THE ROYAL SOCIETY

The localization and interactions of huntingtin

A. Lesley Jones

Institute of Medical Genetics, University of Wales College of Medicine, Cardiff CF4 4XN, UK (jonesl1@cf.ac.uk)

Huntingtin was localized by using a series of antibodies that detected different areas of the protein from the immediate N-terminus to the C-terminal region of the protein. The more C-terminal antibodies gave a cytoplasmic localization in neurons of the brain in controls and cases of Huntington's disease (HD). The N-terminal antibody, however, gave a distinctive pattern of immunoreactivity in the HD brain, with marked staining of axon tracts and white matter and the detection of densely staining intranuclear inclusions. This implies some processing differences between mutated and normal huntingtin. We have also localized two interacting proteins, cystathionine β-synthase and the nuclear receptor co-repressor (N-CoR), in brain. Cystathionine β-synthase was not relocalized in HD brain, but the N-CoR was excluded from neuronal nuclei in HD brain, and a further protein that exists in the same repression complex, mSin3, was similarly excluded. We conclude that the co-repressor might have a part in HD pathology.

Keywords: Huntington's disease; localization; cystathionine β-synthase; nuclear receptor co-repressor (N-CoR); transcriptional repression

1. INTRODUCTION

Huntington's disease (HD) is an autosomal dominant, incurable, progressive neurodegeneration. The disease is associated with an expanded CAG repeat giving expanded polyglutamine in the protein product, huntingtin. Such expanded polyglutamine tracts give rise to a number of inherited neurodegenerations (La Spada *et al*. 1991; Huntington's Disease Collaborative Research Group 1993; Orr *et al*. 1993; Koide *et al*. 1994; Nagafuchi *et al*. 1994; Kawaguchi *et al*. 1994; Imbert *et al*. 1996; Zhuchenko *et al*. 1997; David *et al*. 1997) distinguished by the presence of neuronal inclusions found in cells known to degenerate in six of the eight such diseases characterized (Davies *et al*. 1997; DiFiglia *et al*. 1997; Paulson *et al*. 1997; Skinner *et al*. 1997; Igarashi *et al*. 1998; Li *et al*. 1998; Lunke & Mandel 1998) although the relationship of the inclusions to the aetiology of the disease is unknown (Saudou *et al*. 1998; Klement *et al*. 1998). Each disease shows a characteristically distinct, although overlapping, pattern of neuronal degeneration (Ross 1995); in HD it is the medium spiny neurons of the basal ganglia that are most vulnerable (Hedreen & Folstein 1995). Possible reasons for such specificity include the protein context of the repeat, cell-specific post-translational modifications and interactions with other proteins.

Huntingtin has been immunolocalized in a series of studies that have shown a strong cytoplasmic immunoreactivity (Trottier *et al*. 1995; DiFiglia *et al*. 1995; Gutekunst *et al*. 1995; Sapp *et al*. 1997), although there have been reports of nuclear localization (De Rooij *et al*. 1996). However, the intranuclear inclusions have shown immunoreactivity associated only with epitopes in the furthermost N-teminal regions of huntingtin, the region within which the polyglutamine sequence resides (DiFiglia *et al*. 1997; Becher *et al*. 1998). Antibodies detecting more C-terminal epitopes of huntingtin have not been localized to inclusions.

To investigate whether proteins interacting with huntingtin are important in HD pathology and contribute to the specificity of the observed neurodegeneration, we have been looking for interactions of the polyglutamine-bearing N-terminal region of huntingtin in the yeast two-hybrid system. A number of such associations have been detected previously, either by using the yeast two-hybrid or other affinity-based methods, and include huntingtin-associated protein 1 (Li *et al*. 1995), glyceraldehyde-3-phosphate dehydrogenase (Burke *et al*. 1996), huntingtin-interacting protein 1 (Wanker *et al*. 1997; Kalchman *et al*. 1997), a ubiquitin-conjugating enzyme (Kalchman *et al*. 1996), a series of WW domain proteins (Faber *et al*. 1998) and an SH3-domain-bearing protein, SH3GL3 (Sittler *et al*. 1998). We have detected two further interactions of huntingtin, with cystathionine β-synthase (CBS) (Boutell *et al*. 1998) and the nuclear receptor co-repressor (N-CoR) (Jones *et al*. 1997).

We have now immunolocalized both of these proteins in human control and HD brain, along with a comprehensive localization of huntingtin itself, by using an affinity-purified polyclonal serum against the N-terminal region of huntingtin and a series of monoclonal antibodies against three different, more C-terminal, regions.

