Systematic Uncovering of Multiple Pathways Underlying the Pathology of Huntington Disease by an Acid-cleavable Isotope-coded Affinity Tag Approach*§

Ming-Chang Chiang‡§¶, Chiun-Gung Juo‡¶, Hao-Hung Chang‡, Hui-Mei Chen‡, Eugene C. Yi¶, and Yijuang Chern‡§**

Huntington disease (HD) is an autosomal dominant neurodegenerative disease that results from a CAG (glutamine) trinucleotide expansion in exon 1 of huntingtin (Htt). The aggregation of mutant Htt has been implicated in the progression of HD. The earliest degeneration occurs in the striatum. To identify proteins critical for the progression of HD, we applied acid-cleavable ICAT technology to quantitatively determine changes in protein expressions in the striatum of a transgenic HD mouse model (R6/2). The cysteine residues of striatal proteins from HD and wild-type mice were labeled, respectively, with the heavy and light forms of the ICAT reagents. Samples were trypsinized, uncovered by avidin affinity chromatography, and analyzed by nano-LC-MS/MS. Western blot analyses were used to confirm and to calibrate the ICAT ratios. Linear regression was used to uncover a group of proteins that exhibited consistent changes. In two independent ICAT experiments, we identified 427 cysteine-containing striatal proteins among which ~66% (203 proteins) were detected in both ICAT experiments. Approximately two-thirds of proteins identified in each ICAT experiment were detected in both ICAT experiments. In total, 68 proteins with altered expressions in HD mice were identified. Elevated expressions of two down-regulated proteins (14–3-3z and FKBP12) effectively reduced Htt aggregates in a striatal cell line, supporting the functional relevance of the above findings. Collectively by using a well defined protocol for data analysis, large scale comparisons of protein expressions by ICAT can be reliable and can provide valuable clues for identifying proteins critical for pathophysiological functions.

Molecular & Cellular Proteomics 6:781–797, 2007.

Huntington disease (HD)¹ is a hereditary neurodegenerative disease characterized by dementia, chorea, and psychiatric symptoms. The causative mutation is a CAG (glutamine) trinucleotide expansion in exon 1 of the huntingtin (Htt) gene. The normal Htt gene has 35 or fewer repeats in the N-terminal region, whereas the appearance of neurological symptoms is associated with 36 or more CAG repeats (1). The major characteristic of HD is regional degeneration of neurons in the striatum and cortex that leads to movement disorders and dementia (2, 3). The toxicity of Htt in specific neurons correlates with the length of polyglutamine expansion (4). The aggregate formation of mutant Htt with poly(Q) expansion causes a wide variety of dysfunctions (5, 6). For example, insufficient protein degradation has been proposed as playing a major role (7). Htt aggregates were found to recruit components of protein folding and proteolytic pathways and therefore may suppress functions of the proteasome and heat shock proteins (8–11). In addition, transcriptional dysfunction caused by mutant Htt is critical for polyglutamine diseases (12, 13). Mutant Htt with poly(Q) expansion was shown to sequester and/or interfere with proteins important for the transcriptional machinery including p53, CREB, CREB-binding protein, TAFII130, and SP1 (14–19). These changes are specific because microarray analyses have revealed that expressions of a great number of other genes are not altered (20). It should be noted that the mechanism underlying the toxicity caused by mutant Htt remains largely controversial (21). Although aggregation is correlated with HD pathogenesis, and many beneficial treatments reduce aggregate formation (22, 23), evidence from different laboratories suggests that aggregate formation might confer protective effects against the toxicity induced by soluble mutant Htt with poly(Q) expansion (24, 25). Consistent with the above hypothesis, Schaffar et al. (26) demonstrated that monomers or small soluble oligomers of poly(Q)-expanded mutant Htt were suf-

From the ‡Institute of Biomedical Sciences, Academia Sinica, Taipei 11529, Taiwan, §Institute of Neuroscience, National Yang Ming University, Taipei 112, Taiwan, and ¶Institute of Systems Biology, Seattle, Washington 98103-8904.

Received, September 12, 2006, and in revised form, January 3, 2007.

Published, MCP Papers in Press, January 31, 2007, DOI 10.1074/mcp.M600356-MCP200

¹ The abbreviations used are: HD, Huntington disease; Htt, huntingtin; PKC, protein kinase C; FKBP12, FK506-binding protein, 12 kDa; PrxV, peroxiredoxin 5; CSPG, chondroitin sulfate proteoglycan protein; CREB, cAMP-response element-binding protein; WT, wild-type; RSD, relative standard deviation; hrGFP, humanized Renilla green fluorescent protein; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; 2DGE, two-dimensional gel electrophoresis; PDE, phosphodiesterase.
icient to inactivate TATA box-binding protein, an important transcription factor. Collectively the role of Htt aggregates at different stages of HD is complex and remains to be further elucidated.

Present large scale analyses of biochemical changes in HD have mostly been focused at the level of genes. Only a handful of proteomics analyses of brain proteins using two-dimensional gel electrophoresis-based techniques have been conducted on HD (27). We thus set out to examine the global protein expression profiles in the striatum of HD mice using a quantitative proteomics approach (ICAT). This method uses two labeling reagents whose weights are only 8 Da apart for comparative studies (28, 29). Recently an acid-cleavable ICAT reagent has begun to be used that avoids several shortcomings of the first generation ICAT reagent (30, 31).

In the present study, 427 cysteine-containing striatal proteins were detected from two independent ICAT experiments. Western blot analyses were used to confirm and to calibrate the ICAT data followed by a linear regression analysis to remove the outliers. In total, changes in the expressions of 68 proteins were found. From this it was determined that 6% (four proteins) were up-regulated, whereas 94% (64 proteins) were down-regulated. Among them, disease stage-dependent alterations of 10 proteins in the striatum of HD mice were investigated using Western blot analysis. The functional relevance of two down-regulated proteins (14-3-3s and FKBP12) was also demonstrated by their abilities to reduce Htt aggregates. Our study shows that large scale proteomics analysis using ICAT is a reliable approach for systematically identifying proteins critical for the development of HD.

EXPERIMENTAL PROCEDURES

Reagents—All reagents were purchased from Sigma except where specified. Dulbecco’s modified Eagle’s medium and fetal bovine serum were obtained from Invitrogen.

Animals—Male R6/2 mice and littermate controls were originally obtained from The Jackson Laboratory (Bar Harbor, ME) and were mated to female control mice (B6CBAF1/J). Offspring were identified as R6/2 transgenic mice and 25 wild-type (WT) littermate control mice (B6CBAF1/J) by the Academia Sinica Institutional Animal Care and Utilization Committee, Taiwan.

Nucleus-enriched Protein Preparation—In total, 27 R6/2 transgenic mice and 25 WT littermate control mice were used in this study. Animals were housed at the Institute of Biomedical Sciences Animal Care Facility under a 12-h light/dark cycle. Animal experiments were performed using protocols approved by the Academia Sinica Institutional Animal Care and Utilization Committee, Taiwan.

Nucleus-enriched protein extracts were first collected using a Dounce homogenizer in buffer A (50 mM HEPES (pH 8), 150 mM MgCl2, 10 mM KCl, 50 mM dithiothreitol, 1 mM Na3VO4, 20 mM NaF, 100 mM okadaic acid, and 25% glycerol) and incubated for 60 min on ice. Protein extracts were next collected by centrifugation at 19,275 × g for 5 min at 4 °C to remove the debris. The protein concentration was measured using a Bio-Rad protein assay reagent. This protocol was adopted from earlier studies using the same mouse model of HD at similar disease stages (32) or cell models with large aggregates (19). The majority of Htt aggregates were detected in the nucleus-enriched fraction, not in the cytosolic fraction or the debris (Supplemental Figs. S2 and S3). In addition, Western blot analyses using antibodies against a nuclear marker (lamin A/C) and a cytosolic marker (α-tubulin) demonstrated the successful enrichment of the nuclear fraction. Only slight contamination of the cytosolic fraction was observed (Supplemental Fig. S1). From striatal tissues of one mouse, ~0.4 mg of nucleus-enriched proteins at a concentration of 0.9–1 mg/ml was collected.

ICAT—In total, 12 R6/2 mice and 10 WT littermate control mice (at 10.5 weeks old) were used in two independent ICAT analyses. The nucleus-enriched protein fractions (1 mg) were prepared as described above and labeled with cleavable ICAT reagents (Applied Biosystems, Foster City, CA) following the manufacturer’s protocol. Briefly striatal proteins collected from WT and HD mice were labeled, respectively, with isotopically light and heavy ICAT reagents. The labeled preparations were combined and digested with trypsin (Promega, Madison, WI) overnight at 37 °C using an enzyme-to-protein ratio of 1:50 (w/w). The resulting peptide mixture was fractionated by three-step chromatography. Samples were first separated using a Polysulfoethyl A column (Poly LC, Columbia, MD; 2.1 mm × 20 cm) at a flow rate of 200 μl/min. Peptides were eluted with 100% buffer A (5 mM KH2PO4 and 25% acetonitrile, pH 3.0) for 2 min followed by a linear gradient from buffer A to buffer B (5 mM KH2PO4, 350 mM NaCl, and 25% acetonitrile, pH 3.0) over 48 min. Fractions were collected (one fraction/min) and subjected to affinity purification using a monomeric avidin cartridge (Applied Biosystems). The purified samples were then treated with acid to cleave the tag and separated by reverse-phase capillary liquid chromatography (Magic C18AQ, Michrom Bioreources, Auburn, CA; 75 μm × 11 cm) at a flow rate of 200 nl/min. The eluent was directly analyzed by ion trap mass spectrometry (LCQ Deca XP, Thermo Finnigan, San Jose, CA) under conditions described elsewhere (33). A survey scan followed by three CID events was used. The tolerances of the precursor ion and fragment ion were 2 and 1.5 amu, respectively. Peptide identification by CID was carried out in automated mode using the 3-min dynamic exclusion option.

