Saitohin, Which Is Nested in the tau Locus and Confers Allele-specific Susceptibility to Several Neurodegenerative Diseases, Interacts with Peroxiredoxin 6*

Received for publication, June 6, 2005, and in revised form, August 19, 2005 Published, JBC Papers in Press, September 26, 2005, DOI 10.1074/jbc.M506116200

Lei Gao†, Sze-Wah Tse§, Christopher Conrad¶, and Athena Andreadis∗†‡

From the 1Shriver Center at University of Massachusetts Medical School, Waltham, Massachusetts 02452, the 2Massachusetts General Hospital, Department of Neurology/Alzheimer’s Unit, Charlestown, Massachusetts 02129, and the 3Department of Cell Biology, University of Massachusetts Medical School, Worcester, Massachusetts 01655

Saitohin is a gene unique to humans and their closest relatives, the function of which is not yet known. Saitohin contains a single polymorphism (Q7R), and its Q and R alleles belong to the H1 and H2 tau haplotype, respectively. The Saitohin Q allele confers susceptibility to several neurodegenerative diseases. To get a handle on Saitohin function, we used it as a bait in a yeast two-hybrid screen. By this assay and subsequent co-immunoprecipitation and glutathione S-transferase pull-down assays, we discovered and confirmed that Saitohin interacts with peroxiredoxin 6, a unique member of that family that is bifunctional and the levels of which increase in Pick disease. The strength of the interaction appeared to be allelespecific, giving the first distinction between the two forms of Saitohin.

Saitohin (STH),2 an intronless gene encoding an open reading frame of 128 amino acids, is located in the intron between exons 9 and 10 of the human tau gene (1). It bears no obvious homology to any known protein or motif, and its expression pattern is very similar to that of tau in human tissues. The DNA sequences homologous to the human STH gene reveal an intact, highly conserved open reading frame in the primates most closely related to humans (chimpanzee, bonobo, and gorilla) but not in other primates or in rodents (2, 3). STH provides an evolutionary loci that separates humans and their closest relatives from other mammals.

A single nucleotide polymorphism of human STH has been identified that changes glutamine residue 7 to arginine (Q7R (1)). This polymorphism is associated with the two tau gene haplotypes: the Q allele with H1, the R allele with H2 (2, 4). The Q allele is the most common haplotype in all nonhuman primates and is homozousy for the R allele, which makes the Q allele a human-specific marker (2, 3). Hence, the R allele is the ancestral haplotype, whereas the Q allele evolved after the human lineages separated from the other primates.

In addition to evolution studies, the R allele of STH was initially found to be associated with Alzheimer disease (1), although subsequent data showing association of the R allele with Alzheimer disease have been inconsistent (2, 4–10). Recent work may have resolved the conflicting results by indicating that the Q allele is associated with Alzheimer disease specifically resulting from the ApoE4 susceptibility factor (11). Furthermore, the Q allele has been shown to be over-represented in several neurodegenerative diseases: progressive supranuclear palsy (12, 13), frontotemporal dementia (4), and Parkinson disease (14). Taken together, these results suggest that identifying a function of STH could implicate several proteins and/or pathway(s) as potential contributors in these neurodegenerative diseases as well.

To discover putative functions of STH, we used it as a bait in a yeast two-hybrid screening. We discovered that it interacts with a unique member of the peroxiredoxin family, peroxiredoxin 6 (Prdx6), which has both an antioxidant function and phospholipase activity (15, 16). In addition to its role in normal cells, Prdx6 is elevated in Pick disease (17), a disorder related to progressive supranuclear palsy and frontotemporal dementia, illustrating another potential connection to STH.

EXPERIMENTAL PROCEDURES

Yeast Two-hybrid Screening and Assays—We constructed the bait vector (pLexA/HK) from the LexA fragment of vector pHybLex/Zeo and the kanamycin region of vector pHybcI/HK using AlwNI and NotI (Invitrogen). The STH cDNA was cut with BamHI plus Klenow from pEGFP-STH and cloned 3’ to LexA in the bait vector cut with Bsp120I plus Klenow to generate bait pLexA-STH. The pLexA-STH bait was transformed into yeast strain EGY48 bearing a reporter plasmid (pSH18–34) followed by transformation of a human fetal brain library cloned in vector pG4–5 (Clontech).

