2p−Erf4p complex recognize distinct substrates. The Erf2p−Erf4p complex palmitoylates cysteine adjacent to a farnesylated cysteine, whereas Akr1p appears to be capable of palmitoylating cysteine in the absence of other modifications. Both classes of palmitoyltransferases might be expected from the diverse examples of palmitoylated proteins summarized in Table 1. The yeast

(*S. cerevisiae*) genome harbors seven DHH(Y)C-CRD genes [*ERF2*, *YDR126w* (*SWF1*/*PSL10*), *YDR459c*, *YOL003c*, *YNL326c*, *AKR1*, and *AKR2*]. To date, only Erf2p and Akr1p have been shown to have palmitoyltransferase activity. The family of DHHC-CRD proteins is quite large. Mouse and human genomes appear to have approximately 23 DHHC-CRD genes, and it is clearly too early to assume that all DHHC-CRD proteins are *S*-acyl transferases; however, on the basis of the work on Erf2p and Akr1p, the possibility certainly warrants investigation.

*Mammalian DHHC-CRD Proteins*. DHHC-CRD proteins have been uncovered in several yeast two-hybrid screens. In one case, a Golgi-localized mouse DHHC-CRD protein, GODZ, was found to interact with the carboxyl-terminal tail of the GluRα1 receptor (*50*). Mouse GODZ is 97% identical to a human DHHC-CRD protein (AF441791). Expression of GODZ in COS-7 cells resulted in an increased level of Golgi localization of the GluRα1 receptor. In a second study, cAbl was found to associate with Abl-philin 2 (Aph2), which has a DHHC-CRD domain (*51*). Aph2 is localized in the ER, and overexpression of Aph2 results in the induction of apoptosis in COS-7 and NIH3T3 cells. The pro-apoptotic activity requires the DHHC-CRD region of the protein, but it remains to be determined whether palmitoylation is involved. Finally, a mammalian homologue of yeast Akr1p, *HIP14*, was found in a two-hybrid assay to interact with huntingtin (htt) (*52*). Interestingly, the interaction strength is inversely correlated with the poly(Q) length in htt, suggesting that a decreased level of interaction between HIP14 and htt could be involved in the neuronal dysfunction in Huntington disease.

Perhaps the most interesting connection between a DHHC-CRD protein and human disease comes from a study of a locus associated with susceptibility to schizophrenia (*53*). Candidate genes within this locus include *KIAA1292* [GI17484904 (ZDHHC8)], which encodes a DHHC-CRD protein that is significantly homologous to yeast Erf2p. Further work is necessary to determine if the linkage to ZDCCH8 is causally associated with schizophrenia.

*Discovery of N-Palmitoyltransferases in Drosophila*. The hedgehog signaling pathway presents a new twist on palmitoylation. In *Drosophila*, the 45 kDa precursor of the extracellular ligand hedgehog (Hh) is autoproteolytically processed to generate a 20 kDa amino-terminal and a 25 kDa carboxyl-terminal fragment. The cleavage reaction proceeds through a thioester intermediate which is cleaved *in vivo* by cholesterol to form an amino-terminal Hh product with a carboxyl-terminal attached cholesterol (*54*). Hedgehog proteins are also palmitoylated. This was first demonstrated for the human homologue of Hh, sonic hedgehog (Shh). Shh is palmitoylated at Cys-24 in a reaction that requires both a free thiol and a free $NH_2$ group. It has been suggested that palmitoylation of Cys-24 involves thioesterification followed by transfer to the $NH_2$ terminus through a cyclic intermediate (*55*).

Recently, three reports implicate a putative transmembrane acyltransferase in the palmitoylation of Hh (*12*−*14*). The corresponding gene has been called skinny hedgehog (ski), sightless (sit), or rasp by the three groups. The involvement of ski in hedgehog signaling was originally suggested by mutant phenotypes (*12*). Mutations in skinny hedgehog (ski) resembled the phenotype of loss of hedgehog or wingless signaling. Hedgehog signaling is compromised in the ski mutant, but the hedgehog message and protein were unaffected. This suggested that ski might regulate hedgehog through a posttranscriptional mechanism. The ski transcript encodes a 500-amino acid protein with a segment that is short but significantly homologous to membrane-associated *O*-acyltransferases. Analysis of the hedgehog protein isolated from wild-type and ski mutant flies revealed a decrease in the extent of palmitoylation in the mutant consistent with, but not directly demonstrating, that ski encodes the hedgehog palmitoyltransferase (*12*). Among the questions to be addressed is how the N-linked palmitoylation of Hh occurs. It has been suggested that it may involve the formation of an S-acylated intermediate. It should therefore not be a surprise to see no apparent homology between ski and the DHHC-CRD proteins.

