Identification of a Ras Palmitoyltransferase in Saccharomyces cerevisiae*

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Most Ras proteins are posttranslationally modified by a palmitoyl lipid moiety through a thioester linkage. However, the mechanism by which this occurs is not known. Here, evidence is presented that the Ras2 protein of Saccharomyces cerevisiae is palmitoylated by a Ras protein acyltransferase (Ras PAT) encoded by the ERF2 and ERF4 genes. Erf2p is a 41-kDa protein localized to the membrane of the endoplasmic reticulum and contains a conserved DHHC cysteine-rich domain (DHHC-CRD). Erf2p co-purifies with Erf4p (26 kDa) when it is expressed in yeast or in Escherichia coli. The Erf2p/Erf4p complex is required for Ras PAT activity, and mutations within conserved residues (Cys185, His201, and Cys203) of the Erf2p DHHC-CRD domain abolish Ras PAT activity. Furthermore, a palmitoyl-Erf2p intermediate is detected suggesting that Erf2p is directly involved in palmitate transfer. Erf2 and Erf4 are the first genes identified that encode a palmitoyltransferase for a Ras GTPase.

Dozens of cellular and viral proteins are posttranslationally modified with palmitate or other long-chain fatty acids through a reversible thioester linkage (1, 2). Examples are cell surface receptors, viral glycoproteins, and signal transducers including Ras, heterotrimeric G proteins, and nonreceptor tyrosine kinases. Palmitoylation is almost exclusively a property of membrane proteins and can occur on intracellular membranes early in the secretory pathway or at the plasma membrane. Although this modification was first described over 30 years ago, the molecular mechanism of palmitate addition has not been elucidated and has been a matter of controversy. A palmitoyltransferase activity assayed using mammalian H-Ras as a substrate was purified and identified as thiolase A, an enzyme required for fatty acid β-oxidation (3, 4). The localization of this enzyme in peroxisomes makes it an unlikely candidate for a physiological regulator of Ras palmitoylation. Palmitoyltransferase activities assayed using viral glycoproteins, the nonreceptor tyrosine kinase p59fyn, or G-protein heterotrimer as substrates have been detergent-solubilized, but the instability of the activity has hampered purification and molecular identification (5–7). A candidate palmitoyltransferase for Drosophila hedgehog was recently identified as skinny hedgehog/sightless (5, 6). Hedgehog is modified by cholesterol at the C terminus and palmitoylated through an atypical cysteine amide linkage at the N terminus. The failure to identify a palmitoyltransferase that acylates through a conventional thioester linkage, coupled with the observation that spontaneous and efficient transfer of fatty acid from acyl-CoA to proteins occurs in vitro, has lead to the suggestion that proteins autoacylate in vivo (7–9).

Plasma membrane localization of Ras requires farnesylation of the CaaX box cysteine via a thioether linkage, -aaX proteolysis, and carboxymethylation (see reviews in Refs. 10, 11). With the exception of K-Ras-4b, human Ras proteins are also palmitoylated on one or more neighboring cysteines via a thioester linkage. Palmitoylation is required for efficient plasma membrane localization and transforming activity of oncogenic forms of Ras (12). Previously, we described palmitoylation-dependent alleles of yeast RAS2 and a genetic screen designed to identify mutations that render cells inviable if Ras2p is not palmitoylated (13, 14). Mutations in two genes, ERF2 and ERF4/SHR5, were identified that resulted in diminished palmitoylation of Ras2p and mislocalization of GFP-Ras2p (14, 15). Erf2p is a 41-kDa integral membrane protein localized at the ER, which contains a conserved Asp-His-His-Cys cysteine-rich domain (DHHC-CRD) between residues 164–228. The DHHC-CRD domain, also referred to as the NEK1 or zf-DHHC domain (PF01529) is found in a large family of membrane proteins ranging from unicellular eukaryotes to humans (16, 17). Genes encoding DHHC-CRD proteins in yeast include ERF2, AKR1, AKR2, PSL10, YOL003c, YNL326c, and YDR459c. Erf4p is a 26-kDa peripheral ER membrane protein. The palmitoylation defect observed in erf2a and erf4a strains could affect palmitoylation either directly or indirectly by perturbing Ras2p trafficking and thereby preventing efficient interaction with a Ras palmitoyltransferase. Until now, it has not been possible to distinguish between these possibilities. In this study, we utilize an in vitro palmitoylation assay to demonstrate that Erf2p and Erf4p constitute a protein acyltransferase (Ras PAT) responsible for palmitoylation of yeast Ras2p.

