Annu. Rev. Genomics Hum. Genet. 2000. 1:281–328

TRINUCLEOTIDE REPEATS: Mechanisms and Pathophysiology

C. J. Cummings[1] and H. Y. Zoghbi[2]
[1]Program in Cell and Molecular Biology, Department of Pediatrics, and [2]Departments of Molecular and Human Genetics and Pediatrics, Howard Hughes Medical Institute, Baylor College of Medicine, Houston, Texas 77030; e-mail: cc691520@bcm.tmc.edu; e-mail: hzoghbi@bcm.tmc.edu

Key Words dynamic mutations, repeat expansion, polyglutamine disease, protein aggregates, neurodegenerative disease

■ **Abstract** Within the closing decade of the twentieth century, 14 neurological disorders were shown to result from the expansion of unstable trinucleotide repeats, establishing this once unique mutational mechanism as the basis of an expanding class of diseases. Trinucleotide repeat diseases can be categorized into two subclasses based on the location of the trinucleotide repeats: diseases involving noncoding repeats (untranslated sequences) and diseases involving repeats within coding sequences (exonic). The large body of knowledge accumulating in this fast moving field has provided exciting clues and inspired many unresolved questions about the pathogenesis of diseases caused by expanded trinucleotide repeats. This review summarizes the current understanding of the molecular pathology of each of these diseases, starting with a clinical picture followed by a focused description of the disease genes, the proteins involved, and the studies that have lent insight into their pathophysiology.

INTRODUCTION

In 1991, the gene responsible for fragile X syndrome (FRAXA) was found to contain an unstable, expanded $(CGG)_n$ trinucleotide repeat in its 5′ untranslated region (UTR) (79). That same year, spinobulbar muscular atrophy (SBMA), also known as Kennedy's disease, was found to be associated with an unstable expanded $(CAG)_n$ trinucleotide repeat within the coding region of the androgen receptor (*AR*) gene (153). With the convergence of these two discoveries, the expansion of unstable trinucleotide repeats became a novel genetic paradigm for disease and thereby provided an explanation for an unusual pattern of inheritance that leads to a worsening phenotype in each subsequent generation of families affected by the disease. In the following years came the identification of five other neurological diseases caused by untranslated triplet repeats found in the 3′ [myotonic dystrophy

(DM)], the 5′ [fragile XE mental retardation (MR) and spinocerebellar ataxia (SCA) type 12], the intronic [Friedreich ataxia (FRDA)], and even in potential antisense sequences [SCA type 8 (SCA8)]. Additionally, seven other neurodegenerative diseases are now known to result from expansion of (CAG)n repeats coding for polyglutamine tracts in the corresponding proteins: Huntington disease (HD), dentatorubropallidoluysian atrophy (DRPLA), and the SCA types 1, 2, 3, 6, and 7. In general, trinucleotide repeat disorders are either dominantly inherited or X-linked, the one exception being FRDA, which is autosomal recessive. The mutant repeats show both somatic and germline instability and they expand more frequently than they contract in successive transmissions. The molecular basis of repeat instability is not well understood nor is it known whether somatic and germline instabilities share common mechanisms of expansion (for review, see 284). Increased severity of the phenotype and earlier age of onset in successive generations (anticipation) are generally associated with larger repeat length. The parental origin of the disease allele can influence anticipation; for most of these disorders there is greater risk of repeat expansion upon paternal transmission, whereas, in FRAXA, FRDA, and congenital forms of DM, the maternally transmitted alleles are more prone to cause the most severe phenotypes. Some of the features of each of the known trinucleotide repeats are summarized in Table 1 and are discussed in greater detail in the following sections; Table 2 lists mechanisms of pathophysiology.

COMMON FEATURES OF NONCODING DISORDERS

The mechanism of pathogenesis in noncoding-repeat diseases varies, depending on the consequences of loss of function of the respective proteins or, in some cases, acquired function of a toxic triplet repeat transcript. The trinucleotide sequence and its location in relationship to a gene may play a prominent role in dictating the unique mechanism of pathogenesis for each disorder. Despite these inherent differences, many similarities exist within this group of diseases. First, the noncoding-repeat diseases are typically multisystem disorders involving the dysfunction/degeneration of many different tissues. In fact, phenotypes within a disorder are also often variable, perhaps owing to a more pronounced degree of somatic heterogeneity in the noncoding repeats compared with the coding repeat tracts; this is especially evident in fragile X and DM. Next, the size and variation of the repeat expansions are much greater in the noncoding-repeat diseases than in the polyglutamine repeat diseases. Finally, many of the noncoding-repeat disorders can be associated with a small pool of clinically silent, intermediate-size expansions, or premutations, that may expand to the full mutation after germline transmission (one notable exception to this rule at this time is FRDA). Some other common motifs are highlighted in the following sections.

TABLE 1 Summary of trinuceotide repeat disorders

Disease	Gene	Locus	Protein	Repeat	Repeat Size		Repeat location
					Normal	Disease	
Fragile X syndrome	*FMR1*(FRAXA)	Xq27.3	FMR-1 protein (FMRP)	CGG	6–53	60–200 (pre) >230 (full)	5′-UTR
Fragile XE syndrome	*FMR2* (FRAXE)	Xq28	FMR-2 protein	GCC	6–35	61–200 (pre) >200 (full)	5′-UTR
Friedreich ataxia	*X25*	9q13-21.1	Frataxin	GAA	7–34	34–80 (pre) >100 (full)	Intron 1
Myotonic dystrophy	*DMPK*	19q13	Myotonic dystrophy protein kinase (DMPK)	CTG	5–37	50–thousands	3′-UTR
Spinobulbar muscular atrophy (Kennedy disease)	*AR*	Xq13-21	Androgen receptor (AR)	CAG	9–36	38–62	Coding (amino terminal)
Huntington disease	*HD*	4p16.3	Huntington	CAG	6–35	36–121	Coding (amino terminal)
Dentatorubral-pallidoluysian atrophy (Haw River syndrome)	*DRPLA*	12p13.31	Atrophin-1	CAG	6–35	49–88	Coding (amino terminal)
Spinocerebellar ataxia type 1	*SCA1*	6p23	Ataxin-1	CAG	6–44	39–82	Coding (amino terminal)
Spinocerebellar ataxia type 2	*SCA2*	12q24.1	Ataxin-2	CAG	15–31	36–63	Coding (amino terminal)
Spinocerebellar ataxia type 3 (Machado-Joseph disease)	*SCA3* (*MJD1*)	14q32.1	Ataxin-3	CAG	12–40	55–84	Coding (carboxy terminal)
Spinocerebellar ataxia type 6	*SCA6*	19p13	α_{1A} -Voltage-dependent calcium channel subunit	CAG	4–18	21–33	Coding (carboxy terminal)
Spinocerebellar ataxia type 7	*SCA7*	3p12-13	Ataxin-7	CAG	4–35	37–306	Coding (amino terminal)
Spinocerebellar ataxia type 8	*SCA8*	13q21	None	CTG	16–37	110 to <250?	3′-Terminal exon (antisense?)
Spinocerebellar ataxia type 12	*SCA12*	5q31-33	PP2A-PR55β(PPP2R2B)	CAG	7–28	66–78	5′-UTR

TABLE 2 Proposed mechanisms of pathophysiology in trinucleotide repeat diseases

Disease	Effect of mutation	Mechanism of disease
Fragile X syndrome	Loss of function	Reduced FMR1 triggering abnormal RNA metabolism (misregulation of localized protein synthesis)
Fragile XE syndrome	Loss of function	Reduced FMR2 causing abnormal neuronal gene regulation
Friedreich ataxia	Loss of function	Reduce frataxin causing altered iron homeostasis and mitochondria dysfunction
Myotonic Dystrophy	Loss of function Gain of function	1) haploinsufficiency of DMPK causing altered kinase activity (muscle defects?) 2) cis effects on neighbor gene expression (cognitive/dysmorphology?) 3) dominant effect on RNA metabolism with loss of CUG-BP function
SCA8	Loss/gain of function?	Abnormal antisense regulation?
SCA12	Loss of function?	Abnormal phosphatase activity?
HD, SBMA, DRPLA, SCA1,2,3,6,7	Gain of function	Protein misfolding; abnormal protein-protein interactions; altered proteolysis; altered gene expression

Fragile X Syndrome (FRAXA): Abnormal RNA Metabolism

Clinico Pathology FRAXA is an X-linked disorder that typically presents in males. Symptomatic females are generally less severely affected, depending on the ratio of cells with the normal X chromosome active (1, 222). Although MR is the most common feature of FRAXA, phenotypes vary and may include postpubescent macroorchidism, long and prominent ears and jaws, high-pitched speech, hyperactivity, poor eye contact, and stereotypic hand movements such as hand flapping and hand biting. FRAXA has a prevalence rate of 1 in 4000 and accounts for ~30% of all X-linked MR (241, 270). The prevalence rate may be substantially higher given the broad spectrum of physical and behavioral features in patients who do not have MR (96).

Mutation As its name implies, FRAXA was originally identified through its association with a folate-sensitive fragile site at Xq27.3 in a proportion of affected patients. Sequence analysis of cosmids and cDNAs coincident with this fragile site revealed a polymorphic $(CGG)_n$ repeat present in the 5′-UTR of the fragile X MR gene (*FMR1*) (79, 273). Normal alleles contain from 6 to 53 CGG repeats punctuated by one or more AGGs, which seem to stabilize the CGG tract (71, 79, 195, 273). Loss of the interspersed AGGs and expansion of the CGG repeat beyond 230 trinucleotides cause disease and hypermethylation of the CGG repeat as well as a CpG island within the *FMR1* promoter region (14, 71, 79, 98, 212, 254).

This hypermethylation results in the recruitment of transcriptional silencing machinery to the *FMR1* regulatory region, followed by loss of its transcription. A number of observations in cells from patients with FRAXA support this model, including (*a*) altered chromatin structure at the 5′ end of *FMR1* (70), (*b*) absent protein-DNA footprint profiles at the *FMR1* locus (66, 236), and most recently (*c*) loss of acetylated histones associated with the 5′ end of *FMR1* (45). Hyperexpansion is associated exclusively with maternal transmission, and the risk of expansion to >230 repeats is dependent on the size of the premutation in female carriers. For example, females with premutations below 60 repeats have a low probability of having affected offspring, whereas premutations beyond 90 repeats indicate a high risk of expansion to the full mutation with maternal transmission. Further expansion of the repeat in successive generations provided a molecular explanation for the Sherman paradox (increasing penetrance through subsequent generations in FRAXA) (242, 243).

Gene Product and Pathophysiology The *FMR1* gene product FMRP is a selective RNA-binding protein that contains two K homology domains and an RGG box; it shuttles between the nucleus and the cytoplasm (8, 63, 87, 245, 272). In the cytoplasm, FMRP forms messenger ribonucleoprotein complexes and associates with polyribosomes or endoplasmic reticulum-associated ribosomes (49, 132). FMRP binds to an estimated 4% of brain messenger RNA (mRNA) and may, therefore, be involved in nuclear export and ribosomal targeting of a select group of transcripts (8). *FMR1* is widely expressed in both fetal and adult tissues, with highest levels in brain (63). In neurons, FMRP's association with translational machinery in dendritic spines suggests that it may play a role in modulating the localization and/or translation of its target mRNAs (49, 132). Regulation of localized protein synthesis is known to be important in cell growth, polarity, and the management of synaptic plasticity that accompanies learning- and memory-related events such as long-term potentiation.

The mutation in FRAXA results in a loss of the normal function of FMRP, because pathologically expanded repeats are associated with decreased transcription of *FMR1* and loss of gene product (165, 212, 254). Furthermore, those few cases known to have deletions of *FMR1* show the same clinical phenotype (267). The inverse relationship between the size of the repeat and FMRP expression level correlates with disease severity. The biochemical abnormality leading to MR in FRAXA may result from failure of translation of key proteins during synaptic development or maintenance. The other phenotypic manifestations associated with FRAXA could be expected from the potential pleiotropic effects caused by abnormal RNA metabolism. This model has been supported by studies in mice lacking the *Fmr1* gene (47). These mice exhibit cognitive and behavioral deficits, macroorchidism, and hyperactivity—abnormalities comparable with those of human fragile X patients.

A better understanding of the pathogenetic mechanism in FRAXA will come from studies delineating the FMRP-interacting proteins, the target RNAs regulated by FMRP, and the role FMRP plays in RNA metabolism. *FMRP* is a member

of a gene family that contains two autosomally encoded fragile X–related proteins, FXR1P and FXR2P. All three proteins show significant homology (60%), including conservation of the functional RNA-binding domains (135, 246). Although overlap of expression of the three proteins is not always the rule, they are capable of interacting as homo- and heterodimers and can be coimmunoprecipitated as a complex, suggesting a functional role as a ribonucleoprotein complex (258, 294). At present, the functions of FXR1P and FXR2P—and whether these functions could compensate for some of those lost in unaffected FRAXA cells—remain a mystery (131). Nucleolin and nuclear FMRP-interacting protein are two other FMRP-associated proteins recently identified (11, 35). The physiological significance of these new interactors is not yet known, but they may function as part of the FMRP-ribonucleoprotein complex that regulates specific RNA metabolism.

Fragile XE Mental Retardation (FRAXE): Alterations in Neuronal Gene Regulation

Clinicopathology Although phenotypes may vary, Fragile XE MR (FRAXE) patients have mild MR, have learning deficits, and may be developmentally delayed (190). Some FRAXE individuals present with behavioral abnormalities, which may include attention deficits and hyperactivity. The prevalence is 1%–4% that of FRAXA and, as expected for an X-linked trait, the cognitive deficits are milder in females (25). Physical phenotypes such as those in FRAXA have not been described in FRAXE patients.

Mutation FRAXE is caused by an expansion of a polymorphic $(GCC)_n$ repeat in a folate-sensitive fragile site immediately adjacent to a CpG island, 600 kilobases distal to the FRAXA locus (40, 138). The repeat size varies from 6 to 35 copies on normal alleles that are relatively stable on transmission, whereas repeat sizes in the premutation range (61–200 copies) may expand to the full mutation (>200 copies). Parent-of-origin effects are similar to those described above with FRAXA, but reductions in repeat number are frequently observed in FRAXE upon germline transmission, and males with the full mutation do have affected daughters with full mutations (139).

Gene Product and Pathophysiology Similar to the FRAXA expansion, the FRAXE GCC repeat resides in the promoter region of a gene termed *FMR2* (40, 82, 92). The expanded repeats are also abnormally methylated, leading to transcriptional silencing of *FMR2*. The *FMR2* gene product has amino acid motifs that show similarity to two other human proteins, AF4 and LAF-4 (20, 81, 167). All three proteins exhibit nuclear localization, DNA-binding capacity, and transcription transactivation potential (20, 81, 167). *FMR2* transcripts have been detected in multiple tissues including, but not limited to, adult and fetal brain, placenta, lung, and kidney (40, 83). Within the brain, highest expression levels are in the hippocampus and the amygdala (39, 40).

The cognitive and behavioral deficits in FRAXE likely result from transcriptional silencing of the *FMR2* gene and subsequent loss of FMR2 protein function. This is supported by studies in patients who have deletions within the *FMR2* gene resulting in loss-of-function mutations and phenotypes not unlike FRAXE individuals carrying an expanded repeat (84). Although FMR2's precise function(s) are unknown, its putative role as a transcriptional activator and its high level of expression in areas of the brain involved in learning, memory, and emotion suggest that the pathogenic mechanism in FRAXE is caused by alterations in neuronal gene regulation.

Friedreich Ataxia (FRDA): Abnormal Iron Homeostasis

Clinicopathology FRDA, the most prevalent inherited ataxia, is autosomal recessive and therefore does not show the anticipation common to other triplet repeat disorders (99, 220). FRDA is characterized by unremitting gait, limb, and truncal ataxia, diminished tendon reflexes, loss of position and vibratory senses, dysarthria, cardiomyopathy, and diabetes mellitus; less commonly nystagmus, optic atrophy, scoliosis, and/or skeletal abnormalities are evident. As indicated by the range of clinical manifestations, FRDA is a multisystem disorder characterized pathologically by degeneration of the posterior columns of the spinal cord, invariable loss of large primary sensory neurons of the dorsal root ganglia, and atrophy of several peripheral sensory systems. In contrast to many of the other inherited ataxias, the cerebellar cortex shows only mild, late-stage degeneration. The age of onset is typically early childhood, and the combination of neurological impairment, cardiomyopathy, and occasionally diabetes inevitably shortens life span.