Data Analysis of ICAT—ICAT-labeled peptides were first analyzed using SEQUEST in Bioworks 3.1 (Thermo Finnigan) (34). The tolerances of the precursor ion and fragment ion were the same as described above. The database was downloaded from the website of the National Center for Biotechnology Information (NCBI; mouse; April 12, 2004; 84,599 entries) and then analyzed by Protein Prophet Version 1.0 (35). INTERACT_15-10-2004 (36), and Protein Prophet.pl Version 2.0 (37). Peptide prophet was used to increase the accuracy of peptide identification, and Protein Prophet was used to reduce the redundancy of the search results. The cutoff scores of Peptide Prophet and Protein Prophet were ≈0.9. The false-positive error rate for both ICAT experiments was 0.008. Relative quantification was performed using ASAPRatio 3.0 (38). The above four software packages were kindly provided by Institute for Systems Biology Proteomics Windows Software Distribution. The resulting peptide spectra of proteins were manually checked for qualitative and quantitative results. For protein identification, the peptide sequences were first blasted through the NCBI databases to obtain the corresponding protein sequences, which were then transferred into UniProt to obtain the accession numbers using WU-Blast2 (a tool that effectively finds regions of sequence similarity; www.ebi.ac.uk/blast2). Through the process, 14 proteins were removed due to redundancy or
changes in annotation. In three instances where the detected peptides were located in common domains of certain protein families, family names were assigned. The UniProt primary accession numbers of the remaining 203 proteins are listed in Table II and Supplementary Table S2 to minimize the redundancy. Note that as observed in quite a few published studies and summarized in a recent review (88), a low number of detected peptides per protein is commonly found in quantitative proteomics approaches using chemical tagging-enrichment strategies. In our ICAT study where cytochrome oxidase was used to tag the proteins, 66 of 203 proteins (−33%) were identified by only one peptide (sequence). This is consistent with other studies using chemical tagging-enrichment reactions and does not reflect the quality of our mass data. Names of the proteins for which only one peptide (sequence) was detected in each ICAT experiment are labeled in green (Table II and Supplemental Table S2). Identification of these proteins needs to be validated using orthogonal methods before reaching a conclusion (Table II and Supplemental Table S2).

Six protein ratios determined by Western blot analysis with a relative standard deviation (RSD) of <±0.06 were used to calibrate the ICAT ratios as described in detail in the Supplemental Experimental Procedures. Correlations of different batches of samples were calculated using SAS/STAT, Version 8.0 (SAS Institute, Cary, NC).

**Western Blot Analysis**—In total, 15 R6/2 mice and 15 WT littermates were used in the Western blot analyses. There were seven and eight mice in the groups that were 7 and 10.5 weeks old, respectively. Three to six independent preparations of nucleus-enriched proteins for each condition were prepared from two to three striatal tissues as described above and used for the Western blot analyses. Equal amounts of protein were separated by SDS-PAGE using 10% polyacrylamide gels according to the method of Laemmli (39). The resolved proteins were electroblotted onto Immobilon polyvinylidene difluoride membranes (Millipore, Bedford, MA). Membranes were blocked with 5% skim milk in PBS and incubated with an anti-β-actin antibody (1:2000 dilution; Santa Cruz Biotechnology), anti-PKCα (1:1000 dilution; Transduction Laboratories, Lexington, KY), anti-14-3-3ɛ antibody (1:1000 dilution; Santa Cruz Biotechnology), anti-FKBP12 antibody (1:1000 dilution; Santa Cruz Biotechnology), anti-adducin β antibody (1:1000 dilution; Santa Cruz Biotechnology), anti-casein kinase IIβ antibody (1:1000 dilution; Santa Cruz Biotechnology), anti-CSPG antibody (1:1000 dilution; Chemicon International, Temecula, CA), anti-Gp antibody (1:1000 dilution; Santa Cruz Biotechnology), anti-G2y antibody (1:1000 dilution; Santa Cruz Biotechnology), anti-PnxV antibody (1:1000 dilution; Santa Cruz Biotechnology), anti-lamin A/C antibody (1:2000 dilution; Santa Cruz Biotechnology), anti-α-tubulin antibody (1:3000 dilution; Sigma), anti-Htt antibody (1:1000 dilution; Chemicon International), and anti-V5 antibody (1:2500; Invitrogen) at 4 °C overnight followed by the corresponding secondary antibody for 1 h at room temperature. Immunoreactive bands were detected by enhanced chemiluminescence (Pierce) and recorded using Kodak XAR-5 film.

**RNA Isolation and Quantitative Real Time RT-PCR**—Total RNA was isolated using the Trizol reagent kit (Molecular Research Center, Cincinnati, OH), treated with RNase-free DNase (RQ1, Promega) to remove the potential contamination of genomic DNA, and then transcribed into cDNA using Superscript II reverse transcriptase. Real time quantitative PCR was performed using a TaqMan kiiiet (PE Applied Biosystems) on a TaqMan ABI 7700 Sequence Detection System (PE Applied Biosystems) using heat-activated Taq DNA polymerase (AmpliTaq Gold, PE Applied Biosystems), The sequences of primers used are as follows: for 14-3-3ɛ (the target gene), 5′-GGAGGAAGACCTTCTCTCGTGTGCACTTT-3′ and 5′-CCTCGTACTGTCATGTTCTG-3′; for FKB12 (the target gene), 5′-GGCTGAAGATGGAAA-GAAATTTGAT-3′ and 5′-GCTCGTACTGTCATGTTCTG-3′; for FKB12 (the target gene), 5′-GGCTGAAGATGGAAA-GAAATTTGAT-3′ and 5′-GCTCGTACTGTCATGTTCTG-3′; for FKB12 (the target gene), 5′-GGCTGAAGATGGAAA-GAAATTTGAT-3′ and 5′-GCTCGTACTGTCATGTTCTG-3′; for FKB12 (the target gene), 5′-GGCTGAAGATGGAAA-GAAATTTGAT-3′ and 5′-GCTCGTACTGTCATGTTCTG-3′; for FKB12 (the target gene), 5′-GGCTGAAGATGGAAA-GAAATTTGAT-3′ and 5′-GCTCGTACTGTCATGTTCTG-3′. Independent reverse transcription-PCRs were performed as described elsewhere (19). The relative transcript amount of the target gene, which was calculated using standard curves of serial RNA dilutions, was normalized to that of GAPDH of the same cDNA.

**RESULTS AND DISCUSSION**

To systematically identify proteins important for the disease progression of HD, we applied ICAT technology to quantitatively assess the overall protein expression profiles in the striatum of 10.5-week-old HD mice, an age at which several symptoms of HD have already become manifest (e.g. deficiency in motor coordination, body wasting, and Htt aggregate formation) (23). Previous studies using two-dimensional gel electrophoresis (2DGE) also showed that the levels of oxidatively modified proteins are decreased in the brains of 10-week-old HD mice (27). To reduce the complexity and thus improve the ICAT quality as reported earlier (42), we chose to determine only the nucleus-enriched fraction of the striatum.
The nuclear preparation method used in the present study has been widely used in similar studies of HD mice at a late stage of disease progression and for cell models with large aggregates. Western blot analyses using antibodies against a nuclear marker (lamin A/C) and a cytosolic marker (α-tubulin) demonstrated successful enrichment of the nuclear fraction. Only slight contamination by the cytosolic fraction was observed (Supplemental Fig. S1). Western blot analyses revealed that there was no change in the protein level of lamin A/C in the striatum of R6/2 mice (98.9 ± 2.6% of those in WT mice, mean ± S.D. values from eight independent preparations); we therefore used lamin A/C as an internal control for the following Western blot analyses.

Two independent ICAT analyses of the nucleus-enriched, striatal proteins collected from R6/2 and wild-type mice were carried out. Approximately 380 proteins were identified in each experiment. Nearly two-thirds of the identified proteins in each ICAT experiment were found in both experiments (Fig. 1A). In total, 513 unique proteins were identified. Due to nonspecific binding of peptides in affinity chromatography, only 83% (427 of 513) of the identified peptides contained cysteine(s). The numbers of cysteine-containing proteins identified in experiments I and II were 307 and 323, respectively. Quantitation was only performed for proteins containing cysteine residues. Again nearly two-thirds (203 proteins; Supplemental Table S2) of cysteine-containing proteins were detected in both experiments (Fig. 1B) and were further analyzed using an automated algorithm (ASAPRatio (38)) to produce a ratio value (WT/HD) for each protein (designated RatioASAP). The average RSD of the RatioASAP for experiment I and experiment II was 0.23.

Proper normalization procedures are critical for high throughput, quantitative proteomics approaches (such as ICAT). To the present, the most widely adapted algorithms (including the ASAPRatio) are designed based on the assumption that the major protein population in a proteome is unchanged, which allows normalization by aligning the center of the peptide ratio distribution peak to 1 (or log₁₀ Ratio = 0; Supplemental Fig. S4A). However, in more severe degenera-
plication of ICAT analyses is highly recommended and that correlation between two sets of ICAT data might provide an additional means to increase the confidence level.

