

10. van Soest, S. S., de Wit, G. M., Essing, A. H., ten Brink, J. B., Kamphuis, W., de Jong, P. T., and Bergen, A. A. (2007) Comparison of human retinal pigment epithelium gene expression in macula and periphery highlights potential topographic differences in Bruch's membrane. *Mol. Vis.* 13, 1608-1617

11. Nishizawa, Y., Komori, N., Usukura, J., Jackson, K. W., Tobin, S. L., and Matsumoto, H. (1999) Initiating ocular proteomics for cataloging bovine retinal proteins: microanalytical techniques permit the identification of proteins derived from a novel photoreceptor preparation. *Exp. Eye. Res.* 69, 195-212

12. Alge, C. S., Suppmann, S., Priglinger, S. G., Neubauer, A. S., May, C. A., Hauck, S., Welge-Lussen, U., Ueffing, M., and Kampik, A. (2003) Comparative proteome analysis of native differentiated and cultured dedifferentiated human RPE cells. *Invest. Ophthalmol. Vis. Sci.* 44, 3629-3641

13. Cavusoglu, N., Thierse, D., Mohand-Said, S., Chalmel, F., Poch, O., Van-Dorselaer, A., Sahel, J. A., and Leveillard, T. (2003) Differential proteomic analysis of the mouse

retina: the induction of crystallin proteins by retinal degeneration in the rd1 mouse. *Mol. Cell. Proteomics*. 2, 494-505

14. West, K. A., Yan, L., Shadrach, K., Sun, J., Hasan, A., Miyagi, M., Crabb, J. S., Hollyfield, J. G., Marmorstein, A. D., and Crabb, J. W. (2003) Protein database, human retinal pigment epithelium. *Mol. Cell. Proteomics*. 2, 37-49

15. Ethen, C. M., Reilly, C., Feng, X., Olsen, T. W., and Ferrington, D. A. (2006) The proteome of central and peripheral retina with progression of age-related macular degeneration. *Invest. Ophthalmol. Vis. Sci.* 47, 2280-2290

16. Stafford, T. J. (1974) Maculopathy in an elderly sub-human primate. *Mod. Probl. Ophthalmol.* 12, 214-219

17. El-Mofty, A., Gouras, P., Eisner, G., and Balazs, E. A. (1978) Macular degeneration in rhesus monkey (*Macaca mulatta*). *Exp. Eye. Res.* 27, 499-502

18. Stafford, T. J., Anness, S. H., and Fine, B. S. (1984) Spontaneous degenerative

maculopathy in the monkey. *Ophthalmology* 91, 513-521

19. Ishibashi, T., Sorgente, N., Patterson, R., and Ryan, S. J. (1986) Pathogenesis of drusen in the primate. *Invest. Ophthalmol. Vis. Sci.* 27, 184-193

20. Monaco, W. A., and Wormington, C. M. (1990) The rhesus monkey as an animal model for age-related maculopathy. *Optom. Vis. Sci.* 67, 532-537

21. Hope, G. M., Dawson, W. W., Engel, H. M., Ulshafer, R. J., Kessler, M. J., and Sherwood, M. B. (1992) A primate model for age related macular drusen. *Br. J. Ophthalmol.* 76, 11-16

22. Nicolas, M. G., Fujiki, K., Murayama, K., Suzuki, M. T., Mineki, R., Hayakawa, M., Yoshikawa, Y., Cho, F., and Kanai, A. (1996) Studies on the mechanism of early onset macular degeneration in cynomolgus (*Macaca fascicularis*) monkeys. I. Abnormal concentrations of two proteins in the retina. *Exp. Eye. Res.* 62, 211-219

23. Nicolas, M. G., Fujiki, K., Murayama, K., Suzuki, M. T., Shindo, N., Hotta, Y., Iwata, F.,

Fujimura, T., Yoshikawa, Y., Cho, F., and Kanai, A. (1996) Studies on the mechanism of early onset macular degeneration in cynomolgus monkeys. II. Suppression of metallothionein synthesis in the retina in oxidative stress. *Exp. Eye. Res.* 62, 399-408

24. Suzuki, M. T., Terao, K., and Yoshikawa, Y. (2003) Familial early onset macular degeneration in cynomolgus monkeys (*Macaca fascicularis*). *Primates* 44, 291-294

25. Umeda, S., Ayyagari, R., Suzuki, M. T., Ono, F., Iwata, F., Fujiki, K., Kanai, A., Takada, Y., Yoshikawa, Y., Tanaka, Y., and Iwata, T. (2003) Molecular cloning of ELOVL4 gene from cynomolgus monkey (*Macaca fascicularis*). *Exp. Anim.* 52, 129-135

