Biochemical and Biophysical Research Communications **264,** 37–41 (1999)
Article ID bbrc.1999.1386, available online at http://www.idealibrary.com on IDEAL®

Unique Characteristics of Ubiquitin-Bonded Complex Play a Pathological Role in Dentatorubral–Pallidoluysian Atrophy

Ikuru Yazawa

Department of Neurology, Toranomon Hospital, 2-2-2 Toranomon, Minato-ku, Tokyo 105-8470, Japan; and Okinaka Memorial Institute for Medical Research, 2-2-2 Toranomon, Minato-ku, Tokyo 105-8470, Japan

Received August 16, 1999

Abnormal complex formation of dentatorubral–pallidoluysian atrophy (DRPLA) protein and pathological ubiquitination of abnormal complex are two pathological processes involved in DRPLA neurodegeneration. Pathological ubiquitination and solubility in SDS and reducing agent are two unique characteristics of the DRPLA protein complex. Ubiquitination of abnormal DRPLA protein complex in DRPLA brain tissue is heat-resistant and stronger than that in control brain tissue. Pathological ubiquitination of DRPLA protein complex correlates with the onset of symptoms and the size of an expanded glutamine repeat in brain tissue of patients with DRPLA. Pathological ubiquitination plays an important role in DRPLA pathology. DRPLA protein complex is water-insoluble but soluble in SDS and reducing agent, and displays no difference in water insolubility between control and DRPLA brain tissue. Abnormal insoluble complex formation is not developed by a qualitative change in water insolubility of DRPLA protein complex but is developed by a spontaneous accumulation of an abnormally large amount of the DRPLA protein complex.

Dentatorubral–pallidoluysian atrophy (DRPLA) is a hereditary neurodegenerative disorder caused by expansion of a glutamine repeat in DRPLA protein (1–4). DRPLA protein undergoes greater complex formation in DRPLA brain tissue and expanded glutamine repeat enhances complex formation of DRPLA protein (5). DRPLA protein complex is ubiquitinated in DRPLA brain tissue and pathological ubiquitination of DRPLA protein complex is found selectively in affected lesions of brain tissue of patients with DRPLA (6). Immunohistochemical studies of DRPLA brain tissue using an anti-DRPLA protein antibody showed that immunoreactivity of DRPLA protein appearing as cluster formations corresponds to complex formation of DRPLA protein in immunoblotting and that DRPLA protein is co-localized with ubiquitin in DRPLA afflicted neurons (6). Characteristic neuronal cytoplasmic inclusions are identified in DRPLA dentate nuclei using immunohistochemical studies (6). DRPLA protein complex is found in the core of the inclusions and this core is surrounded by ubiquitin-bonded proteins. This is evidence for disease-specific abnormal ubiquitination of DRPLA protein complex. Thus, abnormal complex formation of DRPLA protein and pathological ubiquitination of abnormal complex are two pathological processes involved in DRPLA neurodegeneration. To elucidate the pathological mechanism of DRPLA, biochemical characteristics of DRPLA protein complex were examined using immunoblotting studies of DRPLA brain tissue under reducing and non-reducing conditions in sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and in native-PAGE.

Abnormal accumulations of ubiquitinated proteins are found in intracellular inclusions in various neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and motor neuron diseases (7–11). Ubiquitin accumulations are related to the process of neurodegeneration (12). Because pathological ubiquitination is not found when DRPLA protein is separated into a monomer form but found when DRPLA protein forms DRPLA protein complex in DRPLA brain tissue (6), pathological ubiquitination of DRPLA protein complex may be different from those of other diseases that cause neuronal ubiquitinated inclusions. This study clearly elucidates two biochemical characteristics of DRPLA protein complex, which relate to pathological processes in DRPLA. First, to determine what roles ubiquitination of DRPLA protein plays in DRPLA neurodegeneration, this study assesses the nature of ubiquitination of DRPLA protein complex in DRPLA brain tissue. By comparing ubiquitination of DRPLA protein complex in human control brain tissue and DRPLA