We thus conducted a linear regression analysis of the log-transformed WT/HD ratios (i.e., RatioC) of experiment I and experiment II (Fig. 3A). The Pearson correlation coefficient was 0.63, and the p value was < 0.0001. The ratios of the two independent ICAT experiments were thus significantly positively correlated. The p value from Student’s t test was 0.77 (>0.05), suggesting that results from experiment I and experiment II did not significantly differ. We also performed a linear regression on the ratios of both experiments ($r^2 = 0.5070$). When $\pm 1.96 \sigma$ was set as the upper and lower limits, 18 outliers were found. Among them, six proteins exhibited opposite expression ratios in the two ICAT experiments, four proteins showed alteration in only one ICAT experiment, whereas the remaining eight proteins were reproducibly regulated toward the same direction in both ICAT experiments. The reason that the latter eight proteins are considered to be outliers is because their expression ratios in the two ICAT experiments were thus significantly posi-

| No | protein | Ratio ASAP | Ratio C | Ratio ASAP | Ratio C | Ratio ASAP | Ratio C | Ratio ASAP | Ratio C | Ratio ASAP | Ratio C |
|----|---------|------------|---------|------------|---------|------------|---------|------------|---------|------------|---------|
| 1  | Chondroitin sulfate proteoglycan protein | 0.65 | 0.06 | 0.61 | 0.12 | 0.30 | 0.63 | 0.74 | 0.26 |
| 2  | G protein, γ 2 subunit (Gγ2) | 0.69 | 0.03 | 0.59 | 0.69 | 0.19 | 0.58 | 0.69 | 0.21 |
| 3  | Peroxiredoxin V (PrxV) | 0.69 | 0.01 | 0.54 | 0.63 | 0.19 | 0.51 | 0.60 | 0.47 |
| 4  | G protein N, β-1 subunit (GBP1) | 0.74 | 0.05 | 0.67 | 0.79 | 0.51 | 0.77 | 0.91 | 0.17 |
| 5  | Adducin-1 α | 1.14 | 0.03 | 1.04 | 1.22 | 0.14 | 0.87 | 1.03 | 0.07 |
| 6  | Casein kinase II, β subunit (CKII β) | 1.44 | 0.06 | 1.10 | 1.29 | 0.18 | 1.11 | 1.31 | 0.23 |
| 7  | Protein kinase C, β (PKCβ) | 2.64 | 0.11 | 3.00 | 3.52 | 0.19 | 1.20 | 1.42 | 0.35 |
| 8  | β-Actin | 3.82 | 0.25 | 1.20 | 1.41 | 0.05 | 1.21 | 1.43 | 0.30 |
| 9  | FK506 binding protein N-12 KD (FKBP12) | 4.46 | 0.21 | 1.27 | 1.49 | 0.10 | 1.42 | 1.68 | 0.23 |
| 10 | 14-3-3 ε | 4.52 | 0.15 | 1.21 | 1.42 | 0.28 | 1.74 | 2.06 | 0.29 |

After removing 10 outliers as described above, we analyzed the remaining 193 proteins that showed alterations in the same direction in the two independent ICAT experiments for meaningful changes in their expression levels. Based on the results of Western blot analyses of 10 different proteins, we found that a 1-fold average RSD (0.23) was an appropriate criterion to define a meaningful change (Table I). We therefore listed proteins with a RatioC of either $\geq 1.23$ or $\leq 0.77$ in Table II. A few proteins, with little possibility of existing in the nucleus and that might have been present due to contamination from other cellular fractions, were removed from the table. Of the 68 proteins, four proteins were up-regulated, whereas the remaining 64 proteins were down-regulated in the striatum of HD mice. To the best of our knowledge, only seven of them (PKCβII, calcium/calcmodulin-dependent protein kinase type II, enolase, triose-phosphate isomerase, aconitase, creatine kinase, and cytochrome c) have been implicated in HD (27, 43–46). Based on information obtained from protein knowledgebases of Swiss-Prot and TrEMBL (www.expasy.org), NCBI (www.ncbi.nlm.nih.gov/protein), European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI) (www.ebi.ac.uk/InterProScan), PSORT II (89), and PubMed (www.ncbi.nlm.nih.gov/PubMed), the differentially regulated proteins fall into 11 categories, excluding six proteins whose functions are unknown at this time (Table II). In total, 64 down-regulated proteins and four up-regulated proteins were found in the striatum of 10.5-week-old HD mice. This finding is consistent with an earlier study using a microarray in which the number of down-regulated transcripts greatly outnumbered up-regulated transcripts (20). These results support the hypothesis that at the symptomatic stage of HD, alterations in protein expressions are tilted toward the side of down-regulation. The major categories in the down-regulated groups were signaling molecules and those involved in general metabolism (Table II and Fig. 4). In a recent report by Perluigi et al. (27), nine proteins from the striatal lysate of HD (R6/2) mice aged 10 weeks were identified using a combination of 2DGE and MS. Among them, six proteins were oxidized and inhibited. Interestingly levels of three suppressed proteins (enolase, aconitase, and creatine kinase) were also found to be reduced in our study (Table II). Consistent with prior reports that ICAT is capable of identifying more proteins than 2DGE (47), we found ~22-fold more striatal proteins by Western blot analysis; RatioASAP, ratio adjusted by the ASAPRatioC; RatioC, RatioASAP calibrated by the RatioW; RSD, S.D./ratio. If applicable, the abbreviation for each protein used in the text is shown in parentheses.  

Molecular & Cellular Proteomics 6.5 785
the present study using ICAT (203 proteins; Supplemental Table S2). In addition, the number of striatal proteins with changes herein was 68 (Table II) and was about 11-fold that reported in the study using 2DGE (six proteins (47)). Note that the age of the HD mice in the earlier study was slightly younger than that used in the present study and that both approaches (ICAT and 2DGE) have their unique limitations that prohibit them from identifying all proteins present. In the

Fig. 2. Confirmation of ICAT results by Western blot analyses. Striatal proteins (50 μg/lane) collected from the indicated 7- and 10.5-week (w)-old mice were subjected to Western blot analysis using the corresponding antibodies. Representative images of three to six independent experiments are shown. The relative amounts of the indicated proteins were normalized with an internal control (lamin A/C) and were compared with those of R6/2 mice in each experiment. Data represent the mean ± S.E. values from four independent experiments and are shown at the top of the corresponding lane. * and **, specific comparison with R6/2 mice (p < 0.05 and p < 0.001, respectively; one-way analysis of variance). Western blot analyses of these 10 proteins are arranged from A to J in the descending order according to their WT/HD ratios at the age of 10.5 weeks old.
future, it would be very interesting to combine these two compensatory approaches and identify more proteins exhibiting changes during HD progression.

As described above, we performed Western blot analyses of 10 proteins to verify and calibrate the expression profiles revealed by ICAT. Among them, results from both ICAT and Western blot analyses suggested that three (CSPG, Gγ2, and PrxV) were up-regulated, whereas five (PKCβ, β-actin, FKBPl2, 14-3-3ζ, and casein kinase IIβ) were down-regulated in the striatum of 10.5-week-old HD mice (Table I). Slight changes in two proteins (Gβ1 and adducin 1α) were detected using Western blot analyses but were not evident in the ICAT analyses when a 1-fold average RSD (0.23) was used as the criterion to define a meaningful change. It appears that the ICAT approach as analyzed and calibrated herein is not suitable for the identification of proteins exhibiting very limited changes.

We next determined the functional relevance of the above findings with ICAT. Western blot analyses revealed that, except for FKBPl2, changes in none of the other proteins in the striatum HD mice were observed at the age of 7 weeks when most symptoms are not yet evident. Down-regulation of FKBPl2 in HD mice was moderate at 7 weeks old and was reduced to only ~20% of that of WT mice at the age of 10.5 weeks. Regulation of these proteins in the striatum therefore appears to be closely associated with disease progression of HD.

Among these proteins, 14-3-3ζ is of the greatest interest because it is a scaffold that interacts with more than 100 proteins important for transcriptional/translational control, the cell cycle, apoptosis, intracellular trafficking, ion channel regulation, and synaptic transmission (48, 49). Suppression of 14-3-3ζ as found in the present study therefore might jeopardize multiple functions in mutant Htt-expressing cells. Most
TABLE II

Lists of striatal proteins up-regulated or down-regulated in 10.5-week-old HD mice compared with WT mice revealed by ICAT

| Protein name | Group | Ratio_WT/HD | Ratio_WT/HD | Ratio_WT/HD | Ratio_WT/HD | Ratio_WT/HD | Ratio_WT/HD | Spectrum No. | Peptide sequence | Precursor ion change |
|--------------|-------|-------------|-------------|-------------|-------------|-------------|-------------|--------------|------------------|--------------------|
| GSK3B       | 1.00  | 1.73        | 2.02        | 0.12        | 1.23        | 1.45        | 0.19        | 1            | AHGQCMVNTS   | 2                  |
|              |       |             |             |             |             |             |             | 2            | AHGQCMVNTS   |                    |
|              |       |             |             |             |             |             |             | 3            |                  |                    |
|              |       |             |             |             |             |             |             | 4            |                  |                    |
|              |       |             |             |             |             |             |             | 5            |                  |                    |
|              |       |             |             |             |             |             |             | 6            |                  |                    |
|              |       |             |             |             |             |             |             | 7            |                  |                    |
|              |       |             |             |             |             |             |             | 8            |                  |                    |
|              |       |             |             |             |             |             |             | 9            |                  |                    |
|              |       |             |             |             |             |             |             | 10           |                  |                    |
|              |       |             |             |             |             |             |             | 11           |                  |                    |
|              |       |             |             |             |             |             |             | 12           |                  |                    |
|              |       |             |             |             |             |             |             | 13           |                  |                    |
|              |       |             |             |             |             |             |             | 14           |                  |                    |
|              |       |             |             |             |             |             |             | 15           |                  |                    |
|              |       |             |             |             |             |             |             | 16           |                  |                    |
|              |       |             |             |             |             |             |             | 17           |                  |                    |
|              |       |             |             |             |             |             |             | 18           |                  |                    |
|              |       |             |             |             |             |             |             | 19           |                  |                    |
|              |       |             |             |             |             |             |             | 20           |                  |                    |
|              |       |             |             |             |             |             |             | 21           |                  |                    |
|              |       |             |             |             |             |             |             | 22           |                  |                    |
|              |       |             |             |             |             |             |             | 23           |                  |                    |
|              |       |             |             |             |             |             |             | 24           |                  |                    |
|              |       |             |             |             |             |             |             | 25           |                  |                    |
|              |       |             |             |             |             |             |             | 26           |                  |                    |
|              |       |             |             |             |             |             |             | 27           |                  |                    |
|              |       |             |             |             |             |             |             | 28           |                  |                    |
|              |       |             |             |             |             |             |             | 29           |                  |                    |
|              |       |             |             |             |             |             |             | 30           |                  |                    |
|              |       |             |             |             |             |             |             | 31           |                  |                    |
|              |       |             |             |             |             |             |             | 32           |                  |                    |
|              |       |             |             |             |             |             |             | 33           |                  |                    |
|              |       |             |             |             |             |             |             | 34           |                  |                    |
|              |       |             |             |             |             |             |             | 35           |                  |                    |
|              |       |             |             |             |             |             |             | 36           |                  |                    |
|              |       |             |             |             |             |             |             | 37           |                  |                    |
|              |       |             |             |             |             |             |             | 38           |                  |                    |
|              |       |             |             |             |             |             |             | 39           |                  |                    |
|              |       |             |             |             |             |             |             | 40           |                  |                    |
|              |       |             |             |             |             |             |             | 41           |                  |                    |
|              |       |             |             |             |             |             |             | 42           |                  |                    |
|              |       |             |             |             |             |             |             | 43           |                  |                    |
|              |       |             |             |             |             |             |             | 44           |                  |                    |
|              |       |             |             |             |             |             |             | 45           |                  |                    |
|              |       |             |             |             |             |             |             | 46           |                  |                    |
|              |       |             |             |             |             |             |             | 47           |                  |                    |
|              |       |             |             |             |             |             |             | 48           |                  |                    |
|              |       |             |             |             |             |             |             | 49           |                  |                    |
|              |       |             |             |             |             |             |             | 50           |                  |                    |
|              |       |             |             |             |             |             |             | 51           |                  |                    |
|              |       |             |             |             |             |             |             | 52           |                  |                    |
|              |       |             |             |             |             |             |             | 53           |                  |                    |
|              |       |             |             |             |             |             |             | 54           |                  |                    |
|              |       |             |             |             |             |             |             | 55           |                  |                    |
|              |       |             |             |             |             |             |             | 56           |                  |                    |
|              |       |             |             |             |             |             |             | 57           |                  |                    |
|              |       |             |             |             |             |             |             | 58           |                  |                    |
|              |       |             |             |             |             |             |             | 59           |                  |                    |
|              |       |             |             |             |             |             |             | 60           |                  |                    |
|              |       |             |             |             |             |             |             | 61           |                  |                    |
|              |       |             |             |             |             |             |             | 62           |                  |                    |
|              |       |             |             |             |             |             |             | 63           |                  |                    |
|              |       |             |             |             |             |             |             | 64           |                  |                    |
|              |       |             |             |             |             |             |             | 65           |                  |                    |