26. Umeda, S., Ayyagari, R., Allikmets, R., Suzuki, M. T., Karoukis, A. J., Ambasudhan, R., Zernant, J., Okamoto, H., Ono, F., Terao, K., Mizota, A., Yoshikawa, Y., Tanaka, Y., and Iwata, T. (2005) Early-onset macular degeneration with drusen in a cynomolgus monkey (*Macaca fascicularis*) pedigree: exclusion of 13 candidate genes and loci. *Invest. Ophthalmol. Vis. Sci.* 46, 683-691

27. Umeda, S., Suzuki, M. T., Okamoto, H., Ono, F., Mizota, A., Terao, K., Yoshikawa, Y.,

Tanaka, Y., and Iwata, T. (2005) Molecular composition of drusen and possible involvement of anti-retinal autoimmunity in two different forms of macular degeneration in cynomolgus monkey (*Macaca fascicularis*). *FASEB. J.* 19, 1683-1685

28. Butt, R. H., Pfeifer, T. A., Delaney, A., Grigliatti, T. A., Tetzlaff, W. G., and Coorsen, J. R. (2007) Enabling coupled quantitative genomics and proteomics analyses from rat spinal cord samples. *Mol. Cell. Proteomics.* 6, 1574-1588

29. Hauck, S. M., Schoeffmann, S., Deeg, C. A., Gloeckner, C. J., Swiatek-de Lange, M., and Ueffing, M. (2005) Proteomic analysis of the porcine interphotoreceptor matrix. *Proteomics* 5, 3623-3636

30. Wang, Y. D., Wu, J. D., Jiang, Z. L., Wang, Y. B., Wang, X. H., Liu, C., and Tong, M. Q. (2007) Comparative proteome analysis of neural retinas from type 2 diabetic rats by two-dimensional electrophoresis. *Curr. Eye. Res.* 32, 891-901

31. Sakuma, H., Inana, G., Murakami, A., Higashide, T., and McLaren, M. J. (1996) Immunolocalization of X-arrestin in human cone photoreceptors. *FEBS. Lett.* 382,

32. Melov, S. (2000) Mitochondrial oxidative stress. Physiologic consequences and potential for a role in aging. *Ann. N. Y. Acad. Sci.* 908, 219-225

33. Ji, H., Liu, Y. E., Jia, T., Wang, M., Liu, J., Xiao, G., Joseph, B. K., Rosen, C., and Shi, Y. E. (1997) Identification of a breast cancer-specific gene, BCSG1, by direct differential cDNA sequencing. *Cancer. Res.* 57, 759-764

34. Bennaars-Eiden, A., Higgins, L., Hertzell, A. V., Kapphahn, R. J., Ferrington, D. A., and Bernlohr, D. A. (2002) Covalent modification of epithelial fatty acid-binding protein by 4-hydroxynonenal in vitro and in vivo. Evidence for a role in antioxidant biology. *J. Biol. Chem.* 277, 50693-50702

35. Schevzov, G., Vrhovski, B., Bryce, N. S., Elmir, S., Qiu, M. R., O'Neill G. M., Yang, N., Verrills, N. M., Kavallaris, M., and Gunning, P. W. (2005) Tissue-specific tropomyosin isoform composition. *J. Histochem. Cytochem.* 53, 557-570

36. Sueoka, E., Goto, Y., Sueoka, N., Kai, Y., Kozu, T., and Fujiki, H. (1999) Heterogeneous nuclear ribonucleoprotein B1 as a new marker of early detection for human lung cancers. *Cancer. Res.* 59, 1404-1407
37. Stone, J. R., and Collins, T. (2002) Rapid phosphorylation of heterogeneous nuclear ribonucleoprotein C1/C2 in response to physiologic levels of hydrogen peroxide in human endothelial cells. *J. Biol. Chem.* 277, 15621-15628
38. Hall, S. W., and Kuhn, H. (1986) Purification and properties of guanylate kinase from bovine retinas and rod outer segments. *Eur. J. Biochem.* 161, 551-556
39. Farber, D. B. (1995) From mice to men: the cyclic GMP phosphodiesterase gene in vision and disease. The Proctor Lecture. *Invest. Ophthalmol. Vis. Sci.* 36, 263-275
40. Surguchov, A., McMahan, B., Masliah, E., and Surgucheva, I. (2001) Synucleins in ocular tissues. *J. Neurosci. Res.* 65, 68-77
41. George, J. M. (2002) The synucleins. *Genome. Biol.* 3, REVIEWS3002

