NAPE-PLD determines the uterine anandamide levels

*N-acylphosphatidylethanolamine-hydrolyzing Phospholipase D is an Important Determinant of Uterine Anandamide Levels during Implantation.*

Yong Guo¹[^, Haibin Wang¹[^, Yasuo Okamoto², Natsuo Ueda², Philip J. Kingsley³, Lawrence J. Marnett³, Harald H. O. Schmid⁴, Sanjoy K. Das⁴[^, Sanjoy K. Das¹[^, and Sudhansu K. Dey¹,⁶,⁷[^*

From the Departments of Pediatrics¹, Biochemistry, Institute of Chemical Biology³, Cancer Biology⁵, Cell & Developmental Biology⁶ and Pharmacology⁷, Vanderbilt University Medical Center, Nashville, TN 37232, USA; Department of Biochemistry², Kagawa University School of Medicine, Kagawa 761-0793, Japan; and The Hormel Institute⁴, Austin, MN 55912, USA

[^These authors contributed equally to this work.

Running Title: NAPE-PLD determines uterine anandamide levels

*Address correspondence to S. K. Dey (sk.dey@vanderbilt.edu)

Implantation requires reciprocal interaction between blastocysts and a receptive uterus. In mice, one important player in this dialogue involves endocannabinoid signaling via cannabinoid receptor CB1. Anandamide is an endogenous cannabinoid ligand and its levels are spatiotemporally regulated in the uterus during early pregnancy, showing lower levels in the receptive uterus and at the implantation site. However, the mechanism by which differential uterine anandamide gradients are established under different pregnancy status is not clearly understood. Using multiple approaches, we show here that uterine anandamide levels conducive to implantation are primarily regulated by spatiotemporal expression of Nape-Pld, the gene encoding N-acylphosphatidyl-ethanolamine-hydrolyzing phospholipase D (NAPE-PLD) that generates anandamide. The expression is well correlated with its activity and anandamide levels. This study is clinically relevant, since elevated anandamide levels in peripheral circulation are associated with spontaneous pregnancy failure in women.

Natural and endogenous cannabinoids show diverse central and peripheral effects that are primarily mediated via G-protein coupled cannabinoid receptors CB1 and CB2¹ (1,2). We have previously shown that cannabinoid/endocannabinoid signaling via CB1 profoundly influences female reproduction during early pregnancy in mice (3). For example, functional expression of CB1 in preimplantation embryos (4,5), synthesis of an endocannabinoid anandamide in the uterus (6,7), and its dose and stage-specific effects on embryo development and implantation
suggest that endocannabinoid signaling is operative very early in pregnancy (8,9). Further, uterine anandamide levels and blastocyst CB1 are coordinately downregulated with the attainment of uterine receptivity and blastocyst activation prior to implantation as opposed to their higher levels in delayed implanting uterus and dormant blastocysts (6,7). These results suggest that lower levels, but not higher levels, of anandamide and CB1 are beneficial to implantation. Indeed, while a low dose of anandamide stimulates blastocyst outgrowth in culture, a higher dose inhibits this event (9). This is further supported by our findings that anandamide within a very narrow range regulates blastocyst activation and implantation by differentially modulating MAP kinase signaling and Ca\(^{++}\) channel activity via CB1(10). Collectively, these results suggest that endocannabinoid signaling involving uterine anandamide and embryonic CB1 is one of the signaling pathways that determine the fate of embryo implantation. However, the underlying mechanism by which differential uterine anandamide levels are spatiotemporally established under different pregnancy status remains unknown.

