Prenylcysteine Lyase Deficiency in Mice Results in the Accumulation of Farnesylcysteine and Geranylgeranylcysteine in Brain and Liver*

Received for publication, May 28, 2002, and in revised form, July 2, 2002
Published, JBC Papers in Press, July 31, 2002, DOI 10.1074/jbc.M205183200

Anne Beigneux‡‡, Shannon K. Withycombe‡, Jennifer A. Digits‡, William R. Tschantz‡, Carolyn A. Weinbaum‡, Stephen M. Griffey‡‡‡, Martin Bergo‡§, Patrick J. Casey‡, and Stephen G. Young‡‡‡‡

From the ‡†Gladstone Institute of Cardiovascular Disease and the §Cardiovascular Research Institute, University of California, San Francisco, California 94141-9100, the ‡Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina 27710-3813, the **Comparative Pathology Laboratory, University of California, Davis, California 95616, and the ‡‡Department of Medicine, University of California, San Francisco, California 94141-9100

In in vitro experiments, prenylcysteine lyase (Pcly) cleaves the thioether bond of prenylcysteines to yield free cysteine and the aldehyde of the isoprenoid lipid. However, the importance of this enzyme has not yet been fully defined at the biochemical or physiologic level. In this study, we show that Pcly is expressed at high levels in mouse liver, kidney, heart, and brain. To test whether Pcly deficiency would cause prenylcysteines to accumulate in tissues and result in pathologic consequences, we produced Pcly-deficient cell lines and Pcly-deficient mice (Pcly–/–). Pcly activity levels were markedly reduced in Pcly–/– cells and tissues. Pcly–/– fibroblasts were more sensitive than wild-type fibroblasts to growth inhibition when prenylcysteines were added to the cell culture medium. To determine if the reduced Pcly enzyme activity levels led to an accumulation of prenylcysteines within cells, mass spectrometry was used to measure farnesylcysteine and geranylgeranylcysteine levels in the tissues of Pcly–/– mice and wild-type controls. These studies revealed a striking accumulation of both farnesylcysteine and geranylgeranylcysteine in the brain and liver of Pcly–/– mice. This accumulation did not appear to be accompanied by significant pathologic consequences. Pcly–/– mice were healthy and fertile, and surveys of more than 30 tissues did not uncover any abnormalities. We conclude that prenylcysteine lyase does play a physiologic role in cleaving prenylcysteines in mammals, but the absence of this activity does not lead to major pathologic consequences.

A wide variety of cellular proteins are posttranslationally modified by cholesterol biosynthetic intermediates, a process generally termed protein prenylation (1–3). Protein prenylation involves the covalent attachment of a 15-carbon farnesyl or a 20-carbon geranylgeranyl lipid to cysteine residue(s) at or near the carboxyl terminus of a protein via a thioether bond. There are two categories of prenylated proteins in mammalian cells, the Rab proteins and the CAAX proteins. The Rab proteins are geranylgeranylated at a pair of carboxy-terminal cysteines (4, 5). CAAX proteins are either farnesylated or geranylgeranylated at the carboxy-terminal cysteine (the C of the CAAX motif) (1–3). After prenylation, CAAX proteins generally undergo two additional modifications. First, the last three residues of the protein (i.e., the AAX of the CAAX motif) are endoproteolytically released. Second, the newly exposed isoprenylcysteine residue is methylated, converting the carboxylate anion of the isoprenylcysteine residue to an α-carboxyl methyl ester (6).

S-Prenylation of cysteines within proteins is a highly stable posttranslational modification. The stability of the modification and the abundance of isoprenylated proteins in cells suggested the existence of an enzymatic mechanism for degrading and disposing of prenylcysteine residues. Such an enzyme, termed prenylcysteine lyase (Pcly), was recently identified (7–9). Pcly is a 505-amino acid flavin adenine dinucleotide-dependent thioether oxidase and is located within lysosomes (8, 9). Pcly recognizes both farnesylcysteine and geranylgeranylcysteine and their methyl esters with high affinity and cleaves the thioether bond to yield free cysteine (or cysteine methyl ester) and the aldehyde of the isoprenoid lipid (7). Interestingly, Pcly is only active against free prenylcysteines and not prenylcysteine residues within prenylated proteins or peptides (7). In humans, PCLY is expressed ubiquitously, with particularly high levels in brain, liver, kidney, and heart (7).

