Journal of Bioenergetics and Biomembranes, Vol. 36, No. 4, August 2004 (© 2004)

Mitochondrial Degeneration in Amyotrophic Lateral Sclerosis

Zuoshang Xu,[1,6] Cheowha Jung,[2] Cynthia Higgins,[3] John Levine,[4] and Jiming Kong[5]

Received March 12, 2004; accepted May 7, 2004

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder that causes motor neuron degeneration, progressive skeletal muscle atrophy, paralysis, and death. To understand the mechanism of motor neuron degeneration, we have analyzed the clinical disease progression and the pathological changes in a transgenic mouse model for ALS. We found massive mitochondrial vacuolation at the onset of disease. By detailed morphological observations, we have determined that this mitochondrial vacuolation is developed from expansion of mitochondrial intermembrane space and extension of the outer membrane and involves peroxisomes. Lysosomes do not actively participate at all stages of this vacuolation. We conclude that this mitochondrial vacuolation is neither classical mitochondrial permeability transition nor autophagic vacuolation. Thus, this appears to be a new form of mitochondrial vacuolation and we term this as mitochondrial vacuolation by intermembrane space expansion or MVISE.

KEY WORDS: Mitochondria; mitochondrion; amyotrophic lateral sclerosis; motor neuron; motoneuron; spinal cord; neurodegenerative disease.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is an age-dependent neurodegenerative disease that causes motor neuron degeneration, progressive skeletal muscle atrophy, paralysis, and death (Rowland and Shneider, 2001). The etiology in most cases is not clear. Studies have implicated redox imbalance, protein aggregation, cytoskeleton disorganization, defective axonal transport, and chronic ischemia playing a role in motor neuron death (Cleveland and Rothstein, 2001). Environmental toxins and lack of dietary vitamin E have also been suggested to cause this disease (Cox *et al*., 2003; De la Rua-Domenech *et al*., 1997).

One crucial question in understanding the disease mechanism is how these different noxious assaults lead to motor neuron degeneration. Are there common, converging cellular pathways? Early studies on humans were limited to pathological examination of autopsy specimens. Those studies revealed gigantic neurofilament swellings in proximal axons (Carpenter, 1968). For some years the neurofilament abnormalities had been the focus for studying the disease mechanism (Hirano, 1991).

In 1993, the first genetic cause for ALS was discovered (Rosen *et al*., 1993). Mutations in Cu, Zn superoxide dismutase (SOD1) cause a subset of familial ALS. Soon after, several animal models for ALS were constructed by expressing the disease-associated SOD1 mutants in transgenic mice (Bruijn *et al*., 1997; Gurney, 1994; Ripps *et al*., 1995; Wong *et al*., 1995). These transgenic mice have allowed studies of motor neuron degeneration process in unprecedented detail. It is from these studies that mitochondrial degeneration and dysfunction have emerged as an important early event in motor neuron degeneration process. This review summarizes efforts from our laboratory in characterizing mitochondrial degeneration in an ALS animal model. Readers who are interested in learning

[1] Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, 364 Plantation St, Worcester, Massachusetts 01605.
[2] Department of Biological Sciences, University of Massachusetts, University Avenue, Lowell, Massachusetts.
[3] Department of Neurology, University of Massachusetts Medical School, Worcester, Massachusetts.
[4] Department of Child Psychiatry, Cambridge Hospital, Harvard Medical School, Cambridge, Massachusetts.
[5] Department of Human Anatomy and Cell Science, University of Manitoba, Winnipeg, Canada.
[6] To whom correspondence should be addressed; e-mail: zuoshang.xu@umassmed.edu.

0145-479X/04/0800-0395/0

more about mitochondrial degeneration in ALS are referred to several broad reviews that are published recently (Julien, 2001; Menzies *et al*., 2002b).