*N-Palmitoylation of $G_{s\alpha}$*. Another variation of N-palmitoylation is found in the G-protein α subunit, $G_{s\alpha}$. The N-terminal sequence of $G_{s\alpha}$ is M-G-C. Most proteins harboring this sequence motif undergo cleavage of the initiator methionine and N-myristoylation of the exposed Gly residue and, finally, are S-palmitoylated at Cys-3. $G_{s\alpha}$ is an exception in that it is palmitoylated through a thioester linkage at Cys-3, but is not N-myristoylated at Gly-2. However, there was evidence that an additional hydrophobic modification was present at or near the amino terminus (*56*). This modification has recently been identified as amidation of the N-terminal glycine residue with palmitate (*57*). How the amide-linked palmitate is added is unknown. One possibility is that $G_{s\alpha}$ is first acylated at Cys-3 and the palmitate is transferred to the amino group of Gly-2 through a cyclic intermediate as is postulated for hedgehog. A second possibility is that NMT2, a second mammalian *N*-myristoyltransferase, can transfer palmitate to proteins. NMT1 has strict acyl-CoA substrate selectivity and cannot transfer palmitate (*5*). However, the acyl-CoA specificity of NMT2 has not been characterized and thus is a candidate for the N-terminal modification of $G_{s\alpha}$.

## *Palmitoylthioesterases*

The finding that the rate of palmitate turnover exceeds that of the protein itself for many palmitoylated substrates predicts that protein palmitoyl thioesterases are present in eukaryotic cells. Two such enzymes have been identified and extensively characterized, a lysosomal hydrolase, protein palmitoylthioesterase I (PPT 1), and the cytoplasmic enzyme acylprotein thioesterase 1 (APT1). The lysosomal localization of PPT1 argues for a role in the catabolism of palmitoylated proteins, whereas the presence of APT1 in the cytoplasm anticipates its function in mediating palmitate turnover on cytoplasmically exposed palmitoylated substrates. However, it is not clear whether APT1 represents the only palmitoylthioesterase for palmitoylated proteins. This issue will be revisited after the structure, function, and molecular genetics of PPT1 and APT1 have been described.

*Identification and Cloning of PPT1, a Lysosomal Hydrolase*. Camp and Hofmann purified PPT1 33000-fold from a soluble extract of bovine brain using an assay that monitored depalmitoylation of radiolabeled H-Ras and G-protein α subunits (*58*). Cloning of the cDNA and additional analysis of the purified enzyme yielded the unexpected finding that

PPT1 is synthesized with a cleavable signal sequence and is modified with N-linked oligosaccharides (*59*). This placed PPT1 in the lumen of intracellular organelles during its biogenesis and suggested that its targets were also localized there or were extracellular. Subsequent analysis revealed that PPT1 is a classical lysosomal hydrolase that is targeted to that organelle by modification of its oligosaccharides with mannose 6-phosphate (*60*−*62*). Lysosomal PPT1 contributes to the degradation of palmitoylated proteins by deacylating cysteine thioesters.

A related enzyme, PPT2, whose amino acid sequence is 28% identical to that of PPT1, was identified in a database search for homologues of PPT1 (*63*). PPT2 is also a lysosomal enzyme with a substrate profile that overlaps with that of PPT1. Both enzymes hydrolyze acyl-CoA, with a preference for acyl chains of 14−18 carbons. Consistent with its role in the catabolism of palmitoylated proteins, PPT1 deacylates cysteine thioesters in a variety of contexts, including intact proteins (palmitoylated H-Ras, $G_\alpha$, and albumin), palmitoylated peptides, and palmitoyl-cysteine. PPT2 is inactive with the same panel of substrates, suggesting that it has a role in degrading other types of lipid thioesters.

*Molecular Genetics of PPT1 and PPT2 Deficiencies*. Interest in PPT1 was heightened by the discovery that defects in the enzyme were responsible for a severe neurodegenerative disorder, infantile neuronal ceroid lipofuscinosis (INCL) (*64*). There is selective loss of cortical neurons in patients with INCL, with the brainstem and spinal cord remaining intact (reviewed in ref *65*). This has devastating consequences clinically in that patients may persist for 5−8 years in a vegetative state with death occurring at 8−11 years of age. In neuronal ceroid lipofuscinosis, there is an accumulation of autofluorescent material in all tissues, including brain. The discovery that PPT1 was a lysosomal enzyme allowed INCL to be classified as a lysosomal storage disorder (*60*, *62*).

The function of PPT1 in the degradation of palmitoylated proteins is underscored in a cell culture model utilizing fibroblasts and lymphoblasts from patients with INCL (*66*). Lipidated thioesters derived from acylated proteins accumulate in cells from patients with INCL, but not from normal controls. The accumulation of these species is dependent on protein synthesis, suggesting that the lipidated thioesters are derived from thioacylated proteins. PPT1 corrects this phenotype. When delivered into cells through uptake by the mannose 6-phosphate receptor, recombinant PPT1 reverses the accumulation of lipid thioesters in INCL lymphoblasts. Furthermore, PPT1 can hydrolyze the lipidated thioesters *in vitro*. Whereas PPT1 can correct the metabolic defect in INCL lymphoblasts, PPT2 cannot (*63*), reinforcing the idea that PPT1 and PPT2 have distinct substrate specificities.

Recently, a mouse model for INCL was created by ablation of the PPT1 gene (*67*). The mice are healthy at birth and fertile. However, 100% of the mice develop neurological abnormalities by 8 months and exhibit decreased survival. The neurological phenotypes include an abnormal clasping behavior and seizures and are undoubtedly related to the loss of neurons due to apoptosis. Like those of patients with PPT1 deficiency, the brains of these animals exhibit an accumulation of autofluorescent material. The spectrum of phenotypes displayed by PPT1-deficient mice makes it an excellent model for the human disease with potential for testing therapies.