The discovery of anandamide provoked intense investigation regarding its biosynthetic pathways (11). One suggested route of anandamide synthesis is the direct \(N\)-acylation of ethanolamine by "anandamide synthase" activity (5,12). However, this \(N\)-acylation reaction has requirements for an alkaline pH and relatively high \(Km\) for both arachidonic acid and ethanolamine (12), questioning the physiological relevance of this pathway. The current consensus is that anandamide is released \textit{in vivo} from hydrolysis of a phospholipid precursor \(N\)-acylphosphatidylethanolamine (NAPE) in the cell membrane by the NAPE-selective phospholipase D (13). Recently, \textit{Nape-pld} gene encoding this enzyme was cloned in rodents and humans (14), and overexpression of this gene in mammalian cell lines generates \(N\)-acylethanolamines (NAEs) including anandamide from NAPE (15). Once synthesized, anandamide is released from the cell into the extracellular space where it acts as an autocrine or paracrine factor. The effectiveness of anandamide depends on its concentration in the extracellular space, which is regulated by intracellular degradation by fatty-acid amide hydrolase (FAAH). It is suggested that embryonic and/or uterine FAAH is a major player in regulating uterine anandamide levels during implantation (16,17). Considering the low abundance of uterine FAAH in mice (17), we speculated that uterine anandamide levels are primarily regulated by changing anandamide synthetic status involving NAPE-PLD. Using molecular, biochemical and physiological approaches, we demonstrate here that uterine NAPE-PLD expression and activity change in a spatiotemporal manner determining anandamide levels at the implantation site and during various states of uterine receptivity for implantation.
EXPERIMENTAL PROCEDURES

Animals and Treatments-Mice were housed at Vanderbilt Animal Care Facility according to NIH and institutional guidelines for laboratory animals. Adult CD-1 female mice (Charles River Laboratories) were mated with fertile or vasectomized males of the same strain to induce pregnancy and pseudopregnancy, respectively (day 1=vaginal plug). Conditions of delayed implantation were induced by ovariectomizing the females on day 4 morning, and maintained with daily subcutaneous injections of progesterone (P4, 2 mg/mouse) from days 5-7. To activate dormant blastocysts and initiate implantation, P4-primed mice were injected with estradiol-17β (E2, 25 ng/mouse). Implantation sites were visualized by an intravenous injection of Chicago blue dye solution (18). Uterine tissues were processed for Northern and in situ hybridization, enzyme activity and anandamide levels. To determine whether Nape-pld was regulated by E2 and P4, ovariectomized wild-type, PR(-/-) or ERβ(-/-) mice were injected with oil (control), E2 (100 ng/mouse), P4 (2 mg/mouse), or a combination of E2 and P4. Mice were killed at indicated times, and uteri processed for mRNA analysis by Northern and in situ hybridization, enzyme activity and anandamide levels.

Analysis of anandamide-Uteri pooled from 3-5 pregnant mice in each group (N=5) were assayed for anandamide as previously described (19,20).

Enzyme Assay-NAPE-PLD activity was measured by the release of anandamide or N-palmitoylethanolamine from N-[14C]arachidonoyl-PE (NArPE) or N-[14C]palmitoyl-PE (NPPE), respectively, as described (14). Uteri pooled from 10-12 mice in each group (N= 3-4) were homogenized and the supernatants (800g) were extracted for NAPE-PLD assay. Assays were performed in 3-5 replicates for each sample.

In situ hybridization-Frozen sections (10 μm) were hybridized with a 35S-labeled cRNA probe to mouse Nape-pld as described (21). Sections hybridized with the sense probes served as negative controls and showed no positive signals.

Northern Hybridization-Poly(A+) RNA (2.0μg) was denatured, separated by formaldehyde-agarose gel electrophoresis, and transferred onto nylon membranes. Cross-linked blots were prehybridized, hybridized, and washed as previously described (21). The hybrids were detected by autoradiography.

Quantitative RT-PCR. Reverse transcription (RT) reaction was performed using SuperScript II RT kit (Invitrogen). Real-time quantitative PCR was performed in the Bio-Rad iCycler using iQTM SYBR Green Supermix according to the manufacturer’s protocol. The primers used were: mGAPDH, 5’-GCC TTC CGT GTT CCT ACC C-3’ and 5’-TGC CTG CTT CACCAC CTT C-3’; and
NAPE-PLD determines the uterine anandamide levels

NAPE-PLD, 5'-GTC CTC ATC AGT CAC AAC C-3' and 5'-AGC TCA ATC ACG TTC TCG-3'. mGAPDH served as the reference gene for analyzing the relative abundance of NAPE-PLD mRNA.