MITOCHONDRIAL DEGENERATION IS AN EARLY EVENT IN MOTOR NEURON DEGENERATION PATHWAY

Early observations on mutant SOD1 transgenic mice reported mitochondrial vacuolation as well as neurofilament accumulation in motors neurons (Dal Canto and Gurney, 1995; Julien, 2001; Wong *et al*., 1995). The neurofilament pathology was expected but the severe mitochondrial abnormalities were a surprise. Were these pathologies revealing crucial steps in motor neuron degeneration pathway or were they simply representing byproducts of degenerative process—a mere consequence of cellular degeneration? This question motivated us to conduct a systematic study. We reasoned that abnormalities that occurred early in the disease process were more likely to contribute to the progression of the degeneration while the abnormalities that appeared late in the disease stages, when substantial neuronal death had already been well under way, were more likely to be a consequence of early degenerative process. The early events are also more intresting in terms of therapeutic intervention, because stopping disease progression early in its track enhances the probability of motor neurons being rescued. Thus, a study that correlates the clinical progression and the sequence of pathological events (the kind of study that is impossible to conduct in humans) would be particularly revealing.

We conducted our studies in a low expresser line of mutant SOD1G93A that was generously made available by Gurney *et al*. (1994). Because the onset and progression of the disease in the mutant SOD1 transgenic animals were heterogeneous, we first used a behavioral assay to determine the process of clinical disease progression. By this assay the disease progression was divided into four stages according to the relative muscle strength measured from the mice: a premuscle weakness (PMW) stage during which the muscle strength remained steady in mutant mice and was indistinguishable from wild-type mice; a rapid declining (RD) stage during which the muscle strength declined suddenly and precipitously; a slow declining (SD) stage during which the muscle strength declined gradually in a prolonged period; and finally the paralysis stage during which one or multiple limbs became totally immobile (Kong and Xu, 1998; Fig. 1). Interestingly, similar patterns of clinical progression, particularly the rapid decline of muscle strength at the onset of ALS, have also been reported in human longitudinal observations (Aggarwal and Nicholson, 2002; Kasarskis and Winslow, 1989).

By collecting tissues from mice at different disease stages, we studied populations of mutant SOD1 transgenic mice synchronized for their disease stages. These studies revealed several surprises. First, at the RD stage when the disease began (60–90 days before paralysis), the loss of motor neurons was minor (less than 10%). The largest loss of motor neuron occurred at the paralysis stage (Fig. 1). This pattern correlated with the changes in astrogliosis, which also sharply rose at the paralysis stage (Levine *et al*., 1999; Fig. 1). This suggested that early therapeutic intervention after onset of ALS may rescue the majority of motor neurons. Second, deliberate searching for prominent neurofilament abnormalities revealed few sites of neurofilament accumulation. These minor changes occured mostly in late SD and paralysis stages. In contrast, widespread vacuoles in the spinal cord were easily observed without deliberate searches. By quantitative measurements, the number of these vacuoles peaked at the RD stage. As the disease further progresses towards the paralysis stage, the number of vacuoles declined (Kong and Xu, 1998; Fig. 1).

The peaking of vacuoles in the ventral horn was particularly interesting because it represented a dominant early pathological event. The follow-up detailed microscopic observations confirmed that these vacuoles were derived from vacuolated mitochondria (Kong and Xu, 1998). Tracing back before the onset of the disease and massive mitochondrial vacuolation, we found a large number of abnormal mitochondria associated with neuronal processes, predominantly in dendrites (Kong and Xu, 1998). This result indicated that neuronal mitochondrial damage began early, prior to the clinical onset of ALS. Indeed, functional measurement of mitochondrial complex I activity detected a decline at age 60 days, the earliest age that we measured (Jung *et al*., 2002; Fig. 1).

MITOCHONDRIAL VACUOLATION BY INTERMEMBRANE SPACE EXPANSION (MVISE)

How do mitochondria become vacuolated in the SOD1G93A mutant mice? Our electron microscopic observations suggested a pattern of progression in mitochondrial vacuolation (Higgins *et al*., 2003; Fig. 2). Initially mitochondria are swollen and their cristae are disorganized. But they maintain the general structure of a mitochondrion. Then the outer membrane folds at a focal point, forming a small protrusion on the mitochondrial