Mutation FRDA is primarily caused by a large intronic GAA repeat expansion located in the center of an Alu repeat in the *X25* gene (also known as *frataxin*) (32). FRDA is unique among the triplet repeat disorders, not only because of the repeat location (intronic) and nucleotide sequence (AT-rich unlike GC-rich triplets), but also because affected individuals carry two mutant alleles. Also remarkable is that the GAA repeat expansion is the most common to date, which explains the high carrier frequency of 1 in 85 (51). Normal alleles contain 7–34 repeats, whereas disease-causing alleles contain $\geq$100 repeats. There are a few large normal alleles that contain 33–55 trinucleotides; GAG interruptions in these large "normal" alleles are thought to restrain the repeats from expanding into the disease-causing range (51, 187). Intermediate premutation alleles containing 34–65 pure GAA repeats have been found to expand to 300–650 repeats in a single generation (51, 187). The unstable GAA repeat undergoes both expansions and contractions after maternal transmission but predominantly contracts when passed through the father (60, 186).

Gene Product and Pathophysiology The *X25* gene product, frataxin, is a 210-amino-acid protein that has well-conserved homologs in many lower organisms (86). With highly conserved mitochondrial targeting signal sequences and

mitochondrial membrane localization in yeasts and humans, frataxin is thought to be involved in iron homeostasis and respiratory function (9, 31, 77, 149, 287). Expression studies in mice and humans show that gene expression correlates in part with the primary sites of pathology in FRDA, including the dorsal root ganglia, spinal cord, sensory nerves, and non-neuronal tissues such as the heart and pancreas (32, 149). Several other tissues unaffected by disease also express significant levels of frataxin. A commonality among all frataxin-expressing tissues is that they are rich in mitochondria. Those that are most affected, however, are postmitotic (e.g. neurons, cardiocytes, and β cells), suggesting that tissues with renewal potential have an advantage in FRDA pathogenesis.

Expanded GAA repeats are associated with reduced *X25* expression (32, 50). Unlike fragile X, the expanded AT-rich sequence does not mediate transcriptional silencing through hypermethylation; rather, it has been hypothesized that transcriptional interference is via a self-association of the GAA/TTC tract, thereby stabilizing the DNA in a higher-order triplex structure (225). There is no in vivo evidence for missplicing or altered RNA processing; RNase protection assays and in vitro transcription experiments show that all portions of frataxin mRNA are reduced (19, 50). Moreover, heterozygous carriers show an intermediate level of mRNA expression between those carrying the wild type and affected individuals (18, 50). Reduced *X25* mRNA results in decreased frataxin levels, suggesting that FRDA results from a partial loss of frataxin function (19, 31, 50, 196). In support of this notion, patients who carry only one expanded allele and one truncating point mutation on the second allele have all of the clinical features of typical FRDA (12, 32, 50).

Disruption of the yeast *frataxin* homolog (*YFH1*) resulted in abnormal accumulation of mitochondrial iron, loss of mitochondrial DNA, respiratory dysfunction, multiple iron-sulfur–dependent enzyme deficiencies, and increased sensitivity to oxidative stress (9, 31, 77, 149, 287). In hearts of FRDA patients, increased iron content has been reported (154) and studies in endomyocardial biopsies of two unrelated FRDA patients demonstrated deficient activities of proteins involved in iron-sulfur homeostasis, including aconitase (221). More recently, FRDA fibroblasts in culture were shown to be hypersensitive to iron and hydrogen peroxide stress, and the kinetics of cell death correlated well with the concentrations of iron and H_2O_2 (288). These results are consistent with the following model for FRDA: frataxin insufficiency results in abnormal iron-sulfur homeostasis and, in turn, mitochondrial dysfunction, free-radical production, oxidative stress, and cellular degeneration. This model is particularly relevant to the degeneration of cells with exclusively aerobic metabolism (e.g. neurons and cardiocytes), which makes them more sensitive to mitochondrial defects.

Myotonic Dystrophy (DM): Multiple Mechanisms in DM Pathogenesis

Clinicopathology DM is an autosomal dominant, multisystem disorder with highly variable manifestations and anticipation (100, 107). Myotonia, muscle

weakness, and progressive muscle wasting primarily characterize classical adult-onset DM. Other features may include facial dysmorphology, presenile cataracts, testicular atrophy, premature balding in males, kidney failure, hyperinsulin secretion, and cardiac conduction abnormalities. There is also variable loss of mental function, but this is more common with congenital DM, which is the most severe form of the disorder (and may also be associated with hypotonia, respiratory distress at birth, and developmental abnormalities) (100). Although variation in prevalence exists between different ethnic groups, the estimated global incidence is 5 in 100,000 with ~1 in 8000 affected individuals in North American and western European populations, making it one of the most frequently inherited neuromuscular diseases (100).

Mutation The mutation in DM is an expanded CTG trinucleotide repeat tract in the 3′-UTR of a protein kinase gene, *DMPK* (24, 30, 80, 170). Wild-type chromosomes with a stable but polymorphic repeat have 5–37 CTGs, whereas all DM patients have expanded repeats ranging from 50 in adult-onset cases to several thousand repeats in some congenital cases. DM and FRAXA thus present the most striking expansions in the trinucleotide repeat family. Also unique in DM is the extreme degree of somatic heterogeneity. Although the length of CTG expansion often correlates with severity of symptoms, mitotic somatic events and germline variation contribute substantially to CTG-length mosaicism and phenotypic variability (5, 7, 108, 134, 260, 269).

Gene Product and Pathophysiology The underlying molecular mechanism by which the expanded repeat causes the DM phenotype remains unknown. The dominant inheritance and variable expressivity suggest that DM has a complex multifaceted pathophysiology, which may involve a combination of molecular defects that have different consequences for different tissues at different developmental stages. Given that the repeat expansion is located outside the coding region, a gain of function mechanism at the protein level is improbable. A number of other mechanisms could contribute either mutually or exclusively to disease; these include (*a*) haploinsufficiency of DMPK protein, (*b*) local chromatin effects on the expression of neighboring genes, and (*c*) novel gain of function(s) conferred on the expanded *DMPK* mRNA. Each mechanism is described in more detail below.

Although the exact physiological role of this kinase is unknown, DMPK is predicted to have several functions based on putative substrates and interacting proteins, including the modulation of skeletal muscle sodium channels, Ca^{2+} homeostasis, RNA metabolism, and cell stress response (reviewed in 262). The CTG repeat could indirectly alter *DMPK* protein levels by interfering with DMPK transcription, RNA processing, and/or translation (78, 151). The result would be abnormal phosphorylation of downstream substrates, depending on the DMPK kinase activity. The effect of the CTG expansion on *DMPK* levels and the protein's role in disease manifestation are, however, controversial. Quantitation of *DMPK* mRNA

and protein levels in DM patient material has demonstrated increases, decreases, and no change compared with the wild type (17, 33, 78, 104, 142, 151, 169, 224). A possible explanation for the different results may stem from the finding that transcripts from the expanded allele are abnormally retained in the nucleus, within discrete nuclear foci (59, 97, 259). The nuclear retention of expansion-derived transcripts may not only explain a loss-of-function mechanism, but may also support a gain of function for the nuclear-retained transcripts (see below). Unlike FRDA, no confirmed case of DM has been attributed to a mutational defect other than the expansion of the CTG repeat. Additionally, patients who are homozygous for the DM mutation do not differ in clinical expression from typical DM heterozygotes (44, 174). Moreover, mice lacking *DMPK* develop minor myopathy and muscle weakness, whereas those overexpressing *DMPK* develop hypertrophic cardiomyopathy and neonatal lethality, suggesting that changes in DMPK levels alone are not sufficient to cause the full spectrum of the DM phenotype (119, 216).

The identification of two other genes flanking *DMPK* raised the possibility that CTG expansion may have *cis*-chromatin effects that could alter neighboring gene expression (21). This hypothesis was initially supported by the finding that the size of the CTG repeat correlates with enhanced nucleosomal packaging and lost DNase 1 hypersensitivity adjacent to the repeat (202, 277, 278). Sequence analysis of this region revealed that the CTG repeat is located within the promoter of a downstream homeobox gene, termed DM locus-associated homeodomain protein [*DMAHP*, also known as *SIX5* (21, 137, 261)]. Furthermore, *DMAHP/SIX5* expression was reduced, in a repeat-dependent manner, in DM patient fibroblasts, myoblasts, muscle, and myocardium (137, 261). These findings are intriguing and suggest that haploinsufficiency of *DMAHP/SIX5* may participate in the pathophysiology of the developmental abnormalities seen in some DM patients. As with variable *DMPK* levels in DM, it is not clear what happens to *DMAHP* expression (2, 97). A clearer picture may stem from future studies in *Six5*-deficient and *Six5/Dmpk* doubly deficient mice.

The second gene in the myotonic-dystrophy locus, gene 59 (also known as *DMWD*), is located immediately upstream of *DMPK* (118, 239). The murine homolog, *DMR-N9*, shows high expression in brain, testis, heart, and kidney, but is absent in skeletal muscle (118). Conflicting data also exist regarding the levels of *DMWD* in DM patient material (2, 97). Nevertheless, the tissue-specific expression profile of *DMWD* makes this another candidate whose loss of function could contribute to some of the features in DM, particularly the MR, testicular atrophy, and kidney failure.

Another hypothesis to explain the variety of clinical features seen in DM proposes a gain-of-function model (276). It is based on a *trans*-dominant effect of the CTG-expanded transcript that interferes with the normal processing and/or metabolism of numerous RNAs (151, 188, 259, 276). Altered metabolism of tissue-specific RNAs could account for the various symptoms seen in DM patients. Timchenko and coworkers proposed that the expanded CUG repeat

sequesters RNA-binding proteins, thereby interfering with their normal role in regulating RNA processing in multiple tissues (218, 263, 264). This hypothesis is supported by the identification of a CUG-binding protein (CUG-BP or hNab50) that could mediate this *trans*-dominant effect (211). It now seems likely that a family of proteins that bind CUG repeats, as well as their CUGBP1-dependent RNAs, might be affected by the DM expansion (3, 16, 164, 211, 263).

Spinocerebellar Ataxia Type 8 (SCA8): Altered RNA Regulation?

Clinicopathology SCA8 is a member of the dominantly inherited SCAs. These are a heterogeneous group of neurologic disorders characterized by variable degrees of degeneration of the cerebellum, spinal tracts, and brain stem (91, 141). Many clinical features such as gait ataxia and dysarthria are common to all of the SCAs, making it difficult to differentiate among them simply by clinical or neuroimaging studies. A limited number of variable features, seen either in some subtypes or in some families with a specific subtype, help distinguish certain ataxias. SCA8 is a slowly progressive ataxia with cerebellar atrophy, decreased vibration sense, and sometimes brisk reflexes. The age of onset is typically in the third to fourth decade and patients have a normal life span.

Mutation When the mutation in SCA8 was shown to be an expansion of a noncoding CTG expansion, as is the case for DM, it not only distinguished SCA8 from the other SCAs, but it introduced a possible novel pathogenic mechanism in a triplet repeat disease (146). Using a technique termed "repeat expansion detection" (RED) to identify expanded CAG repeats (232), Koob et al (146) found a CAG/CTG repeat cosegregating with disease in a family with an unassigned dominant ataxia. It is interesting that no CAG-containing RNA transcripts were identified nor were any significant open reading frames found to extend through the repeat expansion, confirming that SCA8 is not a polyglutamine disorder. Moreover, reverse transcriptase-polymerase chain reaction analysis showed that the SCA8 repeat is transcribed in the CTG orientation and is present in the 3′ terminal exon of a processed transcript (104 base pairs from the polyA signal sequence). A second mRNA was isolated that was transcribed in an orientation opposite to that of the SCA8 transcript. This "sense" transcript encodes for a protein that shares strong homology to the *Drosophila KELCH* gene [named Kelch-like 1 or *KLHL1* (145)]. The region of overlap is limited to the 5′ terminal of the SCA8 transcript; therefore, the CTG repeat is present in the antisense but not the sense *KLHL1* transcript. These data suggest that the SCA8 transcript may be an endogenous antisense RNA that may regulate the expression of *KLHL1*. The normal function of KLHL1 in the brain is not fully known. The protein has POZ/BTB protein-protein domains, a conserved Kelch actin-binding domain, and similarities to a brain-specific gene, *NRP/B*, believed to be involved in neuronal process formation (133).

Pathophysiology The role of the CTG repeat in SCA8 pathology is not yet well understood. It is possible that SCA8 and DM share a similar pathogenic mechanism. The clinical differences between these diseases, however, may be caused by the different expression profiles of the SCA8 and DM transcripts and/or different functions of the proteins encoded by the transcripts that are ultimately affected by the expanded CUG-containing RNA. The limited expression of SCA8 (it is found primarily in brain tissue) correlates well with the phenotype (120). A characteristic not seen in DM or any other triplet diseases is that SCA8 may have a CTG repeat interval that is pathogenic (∼110–250 repeats), with shorter and larger repeats not resulting in disease (214). Whereas it is intuitive that repeats of <100 are not associated with disease, it is intriguing that patients with 800 CTG repeats have no history of ataxia (214). Although this phenomenon is not yet fully understood, it is possible that these very large repeats interfere with SCA8 expression or confer altered RNA processing and/or stability, so that the toxic gain of function does not eventuate.

Spinocerebellar Ataxia Type 12 (SCA12): Altered Phosphastase Regulation?

SCA12 is the most recent addition to the trinucleotide repeat disease family. A rare disease with only one large pedigree described to date, SCA12 is caused by a non-coding CAG trinucleotide repeat expansion in the 5′ UTR of the *PPP2R2B* gene (or *PP2A-PR55*β) (106). *PPP2R2B* encodes a brain-specific regulatory subunit of protein phosphatase 2A. Protein phosphatase 2A has many important and diverse cellular functions; however the precise role of PR55β in one or more of these functions has yet to be determined (183). Although the repeat is flanked by transcriptional start sites and conserved promoter elements, it is not yet known whether the expanded CAG tract is associated with transcriptional interference of *PPP2R2B*, an issue complicated by the absence of patient tissue that is known to express this gene. Further molecular characterization and expression analysis of *PPP2R2B* will provide essential clues toward understanding the pathophysiology of SCA12.

DISEASES INVOLVING CODING REPEATS: POLYGLUTAMINE DISEASES

The second group of triplet repeat disorders are all caused by exonic CAG repeat expansions that are translated into a long polyglutamine tract. Although these proteins do not share any homology aside from the polyglutamine tract, several salient features are shared by the polyglutamine disorders. First, these diseases are all characterized by progressive neurodegeneration, typically striking in mid-life and causing increasing neuronal dysfunction and eventual neuronal loss 10–20 years after onset of symptoms. Second, the polyglutamine repeats confer a gain of function when they are pathologically expanded, suggesting a common

mechanism of pathogenesis. Disease develops when the number of uninterrupted repeats exceeds ~35 glutamines, except for SCA6, which is associated with expansions above 21 repeats. Third, intergenerational instability is more pronounced in paternal transmissions for all of these disorders. Fourth, despite the widespread expression of all eight genes throughout the brain and other tissues, only a certain subset of neurons is vulnerable to dysfunction in each of these diseases. The variability in cell-specific degeneration despite overlapping expression patterns is lost, however, when the expansions are very large, leading to severe juvenile-onset disease. In these cases, there is significant overlap in the phenotypes. This loss of cell specificity with large expansions is interesting, and it hints that toxicity is probably much more widespread through neuronal and non-neuronal cells that are normally spared when the repeat sizes are moderately expanded. It may also reinforce the idea that repeat expansions are smaller in the coding repeat disorders because of selective pressure against the very large expansions, which are likely to be embryonically lethal.

One other shared pathological hallmark of the polyglutamine disorders is the presence of protein aggregates or nuclear inclusions (NIs). Aggregates of disease-causing protein, primarily found in the nucleus, have now been reported for all eight diseases; in SCA6 the aggregates are exclusively found in the cytoplasm. The predominant nuclear localization of the inclusions is intriguing given the various subcellular localizations of the soluble forms of these proteins. The inclusions are often, but not always, found in the tissues that are most susceptible to degeneration despite the ubiquitous expression of the expanded proteins. The factors contributing to protein aggregation and the role that these aggregates may play in disease are discussed in greater detail below.

Spinobulbar Muscular Atrophy (SBMA or Kennedy Disease)

Clinical Characterization SBMA, the only triplet repeat disease showing an X-linked recessive pattern of inheritance, is characterized pathologically by selective degeneration of the anterior horn, bulbar region, and dorsal root ganglion (250). Male patients often present with muscle cramping that leads to proximal muscle weakness and atrophy. The eponymous bulbar degeneration leads to difficulties in speech, articulation, and swallowing. Patients may also show fasciculations of the tongue, lips, or perioral region of the face. No signs of upper-motor-neuron disease, such as hyperreflexia or spasticity, are evident. Signs of mild androgen insensitivity are typically seen at adolescence, including gynecomastia and testicular atrophy (6).