CaMKII, calcium/calmodulin-dependent kinase II; SH3, Src homology 3.

Note that these proteins were identified from a nucleus-enriched preparation and are likely to be located in the nucleus. Nevertheless their nuclear localizations need to be further validated.

Ratios adjusted by the ASAPRatio; RatioW, RatioASAP calibrated by the RatioW; RatioV, ratio detected by Western blot analysis (Table I); RSD, S.D./ratio; C*, cysteine labeled with heavy reagent; M#, oxidized methionine. The spectrum for each peptide is illustrated in Supplemental Table S3.
TABLE II—continued

| UniProt   | Protein name           | Group probability | Ratio P1/P2 | Ratio P1/P3 | Ratio P2/P3 | Spectrum | Peptide sequence | Precursor lon charge |
|-----------|------------------------|-------------------|-------------|-------------|-------------|----------|----------------|---------------------|
| 692828    | N-acetylglutamate dehydrogenase | 1.00              | 1.19        | 1.18        | 1.00        | 86       | LQGYSVRLK        | 2                   |
| 692827    | N-acetylglutamate dehydrogenase | 1.00              | 1.18        | 1.17        | 1.00        | 87       | LQGYSVRLK        | 2                   |
| 692826    | N-acetylglutamate dehydrogenase | 1.00              | 1.17        | 1.16        | 1.00        | 88       | LQGYSVRLK        | 2                   |
| 692825    | N-acetylglutamate dehydrogenase | 1.00              | 1.16        | 1.15        | 1.00        | 89       | LQGYSVRLK        | 2                   |
| 692824    | N-acetylglutamate dehydrogenase | 1.00              | 1.15        | 1.14        | 1.00        | 90       | LQGYSVRLK        | 2                   |
| 692823    | N-acetylglutamate dehydrogenase | 1.00              | 1.14        | 1.13        | 1.00        | 91       | LQGYSVRLK        | 2                   |
| 692822    | N-acetylglutamate dehydrogenase | 1.00              | 1.13        | 1.12        | 1.00        | 92       | LQGYSVRLK        | 2                   |
| 692821    | N-acetylglutamate dehydrogenase | 1.00              | 1.12        | 1.11        | 1.00        | 93       | LQGYSVRLK        | 2                   |
| 692820    | N-acetylglutamate dehydrogenase | 1.00              | 1.11        | 1.10        | 1.00        | 94       | LQGYSVRLK        | 2                   |

Molecular chaperone

| UniProt   | Protein name           | Group probability | Ratio P1/P2 | Ratio P1/P3 | Ratio P2/P3 | Spectrum | Peptide sequence | Precursor lon charge |
|-----------|------------------------|-------------------|-------------|-------------|-------------|----------|----------------|---------------------|
| 702777    | DnaJ homolog 3, mitochondrial | 1.00              | 1.19        | 1.18        | 1.00        | 106      | LQGYSVRLK        | 2                   |
| 702776    | DnaJ homolog 3, mitochondrial | 1.00              | 1.18        | 1.17        | 1.00        | 107      | LQGYSVRLK        | 2                   |
| 702775    | DnaJ homolog 3, mitochondrial | 1.00              | 1.17        | 1.16        | 1.00        | 108      | LQGYSVRLK        | 2                   |
| 702774    | DnaJ homolog 3, mitochondrial | 1.00              | 1.16        | 1.15        | 1.00        | 109      | LQGYSVRLK        | 2                   |
| 702773    | DnaJ homolog 3, mitochondrial | 1.00              | 1.15        | 1.14        | 1.00        | 110      | LQGYSVRLK        | 2                   |
| 702772    | DnaJ homolog 3, mitochondrial | 1.00              | 1.14        | 1.13        | 1.00        | 111      | LQGYSVRLK        | 2                   |
| 702771    | DnaJ homolog 3, mitochondrial | 1.00              | 1.13        | 1.12        | 1.00        | 112      | LQGYSVRLK        | 2                   |
| 702770    | DnaJ homolog 3, mitochondrial | 1.00              | 1.12        | 1.11        | 1.00        | 113      | LQGYSVRLK        | 2                   |

Signaling molecules

| UniProt   | Protein name           | Group probability | Ratio P1/P2 | Ratio P1/P3 | Ratio P2/P3 | Spectrum | Peptide sequence | Precursor lon charge |
|-----------|------------------------|-------------------|-------------|-------------|-------------|----------|----------------|---------------------|
| 702779    | Protein kinase C, beta | 1.00              | 1.19        | 1.18        | 1.00        | 120      | LQGYSVRLK        | 2                   |
| 702778    | Protein kinase C, beta | 1.00              | 1.18        | 1.17        | 1.00        | 121      | LQGYSVRLK        | 2                   |
| 702777    | Protein kinase C, beta | 1.00              | 1.17        | 1.16        | 1.00        | 122      | LQGYSVRLK        | 2                   |
| 702776    | Protein kinase C, beta | 1.00              | 1.16        | 1.15        | 1.00        | 123      | LQGYSVRLK        | 2                   |
| 702775    | Protein kinase C, beta | 1.00              | 1.15        | 1.14        | 1.00        | 124      | LQGYSVRLK        | 2                   |
| 702774    | Protein kinase C, beta | 1.00              | 1.14        | 1.13        | 1.00        | 125      | LQGYSVRLK        | 2                   |
| 702773    | Protein kinase C, beta | 1.00              | 1.13        | 1.12        | 1.00        | 126      | LQGYSVRLK        | 2                   |
| 702772    | Protein kinase C, beta | 1.00              | 1.12        | 1.11        | 1.00        | 127      | LQGYSVRLK        | 2                   |
| 702771    | Protein kinase C, beta | 1.00              | 1.11        | 1.10        | 1.00        | 128      | LQGYSVRLK        | 2                   |

Molecular & Cellular Proteomics 6.5 789
ICAT Analysis of Striatum of Mice with Huntington Disease

