

Introduction



Over fifty years ago, in riding with my father on his professional rounds, I saw my first cases of “that disorder,” which was the way in which the natives always referred to the dreaded disease. I recall it as vividly as though it had occurred but yesterday. It made a most enduring impression upon my boyish mind, an impression which was the very first impulse to my choosing chorea as my virgin contribution to medical lore. Driving with my father through a wooded road leading from East Hampton to Amagansett, we suddenly came upon two women, mother and daughter, both bowing, twisting, grimacing. I stared in wonderment, almost in fear. What could it mean? My father paused to speak with them and we passed on. Then my Gamaliel-like instruction began; my medical education had its inception. From this point on my interest in the disease has never ceased. (Huntington 1872)

Chapter 1

Introduction

1.1. Historical background

George Huntington's single contribution to medical literature in 1872 is where the Huntington's disease (HD) story officially begins. His original paper described and outlined a series of symptoms and clinical observations made by three generations of physicians; grand-father, father and son adding to the awareness of the disease now bearing their name (Huntington 1872). It appears to have made an immediate impact on the neuroscience community of the time as it was translated into German (Kusmaul & Nothnagel 1872) and read by many eminent physicians throughout Europe, including Camillio Golgi in 1873 (Mazzarello 1996). Prior to the disorder being classified as HD it had been grouped together with other neuromotor disorders collectively called 'chorea' derived from the Latin *choreus* meaning 'to dance'. This refers to the hyperkinetic involuntary movements, which can appear to be dance-like defined by Paracelsus in the sixteenth century and later referred to by Thomas Sydenham in 1686. Before this time there is mention of dancing mania throughout the Middle Ages when patients were thought to be possessed by spirits or the devil, taking the name of St John's or St Vitus' dance who became the patron saint of those afflicted with the 'dancing plague'.

There is evidence in the ancient papyri of Egypt that a loss of voluntary movement was ascribed to brain dysfunction (Edwin Smith papyrus, case 31, 3000 B.C.), Paracelsus realised that the excessive movements could be due to an underlying disease of the central nervous system some 4500 years later, the clinical symptoms of which Sydenham chronicled until Huntington realised that there was an inherited component to this disease. In 1982 researchers picked up the trail and using Restriction Fragment Linked Polymorphism (RFLP) analysis linked the disease to band 16.3 on the short arm of chromosome 4 (Gusella, *et al.* 1984) setting the stage for intensive molecular investigations to

answer the multitude of questions posed by the disease and the alluring hope of a cure.

1.2. Epidemiology

HD has a global prevalence of 7-10 per 100,000 with the UK estimated at 4-8 per 100,000 and North America at around 4.5 per 100,000. The lowest prevalence is found in Japan and Hong Kong where it is 0.11-0.65 per 100,000 (Nakashima *et al.* 1996 & Chang *et al.* 1994). There are geographical clusters of patients worldwide where there is a higher than average incidence. Studying these foci, such as a very large pedigree in Venezuela, has led to the discovery of the gene and understanding the nature of the genetics. In the UK there are such clusters in the Moray Firth, East Anglia and in South Wales. The clusters of cases in the USA proved to be more interesting as they were all populations that had originally migrated from countries in north-western Europe bringing with them the mutated gene establishing a clear founder effect, the most famous of these was a group in New England where some of the afflicted were accused of witchcraft in the Salem witch-hunt. The epidemiology is handled in a very thorough and extensive manner in three books on HD Hayden 1981, Harper 1996 and its updated version Bates, Harper & Jones 2002.

1.3. Aetiology

By studying the pedigrees it was discovered that HD was an autosomal dominantly inherited disease (Davenport & Muncey 1916). A single copy of the mutated gene inherited from either parent is enough to cause disease in the resulting offspring therefore there is a 50% chance of the offspring of an affected parent being affected by the disease. There is an equal incidence and an equal level of transmission of the disease in males and females. Unaffected offspring are not at risk of the disease or transmitting it to their offspring. It was also found that the phenomenon of anticipation is occurring in the disease.

Anticipation is defined as increase in severity of disease with an earlier age of onset of symptoms in successive generations, it is often influenced by the sex of the transmitting parent and in the case of most CAG repeat diseases is more severe when paternally transmitted (Koshy & Zoghbi 1997).

1.4. Disease symptoms

The onset of the disease typically occurs in middle age around the age of 40-50 years, these first symptoms are of a neuropsychiatric nature, typically erratic anti-social behaviour, hypersexuality, aggression and depression (Pflanz *et al.* 1991). As the disease progresses the patients exhibit involuntary dance-like or choreiform movements, slow writhing motion of the limbs or athetosis and saccadic eye movements (Leigh *et al.* 1983). These movements may initially become incorporated into day-to-day life but as the disease progresses become more exaggerated and uncontrollable. The only time that patients appear to be free of these movements beyond their control is during sleep, which is also seen in Parkinson's disease where disease sufferers lose rigidity and tremor during sleep. At the end stage of HD patients have severely compromised cognitive ability and often have dementia. At the end of the duration of the disease which can be anytime between 10-20 years patients often end their lives rigid and akinetic in complete contrast to the hyperkinesia shown when symptoms begin (Harper 1996).

HD is not always a disease affecting middle aged people but sometimes is seen during childhood, the juvenile form. George Huntington failed to recognise this precocious form of the disease and stated emphatically that HD was a disease that manifests itself in adult life only. The clinical picture is very different in juvenile patients with brief mild chorea as an early sign giving way quickly to the predominant motor abnormality, which is rigidity and tremor (Markham & Knox 1965), reminiscent more of Parkinson's disease. Again the first symptoms to be picked up are intellectual deterioration and difficulty in

concentration, with some children suffering psychosis, often severe behavioural disturbances requiring legal or medical intervention. Late in the disease children have severe dystonia; abnormal muscle tone causing strange postures with some developing a fine postural tremor of the trunk and limbs, often bed bound and totally dependent on carers (Nance & Myers 2001).

Juvenile cases, that is cases with onset before the age of 20, account for 10% of all HD patients and of these 2% are symptomatic before the age of 10 (Rasmussen *et al.* 2000); with the youngest patient described showing symptoms at the age of 2 (HDCRG 1993; Hayden 1981). In keeping with the inverse correlation of age of onset and the size of repeat length these cases tend to have the largest repeat sizes commonly exceeding 50, which is dealt with in further detail in section 1.8 of this introduction (Telenius *et al.* 1993 & Sanchez *et al.* 1996). The longest repeat size described in the literature was from a juvenile patient with 250 repeats (Nance *et al.* 1999)

As well as the juvenile form there is also another clinical variant called the Westphal variant, the original description was of rigid not overtly choreic phenotype of an 18 year old patient from a known HD family (Westphal 1883). As knowledge of juvenile rigid forms of the disease increased this variant became used more for the adult cases that manifested themselves as rigid and not choreic forms of the disease. It is currently thought that 10% of adult onset cases are of this type. Patients are typically in their twenties and rarely in their thirties. Again this form of the disease seems to indicate that a long repeat size is associated with the more rigid and dystonic severe form of the disease.

Patients are known to have an increased appetite and seem to increase their calorific intake significantly whilst at the same time demonstrate weight loss or cachexia, this was thought to be due to the hyperkinesia but now thought to be independent of that (Pratley *et al.* 2000). High calorie diets do not have much effect on this integral manifestation of the disease as shown by recent studies where patients were evaluated in sensitive calorimeters to measure this phenomenon found that there was not much difference in the patients in

constant writhing motion and those who were rigid (Pratley *et al.* 2000). Urinary incontinence is often a problem with HD patients and EMG studies have shown that there are involuntary movements of the perineal musculature as well as more obvious limb muscles may be causing incontinence. Fertility and genetic fitness it appears are not affected considerably in HD (Harper 1996).

1.5. Current therapies

Currently there are only medications to relieve some symptoms of HD; these are a variety of antidepressants, mood stabilisers, anti-psychotic and anti-anxiety medications are used to manage the whole range of psychiatric problems (Harper 1996 & Wood *et al.* 2002). The chorea can be reduced using dopamine depleting or blocking agents though these are not of use to juvenile patients however, whose symptoms are completely different as discussed above. A small number of children are treated with anti-Parkinsonian or anti-spasticity medications for severe rigidity. Some dystonic features can be alleviated with *botulinum* toxin to improve the quality of life (Nance *et al.* 2001).

Recently several candidate compounds have come to light through the extensive efforts of researchers from the 'Coalition For a Cure' initiative set up by the Huntington's Disease Society of America (HDSA). This initiative involved high throughput screens in cell culture systems, as well as in *Drosophila*, *C.elegans* and mouse models. Some of these compounds are drugs already used in the treatment of other diseases such as anti-cancer and anti-epilepsy drugs (Grove *et al.* 2000), these have already been deemed safe to use in humans and have had dosage studies carried out for them making them prime candidates for small clinical studies in HD patients beginning during 2003 the long term findings of which have yet to be published.

Surgical procedures, where controversially, foetal striatal tissue has been transplanted into patients' *striatum* have been performed recently, but the success of such procedures appears not to be evident (Bachoud-Levi *et al.* 2006

& Keene *et al.* 2007). Procedures using stem cells have recently been tried on HD patients, however the long-term effects of these operations remains to be seen (Freeman *et al.* 2000). Surgery therefore appears to be a very drastic measure with relatively little improvement in return for painful and often expensive treatment (Greenamyre *et al.* 2002).

Far more important than any surgery or medication regimes are the occupational health services, speech therapy and support programmes for both patients and carers run by charities such as the HDSA and Huntington's disease Association in the UK, for improved quality of life of patients. There is as yet no treatment for HD.