We selected interactors on SD (minimal synthetic medium) yeast plates lacking histidine (bait), tryptophan (library), uracil (reporter), and leucine (interaction marker) and verified them independently by the 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) test. We rescued the plasmids of interactors by PCR with primers JG-5 and YUN (shown in TABLE ONE) and classified them by digestion of the PCR products with AluI and HinfI. Unique plasmids were cloned into vector pCR4-TOPO (Invitrogen) and sequenced in an Applied Biosystems fluorescent sequencer. The DNA sequences were analyzed using the BLAST program.

Expression Constructs of STH and Prdx6—The cDNA of Prdx6 was digested with EcoRI and XhoI from the pCR4-TOPO background and cloned into pFLAG-CMV-6c (Strategene). The STH gene was cloned into pEGFP-C1 (Clontech) and into pGEX-4T1 (Amersham Biosciences) by BamHI digests. Western blots using as primaries monoclonal antibodies against FLAG (Sigma, mouse) and GFP (Molecular Probes, rabbit) show that all constructs express proteins of the correct size at equivalent amounts (see Fig. 2, A and B).

We transformed the GST-STH fusion plasmids into BL21(DE3)/RIPL cells (Strategene) and produced recombinant protein according to...
the Novagen instructions. Coomassie Blue staining shows that the fusion protein is expressed and stable (see Fig. 3 A).

Site-directed Mutagenesis—We created both the 7Q and the 7R STH alleles and two mutants of Prdx6 that affect its functions (S32A and C47S (16)) by site-directed mutagenesis using the QuikChange kit (Stratagene) as described previously (18). The mutagenic primers are shown in TABLE ONE.

**Cell Culture, Transfections, and RNA Preparation**—COS (monkey kidney) cells were maintained in Dulbecco’s modified Eagle’s medium plus 10% fetal calf serum. SKN (human neuroblastoma) cells were maintained in minimum Eagle’s medium, 10% fetal calf serum, and 1 × minimal essential amino acids. Plasmid DNA was prepared by Qiagen Tips-50s and introduced into cells by lipofection (LT1, Panvera). SP/10L has been described previously (18). We used 3 μg of each plasmid for transfections. Total RNA was isolated by the TRIzol method (Invitrogen).

Reverse Transcription and PCR Reactions—For analysis of tau construct RNA, we performed reverse transcription and reverse transcription-PCR as described previously (18) using the primer pair SPL-LS/SPL-LN (TABLE ONE). The isoform ratio was calculated by scanning the bands from three independent transfections using the One-Dscan program and the Scanalytics IPLab software.

For analysis of endogenous Prdx6 levels, we used the Ambion quantitative method. 1 μg of poly(A)+ RNA (Clontech) from fetal brain, adult cortex, cerebellum, hippocampus, spinal cord, skeletal muscle, and heart was reverse-transcribed with RNase H- Superscript II (Invitrogen) in 20 μl for 1 h at 42 °C. 1 μl of this mix was then added to one Ready-to-Go PCR bead (Amersham Biosciences) in a volume of 25 μl, and the mixture was amplified for 25 cycles. The PCR conditions were: denaturation 94 °C/1 min, annealing 60 °C/1 min, extension 72 °C/1 min. The primer pair was Prdx6-PS/Prdx6-DN, and a ratio of 3:7 18 S primers to 18 S competimers (Ambion) was used as an internal control.

Co-immunoprecipitations—EGFP-STH and FLAG-Prdx6 were cotransfected in COS and SKN cells. After 48 h, we prepared crude protein lysates using lysis buffer (50 mM Tris HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, and 1% Triton X-100). 100 μl of cell lysate was mixed with 40 μl of anti-FLAG agarose beads (Sigma) and incubated overnight at 4 °C. The beads were washed four times with 1 ml of wash buffer (50 mM Tris HCl, pH 7.4, 150 mM NaCl), mixed with 50 μl of loading buffer, boiled for 10 min, run on SDS-PAGE, and transferred to nitrocellulose filters (Osmonics). The filters were probed with anti-GFP antibody (Molecular Probes).