**TABLE II—continued**

| UnitProt | Protein name | Group probability | Ratio 1 | Ratiom 1 | RSD 1 | Ratio 2 | Ratiom 2 | RSD 2 | Spectrum No | Peptide sequence | Precursor | precursor ion charge |
|----------|--------------|-------------------|--------|---------|------|--------|---------|------|------------|------------------|-----------|-------------------|
| G530F1 | unknown       | 0.90              | 2.47   | 3.68    | 0.28 | 3.49   | 4.11    | 0.18 | 154        | ALPCCCLLSXYHLRE | 2         |                   |
| P11709 | unknown       | 0.98              | 1.98   | 2.15    | 0.24 | 2.37   | 2.79    | 0.23 | 155        | STRYAMSMGAF       | 2         |                   |
| G075F2 | CAP-assoc.    | 1.00              | 1.98   | 1.68    | 0.29 | 1.87   | 2.21    | 0.17 | 156        | ISTYAMGMHR         | 2         |                   |
| G078E3 | CAP-assoc.    | 1.00              | 1.48   | 1.73    | 0.11 | 1.72   | 2.52    | 0.31 | 157        | STRYAMHGGR         | 2         |                   |
| G080E3 | CAP-assoc.    | 1.00              | 1.34   | 1.06    | 0.28 | 1.76   | 2.07    | 0.20 | 158        | STRYAMGHR          | 2         |                   |
| G092G3 | CAP-assoc.    | 1.00              | 1.28   | 1.56    | 0.13 | 1.34   | 1.83    | 0.19 | 159        | STRYAMHR           | 2         |                   |
| P132D3 | NIM1 domain   | 1.00              | 1.27   | 1.46    | 0.16 | 1.42   | 1.66    | 0.23 | 160        | STRYAMHR           | 2         |                   |
| P134D3 | NIM1 domain   | 1.00              | 1.21   | 1.46    | 0.28 | 1.76   | 2.05    | 0.29 | 161        | STRYAMHR           | 2         |                   |
| G032H3 | G-protein C-actin fil  | 1.00              | 0.98   | 0.89    | 0.19 | 0.98   | 0.89    | 0.21 | 162        | STRYAMHR           | 2         |                   |
| G033H3 | G-protein C-actin fil  | 1.00              | 1.19   | 1.36    | 0.28 | 1.09   | 1.26    | 0.19 | 163        | STRYAMHR           | 2         |                   |
| G056E2 | Dihydoxyacetone    | 1.00              | 1.11   | 1.29    | 0.16 | 1.32   | 1.65    | 0.06 | 164        | STRYAMHR           | 2         |                   |
| G052D2 | Dihydoxyacetone    | 1.00              | 1.36   | 1.53    | 0.33 | 1.87   | 2.17    | 0.11 | 165        | STRYAMHR           | 2         |                   |
| G052D2 | Dihydoxyacetone    | 1.00              | 1.32   | 1.56    | 0.26 | 1.47   | 1.74    | 0.14 | 166        | STRYAMHR           | 2         |                   |
| G052D2 | Dihydoxyacetone    | 1.00              | 1.91   | 1.78    | 0.18 | 1.33   | 1.96    | 0.18 | 167        | STRYAMHR           | 2         |                   |
| G052D2 | Dihydoxyacetone    | 1.00              | 1.85   | 1.77    | 0.25 | 1.45   | 1.73    | 0.26 | 168        | STRYAMHR           | 2         |                   |
| G052D2 | Dihydoxyacetone    | 1.00              | 1.40   | 1.49    | 0.15 | 1.21   | 1.62    | 0.17 | 169        | STRYAMHR           | 2         |                   |
| G052D2 | Dihydoxyacetone    | 1.00              | 1.14   | 1.33    | 0.15 | 1.85   | 1.35    | 0.18 | 170        | STRYAMHR           | 2         |                   |
| G052D2 | Dihydoxyacetone    | 1.00              | 1.86   | 2.15    | 0.26 | 1.56   | 1.64    | 0.22 | 171        | STRYAMHR           | 2         |                   |

**Structure-associated protein**

| G350D4 | Spinal nerve 3  | 1.00              | 1.71   | 1.99    | 0.12 | 1.31   | 1.54    | 0.26 | 172        | ALPCCCLLSXYHLRE | 2         |                   |
| G350D4 | Spinal nerve 3  | 1.00              | 1.91   | 1.76    | 0.18 | 1.33   | 1.96    | 0.18 | 173        | ALPCCCLLSXYHLRE | 2         |                   |
| G350D4 | Spinal nerve 3  | 1.00              | 1.40   | 1.73    | 0.25 | 1.45   | 1.73    | 0.26 | 174        | ALPCCCLLSXYHLRE | 2         |                   |
| G350D4 | Spinal nerve 3  | 1.00              | 1.26   | 1.49    | 0.15 | 1.21   | 1.62    | 0.17 | 175        | ALPCCCLLSXYHLRE | 2         |                   |
| G350D4 | Spinal nerve 3  | 1.00              | 1.14   | 1.33    | 0.15 | 1.85   | 1.35    | 0.18 | 176        | ALPCCCLLSXYHLRE | 2         |                   |
| G350D4 | Spinal nerve 3  | 1.00              | 1.86   | 2.15    | 0.26 | 1.56   | 1.64    | 0.22 | 177        | ALPCCCLLSXYHLRE | 2         |                   |
intriguingly, 14-3-3\textsubscript{H9269} was found to be associated with molecular chaperons such as heat shock proteins (50–52). Elevation of several heat shock proteins has been found to reduce the aggregation and toxicity of mutant Htt (8, 53). We thus hypothesized that the expression level of 14-3-3\textsubscript{H9269} might play an important role in the extent of Htt aggregate formation. Indeed results of the filter assay demonstrated that elevated expression of 14-3-3\textsubscript{H9269} markedly reduced the aggregation of mutant Htt with expanded poly(Q) (Fig. 5, A and B) and might lead to beneficial effects on HD progression. Indeed in a recent review by Kaneko and Hachiya (54), 14-3-3\textsubscript{H9269} was hypothesized as selectively recognizing and segregating misfolded proteins and therefore can be used to protect cells against toxicity caused by aggregation. Another protein that caught our attention was FKBP12 whose degeneration in the striatum of HD mice was detected as early as 7 weeks old (Fig. 2B). FKBP12 is a binding protein of FK506 that is able to protect neurons against glutamate excitotoxicity and transient oxygen-glucose deprivation via an antiexcitotoxic effect (55, 56). In addition, like other FKBP members (FKBP51 and FKBP52), FKBP12 might also function as a chaperone and regulate the expressions of heat shock proteins (57). To assess its functional importance, we expressed FKBP12 in a striatal cell line harboring a mutant Htt with expanded poly(Q). Elevated FKBP12 expression reduced the formation of mutant Htt aggregates (Fig. 5, C and D). In contrast, expression of Lim (a molecule important for early development (58)) did not affect Htt aggregate formation (Supplemental Fig. S5). The mechanisms used by 14-3-3\textsubscript{H9269} and FKBP12 to reduce aggregates are currently unknown, and determining these was outside the scope of this study. Instead the above findings demonstrate that the list of molecules revealed by ICAT (Table II) is likely to be functionally relevant and may provide valuable information for the development of HD therapies.

| TABLE II—continued |

| Uniprot | Protein name | Group | Probability | Ratio Error | RSD | Ratio Error | RSD | Spectrum No. | Peptide sequence | Precursor mass/charge |
|---------|-------------|-------|-------------|-------------|-----|-------------|-----|--------------|-------------------|---------------------|
| P21817 | Synaptotagmin | 1.00 | 2.18 | 2.52 | 0.42 | 1.85 | 2.24 | 0.37 | LEDLGDRPR | 2 |
| D27242 | Alpha-actinin | 2.00 | 2.32 | 1.11 | 0.12 | 1.31 | 0.31 | 2 |
| P81208 | Synaptotagmin | 1.00 | 2.11 | 2.25 | 0.40 | 1.46 | 0.48 | 1.36 | 0.36 |
| G81714 | Bipath | 1.00 | 2.14 | 2.31 | 0.40 | 1.46 | 0.48 | 1.36 | 0.31 |
| P82659 | Bipath | 1.00 | 1.21 | 1.10 | 0.15 | 1.30 | 0.30 | 2 |
| G81714 | Bipath | 1.00 | 1.24 | 1.20 | 0.17 | 1.31 | 0.33 | 1.23 | 0.23 |
| P82659 | Bipath | 1.00 | 1.24 | 1.20 | 0.17 | 1.31 | 0.33 | 1.23 | 0.23 |
| G81714 | Bipath | 1.00 | 1.24 | 1.20 | 0.17 | 1.31 | 0.33 | 1.23 | 0.23 |

In addition, like other FKBP members (FKBP51 and FKBP52), FKBP12 might also function as a chaperone and regulate the expressions of heat shock proteins (57). To assess its functional importance, we expressed FKBP12 in a striatal cell line harboring a mutant Htt with expanded poly(Q). Elevated FKBP12 expression reduced the formation of mutant Htt aggregates (Fig. 5, C and D). In contrast, expression of Lim (a molecule important for early development (58)) did not affect Htt aggregate formation (Supplemental Fig. S5). The mechanisms used by 14-3-3\textsubscript{H9269} and FKBP12 to reduce aggregates are currently unknown, and determining these was outside the scope of this study. Instead the above findings demonstrate that the list of molecules revealed by ICAT (Table II) is likely to be functionally relevant and may provide valuable information for the development of HD therapies.
Besides the intriguing functional role of 14-3-3 and FKBP12, the ICAT analyses also provided a list of proteins that might play critical roles in the pathology of HD. For example, the reduced expression of PKCβII protein revealed by ICAT is in agreement with previous studies demonstrating that the transcription of PKCβII is reduced in the striatum of both HD patients and HD mice (44, 59). Low PKC levels might cause deficits in long term potentiation and impairments in corticostriatal synaptic plasticity in HD (60, 61). Another very interesting observation was the reduced expression of nuclear actin, which plays a critical role in RNA polymerase II-based transcription. Down-regulation of nuclear actin might alter the nucleoskeleton and cause defective chromatin remodeling (62, 63). In HD, microtubule destabilization is a primary event that precedes transcriptional dysfunction. Prevention of cytoskeletal dysfunction improves cell survival upon Htt-induced toxicity (64, 65). Consistent with the proposed importance of the nuclear structure and cytoskeleton, results of our ICAT analyses suggested that the expressions of at least eight other structurally related proteins were also reduced (Table II). Functional annotation suggests that these molecules might act as cytoskeleton-associated proteins (66–68) and that their reduction might exacerbate the microtubule disability induced by mutant Htt.