1.6. Pathology

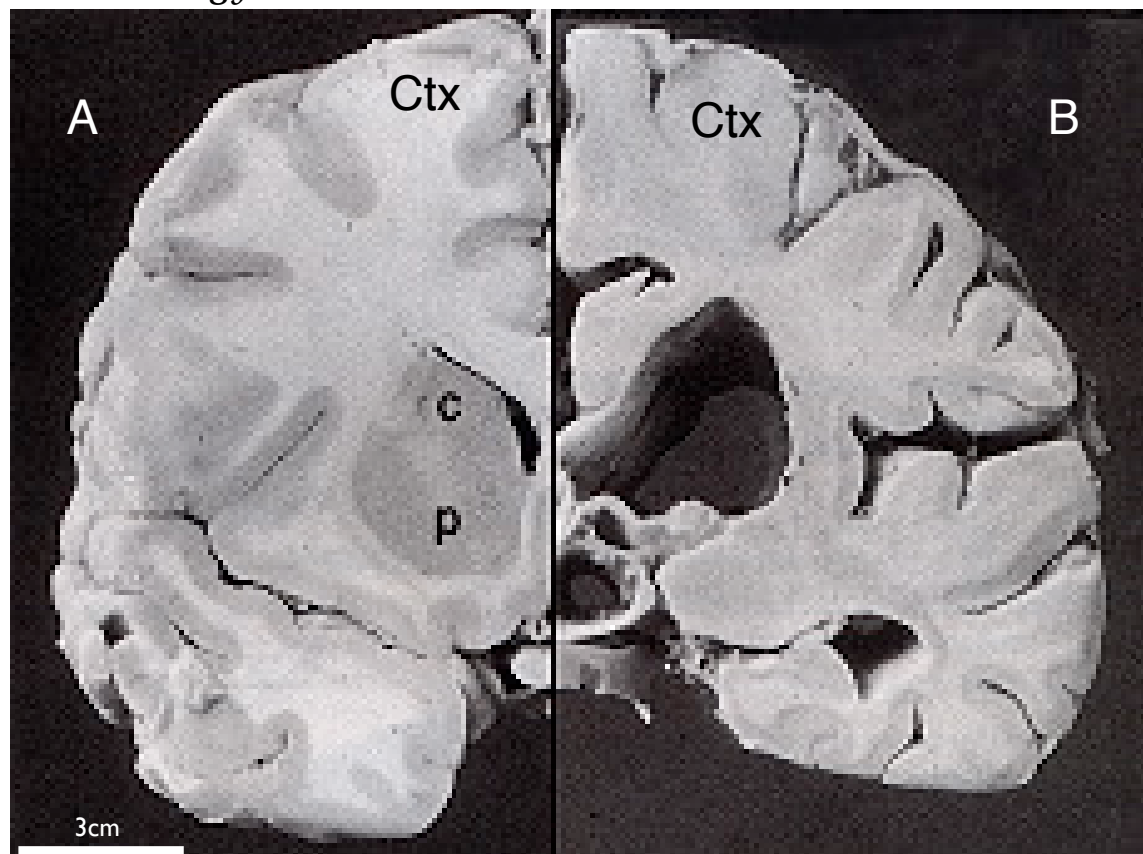


Figure 1.1: Plate showing gross pathology of HD in human brain. Panel A shows a healthy brain and B a HD brain sections cut at the level of the *basal ganglia*.

The photo above illustrates perfectly the gross pathology of Huntington's disease, the hemisection of brain on the right is the diseased brain and the one on the left is a control (from an individual 10 years older). The main points to note are the excessively enlarged lateral ventricle, and the massive reduction in

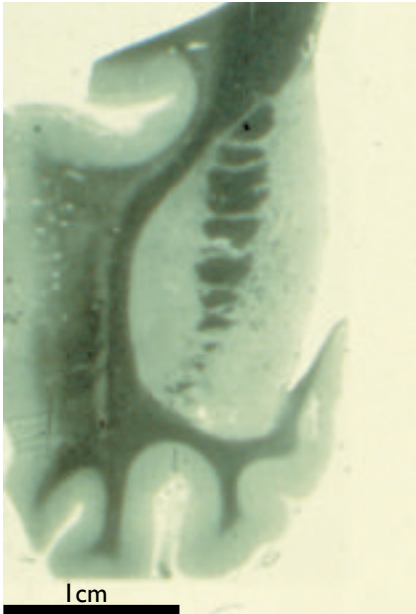
the *caudate* and *putamen* (labelled **c** and **p**) into a thin band, whereas on the control side it is rounded and limits the size of the lateral ventricle. The cortical ribbon (labelled Ctx) is much thinner than in the control with the sulci being more pronounced, and there is thinning also of the subcortical white matter. Finally the overall size of the brain is much smaller, around 20% smaller in size than the control. This photo emphasises the damage that is caused to the brain in Huntington's disease. Although changes can readily be seen in many areas of the CNS published descriptions of cell death and pathological change have focussed almost exclusively on the *neostriatum*, as this is the area of the brain that consistently shows the most severe change. Pathological grading is divided into five stages illustrating the disease progression at *post-mortem* (Vonsattel *et al.* 1985).

Grade 0 patients show no sign of gross brain pathology, but have begun to show some of the early psychiatric symptoms of the disease. When the striatum is subjected to immunocytochemical analysis however the astrocyte marker glial fibrillary acidic protein (GFAP) shows reactive astrocytosis in the striosome or patch compartment (Hedreen & Folstein 1995), indicating that this is the first site of neurodegeneration. Reactive astrocytosis eventually fills the matrix compartment as well, finally affecting the entire *striatum*.

Figure 1.2: Pathology of different stages of HD
courtesy of E.Bird



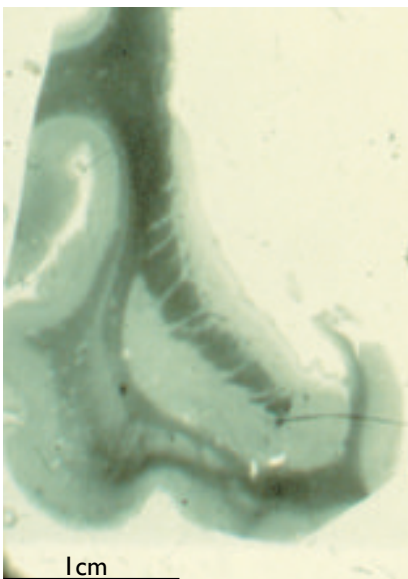
Grade I brains show some shrinkage and no other gross brain pathology. On histological analysis it is shown that up to 50% of the striatal projection neurons have been lost.



Grade II brains show a suggestion of HD. The head of the *caudate* nucleus is atrophic but retains its medial convex outline, as does the internal capsule. The shape of the head of the *caudate* nucleus is normal but the size appears slightly reduced.



Grade III The head of the *caudate* nucleus is shrunken to a narrow ribbon with corresponding dilatation of the anterior horn of the lateral ventricle. The medial outline of the head of the *caudate* nucleus and that of the internal capsule are straight or almost straight. The *putamen* and the *globus pallidus* are also shrunken.



Grade IV The head of the *caudate* nucleus is visible only as an extremely thin concave ribbon with corresponding marked widening of the anterior horn of the lateral ventricle. The internal capsule is medially concave and reduced in width. The *putamen* and the *globus pallidus* are severely shrunken. There is a pronounced overall atrophy, and an astroglial response, most marked is the cell death of projection neurons.

Analysis of homogenates of HD *striatum* shows that there are reduced levels of glutamic acid decarboxylase (GAD) (Bird & Iversen, 1974) and γ -aminobutyric acid (GABA), also reduced in the dorsal *striatum* are substance P and enkephalin. Striatal levels of dopamine, serotonin and noradreneline were not found to be reduced, suggesting that neurons of the *substantia nigra*, *raphe nucleus* and the *locus coeruleus* are spared in HD. Studies have shown that somatostatin levels are raised by five times that of controls (Aronin *et al.* 1983), this increase is due to the survival of NADPH-d neurons in a dramatic sparing of this population which normally accounts for less than 2% to 10-30% of the remaining cells as the disease progresses. The giant cholinergic cells are also spared the AChE levels are maintained, however the ChAT levels show a significant decrease, that may be due to loss of synapses with projection cells in the disease (Ferrante *et al.* 1987). Reasons for some subpopulations in the *striatum* being spared during HD is not known, it has been thought that the projection neurons are more vulnerable to stress due to the metabolic demands of maintaining long axonal processes, however this hypothesis fails to explain why the *striatum* is susceptible to destruction in HD.

The *cortex* is also greatly affected in the disease process as the gross pathology shows a dramatic thinning of the cortical ribbon and the underlying white matter but so far relatively little research has been conducted into this matter. However very recent findings by Rosas *et al.* (2002 & personal communication) at Massachusetts General hospital in Boston (USA), conducting an MRI imaging study of the brains of both symptomatic and presymptomatic patients has shown a substantial thinning of the cortical ribbon in many brain regions of both. As the disease progresses there is significant thinning even in presymptomatic patients at a quite early time point in the disease progression, additionally there is a trend of the degeneration occurring from the more posterior region to the anterior cortical regions.

Neurons in layers III and V are involved in cortico-striatal communications and it appears that only this select sub-population is affected in HD. This

phenomenon has been reported in *postmortem* HD brain tissue with layer VI affected extensively too (Ferrante *et al.* 1991; Sotrel *et al.* 1993; Kowall *et al.* 1993 & Hedreen *et al.* 1995). The selective vulnerability of pyramidal neurons of the *cortex* as been further highlighted by a study in which up to 55% of these cells were shown to be lost in the *angular gyrus*, a region projecting to the dorsal part of the *caudate* (Macdonald *et al.* 1997 & 2002).