Although the ability of recombinant PCLY to cleave prenylcysteines in vitro has been amply established (7–9), neither the biochemical importance nor the physiologic relevance of this enzyme has been fully defined. We do not know, for example, whether the absence of this enzyme would cause prenylcysteines to accumulate in mammalian cells since an alternate “disposal method,” transport out of cells by cell-surface P-glycoprotein, has been suggested by the finding that prenylcys-

CAAX is a sequence motif found at the carboxyl terminus of many prenylated proteins, representing a cysteine (C), a pair of aliphatic amino acids (A), and then a final amino acid residue (X).

The abbreviations used are: Pcly, prenylcysteine lyase; BAC, bacterial artificial chromosome; ES, embryonic stem; PCME, farnesylcysteine methyl ester; GGCME, geranylgeranylcysteine methyl ester; FC, farnesylcysteine; GC, geranylcysteine; GGC, geranylgeranylcysteine; PPT, palmitoyl protein thioesterase.
teines are substrates for that transporter (10). If prenylcysteine were to accumulate, would they cause cell toxicity or tissue pathology? Also located within lysosomes is a thioesterase, palmitoyl-protein thioesterase 1 (PPT1), that cleaves fatty acids from acylated cysteines within proteins. Mutations in PPT1 cause a lysosomal storage disease in humans, infantile neuronal ceroid lipofuscinosis (Batten disease) (11). By analogy, Tschantz et al. (8, 9) have speculated that the absence of prenylcyesteine lyase might also cause a lysosomal storage disease. To address each of these issues and to better define the in vivo importance of this enzyme, we produced and analyzed Pcy-/- deficient mice and Pcy-deficient cell lines.

EXPERIMENTAL PROCEDURES

Analysis of PCLY Mutants—Site-directed mutagenesis (12) was used to introduce a variety of missense mutations into PCLY cDNA. The mutant PCLY cDNAs were used to produce recombinant baculoviruses, which were then used to infect Sf9 cells (8). A total of 2.5 × 10^6 infected Sf9 cells were harvested and resuspended in 10 ml Tris buffer (pH 7.7) containing 0.2% Triton X-100 and protease inhibitors (8). The cells were incubated on ice for 15 min and then disrupted by drawing the solution up and down ten times through a 27-gauge needle. Samples were centrifuged at 10,000 × g for 90 min at 4 °C. The supernatant fluids (detergent extract) were collected, and the protein concentrations were determined by a Lowry assay (13). All mutant proteins were expressed at similar levels as judged by immunoblot analysis with a PCLY-specific antibody (8).

PCLY Activity Measurements—PCLY activities in cell or tissue extracts were assessed by thin-layer chromatography (8). Briefly, 10 µg of protein was incubated for 30 min at 37 °C in 50 ml Tris (pH 7.7) containing 10 µM [35S]farnesylcysteine (≈40,000 dpm/reaction) in a final volume of 20 µl. The reaction was stopped by adding 10 µl of the thin-layer chromatography solvent (n-propanol:NH4OH:H2O (6:3:1, v/v/v)). Samples were processed by silica-gel thin-layer chromatography with a method that separates the reaction product, [35S]cytisteine, from the substrate, [35S]farnesylcyesteine (8). To assess the amount of [35S]cysteine in each reaction, the plates were exposed to x-ray film.

A Pcy Gene-targeting Vector—A human PCLY cDNA (GenBank™ accession number AF181490) was used to identify a bacterial artificial chromosome (BAC) clone that spanned the mouse Pcy gene. A 9.8-kb XhoI fragment containing Pcy exons 4–6 was subcloned into pCR-XL-TOPO (Invitrogen, Carlsbad, CA). The XhoI fragment was used to construct a gene-targeting vector designed to delete exon 5 and replace it with a neomycin-resistance marker. The vector was linearized with AscI and introduced by electroporation into mouse ES cells. Clones were expanded in selective medium and screened by Southern blot analysis. ES cell lines were cultured on mitomycin C-treated STO feeder cells in vitro. The sequence-replacement gene was linearized with I and introduced by electroporation, and ES cell clones by homologous recombination were isolated. ES cell clones were expanded in selective medium and screened by a neomycin-resistance marker.

PCLY-/- Embryonic Fibroblasts—Timed matings were established between Pcly-/+ mice, and pregnant females were sacrificed on day 17 of gestation. Post coitum embryos were dissected in 0.5% trypsin-EDTA (Invitrogen, Carlsbad, CA) at 4 °C. The next morning, embryos were mechanically disrupted in 5 ml of Dulbecco's modified Eagle's medium supplemented with fetal bovine serum, L-glutamine, nonessential amino acids, penicillin, streptomycin (Invitrogen, Carlsbad, CA), and 2-mercaptoethanol (Sigma). After removal of the debris, the cells were then plated in T175 flasks. Fibroblast cell lines were immortalized by serial passaging (18).