In addition, the level of nuclear cytochrome c in the striatum of HD mice was reduced (Table II). Nuclear accumulation of cytochrome c has been implicated in the remodeling of chromatin and acetylation of histone proteins (69). Reduced nuclear cytochrome c thus might contribute to the aberrant acetylation/deacetylation of histone proteins observed in HD (70, 71). The marked decrease in nuclear phosphodiesterase 2A (PDE2A) is also of great interest because PDE2A catalyzes the breakdown of cAMP and cGMP. Selective suppression of other phosphodiesterase isozymes (PDE10A and PDE1B) in HD was reported earlier (72). Reduced expression of PDEs in HD is thought to cause dysfunction of cAMP- and cGMP-regulated functions. Several lines of evidence also strongly imply that energy dysfunction exists in HD. Altered expressions of several proteins involved in energy metabolism were found. As shown in Table II, dysregulated energy proteins include those involved in glucose metabolism (e.g., enolase and triose-phosphate isomerase), the tricarboxylic acid cycle (aconitase), and ATP production (hexokinase and creatine kinase) (27, 46, 73). The impairment of energy metabolism and glycolysis appears to contribute to the neurodegenerative processes of HD. The dramatic decrease in the level of a protein similar to glyceraldehyde-3-phosphate dehydrogenase (GAPDH-like protein) was equally intriguing because GAPDH (a glycolytic enzyme) was the first Htt-interacting protein reported, and inhibition of GAPDH causes striatal lesion (74, 75). Note that GAPDH seems to exhibit functions other than glycolysis. For example, translocation of GAPDH

---

**Fig. 4. Classification of down-regulated striatal proteins in HD mice.** As listed in Table II, proteins with reduced levels of striatal expression in HD mice at the age of 10.5 weeks old when compared with those of wild-type mice were included. For proteins with multiple functions, the best known function is assigned.
into the nucleus occurs during apoptosis (76). In addition, overexpression of GAPDH enhances the nuclear translocation and toxicity of mHtt (77). The GAPDH-like protein (156 amino acids) is much smaller than GAPDH (333 amino acids) with which it shares high homology (83% identity in amino acids). Further experiments are required to define the function/activity of the GAPDH-like protein and understand the functional implications of its marked suppression in HD mice. Collectively our ICAT studies provide valuable information for further functional characterization.

Ample evidence suggests that formation of mutant Htt results in transcriptional dysregulation by either forming aggregates or causing aberrant protein–protein interactions with a number of important transcription factors or coactivators (14–19). Indeed results of the quantitative RT-PCR analyses demonstrated that the transcription of 14-3-3ζ and FKBP12 were also down-regulated in the striatum of 10.5-week-old R6/2 mice (Fig. 6), suggesting that suppression of gene expression might contribute to the reduced protein expression in the nucleus. Consistent with this hypothesis, the protein levels of 14-3-3ζ and FKBP12 in the cytosolic fractions were also reduced (Supplemental Fig. S3) indicating an overall reduction...
in their protein levels. We also compared our ICAT experiments with an earlier study in which microarray analyses of the striatum of 6- and 12-week-old R6/2 mice were conducted. Consistent with our finding of a reduced level of PKC\(\beta\) in the striatum of 10.5-week-old R6/2 mice (Fig. 2 and Table II), the transcript level of PKC\(\beta\) was also reduced in the striatum of 12-week-old HD mice. In contrast, the transcript levels of several proteins (including \(\beta\)-actin, triose-phosphate isomerase, lamin B2, calcium/calmodulin-dependent kinase II\(\beta\), casein kinase II\(\beta\), hexokinase, and creatine kinases) that showed downregulated nuclear expression in the present ICAT study were not altered (20). Additional mechanisms such as a disturbance in nuclear-cytoplasmic shuttling, a deficient protein degradative system, sequestration by Htt aggregates, and malfunctions of other post-transcriptional regulations might mediate the reduced nuclear expression of proteins reported herein.

Although the presence of one or more cysteines is a prerequisite for detection by ICAT, we found that more than 92% of the identified peptides contained only one cysteine, whereas those with three cysteines comprised less than 1% (data not shown). The low ratio of peptides with multiple cysteines might have been due to the difficulty of elution in the affinity purification step. In addition, the length distributions of peptides identified by ICAT are shown in Fig. 7A. The average lengths of peptides identified by ICAT were 13.8 and 13.2 amino acid residues/particle in experiment I and experiment II, respectively. Approximately 80% of the identified peptides were in the range of 8–18 residues. The peptide length profile of our study is very similar to the average length of tryptic peptides of the human proteome (78). Peptides shorter than eight amino acids are not specific enough for protein identification and can easily be lost in the separation when using multidimensional liquid chromatography. For peptides longer than 18 amino acids, the collision energy for fragmentation in MS/MS is insufficient and usually results in poor product ion spectra for subsequent identification. The observations that ICAT detects mostly peptides containing one cysteine and that 8–18 amino acids long might explain, at least partially, why there were so few factors involved in the transcription machinery in the list of ICAT data. In addition to the relatively low abundance of most transcription factors and cofactors, their tryptic peptides might not contain the right number of cysteines. Hypothetical tryptic profiles of four transcriptional factors/coactivators (i.e. 14-3-3\(\zeta\), SP1, c-Fos, and STAT3) are illustrated in Fig. 7B. Based on the criteria described above, 14-3-3\(\zeta\) was detected because it contains four peptides in the detectable range. In contrast, SP1 contains no detectable peptides, and c-Fos has only two detectable peptides that can easily be missed in a complex peptide mixture. STAT3 contains many peptides in the detectable range. However, we did not detect STAT3 using ICAT in our study, probably due to its low abundance. Because ICAT has been reported to be strongly biased toward acidic proteins (79), basic proteins, such as CREB-binding protein, c-Jun, and BTEB3, are expected to be absent from the ICAT database as reported herein. Complementary approaches (i.e. 2DGE) and/or further enrichment (80) are necessary if the goal is to globally characterize the transcription machinery.

Among the 68 proteins with altered expressions observed by ICAT, only four (CSPG, G\(\gamma\)2, PrxV, and lamin B2) were up-regulated in the striatum of R6/2 mice, whereas the other 64 proteins were down-regulated. This distinct difference between the numbers of proteins up-regulated and down-regulated might be due to the overall degeneration at the symptomatic stage of HD, and this certainly caused difficulties in normalizing the HD proteome as described above. For up-regulated proteins, CSPG has been shown to enhance neurite outgrowth of cortical neurons through the phosphatidylinosi-
tol 3-kinase and PKC pathways (81). Its up-regulation in the striatum of HD mice might reflect a compensatory regulation in degenerated neurons. Another up-regulated protein, PrxV, has been shown to exhibit an antioxidant effect and is able to protect cells against elevated oxidative stress and DNA damage (82, 83). Elevation of PrxV in the striatum of HD mice during disease progression therefore is likely to be part of a defense mechanism against excitotoxicity in HD mice. It was intriguing to find that Gβ1 and Gγ2 (from the Western blot analyses; Fig. 2, G and I) are increased in the striatum of HD mice. Both G protein subunits are primarily located in the plasma membrane and mediate signal transduction of G protein-coupled receptors. Nonetheless nuclear G proteins have been implicated in the regulation of mitosis, transcription, and nuclear signal transduction (84, 85). Immunostaining of the striatum with an anti-Gβ1 antibody clearly demonstrated localization of Gβ1 in the nucleus of striatal cells (Supplemental Fig. S6). It will be of great interest to characterize the roles of elevated G protein subunits in the striatum of HD mice.

In summary, we performed the first ICAT analysis of the striatum of HD mice. Calibration using results obtained from Western blotting plus a linear regression analysis of two independent ICAT experiments greatly improved the data reliability. Our analyses have disclosed a list of proteins whose expression levels are altered by mutant Htt and thus might contribute to the pathology of HD (86, 87). Recent reports suggest that combinations of various beneficial agents with different underlying mechanisms might prove to be promising for HD patients. The present study extends our current knowledge of the multiple pathways underlying HD pathologies and might eventually lead to the development of combination treatments based on different mechanisms.

Acknowledgments—We are grateful to Drs. Ruedi Aebersold and Xiaojun Li for help in establishing the ICAT method. We thank Ya-Ping Lin, Show-Rong Ma, and Kuo-Jung Huang for the ICAT experiment analysis, Yu-Jen Liang and Dr. Cathy S.-J. Fann for statistical analysis, and D. Chamberlin for editing the manuscript.

* This work was supported by grants from the National Science Council, Taiwan (Grant NSC-93-2321-B-001-012) and the Institute of Biomedical Sciences (Clinical Research Center projects), Academia Sinica, Taipei, Taiwan. The costs of publication of this article were cleared in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

** To whom correspondence should be addressed: Inst. of Biomedical Sciences, Academia Sinica, Taipei 11529, Taiwan. Tel.: 886-2-27829143; Fax: 886-2-27829143; E-mail: bmychern@ibms.sinica.edu.tw.

REFERENCES

1. The Huntington’s Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington’s disease chromosomes. The Huntington’s Disease Collaborative Research Group. Cell 72, 971–983

2. Martin, J. B., and Gusella, J. F. (1986) Huntington’s disease. Pathogenesis and management. N. Engl. J. Med. 315, 1267–1276

3. Vonsattel, J. P., Myers, R. H., Stevens, T. J., Ferrante, R. J., Bird, E. D., and Richardson, E. P., Jr. (1985) Neuropathological classification of Huntington’s disease. J. Neuropathol. Exp. Neurol. 44, 559–577

4. Davies, S. W., Turmaine, M., Cozens, B. A., DiFiglia, M., Sharp, A. H., Ross, C. A., Scherzering, E., Wanker, E. E., Mangiarini, L., and Bates, G. P. (1997) Formation of neuronal intranuclear inclusions underlies the neurodegenerative dysfunction in mice transgenic for the HD mutation. Cell 90, 537

5. Reddy, P. H., Williams, M., and Tagle, D. A. (1999) Recent advances in understanding the pathogenesis of Huntington’s disease. Trends Neurosci. 22, 248–255

6. DiFiglia, M., Sapp, E., Chase, K. O., Davies, S. W., Bates, G. P., Vonsattel, J. P., and Aronin, N. (1997) Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science 277, 1990–1993

7. Waelter, S., Boedrich, A., Lurz, R., Scherzering, E., Lueder, G., Lehrach, H., and Wanker, E. E. (2001) Accumulation of mutant huntingtin fragments in aggresome-like inclusion bodies as a result of insufficient protein degradation. Mol. Biol. Cell 12, 1393–1407

8. Jana, N. R., Tanaka, M., Wang, G.-h., and Nukina, N. (2000) Polyglutamine length-dependent interaction of Hsp40 and Hsp70 family chaperones with truncated N-terminally huntingtin: their role in suppression of aggregation and cellular toxicity. Hum. Mol. Genet. 9, 2009–2018

9. Wytenbach, A., Swartz, J., Kita, H., Thykjaer, T., Carmichael, J., Bradley, J., Brown, R., Maxwell, M., Schapira, A., Orntoft, T. F., Kato, K., and Rubinsztein, D. C. (2001) Polyglutamine expansions cause decreased CRE-mediated transcription and early gene expression changes prior to cell death in an inducible cell model of Huntington’s disease. Hum. Mol. Genet. 10, 1829–1845.