*Structure of PPT1*. The crystal structure of bovine PPT1 has been determined for three different forms of the enzyme: with and without bound palmitate (*68*) and after inactivation with the inhibitor hexadecylsulfonylfluoride (HDSF) (*69*). The enzyme adopts an $\alpha/\beta$ hydrolase fold with a catalytic triad composed of Ser-115, His-289, and Asp-233 (*68*). $\alpha/\beta$ hydrolase fold proteins comprise a diverse group of hydrolytic enzymes (reviewed in ref *70*). The canonical $\alpha/\beta$ fold is comprised of eight $\beta$-strands ($\beta1-\beta8$) and six $\alpha$-helices ($\alpha A-\alpha F$) and is extremely versatile, accommodating large insertions into a single-domain protein. PPT1 contains several features of the canonical fold, including a central six-stranded parallel $\beta$-sheet that corresponds to $\beta3-\beta8$ in the canonical fold, as well as the interspersed $\alpha$-helices, $\alpha A-\alpha C$ and $\alpha F$ (*70*). A defining characteristic of $\alpha/\beta$ hydrolase proteins is the nucleophilic elbow, a tight turn after $\beta5$ that harbors the catalytic serine in PPT1.

The structure of the acyl−enzyme complex was obtained by crystallization of PPT1 with palmitoyl-CoA (*68*). This structure reveals the hydrophobic groove that accommodates the fatty acid chain and confirms that Ser-115 is the nucleophile. PPT1 is unusual among serine lipases in being resistant to the serine-modifying agents phenylmethanesulfonyl fluoride (PMSF) and diisopropylfluorophosphate (DFP) (*58*). However, a substrate analogue of PMSF, HDSF, is an inhibitor (*69*). The 2.4 Å structure of the inactive form of PPT1 reveals that HDSF modifies serine 115 and occupies the palmitate-binding pocket. HDSF is able to navigate a narrow hydrophobic groove that would not accommodate the bulkier aromatic ring of PMSF. This study provides a structural basis for the resistance of PPT1 to commonly used serine-modifying reagents (*69*).

The crystal structure provides a context for understanding how mutations in PPT1 cause INCL (*68*). More than 36 mutations in the PPT1 gene have been identified (*71*), but the R122W missense mutation is responsible for most of the cases (*64*). The R122W mutation and others associated with the most severe disease are predicted to distort the active site or palmitate binding pocket or disrupt folding of the PPT1 core. In contrast, the T75P and D79G mutations that are associated with the less severe juvenile NCL are removed from the enzyme core. Consistent with a local perturbation of enzyme structure, the kinetic parameters of recombinant enzymes harboring the T75P or D79G mutation are not dramatically different from those of the wild-type enzyme (*71*). Lymphoblasts from patients harboring T75P and D79G missense mutations exhibit low, but measurable, enzyme activity (∼2% of that of the wild-type enzyme) in contrast to other missense alleles that are catalytically inactive. All PPT1 missense mutant proteins are associated with severely reduced levels of immunoreactive protein and accumulate early in the secretory pathway. These are hallmarks of misfolded proteins that are substrates for ER-associated degradation.

*Identification and Characterization of APT1, a Cytoplasmic Acylprotein Thioesterase*. Duncan and Gilman identified a palmitoyl protein thioesterase activity in rat liver cytosol and purified the enzyme 18000-fold (*72*). This activity was distinct from PPT1 and was named acylprotein thioesterase 1 (APT1). Peptide sequences derived from purified APT1

matched those found in a lysophospholipase that had been described previously (*73*−*75*). Subsequent analysis of the native enzyme showed that APT1 cleaves thioesters in acyl-CoAs and acylproteins, as well as oxyesters in lysolipids (*72*). These results were confirmed with recombinant enzyme purified from *Escherichia coli*, demonstrating that the ability to cleave both oxyesters and thioesters resides in the same polypeptide. Biochemical and genetic analyses point to a physiological role for the protein as an acylprotein thioesterase (see below). APT1 orthologs have been identified in a large number of species, ranging from *S. cerevisiae* to humans.

*Biochemical Properties of APT1*. Native and recombinant APT1 from rat and recombinant APT1 from yeast have been purified to apparent homogeneity and extensively studied (*72*, *76*). Despite only 33% sequence similarity, rat and yeast APT1 have remarkably similar chromatographic properties. The yeast and rat enzymes behave as monomers in solution, with an apparent molecular mass of 29 kDa (predicted molecular mass of 24.7 kDa). Kinetic analysis of the rat and yeast enzymes using lysophosphatidylcholine, palmitoyl-CoA, and palmitoyl-$G_{i\alpha1}$ as substrates reveals a marked preference for palmitoyl-$G_{i\alpha1}$. For the rat enzyme, a $K_M$ of 1.1 mM was measured for lysophosphocholine and palmitoyl-CoA, compared to a $K_M$ of 12 $\mu$M for palmitoyl-$G_{i\alpha1}$. Similar results were observed with the yeast enzyme. The pronounced substrate preference for acylproteins over lipid substrates is consistent with a role for APT1 as a regulator of protein thioacylation and not as a regulator of lipid metabolism.