RESULTS AND DISCUSSION

Nape-pld shows spatiotemporal expression in the periimplantation uterus-As described above, endocannabinoid signaling is primarily mediated by interactions between uterine anandamide and embryonic CB1, directing embryo development and implantation in a stage and dose dependent manner in mice. However, it remains unknown how appropriate anandamide levels are spatiotemporally created for synchronizing preimplantation embryo development and uterine receptivity for implantation. The objective of this study was to explore the cell-specific uterine expression of Nape-pld prior to, during and after embryo implantation. As shown in Fig. 1A, in situ hybridization shows that Nape-pld is primarily expressed in the luminal and glandular epithelia with lower levels of expression in the stroma on days 1-4 of pregnancy, indicating that the epithelium is the major source of uterine anandamide prior to implantation. With the initiation and progression of implantation and decidualization on days 5-7, the expression is primarily restricted to the luminal and glandular epithelia located at the inter-implantation region (Fig. 1A), albeit at low levels in the stroma and deciduoma at the implantation site. However on day 5 with the onset of implantation, we observed an interesting expression pattern: while Nape-pld expression is very low in the luminal epithelium close to the implanting blastocyst, the expression is much higher in the luminal epithelium further to the blastocyst within the same implantation chamber and as expected at the interimplantation site (Fig. 1A). These observations suggest a potential role of implanting embryo in regulating uterine Nape-pld expression and anandamide levels. This dynamic expression pattern of Nape-pld in the periimplantation uterus is consistent with our Northern blot experiments, showing lower levels of expression at the implantation sites in contrast to higher expression at the inter-implantation sites on days 5-7 (Fig. 1B). These results suggest that NAPE-PLD is a major player in regulating the dynamic levels of anandamide in the uterus during early pregnancy. Thus, we next examined NAPE-PLD activity and anandamide levels in the periimplantation uterus.

NAPE-PLD activity correlates with uterine anandamide levels during implantation- As previously shown (7), uterine anandamide levels fluctuate with the state of pregnancy with higher levels at the interimplantation site, but lower at the site of implantation (Fig. 1C). Because levels of FAAH expression are very low in the mouse uterus (17), we speculated that NAPE-PLD activity primarily contributes to create an anandamide gradient in the uterus during early pregnancy. Indeed, consistent with the gene expression pattern on days 5-7, NAPE-PLD enzymatic activity, as
NAPE-PLD determines the uterine anandamide levels measured by the release of anandamide or \(N\)-palmitoylethanolamine from \(^{14}\text{C}\) labelled NArPE or NPPE, respectively (14), exhibited a similar pattern showing higher activity at the interimplantation site, but lower activity at the implantation site (Fig. 1D).

Although these results are consistent with our previous findings regarding the pattern of fluctuating uterine anandamide levels during early pregnancy, we were surprised to see that overall levels of uterine anandamide are much lower in pregnant CD1 mice that we currently use at Vanderbilt University (Nashville, TN) compared with our previous observation of very high levels of uterine anandamide in the same strain of mice at Kansas University Medical Center (KUMC, Kansas City, KS) about a decade ago (7). These changes are not due to procedural differences in measuring anandamide levels, since comparable low levels of uterine anandamide were also detected in Vanderbilt-housed mice in the laboratory of Harald Schmid, a co-author of the present and earlier report (8). The major changes between these two animal facilities include differences in mouse diets, caging and bedding systems, environmental conditions, viral load and presumably water quality. More importantly, mice at Vanderbilt are housed in a pathogen-free environment as compared to conventional environment that prevailed a decade ago at KUMC. Although we do not know whether higher uterine anandamide levels would have correlated with elevated NAPE-PLD activity in our earlier studies, it is possible that NAPE-PLD expression and/or activity are sensitive to varying experimental and environmental conditions. Nonetheless, our current results provide direct molecular and biochemical evidence that NAPE-PLD is a major determinant of uterine anandamide levels during implantation. This tight regulation of NAPE-PLD in the uterus perhaps contributes to the biphasic anandamide signaling during implantation (7,10). Ovarian estrogen and progesterone are the primary regulators of uterine receptivity for implantation in mice (22). To better understand the physiological relevance of differential anandamide gradients during early pregnancy, we asked whether uterine NAPE-PLD is regulated by these steroids.

**Estrogen and progesterone downregulate uterine Nape-pld expression**- Since steroid hormonal profiles are complex during early pregnancy in mice, we surmised that the expression of this gene is influenced by ovarian steroid hormones, estrogen and progesterone (P4) singly or in combination. To address this question, we examined *Nape-pld* expression in a defined system, *i.e.* in ovariectomized mice after steroid hormone treatment. **Quantitative RT-PCR analysis** of total uterine RNA was performed to determine the expression of *Nape-pld* in response to estradiol-17\(\beta\) (E\(_2\)) and/or P4. We observed that E\(_2\), P4, or a combined injection of E\(_2\) plus P4 downregulates the expression of *Nape-pld* both at 6 h and 24 h, albeit little less inhibition by P4 alone (Fig.
NAPE-PLD determines the uterine anandamide levels

2A). These results suggest that ovarian steroid hormones individually or in combination influence the expression of Nape-pld.