Cell Proliferation Assay—Pclyl+/+, Pclyl-/-, and Pclyl-/- cells were plated on 96-well plates (5,000 cells/well, 12 wells/cell) line, two independent cell lines/genotype) and incubated for 24, 48, 72, 96, and 120 h. At each time point, cell density was determined with the CellTiter 96 colorimetric assay (Promega, Madison, WI).

Measurement of Prenylcysteines by Mass Spectrometry—Nine-month-old Pclyl-/- and Pclyl-/- mice were anesthetized with avertin, and samples of blood, liver, and brain were obtained. Tissues or blood samples were pooled from 8–10 mice of each genotype. Levels of farnesylcysteine (FC) and geranylgeranylcysteine (GGC) were determined by high-performance liquid chromatography/tandem mass spectrometry analysis. Samples were mixed for 5 min at room temperature and centrifuged for 5 min at 5000 × g in a tabletop centrifuge, and the supernatant fluids were transferred to a 0.2-ml polypropylene injection vial and capped. Samples were then injected into a PerkinElmer Life Sciences Sciex API3000 Triple Quadrupole liquid chromatography/tandem mass spectrometry mass spectrometer fitted with a C18 separation column. The column was developed with a gradient of water to acetonitrile; both solvents contained 0.1% formic acid for ion-suppression during the chromatography. In preliminary trial and calibration separations, the elution positions for FC, GGC, and water (mobile phase A) were synchronized (19). Analysis was performed using electrospray ionization mass spectrometry analysis. Samples were mixed for 5 min at room temperature and centrifuged for 5 min at 5000 × g in a tabletop centrifuge, and the supernatant fluids were transferred to a 0.2-ml polypropylene injection vial and capped. Samples were then injected into a PerkinElmer Life Sciences Sciex API3000 Triple Quadrupole liquid chromatography/tandem mass spectrometry mass spectrometer fitted with a C18 separation column. The column was developed with a gradient of water to acetonitrile; both solvents contained 0.1% formic acid for ion-suppression during the chromatography. In preliminary trial and calibration separations, the elution positions for FC, GGC, and water (mobile phase A) were synchronized (19). Analysis was performed using electrospray ionization mass spectrometry analysis. Samples were mixed for 5 min at room temperature and centrifuged for 5 min at 5000 × g in a tabletop centrifuge, and the supernatant fluids were transferred to a 0.2-ml polypropylene injection vial and capped. Samples were then injected into a PerkinElmer Life Sciences Sciex API3000 Triple Quadrupole liquid chromatography/tandem mass spectrometry mass spectrometer fitted with a C18 separation column. The column was developed with a gradient of water to acetonitrile; both solvents contained 0.1% formic acid for ion-suppression during the chromatography. In preliminary trial and calibration separations, the elution positions for FC, GGC, and water (mobile phase A) were synchronized (19). Analysis was performed using electrospray ionization mass spectrometry analysis. Samples were mixed for 5 min at room temperature and centrifuged for 5 min at 5000 × g in a tabletop centrifuge, and the supernatant fluids were transferred to a 0.2-ml polypropylene injection vial and capped. Samples were then injected into a PerkinElmer Life Sciences Sciex API3000 Triple Quadrupole liquid chromatography/tandem mass spectrometry mass spectrometer fitted with a C18 separation column. The column was developed with a gradient of water to acetonitrile; both solvents contained 0.1% formic acid for ion-suppression during the chromatography. In preliminary trial and calibration separations, the elution positions for FC, GGC, and water (mobile phase A) were synchronized (19). Analysis was performed using electrospray ionization mass spectrometry analysis.
Analysis of Pcly–/– Cells and Tissues—Full necropsies were performed on four different 4- and 10-month-old Pcly–/– mice and littermate Pcly+/+ controls, and more than 30 tissues were examined by routine histology. To determine whether the brains of 10-month-old Pcly–/– mice contained increased amounts of lipofuscin, we examined brain tissue for autofluorescence as described recently for mice lacking palmitoyl protein thioesterase I (19). For these studies, 10-month-old Pcly+/+, Pcly+/–, and Pcly–/– mice were anesthetized with avertin and perfusion-fixed with 4% paraformaldehyde. Brains were removed and immersed in formalin for 24 h. Samples were processed for paraffin embedding, and sagittal sections (5–10 μm) were mounted on polylysine-coated slides. Deparaffinized sections from each sample were examined with a UV-equipped Eclipse E600 microscope (Nikon) at 470 nm (excitation)/525 nm (emission). Finally, to determine if Pcly activity levels in the 4-kb transcript, a 5-kb transcript was detected in liver, and brain of 10-month-old Pcly–/– mice (50- and 40-fold increase, respectively) (Fig. 4B). Geranylgeranylcysteine also accumulated in liver (10-fold) and brain (30-fold) of Pcly–/– mice (Fig. 4C). We did not observe an accumulation of prenylcysteines in the blood of Pcly–/– mice, perhaps because few prenylcysteines are released into the blood or because those substances are cleared rapidly from that compartment.