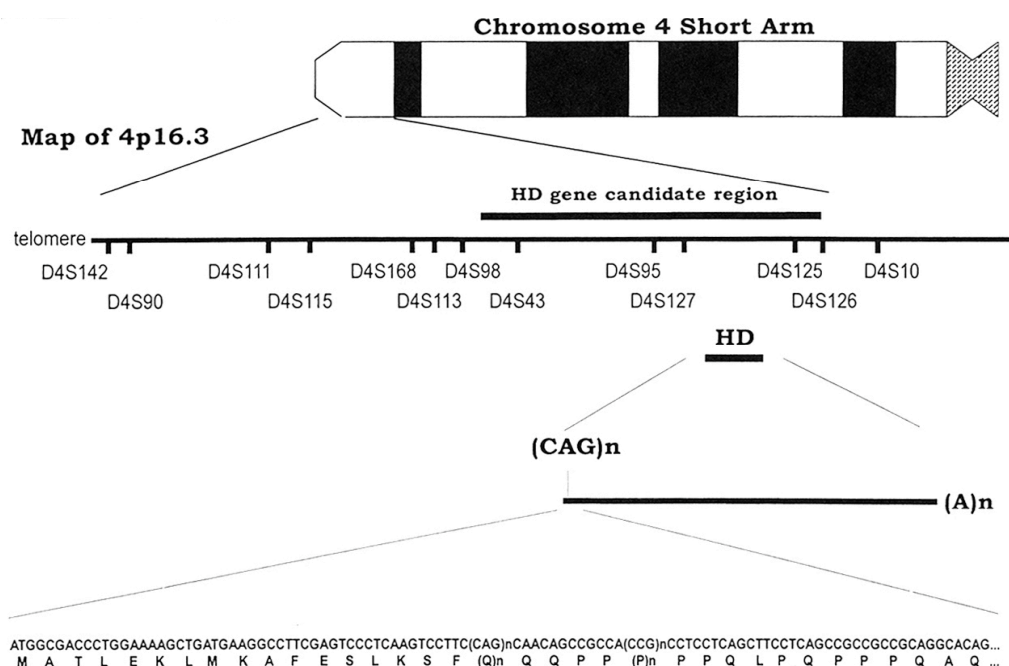
There are some reports of cerebellar involvement in HD pathology in the literature however this is variable and ranges from cerebellar atrophy and Purkinje cell loss to a thinning of the granular cell layer (Byers *et al.* 1973; Castaigne *et al.* 1976; Rodda 1981 & Hattori *et al.* 1984). One particular study found a 50% reduction in the density of Purkinje cells (Jeste *et al.* 1984). What does become apparent from the literature that cerebellar pathology is very much a part of juvenile HD, however this aspect of pathology has been insufficiently studied to give any real insights.

Changes in the *hippocampus* are similarly poorly investigated. An early study suggests a reduction of 20% in the hippocampal area (de la Monte *et al.* 1988), while a later study looking at cell density changes in different regions of the *hippocampus* found that the most dramatic reductions of 35% were in the CA1 (Spargo *et al.* 1993). CA1 degeneration has been observed in other similar diseases but the significance of this finding has yet to be fully understood.

1.7. The HD gene mutation

RFLP analysis led to the pinpointing the location of the HD gene on the tip of the short arm of chromosome 4, this method exploited the presence of polymorphism of sequences in the population, markers linked to these were used in large known pedigrees to track down the exact location (Gusella *et al.* 1983). Meanwhile the gene for myotonic dystrophy (Brook *et al.* 1992) and Friedreich's ataxia (Campuzano *et al.* 1996) had been found and both were found to contain large triplet repeat expansions. By means of extrapolation it was speculated that perhaps in HD too there may be a significance of a triplet

repeat sequence. Both of these findings together helped to drive the hunt for the gene responsible for HD. Additionally conditions known to result from abnormalities of the tip of chromosome 4 were scrutinised to see if they bore any resemblance to HD and whittle down the exact location of the gene responsible. When this area is involved in a balanced translocation there are surprisingly no HD like symptoms (Asamoah *et al.* 1998), also in the paediatric condition Wolf-Hirschorn syndrome where the top of chromosome 4 is completely deleted again there are no symptoms resembling HD (Battaglia *et al.* 2001). Therefore it was concluded that this must be a 'gain of function' mutation of the gene as its absence does not generate a similar disease condition. Work continued on this region of chromosome 4 until a 210kb region labelled IT15 (interesting transcript 15), was isolated by the Huntington's Disease Collaborative Research Group, (HDCRG 1993) this transcript contained an expanded triplet repeat and therefore had a good chance of being the gene for HD. It was found that it was indeed in the coding sequence and the gene that was being sought after.



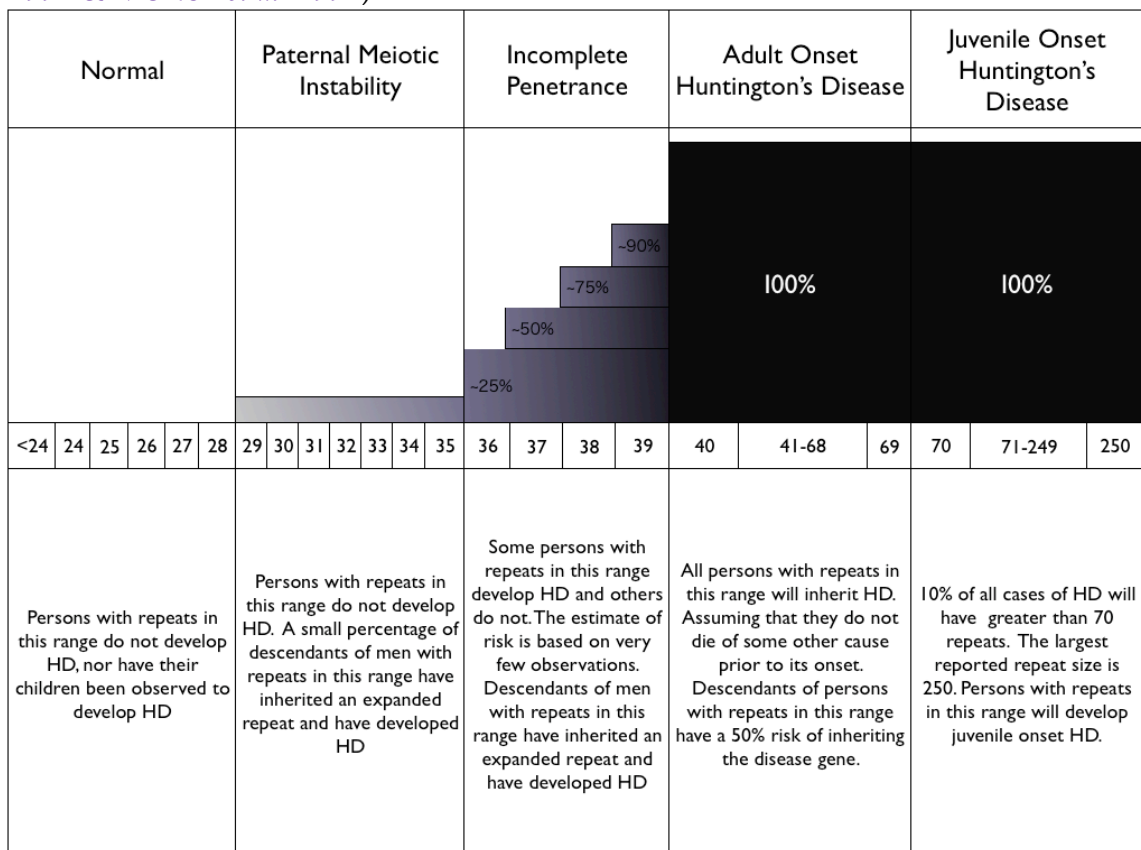
Taken from Gusella *et al.* 1996.

Figure 1.3: A schematic diagram showing the HD gene on the short arm of chromosome 4.

The gene was found to consist of 67 exons of between 43 to 341 base pairs in size spanning 180kb of genomic DNA (Ambrose *et al.* 1994), with an expansion of the CAG repeats in the first exon at the 5' end encoding polyglutamines. The protein product eventually named huntingtin (*htt*).

1.8. Consequences of (CAG)*n* repeat copy numbers in HD

Various studies have shown that the average repeat size in different populations varies; individuals in Boston have repeats from 6-34 and HD patients from 37-100, in Cardiff the unaffected range is 9-34 and the HD range from 30-70. The range between 30-39 can be seen as a grey area which may be totally unaffected (McNeil *et al.* 1997). However this confusion has now been resolved, such that it is now generally assumed that anything below 35 is normal and above 40 is HD (Rubinsztein *et al.* 1996). The area in between could develop HD in very old age and these individuals could give rise to new mutations and in effect start new HD pedigrees (Myers *et al.* 1993; Kremer *et al.* 1994 & McNeil *et al.* 1997).



Adapted from Myers *et al.* 1998

Figure 1.4: Diagram showing the relative incidence of the forms of HD in relation to the CAG repeat number in an individual.

Anticipation is observed when the CAG repeat numbers have been shown to significantly increase in the progeny of affected males giving rise to a more severe form of the disease in the offspring. This is due to the repeat instability in the transmission of the gene. Studies of HD families revealed an increase of up to 7 repeats in germline transmission in both sexes. Much larger increases were seen in offspring of affected males especially ones which had large repeats themselves (Ranen *et al.* 1995 & Trottier *et al.* 1994). As well as CAG repeat sizes increasing in a small number of transmissions the size is also seen to decrease (Kremer *et al.* 1994). It was found that most cases of juvenile HD had inherited the mutation from their affected fathers. As well as this it was found that the age of the fathers at the time of conception is important, as older fathers would have compromised genetic fitness and could have erratic increases in the repeat sizes carried by their gametes (Kremer *et al.* 1994).

An important phenomenon that has come to light as a result of these genetic studies; the correlation between repeat sizes and the age of onset. It appears very clearly that as the repeat sizes increase the age of onset gets younger, this trend is particularly pronounced in the juvenile HD patients studied (Duyao *et al.* 1993; Trottier *et al.* 1994 & Brinkman *et al.* 1997). Studies in other CAG repeat disorders also seem to be supporting these findings, where the larger repeat sizes give rise to earlier presentation of symptoms, in fact it is particularly striking as to how similar this trend is (please see *Figure 1.5*).