Mutation and Gene Product The expanded polymorphic CAG repeat tract in SBMA patients is located in the first coding exon of the androgen receptor (*AR*) gene. The AR is a steroid hormone-activated transcription factor composed of hormone and DNA-binding domains. Upon binding of ligand in the cytoplasm, the receptor dissociates from a number of accessory proteins (chaperones or heat

shock proteins), translocates into the nucleus, dimerizes, and then transactivates androgen-responsive genes (295). The function of the polyglutamine tract in AR is unknown. Although it is located in one of the transactivation domains, an AR lacking the polyglutamine tract is still able to transactivate hormone-responsive genes (121). Expansion of the glutamine repeat has no effect on hormone binding and only slightly reduces its ability to transactivate (41, 182, 191). The expansion may cause partial loss of receptor function that could be responsible for some symptoms of androgen insensitivity in affected males; however, complete absence of AR leads to testicular feminization rather than a motor neuron disease (168, 297). Because SBMA occurs only in males, heterozygous SBMA females may be protected by X-inactivation patterns or by low androgen levels, assuming that the toxic effect is ligand dependent.

Nuclear inclusions containing mutant AR have been reported in motor neurons within the pons, medulla, and spinal cord (157, 158). Although the NIs were absent in unaffected neural tissues, they were present in a select group of unaffected non-neuronal tissues including scrotal skin, dermis, kidney, and, less frequently, heart and testis. They were not detected in spleen, liver, and muscle. It is difficult at this time to reconcile the selective presence of NIs among the various non-neuronal tissues. It is interesting that the postmortem tissue used in this study was from patients with typical adult-onset SBMA (death was between 54 and 82 years), which undermines the contention that loss of tissue specificity, at least in regard to NI formation, occurs only in juvenile-onset cases.

Huntington Disease (HD)

Clinicopathology Slight and sporadic motor disability, involving both involuntary and voluntary movements, becomes progressively exaggerated until full-blown chorea develops within 10–20 years. The motor disorder is often preceded or accompanied by memory deficits, cognitive decline, affective disturbance, and/or changes in personality; other forms of motor dysfunction such as rigidity and dystonia may also be present. Juvenile onset patients show rigidity, bradykinesia, epilepsy, severe dementia, and an accelerated disease course.

Atrophy of the caudate and putamen is visible in brain-imaging scans, and diffuse degeneration of the neostriatum is the pathologic hallmark of HD (275). Medium-sized spiny striatal neurons containing γ-aminobutyric acid are the most severely affected, whereas other somatostatin-containing neurons are particularly spared. Juvenile-onset cases often show loss of cerebellar Purkinje cells along with more pronounced atrophy of the brain (274).

Mutation and Gene Product The CAG repeat in the first exon of the *HD* gene is highly polymorphic and varies from 6 to 35 repeats on unaffected chromosomes and from 36 to 121 repeats in the disease state (223). Adult onset typically occurs when the repeat contains 40–50 units, whereas alleles containing >70 repeats result in the more severe juvenile form of the disease. *HD* homozygote patients have

phenotypes similar in severity to their heterozygote siblings, suggesting that HD is a true dominant disorder (285). Genetic evidence in both humans and mice confirms that loss of huntingtin function does not result in HD (4, 69, 192, 292). HD knockout mice are developmentally retarded and die in gestation between days 8.5 and 10.5, suggesting that huntingtin has, as of yet, an unknown function that is crucial for embryonic development and neurogenesis. Notably, the embryonic function of huntingtin is not impaired when the wild-type *Hdh* alleles are replaced with *Hdh* alleles containing an expanded polyglutamine tract (50 Gln) (286).

The precise function of huntingtin remains elusive, and it has few similarities with known proteins. A number of HD homologs have been isolated, including mouse, rat, pufferfish, zebrafish, and fruit fly. The murine homolog, *Hdh*, shares 90% homology with human *HD* (161), and the putative *Drosophila* ortholog has an overall identity of 24% and similarity of 49% (160). Despite this overall high level of conservation, the mouse protein has only seven consecutive glutamines, and the fly protein lacks both the glutamine stretch and a polyproline stretch that is present in its mammalian counterparts.

HD is expressed throughout the brain, primarily as a cytoplasmic protein (64, 208, 266). It is relatively abundant in large striatal interneurons, medium spiny neurons, cortical pyramidal cells, and cerebellar Purkinje cells (274). Subcellular-localization studies reveal huntingtin staining in somatodendritic regions and in axons. It is associated with microtubules in dendrites and with synaptic vesicles in axon terminals, perhaps serving some function in synaptic transmission. In cell lines, huntingtin has been reported to be located in both the cytoplasm and nucleus, suggesting a potential nuclear function (61).

Abnormal nuclear and neuropil aggregation of mutant huntingtin has been reported in postmortem HD brain (13, 65, 94, 219). Neuropil aggregates, occurring in dendrites and dendritic spines, are similar in structure but are far more common in HD brain than neuronal NIs (94). Moreover, the progressive appearance of the neuropil aggregates is correlated with neurological symptoms in patients and *HD* mice, suggesting that neuropil aggregates may play a role in HD pathogenesis by affecting nerve terminal function (94, 156). In contrast, the NIs form primarily in the cortex but are rarely detected in the area most affected in HD, the striatum (94, 152). All huntingtin aggregates are recognized only with antibodies that are specific for the N terminus of huntingtin (65, 94). Antibodies to internal regions (e.g. amino acids 549–679) or the C terminus do not recognize the aggregates (65, 93, 238, 268). These data support an emerging theme that proteolytic cleavage and release of a toxic expanded polyglutamine protein may be an important step in pathogenesis.

A number of proteins are known to interact with huntingtin. Huntingtin-associated protein 1 (HAP1) was the first such protein isolated (159); it is interesting that the interaction strengthens with increasing polyglutamine length. Although HAP1 is brain specific, it is not particularly enriched in areas that degenerate in HD. HAP1 associates with proteins in the cytoskeleton, supporting a possible role for huntingtin in cytoplasmic protein trafficking (46, 74). Burke et al found

an interaction between huntingtin and the enzyme glyceraldehyde-3-phosphate dehydrogenase (26). If relevant to pathogenesis, this interaction supports a hypothesis that pathology in HD involves a disruption of neuronal energy production. Huntingtin also interacts with an E2-25K, a ubiquitin-conjugating enzyme (126). This interaction is not modulated by the polyglutamine length and might indicate a catabolic route for huntingtin. Huntingtin-interacting protein 1 (HIP1) colocalizes with huntingtin, and its interaction is inversely correlated to the polyglutamine chain length (127). The yeast HIP1 homolog, Sla2p, is essential for proper function of the cytoskeleton, adding further support that huntingtin may play a role in cytoskeletal function in the brain. One recent study showed a repeat-dependent interaction of N-terminal huntingtin and the nuclear receptor corepressor (N-CoR) (22). Complexing with other factors, the nuclear receptor corepressor represses transcription from ligand-activated receptors. Subcellular localization studies demonstrated relocalization of the nuclear receptor corepressor and other associated corepressors in HD brain, suggesting that altered transcriptional regulation is involved in HD (see "Nuclear Localization and Gene Expression" below). Faber et al demonstrated a repeat-length–dependent interaction with members of the WW domain family of proteins, which seem to play a critical role in a number of cellular processes such as nonreceptor signaling, channel function, protein processing, and pre-mRNA splicing (75). Cystathionine β-synthase and an SH3GL3 protein are two additional huntingtin interactors (23, 247). Although the normal function of these interactions is not yet clear, the former may have a pathological role in initiating excitotoxic cell death whereas the latter may specifically promote the aggregation of the truncated mutant protein. Thus, based on the variety of huntingtin-interacting proteins identified, huntingtin may play a role in a number of nuclear and cellular events.

Spinocerebellar Ataxia Type 1 (SCA1)

Clinicopathology The primary clinical features in SCA1 include progressive cerebellar ataxia, dysarthria, and eventual bulbar dysfunction; patients may also present with pyramidal signs and peripheral neuropathy. SCA1 is characterized pathologically by cerebellar atrophy with severe loss of Purkinje cells, dentate nucleus neurons, and neurons in the inferior olive and cranial nerve nuclei III, IV, IX, X, and XII. Neuropathologic studies also reveal gliosis of the cerebellar molecular layer and, within the internal granular layer, eosinophilic spheres or "torpedoes" are present in axons of degenerating Purkinje cells.

Mutation and Gene Product The highly polymorphic CAG repeat in the coding region of the *SCA1* gene results in the synthesis of a polyglutamine track located in the amino-terminal half of its protein product, ataxin-1. Normal alleles contain 6–44 repeats. The larger "normal" alleles (>20) are stabilized by interruptions of 1–4 CAT repeat units encoding histidine (42). Disease alleles contain a pure stretch

of uninterrupted CAG trinucleotides that range from 39 to 82 repeats (223). Large deletions in 6p22-23 spanning the *SCA1* gene do not result in ataxia, confirming that haploinsufficiency of ataxin-1 does not give rise to SCA1 (56).

Ataxin-1 is a novel protein that has a nuclear localization signal (136). The murine homolog shares high identity (89%) with human ataxin-1, but it contains only two glutamines instead of a long glutamine track (10). The wild-type protein has a predicted molecular mass of 87 kDa and is expressed in the central nervous system at two- to fourfold the levels found in peripheral tissues (148, 237). In peripheral cells, that is, heart, skeletal muscle, lymphoblasts, and liver, the protein is localized to the cytoplasm. In neurons, however, it is predominantly nuclear, with limited cytoplasmic staining in Purkinje cells and brain stem nuclei (52, 148, 237, 248). In SCA1 patients, mutant ataxin-1 localizes to a single ubiquitin-positive nuclear inclusion in brain stem neurons (248). In the cerebellum, which is the primary region affected by disease, NIs are not detected; this may be due to severe pathology in the postmortem brain, leaving few Purkinje cells behind to be visualized. It may also hint that NIs are present in the neurons, as was seen in HD, that are more likely to resist neurodegeneration.

Sca1-null mice display impaired spatial and motor learning and decreased paired-pulse facilitation in the CA1 area of the hippocampus (176). In the central nervous system, ataxin-1 may therefore have a role in synaptic plasticity and neuronal functions underlying learning. Moreover, the fact that mice that are homozygous for the null mutation do not develop ataxia confirms that SCA1 is not caused by loss of normal ataxin-1 function.

Further studies to investigate ataxin-1's normal and pathological functions led to the discovery of an ataxin-1–interacting protein called leucine-rich acidic nuclear protein (LANP) (175). The LANP–ataxin-1 interaction is strengthened in a glutamine repeat length-dependent fashion. Although the function of LANP is unknown, it belongs to the family of leucine-rich repeat proteins that mediate protein-protein interactions that are pivotal to processes as varied as morphogenesis, cell adhesion, and signaling. *LANP* is abundantly expressed in Purkinje cells, the primary site of SCA1 pathogenesis. The murine homolog, *Lanp*, is highly expressed in Purkinje cells at postnatal day 14, parallel to a transient burst of *Sca1* expression in the mouse (10, 178). Furthermore, in transfected cells, subcellular distribution of LANP is altered by mutant ataxin-1. Therefore, the tissue-specific degeneration seen in SCA1 may result from mutant ataxin-1 binding, sequestering, and interfering with the normal activity of LANP and/or other nuclear factors.

Spinocerebellar Ataxia Type 2 (SCA2)

Clinicopathology Extremely slow saccadic eye movements, hypo- or areflexia, and, in some patients, ophthalmoparesis are features that can help distinguish SCA2 from the other SCAs. The cerebellum and brain stem show degeneration

with loss of Purkinje and granule cells. Atrophy of the frontotemporal lobes, degeneration of the substantia nigra, and gliosis in the inferior olive and pons have all been reported (67, 201).

Mutation and Gene Product The CAG repeat located in the N-terminal coding region of *SCA2* is less polymorphic than the repeats in all of the 13 other diseases described; 95% of alleles contain 22 or 23 repeats (115, 213, 217, 227). Normal alleles may contain 15–31 repeats and are normally interrupted by two CAA sequences. Disease alleles contain a perfectly uninterrupted CAG repeat tract that ranges between 36 and 63 units.

The *SCA2* gene product, ataxin-2, is a novel cytoplasmic protein with a predicted molecular mass of 140 kDa. Although its function is presently unknown, ataxin-2 contains Sm1 and Sm2 motifs previously found in proteins that are involved in RNA splicing and protein-protein interaction (194). Human ataxin-2 does share sequence homology with the mouse *Sca2* gene product; however, the mouse has only one CAG unit (193). *SCA2* is widely expressed throughout the body and brain, with high levels in cerebellar Purkinje cells (110, 115, 213, 227). Ataxin-2 immunoreactivity is more intense in SCA2 brains than in normal brains, suggesting altered catabolism of the mutant protein (110). Recently, mutant ataxin-2 was shown to aggregate in ubiquitin-positive NIs, but only 1%–2% of the surviving neurons were NI positive (150). As with SCA1, the cerebellum was completely devoid of NIs despite extensive neuronal degeneration (110, 150).

Machado-Joseph Disease: Spinocerebellar Ataxia Type 3 (SCA3)

Clinicopathology Bulging eyes, facial and lingual fasciculation, and rigidity together with progressive ataxia suggest SCA3/Machado-Joseph Disease (MJD), but clinical diagnosis is hampered by variability in clinical and pathological presentation. Degeneration is most prominent in the basal ganglia, brain stem, spinal cord, and dentate neurons of the cerebellum. Purkinje cell loss is mild, and the inferior olives are typically spared (68, 203, 257, 290).

Mutation and Gene Product The polyglutamine repeat in the *SCA3/MJD1* gene product is near the C terminus. Normal individuals have between 12 and 40 glutamines, and in disease the repeat expands to a length of 55–84 glutamines. The *MJD1* gene encodes a ubiquitously expressed protein that has no known function or homology with other proteins. With a predicted molecular mass of 42 kDa, it represents the smallest protein in the polyglutamine disorders (129). The *MJD1* gene product ataxin-3 is predominantly a cytoplasmic protein, but is also known to aggregate in the nucleus of affected neurons in SCA3 patients (204, 205). Ataxin-3 is predicted to contain a nuclear localization signal (NLS) and it was reported to associate with the nuclear matrix (255). The nuclear matrix-bound ataxin-3 is

proposed to adopt an altered conformation, an event that may be significant in disease initiation (207; see below).

Spinocerebellar Ataxia Type 6

Clinicopathology Clinical features in SCA6 consist predominantly of cerebellar dysfunction (85, 123, 235, 296). The disease is characterized by very slow progressive or episodic ataxia. Neuropathologic findings in SCA6 include marked cerebellar atrophy with loss of cerebellar Purkinje cells and only moderate loss of cerebellar granule cells, dentate nucleus neurons, and neurons of the inferior olive (114, 296).

Mutation and Gene Product The CAG repeat in SCA6 is relatively stable and exceptionally small, ranging from 21 to 33 repeats in the disease state and <18 repeats on normal chromosomes (189, 235, 296). This may explain why intergenerational instability is rare in SCA6 (177). The *SCA6* gene codes for the P/Q-type calcium channel α_{1A}-subunit (*CACNA1A*). Voltage-sensitive calcium channels are multimeric complexes made of the pore-forming α_{1A} subunit and several regulatory subunits (34). These channels mediate the entry of calcium into excitable cells and thereby play roles in a variety of neuronal functions that include neurotransmitter release and gene expression. The α_{1A} subunit gene is abundantly expressed in the the central nervous system, with highest levels in the cerebellar Purkinje cells (76, 116, 163, 293). Recently, Ishikawa and colleagues (116) reported that mutant α_{1A} calcium channels aggregate in the cytoplasm of SCA6 Purkinje cells. These inclusions were found in the peripheral perikaryal cytoplasm and in the proximal dendrites but were never localized to the nucleus. The inclusions were ubiquitin negative but could be identified with both amino- and carboxy-terminal antibodies, suggesting that the full-length protein is within the aggregates.