Rat and yeast APT1 exhibit different catalytic efficiencies toward palmitoyl-protein substrates (*76*). Proteins containing three different palmitoylation motifs were studied: $G_{i\alpha1}$, which is myristoylated at its amino terminus and palmitoylated at an adjacent cysteine (*77*), RGS4, which is palmitoylated at two cysteine residues near its amino terminus (C2 and C12) (*78*) and a cysteine residue in the RGS core domain (C95) (*79*), and H-Ras, which is palmitoylated at two cysteine residues immediately upstream of its farnesylated and carboxylmethylated C-terminus (*80*). The relative catalytic efficiencies were ordered $G_{i\alpha1}$ > RGS4 > H-Ras for rat APT1, with only a 10-fold difference between the best and worst substrates (*76*). In contrast, the yeast enzyme demonstrated a marked preference for palmitoyl-$G_{i\alpha1}$ over RGS4 and H-Ras, with 35- and 70-fold differences, respectively. The substrates used in this study were of mammalian origin. It will be interesting to determine if yeast APT1 exhibits the same substrate preference when tested with yeast substrates $G_\alpha$ (Gpa1p) and Ras1p and Ras2p.

*Structure of the Apoenzyme.* As noted above, APT1 was originally described independently by two groups as a lysophospholipase activity (lysophospholipase I or Lyso PLA I) (*73*, *75*). Structural modeling and mutational analysis lead to the prediction that APT1/Lyso PLA I is a member of the serine hydrolase, $\alpha/\beta$ fold family with a catalytic triad of Ser-119, Asp-174, and His-208 (*73*−*75*). With the recently reported structure of human APT1 (*81*), these predictions have been validated. APT1 shares most features of the canonical $\alpha/\beta$ hydrolase fold. However, it lacks the $\beta$1 strand and contains a large insertion following the $\beta$4 strand of the parallel $\beta$-sheet. The $\alpha$D helix is replaced by a short helical segment. Ser-114[3] is located at the apex of the nucleophilic elbow, and Asp-169 and His-203 are positioned in the structure to participate in the charge relay mechanism typical for chymotrypsin-like serine hydrolases.

Both APT1 and PPT1 are soluble enzymes with lipophilic substrates. This raises the important question of whether they access substrate directly from the membrane (interfacial enzyme) or from the aqueous phase (noninterfacial enzyme) (reviewed in ref *82*). Certain lipases that undergo interfacial activation have a moveable lid that covers the active site of the enzyme (*83*). The structures of PPT1 in the palmitate-bound and -free states are very similar and do not provide any evidence for a surface loop covering the active site, nor is there any kinetic evidence for interfacial activation of PPT1 (*68*). However, this issue has not been addressed for APT1. Interestingly, APT1 appears as a homodimer in the crystal structure of the apoenzyme, with the active site of APT1 occluded by the dimer interface. This unexpected finding led the authors to propose that dimeric APT1 must dissociate upon substrate binding (*81*). This hypothesis remains to be tested but raises the interesting possibility that the exposed dimer interface could bind to the membrane surface to access substrate.

*APT1 Regulation of Palmitate Turnover.* Support for the role of APT1 as a physiological regulator of the G-protein palmitoylation cycle comes from studies of APT1 expression in mammalian cells and in yeast in which the *APT1* gene has been deleted. In mammalian cells, the basal rate of turnover of the thioester-linked fatty acid on $G_{s\alpha}$ occurs with a $t_{1/2}$ of 20−90 min (*84*−*86*). Palmitate turnover is accelerated more than 10-fold when $G_{s\alpha}$ is activated by an agonist-occupied receptor or when locked in the GTP-bound form by mutation or cholera toxin-mediated ADP ribosylation. In stable cell lines expressing APT1, the rate of palmitate removal from $G_{s\alpha}$ is faster than that of control lines, suggesting that G-protein $\alpha$ subunits are substrates for APT1 *in vivo* as well as *in vitro* (*72*). Although APT1 has acyl-CoA hydrolase activity, its expression did not significantly increase acyl-CoA hydrolase activity in cell extracts, nor did it perturb incorporation of [$^3$H]palmitate into cellular lipids in cells overexpressing APT1. Thus, the effects of APT1 on palmitate turnover on $G_{s\alpha}$ are not due to effects on the rate of turnover of palmitoyl-CoA. Cell lines expressing high levels of APT1 were not obtained. It is not surprising that overproduction of an enzyme with lysophospholipase activity might be toxic to cells, given the detergent-like properties of lysolipids.

The characteristics of the palmitoylation cycle of $G_{s\alpha}$ in intact cells suggest that $G_{s\alpha}$ is palmitoylated when bound to $G_{\beta\gamma}$ and becomes a substrate for a thioesterase upon activation and dissociation from $G_{\beta\gamma}$. This model is supported by biochemical reconstitution of purified APT1 and the $G_s$ heterotrimer (*72*). Free $G_{s\alpha}$ is depalmitoylated at a faster rate than that bound to $G_{\beta\gamma}$. APT1 is indifferent to the activation state of free $G_{s\alpha}$ because GDP-bound $G_{s\alpha}$ is deacylated at the same rate as the GTP$\gamma$S-bound form. Thus, subunit dissociation is associated with the enhanced rate of depalmitoylation and not the activated conformation of $G_{s\alpha}$. The cycle of subunit association and dissociation appears to account for the regulation of thioacylation of $G_{s\alpha}$ *in vivo*.

[3] The numbering of residues in the human APT1 protein begins at the second methionine in GenBank entry C31610.