GST Pull-downs—COS cells transfected with FLAG-Prdx6 were grown for 48 h, and cell extracts were prepared as described above. GST-STH was expressed in *Escherichia coli* BL21 and bound to glutathione-agarose beads (Novagen). 50 μl of the COS cell extracts were incubated with the beads bearing immobilized GST-STH fusion protein overnight at 4 °C. After washing, the beads were mixed with 50 μl of loading buffer, boiled 10 min, run on SDS-PAGE, and transferred to nitrocellulose filters (Osmonics). The filters were probed with anti-GFP antibody (Sigma).

**Immunocytochemistry**—COS and SKN cells co-transfected with EGFP or EGFP-STH and FLAG-Prdx6 were grown on slides. 24 h after the change of medium, cells were rinsed three times with PBS and then fixed in PBS containing 4% paraformaldehyde at 37 °C for 20 min. After fixation, we rinsed the cells once with PBS, permeabilized them with PBS containing 0.3% Triton X-100 and 0.3% glutaraldehyde for 5 min at room temperature, and blocked them in PBS containing 10% normal goat serum at room temperature for 1 h.

After blocking, cells were incubated with mouse-anti-FLAG (1:500, Sigma) and rabbit-anti-GFP (1:500, Molecular Probes) for 1 h at room temperature, washed with PBS 5 × 5 min, and then double-stained with Alexa Fluor 488 goat-anti-rabbit IgG (1:500, Molecular Probes) and Alexa Fluor 555 goat-anti-mouse IgG (1:500, Molecular Probes) for 1 h at room temperature. All antibodies were diluted in blocking solution. The slides were then washed 5 × 5 min with PBS, drained, dried, mounted with Gel Mount (Biomedia), and observed under a fluorescent microscope.

**RESULTS**

*Prdx6 Interacts with STH in a Yeast Two-hybrid Assay*—To isolate proteins that interact with STH, we performed a yeast two-hybrid screen using LexA-STH as bait to screen a human fetal brain library. One of the clones identified in the yeast two-hybrid assay contained the complete cDNA of peroxiredoxin 6. GST pull-downs—COS cells transfected with FLAG-Prdx6 were grown for 48 h, and cell extracts were prepared as described above. GST-STH was expressed in *Escherichia coli* BL21 and bound to glutathione-agarose beads (Novagen). 50 μl of the COS cell extracts were incubated with the beads bearing immobilized GST-STH fusion protein overnight at 4 °C. After washing, the beads were mixed with 50 μl of loading buffer, boiled 10 min, run on SDS-PAGE, and transferred to nitrocellulose filters (Osmonics). The filters were probed with anti-GFP antibody (Molecular Probes).

**TABLE ONE**

| Name | Strand | Location | Length | Sequence |
|------|--------|----------|--------|----------|
| JG-S | S      | In JG4–5 vector | 24 | GTTAACGTACCAGCCTGGTGTG |
| YUN | A      | In JG4–5 vector | 20 | GGCGATCCGGTATAGGTTG |
| SPL-LS | S | In SPL3 vector | 27 | TCTGATGTCACCTGGACAACCTCAAAGG |
| SPL-LN | A | In SPL3 vector | 27 | ATCTCACTGTATTTGTGGACAGGGAAG |
| Prdx6-PS | S | Starts on Prdx6 ATG | 24 | ATGCCGGAGGTCTGCTTCTCGGG |
| Prdx6-DN | A | Ends on Prdx6 TAA | 24 | TTAAGGCTGGGGTGATAGCAGGGAAG |
| Point mutation constructs* | | | | |
| STH(Q7R)S | S | nt 20 of STH cDNA | 20 | GGTGGAGGCCAGGATCATAG |
| Prdx6(S32A)S | S | nt 94 of Prdx6 cDNA | 21 | CTTGGGAGACAGCCATGGGAT |
| Prdx6(C47S)S | S | nt 140 of Prdx6 cDNA | 21 | ACCCCAGTTGATCCACACAGG |

* For creating point mutations, we used pairs of these primers and their reverse complements (not shown). Changes from the wild type are in bold. Fig. 1 B shows the two residues altered in Prdx6. S = sense, A = antisense; nt = nucleotides.
and C47S) were co-transfected into COS cells. Fig. 2, A and B, show that all fusion proteins express stable full-length proteins at equivalent amounts.