However CAG repeat number cannot be the only factor at work as there is a surprising amount of variance in the age of onset of a given repeat length, particularly in the small and medium repeat lengths, suggesting that they are extremely unstable, which in turn makes predicting the nature of the disease difficult. This also suggests that there is more to HD than simply the size of repeats, other as yet unknown and subtle factors appear to be contributing to the disease profile. The US-Venezuelan Collaborative Research Project has shown environmental factors to be affecting the age of onset in Venezuelan HD families (Wexler *et al.* 2004)

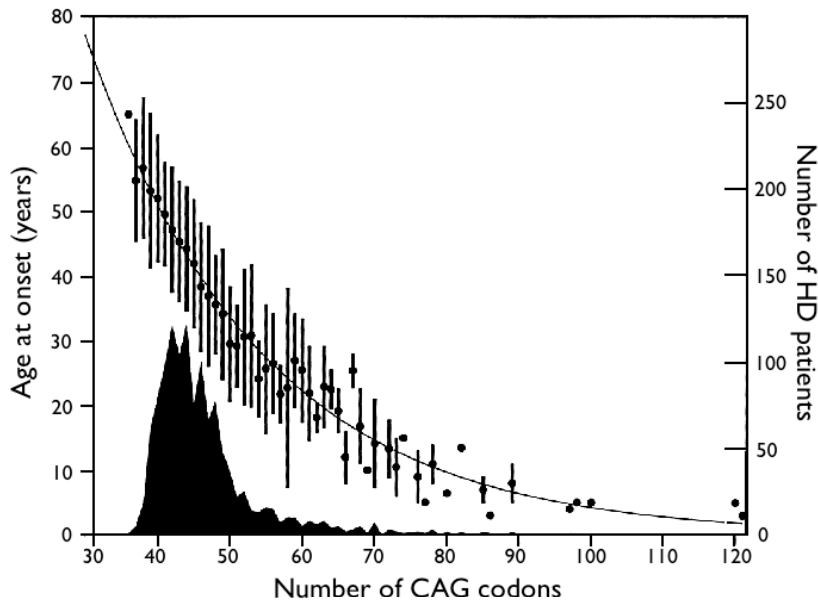
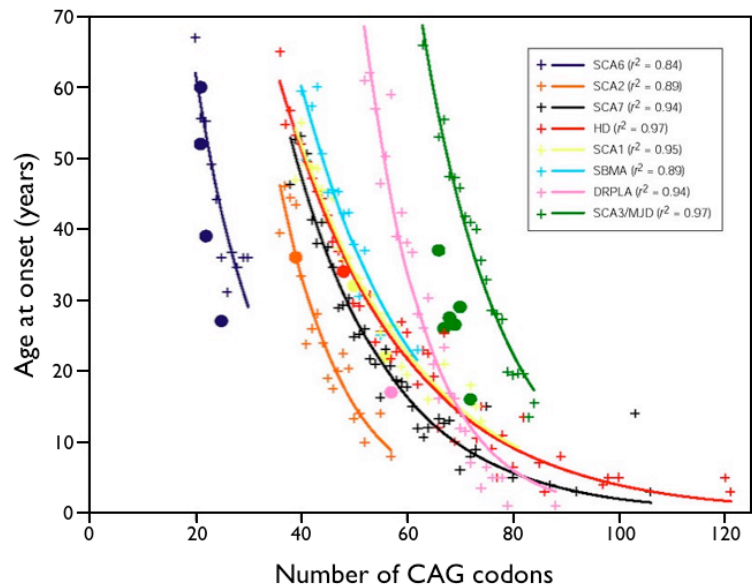


Figure 1.5: Graph showing the variability in the age of onset in relation to the size of the CAG repeat carried by the patient.

Taken from Gusella et al. 1996

Figure 1.6: Graph showing the relationship between age of onset and repeat sizes in all the known CAG repeat diseases, the coloured spots indicate homozygote individuals.



Taken from Gusella & MacDonald 2000.

1.9. Huntingtin

Htt is a large protein ~350 kD in size and is made up of 3144 amino acids. More than a decade after discovery the function of *htt* is still speculated and remains unknown. It is currently thought that it may be a mediator in various cell signalling pathways involved in transcription, mRNA processing and the processing of proteins (MacDonald 2003). It is widely accepted is that it is expressed extensively and is present in most tissues (Sharp et al. 1995). Clearly it has an important role in early development as *htt* knock out animal models have never been carried to term and appear to have been aborted as early as

gastrulation and neurulation events (Duyao *et al.* 1995; Nasir *et al.* 1995 & Zeitlin *et al.* 1995).

1.9.1. Normal Huntingtin

The regional localisation of this *htt* has been the focus of many studies however few studies have addressed the localisation of normal *htt* and contrasted with mutant *htt* in the HD brain. Immunocytochemical studies have indicated that normal *htt* is widely expressed in the cytoplasm of neurons throughout the brain with high levels found in cortical pyramidal cells, Purkinje cells of the *cerebellum* and large striatal interneurons. The expression is heterogenous and it has been shown that the regions and neurons most vulnerable in disease express the highest amounts of *htt* naturally (Ferrante *et al.* 1997). This may in part explain the selective nature of the vulnerability seen in the pathology, but as some brain regions express high levels and remain relatively spared in the disease process, therefore this cannot be the only explanation for this phenomenon. Normal *htt* associates with microtubules, vesicular organelles and mitochondria and has been implicated in organelle transportation (Gutekunst *et al.* 1995, 1998 & DiFiglia *et al.* 1995).

1.9.2. Abnormal Huntingtin

Cleavage of mutant *htt* produces N-terminal fragments, which are able to aggregate and recruit other proteins (Goldberg *et al.* 1996 & Kim *et al.* 2001). These aggregates are found in many compartments of the neuron; the nucleus cytoplasm and neuritic processes.

With the gene and its product identified the field exploded into activity, it was now possible to produce transgenic animal models to probe into the nature of the disease further. Several attempts at a murine model of the disease were made by many different groups. Of these the R6 lines (Mangiarini *et al.* 1996) remarkably exhibited symptoms very much like those seen in patients with HD. Work on these mice revealed that the mutant gene product was being deposited

in the nuclei of neurons (Davies *et al.* 1997). This is now known to be a major part of the disease pathology of not only HD but all the polyglutamine repeat disorders, which at the last count were 9.

1.10. Trinucleotide repeat diseases

In the light of the advances made with understanding HD it was now possible to extrapolate this knowledge further to understanding of other diseases that share a common mechanism. These diseases exhibited a repeat sequence similar to the CAG repeats in HD which led to neurodegenerative disorders too, making the research of one relevant to all those in the family, these known diseases are shown in *Table 1.1* below.

Disorder	Gene	Locus	Repeat	Coding	Normal	Expansion	Neurodegeneration	Reference
HD	huntingtin	4p16.3	CAG	Poly Q	6-35	36-121+	Basal ganglia & cortical	HDCRG 1993
HDL2	junctophilin-3	16q23	CTG	Poly L	7-26	44-57	Basal ganglia & cortical	Holmes 2001a
DRPLA	atrophin-1	12p13.31	CAG	Poly Q	3-35	49-88	Dentate & red nucleus cerebellum & brainstem	Koide <i>et al.</i> 1994
SBMA	Androgen receptor	Xq11-12	CAG	Poly Q	9-36	38-62	Spinal & bulbar motor neurones	La Spada <i>et al.</i> 1991
SCA 1	SCA 1/ ataxin-1	6p23	CAG	Poly Q	6-38	39-83	Cerebellum & brain stem especially PC	Orr <i>et al.</i> 1993
SCA 2	SCA 2/ ataxin-2	12q24	CAG	Poly Q	14-31	32-77	Cerebellum, brainstem & thalamus	Imbert <i>et al.</i> 1996
SCA 3/MJD	MJD/ ataxin-3	14q21	CAG	Poly Q	12-53	54-86	Cerebellum & substantia nigra	Kawaguchi <i>et al.</i> 1994
SCA 6	CACNA 1A Ca channel subunit	19p13	CAG	Poly Q	4-19	20-30	Predominantly cerebellar & brainstem Ca channel subunit	Zhuchenko 1997
SCA 7	SCA 7	3p21.1p12	CAG	Poly Q	4-35	37-200	Cerebellar cortex, brainstem & retinopathy	Lindblad <i>et al.</i> 1996
SCA 8	SCA 8/ KLHL1	13q21	CTG	5'UTR	16-91	107-127	Cerebellar cortex & vermis	Koob <i>et al.</i> 1999
SCA 10	SCA 10	22q13	ATTCT	Intron	10-22	750-4500	Cerebellum & cortical	Matsuura <i>et al.</i> 2000
SCA 12	PPP2R2B	5q31-33	CAG	5'UTR	7-28	66-78	Cerebellum & cortical	Holmes <i>et al.</i> 2001b
SCA 17	TBP	6q27	CAG	Poly Q	29-42	47-63	Cerebellum	Nakamura <i>et al.</i> 2001
Fragile X	FMRI/FMRP	Xq	CGG	5'UTR	29-30	>200	None observed	Oberle <i>et al.</i> 1991
Fragile X premutation	FMRI/FMRP	Xq	CGG	5'UTR	29-30	50-200	Cerebral cortex & cerebellar	Hagerman & Hagerman 2002

A large subset of these disorders have an expanded CAG tract and are known as the polyglutamine repeat diseases, despite having widely different genes and protein sequences they share the polyglutamine expansion in common, which appears to be of direct relevance to the pathogenesis of the central nervous system degeneration. As well as these diseases there are an increasing number

of spinocerebellar ataxias (SCAs) coming to light that are not strictly speaking trinucleotide diseases. As these are so new little is known of them and often only one pedigree is known, but their symptoms are similar and perhaps as more is discovered they may join these diseases in the future.

1.11. Disease model systems

In the past the most common model for neurodegenerative diseases was the excitotoxic animal model, which involved lesioning or injecting substances such as quinolinic acid to the particular area of interest to try and simulate the disease of interest (DiFiglia *et al.* 1990 & Ferrante *et al.* 1993). This excitotoxic model hypothesised that neurons expressing glutamate receptors exposed to high levels of the excitatory amino acid, glutamate, can be excited to death. The cells become depolarised to such a state that the internal calcium increases to a level where internal homeostasis can no longer be maintained and the neuron dies (Olney 1969 & Olney *et al.* 1971). Experimental evidence suggests that neurons exposed to high levels of glutamate initially exhibit dendritic swelling followed by somatic swelling and rupture of the cell body and ultimately death. The mechanism employed to induce toxicity appears to be mediated by excessive movement of calcium (Ca^{2+}) and sodium (Na^{+}) into the cell. Additionally Ca^{2+} has been shown to disrupt normal mitochondrial function and is capable of activating enzymes that can produce free radicals, and both of these mechanisms are implicated in the death of the neuron (Whetsell 1996). Recently this theory has regained favour after having spent the last decade in the wilderness, work done by Panov *et al.* (2002) have all shown that there is a movement of calcium capable of major cell disruption and inducing death.