Our next goal was to examine whether P₄ and estrogen regulation of Nape-pld is mediated via their cognate nuclear receptors. We used mice lacking nuclear estrogen receptor-α (ERα) or progesterone receptor (PR) to further address this question. While the inhibitory effects of P₄ remained unaltered in ERα(-/-) mice, the downregulation of Nape-pld that we observed in E₂-treated wild-type mice remained virtually unaltered (Fig. 2B). On the other hand, downregulation of Nape-pld expression was not observed when the ovariectomized PR(-/-) females were given an injection of P₄; however, downregulation by E₂ persisted in PR(-/-) females (Fig. 2B). These results provide genetic evidence that Nape-pld is regulated by ovarian steroid hormones via their nuclear receptors.

**Uterine Nape-pld expression and activity are correlated with uterine receptivity for implantation**—In mice, the prereceptive, receptive, and nonreceptive phases of the uterus during pregnancy or pseudopregnancy are sequentially programmed by ovarian P₄ and estrogen (22). During normal pregnancy, blastocysts implant only in the receptive uterus within a limited time span known as the "window" of uterine receptivity for implantation. In mice, the P₄-primed uterus becomes receptive on day 4 when it is superimposed with preimplantation ovarian estrogen secretion (18). The receptive uterus then automatically proceed to the nonreceptive state on day 6 when blastocysts fail to implant (18,23). Various factors including cytokines, growth factors, transcription factors and lipid signaling molecules participate in these processes through autocrine, paracrine, and/or juxtacrine manner (22). With respect to endocannabinoid signaling, we provided evidence that downregulation of anandamide levels is associated with uterine receptivity, while its upregulation is correlated with uterine refractoriness to embryo implantation (6,7). Consistent with these observations, we also noted in the present study that uterine anandamide levels on day 6 of pseudopregnancy are significantly higher than those on day 4 (Fig. 3A), reinforcing the idea that the regulation of anandamide levels correlate with uterine receptivity and implantation. We then asked whether alteration in uterine Nape-pld expression accounts for the fluctuated anandamide levels during various states of uterine receptivity. As shown in Fig. 3B, Nape-pld mRNA levels, as assessed by Northern hybridization, were higher in the nonreceptive uterus on day 6 compared with the levels in the receptive uterus on day 4 of pseudopregnancy. Furthermore, this upregulated Nape-pld expression in the nonreceptive uterus was translated into higher NAPE-PLD activity. For example, a significantly higher activity was detected in the uterus on day 6 as opposed to day 4 of pseudopregnancy (Fig. 3C). This correlation of NAPE-PLD expression, activity and anandamide levels with the receptive and nonreceptive uterine phases further
NAPE-PLD determines the uterine anandamide levels

emphasizes that NAPE-PLD is an important determinant of uterine anandamide signaling during early pregnancy.

**Blastocyst's state of activity influences uterine Nape-pld expression**—A reciprocal interaction between the implantation-competent blastocyst and the receptive uterus is required for successful implantation. Our recent observation shows that uterine anandamide within a very narrow range regulates blastocyst function and implantation by differentially modulating MAPK signaling and Ca\(^{++}\) channel activity (10). It is also suggested that embryonic and/or uterine FAAH, which is activated by an unidentified embryo-derived lipid molecule, plays roles in creating optimal levels of anandamide beneficial to implantation (16,17). However, it remains unknown whether the implanting embryo influences anandamide synthesis via NAPE-PLD. Our results showing absence of Nape-pld expression in the luminal epithelium adjacent to the implantation site (Fig. 1A) suggests that this gene is locally regulated in the uterus by implanting blastocysts. Thus, we further examined the expression of this gene in a physiologically relevant delayed implantation model. In the delayed implanting uterus, blastocysts undergo dormancy and fail to implant. However, this condition is terminated by an estrogen injection with activation of dormant blastocysts, attainment of uterine receptivity and implantation (18). We observed that Nape-pld expression is much higher in the luminal epithelium surrounding the dormant blastocyst (Fig. 3D), whereas reactivation of blastocysts by estrogen significantly downregulates the expression in the adjacent luminal and stromal cells with the initiation of implantation (Fig. 3D). This finding is consistent with our results during normal implantation on day 5 (Fig. 1A).