We incubated cell lysates with anti-FLAG agarose and analyzed the immunoprecipitates by anti-GFP antibody in Western blots. The supernatants (not shown) contained equal amounts of STH. The Q version of STH interacts with all three Prdx6 variants, although the interaction with mutant S32A is slightly weaker (Fig. 2C). The R version of STH also interacts with all three Prdx6 variants, with the two mutants showing slightly stronger interaction than the wild type (Fig. 2D).

To further confirm the interaction between STH and Prdx6, we performed GST pull-down assays. We expressed GST-STH fusion proteins of both Q and R alleles (Fig. 3A) and bound them to glutathione beads. We then incubated the GST-STH bound to beads with cell lysates transfected with FLAG-Prdx6 (wild type and both mutants) and performed immunoblotting of the material retained by the beads (after washing) using anti-FLAG antibody.

Both the Q and the R alleles of STH brought down Prdx6, although they showed some differences. The R allele seemed to interact with all Prdx6 variants more strongly than the Q allele, but the relative strength of interaction was the same for both Q and R; C47S is the strongest, and S32A is the weakest (Fig. 3B). The weaker interaction of S32A correlates with the slightly reduced interaction also seen in immunoprecipitations (Fig. 2C). However, the differences in the GST pull-down were exaggerated when compared with those seen in cells, suggesting that the interaction may partly depend on post-translational modifications absent in bacterial cells.

**STH Co-localizes with Prdx6 in Cells**—To examine the localization of the two interacting proteins in cells, we co-transfected EGFP-STH and FLAG-Prdx6 into COS (Fig. 4A) and SKN (Fig. 4B) cells and tracked them using immunofluorescence. In both COS and SKN cells, Prdx6 localizes mostly in the cytoplasm, showing a mesh-like distribution with higher concentration around the nucleus (Fig. 4, A and B, left panels). There are also a few focus-like areas in the nucleus. The distribution is consistent with results from other groups, who report that Prdx6 is found predominantly in the cytoplasm but that some also localizes to the nucleus (23). STH shows a distribution similar in location and pat-
tern to that of Prdx6 in COS cells (Fig. 4, A and B, middle panel). The merged scan (Fig. 4, A and B, right panel) shows that the two indeed largely overlap.

**STH Combined with Prdx6 Activates Splicing of tau Exon 10—STH is nested in the tau locus, its expression profile is congruent with that of tau (1), and its Q allele confers susceptibility to several diseases (2–10) that often show errors in tau splicing regulation (24, 25). For all these reasons, we were curious to see whether STH, alone or in combination with its Prdx6 ligand, influences tau exon 10, which is misspliced in several neurodegenerative diseases (24, 25).

STH increases inclusion of exon 10 by itself, the Q allele weakly, and the R allele moderately (Fig. 5, lanes 2 and 3, respectively). Wild-type or mutant S32A Prdx6 does not alter inclusion of exon 10 (lanes 4 and 7), but mutant C47S moderately increases exon 10 inclusion (lane 10). When combined, STH and Prdx6 strongly activated exon 10 inclusion independently of whether Prdx6 is wild-type or mutant. The distinction between Q and R is retained; in all combinations, the Q allele is a weaker activator (lanes 5, 8, and 11) than its R counterpart (lanes 6, 9, and 12). Interestingly, the C47S Prdx6 mutant, which interacts most strongly with STH, also showed the highest relative activation of exon 10 splicing.

**DISCUSSION**

Given its location, restricted evolutionary profile, and allele-specific correlations with neurodegenerative diseases, Saitohin is a truly intriguing molecule. Because of its shortness and lack of obvious motifs, its function has been elusive. Our study has identified an STH interactor that for the first time highlights molecular differences between the two STH alleles and gives some preliminary clues to the possible role(s) that STH may play in the cell.

There are six peroxiredoxins in mammals, with broad but distinct expression ranges (23). They are antioxidant enzymes that protect cells from damage caused by reactive oxygen species (19, 26–28). Prdx6 is mostly cytosolic but is also found in the nucleus (23). Its promoter is regulated by factors responding to oxidative stress (21, 26, 29).

Several characteristics of Prdx6 make it unique. Prdx6 is the longest peroxiredoxin and has a unique C-terminal domain used for dimerization (15, 30, 31) and for nuclear targeting (19, 28). Although the other peroxiredoxins have two cysteine residues, Prdx6 has only one (at position 47, changed in mutant C47S, which lacks peroxidase activity). It is also bifunctional; in addition to its peroxidase activity, it has a Ca²⁺-independent phospholipase A2 activity (15, 16). This activity has been localized to residue 32, identified by mutant S32A.