EXPERIMENTAL PROCEDURES

Yeast and Bacterial Strains—The construction of yeast strains RJY277 (MATa leu2 ura3 trp1 ade2 ade8 lys2 ras1::HIS3 erf2::TRP1 RAS2(CSet) (Ycp52-RAS2)) and RJY943 (MATa ura3 lys2 ade2 trp1 his3 leu2) has been described elsewhere (14). GST-Erf4p and GST-Ras2p were expressed in yeast strain RJY543. Overexpression of FLAG-Erf2p was performed from the plasmid pESC (Stratagene) also in RJY543. FLAG-Erf2p and GST-Erf4p were expressed in bacterial...
strain XL1 Blue (Stratagene) from an operon fusion constructed from the pFLAG-MAC expression vector (Sigma). The FLAG epitope tag and GST moiety were fused in frame to the N terminus of Erp2p and Erp4p, respectively.

Expression Plasmid Construction and Purification of Protein Substrates—All GST fusions were constructed from the galactose-inducible vector pGEX-4X (18). GST-Ras2CCaaX represents the complete Ras2 protein (38 kDa) fused to GST, GST-HVCCaaX consists of the C-terminal 35 amino acid residues of Ras2p fused to GST, and GST-(HV)CCaaX is the same construct except that Cys318 has been mutated to Ala. GST-CCaax consists of the C-terminal 5 amino acid residues of Ras2p fused to GST. Expression and purification of GST fusion proteins in yeast were performed as follows. Strain RY3453 was co-transformed with pGEX-4X (1000 p.s.i.) and pGEXMA210, a derivative of pGEX-4X which expresses GAL4 under the control of the ADH1 promoter (19). The culture was grown to an A600 of 0.4–0.6 in synthetic medium containing 2% ethanol, 2% glycerol as the carbon source. The cells were induced by addition of galactose (4% final concentration) and incubated overnight at 25 °C. Cells were centrifuged (750 × g for 10 min) and lysed in a solution of Y-PER® (Pierce) containing 1 mm DTT, 1 mm EDTA, 0.1% Triton X-100, and 0.13 mM phenylmethylsulfonyl fluoride at room temperature (30–40 min). The yeast lysate was centrifuged at 2,000 rpm (750 × g) (10 min), and GST-agarose (Pierce) beads were added to the supernatant. The GST-agarose beads were eluted (1 h, room temperature) with 20 mM glutathione in 50 mM Tris-HCl (pH 7.4), 0.02% Triton X-100, 10% glycerol (buffer B). Expected, GST-Ras2CCaaX fusions were membrane-associated due to the prenylation of the CaaX box cysteine. Analysis of the Ras substrate by SDS-PAGE revealed that the GST-Ras protein is the major band present in the preparation. The minor binds cross-react with anti-GST antibody and are either free GST or a proteolytic product of the GST-Ras fusion (data not shown).

H-Ras was expressed as an N-terminal His6-tagged fusion in SF2 cells and purified from detergent extracts of membranes using nickel chelate affinity chromatography (20). The stoichiometry of palmitoylation on the purified substrate has not been determined, but a significant fraction is probably lost during purification due to the action of thioesterases and the presence of reducing agents in the buffers. Myristoylated Gi probably lost during purification due to the action of thioesterases and requires both Erf2p and Erf4p. Removal of either Erf2p or Erf4p results in a nonviable strain (22). Efficient purification of Erf2p/Erf4p, 0.14 pmol/min of palmitate was incorporated into the GST-Erf4p/FLAG-Erf2p prior to substrate adduction.

RESULTS

Deletion of either ERF2 or ERF4 results in a nonviable strain when a palmitoylation-dependent Ras2 allele is the only Ras gene expressed (13) (Fig. 1A, lower). The DHHC-CRD is required for Erp2 function; mutation of Cys318 to Ser or His318 to Ala abolishes Erp2 function. The phenotypes of single and double deletions of ERF2 and ERF4 mutant strains are indistinguishable suggesting that they function at the same step. Consistent with this prediction, Flag-tagged Erp2 co-purifies with GST-Erf4p on a GST-agarose affinity column (Fig. 1B) and GST-Erf4 co-purifies with Flag-Erf2 using anti-FLAG antibody-agarose beads (data not shown). The level of Erp2 that is detected is reduced when isolated from erf4Δ cells, suggesting either that Erp4 is involved in the stability of Erp2 or our ability to extract Erp2 (Fig. 1B). Of the two loss-of-function alleles described above, erf2C189S p, fails to interact with Erp4p, and the level of mutant protein detected is reduced. The other non-functional mutant protein, Erp2H201A p, is still able to interact with Erp4p (Fig. 1B).