0006-291X/99 $30.00

TABLE 1

Clinical Findings of Five DRPLA Patients

Patient	Sex	Age at onset (year)	CAG repeat size
1	Female	4	64/10
2	Male	10	64/13
3	Female	21	62/16
4	Male	43	60/19
5	Male	49	59/19

brain tissue, this study provides evidence that ubiquitination of DRPLA protein complex in DRPLA brain tissue has a pathogenic character. Second, to understand pathological process of abnormal DRPLA protein complex formation, this study examines solubility of DRPLA protein complex in SDS and reducing agent. In Alzheimer's disease, abnormal ubiquitinated paired helical filaments that accumulate progressively in afflicted neurons have unusual insolubility characteristics, including insolubility in both SDS and reducing agent (13, 14). This study compares solubility characteristics of abnormal accumulated proteins in both DRPLA and Alzheimer's disease. Two unique pathological characteristics of ubiquitin-bonded DRPLA protein complex have a close relationship to each other in DRPLA neurodegeneration.

MATERIALS AND METHODS

Sample preparation. Postmortem brain tissue samples from five DRPLA patients (24–69 years old), whose disease had been diagnosed genetically by the PCR analysis and confirmed pathologically, and brain tissue samples from four control subjects (59–79 years old) were examined. Onset of symptoms ranged from 4 to 49 years (Table 1) (3, 15). Tissue samples (2 g) from cerebral frontal cortices of four human control subjects and five DRPLA patients were homogenized separately in 5 volumes of tris-saline buffer with protease inhibitors (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 μg/ml Aprotinin, 1 mM EDTA, 10 μg/ml Leupeptin, 0.5 mM Pefabloc SC, and 10 μg/ml Pepstatin). Samples were stored at −80°C until used for gel electrophoresis.

Native-polyacrylamide gel electrophoresis (PAGE) and SDS-PAGE. Protein concentrations of the brain tissue homogenates were measured by the bicinchoninic acid (BCA) protein assay (Pierce). Each sample of brain tissue was mixed with an equal volume of four types of sample buffers: 1) sample buffer containing SDS (4% SDS, 160 mM Tris-HCl, pH 6.8, 20% glycerol) with a reducing agent (10% 2-mercaptoethanol, ME), 2) sample buffer containing SDS without a reducing agent, 3) sample buffer without SDS (160 mM Tris-HCl, pH 6.8, 20% glycerol) with a reducing agent (10% 2-mercaptoethanol), and 4) native sample buffer (160 mM Tris-HCl, pH 6.8, 20% glycerol). Native sample buffer was used for native-PAGE and the other three types of sample buffers were used for SDS-PAGE. After tissue samples were mixed with each type of sample buffer, they were placed in boiling water for 8 min. To assess the effect of heat upon bonding of ubiquitin with DRPLA protein complex, samples of brain tissue with and without being subjected to heat were compared using immunoblotting. Electrophoresis was performed using two kinds of polyacrylamide gels: 0.1% SDS-containing polyacrylamide gel for SDS-PAGE and SDS-free polyacrylamide gels for native-PAGE. The acrylamide concentration of the stacking gels was 5% and that of the running gels 6%. Samples (10 μg each) were electrophoresed in the gels, then the gels were subjected to immunoblotting.

Immunoblotting. Proteins in the gels were transferred electrophoretically to a polyvinylidene difluoride (Immobilon) membrane (Millipore), which then was blocked with the solution that contained 4% nonfat milk powder and left undisturbed for 16 h at 4°C with anti-DRPLA protein polyclonal antibody (C580) and anti-ubiquitin polyclonal antibody (DAKO). C580 that was produced in rabbits against synthetic peptides corresponding to the C-terminal end of human DRPLA protein (amino acids 1171–1184) was purified by affinity chromatography as described elsewhere (3, 16). The Immobilon membrane was incubated for 1 h at room temperature with anti-rabbit secondary antibodies. In order to visualize the immunoreaction, an enhanced chemiluminescence (ECL) western blotting system (Amersham Pharmacia Biotech) was used.