2. THE LOCALIZATION OF HUNTINGTIN

The characteristics of the antibodies used to localize huntingtin are given in table 1, and the characteristics of the HD tissue used are shown in table 2. Figure 1 demonstrates that the more C-terminal monoclonal

Table 1. *Anti-huntingtin antibody characteristics*

(pAb, polyclonal antibody.)

antibody	type	huntingtin sequence immunogen	epitope detected	source
N-675	pAb	residues 1–17	unknown	in house
HDA3E10	mAb	residues 997-1276	1173-7	MRIC
HDB4E10	mAb	residues 1841-2131	unknown	MRIC
HDC8A4	mAb	residues 2703-2911	unknown	MRIC
ubiquitin	mAb	—	—	Chemicon Int.
GFAP	pAb	—	—	Dr J. Newcombe

Table 2. *Details of HD brains used for immunohistochemistry*

no.	Vonsattel grade	repeats	age at death (yr)
1	3	42/18	41
2	3	42/8	52
3	3	44/16	38
4	4	39/18	66
5	4	43/19	49
6	3	44/19	47

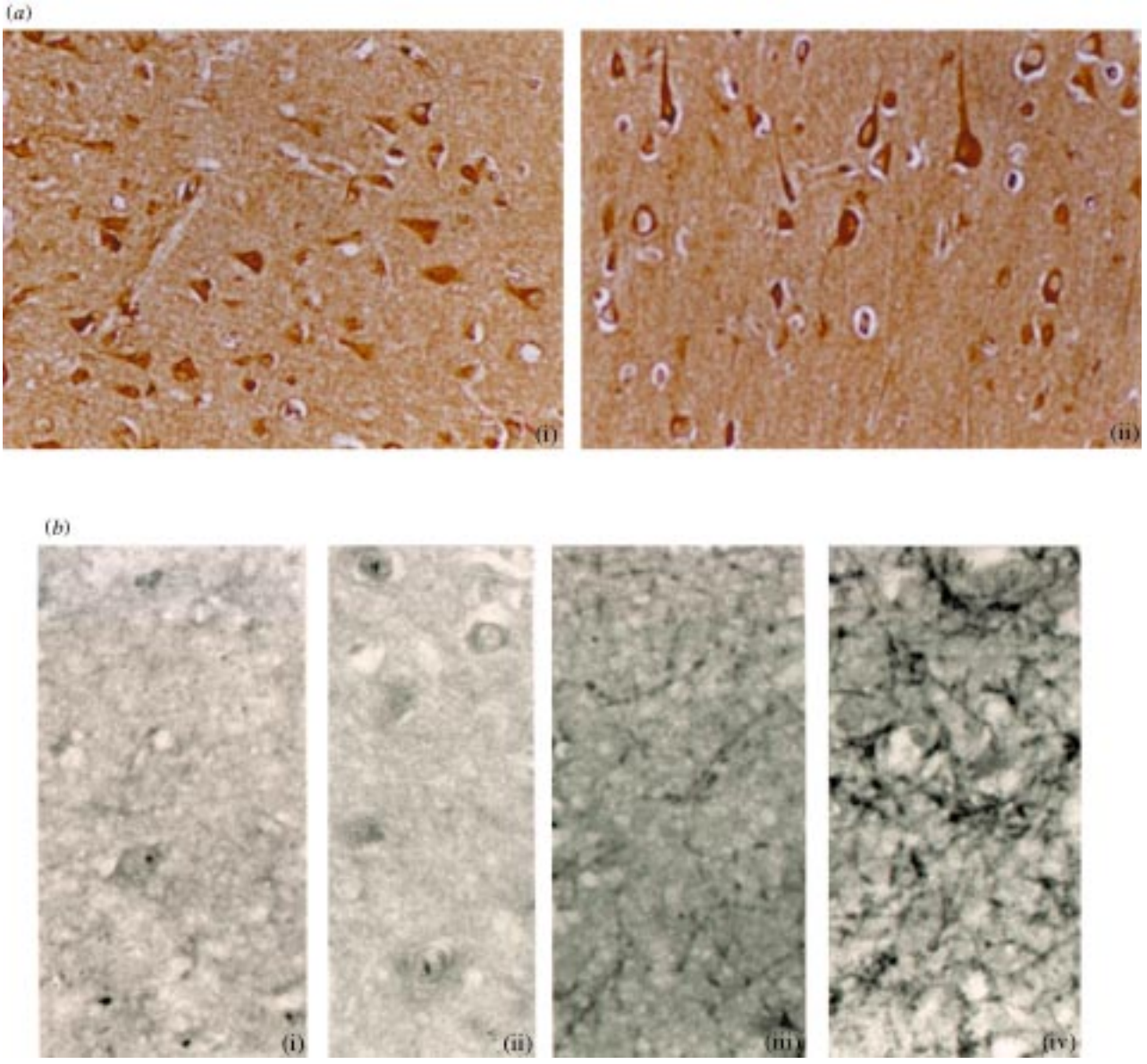

Figure 1. (*a*) Immunostaining HD cortex with monoclonal antibodies (i) HDA3E10 and (ii) HDC8A4. Magnification ×100. (*b*) Immunostaining of HD cortex with N-675 and ubiquitin: (i) staining with N675 demonstrates the presence of inclusions; (ii) staining with ubiquitin (see table 1); (iii) control; (iv) HD brain in cortical layer VI immediately above the white matter, showing strong staining of processes and increased intensity of such staining in HD brain.