We also attempted to quantify levels of prenylcysteine methyl esters in tissues by mass spectrometry, but were unable to detect these compounds due to the lability of these methyl esters in tissue extracts. When FCME and GGCMME were added exogenously to tissue extracts, they were rapidly and completely converted to the corresponding demethylated compounds (i.e. FC, GGC) (not shown). Hence, we believe that the analysis of FC and GGC in the tissue extracts accurately reflected the total level of farnesylated and geranylgeranylated cysteines in these tissues.

Pathologic Analysis of Pcly–/– Mice—The accumulation of prenylcysteine residues did not appear to result in significant consequences. Blood chemistries including calcium, phosphate, glucose, cholesterol, triglycerides, alanine aminotransferase, aspartate aminotransferase, and creatinine were normal in Pcly–/– mice; serum lipids in Pcly–/– and Pcly+/+ mice did not differ on a high-fat diet (not shown). In addition, we analyzed more than 30 different tissues from 4- and 10-month-old Pcly–/– mice with routine histologic stains, and observed no abnormalities.

In the case of palmitoyl protein thioesterase I-deficient mice, the presence of lipofuscin was particularly apparent by fluorescence microscopy (19). To determine if increased levels of prenylcysteines might lead to increased autofluorescence within tissues, we examined brain sections from 10-month-old Pcly+/+ and Pcly–/– mice with a UV-equipped microscope. Regardless of the genotype, autofluorescence levels were highest in the cerebellum, but lower levels of autofluorescence could also
be detected in pons, hippocampus, and frontal cortex. However, we did not observe any differences in the levels of autofluorescence in brains of Poly+/− and Poly−/− mice (not shown).

To look for abnormalities in intracellular organelles, we examined Poly+/+ and Poly−/− ES cells by transmission electron microscopy, after staining the cells with imidazole-osmium tetroxide (which stains unsaturated lipids). No differences were observed in Poly−/− and Poly+/+ ES cells. The lysosomes appeared normal, and there was no increase lipid-staining material anywhere within the cell (not shown).
FIG. 2. PCl activity measurements in PCl+/+, PCl+/−, and PCl−/− cells. A and C, Northern blots with a PCl cDNA probe (corresponding to exon 4–6 sequences) in ES cells (A) and embryonic fibroblasts (C). B and D, PCl activity levels in ES cells (B) and embryonic fibroblasts (D). Results are expressed as the percentage of activity in wild-type cells. Activity data represent the average of duplicate determinations; each experiment was repeated twice, with virtually identical results.

Our studies establish that PCl is the main enzymatic activity for degrading prenylcysteines in mammalian cells. Prenylcysteine lyase activity was markedly reduced in PCl−/− ES cells, in PCl−/− fibroblasts, and in the tissues of PCl−/− mice. In the majority of our experiments, enzymatic activities in PCl−/− cells and tissues were at or just slightly above the background levels for our assay. Therefore, we cannot exclude the possibility that very low levels of a redundant bioactivity exist in mammalian cells. We have looked for a PCl family member within the expressed sequence tag and genomic databases, for it is conceivable that another yet-to-be-identified gene encodes a protein that can metabolize prenylcysteines in the setting of PCl deficiency. In the course of this data mining, we found a hypothetical human protein (GI:13278789) that exhibits 35% homology with a 330-amino acid segment of human PCLY. It would be of interest to determine whether that clone encodes an enzyme with some activity against prenylcysteines (i.e., a PCLY2). This possibility may not be farfetched. PPT1 is a lysosomal enzyme that removes palmitoyl groups from cysteines in proteins, and its absence causes infantile neuronal ceroid lipofuscinosis (11). Recently, cloning of a PPT2 gene has been reported (22). Despite the fact that PPT1 and PPT2 share only 18% identity at the amino acid level, PPT1 and PPT2 have comparable palmitoyl-CoA thioesterase activities. Interestingly, PPT2 appears to display a distinct substrate specificity in cells, and its expression does not correct the metabolic defect in PPT1-deficient cells (22).