RESULTS

Characterization of Ubiquitin Bonding with DRPLA Protein Complex

To assess the nature of chemical bonding of ubiquitin with DRPLA protein complex, DRPLA protein in four samples of human control brain tissue and five samples of DRPLA brain tissue was examined using immunoblotting studies under reducing and non-reducing conditions. To understand the influence of heat upon chemical bonding of ubiquitin with DRPLA protein complex, ubiquitinated DRPLA protein complex was compared using immunoblotting with and without being subjected to heat under non-reducing conditions. Immunoblots of samples of control brain tissue under non-reducing conditions stained with an anti-ubiquitin antibody show, when the samples are subjected to heat after being mixed with sample buffer containing SDS without a reducing agent, there is no immunoreactivity of DRPLA protein complex with apparently high molecular weight at the top of stacking gels (Fig. 1A, lane 3). Although immunoblots of samples of control brain tissue without being subjected to heat show that there appears definite immunoreactivity of DRPLA protein complex at the top of stacking gels (Fig. 1A, lane 1). The data indicate that when subjected to heat the immunoreactivity of DRPLA protein in control brain tissue is completely eliminated, and that ubiquitin bonding formation with DRPLA protein complex is easily broken by heat treatment of samples of control brain tissue. Heat treatment of samples of DRPLA brain tissue had no effect on immunoreactivity of DRPLA protein complex under non-reducing conditions when stained with an anti-ubiquitin antibody (Fig. 1A, lanes 2, 4). This indicates that bonding of ubiquitin with DRPLA protein complex in DRPLA brain tissue is resistant to heat and is stronger than that in control brain tissue.

To understand the pathological influence of expanded glutamine repeat upon ubiquitin-bonded DRPLA protein complex, ubiquitin-bonded DRPLA protein complex is examined using immunoblotting of

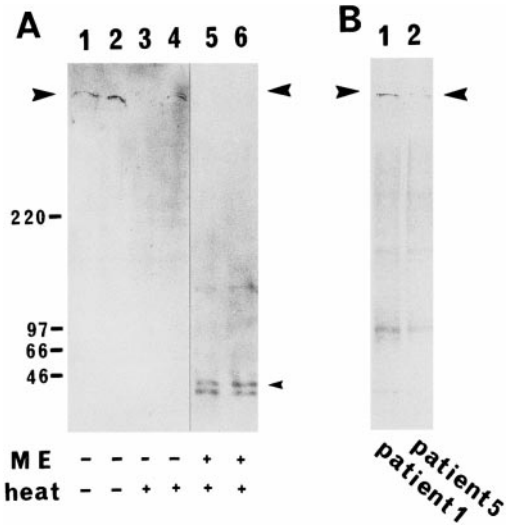

FIG. 1. (A) Immunoblots of a sample of human control brain tissue (lanes 1, 3, 5) and a sample of DRPLA brain tissue (lanes 2, 4, 6) (10 μg protein per lane) under non-reducing (lanes 1-4) and reducing (lanes 5, 6) conditions, stained with an anti-ubiquitin antibody. Samples (lanes 3-6) were subjected to heat and samples (lanes 1, 2) were not subjected to heat, then they were electrophoresed on SDS-polyacrylamide gels. Stained with an anti-ubiquitin antibody, immunoblot of a sample of DRPLA brain tissue when the sample is subjected to heat after being mixed with sample buffer containing SDS under nonreduced conditions shows the same complex band (large arrowhead) as that stained with C580 located at the top of stacking gel (lane 4), whereas the immunoblot of a sample of control brain tissue shows no immunoreactivity of a DRPLA protein complex band at the top of stacking gel (lane 3). In contrast, when samples of control brain tissue and DRPLA brain tissue are not subjected to heat, immunoblots show definite immunoreactivities of DRPLA protein complex bands at the top of stacking gels (lanes 1, 2), indicating that when DRPLA protein complex is subjected to heat, its band becomes undetectable on the immunoblot of a sample of control brain tissue under nonreducing conditions. Immunoblots of samples of control brain tissue and DRPLA brain tissue under reducing conditions when stained with an anti-ubiquitin antibody show immunoreactivities of multiple ubiquitin bands (small arrowhead), showing the absence of immunoreactivity of DRPLA protein complex band (lanes 5, 6). ME is 2-mercaptoethanol. The molecular weight markers are indicated on the left. (B) A comparison of ubiquitin-bonded DRPLA protein complex between brain tissue samples of two DRPLA patients: patient 1 who showed the earliest onset of symptoms and the largest size of expanded CAG repeat, and patient 5 who showed the latest onset and the smallest size, among the five DRPLA patients. Immunoblots of brain tissue samples of patient 1 (lane 1) and of patient 5 (lane 2) with being subjected to heat under non-reducing conditions were stained with an anti-ubiquitin antibody. Immunoblot of a brain tissue sample of patient 1 shows greater immunoreactivity of ubiquitin-bonded DRPLA protein complex than that of patient 5 (large arrowhead).