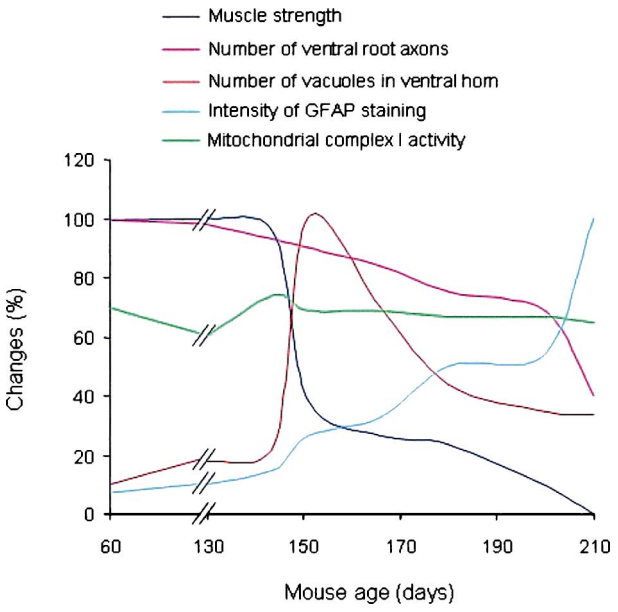

Fig. 1. Sequence of pathological events leading to motor neuron death in mutant SODIG93A mice. This plot is based on data in Kong and Xu (1998), Levine et al. (1999) and Jung et al. (2003).

surface and creating a small space between the outer and inner membranes. This might be caused by damage to the attachment structure between the inner and the outer mitochondrial membranes. Following the formation of this small protrusion is a further detachment between the inner and the outer membranes and expansion of the intermembrane space. As the space becomes increasingly large, the inner membrance components disintegrate, forming the inner membrane remnants inside the mitochondrial vacuole (Higgins *et al*., 2003; Fig. 2).

This model for mitochondrial vacuolation was plausible because several other studies have shown that both wild-type and mutant SOD1 exist in mitochondrial intermembrane space (Higgins *et al*., 2002; Mattiazzi *et al*., 2002; Okado-Matsumoto and Fridovich, 2001; Sturtz *et al*., 2001) and expansion of intermembrane space had been suggested (Bendotti *et al*., 2001; Jaarsma *et al*., 2001).

To test this model, we localized markers for various mitochondrial components in the vacuoles. We took the advantage of our early finding that mutant SOD1 was present at the boundary of vacuoles (Levine *et al*., 1999) and marked vacuoles using anti-SOD1 antibodies. By double immunofluorescent staining, we demonstrated that the inner mitochondrial membrane marker cytochrme *c* oxidase was located with the inner membrane remnants inside the vacuole. The outer mitochondrial membrane markers, transporter of outer membrane TOM20 and TOM40, were located on the outer vacuolar membrane. Cytochrome *c*, an intermembrane space marker, colocalized with SOD1 in mitochondria at the beginning stage of the vacuolation, but disappeared when the vacuoles enlarged. The disappearance of cytochrome *c* in large vacuoles could be either due to a dilution of cytochrome *c* as the intermembrane space expands or due to leakage out of mitochondrial intermembrane space. Our observation that the outer membranes of the large vacuoles were often porous supported the latter possibility (Higgins *et al*., 2003).

As we further looked for other organelles that might participate in mitochondrial vacuolation, we were surprised to find abundant peroxisomes inside the vacuoles and that lysosomes were not associated with the vacuoles (Higgins *et al*., 2003). The features of this mitochondrial vacuolation, including the expansion of the intermembrane space (instead of mitochondrial matrix) and the presence of peroxisomes (instead of lysosomes), suggest that the mutant SOD1-induced mitochondrial vacuolation is neither the classical mitochondrial permeability transition (which involves expansion of the mitochondrial matrix) nor autophagic vacuolation (which involves lysosomes), but rather, a vacuolation by intermembrane space expansion or MVISE (Fig. 2).