Levels of A2 phospholipases increase during neurodegeneration (32). Specifically, alterations in Prdx6 levels are associated with Pick disease, dementia with Lewy body disease, and sporadic Creutzfeldt-Jacob disease (17, 33, 34). Most interesting in connection with brain function is the finding that Prdx6 is elevated in Pick disease, a type of neurodegeneration related to progressive supranuclear palsy and frontotemporal dementia, both of which correlate with the STH Q allele (4, 12, 13). Quite intriguingly, all these types of dementia are tangle-only tauopathies. Moreover, some familial Pick disease and frontotemporal dementia pedigrees result directly from changes in ratios of tau exon 10 (24, 25), which are apparently also affected by STH and Prdx6 (Fig. 5).

Our results showed that Prdx6 interacts with STH and that the two STH alleles show slight differences in this interaction. STH 7R interacted better with both mutants when compared with wild-type Prdx6. Prdx6 mutant S32A, which abolishes phospholipase activity (16), is the weakest interactor of STH 7Q in cells and in vitro. Additionally, STH and Prdx6 together (particularly the 7R and C47S variants, respectively) significantly increased splicing of tau exon 10 by a yet undefined mechanism.

The evidence is circumstantial but highly suggestive that STH, through its Prdx6 ligand, may be linked to the cascade of events that lead from tau splicing to neurodegeneration. Chimpanzees, which exclusively have the R STH allele, appear resistant to neurodegeneration, whereas the Q allele confers susceptibility to several tangle-only dementias. However, the picture is complicated by the fact that in humans, the R allele may be connected to the etiology of Alzheimer disease, which could correlate with its relative rarity in populations. The fact that STH Q allele is unique to humans makes it an invaluable tool to understand why dementia seems to have singled out our species for preferential treatment.

Acknowledgment—We thank Dr. Peter Davies for scientific and moral support and for helpful comments on the manuscript.