To examine if the Erp2p and Erp4p complex is directly involved in the palmitoylation of Ras, we performed an in vitro palmitoylation assay on the GST-agarose-enriched Erp2p/Erp4p complex. A prenylated Ras substrate protein, Ras2-(HV)CCaaX was purified from yeast as a GST fusion protein. The CaaX box of GST-Ras2(HV)CCaaX is prenylated, aax-proteolyzed, and carboxyl-methylated (25). Incubation of Ras2p with bead-bound Erp2p/Erp4p in the presence of [3H]palmitoyl-CoA led to incorporation of the label onto the Ras2p substrate. (Fig. 1C). As expected of a protein-mediated reaction, incorporation of [3H]palmitate is prevented by heating the beads (100 °C, 15 min) prior to adding the substrates. In the presence of Erp2p/Erp4p, 0.14 pmol/min of palmitate was incorporated into the Ras2p substrate. This represents an acceleration of the spontaneous rate of ~160-fold. Consistent with radiolabeling studies in vivo (14), palmitoylation in vitro requires both Erp2p and Erp4p. Removal of either from the assay abolished Ras2p palmitoylation. The DHHC-CRD domain of Erp2p is also important for the palmitoylation
reaction. Mutating Cys\textsuperscript{189} or His\textsuperscript{301} residues within the conserved DHHC-CRD of Erf2p abolished Ras PAT activity (Fig. 1C). The loss of Ras PAT activity observed with the Erf2p(C189S)p mutant can be attributed to its absence from the complex (Fig. 1B). However, the loss of Ras PAT activity in the Erf2p(H201A)p suggests that the conserved histidine of the DHHC signature sequence might play a more direct role catalyzing palmitate transfer. Mutation of Cys\textsuperscript{318}, the residue palmitoylated on Ras \textit{in vivo}, to Ala also abolished palmitoylation (13) (top). The specificity of Erf2p/Erf4p complex comprises the major proteins on the GSH beads (data not shown). It was not possible to detect Ras PAT activity above background in a strain expressing endogenous levels of Erf2p and Erf4p. However overexpression of GST-Erf4p and FLAG-Erf2p from high copy inducible plasmids results in detectable Ras PAT activity with a specific activity of 37 pmol/min/mg. Partial purification by GSH affinity beads increases the specific activity to 1300 pmol/min/mg, representing a 35-fold purification. The specific activity of the partially purified complex is an underestimate because GST-Erf4p is complexed with FLAG-Erf2p under these conditions. We often see a slower migrating species in Erf2p immunoblots (Fig. 2, lane 5). The electrophoretic mobility of this slower migrating form is not sensitive to hydroxylation and therefore does not represent a palmitoylated species. The cause of the slower migrating species that presumably arises by posttranslational modification is under investigation. To further assess the purity of the Erf2p/Erf4p complex we also performed immunoblots for Sec61p, the major integral membrane protein of the ER and Ras2p. Neither appeared to co-purify with Erf2p/ Erf4p, demonstrating that the GST-Erf4p/FLAG-Erf2p complex comprises the major proteins on the GSH beads (data not shown). It is important to note that Ras PAT activity above background was seen in strain expressing endogenous levels of Erf2p and Erf4p. However, overexpression of GST-Erf4p and FLAG-Erf2p from high copy inducible plasmids results in detectable Ras PAT activity with a specific activity of 37 pmol/min/mg. Partial purification by GSH affinity beads increases the specific activity to 1300 pmol/min/mg, representing a 35-fold purification. The specific activity of the partially purified complex is an underestimate because GST-Erf4p is present in large excess over Erf2p. These data are consistent with the conclusion that the Erf2p/Erf4p complex itself constitutes the Ras PAT activity.