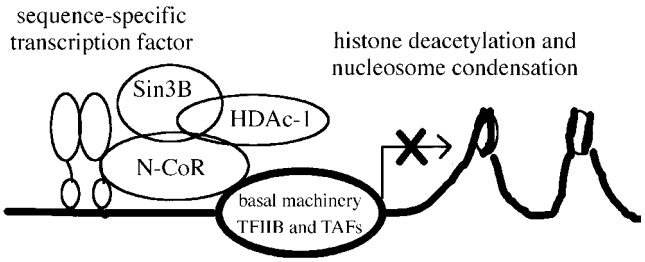

Figure 2. Simplified diagram showing the interactions of N-CoR and other proteins thought to be involved in the repression complex.

antibodies (mAbs) HDA3E10, HDB4E10 and HDC8A4 show a similar pattern of immunoreactivity, with strong cytoplasmic staining of neurons, sparing of nuclei and some staining of neuronal processes (figure 1*a*(i,ii)). This is similar to observations made previously (DiFiglia *et al*. 1995; Trottier *et al*. 1995; Gutekunst *et al*. 1995). The N-terminal polyclonal antisera, N-675, shows a different staining pattern (figure 1*b*), with only faint cytoplasmic staining (figure 1*b*(i,ii)), although this is similar to that observed with the more C-terminal mAbs. What this antibody does reveal, however, is the presence of strongly huntingtin-immunoreactive intranuclear inclusions (NIIs) (figure 1*b*(i)), a subset of which are also ubiquitin immunoreactive (figure 1*b*(ii)), as observed previously (DiFiglia *et al*. 1997; Becher *et al*. 1998). These NIIs are never observed in control brain tissue. The other notable difference between the immunoreactivity revealed by this antisera and the more C-terminal antibodies is the immunostaining of neuronal processes (figure 1*b*(iii,iv)); the more C-terminal sera do not give strong immunoreactivity in the axon tracts of the subcortical white matter nor such intense staining of processes within the grey matter. This immunoreactivity is less intense in control than HD cortex (compare figure 1*b*(iii) and (iv)). HD caudate, at this point in the disease (Vonsattel grade III or IV), has such an altered morphology compared with normal caudate that it is difficult to provide a meaningful comparison of these tissues. However, the most notable features are that the reactive astrocytes in HD caudate do show huntingtin immunoreactivity with all the more C-terminal antibodies (results not shown), confirming our initial observation of such immunoreactivity (Singhrao *et al*. 1998), the few neurons surviving show cytoplasmic immunoreactivity with the more C-terminal antibodies, and the N-terminal antiserum demonstrates the presence of NIIs (figure 1*b*(i)). Inclusions in the caudate are less frequent than in the cortex. This could be a reflection of the earlier atrophy in the caudate than in the cortex (Hedreen & Folstein 1995), with affected inclusion-containing cells cleared from the tissue or the caudate neurons dying from lack of input from the inclusion-bearing cortical neurons. The cortical neurons with inclusions are mainly in the lower cortical layers, particularly layer V, which have input into the caudate. Conversely, the death of the caudate neurons might be preventing retrograde messages to the neurons with cortical inputs into the caudate, which could lead to cortical atrophy and the formation of nuclear inclusions.

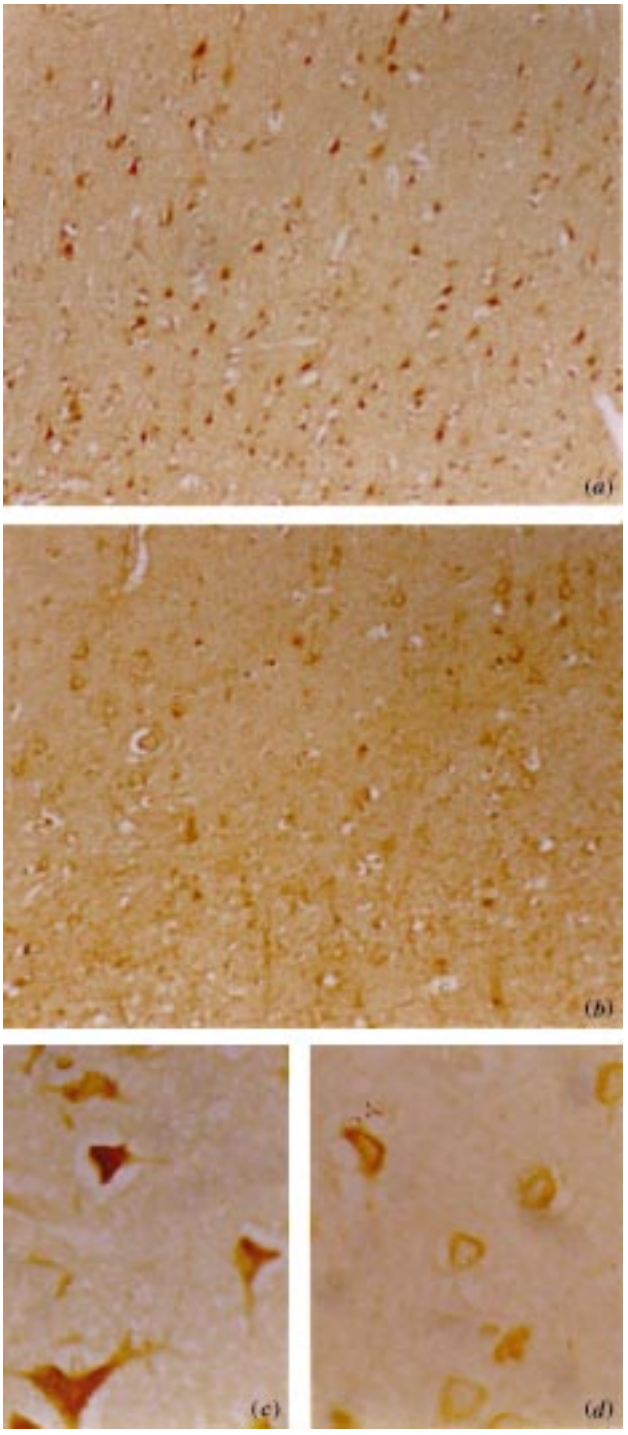

Figure 3. (*a*,*b*) Control (*a*) and HD (*b*) brain immunostained with N-CoR C20 (purchased from Santa Cruz Biotechnology), showing the lack of nuclear immunostaining in the HD brain for N-CoR. Magnification ×40. (*c*,*d*) Control (*c*) and HD (*d*) brain immunostained with mSin3A polyclonal serum (AK12), again demonstrating no immunoreactivity in the nuclei in HD brain for mSin3A. Magnification ×200.