In yeast, deletion of the APT1 gene impacts palmitoylprotein metabolism, but has no obvious effect on lipid metabolism. Cell extracts derived from *apt1* null cells exhibit no reduction in lysophospholipase activity *in vitro*. Acyl-CoA metabolism as assessed by incorporation of radiolabeled fatty acids into cellular lipid pools appears to be normal. However, *apt1* null cells do exhibit perturbations in the palmitate cycle of Gpa1p, the $G_\alpha$ subunit of the pheromone response pathway. Gpa1p is most similar to mammalian $G_\alpha$ subunits of the $G_{i\alpha}$ family, sharing an amino-terminal N-myristoylation, S-palmitoylation motif. In metabolic radiolabeling studies of *apt1* null cells, palmitate turnover on Gpa1p is not detectable, whereas in wild-type cells, Gpa1p exhibits a measurable loss of radiolabeled palmitate. Cell extracts from *apt1* null cells exhibit almost no acylprotein thioesterase activity toward palmitoyl-$G_{i\alpha1}$. With the caveat that Gpa1p has not been tested as a substrate for yeast Apt1p *in vitro*, these studies strongly suggest that Apt1p is the enzyme that deacylates Gpa1p *in vivo*. However, deletion of *APT1* does not obviously perturb the pheromone response pathway as assessed by a transcriptional reporter for the pathway or in the cells' ability to adapt to pheromone. Whether *APT1* plays a modulatory role in the pheromone response pathway will require further study.

A second myristoylated, palmitoylated protein that appears to be a target of APT1 is endothelial nitric oxide synthase (eNOS) (*87*). This enzyme is an important regulator of vascular tone and platelet aggregation (*88*). Michel and coworkers showed eNOS was a substrate for APT1 *in vitro* and that turnover of [$^3$H]palmitate on eNOS was accelerated in COS-7 cells transfected with eNOS and APT1. Depalmitoylation of eNOS is potentiated by $Ca^{2+}$-CaM, an important allosteric activator of eNOS. Bovine aortic endothelial cells express native eNOS and APT1. Taken together, these findings suggest that APT1 may be responsible for the agonist-induced depalmitoylation of eNOS in bovine aortic endothelial cells (*87*).

An important question that remains to be addressed is the scope of APT1 function. To date, APT1 has only been tested with a limited range of palmitoylated substrates. It will be interesting to determine whether APT1 can deacylate integral membrane proteins such as G-protein-coupled receptors and how this impacts protein function. A recent study reports that viral glycoproteins are substrates for APT1 *in vitro* (*89*). Recombinant rat APT1 deacylates several viral glycoproteins, including the G-protein of vesicular stomatitis virus and hemagglutinin proteins of influenza A and C virus. Palmitate or stearate was effectively removed by APT1. Interestingly, the E1 protein of Semliki Forest virus was not a substrate for APT1, suggesting that there is specificity *in vitro*. Viral glycoproteins do not undergo palmitate turnover in cells. Thus, APT1 is not likely to be relevant physiologically to the biogenesis of enveloped viruses. Nonetheless, this study indicates the potential for APT1 as a regulator of cell surface receptors that are palmitoylated.

A related question is whether there are other acylprotein thioesterases that regulate palmitate turnover. Two observations suggest that there may be. First, a second lysophospholipase has been purified, cloned, and characterized with respect to its activity on various lipid substrates (*90*, *91*). Lysophospholipase II is 64% identical at the amino acid level to APTI/Lyso PLAI. It is obviously of interest to determine whether this enzyme also harbors acylprotein thioesterase activity. Second, there is low but measurable thioesterase activity in yeast extracts for palmitoyl H-Ras that is not diminished in extracts from *apt1*null cells (*76*). Thus, there may be additional acylprotein thioesterases in yeast that have not been recognized by the similarity of their sequence to those of the known enzymes. Identification of all of the players is required for elucidation of the mechanisms that underlie reversible palmitoylation.

*Perspective*

Recent results have led to a significant advance in our understanding of the mechanisms of palmitoylation and depalmitoylation. The identification of protein palmitoyl acyl transferases (PATs) and protein palmitoylthioesterases (PPTs) opens up numerous avenues of investigation. The utility of genetic manipulation of model organisms is aptly demonstrated by the discovery of DHHC-CRD PATs in yeast and skinny hedgehog in *Drosophila*. PPT1 and APT1 were discovered by traditional biochemical methods, but our understanding of these proteins was rapidly advanced by molecular genetic and structural analyses. This multidisciplinary approach must be extended to protein acyltransferases.

Investigation of the DHHC-CRD proteins is a high priority. It is important to remember that only the Erf2p−Erf4p complex and Akr1p have been shown to have PAT activity. Therefore, testing whether other DHHC-CRD proteins exhibit PAT activity is paramount, and if PAT activity is detected, *in vivo* substrates must be identified. Gene disruptions of other DHHC-CRD proteins in yeast and perhaps other fungal organisms can be performed to assess their functional significance and to identify potential overlap in specificity between putative PATs. Equally important will be the development of reliable enzyme assays. Among the challenges to overcome is the fact that the DHHC-CRD proteins identified to date are integral membrane proteins. In the case of yeast Ras PAT, Erf2p requires the presence of a second protein, Erf4p, whose role is currently not known. Akr1p appears to function without a partner protein. Whether other DHHC-CRD proteins might require additional subunits for PAT activity is an open question.