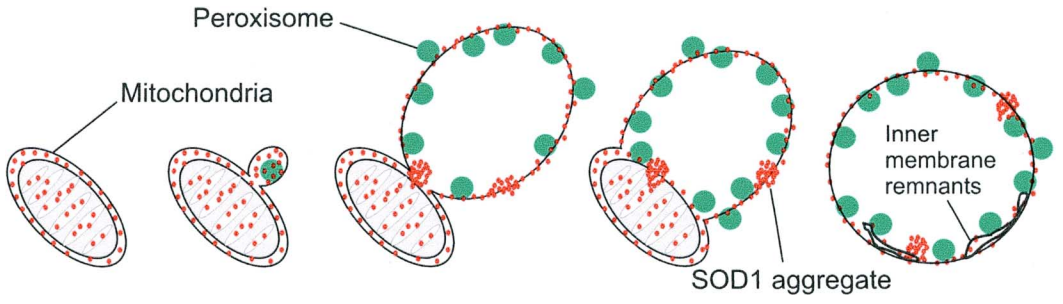

Fig. 2. Mitochondrial vacuolation by intermembrane space expansion (MVISE). This model is based on data published in Higgins et al. (2003).

THE ROLE OF MITOCHONDRIAL DEGENERATION IN MOTOR NEURON DEGENERATION PATHWAY

Experiments in cultured cells and in mice from other investigators support our in vivo observations on mutant SOD1-induced mitochondrial degeneration. Expression of mutant SOD1 in cultured neuroblastoma cells caused a decrease in mitochondrial membrane potential (Carri *et al*., 1997). Similarly, expression of mutant SOD1 in motor-neuron-like cell line NSC34 cells damaged mitochondria and caused mitochondrial dysfunction and cell death (Liu *et al*., 2002; Menzies *et al*., 2002a). Targeting mutant SOD1 to mitochondria in cultured neuroblastoma cells produced heightened toxicity (Takeuchi *et al*., 2002). In vivo, transgenic mice expressing mutant SOD1 showed a loss in mitochondrial mass (Wiedemann *et al*., 2002). Mitochondrial toxin MTPT or a decrease in mitochondrial SOD2 activity significantly exacerbated the clinical progression of ALS in mutant SOD1 transgenic mice (Andreassen *et al*., 2000, 2001).

Is mitochondrial degeneration relevant for human ALS? Although early studies on human autopsy focused on neurofilament accumulation in proximal axons because of their conspicuous presence, careful examination of the published EM micrographs reveals numerous vacolated mitochondria among swirls of disorganized neurofilaments in both sporadic and familial cases (Hirano *et al*., 1984a,b). Other studies have shown vacuolated mitochondria in upper and lower motor neurons (Sasaki *et al*., 1990; Sasaki and Iwata, 1999) as well as hepatocytes (Masui *et al*., 1985) in human ALS cases. These observations suggest that mitochondrial degeneration play a role in human ALS.

Some SOD1 mutants cause motor neuron degeneration without mitochondrial vacuolation (Bruijn *et al*., 1998; Ripps *et al*., 1995; Wang *et al*., 2002). However, whether these mutants cause functional impairment is unclear and remains a future challenge for our investigation. To further understand the role of mitochondrial damage in the motor neuron degeneration pathway, two questions need to be answered: How do SOD1 mutants damage mitochondria and what is the consequence of this damage? Mutant SOD1 may directly damage mitochondria. Both wild-type and mutant SOD1 are found in mitochondria (Higgins *et al*., 2002; Mattiazzi *et al*., 2002), probably in the intermembrane space (Okado-Matsumoto and Fridovich, 2001; Sturtz *et al*., 2001). Mutant SOD1 aggregates in mitochondria and the aggregates are associated with mitochondria membranes (Higgins *et al*., 2003). Therefore, the aggregates could cause direct damage to mitochondrial membranes. Evidence for protein aggregates damaging biomembranes has emerged from studies on Parkinson's disease, where mutant α-synucleins forms aggregates and annular rings on membranes (Lashuel *et al*., 2002), and causing permeabilization of membranes (Volles *et al*., 2001). Mutant SOD1 could damage mitochondria by similar mechanisms.