3. THE LOCALIZATION OF INTERACTING PROTEINS

(a) *Cystathionine β-synthase*

We have detected an interaction of huntingtin with CBS (Boutell *et al*. 1998). CBS is a key enzyme in the generation of cysteine from methionine, catalysing the formation of cystathionine by the condensation of homocysteine and

serine. The absence of CBS activity is associated with homocystinuria, a recessive disorder first recognized in the 1960s (Mudd *et al.* 1964); the gene was isolated in 1990 (Kraus 1990) and a number of mutations have been detected in homocystinureic patients. A detailed account of this disease can be found in Mudd *et al.* (1995). The initial metabolic consequence of CBS deficiency is the intracellular accumulation of the enzyme's substrate, homocysteine, followed by its export from the cell and a rise in the level of homocysteine and its derivatives in plasma, interstitial fluid and urine (Refsum *et al.* 1994). Mental retardation is often the first symptom that brings CBS deficiency to clinical attention through developmental delay in the early years; it is the most frequent abnormality of the central nervous system (CNS) (Mudd *et al.* 1985) and there is a high prevalence of psychiatric disorders including depression, behavioural abnormalities and personality disorders (Abbott *et al.* 1987). Pathology in the brain shows infarcts caused by cerebrovascular inclusions. The possibility that the neurological effects of CBS deficiency might be caused by excitotoxicity in the CNS has been suggested as the basis for the mental retardation and seizures seen in CBS-deficient patients (Schwarz & Zhou 1991). Two of the oxidation products of homocysteine, L-homocysteate and L-homocysteine sulphinate, are known to be potent agonists for *N*-methyl-D-aspartate (NMDA) receptors and thus to exert excitotoxic effects on neurons, which can be blocked by NMDA receptor antagonists (Schwarz *et al.* 1990). Both compounds can be detected in the urine of homocystinureic patients but not in that of normal controls (Omori *et al.* 1972).

One of the hypotheses for the selective neuronal death seen in HD is that it occurs through excitotoxic insult (Beal *et al.* 1991; DiFiglia 1990). There are a number of pieces of evidence that support this idea. HD initially affects the striatal area of the basal ganglia, which receives a major glutamatergic input from the cortex, thalamus and subthalamic nucleus (Hedreen & Folstein 1995). The neurochemical and neuropathological characteristics of HD can be mimicked in animals by using glutamate receptor agonists such as kaininic and quinolinic acids, which are both excitotoxic amino acids. HD is characterized by a loss of striatal projection neurons and a sparing of striatal interneurons containing acetylcholine and somatostatin; this pattern of selective vulnerability is also seen in rodents with quinolinic acid lesions of the striatum (Beal *et al.* 1991). As the oxidation products of homocysteine are known to be powerful excitotoxins, this could provide an explanation for some of the damage seen in HD brain. Huntingtin could therefore bind CBS and inhibit its activity, either directly or by preventing the processing of the enzyme to its active form (Skovby *et al.* 1984).

Although the neuronal death in HD is initially specific, as the disease progresses virtually all neurons in the caudate and putamen are affected and other areas of the striatum and the cortex also atrophy; these are the cells that contain huntingtin-immunoreactive aggregates. Although the inclusions in patients are nuclear, huntingtin-positive dystrophic neurites are also seen in HD brain, and in the transgenic mice aggregation seems to begin in the cytoplasm and be followed by translocation to the nucleus (Davies *et al.* 1997; DiFiglia *et al.* 1997). Although there is strong circumstantial evidence that the aggregation observed in HD and other polyglutamine repeat diseases (Paulson *et al.* 1997) is part of the pathological effect of the polyglutamine expansion, the actual primary pathological event remains unknown. Because of this evidence we examined the localization of CBS in HD brain, in particular whether antibodies against CBS localized to inclusions. We used polyclonal antiserum raised against recombinant human CBS raised in *Escherichia coli*, and a CBS antiserum that was a gift from Professor Jan Kraus. We found CBS in the brain to be ubiquitous; no differences between the distributions in HD and control brain were observed. Neither antiserum localized to NII, nor was any strong staining of neuronal processes seen. As this is a metabolic enzyme its ubiquitous localization is not surprising. This indicates that it is unlikely that CBS is involved in HD pathology, although assays of its function in relation to CAG repeat length should be performed to confirm this.