42. Ninkina, N., Papachroni, K., Robertson, D. C., Schmidt, O., Delaney, L., O'Neill, F., Court, F., Rosenthal, A., Fleetwood-Walker, S. M., Davies, A. M., and Buchman, V. L. (2003) Neurons expressing the highest levels of gamma-synuclein are unaffected by targeted inactivation of the gene. *Mol. Cell. Biol.* 23, 8233-8245

43. Surgucheva, I., Ninkina, N., Buchman, V. L., Grasing, K., and Surguchov, A. (2005) Protein aggregation in retinal cells and approaches to cell protection. *Cell. Mol. Neurobiol.* 25, 1051-1066

44. Surguchov, A., Surgucheva, I., Solessio, E., and Baehr, W. (1999) Synoretin--A new protein belonging to the synuclein family. *Mol. Cell. Neurosci.* 13, 95-103

45. Snyder, H., Mensah, K., Hsu, C., Hashimoto, M., Surgucheva, I. G., Festoff, B., Surguchov, A., Masliah, E., Matouschek, A., and Wolozin, B. (2005) beta-Synuclein reduces proteasomal inhibition by alpha-synuclein but not gamma-synuclein. *J. Biol. Chem.* 280, 7562-7569

46. Medrano, C. J., and Fox, D. A. (1995) Oxygen consumption in the rat outer and inner retina: light- and pharmacologically-induced inhibition. *Exp. Eye. Res.* 61, 273-284

47. Hoang, Q. V., Linsenmeier, R. A., Chung, C. K., and Curcio, C. A. (2002) Photoreceptor inner segments in monkey and human retina: mitochondrial density, optics, and regional variation. *Vis. Neurosci.* 19, 395-407

48. Colombini, M. (1989) Voltage gating in the mitochondrial channel, VDAC. *J. Membr. Biol.* 111, 103-111

49. Adams, V., Griffin, L., Towbin, J., Gelb, B., Worley, K., and McCabe, E. R. (1991) Porin interaction with hexokinase and glycerol kinase: metabolic microcompartmentation at the outer mitochondrial membrane. *Biochem. Med. Metab. Biol.* 45, 271-291

50. Rivera, A., Fisher, S. A., Fritsche, L. G., Keilhauer, C. N., Lichtner, P., Meitinger, T., and Weber, B. H. (2005) Hypothetical LOC387715 is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk. *Hum. Mol. Genet.* 14, 3227-3236

51. Dewan, A., Liu, M., Hartman, S., Zhang, S. S., Liu, D. T., Zhao, C., Tam, P. O., Chan, W. M., Lam, D. S., Snyder, M., Barnstable, C., Pang, C. P., and Hoh, J. (2006) HTRA1 promoter polymorphism in wet age-related macular degeneration. *Science* 314, 989-992
52. Yang, Z., Camp, N. J., Sun, H., Tong, Z., Gibbs, D., Cameron, D. J., Chen, H., Zhao, Y., Pearson, E., Li, X., Chien, J., Dewan, A., Harmon, J., Bernstein, P. S., Shridhar, V., Zabriskie, N. A., Hoh, J., Howes, K., and Zhang, K. (2006) A variant of the HTRA1 gene increases susceptibility to age-related macular degeneration. *Science* 314, 992-993
53. Kanda, A., Chen, W., Othman, M., Branham, K. E., Brooks, M., Khanna, R., He, S., Lyons, R., Abecasis, G. R., and Swaroop, A. (2007) A variant of mitochondrial protein LOC387715/ARMS2, not HTRA1, is strongly associated with age-related macular degeneration. *Proc. Natl. Acad. Sci. U S A* 104, 16227-16232
54. Fritsche, L. G., Loenhardt, T., Janssen, A., Fisher, S. A., Rivera, A., Keilhauer, C. N., and Weber, B. H. (2008) Age-related macular degeneration is associated with an unstable ARMS2 (LOC387715) mRNA. *Nat. Genet.* 40, 892-896