brain tissue samples of five DRPLA patients under non-reducing conditions. Patient 1 had the earliest onset of symptoms and the largest size of expanded CAG repeat, and patient 5 had the latest onset of symptoms and the smallest size of expanded CAG repeat, among the five DRPLA patients (Table 1). For this reason, their DRPLA protein complexes were chosen for further study. On immunoblots of two brain tissue samples under non-reducing conditions stained with an anti-ubiquitin antibody, ubiquitin-bonded DRPLA protein complex at the top of stacking gels shows greater immunoreactivity in patient 1 than in patient 5 (Fig. 1B), indicating that patient 1 had more extent of pathological ubiquitination of DRPLA protein complex. This suggests that the onset of symptoms and the size of expanded glutamine repeat in DRPLA protein correlate with the severity of pathological ubiquitination of DRPLA protein complex in DRPLA brain tissue.

Solubility of DRPLA Protein and DRPLA Protein Complex

The total homogenates of four samples of control brain tissue and five samples of DRPLA brain tissue were analyzed using SDS-PAGE under reducing and non-reducing conditions and native-PAGE. When the samples of control brain tissue and DRPLA brain tissue were treated with sample buffer containing SDS, no precipitation was observed. However, when the samples were treated with native sample buffer and sample buffer without SDS, apparent precipitation was observed, and the supernatant was then examined to study solubility of DRPLA protein and DRPLA protein complex.

Immunoblots of control and DRPLA samples under reducing conditions stained with anti-DRPLA protein antibody (C580), an affinity-purified polyclonal antibody against the C-terminus of DRPLA protein (3), show that wild-type and mutant DRPLA protein bands are detected only when the samples are treated with sample buffer containing SDS, but are not detected when treated with sample buffer without SDS (Fig. 2, lanes 1-4). The results indicate that DRPLA protein is water-insoluble but soluble in SDS, one kind of chemical detergent. There is no difference in solubility between wild-type and mutant DRPLA proteins in samples of DRPLA brain tissue. On immunoblots of samples of control brain tissue that are treated with sample buffer containing SDS under non-reducing conditions stained with C580, DRPLA protein migrates in two bands of a reduced form of DRPLA protein with an apparent molecular weight of approximately 200kDa and DRPLA protein complex with apparently high molecular weight at the top of stacking gels (Fig. 2, lane 5). On immunoblots of samples of DRPLA brain tissue that are treated with sample buffer containing SDS under non-reducing conditions, DRPLA protein migrates in a band of DRPLA protein complex to the top of stacking gels (Fig. 2, lane 6), and the immunoreactivity of DRPLA protein complex in samples of DRPLA brain tissue is greater than that in samples of control brain tissue as previously demonstrated (5). In contrast, when samples of control and DRPLA brain tissue are treated with native sample buffer, immunoblots stained with C580 show little immunoreactivity of