It is presently unknown how such small CAG expansions result in neurodegeneration. A number of hypotheses can be proposed: (*a*) loss of function caused by haploinsufficiency of the polyglutamine-expanded gene product, (*b*) action of the mutant protein in a dominant negative manner, (*c*) gain of function of the mutant protein by a scenario similar to other polyglutamine disorders. It is not unreasonable to hypothesize that even a small glutamine expansion will obstruct or alter the channel's normal function. In fact, quantitative studies in tissue culture cells demonstrated that channels containing pathologically expanded repeats do have altered properties (179). It is interesting that different mutations in this gene produce variable phenotypic effects. Missense and splicing mutations in the α_{1A} voltage-dependent calcium channel have been identified in other neurological disorders, including hereditary paroxysmal cerebellar ataxia (or episodic ataxia type 2) and familial hemiplegic migraine (199). Additionally, the tottering and tottering-leaner mice bear nonexpansion mutations in this subunit (76, 199). The generation and

analysis of transgenic mice that express an allele with pathologically expanded repeats will undoubtedly help clarify the mode of pathogenesis in SCA6.

Spinocerebellar Ataxia Type 7

Clinicopathology SCA7 patients initially present with cerebellar ataxia and/or visual problems, including pigmentary macular degeneration. The minority of patients who first show only visual deficits will likely develop ataxia within a few years. Onset in early childhood or infancy has an especially rapid and severe clinical course, whereas the infantile phenotype is unique in affecting neuronal and non-neuronal tissue (heart) (15, 55, 124). Primary neuronal loss is in the cerebellum, inferior olive, and some cranial nerve nuclei. Hypomyelination of the optic tract and gliosis of the lateral geniculate body and visual cortex are also characteristics of SCA7 (55, 172).

Mutation and Gene Product The polyglutamine tract is near the amino terminus of the *SCA7* gene product, ataxin-7. All normal alleles contain pure CAG tracts without any evidence of interruption (typically 4–35 repeats), whereas adult-onset disease occurs when the repeats exceed 37 (15, 54, 55, 62, 89, 124, 189). The SCA7 mutation is the most unstable in the polyglutamine disease family (54, 252). Moreover, SCA7 manifests the most extreme intergenerational CAG repeat instability, with an enlargement of 263 repeats reported in one father-to-son transmission (15). As with most other triplet repeat diseases, the repeat expansion in SCA7 is markedly associated with paternal transmission. Disease, however, is more frequently observed upon maternal transmission, suggesting that paternally transmitted alleles may be embryonically lethal (15, 89, 185).

The function of ataxin-7 is unknown, and it shares no significant homology with other proteins. It does contain a functional NLS consensus sequence and nuclear and nucleolar localization, and it can bind to the nuclear matrix (54, 130). Cytoplasmic staining in neurons of several brain regions has also been reported (105). *SCA7* shows ubiquitous expression, with the highest levels in the heart, placenta, skeletal muscle, and pancreas. Within the central nervous system, the *SCA7* transcript is most abundant in the cerebellum (54). In SCA7 patients, ataxin-7–positive nuclear aggregates were identified in affected brain regions (brainstem, especially pons and inferior olive), and, in one juvenile-onset patient, they were also found in brain regions not associated with disease (cerebral cortex) (105). The degree of ubiquitin reactivity in the NIs varied spatially and may be correlated with the sites of neuronal degeneration (<1% ubiquitin positive in cortex vs 60% in inferior olive).

Dentatorubropallidoluysian Atrophy (DRPLA)

Clinicopathology Distinguishing clinical characteristics of DRPLA include choreoathetosis, myoclonic epilepsy, dementia, and progressive intellectual

deterioration in juvenile-onset cases (113, 280). The neuropathology of DRPLA is quite extensive, with marked neuronal loss in the cerebral cortex, cerebellar cortex, globus pallidus, striatum, and the dentate, subthalamic, and red nuclei. There is intense gliosis and severe demyelination at many of the sites of neuronal degeneration, and in some families calcification of the basal ganglia is also observed (27, 249, 256).

Mutation and Gene Product The glutamine repeat in DRPLA is found near the C terminus of the *DRPLA* gene product, atrophin-1. The normal repeat range is 6–35 copies; expanded alleles have 49–88 repeats (143, 223). Atrophin-1 is widely expressed and is primarily localized to the cytoplasm in neurons and peripheral cells (140, 291). Atrophin-1 does contain a putative NLS, and nuclear localization has been reported (111, 184, 197).

The function of atrophin-1 is not yet known. Similar to huntingtin, ataxin-1, and AR, atrophin-1 was found to associate with glyceraldehyde-3-phosphate dehydrogenase (26, 147). As discussed above, the significance of the interaction is still not clear. Wood et al identified five putative atrophin-1 interacting proteins (289). Similar to huntingtin, all five interactors contain WW domains. Two of these proteins are novel. The three others contain a HECT domain characteristic of E3-ubiquitin ligases and appear to be closely related to family members of the Nedd-4 ubiquitin ligases. No repeat length dependencies were observed for any of these proteins, suggesting that the physiological importance of these interactions may be more salient to the normal function or catabolism of atrophin-1 rather than its direct role in pathogenesis. Ohamura-Oho and colleagues (197) showed an interaction between atrophin-1 and an insulin receptor tyrosine-kinase substrate, suggesting that atrophin-1 might function in the insulin/insulin growth factor signal transduction pathways. It is interesting that the binding appears to be negatively affected by glutamine expansion (197).

PATHOGENETIC MECHANISMS IN POLYGLUTAMINE DISORDERS

In all of the polyglutamine disorders, disease is more severe with longer repeats, and the mutant proteins are only damaging to a certain population of neurons. A model of pathogenesis posits that the expanded glutamine repeat mediates some undefined toxic gain of function that results in neuronal dysfunction and death. To bring form to this model, a number of points and paradoxes are discussed: (*a*) Is the expansion of the glutamine tract necessary to cause disease? (*b*) What is the role of the glutamine tract in disease? and (*c*) Is pathogenesis solely dependent on the expanded polyglutamine tract or are other sequences in the protein necessary for neurodegeneration?

Importance of the Glutamine Tract in Disease

Studies of mouse models have been the most compelling to show the importance of polyglutamine expansion in causing disease. The first transgenic mice generated to model a polyglutamine disorder were those that overexpressed the human *SCA1* cDNA encoding full-length ataxin-1 (28). Using the regulatory elements of the Purkinje cell-specific promoter from the *Pcp2* gene, lines were made to express high levels of either wild-type ataxin-1 with 30 repeats or mutant ataxin-1 with 82 repeats (82Q). The 82Q-transgenic mice developed severe ataxia and progressive Purkinje cell pathology, whereas mice expressing ataxin-1 with 30 repeats displayed no neurologic abnormalities and were indistinguishable from nontransgenic littermates (43). Mice from one of the 82Q expressing lines (termed B05) have been extensively characterized. These mice developed mild motor skill impairment that progressed to overt ataxia (43). A number of progressive pathological changes develop in these mice before Purkinje cell death, including the formation of Purkinje cell cytoplasmic vacuoles and nuclear aggregates, reduction in the Purkinje cell dendritic arborization, atrophy of the molecular layer, and heterotopic migration of Purkinje cells. These findings challenged the long-lived assumption that neurological phenotypes in SCA patients result from neuronal death; instead, neurological impairment is likely caused by neuronal dysfunction. These studies also demonstrated that pathological changes are induced by the expression of ataxin-1 with an expanded polyglutamine tract, which was very encouraging because it confirmed that mice could be used to model some of the phenotypes of a slow progressive polyglutamine disorder, despite the confines of a much shorter life span compared with humans. It is noteworthy that the severity and disease progression were dependent on transgene expression levels. This was the first clue that not only glutamine length is significant in disease, but also the amount of toxic protein and the time a neuron is exposed to the mutant protein. The question of whether pathogenesis is solely dependent on the expanded glutamine tract was left unanswered.

Studies in mouse models of SCA3 and HD were the first to address this question (112, 171). Ikeda and colleagues generated transgenic mice expressing full-length and truncated versions of the MJD/SCA3 protein, also using the *Pcp-2* promoter (112). Animals expressing truncated fragments of ataxin-3 with 79 repeats developed overt ataxia. In contrast, none of the mice expressing full-length ataxin-3 with 79 repeats or a truncated ataxin-3 with 35 repeats developed ataxia. These data tell us that the expanded polyglutamine tract alone is sufficient to induce neuronal cell death. Without information about the relative expression levels in the various transgenic lines, it is difficult to make conclusions regarding the toxicity of full-length ataxin-3. Based on the available data, however, the authors proposed that cell-specific proteolytic cleavage of the mutant protein liberates an elongated polyglutamine tract that then induces cell death. Assuming that this protease is lacking in Purkinje cells, a cell type not primarily affected in SCA3, then the toxic

fragment would not be produced, and these neurons would be spared. Consistent with this model is the increasing evidence that generation of truncated proteins containing an expanded polyglutamine tract may be a key step in pathogenesis (72, 73, 88, 226, 283; see below).

In the first mouse model for HD, Mangiarini and colleagues generated transgenic mice expressing a truncated huntingtin fragment with 144 repeats (171). Under the control of the *HD* promoter, these mice ubiquitously expressed a polypeptide that included the first 69 amino acids of huntingtin and an expanded glutamine tract. At lower-than-endogenous levels, these mice showed a progressive neurological phenotype. Mice expressing the same peptide with 18 repeats, however, are indistinguishable from nontransgenic controls. One transgenic line extensively studied, the R6/2 line, appear normal at weaning, but gradually develop an irregular gait, resting tremor, stereotypic grooming activity, sudden shuddering movements, and occasional seizures. The neurological phenotypes are accompanied by a progressive decline in body weight, urinary incontinence, sterility, alterations in brain neurotransmitter receptors, and insulin-responsive diabetes (36, 109, 171). Although the diabetes may account for some of the phenotypic features in these mice, such as weight loss, it is unlikely to be the primary cause of the neurological phenotype. It is interesting that, despite the prominent neurological findings in these mice, there is no indication of neuronal degeneration except for diminished brain weights. The R6/2 mice die between 10 and 13 weeks and conceivably don't live long enough to develop the typical HD pathology. These data have been strengthened by similar findings in mice expressing N-terminal huntingtin with 82Q under the strong and ubiquitous prion promoter (234). Together these studies indicate that a small huntingtin fragment with an expanded glutamine tract is toxic to neurons. Additionally, the toxic peptide alone is sufficient to create progressive neurological phenotypes that are quite similar to those in human HD, even when this peptide is expressed at lower-than-endogenous levels. Are these phenotypes merely the result of generalized polyglutamine toxicity, or is the mechanism of degeneration in these neurons a true model of the events that occur in patients?

These questions were partially answered by Ordway and colleagues, who "knocked-in" 146-CAG repeats into a gene that codes for a protein previously not harboring a polyglutamine tract, the mouse hypoxanthine phosphoribosyl transferase (*Hprt*) (200). Similar to the *HD* transgenics, these mice developed seizures, tremors, and motor dysfunction, and they died prematurely. In addition, graded neuropathological abnormalities were evident. The selective neuronal loss that is characteristic of HD, however, is not seen in the HPRT mouse. Thus several lines of compelling evidence confirm that the expanded glutamine tract is toxic. Moreover, the polyglutamine tract is toxic irrespective of the protein context. What is additionally clear from these studies is that the selective pattern of neuropathology in these diseases must be mediated by the amino acid sequence surrounding the polyglutamine tract.

Importance of Protein Context

The importance of protein context is emphasized by several other studies of mice that were engineered to express either full-length *HD* or *DRPLA* with an expanded number of CAG repeats. In the first study, Reddy and colleagues (215) reported that transgenic mice with an expanded CAG tract under the control of the cytomegalovirus promoter developed progressive behavioral abnormalities, urinary incontinence, and decreased locomotor activity. The hypokinesia was accompanied by striatal neuronal loss and gliosis in a pattern similar to that seen in HD patients. Neuronal loss also occurred in the hippocampus, thalamus, and cerebral cortex. Despite widespread expression of the transgene, neuronal degeneration was identified only in areas typically affected in HD. In the second study, Hodgson et al developed a YAC-transgenic mouse model expressing full-length HD with 72 CAG repeats under the control of the endogenous huntingtin promoter (103). These mice became hyperactive and developed progressive electrophysiological dysfunction and degeneration of the medium spiny neurons in the lateral striatum, reminiscent of that see in HD patients. Two other studies of knock-in HD mice, a model that most closely resembles the mutation in humans, demonstrated that mice with an expansion of 72–80 CAG repeats introduced into the *Hd* locus develop behavioral abnormalities and impaired synaptic plasticity (240, 271). These phenotypic changes are similar to some findings in HD patients; however, they arise without any signs of neurodegeneration or NI formation, further supporting a distinction between neuronal dysfunction and cell death. Finally, Sato and colleagues described an animal model for DRPLA (229, 230). Transgenic mice harboring a single copy of a full-length human *DRPLA* gene with 76 CAG repeats and its own promoter were generated to study the molecular mechanisms of CAG repeat instability. Although these mice showed no obvious phenotypes, the authors identified a mosaic mouse that carried mutant DRPLA genes with ~129 and 76 CAG repeats. The hemizygous transgenic offspring expressing atrophin-1 (129 repeats) showed progressive myoclonic movements, epilepsy, and decrease in brain weight, a phenotype quite similar to that seen in juvenile human DRPLA patients. That the mice expressing 76 repeats did not manifest symptoms, whereas those harboring the longer repeat did, stresses the relationship between glutamine repeat length and progression of disease; the life span of the mouse is not sufficient to manifest disease with full-length atrophin-1 (76 repeats) when expressed at endogenous levels. Moreover, these findings, together with data from the full-length *HD* transgenics, demonstrate that selective neuronal loss occurs only when the full-length protein with an expanded glutamine tract is expressed, again suggesting that protein context helps mediate this selective pattern of degeneration.

In summary, expansion of the glutamine repeat is necessary to cause disease, and the expanded glutamine tracts alone are extremely toxic. Transgenic mice expressing full-length huntingtin develop phenotypes that are much slower and milder than those developed by the truncated *HD* transgenic mice, even though the latter have much lower levels of transgene expression. This could be explained

by the smaller size of the peptide fragments, which are more insoluble and prone to aggregate or are more readily transported into the nucleus where they can wreak havoc. The larger proteins may first need to be cleaved before aggregation or nuclear import; this is an event requiring contextual clues surrounding the glutamine repeats as well as interacting proteins. Thus full-length proteins temper the toxicity of the expanded glutamine tract and may also function through protein-protein interactions that could result in a selective pattern of neurodegeneration.

Features of Polyglutamine Tracts That May Render Them Toxic

What are the features inherent in long polyglutamine tracts that might render them and/or the proteins harboring them toxic to neurons? Green proposed that the extended polyglutamine tract is a transglutaminase (tTG) substrate that can become cross-linked via ε-(γ-glutamine) lysine isopeptide bonds (90). The cross-linked products will contain multimers of the polyglutamine proteins as well as other copolymers, leading to the formation of aggregates. Studies in vitro have shown that polyglutamine repeat peptides and the polyglutamine tract within huntingtin are substrates for tTG (48, 125). It has yet to be determined, however, whether transglutamination of polyglutamines occurs in vivo or is even significant in the pathology of these diseases. Several reports recently showed that tissue transglutaminase activity is increased in HD brain (128, 155). Moreover, in cell culture models of DRPLA, tTG inhibition decreased atrophin-1 polymerization and apoptotic cell death (111). Perutz et al proposed an alternate mechanism to explain how polyglutamine repeats might contribute to disease based on the ability of polyglutamine repeats to organize into polar zippers and then aggregate. In this model, antiparallel β-strands of polyglutamine repeats can be linked by hydrogen bonds and undergo multimerization (209). In support of this model, synthetic peptides containing glutamine repeats, shorter than the pathogenic threshold, formed polar zippers that aggregate in solution (253). Scherzinger et al demonstrated that an amino-terminal fragment of huntingtin can aggregate in vitro, forming amyloidlike fibrils (233). They further demonstrated that there was a threshold for aggregation that correlates well with HD. However, the addition of glutathione S-transferase protein to the amino-terminal huntingtin fragment ($>$36 repeats) prevented aggregation, but only as long as the fusion protein was not proteolytically cleaved from the huntingtin polypeptide. These data, together with the finding that full-length huntingtin with an expanded glutamine tract does not aggregate spontaneously in vitro, suggest that, if aggregation of the polyglutamine tract is to occur, it has to first be cleaved from huntingtin. (See "Cleavage, Caspase Activity, and Apoptosis" below.)