It is still formally possible that PAT activity is co-purifying as a minor component of our yeast affinity purification. To rule this out, we partially purified the Erf2p/Erf4p complex from \textit{E. coli} expressing an operon fusion of FLAG-Erf2p and GST-Erf4p driven by the P\textsubscript{lac} promoter (Fig 3A). Proteins are not modified with thiosticker-linked fatty acids in bacteria. As seen in Fig. 3B, Erf2p/Erf4p isolated from \textit{E. coli} is able to carry out palmitoylation of Ras2p. The Erf2p/Erf4p activity purified from extracts exhibits the same heat sensitivity as the activity isolated from yeast. No Ras PAT activity is detected in \textit{E. coli} not expressing \textit{ERF2} and \textit{ERF4}. Total extracts expressing the GST-Erf4p/FLAG-Erf2 operon fusion have Ras PAT activity of 4.5 pmol/min/mg. Purification by GSH-agarose affinity chromatography increases the specific activity to 340 pmol/min/mg or a 76-fold purification. It is not clear at this time if the difference in specific activity between Ras PAT isolated from bacteria compared with that isolated from yeast is significant.

Table I

| Substrate\(^a\) | PAT activity\(^a\) |
|----------------|-----------------|
| GST-RasCCaaX\(^c\) | 940 ± 40 |
| GST-(HV)CaaX\(^c\) | 2720 ± 50 |
| GST-(HV)XaaX\(^c\) | 2720 ± 50 |
| CaaXaaX | 250 ± 10 |
| H-Ras | 40 ± 5 |
| C\textsubscript{aa} | 55 ± 5 |

\(^a\) The PAT activity is the mean ± standard error of triplicate assays for one experiment. All substrates were assayed with the same preparation of Erf2p/Erf4p partially purified from yeast (see Fig. 2).

\(^c\) Palmitoylated cysteine residue is underlined.
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The importance of the signature \(^{200}\)DHHC\(^{203}\) motif of the DHHC-CRD is also evident in experiments with bacterially expressed Erf2p/Erf4p. FLAG-Erf2p(H201A)p and FLAG-Erf2p(C203S)p can be co-purified with GST-Erf4p, but no Ras PAT activity above background levels is detected (Fig. 3).

Erf2p appears to be directly involved in the transfer of palmitate to Ras based on the fact that wild type FLAG-Erf2p becomes labeled in the presence of \[^{[3H]}\text{palmitate}\] (Fig. 4). Incubating Erf2p/Erf4p partially purified from yeast with \[^{[3H]}\text{palmitoyl-CoA}\] and wild type or Erf2p mutant (H201A or C203S) Erf2p/Erf4p complexes, and Ras PAT assays were carried out as described in Fig. 1. The GST-Ras2(HV)CCIIS was added to the Ras PAT reactions as indicated in the reaction scheme. A heat pretreatment (100 °C, 15 min) was performed to determine whether labeling was protein-mediated. Samples were resolved by SDS-PAGE Bis-Tris gels, pH 6.4 (Nu-PAGE), fixed, and fluorography was performed. The dried gel was exposed to film for 1 week. The asterisk indicates the migration position of FLAG-Erf2p. The arrow on the right indicates the migration position of GST-Ras2(HV)CCIISX.

\[^{[3H]}\text{palmitate}\] in the absence of the Ras2(HV)CCIISX substrate results in the formation of an acyl-enzyme intermediate (Fig. 4). Appearance of the tritium-labeled Erf2p was sensitive to heat inactivation, indicating that palmitoylation requires a native conformation of Erf2p. It was shown in Fig. 3 that the DHHC signature in the DHHC-CRD domain of Erf2p is required for Ras PAT activity. This domain is also involved in the formation of the palmitoyl-Erf2p intermediate. Although the Erf4p-Erf2(H201A)p mutant does not display Ras PAT activity, it still forms the \[^{[3H]}\text{palmitoylated}\] Erf2p(H201A)p acyl-enzyme intermediate (Fig. 4). This suggests that His\(^{201}\) may play a role in the transfer of palmitate from the acylated Erf2p to Ras. The conserved cysteine of the DHHC motif (Erf2p(C203S)p) behaves similarly to the Erf2(H201A)p mutant, i.e., stable expression, co-purification with Erf4p, and loss of Ras PAT activity. However, unlike Erf2(H201A)p, the Erf2(C203S)p mutant protein does not form the palmitoylated enzyme intermediate (Fig. 4) suggesting that Cys\(^{203}\) may be the site of palmitate attachment. Together, these results suggest that the Ras PAT reaction involves the formation of a \[^{[3H]}\text{palmitoylated}\] Erf2p acyl-enzyme intermediate prior to transfer of labeled palmitate to the Ras substrate.