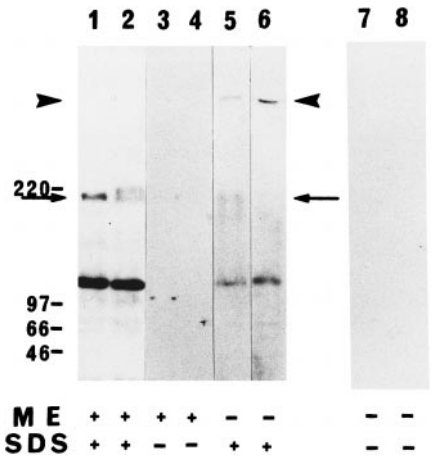

FIG. 2. Immunoblots of the total homogenates of the cerebral cortices from control brain tissue from a subject (lanes 1, 3, 5, 7) and brain tissue from a DRPLA patient (lanes 2, 4, 6, 8) (10 μg protein per lane), stained with an anti-DRPLA protein antibody (C580). Samples were treated with sample buffers containing SDS (lanes 1, 2, 5, 6) and without SDS (lanes 3, 4), under the reducing (lanes 1-4) and non-reducing (lanes 5, 6) conditions, and they were electrophoresed on SDS-polyacrylamide gels. For native-PAGE analysis, the samples were treated with native sample buffer and were electrophoresed on native-polyacrylamide gels (lanes 7, 8). Stained with C580, immunoblots of a sample of control brain tissue and a sample of DRPLA brain tissue under nonreducing conditions (lanes 5, 6) show a complex band with apparently high molecular weight located at the top of stacking gels (arrowhead), but only the immunoblot of a sample of control brain tissue (lane 5) shows a reduced band of DRPLA protein with an apparent molecular weight of approximately 200 kDa (arrow) which is detected on immunoblot of a sample of control brain tissue under reducing conditions (lane 1). Immunoreactivity of the complex band is greater on the immunoblot of a sample of DRPLA brain tissue than on the immunoblot of a sample of control brain tissue. On immunoblots of samples of control brain tissue and DRPLA brain tissue treated with sample buffer without SDS under reducing conditions, C580 detects little immunoreactivities of DRPLA protein and its complex bands (lanes 3, 4). Immunoblots of samples of control brain tissue and DRPLA brain tissue using native-PAGE show no immunoreactivities of DRPLA protein and its complex bands (lanes 7, 8). ME is 2-mercaptoethanol. The molecular weight markers using SDS-PAGE are indicated on the left.

DRPLA protein complex at the top of stacking gels (Fig. 2, lanes 7, 8). The data indicate that DRPLA protein complex both in control and DRPLA brain tissue is water-insoluble but soluble in SDS, showing no difference in water-insolubility of DRPLA protein complex between in samples of control brain tissue and DRPLA brain tissue.

DISCUSSION

Pathological ubiquitination and solubility in SDS and reducing agent are two unique characteristics of DRPLA protein complex, but pathological ubiquitination is the more significant characteristic. A recent study has identified ubiquitin as a component of DRPLA protein complex on immunoblots of DRPLA brain tissue under non-reducing conditions, indicating that DRPLA protein complex is pathologically ubiquitinated in DRPLA brain tissue (6). Immunoblotting studies of samples of control brain tissue and DRPLA brain tissue show that ubiquitination of abnormal DRPLA protein complex is heat-resistant in DRPLA brain tissue. This heat-resistance indicates qualitatively stronger bonding than in control brain tissue. In control brain tissue ubiquitination may be a nonspecific physiological phenomenon, but in DRPLA brain tissue ubiquitination is a disease-specific pathological phenomenon that is produced by abnormal DRPLA protein complex formation. In postmortem human brain tissue, nonspecific ischemic changes may easily occur, and careful assessment of resulting data is necessary to understand pathological mechanisms of any brain disease. Different strength of bonding of ubiquitin with DRPLA protein complex in control and DRPLA brain tissue is important evidence to assess data resulting from the studies of postmortem human brain tissue. Thus, ubiquitination of DRPLA protein complex in DRPLA brain tissue is a pathological phenomenon. Moreover, pathological ubiquitination of DRPLA protein complex correlates with onset of symptoms and size of expanded glutamine repeat in DRPLA protein. Evidence clearly indicates that pathological ubiquitination plays an important role in DRPLA pathology. Abnormal protein interaction caused by expanded glutamine repeat may relate to abnormal protein complex formation in DRPLA protein and it triggers pathological ubiquitination in DRPLA afflicted neurons.