10. Wytenbach, A., Carmichael, J., Swartz, J., Furlong, R. A., Narain, Y., Rankin, J., and Rubinsztein, D. C. (2000) Effects of heat shock, heat shock protein 40 (HDJ-2), and proteasome inhibition on protein aggregation in cellular models of Huntington’s disease. Proc. Natl. Acad. Sci. U. S. A. 97, 2986–2993

11. Suhr, S. T., Senut, M.-C., Whitelegge, J. P., Faull, K. F., Cuizon, D. B., and Gage, F. H. (2001) Identities of sequenced HD proteins in aggregates from cells with induced polyglutamine expression. J. Cell Biol. 153, 283–294

12. Okazawa, H. (2003) Polyglutamine diseases: a transcription disorder? Cell. Mol. Life Sci. 60, 1427–1439

13. Okazawa, H., Rich, T., Chang, A., Lin, X., Waragai, M., Kajikawa, M., Enokido, Y., Komuro, A., Kato, S., and Shibata, M. (2002) Interaction between mutant ataxin-1 and P/QBP-1 affects transcription and cell death. Mol. Genet. Genomics 267, 701–709

14. Steffen, J. S., Kazantsev, A., Spasic-Boskovic, O., Greenwald, M., Zhu, Y. Z., Gohler, H., Wanker, E. E., Bates, G. P., Housman, D. E., and Thompson, L. M. (2000) The Huntington’s disease protein interacts with p53 and CREB-binding protein and represses transcription. Proc. Natl. Acad. Sci. U. S. A. 97, 6763–6768

15. Nucifora, F. C., Jr., Sasaki, M., Peters, M. F., Huang, H., Cooper, J. K., Yu, Y., Tomoshige, M., Takahashi, H., Tsuji, S., Tonomura, Y., L. Li, Dawson, T. M., and Ross, C. A. (2001) Interference by huntingtin and atrophin-1 with c-myc-mediated transcription leading to cellular toxicity. Science 291, 2423–2428

16. Dunah, A. W., Jeong, H., Griffin, A., Kim, Y. M., Standart, D. G., Hersch, S. M., Mouradian, M. M., Young, A. B., Tanese, N., and Krainc, D. (2002) Sp1 and TAFII130 transcriptional activity disrupted in early Huntington’s disease. Science 296, 2238–2243

17. Kegel, K. B., Meloni, A. P., Kim, Y. J., Doyle, E., Culfio, B. G., Sapp, E., Wang, Y., Qin, Z. H., Chen, J. D., Nevins, J. R., Aronin, N., and DiFiglia, M. (2002) Huntingtonin is present in the nucleus, interacts with the transcriptional corepressor C-terminal binding protein, and represses transcription. J. Biol. Chem. 277, 7466–7476

18. Li, S. H., Cheng, A. L., Zhou, H., Lam, S., Rao, M., Li, H., and Li, X. J. (2002) Interaction of Huntington disease protein with transcriptional activator Sp1. Mol. Cell. Biol. 22, 1277–1287

19. Chiang, M.-C., Lee, Y.-C., Huang, C.-L., and Chern, Y. (2005) cAMP-response element-binding protein contributes to suppression of the A2A adenosine receptor promoter by mutant huntingtin with expanded polyglutamine residues. J. Biol. Chem. 280, 14331–14340

20. Luthi-Carter, R., Strand, A., Peters, N. L., Solano, S. M., Hollingsworth, Z. R., Menon, A. S., Frey, A. S., Spektor, B. S., Penney, E. B., Schilling,
ICAT Analysis of Striatum of Mice with Huntington Disease

G., Ross, C. A., Borchelt, D. R., Tapscott, S. J., Young, A. B., Cha, J.-H. J., and Olson, J. M. (2000) Decreased expression of striatal signalizing genes in a mouse model of Huntington’s disease. *Hum. Mol. Genet.* 9, 1259–1271

21. Martindale, D., Hackam, A., Wieczorek, A., Ellerby, L., Wellington, C., McCutcheon, K., Singaraja, R., Kazemi-Esfarjani, P., Devon, R., Kim, S. U., Breder, D. E., Tufto, F., and Hayden, M. R. (1998) Length of huntingtin and its polyglutamine tract influences localization and frequency of intracellular aggregates. *Nat. Genet.* 18, 150–154

22. Tanaka, M., Machida, Y., Niu, S., Ikeda, T., Jana, N. R., Doi, H., Kurosawa, M., Nekooei, M., and Nukina, N. (2004) Trehalose alleviates polyglutamine-mediated pathology in a mouse model of Huntington disease. *Nat. Med.* 10, 148–154

23. Gauthier, L. R., Charrin, B. C., Borrell-Pages, M., Dompierre, J. P., Yi, E. C., Lee, H., Aebersold, R., and Goodlett, D. R. (2003) A statistical model for identifying proteins by tandem mass spectrometry. *Mol. Cell. Biol.* 23, 310–320

24. Arrastae, M., Mitra, S., Schweitzer, E. S., Segal, M. R., and Finkbeiner, S. (2004) Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death. *Nature* 431, 805–810

25. Gauthier, L. R., Borrell-Pages, M., Dompierre, J. P., Ringone, H., Cordelier, F. P., De Mey, J., MacDonald, M. E., Lessmann, V., Humbert, S., and Saudou, F. (2004) Huntingtin controls neurotrophic support and survival of neurons by enhancing BDNF vesicular transport along microtubules. *Cell* 118, 127–138

26. Schaffar, G., Breuer, P., Boteva, R., Behrends, C., Tzvetkov, N., Strippel, N., Sakahira, H., Siegers, K., Hayer-Hartl, M., and Hartl, F. U. (2004) Co-translational polyglutamine expansion: mechanisms of translation. *Cell* 15, 95–105

27. Chou, S. Y., Lee, Y. C., Chen, H. M., Chiang, M. C., Lai, H. L., Chang, H. H., Wu, Y. C., Sun, C. N., Chien, C. L., Lin, Y. S., Wang, S. C., Tung, Y. Y., Chang, C., and Chern, Y. (2005) CGS21680 attenuates symptoms of Huntington’s disease in a transgenic mouse model. *J. Neurochem.* 93, 310–320

28. Link, A. J., Eng, J., Schieltz, D. M., Carmack, E., Mize, G. J., Morris, D. R., Gygi, S. P., Rist, B., Gerber, S. A., Turecek, F., Gelb, M. H., and Aebersold, R. (2001) Protein kinase Cβ, βI, and βII induce aberrant protein-protein associations. *Mol. Cell. Proteomics* 4, 1849–1861

29. Gygi, S. P., Rist, B., Gerber, S. A., Turecek, F., Gelb, M. H., and Aebersold, R. (1999) Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. *Nat. Biotechnol.* 17, 994–999

30. Link, A. J., Eng, J., Schieltz, D. M., Carmack, E., Mize, G. J., Morris, D. R., Gurvik, B. M., and Yates, J. R. (1999) Direct analysis of protein complexes using mass spectrometry. *Nat. Biotechnol.* 17, 676–682

31. Hansen, K. C., Schmitt-Ulms, G., Chalkley, R. J., Hirsch, J., Baidwin, M. A., and Burlingame, A. L. (2003) Mass spectrometric analysis of protein mixtures at low levels using cleavable 13C-isotope-coded affinity tag and multidimensional chromatography. *Mol. Cell. Proteomics* 2, 299–314

32. Zhang, R., Sioma, C. S., Wang, S., and Regnier, F. E. (2001) Fractionation of isotopically labeled peptides in quantitative proteomics. *Anal. Chem.* 73, 5142–5149

33. Scherzinger, E., Lurz, R., Carmack, E., Mize, G. J., Mori, D. R., Gurvik, B. M., and Yates, J. R. (1999) Analysis of complex protein mixtures using mass spectrometry. *Nat. Biotechnol.* 17, 676–682

34. Yam, W., Lee, H., Yi, E. C., Reiss, D., Shannon, P., Kwieciszewski, B. K., Cotpo, O., Li, X. J., Keller, A., Eng, J., Gaittaki, T., Goodlett, D. R., Aebersold, R., and Katze, M. G. (2004) System-based proteomic analysis of the interferon response in human liver cells. *Neuromol. Biol.* 5, R54

35. Deckel, A. W., Gordinier, A., Nuttal, D., Tang, V., Kuwada, C., Freitas, R., and Gary, K. A. (2001) Reduced activity and protein expression of NOS in R6/2 HD transgenic mice: effects of L-NAME on symptom progression. *Brain Res. 919*, 70–81

36. Hanis, A. S., Denovan-Wright, E. M., Hamilton, L. C., and Robertson, H. A. (2001) Protein kinase Cβ, βI, and βII induce aberrant protein-protein associations. *Mol. Cell. Proteomics* 4, 279–314

37. Peters, A., Krieger, M., Otzen, D. E., and De Camilli, P. (2004) Molecular mechanisms of protein transport along microtubules: a sweep of misfolded proteins in disease conditions. *Brain Res. Hypotheses* 1137, 115–117

38. Wang, X., Grammatikakis, N., Siganou, A., Stevenson, M. A., and Calderwood, S. K. (2004) Interactions between extracellular signal-regulated kinase 1, 14-3-3[epsilon], and heat shock protein 70 and cellular prion protein. *J. Neuroatl. Exp. Neurol.* 64, 858–868

39. Guo, X., Dillman, J. F., III, Dawson, V. L., and Dawson, T. M. (2001) Neuroimmunophilins: novel neuroprotective and neuroregeneratve targets. *Adv. Neurol.* 90, 6–16