Protein Aggregation and Nuclear Inclusions

Does the glutamine expansion in the disease-causing proteins destabilize their native conformations, resulting in altered protein folding and aggregation? The

first evidence for this came from the identification of antibodies that preferentially recognize the expanded but not the wild-type protein (268). This was followed by a series of studies of animal models, cell-culture models, and patient tissues that demonstrated that mutant polyglutamine proteins aggregate in NIs and cytoplasmic inclusions. These aggregates were originally identified in the nucleus of neurons from a mouse model of HD and subsequently in HD brains (57, 65, 233). Soon after, the aggregates were identified in the nucleus of affected neurons in SCA3 and SCA1 patient brains as well as *SCA1* transgenic mice (205, 248). Subsequently, they have been identified in postmortem tissue from SBMA (157), SCA2 (150), SCA6 (116), SCA7 (105), and DRPLA (13). All of the inclusions (with the exception of the aggregates in SCA6) are localized to the nucleus and stain positively for ubiquitin, indicating that the proteins are misfolded and targeted for hydrolysis. In addition, protein aggregates have been reported in the neuropil of HD brains and transgenic mice (94, 156). The nuclear and cytoplasmic aggregates have been proposed to cause aberrant protein-protein interactions that affect either nuclear architecture or the normal subcellular distribution and/or function of interacting proteins.

The discovery of the NIs was intriguing because it suggested a common pathogenic mechanism for all polyglutamine disorders. The initial evidence that NIs are present only in vulnerable neurons, which once offered a clue to selective vulnerability, is however not holding true. As described above, the regional distribution of the NIs in SBMA, HD, SCA2, and SCA7 patients does not always correspond to the selective pathology. Studies in animal models also strongly indicate that NIs are neither necessary nor sufficient for neuronal dysfunction. First, the R6/2 *HD* mice have massive numbers of NIs in neuronal and non-neuronal (e.g. muscle) cells, yet show no signs of neurodegeneration, neuropathy, or myopathy—only decreases in tissue mass (57, 228). In full-length *HD* transgenic mice, NIs were detected in several regions, including those typically unaffected by the disease (e.g. Purkinje cells); paradoxically, the frequency of the inclusions was <1% in the striatum, where the neuronal loss was most prominent (103, 215). Most convincingly, Klement and colleagues generated transgenic mice by using ataxin-1 (77 repeats) with amino acids that were deleted from the self-association region found to be essential for ataxin-1 dimerization (136). These mice developed ataxia and Purkinje cell pathology that were similar to the original *SCA1* (82Q) mice, but without apparent nuclear ataxin-1 aggregation. Therefore, we can conclude that visible nuclear aggregates are not necessary for the initiation of polyglutamine diseases in vivo.

Nuclear Localization and Gene Expression

Although nuclear aggregation is not necessary to induce neurodegeneration, nuclear localization of the mutant proteins may be a critical event in polyglutamine disease. The importance of nuclear localization of mutant ataxin-1 has been clearly demonstrated in a transgenic mouse model for SCA1. Klement et al generated

transgenic mice that overexpress expanded ataxin-1 (82Q) with a mutated NLS (136). Although these mice had abundant levels of cytoplasmic mutant ataxin-1, they never developed ataxia or pathology. In cell culture models of HD, the addition of a nuclear export signal to mutant huntingtin forces it to localize to the cytoplasm and thereby significantly suppress aggregation and cell death; the opposite effect is seen with the addition of an NLS (210, 231).

Several lines of evidence suggest that the expanded polyglutamine proteins, once inside the nucleus, may have toxic effects on gene expression. For example, overexpression of full-length ataxin-1 caused the protein to localize to the nucleus and form nuclear-matrix–associated aggregates that redistribute the promyelocytic oncogenic domains (thought to be important in transcriptional regulation) (248). Similarly, overexpression of ataxin-7 in transfected cells resulted in nuclear localization, nuclear-matrix association, and colocalization with a component of the promyelocytic oncogenic domain, promyelocytic leukemia protein (130). Full-length AR with 48 repeats accumulates in a hormone-dependent manner in both cytoplasmic and nuclear aggregates, and these aggregates caused redistribution of the AR-associated steroid receptor coactivator (SRC1), suggesting potential pathological effects on SRC1-mediated transcription pathways (251). Perez et al demonstrated that NIs can sequester other polyglutamine-containing proteins, such as the TATA-binding protein (206). This finding is intriguing especially in light of a recent report describing a patient with cerebellar ataxia and mental deterioration who has a de novo expansion of the CAG repeat of the *TBP* gene (144). Moreover, a polyglutamine tract-binding domain protein (PQBP-1) was identified in a yeast two-hybrid screen and was shown to interact with the glutamine tract in transcription factors and polyglutamine repeat disease proteins; this interaction is strengthened in a length-dependent manner and may affect cell survival in vitro (279). Finally, Lin and colleagues demonstrated that certain neuronal genes involved in signal transduction and calcium homeostasis were specifically downregulated by the expression of expanded ataxin-1 in SCA1 transgenic mice. It is important that the alterations in gene expression required nuclear localization of mutant ataxin-1 and occurred before any detectable pathology (162). Together these results suggest that regulation of gene expression may be an important factor in the pathogenesis of polyglutamine disease—perhaps the earliest molecular player.

Cleavage, Caspase Activity, and Apoptosis

Nuclear localization and aggregation of many of the disease proteins becomes apparent in cell culture only after the expression of truncated forms of these expanded proteins (29, 95, 111, 112, 166, 173, 180, 205). This suggests that, for some polyglutamine proteins, processing of the full-length protein may be necessary to liberate a peptide, which can then translocate to the nucleus and exert its toxic effect. In support of this suggestion, proteolytic fragments have been detected with full-length expanded huntingtin and AR (88, 166, 180). Additionally, studies show

that huntingtin, AR, atrophin-1, and ataxin-3 are substrates for one or more caspases, although the CAG length does not modulate cleavage of any these proteins (283). Amino-terminal huntingtin fragments containing the expanded polyglutamine tract have also been detected in HD brain nuclear extracts, suggesting that huntingtin processing may occur in vivo (65).

How is the cleavage of huntingtin or other polyglutamine proteins involved in pathogenesis? Is the cleavage event necessary to release a toxic, sticky peptide that eventually leads to cell death? Sanchez and colleagues recently suggested a novel mechanism of caspase activation and apoptotic cell death based on the inherent glutinous nature of these proteins (226). These authors proposed that aggregates of polyglutamine repeat proteins can sequester one or more procaspases, which become activated and set off the apoptotic pathway. Partial support for this model comes from the identification of activated caspase-8, found uniquely in the insoluble fraction of affected HD brain extracts but not in controls (226). Additionally, preventing caspase-8–mediated apoptosis inhibited the polyglutamine-induced cell death in primary neurons and cell lines. Along the same lines, Ona et al demonstrated that caspase-1 is activated in HD brains and transgenic mice and that inhibiting its activity reduced endogenous huntingtin cleavage in HD mice (R6/2) (198). This inactivation also slowed the progression of several of the pathological features in this animal model, even though the transgenic construct lacks the known caspase-1 cleavage site. These results raise the possibility that caspase activity has a role in polyglutamine disease progression; however, the underlying mechanism is not clear at this time. Is caspase cleavage of the polyglutamine protein directly involved in disease—or do the polyglutamine polymers act, indirectly, as docking sites for procaspases that in turn trigger apoptosis when sequestered together into aggregates? Does the apoptotic pathway play a role in neuronal dysfunction, or is it the result of a dysfunctional neuron that can no longer cope with increasing amounts of a toxic protein? Future studies will need to reconcile how these intriguing findings relate to the progressive and long-term degeneration seen in patients, which does not always resemble classical apoptosis. Moreover, the formation of large insoluble aggregates does not necessarily correlate with the primary sites of pathology in patients, and, in transfected cells, polyglutamine-induced cell death does not always correlate with aggregate formation (152, 231, 244).

Protein Misfolding and the Ubiquitin Proteasome Pathway

That the NIs are ubiquitin positive raises the possibility that the proteolytic pathway in these neurons might be altered. In addition, if a crucial problem in these diseases is the misfolding and perturbed catabolism of polyglutamine proteins, one might expect to see the affected neurons mounting a chaperone stress response. The first clue that this might be the case came from the observation that components of the 26S proteasome and the molecular chaperones HDJ-2/HSDJ and Hsp70 were redistributed to the sites of ataxin-1 aggregation in SCA1 patient tissue and transgenic mice (52). Similarly, the proteasome and molecular

chaperones colocalized with nuclear aggregates that formed in ataxin-1–transfected tissue culture cells. A marked induction of Hsp70 was seen in the cells harboring the larger ataxin-1 aggregates, suggesting that these cells were mounting a chaperone stress response to the presence of this toxic protein. When overexpressed, the HDJ-2/HSDJ chaperone caused a decrease in both the size and frequency of the ataxin-1 nuclear aggregates. Similar results in cell culture have now been reported with mutant AR and ataxin-3 (37, 251). Chai and colleagues extended the observations by demonstrating a concomitant decrease in toxicity with reduced aggregation (37). Proteasome components and molecular chaperones have now also been identified in the NIs found in SCA3 brains and in *HD* (R6/2) and *SBMA* mice (37, 38, 58, 181). Because molecular chaperones have clearly been implicated in protein folding, ubiquitin-dependent degradation, and suppression of protein aggregation (101, 102), we might infer that the cellular levels of a chaperone could directly mediate the aggregation and/or turnover of a mutant polyglutamine protein.

The presence of ubiquitinated aggregates that contain chaperones and the proteasome supports the view that the glutamine expansion causes the mutant proteins to misfold and subsequently be targeted for degradation by the ubiquitin-proteasome pathway. Expanded polyglutamine proteins may adopt energetically stable structures (as described above), which resist unfolding and thus impede proteasomal degradation. Misfolded and potentially with extended half-lives, these proteins may interact with other proteins in an aberrant fashion and thereby trigger tissue-specific degeneration. If the capacity of the intracellular system handling these proteins is exceeded, then the NIs may develop and cause redistribution of the proteasome to the NIs; proteasome redistribution may contribute to disease progression by disturbing proteolysis and subsequent vital cellular functions. This model is supported by a number of in vitro and in vivo studies. First, polyglutamine-expanded ataxin-1 is considerably more resistant to ubiquitin-mediated degradation in vitro (53). Second, proteasome inhibitors promote aggregation of mutant ataxin-1 and ataxin-3 in cell culture, suggesting proper proteasomal function is important for handling expanded polyglutamine proteins (38, 53). Third, the ubiquitination of mutant huntingtin may play a role in pathogenesis because the expression of a dominant negative ubiquitin-conjugating enzyme in cell culture inhibited the formation of NIs while accelerating huntingtin-induced cell death (231). Finally, to examine the role of the ubiquitin-proteasome pathway in vivo, Cummings et al (53) crossed the *SCA1* transgenic mice with mice that had a targeted deletion of the gene coding for an E3-ubiquitin-protein ligase, *Ube3A* (122). As was seen in tissue culture with the dominant negative ubiquitin-conjugating enzyme, Purkinje cells from the *SCA1/Ube3a* mice had significantly fewer NIs. Nonetheless, the polyglutamine-induced pathology is markedly worse and more rapid in progression in the *SCA1/Ube3a* mice compared with the *SCA1* control littermates (53). One interpretation for these data is that mutant polyglutamine proteins are more toxic if not properly ubiquitinated, turned-over, or possibly sequestered to an NI. These results demonstrate that

impaired proteasomal degradation of mutant polyglutamine proteins may contribute to pathogenesis, but more important, the NIs are not necessary to induce neurodegeneration.

Potential Avenues for Therapy

In light of a possible role for apoptosis in polyglutamine diseases, one avenue of therapy could be the inhibition of caspases. If apoptosis is a downstream event, however, then this would not be an effective treatment, because it would merely prolong the lives of dysfunctional neurons. In addition, given the pleiotropic effects of many caspase inhibitors, it may be necessary to specifically deliver these agents only to those specific cells that are vulnerable to disease; at present this is a formidable task.

In affected neurons, molecular chaperones may be targeted to the NI in an ultimately unsuccessful attempt to maintain the proteins in a conformation that promotes either their refolding or their modification by the ubiquitinating enzymes and subsequent hydrolysis by the 26S proteasome. By overexpressing the chaperones in cultured cells, however, it is possible to augment a cellular response to the presence of misfolded polyglutamine repeat proteins, thereby curbing aggregate formation, accumulation, and toxicity. The present challenge is to confirm that these modifications seen in cell culture will occur, without any adverse effects, in an animal model.

Very encouraging are the recent studies in *Drosophila* animal models for polyglutamine disease. Two *Drosophila* models expressing peptides with expanded polyglutamine tracts have been reported. Warrick and colleagues expressed a C-terminal fragment of ataxin-3 with various repeats in the eye, resulting in high expression of truncated ataxin-3 in the photoreceptor cells (282). Flies expressing mutant ataxin-3 (78 repeats) showed retinal degeneration and developed NIs. Jackson et al expressed an N-terminal fragment of *HD* also with various size repeats in *Drosophila*, by directing fusion of any eye-specific promoter to the huntingtin peptide (117). Photoreceptor neurons expressing these expanded peptides also degenerated and showed numerous nuclear aggregates. In both studies, the age of onset and severity of the phenotype correlated with glutamine repeat length. Both groups also evaluated the effect of the viral antiapoptotic protein P35 on photoreceptor degeneration. With overexpression of P35, some of the mild phenotypes in MJD-Q78 flies were partially rescued, but no protective effect on neuronal degeneration was detected in either model. These models are also invaluable tools to search for genetic modifiers of the polyglutamine-induced degenerative phenotype. Warrick and colleagues recently showed that a dominant negative form of a fly Hsp70 enhances degeneration in the MJD (78-repeat) flies (281). More exciting was the observation that overexpression of Hsp70 suppresses polyglutamine-induced neurodegeneration. It is interesting that this suppression occurred without any effect on NI formation, arguing further against a correlation between polyglutamine-induced toxicity and NI formation.

Notwithstanding the evidence that indicates that polyglutamine-induced toxicity is dissociated from the formation of NIs, it is striking that, in addition to all of the polyglutamine diseases, many other neurodegenerative diseases such as Alzheimer, amyotrophic lateral sclerosis, prion disease, and Parkinson disease involve protein misfolding and aggregation (265). Certainly, the near future will bring a better understanding of how the mutations in polyglutamine diseases, as well as other "proteinopathies," affect protein cleavage, turnover, and protein-protein interactions. These results will provide a platform that, together with studies aimed at enhancing proper folding and degradation, will be invaluable to slow or prevent the disease process.