Mutant SOD1 could also damage mitochondria indirectly. Mutant SOD1 interacts with cellular chaperones (Okado-Matsumoto and Fridovich, 2002; Shinder *et al*., 2001) and inhibits chaperone activity in motor neurons (Batulan *et al*., 2003; Bruening *et al*., 1999). Mitochondrial protein import depends on cytoplasmic as well as mitochondrial chaperone activities (Neupert and Brunner, 2002; Young *et al*., 2003). The reduced chaperone activity may impair mitochondrial function by interfering with protein import into mitochondria.

What is the consequence of mitochondrial damage? Mitochondrial dysfunction can lead to energy deficiency and ionic imbalance (Beal, 1992), elevated reactive oxidative stress and oxidative damage (Andreassen *et al*., 2000), and increased sensitivity of neurons to excitotoxicity (Bittigau and Ikonomidou, 1997; Ikonomidou *et al*., 1996; Kaal *et al*., 2000; Kruman *et al*., 1999). These effects could lead to structural damage to mitochondria, resulting in MVISE. The loss of structural integrity could trigger cell death programs by releasing pro-apoptotic proteins that reside in the mitochondrial intermembrane space, such as cytochrome *c*, AIF, SMAC/DIABLO, endo G, and Htra/Omi (Green and Evan, 2002). Because the majority of vacuoles develop in distal small dendrites (Levine *et al*., 1999), the release of these proapoptotic molecules may not cause typical apoptotic changes in motor neuron cell bodies, such as chromatin condensation and cytoplasmic blebbing. Indeed, the typical changes are not observed by EM (Bendotti *et al*., 2001; Guegan and Przedborski, 2003), but widespread caspase activation is detected in spinal cords (Guegan *et al*., 2001; Guegan and Przedborski, 2003; Pasinelli *et al*., 2000), suggesting the occurrence of a neuritic death program (Mattson and Duan, 1999).

In summary, there is strong evidence that mitochondrial degeneration plays important roles in mutant SOD1-induced motor neuron degeneration pathway. Further challenges in defining this role will be to determine whether SOD1 mutants that do not induce mitochondrial vacuolation impair mitochondrial function and the mechanism whereby all mutant SOD1 damages mitochondria. Because of the complexity of cellular environment, future breakthrough in mechanistic understanding of how mutant SOD1 damages mitochondria will most likely emerge from defined in vitro experimental systems.

ACKNOWLEDGMENT

We thank the agencies that funded this research: The ALS Association, National Institute of Neurological Diseases and Stroke, Worcester Foundation for Biomedical Research, and the Robert Pachard Center for ALS Research at Johns Hopkins. The contents of this report are solely the responsibility of the authors and do not necessarily represent the offical views of NINDS.