To begin to evaluate the protein substrate specificity of the Erf2p/Erf4p Ras PAT, full-length GST-Ras2 was compared with GST fused to the final 28 amino acid residues of the Ras2 hypervariable (HV) region. The HV domain is required for palmitoylation of Ras \(\text{in vivo}\). Erf2p/Erf4p Ras PAT palmitoylated GST(HV)CCIIS to levels similar to full-length GST-Ras2CCIIS (Table I). Consistent with the importance of the hypervariable domain for substrate recognition, \(\text{in vitro}\) palmitoylation of GST-CCIISX is reduced 10-fold compared with GST(HV)CCIISX. Next, we examined whether yeast Ras PAT is able to palmitoylate mammalian H-Ras, which like yeast Ras, is farnesylated on a C-terminal CaaX box and palmitoylated on two adjacent cysteine residues. H-Ras was indeed palmitoylated by yeast Ras PAT but at levels much lower (5%) than the proposed natural substrate, Ras2p. The reduction in labeling could be due to differences between the H-Ras and yeast Ras2 hypervariable domains that we show above is required for Ras PAT activity. Alternatively, it could be due to incomplete farnesylation of the H-Ras purified from SF9 insect.

Fig. 2. Partial purification of Erf2p/Erf4p from yeast extracts. GST-Erf4p and FLAG-Erf2p were produced by galactose induction of RLYY543. Cells were lysed by homogenization in Buffer A and GSH affinity purification was performed as described under “Experimental Procedures.” A total cell extract (16.5 μg) (lane 2) or GSH affinity-purified sample (1.4 μg)(lane 2) were resolved by SDS-PAGE, and the gel stained with Coomassie Blue. Protein molecular mass standards are loaded in lane 1, and their size (kDa) indicated. The GSH bead-enriched fraction was loaded on a second gel for immunoblotting with either anti-GST (lane 4) or anti-FLAG (lane 5) antibody.

Fig. 3. Ras PAT activity of Erf2p/Erf4p expressed and partially purified from E. coli. A. FLAG-Erf2p and GST-Erf4p were expressed together from an operon fusion in the pFLAG-MAC expression vector (Sigma). The gray and black boxes represent the FLAG epitope and GST, respectively. The white boxes represent the open reading frames of ERF2 and ERF4. The cells were lysed by high pressure (25,000 p.s.i.) homogenization, and the GST-Erf4p/FLAG-Erf2p complex was enriched using GSH-agarose beads. B. PAT assays were performed as described under “Experimental Procedures.” Reaction products were analyzed either directly (top panel), or prior to the PAT assay the GST beads were heated (100 °C, 15 min) (bottom panel). C, proteins partially purified by GSH-agarose affinity chromatography were analyzed by immunoblotting with either anti-GST antibody (top panel) or anti-FLAG antibody (bottom panel).

Fig. 4. \[^{[3H]}\text{palmitate labeling of Erf2p}\]. \[^{[3H]}\text{palmitoyl-CoA}\] was added to wild type or Erf2p mutant (H201A or C203S) Erf2p/Erf4p complexes, and Ras PAT assays were carried out as described in Fig. 1. The GST-Ras2(HV)CCIISX was added to the Ras PAT reactions as indicated. A heat pretreatment (100 °C, 15 min) was performed to determine whether labeling was protein-mediated. Samples were resolved by SDS-PAGE Bis-Tris gels, pH 6.4 (Nu-PAGE), fixed, and fluorography was performed. The dried gel was exposed to film for 1 week. The asterisk indicates the migration position of FLAG-Erf2p. The arrow on the right indicates the migration position of GST-Ras2(HV)CCIISX.
duced in a strain expressing to a myristoylated N-terminal glycine. This substrate was pro-

which is normally palmitoylated on a cysteine residue adjacent

in vivo

lated (21) but has not been palmitoylated

activity in the absence of competitor (100%).

lauroyl-CoA (C12:0), myristoyl-CoA (C14:0), palmitoyl-CoA (C16:0),

performed as described in Fig. 1. The substrates for the reactions were

maximal inhibition required palmitoyl or longer acyl chain

lengths. Both saturated and unsaturated fatty acyl-CoAs were

Decanoyl (C10:0) was ineffective at similar concentrations.