(b) *Nuclear receptor co-repressor*

Both the yeast two-hybrid system and studies *in vitro* with His-tagged fusion proteins indicate that C-terminal N-CoR from rat or human brain binds specifically to rat and human huntingtin. This interaction occurred between rat and human N-CoR and rat and human huntingtin, because full-length huntingtin could be attached to recombinant rat or human C-terminal N-CoR (Jones *et al.* 1997). N-CoR acts as a repressor of transcription in a complex that is known to be common to a number of sequence-specific DNA-binding transcriptional repressors, including the unliganded thyroid hormone–retinoic acid–retinoid X receptor dimers, Mad:Max dimers, RevErb and Daxl orphan receptors, Pitl and Ume6 (Alland *et al.* 1997; Heinzel *et al.* 1997; Hassig *et al.* 1997; Kadosh & Struhl 1997; Laherty *et al.* 1997; Nagy *et al.* 1997; Zhang *et al.* 1997; Crawford *et al.* 1998; Xu *et al.* 1998). The *Mus musculus* N-CoR was isolated by a yeast two-hybrid screen with the use of thyroid hormone receptors by Horlein *et al.* (1995) and since then there has been an explosion of work on transcriptional regulation. It seems inevitable that more DNA binding or transcriptional regulatory proteins will prove to be controlled by this repression system (Lavinsky *et al.* 1998). N-CoR and the homologous silencing mediator of retinoid and thyroid hormone receptor, SMRT, repress transcription by linking the sequence-specific DNA-binding moieties with proteins known to possess histone deacetylase activity (Crawford *et al.* 1998; Pazin & Kadonga 1997). This link is mediated by interaction between these co-repressors and the mSin3A and mSin3B proteins, mammalian homologues of the yeast Sin3 protein (Taunton *et al.* 1996; Yang *et al.* 1996) that interact with a series of proteins that include the histone deacetylases 1 and 2 (Alland *et al.* 1997; Heinzel *et al.* 1997; Hassig *et al.* 1997; Kadosh & Struhl 1997; Laherty *et al.* 1997; Nagy *et al.* 1997; Pazin & Kadonga 1997), although N-CoR also interacts directly with the basal transcription factors TFIIB, $TAF_{II}32$ and $TAF_{II}70$ (Muscat *et al.* 1998). Histone deacetylation is thought to repress transcription by condensation of chromatin, thus preventing access of the basal transcription factors to promotors.

The interaction of huntingtin and N-CoR is dependent on repeat length. Mapping of the domains within NCoR

has already revealed that the nuclear receptor interaction domain is located at the C-terminus of the protein (Horlein *et al.* 1995) (figure 2) and this is the domain that interacts with huntingtin. The two major repression domains responsible for the transcriptional silencing are localized to the N-terminal region of the protein, neither of which are included in our constructs; these are the regions known to carry the Sin3/RPD3-interacting domains (figure 2) (Horlein *et al.* 1995). As huntingtin interacts with the C-terminal N-CoR domain, in this modular protein it is possible that the more N-terminal domains remain accessible to their normal binding partners. The binding of N-CoR to mutant huntingtin is likely to have altered dissociation kinetics; if N-CoR is sequestered to huntingtin it might be excluded from its normal function, and the proteins with which it interacts might similarly be prevented from performing their normal role. This hypothesis is made more likely because the N-CoR containing complexes are probably the rate-limiting requirements in the action of specific nuclear receptors (Laherty *et al*. 1997).

Because of this possibility we examined the localization of N-CoR and a number of its associated complex proteins in immunohistochemical studies in HD and control brain. The brains were those characterized in the study of huntingtin localization (table 2). We found that although commercial antibodies were available to a number of the complex proteins, few of these were suitable for immunohistochemistry. Of those that had no cross-reactions on Western blotting of human brain, we illustrate results from N-CoR and mSin3A (purchased from Santa Cruz Biotechnology) (figure 3). Both of these antisera demonstrate localization in the nuclei and cytoplasm of cortical neurons in control brain, but immunoreactivity is notably excluded from the nucleus in HD brain (figure 3*b*,*d*). We did not find that N-CoR immunoreactivity localized to the NII in HD brain, but as the antiserum used was directed against the C-terminus of N-CoR and this is the region that interacts with huntingtin, the appropriate epitope might be masked. mSin3 immunoreactivity occasionally localized to structures that looked like NIIs, but with a much lower frequency than that with which NIIs themselves occurred. It must be noted that all the brain used was late-stage HD.

It is also possible that the interaction with N-CoR has a role in the transport of NII or soluble huntingtin into the nucleus. N-CoR immunoreactivity, like huntingtin immunoreactivity, is markedly increased in HD neuronal processes compared with control brain (figure 1*b*(iii,iv) and figure 3*a*,*b*). Huntingtin is thought to have a role in transport along the cytoskeleton (DiFiglia *et al*. 1995; Block-Galarza *et al*. 1997; Engelender *et al*. 1997; Kalchman *et al*. 1997), and thus this might be blocked at the entrance to the nucleus. The only real way to answer this question is by using cell culture or animal models.

The role of huntingtin and the relation of its interactions to disease pathology remain unclear. Further work on the nature of the interactions and their roles in the normal and pathological function of HD need to be performed in animal and cellular models of the disease. This should give clues to which interactions are important in pathology and which might be suitable targets for therapeutic interventions.

This work was supported by the MRC (UK) and the Welsh Scheme for Health and Social Research. The mAbs HDA3E10, HDB4E10 and HDC8A4 were gifts from Professor G. E. Morris, Ms F. Wilkinson and N. thi Man, North East Wales Institute, Wrexham.