Another unique characteristic of DRPLA protein complex is solubility in SDS and reducing agent. DRPLA protein complex is insoluble in water but soluble in SDS and reducing agent. This complex has similar solubility characteristics as DRPLA protein and there is no difference in water-insolubility of DRPLA protein complex between control brain tissue and DRPLA brain tissue. The findings are consistent with those seen in previous subcellular fractionation experiments that show most of DRPLA proteins are located in the water-insoluble nuclear fraction in control and DRPLA brain tissue (17). Insoluble ubiquitinated neuronal intranuclear inclusions are composed of gene products in patients with glutamine repeat diseases and animal models of glutamine repeat diseases (18–23). It is important to study the difference in biochemical characteristics between wild-type and mutant gene products to gain an understanding of pathological mechanism or mechanisms of glutamine repeat diseases. Previous two-dimensional electrophoretic studies of DRPLA brain tissue showed that isoelectric points of mutant DRPLA protein are similar to those of wild-type DRPLA protein (24). This study also shows there is no difference in water-insolubility of wild-type

and mutant DRPLA protein. Evidence indicates that both wild-type and mutant DRPLA protein shares many similar protein characteristics in DRPLA brain tissue. Then, why does DRPLA protein develop abnormal insoluble complex formation in DRPLA brain tissue? This study suggests that abnormal insoluble complex formation is not developed by a qualitative change in water-insolubility of DRPLA protein complex, but complex formation is developed by a spontaneous accumulation of a quantitatively large amount of DRPLA protein complex. Because abnormal complex is formed by mutant DRPLA protein that possesses similar biochemical characteristics as wild-type DRPLA protein, DRPLA protein spontaneously accumulates to form DRPLA protein complex. Moreover, wild-type DRPLA protein in addition to mutant DRPLA protein is involved in abnormal complex formation in brain tissue of patients with DRPLA.

Pathological ubiquitination and solubility in SDS and reducing agent are two unique characteristics of DRPLA protein complex in DRPLA. In Alzheimer's disease, abnormal ubiquitinated paired helical filaments show insolubility in both SDS and reducing agent (13, 14). Although abnormal accumulations of ubiquitinated proteins are commonly found in both DRPLA and Alzheimer's disease (6–8), this study demonstrates that solubility characteristics of abnormal accumulated proteins are clearly different between DRPLA and Alzheimer's disease. This suggests that two separate and unique processes of neurodegeneration caused by abnormal accumulations of ubiquitinated proteins must be involved in DRPLA and Alzheimer's disease. Abnormal accumulations, called senile plaque and neurofibrillary tangle which contains paired helical filaments, are neuropathological features that are found in Alzheimer's disease but are not found in DRPLA. These findings provide evidence that two diseases have different pathological mechanisms.

ACKNOWLEDGMENTS

The author greatly acknowledges for the cooperation of five families of the patients, Masumi Tanaka for technical assistance, Thomas Hughes for editorial assistance, and Ichiro Kanazawa, Hirofumi Nakase for many helpful discussions. This research was supported in part by a grant from the Takeda Science Foundation, by a grant from the Okinaka Memorial Institute for Medical Research, by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science, Japan, and by a grant from the Pilot International Foundation, U.S.A.