LITERATURE CITED

1. Abrams MT, Reiss AL, Freund LS, Baumgardner TL, Chase GA, Denckla MB. 1994. Molecular-neurobehavioral associations in females with the fragile X full mutation. *Am. J. Med. Genet.* 51:317–27
2. Alwazzan M, Newman E, Hamshere MG, Brook JD. 1999. Myotonic dystrophy is associated with a reduced level of RNA from the DMWD allele adjacent to the expanded repeat. *Hum. Mol. Genet.* 8: 1491–97
3. Amack JD, Paguio AP, Mahadevan MS. 1999. Cis and trans effects of the myotonic dystrophy (DM) mutation in a cell culture model. *Hum. Mol. Genet.* 8:1975–84
4. Ambrose HJ, Byrd PJ, McConville CM, Cooper PR, Stankovic T, et al. 1994. A physical map across chromosome 11q22-q23 containing the major locus for ataxia telangiectasia. *Genomics* 21:612–19
5. Anvret M, Ahlberg G, Grandell U, Hedberg B, Johnson K, Edstrom L. 1993. Larger expansions of the CTG repeat in muscle compared to lymphocytes from patients with myotonic dystrophy. *Hum. Mol. Genet.* 2: 1397–400
6. Arbizu T, Santamaria J, Gomez JM, Quilez A, Serra JP. 1983. A family with adult spinal and bulbar muscular atrophy, X-linked inheritance and associated testicular failure. *J. Neuro. Sci.* 59:371–82
7. Ashizawa T, Dubel JR, Harati Y. 1983. Somatic instability of CTG repeat in myotonic dystrophy. *Neurology* 43:2674–78
8. Ashley CT Jr, Wilkinson KD, Reines D, Warren ST. 1993. FMR1 protein: conserved RNP family domains and selective RNA binding. *Science* 262:563–66
9. Babcock M, de Silva D, Oaks R, Davis-Kaplan S, Jiralerspong S, et al. 1997. Regulation of mitochondrial iron accumulation by Yfh1p, a putative homolog of frataxin. *Science* 276:1709–12
10. Banfi S, Servadio A, Chung M-y, Capozzoli F, Duvick LA, et al. 1996. Cloning and developmental expression analysis of the murine homolog of the spinocerebellar ataxia type 1 gene (*Sca1*). *Hum. Mol. Genet.* 5:33–40
11. Bardoni B, Schenck A, Mandel JL. 1999. A novel RNA-binding nuclear protein that interacts with the fragile X mental retardation (FMR1) protein. *Hum. Mol. Genet.* 8:2557–66
12. Bartolo C, Mendell JR, Prior TW. 1998. Identification of a missense mutation in a Friedreich's ataxia patient: implications for diagnosis and carrier studies. *Am. J. Med. Genet.* 79:396–99
13. Becher MW, Kotzuk JA, Sharp AH, Davies SW, Bates GP, et al. 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.* 4:387–97
14. Bell MV, Hirst MC, Nakahori Y, MacKinnon RN, Roche A, et al. 1991. Physical mapping across the fragile X: hypermethylation and clinical expression of the fragile. *Cell* 64:861–66
15. Benton CS, de Silva R, Rutledge SL, Bohlega S, Ashizawa T, Zoghbi HY. 1998. Molecular and clinical studies in SCA-7 define a broad clinical spectrum and the infantile phenotype. *Neurology* 51:1081–86
16. Bhagwati S, Ghatpande A, Leung B. 1996. Identification of two nuclear proteins which bind to RNA CUG repeats: significance for myotonic dystrophy. *Biochem. Biophys. Res. Commun.* 228:55–62
17. Bhagwati S, Ghatpande A, Leung B. 1996. Normal levels of DM RNA and myotonin protein kinase in skeletal muscle from adult myotonic dystrophy (DM) patients. *Biochim. Biophys. Acta* 1317: 155–77
18. Bidichandani S, Ashizawa T, Patel PI. 1997. Atypical Friedreich ataxia caused by compound heterozygosity for a novel missense mutation and the GAA triplet-repeat expansion. *Am. J. Hum. Genet.* 60:1251–56
19. Bidichandani S, Ashizawa T, Patel PI. 1998. The GAA triplet-repeat expansion in Friedreich ataxia interferes with transcription and may be associated with an unusual DNA structure. *Am. J. Hum. Genet.* 62:111–21
20. Biondi A, Rambaldi A, Rossi V, Elia L, Caslini C, et al. 1993. Detection of ALL-1/AF4 fusion transcript by reverse transcription-polymerase chain reaction for diagnosis and monitoring of acute leukemias with the t(4;11) translocation. *Blood* 82:2943–47
21. Boucher CA, King SK, Carey N, Krahe R, Winchester CL, et al. 1995. A novel homeodomain-encoding gene is associated with a large CpG island interrupted by the myotonic dystrophy unstable (CTG)n repeat. *Hum. Mol. Genet.* 4:1919–25
22. Boutell JM, Thomas P, Neal JW, Weston VJ, Duce J, et al. 1999. Aberrant interactions of transcriptional repressor proteins with the Huntington's disease gene product, huntingtin. *Hum. Mol. Genet.* 8: 1647–55
23. Boutell JM, Wood JD, Harper PS, Jones AL. 1998. Huntingtin interacts with cystathionine beta-synthase. *Hum. Mol. Genet.* 7:371–78
24. Brook JD, McCurrach ME, Harley HG, Buckler AJ, Church D, et al. 1992. Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3′ end of a transcript encoding a protein kinase family member. *Cell* 68:799–808
25. Brown WT. 1996. The FRAXE Syndrome: Is it time for routine screening? *Am. J. Hum. Genet.* 58:903
26. Burke JR, Enghild JJ, Martin ME, Jou Y-S, Myers RM, et al. 1996. Huntingtin and DRPLA proteins selectively interact with the enzyme GAPDH. *Nat. Med.* 2:347–50
27. Burke JR, Wingfield MS, Lewis KE, Roses AD, Lee JE, et al. 1994. The Haw River syndrome: dentatorubropallidoluysian atrophy (DRPLA) in an African-American family. *Nat. Genet.* 7:521–24
28. Burright EN, Clark HB, Servadio A, Matilla T, Feddersen RM, et al. 1995. SCA1 transgenic mice: a model for neurodegeneration caused by an expanded CAG trinucleotide repeat. *Cell* 82: 937–48
29. Butler R, Leigh PN, McPhaul MJ, Gallo JM. 1998. Truncated forms of the androgen receptor are associated with polyglutamine expansion in X-linked spinal and bulbar muscular atrophy. *Hum. Mol. Genet.* 7:121–27
30. Buxton J, Shelbourne P, Davies J, Jones C, Van Tongeren T, et al. 1992. Detection of an unstable fragment of DNA specific to individuals with myotonic dystrophy. *Nature* 355:547–48

31. Campuzano V, Montermini L, Lutz Y, Cova L, Hindelang C, et al. 1997. Frataxin is reduced in Friedreich ataxia patients and is associated with mitochondrial membranes. *Hum. Mol. Genet.* 6:1771–80
32. Campuzano V, Montermini L, Molto MD, Pianese L, Cossee M, et al. 1996. Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. *Science* 271:1423–27
33. Carango P, Noble JE, Marks HG, Funanage VL. 1993. Absence of myotonic dystrophy protein kinase (DMPK) mRNA as a result of a triplet repeat expansion in myotonic dystrophy. *Genomics* 18:340–48
34. Catterall WA. 1995. Structure and function of voltage-gated ion channels. *Annu. Rev. Biochem.* 64:493–531
35. Ceman S, Brown V, Warren ST. 1999. Isolation of an FMRP-associated messenger ribonucleoprotein particle and identification of nucleolin and the fragile X-related proteins as components of the complex. *Mol. Cell Biol.* 19:7925–32
36. Cha JH, Kosinski CM, Kerner JA, Alsdorf SA, Mangiarini L, et al. 1998. Altered brain neurotransmitter receptors in transgenic mice expressing a portion of an abnormal human huntington disease gene. *Proc. Natl. Acad. Sci. USA* 95:6480–85
37. Chai Y, Koppenhafer SL, Bonini NM, Paulson HL. 1999. Analysis of the role of heat shock protein (Hsp) molecular chaperones in polyglutamine disease. *J. Neurosci.* 19:10338–47
38. Chai Y, Koppenhafer SL, Shoesmith SJ, Perez MK, Paulson HL. 1999. Evidence for proteasome involvement in polyglutamine disease: localization to nuclear inclusions in SCA3/MJD and suppression of polyglutamine aggregation in vitro. *Hum. Mol. Genet.* 8:673–82
39. Chakrabarti L, Bristulf J, Foss GS, Davies KE. 1998. Expression of the murine homologue of FMR2 in mouse brain and during development. *Hum. Mol. Genet.* 7:441–48
40. Chakrabarti L, Knight SJ, Flannery AV, Davies KE. 1996. A candidate gene for mild mental handicap at the FRAXE fragile site. *Hum. Mol. Genet.* 5:275–82
41. Chamberlain NL, Driver ED, Miesfeld RL. 1994. The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. *Nucleic Acids Res.* 22:3181–86
42. Chung M-Y, Ranum LPW, Duvick L, Servadio A, Zoghbi HY, Orr HT. 1993. Analysis of the CAG repeat expansion in spinocerebellar ataxia type I: evidence for a possible mechanism predisposing to instability. *Nat. Genet.* 5:254–58
43. Clark HB, Burright EN, Yunis WS, Larson S, Wilcox C, et al. 1997. Purkinje cell expression of a mutant allele of *SCA1* in transgenic mice leads to disparate effects on motor behaviors, followed by a progressive cerebellar dysfunction and histological alterations. *J. Neurosci.* 17: 7385–95
44. Cobo A, Martinez JM, Martorell L, Baiget M, Johnson K. 1993. Molecular diagnosis of homozygous myotonic dystrophy in two asymptomatic sisters. *Hum. Mol. Genet.* 2:711–15
45. Coffee B, Zhang F, Warren ST, Reines D. 1999. Acetylated histones are associated with FMR1 in normal but not fragile X-syndrome cells. *Nat. Genet.* 22:98–101
46. Colomer V, Engelender S, Sharp AH, Duan K, Cooper JK, et al. 1997. Huntingtin-associated protein 1 (HAP1) binds to a Trio-like polypeptide, with a rac1 guanine nucleotide exchange factor domain. *Hum. Mol. Genet.* 6:1519–25
47. Consortium TD-BFX. 1994. Fmr1 knockout mice: a model to study fragile X mental retardation. *Cell* 78:23–33
48. Cooper AJ, Sheu KF, Burke JR, Onodera O, Strittmatter WJ, et al. 1997. Polyglutamine domains are substrates of tissue transglutaminase: does transglutaminase play a role in expanded CAG/poly-Q neurodegenerative diseases? *J. Neurochem.* 69:431–44

49. Corbin F, Bouillon M, Fortin A, Morin S, Rousseau F, Khandjian EW. 1997. The fragile X mental retardation protein is associated with poly(A)+mRNA in actively translating polyribosomes. *Hum. Mol. Genet.* 6:1465–72
50. Cossee M, Campuzano V, Koutnikova H, Fischbeck K, Mandel JL, et al. 1997. Frataxin fracas. *Nat. Genet.* 15:337–38
51. Cossee M, Schmitt M, Campuzano V, Reutenauer L, Moutou C, et al. 1997. Evolution of the Friedreich's ataxia trinucleotide repeat expansion: founder effect and premutations. *Proc. Natl. Acad. Sci. USA* 94:7452–57
52. Cummings CJ, Mancini MA, Antalffy B, DeFranco DB, Orr HT, Zoghbi HY. 1998. Chaperone suppression of aggregation and altered subcellular proteasome localization imply protein misfolding in SCA1. *Nat. Genet.* 19:148–54
53. Cummings CJ, Reinstein E, Sun Y, Antalffy B, Jiang Y-h, et al. 1999. Mutation of the E6-AP ubiquitin ligase reduces nuclear inclusion frequency while accelerating polyglutamine-induced pathology in SCA1 mice. *Neuron* 24:879–92
54. David G, Abbas N, Stevanin G, Durr A, Yvert G, et al. 1997. Cloning of the SCA7 gene reveals a highly unstable CAG repeat expansion. *Nat. Genet.* 17:65–70
55. David G, Durr A, Stevanin G, Cancel G, Abbas N, et al. 1998. Molecular and clinical correlations in autosomal dominant cerebellar ataxia with progressive macular dystrophy (SCA7). *Hum. Mol. Genet.* 7:165–70
56. Davies AF, Mirza G, Sekhon G, Turnpenny P, Leroy F, et al. 1999. Delineation of two distinct 6p deletion syndromes. *Hum. Genet.* 104:64–72
57. Davies SW, Turmaine M, Cozens BA, DiFiglia M, Sharp AH, et al. 1997. Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell* 90:537–48
58. Davies SW, Turmaine M, Cozens BA, Raza AS, Mahal A, et al. 1999. From neuronal inclusions to neurodegeneration: neuropathological investigation of a transgenic mouse model of Huntington's disease. *Philos. Trans. R. Soc. London Ser. B Biol. Sci.* 354:981–89
59. Davis BM, McCurrach ME, Taneja KL, Singer RH, Housman DE. 1997. Expansion of a CUG trinucleotide repeat in the 3' untranslated region of myotonic dystrophy protein kinase transcripts results in nuclear retention of transcripts. *Proc. Natl. Acad. Sci. USA* 94:7388–93
60. De Michele G, Cavalcanti F, Criscuolo C, Pianese L, Monticelli A, et al. 1998. Parental gender, age at birth and expansion length influence GAA repeat intergenerational instability in the X25 gene: pedigree studies and analysis of sperm from patients with Friedreich's ataxia. *Hum. Mol. Genet.* 7:1901–6
61. De Rooij KE, Dorsman JC, Smoor MA, Den Dunnen JT, Van Ommen G-JB. 1996. Subcellular localization of the Huntington's disease gene product in cell lines by immunofluorescence and biochemical subcellular fractionation. *Hum. Mol. Genet.* 5:1093–99
62. Del-Favero J, Krols L, Michalik A, Theuns J, Lofgren A, et al. 1998. Molecular genetic analysis of autosomal dominant cerebellar ataxia with retinal degeneration (ADCA type II) caused by CAG triplet repeat expansion. *Hum. Mol. Genet.* 7:177–86
63. Devys D, Lutz Y, Rouyer N, Bellocq JP, Mandel JL. 1993. The FMR-1 protein is cytoplasmic, most abundant in neurons and appears normal in carriers of a fragile X premutation. *Nat. Genet.* 4:335–40
64. DiFiglia M, Sapp E, Chase K, Schwarz C, Meloni A, et al. 1995. Huntingtin is a cytoplasmic protein associated with vesicles in human and rat brain neurons. *Neuron* 14:1075–81
65. DiFiglia M, Sapp E, Chase KO, Davies SW,

Bates GP, et al. 1997. Aggregation of Huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science* 277:1990–93

66. Drouin R, Angers M, Dallaire N, Rose TM, Khandjian W, Rousseau F. 1997. Structural and functional characterization of the human FMR1 promoter reveals similarities with the hnRNP-A2 promoter region. *Hum. Mol. Genet.* 6:2051–60
67. Durr A, Smadja D, Cancel G, Lezin A, Stevanin G, et al. 1995. Autosomal dominant cerebellar ataxia type I in Martinique (French West Indies): clinical and neuropathological analysis of 53 patients from three unrelated SCA2 families. *Brain* 118:1573–81
68. Durr A, Stevanin G, Cancel G, Duyckaerts C, Abbas N, et al. 1996. Spinocerebellar ataxia 3 and Machado-Joseph disease: clinical, molecular and neuropathological features. *Ann. Neurol.* 39:490–99
69. Duyao MP, Auerbach AB, Ryan A, Persichetti F, Barnes GT, et al. 1995. Inactivation of the mouse Huntington's disease gene homolog *Hdh. Science* 269:407–10
70. Eberhart DE, Warren ST. 1996. Nuclease sensitivity of permeabilized cells confirms altered chromatin formation at the fragile X locus. *Somat. Cell Mol. Genet.* 22:435–41
71. Eichler EE, Holden JJA, Popovich BW, Reiss AL, Snow K, et al. 1994. Length of uninterrupted CGG repeats determines instability in the *FMR1* gene. *Nat. Genet.* 8:88–94
72. Ellerby LM, Andrusiak RL, Wellington CL, Hackam AS, Propp SS, et al. 1999. Cleavage of atrophin-1 at caspase site aspartic acid 109 modulates cytotoxicity. *J. Biol. Chem.* 274:8730–36
73. Ellerby LM, Hackam AS, Propp SS, Ellerby HM, Rabizadeh S, et al. 1999. Kennedy's disease: caspase cleavage of the androgen receptor is a crucial event in cytotoxicity. *J. Neurochem.* 72:185–95
74. Engelender S, Sharp AH, Colomer V, Tokito MK, Lanahan A, et al. 1997. Huntingtin-associated protein 1 (HAP1) interacts with the p150Glued subunit of dynactin. *Hum. Mol. Genet.* 6:2205–12
75. Faber PW, Barnes GT, Srinidhi J, Chen J, Gusella JF, MacDonald ME. 1998. Huntingtin interacts with a family of WW domain proteins. *Hum. Mol Genet.* 7:1463–74
76. Fletcher CF, Lutz CM, O'Sullivan TN, Shaughnessy JD, Hawkes R, et al. 1996. Absence epilepsy in Tottering mutant mice is associated with calcium channel defects. *Cell* 87:607–17
77. Foury F, Cazzalini O. 1997. Deletion of the yeast homologue of the human gene associated with Friedreich's ataxia elicits iron accumulation in mitochondria. *FEBS Lett.* 411:373–77
78. Fu Y, Friedman DL, Richards S, Pearlman JA, Gibbs RA, et al. 1993. Decreased expression of myotonin-protein kinase mRNA and protein in adult. *Science* 260:235–38
79. Fu Y-H, Kuhl DPA, Pizutti A, Pieretti M, Sutcliffe JS, et al. 1991. Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. *Cell* 67:1047–58
80. Fu Y-H, Pizutti A, Fenwick RG, King JJ, Rajnarayan S, et al. 1992. An unstable triplet repeat in a gene related to myotonic muscular dystrophy. *Science* 255: 1256–59
81. Gecz J, Bielby S, Sutherland GR, Mulley JC. 1997. Gene structure and subcellular localization of FMR2, a member of a new family of putative transcription activators. *Genomics* 44:201–13
82. Gecz J, Gedeon AK, Sutherland GR, Mulley JC. 1996. Identification of the gene FMR2, associated with FRAXE mental retardation. *Nat. Genet.* 13: 105–8
83. Gecz J, Oostra BA, Hockey A, Carbonell P, Turner G, et al. 1997. FMR2 expression in families with FRAXE mental retardation. *Hum. Mol. Genet.* 6:435–41