REFERENCES

Abbott, M. H., Folstein, S. E., Abbey, H. & Pyeritz, R. E. 1987 Psychiatric manifestations of homocystinuria due to cystathionine β-synthase deficiency: prevalence, natural history, and relationship to neurologic impairment and vitamin B6-responsiveness. *Am. J. Med. Genet.* **26**, 959–969.

Alland, L., Muhle, R., Hou Jr, H., Potes, J., Chin, L., Schreiber-Agus, N. & DePinho, R. A. 1997 Role for N-CoR and histone deacetylase in Sin3-mediated transcriptional repression. *Nature* **387**, 49–55.

Beal, M. F., Ferrante, R. J., Swartz, K. J. & Kowall, N. W. 1991 Chronic quinolinic acid lesions in rats closely resemble Huntington's disease. *J. Neurosci.* **11**, 1649–1659.

Becher, M. W., Kotzuk, J. A., Sharp, A. H., Davies, S. W., Bates, G. P., Price, D. L. & Ross, C. A. 1998 Intranuclear neuronal inclusions in Huntington's disease and dentatorubral and pallidoluysian atrophy: correlation between the density of inclusions and *IT15* CAG triplet repeat length. *Neurobiol. Dis.* **4**, 387–397.

Block-Galarza, J., Chase, K. O., Sapp, E., Vaughn, K. T., Vallee, R. B., DiFiglia, M. & Aronin, N. 1997 Fast transport and retrograde movement of huntingtin and HAP 1 in axons. *NeuroReport* **8**, 2247–2251.

Boutell, J. M., Wood, J. D., Harper, P. S. & Jones, A. L. 1998 Huntingtin interacts with cystathionine β-synthase. *Hum. Mol. Genet.* **7**, 371–378.

Burke, J. R., Enghild, J. J., Martin, M. E., Jou, Y. S., Myers, R. M., Roses, A. D., Vance, J. M. & Strittmatter, W. J. 1996 Huntingtin and DRPLA proteins selectively interact with the enzyme GAPDH. *Nature Med.* **2**, 347–350.

Crawford, P. A., Dorn, C., Sadovsky, Y. & Milbrandt, J. 1998 Nuclear receptor DAX-1 recruits nuclear receptor corepressor N-CoR to steroidogenic factor 1. *Mol. Cell. Biol.* **18**, 2949–2956.

David, G. (and 18 others) 1997 Cloning of the SCA7 gene reveals a highly unstable CAG repeat expansion. *Nature Genet.* **17**, 65–70.

Davies, S. W., Turmaine, M., Cozens, B. A., DiFiglia, M., Sharp, A. H., Ross, C. A., Scherzinger, E., Wanker, E. E., Mangiarini, L. & Bates, G. P. 1997 Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell* **90**, 537–548.

De Rooij, K. E., Dorsman, J. C., Smoor, M. A., Den Dunnen, J. T. & Van Ommen, G. J. 1996 Subcellular localization of the Huntington's disease gene product in cell lines by immunofluorescence and biochemical subcellular fractionation. *Hum. Mol. Genet.* **5**, 1093–1099.

DiFiglia, M. 1990 Excitotoxic injury in the neostriatum: a model for Huntington's disease. *Trends Neurosci.* **13**, 286–289.

DiFiglia, M. (and 11 others) 1995 Huntingtin is a cytoplasmic protein associated with vesicles in human and rat brain neurons. *Neuron* **14**, 1075–1081.

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

Engelender, S., Sharp, A. H., Colomer, V., Tokito, M. K., Lanahan, A., Worley, P., Holzbaur, E. L. & Ross, C. A. 1997 Huntingtin-associated protein 1 (HAP1) interacts with the p150Glued subunit of dynactin. *Hum. Mol. Genet.* **6**, 2205–2212.

Faber, P. W., Barnes, G. T., Srinidhi, J., Chen, J., Gusella, J. F. & MacDonald, M. E. 1998 Huntingtin interacts with a family of WW domain proteins. *Hum. Mol. Genet.* **7**, 1463–1474.

Gutekunst, C. A., Levey, A. I., Heilman, C. J., Whaley, W. L., Yi, H., Nash, N. R., Rees, H. D., Madden, J. J. & Hersch, S. M. 1995 Identification and localization of huntingtin in brain and human lymphoblastoid cell-lines with anti-fusion protein antibodies. *Proc. Natl Acad. Sci. USA* **92**, 8710–8714.

Hassig, C. A., Fleischer, T. C., Billin, A. N., Schreiber, S. L. & Ayer, D. E. 1997 Histone deacetylase activity is required for full transcriptional repression by mSin3A. *Cell* **89**, 341–347.

Hedreen, J. C. & Folstein, S. E. 1995 Early loss of neostriatal striosome neurons in Huntington's disease. *J. Neuropathol. Exp. Neurol.* **54**, 105–120.

Heinzel, T. (and 14 others) 1997 A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. *Nature* **387**, 43–48.

Horlein, A. J. (and 10 others) 1995 Ligand-independent repression by the thyroid-hormone receptor mediated by a nuclear receptor co-repressor. *Nature* **377**, 397–404.

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

Igarashi, S. (and 18 others) 1998 Suppression of aggregate formation and apoptosis by transglutaminase inhibitors in cells expressing truncated DRPLA protein with an expanded polyglutamine stretch. *Nature Genet.* **18**, 111–117.