However since many of the genes responsible for these neurodegenerative diseases have been identified and characterised, it has become possible to manipulate these and their homologues in other animal genomes. Several attempts were made at generating a mouse model of HD, the first of these

knocked out the gene to ascertain whether it was vital for the development of the animal. All groups showed that when the gene was knocked out it proved lethal for the animals (Duyao *et al.* 1995; Nasir *et al.* 1995 & Zeitlin *et al.* 1995). Closer examination showed that gastrulation had not occurred, suggesting that the normal function of the *htt* in mice and humans may have to do with early developmental events.

The most successful model to emerge was a transgenic mouse model where a fragment taken from a juvenile patient containing the human promoter sequence and exon 1 including the polyglutamine and polyproline sequences were integrated into the mouse genome. This model is known as the R6 lines, the most studied of which are the R6/2 mice. The success of this model has been the extensive phenotype that they exhibit which mirrors the juvenile form of the disease (Mangiarini *et al.* 1996). Immunocytochemical studies of these mice revealed the localisation of the mutant protein which appeared to be present in the neuronal nucleus in large amounts in discrete sites. These were proved conclusively by EM studies not to be the nucleolus, Cajal bodies or female sex chromatin condensations called Barr bodies, but an independent nuclear body which was named the neuronal nuclear inclusions (NII) (Davies *et al.* 1997). The NII has since been seen as the hallmark pathology of HD and was swiftly found in the majority of the polyglutamine repeat diseases (with exception of SCA 2 which has inclusions in secondary pathological regions (Huynh *et al.* 2000) and SCA6 (Ishikawa *et al.* 1999) so far), reinforcing the idea that they do all occur by a similar disease progression mechanism. The HD striatal neurons containing an NII also showed a highly indented nuclear membrane and an observable increase in the number of nuclear pores seen in the mice and human tissue. The R6 lines used in this study are discussed in more detail in the materials and methods.

Since then new transgenic lines are being generated for all the different polyglutamine disorders. The genetic strategies becoming more and more elaborate, at the last count there are ten models of different aspects of HD.

Three of these which pretty much encompass the field are studied extensively in this study to highlight the need for every aspect of the disease process to be taken into account. Their genetics are discussed in more detail in the materials and methods chapter. As well as murine models there appeared to be an evolutionary regression of the field, by making invertebrate models such as the fruit fly *Drosophila melanogaster*, nematode worm *Caenorhabditis elegans* and there are attempts at a zebrafish model the results of which are unpublished (H Paulson, personal communication). The nematode worm has been used to incorporate polyglutamine expansions with green fluorescence protein (GFP) as a reporter protein into the muscle cells of the worm (Satyal *et al.* 2000). These appear to form aggregates and disable the movement of the worms. The *Drosophila* homologue of HD gene has been identified and found to have 29 exons showing a high degree of similarity between it and the vertebrate HD sequence, with around 49% similarity between the fly and human sequences (Li *et al.* 1999). The incorporation of polyglutamine repeats in the fly gene causes neurodegeneration most readily observed in the compound eye of the fly (Jackson *et al.* 1998; Bonini 1999; Kazemi-Esfarjani & Benzer 2000 & Steffan *et al.* 2001) which is composed of a regular trapezoidal arrangement of seven rhabdomeres or light gathering structures produced by the photoreceptor neuron of each ommatidium; this highly organised structure is disrupted so that in transgenes the number rhabdomeres is found to decline. Both of these have proved to be very popular as firstly they show the pathological hallmark NII in affected populations of their cells, secondly their short lifespans allow experimental questions especially those of inheritance answered quickly as disease progression can take a matter of days not months as in mouse models and years in the case of human patients, and thirdly the simplicity of their genome allows easy manipulation. Both these systems are a step up from the *in vitro* cells in culture systems which are important in understanding cellular events in their own right however these diseases are more relevant to *in vivo* whole animal systems. These simple animal models are able to tease out

different event pathways which all occur together in these complex diseases. Recently they have played important roles in finding possible treatment compounds in high throughput tests, the *Drosophila* system enabled identification of histone deacetylase inhibitors such as the compound SAHA and sodium butyrate as possible treatments (Steffan *et al.* 2001). These are currently being tested in the murine models and then eventually will be used in clinical trials.

1.12. Current theories of HD pathogenesis

1.12.1. Protein aggregation

There are a large number of disorders that result from an accumulation of abnormal proteins in the cells in the form of inclusions, some like the polyglutamine repeat diseases have already been discussed, but it is important to appreciate that this phenomenon occurs in many biological systems and therefore the understanding of the cellular mechanisms have a much wider implication. Other hereditary diseases like cystic fibrosis, thalassemia and many globinopathies have excess globin, which precipitates as inclusions and distorts the shape of red blood cells (Amrani *et al.* 1993). Denatured proteins form inclusions in other neurodegenerative diseases like superoxide dismutase (SOD) aggregates seen in amyotrophic lateral sclerosis (ALS), Tau protein in tangles seen in Alzheimer's disease (AD) and α -synuclein in Lewy bodies seen in Parkinson's disease (PD), the latter two being much more prevalent than the polyglutamine repeat expansion diseases. It is currently thought that the pathogenicity arises from the mutant protein's ability to aggregate and recruit other proteins, effectively sequestering them away from normal functions depleting crucial cellular and nuclear proteins. What should be recognised is that misfolded protein bodies can be formed in all compartments of cells and can therefore assert dysfunction via subtly differing routes.

1.12.2. Seeded aggregation theory

There are several different theories put forward as to how the aggregation process arises in a diseased neuron, the simplest of these is a seeding process. The crystallisation process is started with a seed crystal, which allows the crystal to form around it over a certain concentration threshold, it is suggested by some that the inclusion may be formed by a similar mechanism (Lansbury 1997). *In vitro* work done by Busch *et al.* (2003) has shown that mutant *htt* protein is able to seed endogenous protein into spontaneous aggregation, suggesting that perhaps it may be playing a recruitment role and that the combined proteins are what may be the toxic species. It has also been shown that all proteins can be encouraged to form polymers given favourable conditions (Dobson *et al.* 2004), normally this is in the form of fibrils or fibres which is also what has been suggested by Wanker and colleagues, however fibrous structures of *htt* have not been shown *in vivo* to date and these favourable conditions are often not physiologically possible (Scherzinger *et al.* 1999 & Busch *et al.* 2003).

1.12.3. Polar zipper hypothesis

In 1994 Max Perutz (Perutz 1994) put forward a theory whereby polyglutamine tracts of a length over the threshold number of repeats may be able to hydrogen bond with anti parallel opposing lengths of polyglutamine forming a glutamine 'polar zipper'. This was based on synthetic poly-L-glutamine being used for molecular modelling and X-ray diffraction experiments which concluded that they formed hydrogen bonded β -pleated sheets (Perutz *et al.* 1994). He went further to suggest that polyglutamine repeats could have a normal functional role in transcriptional regulation citing *Drosophila* homeobox genes as prime examples. The Abdominal B gene proteins are such genes, one of which is made up of more than 32% glutamine repeats and when part of this protein sequence was deleted transcriptional activity was

reduced by half. Many transcription factors are rich in glutamine repeat regions in their transcriptional activation domains. Glutamine and proline rich domains form a major class of activation domains and acidic domains rich in serine or threonine form the other. In humans and rodents the longest tracts of polyglutamines are found in the TATA binding protein (TBP), N-OCT3 and the glucocorticoid receptor (Gerber 1994). If the hypothesised polar zippers are indeed formed *in vivo* perhaps they interfere with the transcriptional machinery and render the cell incapable of transcriptional regulation.



Photograph taken by A.R. Fersht at the Royal Institute, London 1994

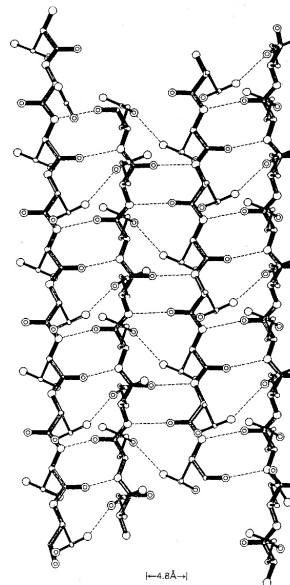


Figure 1.7: Max Perutz and the model of the hypothesised polar zippered β -pleated sheet structure by which *htt* could aggregate.

Taken from Perutz et al. 1996.

1.12.4. The NII

The NII is a circular non-membrane bound structure that has a pale, and both granular and filamentous appearance when seen at the ultrastructural level with TEM. The nuclear membrane is frequently indented and there is an increase in the number of nuclear pores (Davies *et al.* 1997) these later two features are reported to characterise neuronal response to axonal injury (Lieberman *et al.* 1971). Quite incredibly similar structures to the NIIs were first reported in EM studies of human tissue biopsies of HD patients in 1979 by Roizin *et al.*, and nuclear membrane indentation (Roos & Bots 1983) and increased pore density (Tellez-Nagel *et al.* 1974) were also reported much before the importance of these changes was fully comprehended.