55. Liu, Y., Fiskum, G., and Schubert, D. (2002) Generation of reactive oxygen species by the mitochondrial electron transport chain. *J. Neurochem.* 80, 780-787
56. Winkler, B. S., Boulton, M. E., Gottsch, J. D., and Sternberg, P. (1999) Oxidative damage and age-related macular degeneration. *Mol. Vis.* 5, 32
57. Imamura, Y., Noda, S., Hashizume, K., Shinoda, K., Yamaguchi, M., Uchiyama, S., Shimizu, T., Mizushima, Y., Shirasawa, T., and Tsubota, K. (2006) Drusen, choroidal neovascularization, and retinal pigment epithelium dysfunction in SOD1-deficient mice: a model of age-related macular degeneration. *Proc. Natl. Acad. Sci. U S A* 103, 11282-11287
58. Justilien, V., Pang, J. J., Renganathan, K., Zhan, X., Crabb, J. W., Kim, S. R., Sparrow, J. R., Hauswirth, W. W., and Lewin, A. S. (2007) SOD2 knockdown mouse model of early AMD. *Invest. Ophthalmol. Vis. Sci.* 48, 4407-4420
59. Perkins, G. A., Ellisman, M. H., and Fox, D. A. (2003) Three-dimensional analysis of

mouse rod and cone mitochondrial cristae architecture: bioenergetic and functional implications. *Mol. Vis.* 9, 60-73

60. Barron, M. J., Johnson, M. A., Andrews, R. M., Clarke, M. P., Griffiths, P. G., Bristow, E., He, L. P., Durham, S., and Turnbull, D. M. (2001) Mitochondrial abnormalities in ageing macular photoreceptors. *Invest. Ophthalmol. Vis. Sci.* 42, 3016-3022

61. Hopper, R. K., Carroll, S., Aponte, A. M., Johnson, D. T., French, S., Shen, R. F., Witzmann, F. A., Harris, R. A., and Balaban, R. S. (2006) Mitochondrial matrix phosphoproteome: effect of extra mitochondrial calcium. *Biochemistry* 45, 2524-2536

62. Li, Y., Huang, T. T., Carlson, E. J., Melov, S., Ursell, P. C., Olson, J. L., Noble, L. J., Yoshimura, M. P., Berger, C., Chan, P. H., Wallace, D. C., and Epstein, C. J. (1995) Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat. Genet.* 11, 376-381

63. Sandbach, J. M., Coscun, P. E., Grossniklaus, H. E., Kokoszka, J. E., Newman, N. J., and Wallace, D. C. (2001) Ocular pathology in mitochondrial superoxide dismutase

(Sod2)-deficient mice. *Invest. Ophthalmol. Vis. Sci.* 42, 2173-2178

64. Kapphahn, R. J., Giwa, B. M., Berg, K. M., Roehrich, H., Feng, X., Olsen, T. W., and Ferrington, D. A. (2006) Retinal proteins modified by 4-hydroxynonenal: identification of molecular targets. *Exp. Eye. Res.* 83, 165-175

65. Kingma, P. B., Bok, D., and Ong, D. E. (1998) Bovine epidermal fatty acid-binding protein: determination of ligand specificity and cellular localization in retina and testis. *Biochemistry* 37, 3250-3257

66. Krecic, A. M., and Swanson, M. S. (1999) hnRNP complexes: composition, structure, and function. *Curr. Opin. Cell. Biol.* 11, 363-371

67. Dreyfuss, G., Kim, V. N., and Kataoka, N. (2002) Messenger-RNA-binding proteins and the messages they carry. *Nat. Rev. Mol. Cell. Biol.* 3, 195-205

68. Kattapuram, T., Yang, S., Maki, J. L., and Stone, J. R. (2005) Protein kinase CK1 α regulates mRNA binding by heterogeneous nuclear ribonucleoprotein C in

response to physiologic levels of hydrogen peroxide. *J. Biol. Chem.* 280, 15340-15347

69. Rajagopalan, L. E., Westmark, C. J., Jarzembowski, J. A., and Malter, J. S. (1998)

hnRNP C increases amyloid precursor protein (APP) production by stabilizing APP mRNA. *Nucleic Acids Res.* 26, 3418-3423

70. White, R., Gonsior, C., Kramer-Albers, E. M., Stohr, N., Huttelmaier, S., and Trotter, J.

(2008) Activation of oligodendroglial Fyn kinase enhances translation of mRNAs transported in hnRNP A2-dependent RNA granules. *J. Cell. Biol.* 181, 579-586

71. Houle, F., Rousseau, S., Morrice, N., Luc, M., Mongrain, S., Turner, C. E., Tanaka, S.,

Moreau, P., and Huot, J. (2003) Extracellular signal-regulated kinase mediates phosphorylation of tropomyosin-1 to promote cytoskeleton remodeling in response to oxidative stress: impact on membrane blebbing. *Mol. Biol. Cell.* 14, 1418-1432