Collectively, the present work provides molecular, biochemical and physiological evidence that uterine anandamide levels conducive to implantation are primarily regulated by dynamic expression and activity of NAPE-PLD. Thus, an aberrant uterine NAPE-PLD activity may cause implantation failure or defective implantation. This study is clinically relevant, since there is evidence that high peripheral anandamide levels due to low FAAH activity are associated with spontaneous pregnancy loss in women (24).

REFERENCES

1. Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C., and Bonner, T. I. (1990) *Nature* **346**, 561-564
2. Munro, S., Thomas, K. L., and Abu-Shaar, M. (1993) *Nature* **365**, 61-65
3. Paria, B. C., and Dey, S. K. (2000) *Chem Phys Lipids* **108**, 211-220
4. Yang, Z. M., Paria, B. C., and Dey, S. K. (1996) *Biol Reprod* **55**, 756-761
5. Paria, B. C., Das, S. K., and Dey, S. K. (1995) *Proc Natl Acad Sci U S A* **92**, 7

Downloaded from http://www.jbc.org/ on March 24, 2020
NAPE-PLD determines the uterine anandamide levels

6. Paria, B. C., Song, H., Wang, X., Schmid, P. C., Krebsbach, R. J., Schmid, H. H., Bonner, T. I., Zimmer, A., and Dey, S. K. (2001) *J Biol Chem* **276**, 20523-20528
7. Schmid, P. C., Paria, B. C., Krebsbach, R. J., Schmid, H. H., and Dey, S. K. (1997) *Proc Natl Acad Sci U S A* **94**, 4188-4192
8. Paria, B. C., Ma, W., Andreyak, D. M., Schmid, P. C., Schmid, H. H., Moody, D. E., Deng, H., Makriyannis, A., and Dey, S. K. (1998) *Biol Reprod* **58**, 1490-1495
9. Wang, J., Paria, B. C., Dey, S. K., and Armanit, D. R. (1999) *Biol Reprod* **60**, 839-844
10. Wang, H., Matsumoto, H., Guo, Y., Paria, B. C., Roberts, R. L., and Dey, S. K. (2003) *Proc Natl Acad Sci U S A* **100**, 14914-14919
11. Schmid, H. H. (2000) *Chem Phys Lipids* **108**, 71-87
12. Devane, W. A., and Axelrod, J. (1994) *Proc Natl Acad Sci U S A* **91**, 6698-6701
13. Di Marzo, V., Fontana, A., Cadas, H., Schinelli, S., Cimino, G., Schwartz, J. C., and Piomelli, D. (1994) *Nature* **372**, 686-691
14. Okamoto, Y., Morishita, J., Tsuboi, K., Tonai, T., and Ueda, N. (2004) *J Biol Chem* **279**, 5298-5305
15. Okamoto, Y., Morishita, J., Wang, J., Schmid, P. C., Krebsbach, R. J., Schmid, H. H., and Ueda, N. (2005) *Biochem J* (Mar 10, Epub ahead of print)
16. Maccarrone, M., DeFelici, M., Klinger, F. G., Battista, N., Fezza, F., Dainese, E., Siracusa, G., and Finazzi-Agro, A. (2004) *Mol Hum Reprod* **10**, 215-221
17. Paria, B. C., Zhao, X., Wang, J., Das, S. K., and Dey, S. K. (1999) *Biol Reprod* **60**, 1151-1157
18. Paria, B. C., Huet-Hudson, Y. M., and Dey, S. K. (1993) *Proc Natl Acad Sci U S A* **90**, 10159-10162
19. Kingsley, P. J., and Marnett, L. J. (2003) *Anal Biochem* **314**, 8-15
20. Wang, H., Guo, Y., Wang, D., Kingsley, P. J., Marnett, L. J., Das, S. K., DuBois, R. N., and Dey, S. K. (2004) *Nat Med* **10**, 1074-1080
21. Das, S. K., Wang, X. N., Paria, B. C., Damm, D., Abraham, J. A., Klagsbrun, M., Andrews, G. K., and Dey, S. K. (1994) *Development* **120**, 1071-1083
22. Dey, S. K., Lim, H., Das, S. K., Reese, J., Paria, B. C., Daikoku, T., and Wang, H. (2004) *Endocr Rev* **25**, 341-373
23. Song, H., Lim, H., Paria, B. C., Matsumoto, H., Swift, L. L., Morrow, J., Bonventre, J. V., and Dey, S. K. (2002) *Development* **129**, 2879-2889
24. Maccarrone, M., Valensise, H., Bari, M., Lazzarin, N., Romanini, C., and Finazzi-Agro, A. (2000) *Lancet* **355**, 1326-1329