There are two other reasons to think that PCl is the principal route for disposing of prenylcysteines in cells, even if minute amounts of another prenylcysteine-degrading enzyme ex-
ist. First, mass spectrometric analysis showed a significant accumulation of both farnesylcysteine and geranylgeranylcysteine in the brain and liver of Pcly−/− mice compared with tissues from aged-matched control mice. Second, Pcly−/− fibroblasts, when challenged with increasing doses of exogenous prenylcysteines, displayed increased sensitivity to the toxicity of these compounds.

We did not note an accumulation of prenylcysteines in the blood of Pcly−/− mice. We do not know the reason for the absence of prenylcysteine accumulation in blood, although we hypothesize that the production of prenylcysteines is probably minimal in erythrocytes. Alternatively, it is possible that prenylcysteines readily diffuse away from blood cells into the plasma and are rapidly cleared by the kidney.

One could speculate that the accumulation of farnesylcysteine and geranylgeranylcysteine in brain and liver might inhibit the methylation of prenylated proteins by isoprenylcysteine carboxyl methyltransferase (6). However, we do not consider this to be likely, because the concentrations of the prenylcysteines in the Pcly−/− cells were in the picomolar range, significantly below the micromolar concentrations required to inhibit isoprenylcysteine carboxyl methyltransferase (6, 20, 23, 24). In keeping with this prediction, we found that extracts from Pcly−/− cells and Pcly+/+ cells were equally effective in methylethylating recombinant farnesyl-K-Ras.3

We had speculated that the absence of prenylcysteine lyase in mammalian lysosomes might cause, either directly or indirectly, a lysosomal storage disease (8, 9) akin to that occurring in the setting of PPT1 mutations (11, 19, 25). A deficiency in PPT1 leads to the accumulation of a finely granular autofluorescent sudanophilic storage material (lipofuscin) in the brain (19, 25). However, no such pathologic findings were noted in Pcly−/− mice. We were unable to detect histologic abnormalities in a survey of more than 30 tissues from Pcly−/− mice, and there was no increase in autofluorescence within the brains of Pcly−/− mice. A key difference between PPT1 and Pcly is that PPT1 cleaves palmitate residues from proteins, whereas Pcly acts only on free prenylcysteines after the protein has been degraded. The accumulation of free prenylcysteines in the setting of Pcly deficiency (as opposed to palmitoylated proteins in the setting of PPT1 deficiency) apparently has little proclivity to form lipofuscin either directly by inducing aggregation of proteins or indirectly by interfering with the action of other lysosomal enzymes.

The absence of overt pathology in Pcly-deficient mice raises the possibility that the cell might have more than one strategy for dealing with prenylcysteines. In yeast, at least one isoprenylated peptide, a-factor, is transported out of cells by an ABC transporter, Ste6p (26). A related protein, P-glycoprotein, has been shown to be capable of transporting prenylcysteines out of human cells (10). In the setting of Pcly deficiency, it is conceivable that P-glycoprotein-mediated transport of prenylcysteines out of cells partially prevents the intracellular accumulation of prenylcysteines and thereby prevents the pathologic consequences of the enzyme deficiency. However, it is far from clear that P-glycoprotein functions in a meaningful way to dispose of prenylcysteines residues in normal cells. For example, prenylation of proteins is ubiquitous, but most mammalian tissues do not express appreciable levels of P-glycoprotein (27).

In conclusion, our data support the hypothesis that catabolism of prenylcysteines in mammals requires Pcly. In the absence of that enzyme, the cell’s ability to cleave prenylcysteines is severely compromised, and prenylcysteine residues accumulate. Despite this accumulation, Pcly-deficient mice are born at the expected mendelian frequency, are fertile, and are free of obvious pathology.

Acknowledgments—We thank J. Wong and R. Hamilton for transmission electron microscopy, D. Sanan for help with the fluorescence microscopy, and S. Ordway, and G. Howard for criticisms of the manuscript.