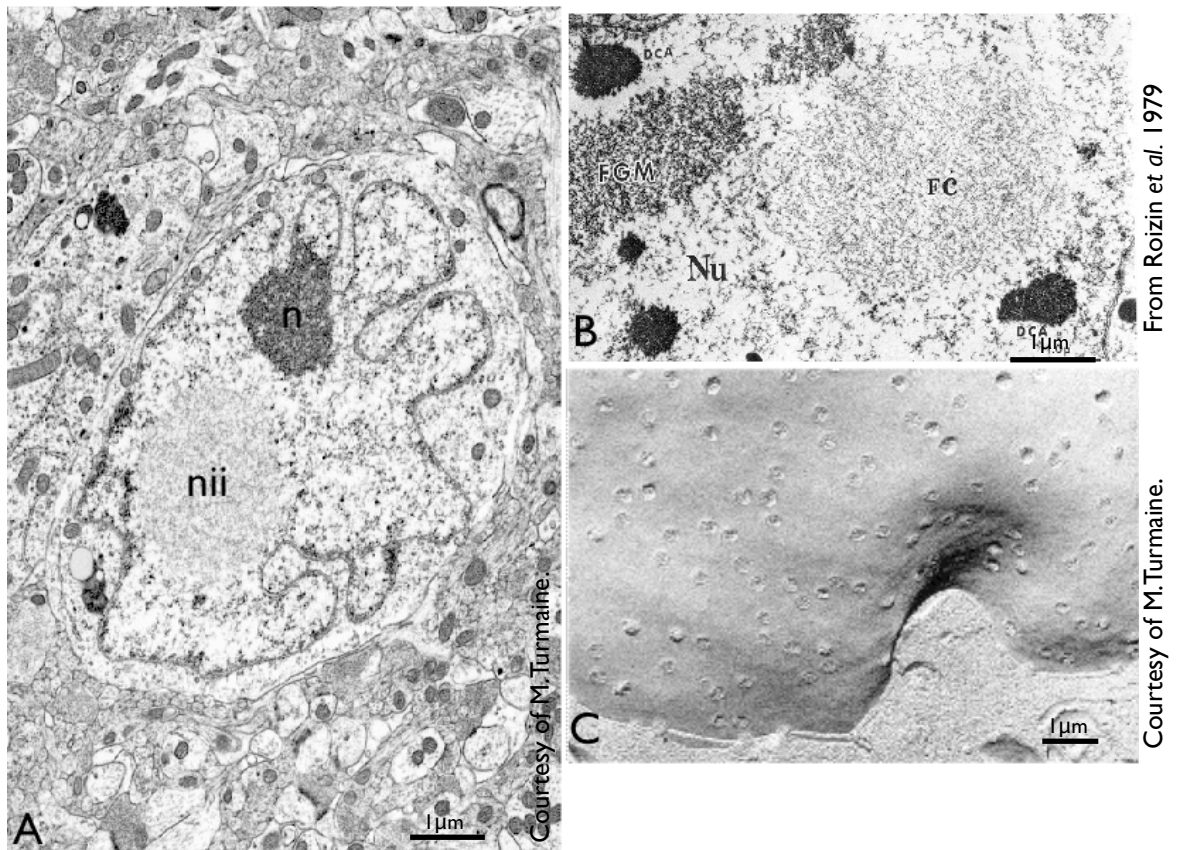
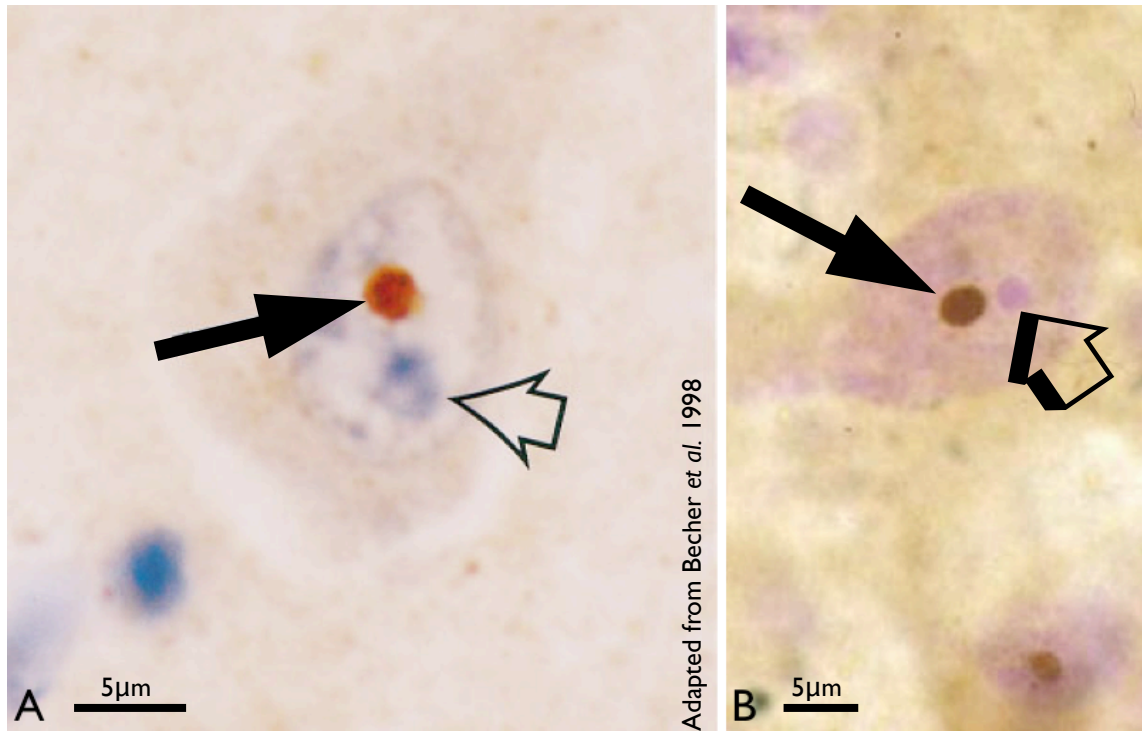


Figure 1.8: A shows an EM of an NII (nii) in a cortical mouse neuronal nucleus containing a distinctly separate nucleolus (n) and with highly indented nuclear membrane. B shows a plate from Roizin's material showing what we now know to be an inclusion (FC) and the nucleolus (Nu). C shows a freeze fracture EM showing the indentation of the nuclear membrane and the increased density of nuclear pores spanning it. Panels B & C are both from *striatum*.

At the light microscopic level it can be visualised now with various antibodies via the process of immunocytochemistry as a dark circular body in each nucleus, and can be discerned with some difficulty with tinctorial stain where it is seen as a clear circle with Nissl staining. Avidin-biotin complex kits are used to amplify the antibody signal and visualise it by developing in DAB which precipitates at the location of the signal in situ showing up as a dark reaction product deposit (these are covered more extensively in the Methods Chapter). All the models of polyglutamine repeat diseases and indeed tissue from patients of these diseases demonstrate that the mutant protein is present in the form of inclusions from before symptoms first arise to the time of death serves to point the finger of guilt on this structure as the cause.

Figure 1.9: Panel A shows an inclusion within a cortical neuron from an HD patient with 74 CAG repeats (arrow) stained with ubiquitin antibody, very distinct from the nucleolus stained with Nissl stain (open arrow). Panel B shows the same arrangement in an R6/2 cortical neuron.



Since its discovery by [Davies et al.](#) in 1997 it has rapidly gained a reputation as a protein black hole sucking all manner of molecules into its structure and therefore increasing in size as the disease progresses. Inclusions were found to be very inert structures and attempts to break them down into their constituent proteins were generally unsuccessful. Methods included boiling them in SDS and in high concentrations of urea. Until a study which identifies the proteins sequestered in an inclusion found amongst others to include cytoskeletal proteins such as actin and lamins, heat shock chaperone proteins, caspases -3, -8 and -9, as well as *htt*, ubiquitin and proteasome subunits ([Suhr et al. 2001](#)), suggesting that several cellular pathways were affected by the presence of the NII. However this study was carried out in transfected cells in culture and not neurons *in situ*, and whereas its findings were important in deciphering the complex proteins present it may bear little relevance to human or indeed mouse neurons *in vivo*. Neurons being highly specialised post mitotic cells would have a different cellular protein environment suited to their activity. Matters are

further complicated by the appearance of neurite inclusions, smaller aggregates seen in the axons and dendrites of the neurons proving that aggregation was not just a nuclear phenomenon as first thought. How exactly the two species of aggregate fit into the disease progression time frame and the mechanisms of their relative formations is still not understood.

It has not yet been decided whether the disease pathology is a direct result of the NII or whether the NII is a result of the pathology. It would appear logical to say that as the mutant protein accumulates and the NII forms and grows for some time before the appearance of any symptoms that they may be causative. However it could equally be argued that the NII is formed as a damage control exercise by the cell to sequester potentially harmful protein in an inert form allowing the cell to function (Cummings *et al.* 1999). After all in HD patients the disease process can take up to 15 years. Blocking inclusion formation in neuronal cultures transfected with *htt* does not prevent disease pathology (Saudou *et al.* 1998), similarly in mice expressing mutant ataxin-1 nuclear aggregate formation was not necessary for pathology (Klement *et al.* 1998). To add to all this evidence, inclusions have never been found in the brain pathology of SCA-2 (Huynh *et al.* 1999). However it is entirely reasonable to suggest that a physical structure of this size would indeed disrupt the normal functions of the cell, more so as there have been studies that implicate involvement in transcription factors and regulatory proteins such as CBP and TATA-binding protein (Perez *et al.* 1998).

What has become increasingly apparent now is that the presence of the NII is not a reliable time-point for disease progression and cannot be used as a quantifiable landmark in drug efficacy trials. In a number of cases now mouse models have responded to treatments and have shown improvement in phenotype. At the same time however the pathological studies have indicated that there has been no changes in the number and size of NIIs (Hockly *et al.* 2002 & Ferrante *et al.* 2003), suggesting that they alone are not responsible for the symptoms but may indeed be a protective measure the cell puts into action.

1.12.5. The DNI

The dystrophic neurite inclusion (DNI) in the HD brain was first described by DiFiglia *et al.* (1997). Intensely stained ovoid and lozenge shaped extra-nuclear structures were seen in the neuropil and shown by immunocytochemistry to contain mutant *htt* and ubiquitin (Sieradzan *et al.* 1999); DNIs stain with anti-ubiquitin antibody as single inclusions often with a tail like process (DiFiglia *et al.* 1997). EM studies showed that these inclusions also had a granulo-filamentous consistency similar to that of the NIIs, differing only in that they are surrounded by a thin layer of cytoplasm containing a high accumulation of organelles especially mitochondria. This species of aggregate may be more deleterious to the neuron, in that an inclusion of this sort and size would seriously obstruct transportation along axons and dendrites, and would eventually cause death as shown to occur in cultured cells (Li *et al.* 2001). Schilling *et al.* (1999) first showed this neuritic pathology in a mouse model of HD, they suggest that they are made up of mutant *htt* fragments, which are as toxic to neurons than the full-length protein like the NIIs. There is a school of thought that suggests that the NII is not involved in the neurodegeneration process and the DNI may be more able to explain the more selective degeneration seen in HD. The pathological role of axonal aggregates is further supported by the reality of axonal damage resulting in neurological conditions such as multiple sclerosis (Arnold 1999) and stroke (Sawhani *et al.* 1997) among others. Clearly DNIs have a role in the disease pathology as they are found in patient tissue and mouse models of HD including the R6 lines, HD94 and Shelbourne (Li *et al.* 2001) models (which are discussed further in the results chapters) but exactly how much of a role remains to be elucidated. A hopeful finding in the HD94 models has shown with the administration of Doxycycline the most recently formed DNIs to dissociate showing that the aggregates are not as inert as once thought and are indeed capable of being dismantled (Yamamoto *et al.* 2000).