REFERENCES

1. Conrad, C., Vianna, C., Freeman, M., and Davies, P. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 7751–7756
2. Conrad, C., Vianna, C., Schultz, C., Thal, D. R., Ghebremedhin, E., Lenz, J., Braak, H., and Davies, P. (2004) J. Neurochem. 89, 179–188
3. Holzer, M., Craxton, M., Jakes, R., Arendt, T., and Goedert, M. (2004) Gene (Amst.) 341, 313–322
4. Verpille, P., Ricard, S., Hannenquin, D., Dobois, B., Boul, J., Camuzat, A., Pradier, L., Frebourg, T., Brice, A., Clerget-Darpoux, F., Delerue, J.-F., and Campion, D. (2002) Ann. Neurol. 52, 829–832
5. Combarros, O., Rodero, L., Infante, J., Pacilio, E., Liorca, J., Fernandez-Viadero, C.,
Pena, N., and Berciano, J. (2003) *Dement. Geriatr. Cogn. Disord.*, **16**, 132–135
6. Cook, L., Brayne, C. E., Easton, D., Evans, J. G., Xuereb, J., Cairns, N. J., and Rubinsztein, D. C. (2002) *Ann. Neurol.*, **52**, 690–691
7. Clark, L. N., Levy, G., Tang, M. X., Mejia-Santana, H., Ciappa, A., Tycko, B., Cote, L. J., Louis, E. D., Mayeux, R., and Marder, K. (2003) *Neurosci. Lett.*, **347**, 17–20
8. Oliveira, S. A., Martin, E. R., Scott, W. K., Nicodemus, K. K., Small, G. W., Schmechel, D. E., Doraiswamy, P. M., Roses, A. D., Saunders, A. M., Gilbert, J. R., Haines, J. L., Vance, J. M., and Pericak-Vance, M. A. (2003) *Neurosci. Lett.*, **347**, 143–146
9. Streffer, J. R., Papassotiropoulos, A., Kurosiński, P., Signorell, A., Wollmer, M. A., Tsolaki, M., Iakovidou, V., Horniš, F., Bosset, J., Gotz, J., Nitsch, R. M., and Hock, C. (2003) *J. Neurol. Neurosurg. Psychiatry*, **74**, 362–363
10. Tanahashi, H., Asada, T., and Tabira, T. (2004) *Neuroreport*, **15**, 175–179
11. Seripa, D., Matera, M. G., D’Andrea, R. P., Gravina, C., Masullo, C., Daniele, A., Bizzarro, A., Rinaldi, M., Antuono, P., Wekstein, D. R., Dal Forno, G., and Fazio, V. M. (2004) *Neurology*, **63**, 1631–1633
12. De Silva, R. D., Hope, A., Pittman, A., Weale, M. E., Morris, H. R., Wood, N. W., and Lee, A. J. (2003) *Neurology*, **61**, 407–409
13. Ezquerra, M., Campdelacreu, J., Munoz, E., Oliva, R., and Tolosa, E. (2004) *J Neurosurg. Psychiatry*, **75**, 155–157
14. Levecque, C., Elbaz, A., Clavel, J., Vidal, J. S., Amouyel, P., Alperovitch, A., Tsourio, C., and Chartier-Harlin, M. C. (2004) *J. Neurol. Neurosurg. Psychiatry*, **75**, 478–480
15. Fisher, A. B., Dodia, C., Manevich, Y., Chen J. W., and Feinstein, S. (1999) *J. Biol. Chem.*, **274**, 21326–21334
16. Chen, J. W., Dodia, C., Feinstein, S. I., Jain, M. K., and Fisher, A. B. (2000) *J. Biol. Chem.*, **275**, 28421–28427
17. Krapfenbauer, K., Ingididawork, E., Cairns, N., Fountoulakis, M., and Lubec, G. (2003) *Brain Res.*, **967**, 152–160
18. Wang, J., Gao, Q. S., Wang, Y., Lafiayı, S., Stamm, S., and Andreadis, A. (2004) *J. Neurochem.*, **88**, 1078–1090
19. Fujii, T., Fujii, J., and Taniguchi, N. (2001) *Eur. J. Biochem.*, **268**, 218–224
20. Leyens, G., Donnay, I., and Knoops, B. (2003) *Comp. Biochem. Physiol. Physiol.*, **136**, 943–955
21. Lee, T. H., Yu, S. L., Kim, S. U., Kim, Y. M., Choi, I., Kang, S. W., Rhee, S. G., and Yu, D. Y. (1999) *Gene*(Amst.), **234**, 337–344
22. Sparling, N. E., and Phelan, S. A. (2003) *Redox Rep.*, **8**, 87–94
23. Wood, Z. A., Schroeder, E., Robin Harris, J., and Poole, L. B. (2003) *Trends Biochem. Sci.*, **28**, 32–40
24. Goedert, M., and Jakes, R. (2005) *Biochim. Biophys. Acta*, **1739**, 240–250
25. Andreadis, A. (2005) *Biochim. Biophys. Acta*, **1739**, 91–103
26. Fatma, N., Singh, D. P., Shinohara, T., and Chylack, L. T. (2003) *J. Biol. Chem.*, **276**, 48899–48907
27. Wang, X., Phelan, S. A., Forsman-Semb, K., Taylor, E. F., Petros, C., Brown, A., Lerner, C. P., and Paigen, B. (2003) *J. Biol. Chem.*, **278**, 25179–25190
28. Salmon, M., Moutier, J. D. L., Wendlers, F., Chiariza, S., Eliaers, F., Remacle, J., Royer, V., Pascal, T., and Toussaint, O. (2004) *FEBS Lett.*, **557**, 26–32
29. Seh, C. K., and Packer, L. (1996) *FASEB J.*, **10**, 709–720
30. Choi, H. J., Kang, S. W., Yang, C. H., Rhee, S. G., and Ryn, S. E. (1998) *Nat. Struct. Biol.*, **5**, 400–406
31. Phelan, S. A. (1999) *Antiox. Redox Signal*, **1**, 571–584
32. Farooqui, A. A., Ong, W. Y., and Horrocks, L. A. (2004) *Neurochem. Res.*, **29**, 1961–1977
33. Krapfenbauer, K., Yoo, B. C., Fountoulakis, M., Mitrova, E., and Lubec, G. (2002) *Electrophoresis*, **23**, 2541–2547
34. Power, J. H., Shannon, J. M., Blumbergs, P. C., and Gai, W. P. (2002) *Am. J. Pathol.*, **161**, 885–894