40. Labrava, C., Velly, L., Canario, B., Guillet, B., Masmejean, F., Nieuillon, A., and Pisano, P. (2006) Neuroprotective effects of tacrolimus (FK506) in a model of ischemic cortical cell cultures: Role of glutamate uptake and FKS06 binding protein 12 kDa. *Neuroscience* 137, 231–239

41. Kaneko, K., and Hachiya, N. S. (2006) The alternative role of 14-3-3 [sigma]. *Cell. Biol.* 279, 49460–49469

42. Wytenbach, A., Sauvageot, O., Carmichael, J., Diaz-Latoud, C., Arrigo, A. P., and Rubinsztein, D. C. (2002) Heat shock protein 27 prevents cellular polyglutamine toxicity and suppresses the increase of reactive oxygen species caused by huntingtin. *Hum. Mol. Genet.* 11, 1137–1151

43. Krust, A., and Hachey, L. S. (2006) The alternative role of 14-3-3 [sigma]. *Cell. Biol.* 279, 49460–49469
ICAT Analysis of Striatum of Mice with Huntington Disease

58. Yu, T. S., Mocetzuma-Anaya, M., Kubo, A., Keller, G., and Robertson, S. (2002) The heart LIM protein gene (Hop), expressed in the developing and adult heart, defines a new tissue-specific LIM-only protein family. Mech. Dev. 116, 187–192

59. Hashimoto, T., Kitamura, N., Saito, N., Komure, O., Nishino, N., and Tanaka, C. (1992) The loss of βII-protein kinase C in the striatum from patients with Huntington’s disease. Brain Res. 585, 303–306

60. Usdin, M. T., Shelbourne, P. F., Myers, R. M., and Madison, D. V. (1999) Impaired synaptic plasticity in mice carrying the Huntington’s disease mutation. Hum. Mol. Genet. 8, 839–846

61. Centonze, D., Gubellini, P., Piccioni, B., Sausle, E., Tolu, M., Bonsi, P., Giacomini, P., and Calabresi, P. (2001) An abnormal striatal synaptic plasticity may account for the selective neuronal vulnerability in Huntington’s disease. Neuron. Sci. 22, 51–62

62. Shumaker, D. K., Kuczmarski, E. R., and Goldman, R. D. (2003) The nucleoskeleton: lamins and actin are major players in essential nuclear functions. Curr. Opin. Cell Biol. 15, 358–366

63. Franke, W. W. (2004) Actin’s many actions start at the genes. Nat. Cell Biol. 6, 1013–1014

64. Trushina, E., Heiderbrant, M. P., Perez-Terzic, C. M., Bortolon, R., Kovtun, I. V., Badjo, J. D., Il, Terzic, A., Estevce, A., Windebank, A. J., Dyer, R. B., Yao, J., and McMurray, C. T. (2003) Microtubule destabilization and nuclear entry are sequential steps leading to toxicity in Huntington’s disease. Proc. Natl. Acad. Sci. U. S. A. 100, 12171–12176

65. Hoffner, G., Kahl, E. P., and Dijan, P. (2002) Perinuclear localization of huntingtin as a consequence of its binding to microtubules through an interaction with beta-tubulin: relevance to Huntington’s disease. J. Cell Sci. 115, 941–948

66. Wang, K., Knipfer, M., Huang, Q. Q., van Heerden, A., Hsu, L. C., Gutierrez, G., Quian, X. L., and Stedman, H. (1996) Human skeletal muscle nuclein sequence encodes a blueprint for thin filament architecture. Sequence motifs and affinity profiles of tandem repeats and terminal SH3. J. Biol. Chem. 271, 4304–4314

67. Schenck, A., Bardoni, B., Langmann, C., Hardin, N., Mandel, J. L., and Giangrande, A. (2003) CYFIP/Sra-1 controls neuronal connectivity in Drosophila and links the Rac1 GTPase pathway to the fragile X protein. Neuron 38, 887–898

68. Yarm, F. R. (2002) Phk phosphorylation regulates the microtubule-stabilizing protein TCTP. Mol. Cell Biol. 22, 6209–6221

69. Nur, E. K. A., Gross, S. R., Pan, Z., Baiklava, Z., Ma, J., and Liu, L. F. (2004) Nuclear translocation of cytochrome c during apoptosis. J. Biol. Chem. 279, 24911–24914

70. Langley, B., Gensert, J. M., Beal, M. F., and Ratan, R. R. (2005) Remodeling chromatin and stress resistance in the central nervous system: histone deacetylases inhibitors as novel and broadly effective neuroprotective agents. Curr. Drug Targets CNS Neurol. Disord. 4, 41–50

71. Ferrante, R. J., Kobilus, J. K., Lee, J., Ryu, H., Beesen, A., Zucker, B., Smith, K., Kowall, N. W., Ratan, R. R., Luthi-Carter, R., and Hersch, S. M. (2003) Histone deacetylase inhibition by sodium butyrate chemotherapy ameliorates the neurodegenerative phenotype in Huntington’s disease mice. J. Neurosci. 23, 9418–9427

72. Hebb, A. L., Robertson, H. A., and Denovan-Wright, E. M. (2004) Striatal phosphodesterase mRNA and protein levels are reduced in Huntington’s disease transgenic mice prior to the onset of motor symptoms. Neuroscience 123, 987–981

73. Kim, J.-w., and Dang, C. V. (2005) Multifaceted roles of glycolytic enzymes. Trends Biochem. Sci. 30, 142–150

74. Matthews, R. T., Ferrante, R. J., Jenkins, B. G., Browne, S. E., Goetz, K., Berger, S., Chen, I. Y., and Beal, M. F. (1997) Iodoacetate produces striatal excitotoxic lesions. J. Neurochem. 69, 285–289

75. Moulder, K. L., Onodera, O., Burke, J. R., Strittmatter, W. J., and Johnson, E. M., Jr. (1999) Generation of neuronal intranuclear inclusions by polyglutamine-GFP: analysis of inclusion clearance and toxicity as a function of polyglutamine length. J. Neurosci. 19, 705–715

76. Mazzola, J. L., and Sirover, M. A. (2002) Alteration of nuclear glyceraldehyde-3-phosphate dehydrogenase structure in Huntington’s disease fibroblasts. Brain Res. Mol. Brain Res. 100, 95–101

77. Bae, B. I., Harai, M. R., Cascio, M. B., Wellington, C. L., Hayden, M. R., Ross, C. A., Ha, H. C., Li, X. J., Snyder, S. H., and Sawa, A. (2006) Mutant huntingtin: nuclear translocation and cytotoxicity mediated by GAPDH. Proc. Natl. Acad. Sci. U. S. A 103, 3406–3409

78. Shevchenko, A., Sunyaev, S., Loboda, A., Shevchenko, A., Bork, P., Ens, W., and Stading, K. G. (2001) Charting the proteomes of organisms with unsequenced genomes by MALDI-quadrupole time-of-flight mass spectrometry and BLAST homology searching. Anal. Chem. 73, 1917–1926

79. Molloy, M. P., Donohoe, S., Brzezinski, E. E., Kilby, G. W., Stevenson, T. I., Baker, J. D., Goodlett, D. R., and Gage, D. A. (2005) Large-scale evaluation of quantitative reproducibility and proteome coverage using acid cleavable isotope coded affinity tag mass spectrometry for proteomic profiling. Proteomics 5, 1204–1208

80. Shio, Y., Eiseinan, R. N., Yi, E. C., Donohoe, S., Goodlett, D. R., and Aebersold, R. (2003) Quantitative proteomic analysis of chromatin-associated factors. J. Am. Soc. Mass Spectrom. 14, 696–703

81. Nakano, M., Aono, S., Hiran, K., Kuroda, Y., Ida, M., Tokita, Y., Matsui, F., and Oohira, A. (2006) Identification of neurite outgrowth-promoting domains of neuroglycan C, a brain-specific chondroitin sulfate proteoglycan, and involvement of phosphatidylinositol 3-kinase and protein kinase C signaling pathways in neuritogenesis. J. Biol. Chem. 281, 24970–24978

82. Bannmeyer, I., Marchand, C., Verhaeghe, C., Vucic, B., Rees, J.-F., and Knoops, B. (2004) Overexpression of human peroxiredoxin 5 in subcellular compartments of chinese hamster ovary cells: effects on cytotoxicity and DNA damage caused by peroxides. Free Radic. Biol. Med. 36, 65–77

83. Kropotov, A. V., Grudinin, P. S., Pleskach, N. M., Gavrilov, B. A., Tomilin, N. V., and Zhivotovsky, B. (2004) Downregulation of peroxiredoxin V stimulates formation of etoposide-induced double-strand DNA breaks. FEBS Lett. 572, 75–79

84. Crouch, M. F., and Simson, L. (1997) The G-protein Gq regulates mitosis but does not DNA synthesis in growth factor-activated fibroblasts: a role for the nuclear translocation of G. FASEB J. 11, 189–198

85. Kino, T., Tiulpakov, A., Ichijo, T., Ohheng, L., Kozasa, T., and Chrousos, G. P. (2005) G protein β interacts with the glucocorticoid receptor and suppresses its transcriptional activity in the nucleus. J. Cell Biol. 169, 885–896

86. Couzin, J. (2004) Huntington’s disease. Unorthodox clinical trials meld science and care. Science 304, 816–817

87. Agrawal, N., Pallos, J., Siepko, N., Apostol, B. L., Bodai, L., Chang, L. W., Chiang, A. S., Thompson, L. M., and Marsh, J. L. (2005) Identification of combinatorial drug regimens for treatment of Huntington’s disease using Drosophila. Proc. Natl. Acad. Sci. U. S. A. 102, 3777–3781

88. Leitner, A., and Lindner, W. (2006) Chemistry meets proteomics: the use of chemical tagging reactions for MS-based proteomics. Proteomics 6, 5418–5434

89. Nakai, K., and Horton, P. (1999) PSORT: a program for detecting the sorting signal of proteins and predicting their subcellular localization. Trends Biochem. Sci. 24, 34–35

Molecular & Cellular Proteomics 6.5 797