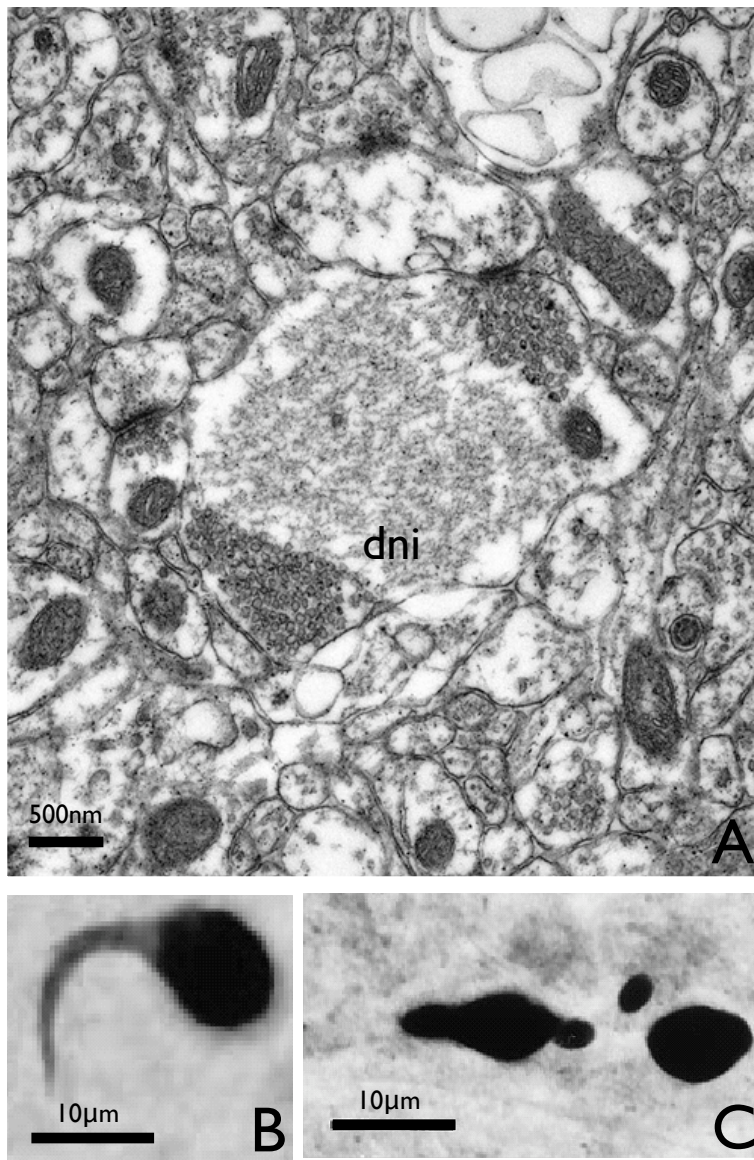


Figure 1.10: A is an EM of a DNI in an R6/2 mouse cortex it appears to be more fibrous in nature than NIIs. B and C are two LM photos showing DNIs in human brain stained with ubiquitin, they show characteristic non-spherical profiles and tails.

1.12.6. Cell Damage Control: The UPS & Chaperones

Protein accumulations in the cell, or in this case the neuron, can pose a very serious threat to the normal function and viability of the cell. This made much worse when the aberrant proteins are of highly abnormal conformations. The inclusions seen in HD and the other polyglutamine diseases are examples of such insoluble accumulations of proteins, though it is not yet been proven that they are responsible for the observed pathology, it is thought that they do play a role in the cellular interactions leading up to the diseased state. These inclusions contain amongst other proteins components of the ubiquitin-proteasome pathway and molecular chaperone molecules which are the two

main protective protein degradation systems operating in eukaryotic cells (Sherman & Goldberg 2001 & Goldberg 2003).

Misfolding is thought to be the cause of degradation of between 30-50% of nascent proteins (Schubert *et al.* 2000 & Turner & Varshavsky 2000). Protein degradation has to be carried out in a highly selective manner. Proteins destined for degradation are tagged with small polypeptide markers like ubiquitin, a chain of four or more ubiquitins to a lysine on the substrate marks it down for rapid digestion by the proteasome complex. This complex is responsible for the degradation of most cell proteins with the exception of those dealt with by the lysosome. The 26S proteasome complex consists of two 19S regulator complex caps on either end of a hollow barrel shaped 20S core structure inside which proteins are degraded down to small peptides by six proteolytic sites which cleave after hydrophobic, basic and acidic residues, and can eventually be hydrolysed by cytosolic peptidases into amino acids (Zwickl *et al.* 1999). The proteasome processes proteins via three different types of degradation; trypsin-like, chymotrypsin-like and peptidylglutamyl-peptide hydrolytic sites all of which are found on the inner surface of the inner rings (green subunits in *Figure 1.11*) (Orlowski & Wilk 2000). Ubiquitin chains bind the substrate to the 19S (aqua and grey subunits in *Figure 1.11*) which contains a ring of ATPases which unfold globular proteins and funnel them into the 20S core (blue and green subunits) to be broken down, the ubiquitin molecules are then released to be recycled in another degradation event.

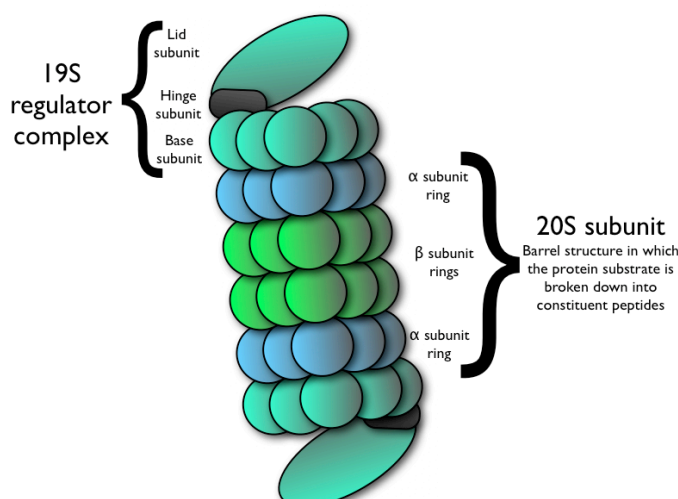
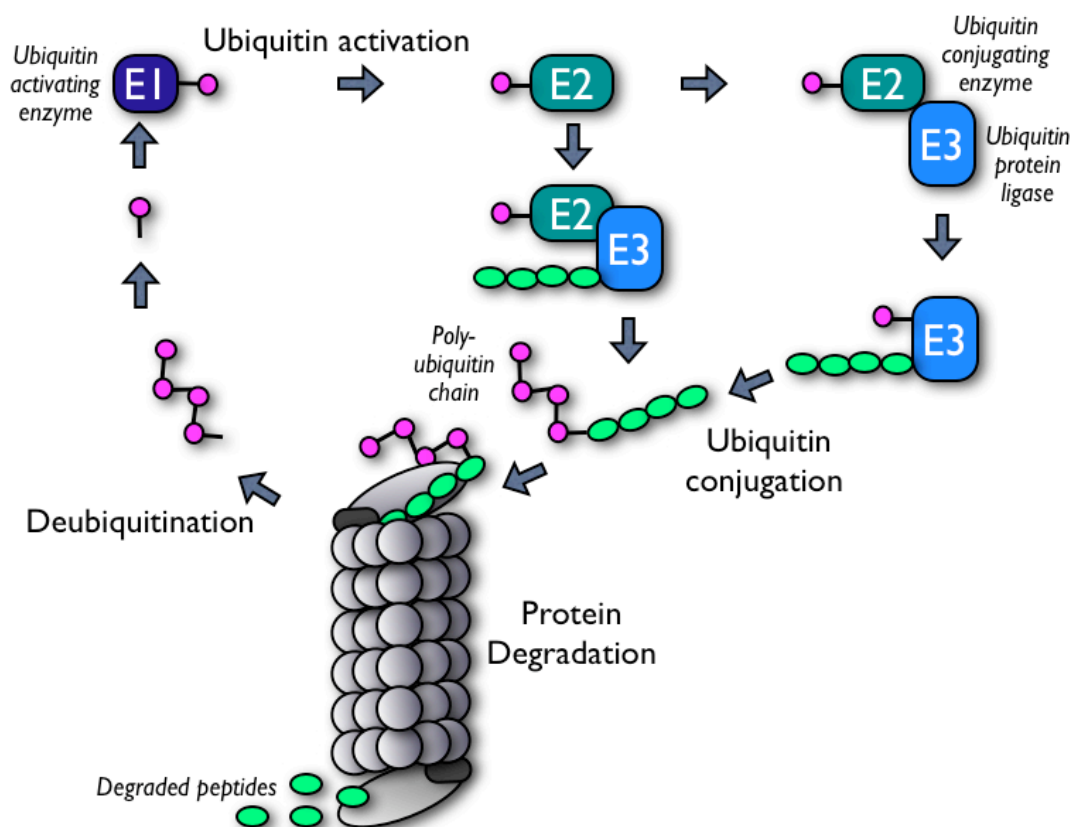


Figure 1.11: Schematic diagram of the proteasome, as the diagram suggests it is made up of two inner and two outer heptameric rings stacked together to form a hollow tube within which degradation can occur. Most proteasome complexes are capped by 19S regulator complexes at either end however there are some where one of these is exchanged for a different one such as PA28 perhaps for unidirectionality.

The free ubiquitin molecule is activated by the enzyme E1, which transfers the molecule to the ubiquitin carrier E2, which in turn transfers the activated ubiquitin molecule to the protein substrate. Mammalian cells have hundreds of highly specific E3 enzymes, which work with specific E2 enzymes to ubiquitinate different proteins. The activity of the ubiquitin molecules is controlled by the ubiquitin conjugating enzymes which serve to further fine tune this highly specified degradation process. More ubiquitin molecules are added to form a polyubiquitin chain which is recognised by the proteasome and causes the unfolding and degradation of the polypeptide releasing free peptides and ubiquitin.