**FOOTNOTES**

1The Abbreviations used are P₄, progesterone; E₂, estradiol-17β; CB1, brain-type cannabinoid receptor; NAE, N-acylethanolamine; PE, phosphatidylethanolamine; NAPE, N-acylphosphatidyl-ethanolamine; NAPE, N-arachidonoylphosphatidyl-ethanolamine; NPPE, N-palmitoylethanolamine- ethanamine; PLD, phospholipase D; FAAH, fatty-acid amide hydrolase; RT-PCR, reverse transcription-PCR.
NAPE-PLD determines the uterine anandamide levels

ACKNOWLEDGMENTS
We thank Dingzhi Wang, Hao Zhang and Susanne Tranguch for their assistance in quantitative RT-PCR, in situ hybridization and animal breeding, respectively. This work was supported in parts by the National Institutes of Health (NIH) Grants (DA06668, HD12304 & HD33994 to S. K. Dey and ES07814 & HD37830 to S. K. Das). S. K. Dey is recipient of Method to Extend Research in Time (MERIT) Awards from the National Institute on Drug Abuse (NIDA) and the National Institute of Child Health and Human Development (NICHD). H Wang is recipient of Solvay/Mortola Research Award from the Society for Gynecologic Investigation.

FIGURE LEGEND

FIG. 1. NAPE-PLD determines uterine anandamide levels during implantation. Uterine expression of Nape-pld was analyzed by in situ (A) and Northern (B) hybridization. Photomicrographs of representative longitudinal uterine sections are shown in panel A. Bar, 250 μm. le, luminal epithelium; ge, glandular epithelium; s, stroma; myo, myometrium; bl, blastocyst; Em, embryo; IS, implantation site; Inter-IS, inter implantation site; M, mesometrial site; AM, anti-mesometrial site. C and D, uterine anandamide levels correlate with NAPE-PLD activity during implantation. Higher anandamide levels (C) and NAPE-PLD activity (D) are noted at Inter-IS vs IS (unpaired t-test, *P<0.05 and **P<0.005).

FIG. 2. Estrogen and progesterone downregulate uterine Nape-pld expression via their nuclear receptors. Steroid hormonal regulation of uterine Nape-pld expression in ovariectomized (A) wild-type, and (B) ERα (-/-) and PR (-/-) mice were analyzed by quantitative RT-PCR. The data presented as fold induction are relative to oil treatment and are mean ± SEM of three independent RNA samples.

FIG. 3. Expression and activity of NAPE-PLD are coordinated with uterine receptivity and blastocyst activation. A-C, Nape-pld expression and activity correlate, showing elevated NAPE-PLD activity and uterine anandamide levels in the nonreceptive uterus on day 6 of pseudopregnancy compared with those in the receptive uterus on day 4 (unpaired t-test, **P<0.005). D, Blastocyst's state of activity influences uterine Nape-pld expression. Representative photomicrographs of in situ hybridization in the uterus during the delay and following estrogen activation are presented. Arrows indicate the location of blastocysts. Bar, 250 μm. le, luminal epithelium; ge, glandular epithelium; s, stroma; myo, myometrium; bl, blastocyst; IS, implantation site; Inter-IS, inter implantation site; M, mesometrial site; AM, anti-mesometrial site.
Fig. 3
N-acylphosphatidylethanolamine-hydrolyzing phospholipase D is an important determinant of uterine anandamide levels during implantation
Yong Guo, Haibin Wang, Yasuo Okamoto, Natsuo Ueda, Philip J. Kingsley, Lawrence J. Marnett, Harald H. O. Schmid, Sanjoy K. Das and Sudhansu K. Dey

*J. Biol. Chem.* published online May 12, 2005

Access the most updated version of this article at doi: 10.1074/jbc.C500168200

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