Determining the subcellular location of protein palmitoylation and depalmitoylation events may provide insight into the function of a PAT or PPT. The lysosomal localization of PPT1 implicates this thioesterase in protein degradation, whereas Apt1 is a soluble protein presumably working on cytosolic proteins, some of which may be involved in signaling. Yeast Ras PAT, Erf2p−Erf4p, is localized primarily in the ER and has been shown to required for the ER to plasma membrane localization of yeast Ras proteins (*44*, *45*). Akr1p is found on the Golgi and has been implicated in intracellular trafficking. Golgi localization has also been shown for the mammalian DHHC-CRD proteins GODZ and *HIP14*. Furthermore, HIP14 has been shown to complement the endocytosis defect of yeast lacking Akr1p. This underscores the role yeast may play in uncovering the function of PATs.

Deciphering the puzzle of protein palmitoylation will require complementary genetic and biochemical approaches. The recent success identifying protein *S*-acyltransferases, a

candidate protein *N*-palmitoyltransferase, and two classes of protein palmitoylthioesterases provides a solid foundation for future studies. The new insights have propelled the study of protein palmitoylation into a new phase.

## REFERENCES


1. Braun, P. E., and Radin, N. S. (1969) *Biochemistry 8*, 4310.
2. Stoffyn, P., and Folch-Pi, J. (1971) *Biochem. Biophys. Res. Commun. 44*, 157.
3. Gagnon, J., Finch, P. R., Wood, D. D., and Moscarello, M. A. (1971) *Biochemistry 10*, 4756.
4. Hackett, M., Guo, L., Shabanowitz, J., Hunt, D. F., and Hewlett, E. L. (1994) *Science 266*, 433.
5. Farazi, T. A., Waksman, G., and Gordon, J. I. (2001) *J. Biol. Chem. 276*, 39501.
6. Milligan, G., Parenti, M., and Magee, A. I. (1995) *Trends Biochem. Sci. 20*, 181.
7. Dunphy, J. T., and Linder, M. E. (1998) *Biochim. Biophys. Acta 1436*, 245.
8. Resh, M. D. (1999) *Biochim. Biophys. Acta 1451*, 1.
9. El-Husseini Ael, D., and Bredt, D. S. (2002) *Nat. Rev. Neurosci. 3*, 791.
10. Silvius, J. R. (2002) *J. Membr. Biol. 190*, 83.
11. Bijlmakers, M. J., and Marsh, M. (2003) *Trends Cell Biol. 13*, 32.
12. Chamoun, Z., Mann, R. K., Nellen, D., von Kessler, D. P., Bellotto, M., Beachy, P. A., and Basler, K. (2001) *Science 2*, 2.
13. Lee, J. D., and Treisman, J. E. (2001) *Curr. Biol. 11*, 1147.
14. Micchelli, C. A., The, I., Selva, E., Mogila, V., and Perrimon, N. (2002) *Development 129*, 843.
15. Lobo, S., Greentree, W. K., Linder, M. E., and Deschenes, R. J. (2002) *J. Biol. Chem. 277*, 41268.
16. Roth, A. F., Feng, Y., Chen, L., and Davis, N. G. (2002) *J. Cell Biol. 159*, 23.
17. Quesnel, S., and Silvius, J. R. (1994) *Biochemistry 33*, 13340.
18. Bharadwaj, M., and Bizzozero, O. A. (1995) *J. Neurochem. 65*, 1805.
19. O'Brien, P. J., St. Jules, R. S., Reddy, T. S., Bazan, N. G., and Zatz, M. (1987) *J. Biol. Chem. 262*, 5210.
20. Duncan, J. A., and Gilman, A. G. (1996) *J. Biol. Chem. 271*, 23594.
21. Mollner, S., Ferreira, P., Beck, K., and Pfeuffer, T. (1998) *Eur. J. Biochem. 257*, 236.
22. Bizzozero, O. A., Bixler, H. A., and Pastuszyn, A. (2001) *Biochim. Biophys. Acta 1545*, 278.
23. Gossett, R. E., Frolov, A. A., Roths, J. B., Behnke, W. D., Kier, A. B., and Schroeder, F. (1996) *Lipids 31*, 895.
24. Leventis, R., Juel, G., Knudsen, J. K., and Silvius, J. R. (1997) *Biochemistry 36*, 5546.
25. Berthiaume, L., Deichaite, I., Peseckis, S., and Resh, M. (1994) *J. Biol. Chem. 269*, 6498.
26. Moore, K. H., Dandurand, D. M., and Kiechle, F. L. (1992) *Int. J. Biochem. 24*, 809.
27. Morel, F., Lauquin, G., Lunardi, J., Duszynski, J., and Vignais, P. V. (1974) *FEBS Lett. 39*, 133.
28. Kawaguchi, A., and Bloch, K. (1976) *J. Biol. Chem. 251*, 1406.
29. Corvi, M., Soltys, C.-L. M., and Berthiaume, L. (2001) *J. Biol. Chem. 276*, 45704.
30. Berger, M., and Schmidt, M. F. (1984) *J. Biol. Chem. 259*, 7245.
31. Mack, D., Berger, M., Schmidt, M. F., and Kruppa, J. (1987) *J. Biol. Chem. 262*, 4297.
32. Berthiaume, L., and Resh, M. D. (1995) *J. Biol. Chem. 270*, 22399.
33. Dunphy, J. T., Greentree, W. K., Manahan, C. L., and Linder, M. E. (1996) *J. Biol. Chem. 271*, 7154.
34. Das, A. K., Dasgupta, B., Bhattacharya, R., and Basu, J. (1997) *J. Biol. Chem. 272*, 11021.
35. Liu, L., Dudler, T., and Gelb, M. H. (1996) *J. Biol. Chem. 271*, 23269.