REFERENCES

Aggarwal, A., and Nicholson, G. (2002). *J. Neurol. Neurosurg. Psychiatry.* **73**, 199–201.
Andreassen, O. A., Ferrante, R. J., Klivenyi, P., Klein, A. M., Dedeoglu, A., Albers, D. S., Kowall, N. W., and Beal, M. F. (2001). *Exp. Neurol.* **168**, 356–363.
Andreassen, O. A., Ferrante, R. J., Klivenyi, P., Klein, A. M., Shinobu, L. A., Epstein, C. J., and Beal, M. F. (2000). *Ann. Neurol.* **47**, 447–455.
Batulan, Z., Shinder, G. A., Minotti, S., He, B. P., Doroudchi, M. M., Nalbantoglu, J., Strong, M. J., and Durham, H. D. (2003). *J. Neurosci.* **23**, 5789–5798.
Beal, M. F. (1992). *Ann. Neurol.* **31**, 119–130.
Bendotti, C., Calvaresi, N., Chiveri, L., Prelle, A., Moggio, M., Braga, M., Silani, V., and De Biasi, S. (2001). *J. Neurol. Sci.* **191**, 25–33.
Bittigau, P., and Ikonomidou, C. (1997). *J. Child Neurol.* **12**, 471–485.
Bruening, W., Roy, J., Giasson, B., Figlewicz, D. A., Mushynski, W. E., and Durham, H. D. (1999). *J. Neurochem.* **72**, 693–699.
Bruijn, L. I., Becher, M. W., Lee, M. K., Anderson, K. L., Jenkins, N.A., Copeland, N. G., Sisodia, S. S., Rothstein, J. D., Borchelt, D. R., Price, D. L., and Cleveland, D. W. (1997). *Neuron.* **18**, 327–338.
Bruijn, L. I., Houseweart, M. K., Kato, S., Anderson, K. L., Anderson, S. D., Ohama, E., Reaume, A. G., Scott, R. W., and Cleveland, D. W. (1998). *Science* **281**, 1851–1854.
Carpenter, S. (1968). *Neurol.* **18**, 841–851.
Carri, M. T., Ferri, A., Battistoni, A., Famhy, L., Cabbianelli, R., Poccia, F., and Rotilio, G. (1997). *FEBS Lett.* **414**, 365–368.
Cleveland, D. W., and Rothstein, J. D. (2001). *Nat. Rev. Neurosci.* **2**, 806–819.
Cox, P. A., Banack, S. A., and Murch, S. J. (2003). *Proc. Natl. Acad. Sci. U.S.A.* 100, 13380–13383.
Dal Canto, M. C., and Gurney, M. E. (1995). *Brain Res.* **676**, 25–40.
De la Rua-Domenech, R., Mohammed, H. O., Cummings, J. F., Divers, T. J., De Lahunta, A., and Summers, B. A. (1997). *Vet. J.* **154**, 203–213.
Green, D. R., and Evan, G. I. (2002). *Cancer Cell* **1**, 19–30.
Guegan, C., and Przedborski, S. (2003). *J. Clin. Invest.* **111**, 153–161.
Guegan, C., Vila, M., Rosoklija, G., Hays, A. P., and Przedborski, S. (2001). *J. Neurosci.* **21**, 6569–6576.
Gurney, M. E. (1994). *N. Engl. J. Med.* **331**, 1721–1722.
Gurney, M. E., Pu, H., Chiu, A. Y., Dal Canto, M. C., Polchow, C. Y., Alexander, D. D., Caliendo, J., Hentati, A., Kwon, Y. W., Deng, H.-X., Chen, W., Zhai, P., Sufit, R. L., and Siddique, T. (1994). *Science* **264**, 1772–1775.
Higgins, C. M., Jung, C., Ding, H., and Xu, Z. (2002). *J. Neurosci.* **22**, RC215.
Higgins, C. M., Jung, C., and Xu, Z. (2003). *BMC Neurosci.* **4**, 16.
Hirano, A. (1991). *Adv. Neurol.* **56**, 91–101.
Hirano, A., Donnenfeld, H., Sasaki, S., and Nakano, I. (1984a). *J. Neuropath. Exp. Neurol.* **43**, 461–470.
Hirano, A., Nakano, I., Kurland, L. T., Mulder, D. W., Holley, P. W., and Saccomanno, G. (1984b). *J. Neuropath. Exp. Neurol.* **43**, 471–480.
Ikonomidou, C., Qin Qin, Y., Labruyere, J., and Olney, J. W. (1996). *J. Neuropath. Exp. Neurol.* **55**, 211–224.
Jaarsma, D., Rognoni, F., van Duijn, W., Verspaget, H. W., Haasdijk, E. D., and Holstege, J. C. (2001). *Acta Neuropathol. (Berl.)* **102**, 293–305.
Julien, J. P. (2001). *Cell* **104**, 581–591.