Imbert, G. (and 14 others) 1996 Cloning of the gene for spinocerebellar ataxia 2 reveals a locus with high sensitivity to expanded CAG/glutamine repeats. *Nature Genet.* **13**, 285–291.

Jones, A. L., Boutell, J. M., Wood, J. D. & Harper, P. S. 1997 Two more interactions of huntingtin. *Am. J. Hum. Genet.* **61**, A311.

Kadosh, D. & Struhl, K. 1997 Repression by Ume6 involves recruitment of a complex containing Sin3 corepressor and Rpd3 histone deacetylase to target promoters. *Cell* **89**, 365–371.

Kalchman, M. A., Graham, R. K., Xia, G., Koide, H. B., Hodgson, J. G., Graham, K. C., Goldberg, Y. P., Gietz, R. D., Pickart, C. M. & Hayden, M. R. 1996 Huntingtin is ubiquitinated and interacts with a specific ubiquitin-conjugating enzyme. *J. Biol. Chem.* **271**, 19 385–19 394.

Kalchman, M. A. (and 13 others) 1997 HIP1, a human homologue of *S. cerevisiae* Sla2p, interacts with membrane-associated huntingtin in the brain. *Nature Genet.* **16**, 44–53.

Kawaguchi, Y. (and 12 others) 1994 CAG expansions in a novel gene for Machado–Joseph disease at chromosome 14q32.1. *Nature Genet.* **8**, 221–228.

Klement, I. A., Skinner, P. J., Kaytor, M. D., Yi, H., Hersch, S. M., Clark, H. B., Zoghbi, H. Y. & Orr, H. T. 1998 Ataxin-1 nuclear localization and aggregation: role in polyglutamine-induced disease in SCA1 transgenic mice. *Cell* **95**, 41–53.

Koide, R. (and 15 others) 1994 Unstable expansion of CAG repeat in hereditary dentatorubral-pallidoluysian atrophy (DRPLA). *Nature Genet.* **6**, 9–13.

Kraus, J. P. 1990 Molecular analysis of cystathionine β-synthase—a gene on chromosome 21. *Prog. Clin. Biol. Res.* **360**, 201–214.

Laherty, C. D., Yang, W. M., Sun, J. M., Davie, J. R., Seto, E. & Eisenman, R. N. 1997 Histone deacetylases associated with the mSin3 corepressor mediate mad transcriptional repression. *Cell* **89**, 349–356.

La Spada, A. R., Wilson, E. M., Lubahn, D. B., Harding, A. E. & Fischbeck, K. H. 1991 Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature* **352**, 77–79.

Lavinsky, R. M. (and 14 others) 1998 Diverse signalling pathways modulate nuclear receptor recruitment of N-CoR and SMRT complexes. *Proc. Natl Acad. Sci. USA* **95**, 2920–2925.

Li, M., Miwa, S., Kobayashi, Y., Merry, D. E., Yamamoto, M., Tanaka, F., Doyu, M., Hashizume, Y., Fischbeck, K. H. & Sobue, G. 1998 Nuclear inclusions of the androgen receptor protein in spinal and bulbar muscular atrophy. *Ann. Neurol.* **44**, 249–254.

Li, X. J., Li, S. H., Sharp, A. H., Nucifora Jr, F. C., Schilling, G., Lanahan, A., Worley, P., Snyder, S. H. & Ross, C. A. 1995 A huntingtin-associated protein enriched in brain with implications for pathology. *Nature* **378**, 398–402.

Lunke, A. & Mandel, J.-L. 1998 A cellular model that recapitulates major pathogenic steps of Huntington's disease. *Hum. Mol. Genet.* **7**, 1355–1361.

Mudd, S. H., Finkelstein, J. D., Irreverre, F. & Laster, L. 1964 Homocystinuria: an enzymatic defect. *Science* **143**, 1443.

Mudd, S. H. (and 13 others) 1985 The natural history of homocystinuria due to cystathionine β-synthase deficiency. *Am. J. Hum. Genet.* **37**, 1–31.

Mudd, S. H., Levy, H. L. & Skovby, F. 1995 Disorders of transsulfuration. In *The metabolic and molecular bases of inherited disease*, 7th edn (ed. C. R. Scriver, A. L. Beaudet, W. S. Sly & D. Valle), pp. 1279–1327. New York: McGraw-Hill.

Muscat, G. E., Burke, L. J. & Downes, M. 1998 The corepressor N-CoR and its variants RIP13a and RIP13Delta1 directly interact with the basal transcription factors TFIIB, TAFII32 and TAFII70. *Nucl. Acids Res.* **26**, 2899–2907.

Nagafuchi, S. (and 22 others) 1994 Dentatorubral and pallidoluysian atrophy expansion of an unstable CAG trinucleotide on chromosome-12p. *Nature Genet.* **6**, 14–18.

Nagy, L., Kao, H. Y., Chakravarti, D., Lin, R. J., Hassig, C. A., Ayer, D. E., Schreiber, S. L. & Evans, R. M. 1997 Nuclear receptor repression mediated by a complex containing SMRT, mSin3A, and histone deacetylase. *Cell* **89**, 373–380.