72. Liu, Q., Tan, G., Levenkova, N., Li, T., Pugh, E. N., Jr., Rux, J. J., Speicher, D. W., and

Pierce, E. A. (2007) The proteome of the mouse photoreceptor sensory cilium complex. *Mol. Cell. Proteomics.* 6, 1299-1317

73. Folkman, J. (1971) Tumor angiogenesis: therapeutic implications. *N. Engl. J. Med.* 285, 1182-1186

74. MacDonald, N. J., Shivers, W. Y., Narum, D. L., Plum, S. M., Wingard, J. N., Fuhrmann, S. R., Liang, H., Holland-Linn, J., Chen, D. H., and Sim, B. K. (2001) Endostatin binds tropomyosin. A potential modulator of the antitumor activity of endostatin. *J. Biol. Chem.* 276, 25190-25196

75. Stamm, S., Casper, D., Lees-Miller, J. P., and Helfman, D. M. (1993) Brain-specific tropomyosins TMBr-1 and TMBr-3 have distinct patterns of expression during development and in adult brain. *Proc. Natl. Acad. Sci. U S A* 90, 9857-9861

76. Vrhovski, B., Schevzov, G., Dingle, S., Lessard, J. L., Gunning, P., and Weinberger, R. P. (2003) Tropomyosin isoforms from the gamma gene differing at the C-terminus are spatially and developmentally regulated in the brain. *J. Neurosci. Res.* 72, 373-383

77. Cooper, J. A. (2002) Actin dynamics: tropomyosin provides stability. *Curr. Biol.* 12,

R523-525

78. Williams, D. S. (1991) Actin filaments and photoreceptor membrane turnover.

Bioessays 13, 171-178

79. Kennedy, C. J., Rakoczy, P. E., and Constable, I. J. (1995) Lipofuscin of the retinal pigment epithelium: a review. *Eye* 9 (Pt 6), 763-771

Figure legends

Figure 1

Resolution of monkey retina proteins on 2D gels stained with SYPRO Ruby (pH3-10)

Peripheral retina and macula extracted proteins (300 μ g each) were isoelectric focused on pH3-10 IPG strip and then separated on 12% SDS-page gels. Spots identified by LC-MS/MS are marked by spot numbers.

Figure 2

Resolution of monkey retina proteins on 2D gels stained with SYPRO Ruby (pH 4-7).

Peripheral retina and macula extract proteins (300 μ g each) were isoelectric focused on pH4-7 IPG strip. Spots identified by LC-MS/MS are marked by the spot number.

Figure 3

Resolution of monkey retina proteins on 2D gels stained with SYPRO Ruby (pH5-8).

Peripheral retina and macula extract proteins (300 μ g each) were isoelectric focused on pH5-8 IPG strip. Spots identified by LC-MS/MS are marked by the spot number.

Figure 4

Resolution of monkey retina proteins on 2D gels stained with SYPRO Ruby (pH7-10).

Peripheral retina and macula extract proteins (300 μ g each) were isoelectric focused on pH 7-10. Spots identified by LC-MS/MS are marked by the spot number.

Figure 5

Western blot of 8 proteins.

Five micrograms of each sample from the peripheral retina and macula were loaded onto SDS-page gel (for γ -synuclein 15 μ g loading). After transferring to PVDF membrane, the

proteins were detected with antibodies specific to Arrestin-C (1), Mn-SOD (2), γ -synuclein (3), E-FABP (4), Tropomyosin1a Br-1,Br-3 (5), Tropomyosin1a TM311 (6), hnRNPs A2/B1 (7), and hnRNPs C1/C2 (8). Lane P; peripheral retina, Lane M; macula.

Figure 6

2D western blotting of Mn-SOD.

Thirty microgram of each sample from the peripheral retina and macula were separated by 2D gel electrophoresis. After transferring to PVDF membrane, the proteins were detected with antibodies specific to Mn-SOD.

Figure 7

Tissue localization of macula enriched proteins.

Four micrometer paraffin section of monkey eye was stained with hematoxylin and eosin (A), other sections were labeled with antibodies specific to Arrestin-C (B), Mn-SOD (C), γ -synuclein (D), E-FABP (E), Tropomyosin Br-1, Br-3 (F), TM311 to Tropomyosin (G), hnRNPs A2/B1 (H), and hnRNPs C1/C2 (I). GCL, ganglion cell layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; PIS, photoreceptor inner segment; POS, photoreceptor outer segment; RPE, retinal pigment epithelial; Ch,

choroid. (Bar; 50 μ)

Figure 1