Jung, C., Higgins, C. M., and Xu, Z. (2002). *J. Neurochem.* **83**, 535–545.
Kaal, E. C., Vlug, A. S., Versleijen, M. W., Kuilman, M., Joosten, E. A., and Bar, P. R. (2000). J. Neurochem. 74, 1158–1165.
Kasarskis, E. J., and Winslow, M. (1989). *Neurology* **39**, 1243–1245.
Kong, J., and Xu, Z. (1998). *J. Neurosci.* **18**, 3241–3250.
Kruman, P. W. A., II, Springer, J. E., and Mattson, M. P. (1999). *Exp. Neurol.* **160**, 28–39.
Lashuel, H. A., Petre, B. M., Wall, J., Simon, M., Nowak, R. J., Walz, T., and Lansbury, P. T., Jr. (2002). *J. Mol. Biol.* **322**, 1089–1102.
Levine, J. B., Kong, J., Nadler, M., Xu, Z. (1999). *Glia* **28**, 215–224.
Liu, R., Li, B., Flanagan, S. W., Oberley, L. W., Gozal, D., and Qiu, M. (2002). *J. Neurochem.* **80**, 488–500.
Masui, Y., Mozai, T., and Kakehi, K. (1985). *J. Neurol.* **232**, 15–19.
Mattiazzi, M., D'Aurelio, M., Gajewski, C. D., Martushova, K., Kiaei, M., Beal, M. F., and Manfredi, G. (2002). *J. Biol. Chem.* **277**, 29626–29633.
Mattson, M. P., and Duan, W. (1999). *J. Neurosci. Res.* **58**, 152–166.
Menzies, F. M., Cookson, M. R., Taylor, R. W., Turnbull, D. M., Chrzanowska-Lightowlers, Z. M., Dong, L., Figlewicz, D. A., and Shaw, P. J. (2002a). *Brain* **125**, 1522–1533.
Menzies, F. M., Ince, P. G., and Shaw, P. J. (2002b). *Neurochem. Int.* **40**, 543–551.
Neupert, W., and Brunner, M. (2002). *Nat. Rev. Mol. Cell. Biol.* **3**, 555–565.
Okado-Matsumoto, A., and Fridovich, I. (2001). *J. Biol. Chem.* **276**, 38388–38393.
Okado-Matsumoto, A., and Fridovich, I. (2002). *PNAS* **99**, 9010–9014.
Pasinelli, P., Houseweart, M. K., Brown, R. H., Jr., and Cleveland, D. W. (2000). *Proc. Natl. Acad. Sci. U.S.A.* **97**, 13901–13906.
Ripps, M. E., Huntley, G. W., Hof, P. R., Morrison, J. H., and Gordon, J. W. (1995). *Proc. Natl. Acad. Sci. U.S.A.* **92**, 689–693.
Rosen, D. R., Siddique, T., Patterson, D., Figlewicz, D. A., Sapp, P., Hentati, A., Donaldson, D., Goto J., O'Regan J. P., and Deng H. X., *et al.* (1993). *Nature* **362**, 59–62.
Rowland, L. P., and Shneider, N. A. (2001). *N. Engl. J. Med.* **344**, 1688–1700.
Sasaki, S., and Iwata, M. (1999). *Neurosci. Lett.* **268**, 29–32.
Sasaki S., Maruyama S., Yamane K., Sakuma H., and Takeishi, M. (1990). *J. Neurol. Sci.* **97**, 233–240.
Shinder, G. A., Lacourse, M.-C., Minotti, S., and Durham, H. D. (2001). *J. Biol. Chem.* **276**, 12791–12796.
Sturtz, L. A., Diekert, K., Jensen, L. T., Lill, R., and Culotta, V. C. (2001). *J. Biol. Chem.* **276**, 38084–38089.
Takeuchi, H., Kobayashi, Y., Ishigaki, S., Doyu, M., and Sobue, G. (2002). *J. Biol. Chem.* **277**, 50966–50972.
Volles, M. J., Lee, S. J., Rochet, J. C., Shtilerman, M. D., Ding, T. T., Kessler, J. C., and Lansbury, P. T., Jr. (2001). *Biochemistry* **40**, 7812–7819.
Wang, J., Xu G., Gonzales, V., Coonfield, M., Fromholt, D., Copeland, N. G., Jenkins, N. A., and Borchelt, D. R. (2002). *Neurobiol. Dis.* **10**, 128–138.
Wiedemann F. R., Manfredi G., Mawrin C., Beal M. F., and Schon, E. A. (2002). *J. Neurochem.* **80**, 616–625.
Wong, P. C., Pardo, C. A., Borchelt, D. R., Lee, M. K., Copeland, N. G., Jenkins, N. A., Sisodia, S. S., Cleveland, D. W., and Price, D. L. (1995). *Neuron* **14**, 1105–1116.
Young, J. C., Hoogenraad, N. J., and Hartl, F. U. (2003). *Cell* **112**, 41–50.