84. Gedeon AK, Meinanen M, Ades LC, Kaariainen H, Gecz J, et al. 1995. Overlapping submicroscopic deletions in Xq28 in two unrelated boys with developmental disorders: identification of a gene near FRAXE. *Am. J. Hum. Genet.* 56:907–14
85. Geschwind DH, Perlman S, Figueroa CP, Karim J, Baloh RW, Pulst SM. 1997. Spinocerebellar ataxia type 6 (SCA6): frequency of the mutation and genotype-phenotype correlations. *Neurology* 49:1247–51
86. Gibson TJ, Koonin EV, Musco G, Pastore A, Bork P. 1996. Friedreich's ataxia protein: phylogenetic evidence for mitochondrial dysfunction. *Trends Neurosci.* 19:465–68
87. Gibson TJ, Rice PM, Thompson JD, Heringa J. 1993. KH domains within the FMR1 sequence suggest that fragile X syndrome stems from a defect in RNA metabolism. *Trends Biochem. Sci.* 18:331–33
88. Goldberg YP, Nicholson DW, Rasper DM, Kalchman MA, Koide HB, et al. 1996. Cleavage of huntingtin by apopain, a proapoptotic cysteine protease, is modulated by the polyglutamine tract. *Nat. Genet.* 13:442–49
89. Gouw LG, Castaneda MA, McKenna CK, Digre KB, Pulst SM, et al. 1998. Analysis of the dynamic mutation in the SCA7 gene shows marked parental effects on CAG repeat transmission. *Hum. Mol. Genet.* 7:525–32
90. Green H. 1993. Human genetic diseases due to codon reiteration: relationship to an evolutionary mechanism. *Cell* 74:955–56
91. Greenfield JG. 1954. *The Spino-cerebellar Degenerations.* Springfield, IL: Thomas. 112 pp.
92. Gu Y, Shen Y, Gibbs RA, Nelson DL. 1996. Identification of FMR2, a novel gene associated with the FRAXE CCG repeat and CpG island. *Nat. Genet.* 13:109–13
93. Gutekunst CA, Levey AI, Heilman CJ, Whaley WL, Yi H, et al. 1995. Identification and localization of huntingtin in brain and human lymphoblastoid cell lines with anti-fusion protein antibodies. *Proc. Natl. Acad. Sci. USA* 92:8710–14
94. Gutekunst CA, Li SH, Yi H, Mulroy JS, Kuemmerle S, et al. 1999. Nuclear and neuropil aggregates in Huntington's disease: relationship to neuropathology. *J. Neurosci.* 19:2522–34
95. Hackam AS, Singaraja R, Wellington CL, Metzler M, McCutcheon K, et al. 1998. The influence of huntingtin protein size on nuclear localization and cellular toxicity. *J. Cell Biol.* 141:1097–105
96. Hagerman RJ. 1996. Physical and behavioral phenotypes. In *Physical and Behavioral Phenotypes*, ed. RJ Hagerman, A Cronister, pp. 283–331. Baltimore: Johns Hopkins Univ. Press
97. Hamshere MG, Newman EE, Alwazzan M, Athwal BS, Brook JD. 1997. Transcriptional abnormality in myotonic dystrophy affects DMPK but not neighboring genes. *Proc. Natl. Acad. Sci. USA* 94:7394–99
98. Hansen RS, Gartler SM, Scott CR, Chen SH, Laird CD. 1992. Methylation analysis of CGG sites in the CpG island of the human FMR1 gene. *Hum. Mol. Genet.* 1:571–78
99. Harding AE. 1981. Friedreich's ataxia: a clinical and genetic study of 90 families with an analysis of early diagnostic criteria and intrafamilial clustering of clinical features. *Brain* 104:589–620
100. Harper PS. 1989. *Myotonic Dystrophy.* London: Saunders. 384 pp.
101. Hartl F. 1996. Molecular chaperones in cellular protein folding. *Nature* 381: 571–79
102. Hayes SA, Dice JF. 1996. Roles of molecular chaperones in protein degradation. *J. Cell Biol.* 132:255–58
103. Hodgson JG, Agopyan N, Gutekunst CA, Leavitt BR, LePiane F, et al. 1999. A YAC mouse model for Huntington's disease with full-length mutant huntingtin,

cytoplasmic toxicity, and selective striatal neurodegeneration. *Neuron* 23:181–92
104. Hofmann-Radvanyi H, Lavedan C, Rabes JP, Savoy D, Duros C, et al. 1993. Myotonic dystrophy: absence of CTG enlarged transcript in congenital forms, and low expression of the normal allele. *Hum. Mol. Genet.* 2:1263–66
105. Holmberg M, Duyckaerts C, Durr A, Cancel G, Gourfinkel-An I, et al. 1998. Spinocerebellar ataxia type 7 (SCA7):a neurodegenerative disorder with neuronal intranuclear inclusions. *Hum. Mol. Genet.* 7:913–18
106. Holmes SE, O'Hearn EE, McInnis MG, Gorelick-Feldman DA, Kleiderlein JJ, et al. 1999. Expansion of a novel CAG trinucleotide repeat in the 5′ region of PPP2R2B is associated with SCA12. *Nat. Genet.* 23:391–92
107. Howeler CJ, Busch HF, Geraedts JP, Niermeijer MF, Staal A. 1989. Anticipation in myotonic dystrophy: fact or fiction? *Brain* 112:779–97
108. Hunter A, Tsilfidis C, Mettler G, Jacob P, Mahadevan M, et al. 1992. The correlation of age of onset with CTG trinucleotide repeat amplification in. *J. Med. Genet.* 29:774–79
109. Hurlbert MS, Zhou W, Wasmeier C, Kaddis FG, Hutton JC, Freed CR. 1999. Mice transgenic for an expanded CAG repeat in the Huntington's disease gene develop diabetes. *Diabetes* 48:649–51
110. Huynh DP, Del Bigio MR, Ho DH, Pulst SM. 1999. Expression of ataxin-2 in brains from normal individuals and patients with Alzheimer's disease and spinocerebellar ataxia 2. *Ann. Neurol.* 45:232–41
111. Igarashi S, Koide R, Shimohata T, Yamada M, Hayashi Y, et al. 1998. Suppression of aggregate formation and apoptosis by transglutaminase inhibitors in cells expressing truncated DRPLA protein with an expanded polyglutamine stretch. *Nat. Genet.* 18:111–17
112. Ikeda H, Yamaguchi M, Sugai S, Aze Y, Narumiya S, Kakizuka A. 1996. Expanded polyglutamine in the Machado-Joseph disease protein induces cell death *in vitro* and *in vivo. Nat. Genet.* 13: 196–202
113. Ikeuchi T, Koide R, Tanaka H, Onodera O, Igarashi S, et al. 1995. Dentatorubral-pallidoluysian atrophy: Clinical features are closely related to unstable expansions of trinucleotide (CAG) repeat. *Ann. Neurol.* 37:769–75
114. Ikeuchi T, Takano H, Koide R, Horikawa Y, Honma Y, et al. 1997. Spinocerebellar ataxia type 6:CAG repeat expansion in alpha1A voltage-dependent calcium channel gene and clinical variations in Japanese population. *Ann. Neurol.* 42:879–84
115. Imbert G, Saudou F, Yvert G, Devys D, Trottier Y, et al. 1996. Cloning of the gene for spinocerebellar ataxia 2 reveals a locus with high sensitivity to expanded CAG/glutamine repeats and high instability. *Nat. Genet.* 14:285–91
116. Ishikawa K, Fujigasaki H, Saegusa H, Ohwada K, Fujita T, et al. 1999. Abundant expression and cytoplasmic aggregations of [alpha]1A voltage-dependent calcium channel protein associated with neurodegeneration in spinocerebellar ataxia type 6. *Hum. Mol. Genet.* 8:1185–93
117. Jackson GR, Salecker I, Dong X, Yao X, Arnheim N, et al. 1998. Polyglutamine-expanded human huntingtin transgenes induce degeneration of Drosophila photoreceptor neurons. *Neuron* 21:633–42
118. Jansen G, Bachner D, Coerwinkel M, Wormskamp N, Hameister H, et al. 1995. Structural organization and developmental expression pattern of the mouse WD-repeat gene DMR-N9 immediately upstream of the myotonic dystrophy locus. *Hum. Mol. Genet.* 4:843–52
119. Jansen G, Groenen PJ, Bachner D, Jap PH, Coerwinkel M, et al. 1996. Abnormal myotonic dystrophy protein kinase levels

produce only mild myopathy in mice. *Nat. Genet.* 13:316–24
120. Janzen ML, Moseley ML, Benzow KA, Day JW, Koob MD, et al. 1999. Limited expression of SCA8 is consistent with cerebellar pathogenesis and toxic gain of function RNA model. *Am. J. Hum. Genet.* 65(Suppl.):A276
121. Jenster G, van der Korput HA, van Vroonhoven C, van der Kwast TH, Trapman J, et al. 1991. Domains of the human androgen receptor involved in steroid binding, transcriptional activation, and subcellular localization. *Mol. Endocrinol.* 5: 1396–404
122. Jiang YH, Armstrong D, Albrecht U, Atkins CM, Noebels JL, et al. 1998. Mutation of the Angelman ubiquitin ligase in mice causes increased cytoplasmic p53 and deficits of contextual learning and longterm potentiation. *Neuron* 21: 799–811
123. Jodice C, Mantuano E, Veneziano L, Trettel F, Sabbadini G, et al. 1997. Episodic ataxia type 2 (EA2) and spinocerebellar ataxia type 6 (SCA6) due to CAG repeat expansion in the CACNA1A gene on chromosome 19p. *Hum. Mol. Genet.* 6:1973–78
124. Johansson J, Forsgren L, Sandgren O, Brice A, Holmgren G, et al. 1998. Expanded CAG repeats in Swedish spinocerebellar ataxia type 7 (SCA7) patients: effect of CAG repeat length on the clinical manifestation. *Hum. Mol. Genet.* 7:171–76
125. Kahlem P, Green H, Djian P. 1998. Transglutaminase action imitates Huntington's disease: selective polymerization of Huntingtin containing expanded polyglutamine. *Mol. Cell* 1:595–601
126. Kalchman MA, Graham RK, Xia G, Koide HB, Hodgson JG, et al. 1996. Huntingtin is ubiquitinated and interacts with a specific ubiquitin-conjugating enzyme. *J. Biol. Chem.* 271:19385–94
127. Kalchman MA, Koide HB, McCutcheon K, Graham RK, Nichol K, et al. 1997. *HIP1*, a human homologue of S. cerevisiae *Sla2p*, interacts with membrane-associated huntingtin in the brain. *Nat. Genet.* 16:44–53
128. Karpuj MV, Garren H, Slunt H, Price DL, Gusella J, et al. 1999. Transglutaminase aggregates huntingtin into nonamyloidogenic polymers, and its enzymatic activity increases in Huntington's disease brain nuclei. *Proc. Natl. Acad. Sci. USA* 96:7388–93
129. Kawaguchi Y, Okamoto T, Taniwaki M, Aizawa M, Inoue M, et al. 1994. CAG expansions in a novel gene for Machado-Joseph disease at chromosome 14q32.1. *Nat. Genet.* 8:221–27
130. Kaytor MD, Duvick LA, Skinner PJ, Koob MD, Ranum LP, et al. 1999. Nuclear localization of the spinocerebellar ataxia type 7 protein, ataxin-7. *Hum. Mol. Genet.* 8:1657–64
131. Khandjian EW, Bardoni B, Corbin F, Sittler A, Giroux S, et al. 1998. Novel isoforms of the fragile X related protein FXR1P are expressed during myogenesis. *Hum. Mol. Genet.* 7:2121–28
132. Khandjian EW, Corbin F, Woerly S, Rousseau F. 1996. The fragile X mental retardation protein is associated with ribosomes. *Nat. Genet.* 12:91–93
133. Kim TA, Lim J, Ota S, Raja S, Rogers R, et al. 1998. NRP/B, a novel nuclear matrix protein, associates with p110(RB) and is involved in neuronal differentiation. *J. Cell Biol.* 141:553–66
134. Kinoshita M, Takahashi R, Hasegawa T, Komori T, Nagasawa R, et al. 1996. (CTG)n expansions in various tissues from a myotonic dystrophy patient. *Muscle Nerve* 19:240–42
135. Kirkpatrick LL, McIlwain KA, Nelson DL. 1999. Alternative splicing in the murine and human FXR1 genes. *Genomics* 59:193–202
136. Klement IA, Skinner PJ, Kaytor MD, Yi H, Hersch SM, et al. 1998. Ataxin-1

nuclear localization and aggregation: role in polyglutamine-induced disease in SCA1 transgenic mice. *Cell* 95:41–53
137. Klesert TR, Otten AD, Bird TD, Tapscott SJ. 1997. Trinucleotide repeat expansion at the myotonic dystrophy locus reduces expression of DMAHP. *Nat. Genet.* 16:402–6
138. Knight SJ, Flannery AV, Hirst MC, Campbell L, Christodoulou Z, et al. 1993. Trinucleotide repeat amplification and hypermethylation of a CpG island in FRAXE mental retardation. *Cell* 74: 127–34
139. Knight SJ, Voelckel MA, Hirst MC, Flannery AV, Moncla A, et al. 1994. Triplet repeat expansion at the FRAXE locus and X-linked mild mental handicap. *Am. J. Hum. Genet.* 55:81–86
140. Knight SP, Richardson MM, Osmand AP, Stakkestad A, Potter NT. 1997. Expression and distribution of the dentatorubral-pallidoluysian atrophy gene product (atrophin-1/drplap) in neuronal and non-neuronal tissues. *J. Neurol. Sci.* 146: 19–26
141. Koeppen AH, Barron KD. 1984. The neuropathology of olivopontocerebellar atrophy. In *The Neuropathology of Olivopontocerebellar Atrophy*, ed. RC Duvoisin, A Plaitakis, pp. 13–38. New York: Raven
142. Koga R, Nakao Y, Kurano Y, Tsukahara T, Nakamura A, et al. 1994. Decreased myotonin-protein kinase in the skeletal and cardiac muscles. *Biochem. Biophys. Res. Commun.* 202:577–85
143. Koide R, Ikeuchi T, Onodera O, Tanaka H, Igarashi S, et al. 1994. Unstable expansion of CAG repeat in hereditary dentatorubral-pallidoluysian atrophy (DRPLA). *Nat. Genet.* 6:9–13
144. Koide R, Kobayashi S, Shimohata T, Ikeuchi T, Maruyama M, et al. 1999. A neurological disease caused by an expanded CAG trinucleotide repeat in the TATA-binding protein gene: a new polyglutamine disease? *Hum. Mol. Genet.* 8:2047–53
145. Koob MD, Moseley ML, Benzow KA, Johnson CM, Nemes JP, et al. 1999. The SCA8 transcript is an antisense RNA to a brain-specific transcript encoding a novel actin-binding protein (KLHL1). *Am. J. Hum. Genet.* 65(Suppl.):A30
146. Koob MD, Moseley ML, Schut LJ, Benzow KA, Bird TD, et al. 1999. An untranslated CTG expansion causes a novel form of spinocerebellar ataxia (SCA8). *Nat. Genet.* 21:379–84
147. Koshy B, Matilla T, Burright EN, Merry DE, Fischbeck KH, et al. 1996. Spinocerebellar ataxia type-1 and spinobulbar muscular atrophy gene products interact with glyceraldehyde-3-phosphate dehydrogenase. *Hum. Mol. Genet.* 5:1311–18
148. Koshy BT, Matilla A, Zoghbi HY. 1998. Clues about the pathogenesis of SCA1 from biochemical and molecular studies of ataxi-1. In *Clues About the Pathogenesis of SCA1 from Biochemical and Molecular Studies of Ataxin-1*, ed. RD Wells, ST Warren, pp. 241–48. San Diego: Academic
149. Koutnikova H, Campuzano V, Foury F, Dolle P, Cazzalini O, et al. 1997. Studies of human, mouse and yeast homologues indicate a mitochondrial function for frataxin. *Nat. Genet.* 16:345–51
150. Koyano S, Uchihara T, Fujigasaki H, Nakamura A, Yagishita S, et al. 1999. Neuronal intranuclear inclusions in spinocerebellar ataxia type 2: triple-labeling immunofluorescent study. *Neurosci. Lett.* 273:117–20
151. Krahe R, Ashizawa T, Abbruzzese C, Roeder E, Carango P, et al. 1995. Effect of myotonic dystrophy trinucleotide repeat expansion on DMPK transcription and processing. *Genomics* 28:1–14
152. Kuemmerle S, Gutekunst CA, Klein AM, Li XJ, Li SH, et al. 1999. Huntington aggregates may not predict neuronal