REFERENCES

1. Koide, R., Ikeuchi, T., Onodera, O., Tanaka, H., Igarashi, S., Endo, K., Takahashi, H., Kondo, R., Ishikawa, A., Hayashi, T., Saito, M., Tomoda, A., Miike, T., Naito, H., Ikuta, F., and Tsuji, S. (1994) *Nature Genet.* **6,** 9–13.
2. Nagafuchi, S., Yanagisawa, H., Sato, K., Shirayama, T., Ohsaki, E., Bundo, M., Takeda, T., Tadokoro, K., Kondo, I., Murayama, N., Tanaka, Y., Kikushima, H., Umino, K., Kurosawa, H., Furukawa, T., Nihei, K., Inoue, T., Sano, A., Komure, O., Takahashi, M., Yoshizawa, T., Kanazawa, I., and Yamada, M. (1994) *Nature Genet.* **6,** 14–18.
3. Yazawa, I., Nukina, N., Hashida, H., Goto, J., Yamada, M., and Kanazawa, I. (1995) *Nature Genet.* **10,** 99–103.
4. Yazawa, I., Nukina, N., Ichikawa, Y., and Kanazawa, I. (1996) *Neurology* **47,** 586–588.
5. Yazawa, I., Hazeki, N., and Kanazawa, I. (1998) *Biochem. Biophys. Res. Commun.* **250,** 22–26.
6. Yazawa, I., Nakase, H., and Kurisaki, H. (1999) *Biochem. Biophys. Res. Commun.* **260,** 133–138.
7. Mori, H., Kondo, J., and Ihara, Y. (1987) *Science* **235,** 1641–1644.
8. Perry, G., Friedman, R., Shaw, G., and Chao, V. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3033–3036.
9. Lowe, J., Blanchard, A., Morrell, K., Lennox, G., Reynolds, L., Billett, M., Landon, M., and Mayer, R. J. (1988) *J. Path.* **155,** 9–15.
10. Lennox, G., Lowe, J., Morrell, K., Landon, M., and Mayer, R. J. (1988) *Neurosci. Lett.* **94,** 211–217.
11. Lowe, J., Lennox, G., Jefferson, D., Morrell, K., McQuire, D., Gray, T., Landon, M., Doherty, F. J., and Mayer, R. J. (1988) *Neurosci. Lett.* **94,** 203–210.
12. Gallo, J. M., and Anderton, B. M. (1989) *Nature* **337,** 687–688.
13. Selkoe, D. J., Ihara, Y., and Salazar, F. J. (1982) *Science* **215,** 1243–1245.
14. Ihara, Y., Abraham, C., and Selkoe, D. J. (1983) *Nature* **304,** 727–730.
15. Yazawa, I., Nukina, N., Goto, J., Kurisaki, H., Hebisawa, A., and Kanazawa, I. (1997) *Neurosci. Lett.* **225,** 53–56.
16. Onodera, O., Oyake, M., Takano, H., Ikeuchi, T., Igarashi, S., and Tsuji S. (1995) *Am. J. Hum. Genet.* **57,** 1050–1060.
17. Yazawa, I., Hazeki, N., and Kanazawa, I. (1998) *Biochem. Biophys. Res. Commun.* **253,** 209–213.
18. Davies, S. W., Turmaine, M., Cozens, B. A., DiFiglia, M., Sharp, A. H., Ross, C. A., Scherzinger, E., Wanker, E. E., Mangiarini, L., and Bates, G. P. (1997) *Cell* **90,** 537–548.
19. Paulson, H. L., Perez, M. K., Trottier, Y., Trojanowski, J. Q., Subramony, S. H., Das, S. S., Vig, P., Mandel, J. L., Fischbeck, K. H., and Pittman, R. N. (1997) *Neuron* **19,** 333–344.
20. DiFiglia, M., Sapp, E., Chase, K. O., Davies, S. W., Bates, G. P., Vonsattel, J. P., and Aronin, N. (1997) *Science* **277,** 1990–1993.
21. Skinner, P. J., Koshy, B. T., Cummings, C. J., Klement, I. A., Helin, K., Servadio, A., Zoghbi, H. Y., and Orr, H. T. (1997) *Nature* **389,** 971–974.
22. Igarashi, S., Koide, R., Shimohata, T., Yamada, M., Hayashi, Y., Takano, H., Date, H., Oyake, M., Sato, T., Sato, A., Egawa, S., Ikeuchi, T., Tanaka, H., Nakano, R., Tanaka, K., Hozumi, I., Inuzuka, T., Takahashi, H., and Tsuji, S. (1998) *Nature Genet.* **18,** 111–117.
23. Mei, L., Miwa, S., Kobayashi, Y., Merry, D. E., Yamamoto, M., Tanaka, F., Doyu, M., Hashizume, Y., Fischbeck, K. H., and Sobue, G. (1998) *Ann. Neurol.* **44,** 249–254.
24. Yazawa, I., Nukina, N., and Kanazawa, I. (1996) *Brain Res.* **732,** 154–158.