36. Liu, L., Dudler, T., and Gelb, M. H. (1999) *J. Biol. Chem. 274*, 3252a.
37. Ueno, K., and Suzuki, Y. (1997) *J. Biol. Chem. 272*, 13519.
38. Gutierrez, L., and Magee, A. I. (1991) *Biochim. Biophys. Acta 1078*, 147.
39. Dunphy, J. T., Schroeder, H., Leventis, R., Greentree, W. K., Knudsen, J. K., Silvius, J. R., and Linder, M. E. (2000) *Biochim. Biophys. Acta 1485*, 185.
40. Fujiyama, A., and Tamanoi, F. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 1266.
41. Deschenes, R. J., and Broach, J. R. (1987) *Mol. Cell. Biol. 7*, 2344.
42. Bhattacharya, S., Chen, L., Broach, J. R., and Powers, S. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 2984.
43. Jung, V., Chen, L., Hofmann, S. L., Wigler, M., and Powers, S. (1995) *Mol. Cell. Biol. 15*, 1333.
44. Bartels, D. J., Mitchell, D. A., Dong, X., and Deschenes, R. J. (1999) *Mol. Cell. Biol. 19*, 6775.
45. Zhao, L., Lobo, S., Dong, X., Ault, A. D., and Deschenes, R. J. (2002) *J. Biol. Chem. 277*, 49352.
46. Pryciak, P. M., and Hartwell, L. H. (1996) *Mol. Cell. Biol. 16*, 2614.
47. Givan, S. A., and Sprague, G. F., Jr. (1997) *Mol. Biol. Cell 8*, 1317.
48. Kao, L. R., Peterson, J., Ji, R., Bender, L., and Bender, A. (1996) *Mol. Cell. Biol. 16*, 168.
49. Feng, Y., and Davis, N. G. (2000) *Mol. Cell. Biol. 20*, 5350.
50. Uemura, T., Mori, H., and Mishina, M. (2002) *Biochem. Biophys. Res. Commun. 296*, 492.
51. Li, B., Cong, F., Tan, C. P., Wang, S. X., and Goff, S. P. (2002) *J. Biol. Chem. 277*, 28870.
52. Singaraja, R. R., Hadano, S., Metzler, M., Givan, S., Wellington, C. L., Warby, S., Yanai, A., Gutekunst, C. A., Leavitt, B. R., Yi, H., Fichter, K., Gan, L., McCutcheon, K., Chopra, V., Michel, J., Hersch, S. M., Ikeda, J. E., and Hayden, M. R. (2002) *Hum. Mol. Genet. 11*, 2815.
53. Liu, H., Abecasis, G. R., Heath, S. C., Knowles, A., Demars, S., Chen, Y. J., Roos, J. L., Rapoport, J. L., Gogos, J. A., and Karayiorgou, M. (2002) *Proc. Natl. Acad. Sci. U.S.A. 99*, 16859.
54. Porter, J. A., Young, K. E., and Beachy, P. A. (1996) *Science 274*, 255.
55. Pepinsky, R. B., Zeng, C., Wen, D., Rayhorn, P., Baker, D. P., Williams, K. P., Bixler, S. A., Ambrose, C. M., Garber, E. A., Miatkowski, K., Taylor, F. R., Wang, E. A., and Galdes, A. (1998) *J. Biol. Chem. 273*, 14037.
56. Kleuss, C., and Gilman, A. G. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 6116.
57. Kleuss, C., and Krause, E. (2003) *EMBO J. 22*, 826.
58. Camp, L. A., and Hofmann, S. L. (1993) *J. Biol. Chem. 268*, 22566.
59. Camp, L. A., Verkruyse, L. A., Afendis, S. J., Slaughter, C. A., and Hofmann, S. L. (1994) *J. Biol. Chem. 269*, 23212.
60. Hellsten, E., Vesa, J., Olkkonen, V. M., Jalanko, A., and Peltonen, L. (1996) *EMBO J. 15*, 5240.
61. Sleat, D. E., Sohar, I., Lackland, H., Majercak, J., and Lobel, P. (1996) *J. Biol. Chem. 271*, 19191.
62. Verkruyse, L., and Hofmann, S. (1996) *J. Biol. Chem. 271*, 15831.
63. Soyombo, A. A., and Hofmann, S. L. (1997) *J. Biol. Chem. 272*, 27456.
64. Vesa, J., Hellsten, E., Verkruyse, L., Camp, L., Rapola, J., Santavuori, P., Hofmann, S., and Peltonen, L. (1995) *Nature 376*, 584.
65. Hofmann, S. L., Lee, L. A., Lu, J. Y., and Verkruyse, L. A. (1997) *Neuropediatrics 28*, 27.
66. Lu, J. Y., Verkruyse, L. A., and Hofmann, S. L. (1996) *Proc. Natl. Acad. Sci. U.S.A. 93*, 10046.
67. Gupta, P., Soyombo, A. A., Atashband, A., Wisniewski, K. E., Shelton, J. M., Richardson, J. A., Hammer, R. E., and Hofmann, S. L. (2001) *Proc. Natl. Acad. Sci. U.S.A. 98*, 13566.
68. Bellizzi, J. J., 3rd, Widom, J., Kemp, C., Lu, J. Y., Das, A. K., Hofmann, S. L., and Clardy, J. (2000) *Proc. Natl. Acad. Sci. U.S.A. 97*, 4573.
69. Das, A. K., Bellizzi, J. J., 3rd, Tandel, S., Biehl, E., Clardy, J., and Hofmann, S. L. (2000) *J. Biol. Chem. 275*, 23847.
70. Heikinheimo, P., Goldman, A., Jeffries, C., and Ollis, D. L. (1999) *Struct. Folding Des. 7*, R141.
71. Das, A. K., Lu, J. Y., and Hofmann, S. L. (2001) *Hum. Mol. Genet. 10*, 1431.
72. Duncan, J. A., and Gilman, A. G. (1998) *J. Biol. Chem. 273*, 15830.
73. Sugimoto, H., Hayashi, H., and Yamashita, S. (1996) *J. Biol. Chem. 271*, 7705.
74. Wang, A., Loo, R., Chen, Z., and Dennis, E. A. (1997) *J. Biol. Chem. 272*, 22030.
75. Wang, A., Deems, R. A., and Dennis, E. A. (1997) *J. Biol. Chem. 272*, 12723.
76. Duncan, J. A., and Gilman, A. G. (2002) *J. Biol. Chem. 277*, 31740.