Figure 1.12: Below is a schematic diagram of the ubiquitin-proteasome pathway, the ubiquitin molecules are in magenta circles and protein substrate in green ovals.



Virtually all cells respond to potentially toxic stressful situations with the induction of a set of highly conserved genes encoding the heat shock proteins (HSPs) forming the main cellular defence against damaged, misfolded and mutant proteins. These molecular chaperones are in place to prevent the aggregation of mutant and damaged proteins, solubilize aggregates if they have managed to form by promoting ubiquitination and degradation. Under normal conditions they ensure that proteins are folded correctly into tertiary structures (Ptitsyn 1991 & Christensen & Pain 1991) and regulate their own expression in the cytosol and *endoplasmic reticulum* (ER) as well as suppress apoptosis. Yeast studies have shown that three particular HSPs play a role in stressed cells; co-chaperones HSP70 and HSP40 work together preventing aggregation and can also function in an ATP-dependent manner to refold denatured molecules into active forms thus providing a rescue system for aggregated proteins if this fails, when working in combination with HSP104 they are able to disassemble intracellular protein aggregates and refold insoluble molecules into soluble native species (Glover & Lindquist 1998).

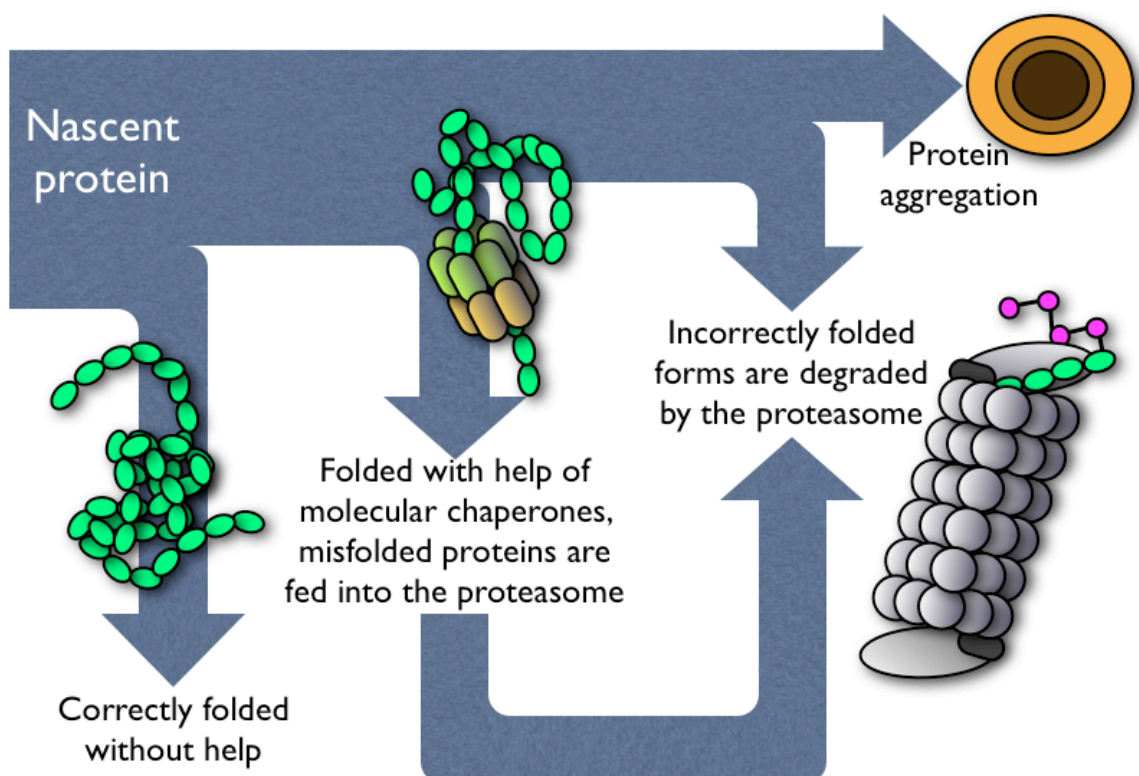


Figure 1.13: Diagram showing protein management in the cell which enables such a fine tuned system. Chaperones and the proteasome intervene when proteins fail to fold correctly and aggregate.

Another class of HSPs are also induced in the heat shock response these are specialised to combat oxygen free radicals and prevent oxidative damage to cell proteins lipids, and more importantly DNA, these include superoxide dismutase, heme oxygenase and catalase.

Both the UPS and the chaperones are coupled and work together as complementary mechanisms, some chaperone molecules serve also as components of the UPS and facilitate ubiquitination and proteolysis just as some ATPases in the 26S proteasome have chaperone like activities and the ability to block aggregation. Some *in vitro* studies have shown the similarities in the functions of members of the UPS and the chaperones (Braun *et al.* 1999 & Strickland *et al.* 2000) and co-purification studies have shown these two protein classes to occur together (Montel *et al.* 1999) Together these two mechanisms are a sophisticated and highly specified cell protection system, which is breached to give rise to various disorders, and becomes inefficient with old age and therefore more prone to disease.

In the transgenic models generated so far all report the presence of components of both the UPS and chaperone molecules discussed earlier, suggesting that the neuron has attempted to fight back and prevent the aggregation of the mutant protein from forming the NIIs (Cummings *et al.* 1999). Additionally it has been shown *in vitro* that aggregation of mutant proteins is able to impair the proteasome (Bence *et al.* 2001) It is very easy to therefore assume that the cellular pathology seen is due to the failure of the cellular damage control system and formation of the NII, however this remains to be proved.

1.12.7. Cell Death

Cell death has always been assumed to be the cause of the disease symptoms in HD and is thought to take place in one of two ways, either by apoptosis or by necrosis. Both these death pathways have different molecules that participate

in them and are used as markers to identify which process is taking place. Apoptosis, when simplified, is described as programmed cell death or cell suicide mechanism in which the cell and nucleus shrink, condense and frequently fragment. The cellular remains are quickly phagocytosed by efficient macrophages, thus avoiding an inflammatory response. In contrast cells that die accidentally via the process of necrosis swell and burst spilling their cytosolic contents into the extracellular space and initiate an inflammatory response. In polyglutamine repeat diseases however neither of these processes appeared to be taking place, but a third death pathway was taking place called dark cell degeneration (Turmaine *et al.* 2000). This process is morphologically and biochemically distinct from apoptosis as it does not have DNA condensations or apoptotic bodies, blebbing of the nucleus or cytoplasm or fragmentation of DNA and does not have TUNEL labelling which is the standard test for apoptosis. Dying neurons in HD *postmortem* tissue and R6/2 mice characteristically show NIIs, a darkening and condensation of both the cytoplasm and the nucleus, ruffling of the plasma membrane while maintaining ultrastructural preservation of cellular organelles. It was assumed that the pathology observed in HD was due to excessive amounts of cell death, but there is relatively little in the way of dead or dying cells in brain tissue before symptoms are apparent in patients and in the R6/2 mice until end stage. This was a surprising result as it had always been thought that dysfunction was caused by severed communication as neurons died. By looking at a whole range of mouse models of polyglutamine diseases and other disorders such as the Cln-3 knock out mouse simulating the paediatric condition of Batten's disease (Mitchison *et al.* 1999 & 2004) and the NK-1 receptor knock out mouse modelling anxiety (De Felipe *et al.* 1998) among others, it was found that there was a pattern emerging. There appeared to be more cell death apparent in models which had a more neuritic pathology and lipofuscin deposits in their neurons. Nuclear pathology such as that seen in the R6/2 mice seemed to have less cell death present in the brains than those with a more neuritic pathology

such as the HD94 mice, suggesting that perhaps the presence of an NII allows the cell to survive longer but in turn causes a more severe phenotype. The dying neuronal profiles are different in different populations also suggesting that maybe there are different death pathways at work within the same afflicted brain. A neuron may be in the advanced stages of dark cell degeneration but an adjacent neuron can appear perfectly healthy unaffected and unaware of the plight of its neighbour.

1.12.8. Glial Response to the presence of NIIs

Golgi remarked, on writing about his pathological study of a HD case, at the massive gliosis that accompanied the degeneration of the brain (Mazzarello 1996). He could visualise using his now famous Golgi black reaction quite clearly the presence of glial cells as well as the neurons. Glial cells provide structural and metabolic support for neurons. There are two major classes of glia; the microglia which respond to nervous system infection or damage and serve a phagocytic or scavenger role, and the macroglia which have a variety of supportive and nutritive functions made up of oligodendrocytes, Schwann cells, astrocytes and ependymal cells.

There is now convincing evidence suggesting that oligodendrocytes, uniquely among cell types in the CNS are dramatically increased in density in the *striatum* throughout the course of HD from presymptomatic through to Vonsattel Grade 4 pathology in human tissue (Myers *et al.* 1991), and this is also seen in the R6/2 transgenic mice at 12 weeks of age (Ma *et al.* 2003), which is end stage for this model. HD represents a picture of abnormal cell densities, which have been present since early life and potentially affect the course of the disease. NIIs are found in oligodendrocytes in human HD tissue but can also be seen in other conditions such as neuronal intranuclear hyaline inclusion disease (NIHID) (Davies *et al.* 2001). In the R6/2 mice there are plenty of inclusions in the oligodendrocytes of the *corpus callosum*, but these are not seen

in the *striatum*. Additionally glial cells associated with dying neurons are not labelled with the traditional antibodies of GFAP, NG2 or F4/80, which suggests that they may be a type of immature astrocyte.

1.13. Aims of this study

Neuropathology of mouse models of HD is a vast field in itself, however I would like to outline here what I hope to accomplish in this study and how I hope to achieve this end.

I have chosen a “transgenic”, a “knock-in” and a “conditional” mouse model to cover the breadth of HD models available. And considered it more valuable for the purposes of this study to concentrate on immunohistochemical studies with an extensive range of antibodies and Golgi impregnation studies.

Previous studies have shown that there is very little cell death and what little is seen is via a novel form known as dark cell degeneration (Turmaine *et al.* 2000), however this process alone is not sufficient to explain the marked atrophy of the brain which is seen. Therefore a detailed morphometric analysis was required to investigate and explain these discrepant findings.

I hope to establish a neuropathological baseline against which any therapeutic studies can be validated in these murine models.