Omori, S., Kodama, H., Ikegami, T., Mizuhara, S., Oura, T., Isshiki, G. & Uemera, I. 1972 Unusual sulfur-containing amino acids in the urine of homocystinuric patients. III. Homocysteic acid, homocysteine sulfinic acid, *S*-(carboxymethylthio)homocysteine, and *S*-(3-hydroxy-3-carboxy-n-propyl)homocysteine. *Physiol. Chem. Phys.* **4**, 286–294.

Orr, H. T., Chung, M. Y., Banfi, S., Kwiatkowski, T. J., Servadio, A., Beaudet, A. L., Mccall, A. E., Duvick, L. A., Ranum, L. P. W. & Zoghbi, H. Y. 1993 Expansion of an unstable trinucleotide CAG repeat in spinocerebellar ataxia type-1. *Nature Genet.* **4**, 221–226.

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. & Pittman, R. N. 1997 Intranuclear inclusions of expanded polyglutamine protein in spinocerebellar ataxia type 3. *Neuron* **19**, 333–344.

Pazin, M. J. & Kadonga, J. T. 1997 What's up and down in histone deacetylation and transcription? *Cell* **89**, 325–328.

Refsum, H., Helland, S. & Ueland, P. M. 1985 Radioenzymic determination of homocysteine in plasma and urine. *Clin. Chem.* **31**, 624–628.

Ross, C. A. 1995 When more is less: pathogenesis of glutamine repeat neurodegenerative diseases. *Neuron* **15**, 493–496.

Sapp, E., Schwarz, C., Chase, K., Bhide, P. G., Young, A. B., Penney, J., Vonsattel, J. P., Aronin, N. & DiFiglia, M. 1997 Huntingtin localization in brains of normal and Huntington's disease patients. *Ann. Neurol.* **42**, 604–612.

Saudou, F., Finkbeiner, S., Devys, D. & Greenberg, M. E. 1998 Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. *Cell* **95**, 55–66.

Schwarz, S. & Zhou, G. Z. 1991 *N*-Methyl-D-aspartate receptors and CNS symptoms of homocystinuria. *Lancet* **337**, 1226–1227.

Schwarz, S., Zhou, G. Z., Katki, A. G. & Rodbard, D. 1990 l-homocysteate stimulates [3H]MK-801 binding to the phencyclidine recognition site and thus is an agonist for the *N*-methyl-D-aspartate operated cation channel. *Neuroscience* **37**, 193–200.

Singhrao, S. K., Thomas, P., Wood, J. D., MacMillan, J. C., Neal, J. W., Harper, P. S. & Jones, A. L. 1998 Huntingtin protein colocalizes with lesions of neurodegenerative disease: an investigation in Huntington's, Alzheimer's and Pick's diseases. *Exp. Neurol.* **150**, 213–222.

Sittler, A., Walter, S., Wedemeyer, N., Hasenbank, R., Scherzinger, E., Eickhoff, H., Bates, G. P., Lehrach, H. & Wanker, E. E. 1998 SH3GL3 associates with the huntingtin exon 1 protein and promotes the formation of polygln-containing protein aggregates. *Mol. Cell* **2**, 427–436.

Skinner, P. J., Koshy, B. T., Cummings, C. J., Klement, I. A., Helin, K., Servadio, A., Zoghbi, H. Y. & Orr, H. T. 1997 Ataxin-1 with an expanded glutamine tract alters nuclear matrix-associated structures. *Nature* **389**, 971–974.

Skovby, F., Kraus, J. P. & Rosenberg, L. E. 1984 Biosynthesis and proteolytic activation of cystathionine β-synthase in rat liver. *J. Biol. Chem.* **259**, 588–593.

Taunton, J., Hassig, C. A. & Schreiber, S. L. 1996 A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. *Science* **272**, 408–411.

Trottier, Y., Devys, D., Imbert, G., Saudou, F., An, I., Lutz, Y., Weber, C., Agid, Y., Hirsch, E. C. & Mandel, J. L. 1995 Cellular-localization of the Huntington's disease protein and discrimination of the normal and mutated form. *Nature Genet.* **10**, 104–110.

Wanker, E. E., Rovira, C., Scherzinger, E., Hasenbank, R., Walter, S., Tait, D., Colicelli, J. & Lehrach, H. 1997 HIP-1: a huntingtin interacting protein isolated by the yeast two-hybrid system. *Hum. Mol. Genet.* **6**, 487–495.

Xu, L. (and 13 others) 1998 Signal-specific co-activator domain requirements for Pit-1 activation. *Nature* **395**, 301–306.

Yang, W. M., Inouye, C., Zeng, Y., Bearss, D. & Seto, E. 1996 Transcriptional repression by YY1 is mediated by interaction with a mammalian homolog of the yeast global regulator RPD3. *Proc. Natl Acad. Sci. USA* **93**, 12 845–12 850.

Zhang, Y., Iratni, R., Erdjument-Bromage, H., Tempst, P. & Reinberg, D. 1997 Histone deacetylases and SAP18, a novel polypeptide, are components of a human Sin3 complex. *Cell* **89**, 357–364.

Zhuchenko, O., Bailey, J., Bonnen, P., Ashizawa, T., Stockton, D. W., Amos, C., Dobyns, W. B., Subramony, S. H., Zoghbi, H. Y. & Lee, C. C. 1997 Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the α1A-voltage-dependent calcium channel. *Nature Genet.* **15**, 62–69.