REFERENCES

1. Casey, P. J. (1992) J. Lipid Res. 33, 1731–1740
2. Casey, P. J., and Seabra, M. C. (1996) J. Biol. Chem. 271, 5289–5292
3. Zhang, F. L., and Casey, P. J. (1996) J. Biol. Chem. 271, 14497–14503
4. Farnsworth, C. C., Seabra, M. C., Ericsson, L. H., Gelb, M. H., and Glomset, J. A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11863–11867
5. Young, S. G., Ambrozik, P., Kim, E., and Clarke, S. (2000) In The Enzymes (Tammou, F., and Sigman, D. S., eds) Vol. 21, pp. 155–213, Academic Press, San Diego, CA
6. Zhang, L., Tschantsz, W. R., and Casey, P. J. (1997) J. Biol. Chem. 272, 23354–23359
7. Tschantsz, W. R., Zhang, L., and Casey, P. J. (1999) J. Biol. Chem. 274, 35802–35808
8. Tschantsz, W. R., Digits, J. A., Pyun, H.-J., Coates, R. M., and Casey, P. J. (2001) J. Biol. Chem. 276, 2321–2324
9. Zhang, L., Sachs, C. W., Fu, H.-W., Fine, R. L., and Casey, P. J. (1995) J. Biol. Chem. 270, 23269–23285
10. Zhang, L., Sachs, C. W., Fu, H.-W., Fine, R. L., and Casey, P. J. (1995) J. Biol. Chem. 270, 23269–23285
11. Vesa, J., Hellsten, E., Verkruyse, L. A., Camp, L. A., Rapola, J., Santavuori, P., Hofmann, S. L., and Peltonen, L. (1995) Nature 376, 584–587
12. Jones, D. H., and Howard, B. H. (1991) BioTechniques 10, 62–66
13. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
14. Bergo, M. O., Leung, G. K., Ambrozik, P., Otto, J. C., Casey, P. J., and Young, S. G. (2000) J. Biol. Chem. 275, 17605–17610
15. McMahon, A. P., and Bradley, A. (1990) Cell 62, 1073–1085
16. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
17. Mortensen, R. M., Conner, D. A., Chao, S., Geisterfer-Lowrance, A. A. T., and Mortensen, R. M. (1996) Mol. Biol. Cell. 12, 2391–2395
18. Todaro, G. J., and Green, H. (1963) J. Cell Biol. 17, 299–313
19. Gupta, P., Soyombo, A. A., Atashband, A., Wissmiewski, K. R., Shelton, J. M., Richardson, J. A., Hammer, R. E., and Hofmann, S. L. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 13566–13571
20. Philips, M. R., Pillinger, M. H., Staud, R., Volker, C., Rosenfeld, M. G., Weissmann, G., and Stock, J. B. (1993) Science 259, 977–980
21. Parish, C. A., Brazil, D. P., and Rando, R. R. (1997) Biochemistry 36, 2686–2693
22. Soyombo, A. A., and Hofmann, S. L. (1997) J. Biol. Chem. 272, 27456–27463
23. Volker, C., Miller, R. A., McCleary, W. R., Staud, R., Poenie, M., Backer, J. M., and Stock, J. B. (1991) J. Biol. Chem. 266, 21515–21522
24. Huzoor-Akbar, W., Wang, W., Kornhauser, R., Volker, C., and Stock, J. B. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 868–872
25. Lu, J. Y., Verkruyse, L. A., and Hofmann, S. L. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 10046–10050
26. Kerbownik, C., and Michaelis, S. (1991) EMBO J. 10, 3777–3785
27. Ambudkar, S. V., Dey, S., Hrycyna, C. A., Ramachandra, M., Pastan, I., and Gottesman, M. M. (1999) Annu. Rev. Pharmacol. Toxicol. 39, 361–398

3 S. Withycombe and A. Beigneux, unpublished data.
Prenylcysteine Lyase Deficiency in Mice Results in the Accumulation of Farnesylcysteine and Geranylgeranylcysteine in Brain and Liver
Anne Beigneux, Shannon K. Withycombe, Jennifer A. Digits, William R. Tschantz, Carolyn A. Weinbaum, Stephen M. Griffey, Martin Bergo, Patrick J. Casey and Stephen G. Young

J. Biol. Chem. 2002, 277:38358-38363. doi: 10.1074/jbc.M205183200 originally published online July 31, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M205183200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 26 references, 17 of which can be accessed free at http://www.jbc.org/content/277/41/38358.full.html#ref-list-1