Lauryl (12:0) and myristoyl (14:0) were more effective, but

The lipid substrate specificity of Ras PAT was investigated by adding increasing amounts of unlabeled acyl-CoAcompetitors to the radioactive Ras PAT reaction. As expected, addition of unlabelled palmitoyl-CoA was the most effective competitor, achieving 90% inhibition at 8 μM, a 16-fold excess (Fig. 5). Decanoyl (C10:0) was ineffective at similar concentrations. Lauryl (12:0) and myristoyl (14:0) were more effective, but maximal inhibition required palmitoyl or longer acyl chain lengths. Both saturated and unsaturated fatty acyl-CoAs were effective inhibitors. Little difference was observed between C16:0 and C16:1, or C18:0 and C18:1. The lack of complete acyl-CoA substrate specificity of Ras PAT is consistent with the finding that thioester-linked fatty acids on protein are heterogeneous in vivo (1).

**DISCUSSION**

Genetic studies have revealed that Erp2p and Erp4p are required for efficient palmitoylation and plasma membrane association of Ras in yeast (14, 15). In this study, we establish that Erp2p/Erp4p can directly mediate palmitate transfer to yeast Ras using palmitoyl-CoA as a donor, thereby acting as a palmitoyltransferase. Erp2p and Erp4p copurify as a complex of unknown stoichiometry. Erp2p appears to have a direct role in palmitate transfer based on its ability to form an acyl-interme-

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diate that is dependent upon the DHHC signature motif in the DHHC-CRD. It is important to note that the putative metazoan homologs of Erp2p were identified based on the presence of the highly conserved DHHC-CRD motif that we have shown is required for Ras PAT activity, leading us to suspect that other members of the DHHC-CRD family too are palmitoyltransferases (14). Consistent with this prediction, preliminary re-

results with two other members of the yeast Erp2p family, Akr1p and YOL003c, indicate that these two DHHC-CRD proteins can also palmitoylate Ras2p, albeit at a lower efficiency than Erp2p. Presumably, Akr1p and YOL003c palmitoylate other substrates preferentially in vivo. It will be interesting to exam-

whether metazoan DHHC-CRD proteins also possess PAT activity. For the most part, there is little functional information known about these other DHHC-CRD proteins (17). However, recently a c-Abl-interacting protein containing a DHHC-CRD motif, Aph2, has been identified by a two hybrid screen (25). Co-expression of Aph2 and c-Abl results in induction of apoptosis in COS-7 cells.

Erp4p is necessary for stable expression or solubilization of Erp2p from yeast cells, suggesting that it may act as a chaper-
one for Erp2p. Whether it has other functions in mediating palmitate transfer is under investigation. Erp4p does not have any obvious protein domains that predict its function, and although putative Erp4p homologs can be found in other fungal genomes, it has not been possible to identify metazoan ho-

mologs based on sequence identity. It is not known at this time whether other DHHC-CRD proteins require association with auxiliary proteins similar to the Erp2p interaction with Erp4p.

At present it is not clear whether Erp2p/Erp4p acts as a typical enzyme or palmitoylates Ras through an unconventional mech-

anism (26). Resolving this issue in the present study is not possible due to several factors, including limitations in the assay (subsaturating concentrations of Ras substrate) and the difficulty of estimating the amount of active Erp2p/Erp4p in the GST-pulldown. Current efforts are focused on obtaining a purified preparation of Erp2p/Erp4p that is amenable to a more detailed mechanistic analysis.

The Erp2p/Erp4p-dependent Ras PAT is distinct in several ways from the recently proposed Hedgehog palmitoyltransferase, skinny hedgehog/sightless (ski) (5). Ski shares a short region of sequence homology with membrane-bound acyltrans-

ferases, including acyl-coenzyme Acholesterol acyltransferase and diacylglycerol acyltransferase, whereas there is no significant homology between Erp2p or Erp4p and this class of en-

zymes. The mechanism of palmitate addition by Ski is unclear. It is predicted to occur through a thioester linkage to the N-terminal cysteine residue, followed by a rearrangement through a cyclic intermediate to form an amide (5). Ras PAT modifies Cys, preferably in the context of a C-terminal-preny-

lated cysteine. The substrate hedgehog is an extracellular protein that is modified either in

the lumen of the secretory system or on the surface of the cell,

whereas Ras is modified by the Erp2p/Erp4p complex, which is

localized to the ER (17). The Erp2p/Erp4p-dependent Ras PAT may therefore represent a new class of enzymes required for the palmitoylation of cellular proteins. Erp2p/Erp4p exhibits a preference for yeast Ras substrates. Based on these results, we propose that other members of the Erp2p DHHC-CRD family carry out thioacylation of other lipid-modified proteins.

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