77. Degtyarev, M. Y., Spiegel, A. M., and Jones, T. L. (1994) *J. Biol. Chem. 269*, 30898.
78. Srinivasa, S. P., Bernstein, L. S., Blumer, K. J., and Linder, M. E. (1998) *Proc. Natl. Acad. Sci. U.S.A. 95*, 5584.
79. Tu, Y., Popov, S., Slaughter, C., and Ross, E. M. (1999) *J. Biol. Chem. 274*, 38260.
80. Hancock, J. F., Magee, A. I., Childs, J. E., and Marshall, C. J. (1989) *Cell 57*, 1167.
81. Devedjiev, Y., Dauter, Z., Kuznetsov, S. R., Jones, T. L., and Derewenda, Z. S. (2000) *Struct. Folding Des. 8*, 1137.
82. Gelb, M. H., Min, J. H., and Jain, M. K. (2000) *Biochim. Biophys. Acta 1488*, 20.
83. Derewenda, Z. S., and Sharp, A. M. (1993) *Trends Biochem. Sci. 18*, 20.
84. Degtyarev, M. Y., Spiegel, A. M., and Jones, T. L. (1993) *J. Biol. Chem. 268*, 23769.
85. Mumby, S. M., Kleuss, C., and Gilman, A. G. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 2800.
86. Wedegaertner, P. B., and Bourne, H. R. (1994) *Cell 77*, 1063.
87. Yeh, D. C., Duncan, J. A., Yamashita, S., and Michel, T. (1999) *J. Biol. Chem. 274*, 33148.
88. Feron, O. (1999) *Curr. Opin. Clin. Nutr. Metab. Care 2*, 291.
89. Veit, M., and Schmidt, M. F. (2001) *Virology 288*, 89.
90. Sunaga, H., Sugimoto, H., Nagamachi, Y., and Yamashita, S. (1995) *Biochem. J. 308* (Part 2), 551.
91. Toyoda, T., Sugimoto, H., and Yamashita, S. (1999) *Biochim. Biophys. Acta 1437*, 182.
92. Papac, D. I., Thornburg, K. R., Bullesbach, E. E., Crouch, R. K., and Knapp, D. R. (1992) *J. Biol. Chem. 267*, 16889.
93. O'Dowd, B. F., Hnatowich, M., Caron, M. G., Lefkowitz, R. J., and Bouvier, M. (1989) *J. Biol. Chem. 264*, 7564.
94. Yang, X., Claas, C., Kraeft, S.-K., Chen, L., Wang, Z., Kreidberg, J., and Hemler, M. (2002) *Mol. Biol. Cell 13*, 767.
95. Schlesinger, M. J., Veit, M., and Schmidt, M. F. G. (1993) in *Lipid Modifications of Proteins* (Schlesinger, M. J., Ed.) pp 2, CRC Press, Boca Raton, FL.
96. Alland, L., Peseckis, S. M., Atherton, R. E., Berthiaume, L., and Resh, M. D. (1994) *J. Biol. Chem. 269*, 16701.
97. Robinson, L. J., and Michel, T. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 11776.
98. Liu, Y., Fisher, D. A., and Storm, D. R. (1993) *Biochemistry 32*, 10714.
99. Topinka, J. R., and Bredt, D. S. (1998) *Neuron 20*, 125.
100. Wilson, P. T., and Bourne, H. R. (1995) *J. Biol. Chem. 270*, 9667.
101. Lane, S. R., and Liu, Y. (1997) *J. Neurochem. 69*, 1864.
102. Gundersen, C. B., Mastrogiacomo, A., Faull, K., and Umbach, J. A. (1994) *J. Biol. Chem. 269*, 19197.
103. DeVries, L., Elenko, E., Hubler, L., Jones, T. L. Z., and Farquhar, M. G. (1996) *Proc. Natl. Acad. Sci. U.S.A. 93*, 15203.

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