death in Huntington's disease. *Ann. Neurol.* 46:842–49
153. La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck H. 1991. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature* 352:77–79
154. Lamarche JB, Cote M, Lemieux B. 1980. The cardiomyopathy of Friedreich's ataxia morphological observations in 3 cases. *Can. J. Neurol. Sci.* 7:389–96
155. Lesort M, Chun W, Johnson GV, Ferrante RJ. 1999. Tissue transglutaminase is increased in Huntington's disease brain. *J. Neurochem.* 73:2018–27
156. Li H, Li SH, Cheng AL, Mangiarini L, Bates GP, et al. 1999. Ultrastructural localization and progressive formation of neuropil aggregates in Huntington's disease transgenic mice. *Hum. Mol. Genet.* 8:1227–36
157. Li M, Miwa S, Kobayashi Y, Merry DE, Yamamoto M, et al. 1998. Nuclear inclusions of the androgen receptor protein in spinal and bulbar muscular atrophy. *Ann. Neurol.* 44:249–54
158. Li M, Nakagomi Y, Kobayashi Y, Merry DE, Tanaka F, et al. 1998. Nonneural nuclear inclusions of androgen receptor protein in spinal and bulbar muscular atrophy. *Am. J. Pathol.* 153:695–701
159. Li X-J, Li S-H, Sharp AH, Nucifora FCJ, Schilling G, et al. 1995. A huntingtin-associated protein enriched in brain with implications for pathology. *Nature* 378:398–402
160. Li Z, Karlovich CA, Fish MP, Scott MP, Myers RM. 1999. A putative Drosophila homolog of the Huntington's disease gene. *Hum. Mol. Genet.* 8:1807–15
161. Lin B, Nasir J, MacDonald H, Hutchinson G, Graham RK, et al. 1994. Sequence of the murine Huntington disease gene: evidence for conservation, alternate splicing and polymorphism in a triplet (CCG) repeat. *Hum. Mol. Genet.* 3:85–92
162. Lin X, Antalffy B, Kang D, Orr T, Zoghbi HY. 2000. Polyglutamine expansion in ataxin-1 downregulates specific neuronal genes before pathogenic changes in spinocerebellar ataxia type 1. *Nat. Neurosci.* In press
163. Llinaw RR, Sugimori M, Cherksey B. 1989. Voltage-dependent calcium conductances in mammalian neurons. The P channel. *Ann. NY Acad. Sci.* 560:103–11
164. Lu X, Timchenko NA, Timchenko LT. 1999. Cardiac elav-type RNA-binding protein (ETR-3) binds to RNA CUG repeats expanded in myotonic dystrophy. *Hum. Mol. Genet.* 8:53–60
165. Lugenbeel KA, Peier AM, Carson NL, Chudley AE, Nelson DL. 1995. Intragenic loss of function mutations demonstrate the primary role of FMR1 in fragile X syndrome. *Nat. Genet.* 10:483–85
166. Lunkes A, Mandel JL. 1998. A cellular model that recapitulates major pathogenic steps of Huntington's disease. *Hum. Mol. Genet.* 7:1355–61
167. Ma C, Staudt LM. 1996. LAF-4 encodes a lymphoid nuclear protein with transactivation potential that is homologous to AF-4, the gene fused to MLL in t(4;11) leukemias. *Blood* 87:734–45
168. MacLean HE, Warne GL, Zajac JD. 1995. Defects of androgen receptor function: from sex reversal to motor neurone disease. *Mol. Cell Endocrinol.* 112:133–41
169. Maeda M, Taft CS, Bush EW, Holder E, Bailey WM, et al. 1995. Identification, tissue-specific expression, and subcellular localization of the 80- and 71-kDa forms of myotonic dystrophy kinase protein. *J. Biol. Chem.* 270:20246–49
170. Mahadevan M, Tsilfidis C, Sabourin L, Shutler G, Amemiya C, et al. 1992. Myotonic dystrophy mutation: an unstable CTG repeat in the 3′ untranslated region of the gene. *Science* 255:1253–55
171. Mangiarini L, Sathasivam K, Seller M, Cozens B, Harper A, et al. 1996. Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a

progressive neurological phenotype in transgenic mice. *Cell* 87:493–506
172. Martin JJ, Van Regemorter N, Krols L, Brucher JM, de Barsy T, et al. 1994. On an autosomal dominant form of retinal-cerebellar degeneration: an autopsy study of five patients in one family. *Acta Neuropathol.* 88:277–86
173. Martindale D, Hackam A, Wieczorek A, Ellerby L, Wellington C, et al. 1998. Length of huntingtin and its polyglutamine tract influences localization and frequency of intracellular aggregates. *Nat. Genet.* 18:150–54
174. Martorell L, Illa I, Rosell J, Benitez J, Sedano MJ, et al. 1996. Homozygous myotonic dystrophy: clinical and molecular studies of three unrelated cases. *J. Med. Genet.* 33:783–85
175. Matilla A, Koshy B, Cummings CJ, Isobe T, Orr HT, et al. 1997. The cerebellar leucine-rich acidic nuclear protein interacts with ataxin-1. *Nature* 389:974–78
176. Matilla A, Roberson ED, Banfi S, Morales J, Armstrong DL, et al. 1998. Mice lacking ataxin-1 display learning deficits and decreased hippocampal paired-pulse facilitation. *J. Neurosci.* 18:5508–16
177. Matsumura R, Futamura N, Fujimoto Y, Yanagimoto S, Horikawa H, et al. 1997. Spinocerebellar ataxia type 6:Molecular and clinical features of 35 Japanese patients including one homozygous for the CAG repeat expansion. *Neurology* 49:1238–43
178. Matsuoka K, Taoka M, Satozawa N, Nakayama H, Ichimura T, et al. 1994. A nuclear factor containing the leucine-rich repeats expresses in murine cerebellar neurons. *Proc. Natl. Acad. Sci. USA* 91:9670–74
179. Matsuyama Z, Wakamori M, Mori Y, Kawakami H, Nakamura S, et al. 1999. Direct alteration of the P/Q-type Ca2+ channel property by polyglutamine expansion in spinocerebellar ataxia 6. *J. Neurosci.* 19:RC14
180. Merry DE, Kobayashi Y, Bailey CK, Taye AA, Fischbeck KH. 1998. Cleavage, aggregation and toxicity of the expanded androgen receptor in spinal and bulbar muscular atrophy. *Hum. Mol. Genet.* 7:693–701
181. Merry DE, Woods J, Walcott J, Bish L, Fischbeck KH, et al. 1999. Characterization of a transgenic model for SBMA. *Am. J Hum. Genet.* 65(Suppl.):A30
182. Mhatre AN, Trifiro MA, Kaufman M, Kazemi-Esfarjani P, Figlewicz D, et al. 1993. Reduced transcriptional regulatory competence of the androgen receptor in X-linked spinal and bulbar muscular atrophy. *Nat. Genet.* 5:184–88
183. Millward TA, Zolnierowicz S, Hemmings BA. 1999. Regulation of protein kinase cascades by protein phosphatase 2A. *Trends Biochem. Sci.* 24:186–91
184. Miyashita T, Matsui J, Ohtsuka Y, Mami U, Fujishima S, et al. 1999. Expression of extended polyglutamine sequentially activates initiator and effector caspases. *Biochem. Biophys. Res. Commun.* 257:724–30
185. Monckton DG, Cayuela ML, Gould FK, Brock GJ, Silva R, et al. 1999. Very large (CAG)(n) DNA repeat expansions in the sperm of two spinocerebellar ataxia type 7 males. *Hum. Mol. Genet.* 8:2473–78
186. Monros E, Molto MD, Martinez F, Canizares J, Blanca J, et al. 1997. Phenotype correlation and intergenerational dynamics of the Friedreich ataxia GAA trinucleotide repeat. *Am. J. Hum. Genet.* 61:101–10
187. Montermini L, Andermann E, Labuda M, Richter A, Pandolfo M, et al. 1997. The Friedreich ataxia GAA triplet repeat: premutation and normal alleles. *Hum. Mol. Genet.* 6:1261–66
188. Morrone A, Pegoraro E, Angelini C, Zammarchi E, Marconi G, et al. 1997. RNA metabolism in myotonic dystrophy: patient muscle shows decreased insulin receptor RNA and protein consistent

with abnormal insulin resistance. *J. Clin. Invest.* 99:1691–98
189. Moseley ML, Benzow KA, Schut LJ, Bird TD, Gomez CM, et al. 1998. Incidence of dominant spinocerebellar and Friedreich triplet repeats among 361 ataxia families. *Neurology* 51:1666–71
190. Mulley JC, Yu S, Loesch DZ, Hay DA, Donnelly A, et al. 1995. FRAXE and mental retardation. *J. Med. Genet.* 32:162–69
191. Nakajima H, Kimura F, Nakagawa T, Furutama D, Shinoda K, et al. 1996. Transcriptional activation by the androgen receptor in X-linked spinal and bulbar muscular atrophy. *J. Neurol. Sci.* 142:12–16
192. Nasir J, Floresco SB, O'Kusky JR, Diewert VM, Richman JM, et al. 1995. Targeted disruption of the Huntington's disease gene results in embryonic lethality and behavioral and morphological changes in heterozygotes. *Cell* 81: 811–23
193. Nechiporuk T, Huynh DP, Figueroa K, Sahba S, Nechiporuk A, et al. 1998. The mouse SCA2 gene: cDNA sequence, alternative splicing and protein expression. *Hum. Mol. Genet.* 7:1301–9
194. Neuwald AF, Koonin EV. 1998. Ataxin-2, global regulators of bacterial gene expression, and spliceosomal snRNP proteins share a conserved domain. *J. Mol. Med.* 76:3–5
195. Oberle I, Vincent A, Abbadi N, Rousseau F, Hupkes PE, et al. 1991. New polymorphism and a new chromosome breakpoint establish the physical and genetic mapping of DXS369 in the DXS98–FRAXA interval. *Am. J. Med. Genet.* 38:336–42
196. Ohshima K, Montermini L, Wells RD, Pandolfo M. 1998. Inhibitory effects of expanded GAA · TTC triplet repeats from intron I of the Friedreich ataxia gene on transcription and replication in vivo. *J. Biol. Chem.* 273:14588–95
197. Okamura-Oho Y, Miyashita T, Ohmi K, Yamada M. 1999. Dentatorubral-pallidoluysian atrophy protein interacts through a proline-rich region near polyglutamine with the SH3 domain of an insulin receptor tyrosine kinase substrate. *Hum. Mol. Genet.* 8:947–57
198. Ona VO, Li M, Vonsattel JP, Andrews LJ, Khan SQ, et al. 1999. Inhibition of caspase-1 slows disease progression in a mouse model of Huntington's disease. *Nature* 399:263–67
199. Ophoff RA, Terwindt GM, Vergouwe MN, van Eijk R, Oefner PJ, et al. 1996. Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the CA^{2+} channel gene CACNL1A4. *Cell* 87:543–52
200. Ordway JM, Tallaksen-Greene S, Gutekunst CA, Bernstein EM, Cearley JA, et al. 1997. Ectopically expressed CAG repeats cause intranuclear inclusions and a progressive late onset neurological phenotype in the mouse. *Cell* 91:753–63
201. Orozco G, Estrada R, Perry TL, Arana J, Fernandez R, et al. 1989. Dominantly inherited olivopontocerebellar atrophy from eastern Cuba. Clinical, neuropathological, and biochemical findings. *J. Neurol. Sci.* 93:37–50
202. Otten AD, Tapscott SJ. 1995. Triplet repeat expansion in myotonic dystrophy alters the adjacent chromatin structure. *Proc. Natl. Acad. Sci. USA* 92:5465–69
203. Paulson HL. 1998. Spinocerebellar ataxia type 3/ Machado-Joseph disease. In *Spinocerebellar Ataxia Type 3/Machado-Joseph Disease*, ed. DC Rubinsztein, MR Hayden, pp. 129–41. Oxford, UK: Bios Sci.
204. Paulson HL, Das SS, Crino PB, Perez MK, Patel SC, et al. 1997. Machado-Joseph disease gene product is a cytoplasmic protein widely expressed in brain. *Ann. Neurol.* 41:453–62
205. Paulson HL, Perez MK, Trottier Y, Trojanowsk JQ, Subramony SH, et al.

1997. Intranuclear inclusions of expanded polyglutamine protein in spinocerebellar ataxia type 3. *Neuron* 19:333–34

206. Perez MK, Paulson HL, Pendse SJ, Saionz SJ, Bonini NM, et al. 1998. Recruitment and the role of nuclear localization in polyglutamine-mediated aggregation. *J. Cell Biol.* 143:1457–70
207. Perez MK, Paulson HL, Pittman RN. 1999. Ataxin-3 with an altered conformation that exposes the polyglutamine domain is associated with the nuclear matrix. *Hum. Mol. Genet.* 8:2377–85
208. Persichetti F, Ambrose CM, Ge P, McNeil SM, Srinidhi J, et al. 1995. Normal and expanded Huntington's disease gene alleles produce distinguishable proteins due to translation across the CAG repeat. *Mol. Med.* 1:374–83
209. Perutz MF, Johnson T, Suzuki M, Finch JT. 1994. Glutamine repeats as polar zippers: their possible role in inherited neurodegenerative diseases. *Proc. Natl. Acad. Sci. USA* 91:5355–58
210. Peters MF, Nucifora FC Jr, Kushi J, Seaman HC, Cooper JK, et al. 1999. Nuclear targeting of mutant Huntingtin increases toxicity. *Mol. Cell Neurosci.* 14:121–28
211. Philips AV, Timchenko LT, Cooper TA. 1998. Disruption of splicing regulated by a CUG-binding protein in myotonic dystrophy. *Science* 280:737–41
212. Pieretti M, Zhang F, Fu Y-H, Warren ST, Oostra BA, et al. 1991. Absence of expression of the *FMR-1* gene in fragile X syndrome. *Cell* 66:817–22
213. Pulst S-M, Nechiporuk A, Nechiporuk T, Gisper S, Chen X-N, et al. 1996. Identification of the SCA2 gene: moderate expansion of a normally biallelic trinucleotide repeat. *Nat. Genet.* 14:269–76
214. Ranum LPW, Moseley ML, Leppert MF, van den Engh G, La Spada AR, et al. 1999. Massive CTG expansions and deletions may reduce penetrance of spinocerebellar ataxia type 8. *Am. J. Hum. Genet.* 65(Suppl.):A466
215. Reddy PH, Williams M, Charles V, Garrett L, Pike-Buchanan L, et al. 1998. Behavioural abnormalities and selective neuronal loss in HD transgenic mice expressing mutated full-length HD cDNA. *Nat. Genet.* 20:198–202
216. Reddy S, Smith DB, Rich MM, Leferovich JM, Reilly P, et al. 1996. Mice lacking the myotonic dystrophy protein kinase develop a late onset progressive myopathy. *Nat. Genet.* 13:325–35
217. Riess O, Schols L, Bottger H, Nolte D, Vieira-Saecker AM, et al. 1997. SCA6 is caused by moderate CAG expansion in the alpha1A-voltage-dependent calcium channel gene. *Hum. Mol. Genet.* 6: 1289–93
218. Roberts R, Timchenko NA, Miller JW, Reddy S, Caskey CT, et al. 1997. Altered phosphorylation and intracellular distribution of a (CUG)n triplet repeat RNA-binding protein in patients with myotonic dystrophy and in myotonin protein kinase knockout mice. *Proc. Natl. Acad. Sci. USA* 94:13221–26
219. Roizin L, Stellar S, Liu JD. 1979. Neuronal nuclear-cytoplasmic changes in Huntingtons chorea: electron microscope investigations. In *Neuronal Nuclear-Cytoplasmic Changes in Huntingtons Chorea: Electron Microscope Investigations*, ed. TN Chase, NS Wexler, A Barbeau, pp. 95–122. New York: Raven
220. Romeo G, Menozzi P, Ferlini A, Fadda S, Di Donato S, et al. 1983. Incidence of Friedreich ataxia in Italy estimated from consanguineous marriages. *Am. J. Hum. Genet.* 35:523–29
221. Rotig A, de Lonlay P, Chretien D, Foury F, Koenig M, et al. 1997. Aconitase and mitochondrial iron-sulphur protein deficiency in Friedreich ataxia. *Nat. Genet.* 17:215
222. Rousseau F, Heitz D, Oberle I, Mandel JL. 1991. Selection in blood cells from female carriers of the fragile X syndrome: inverse correlation between age and

proportion of active X chromosomes carrying the full mutation. *J. Med. Genet.* 28:830–36
223. Rubinsztein DC, Hayden MR, eds. 1998. *Analysis of Triplet Repeat Disorders.* Oxford, UK: Bios Sci.
224. Sabouri LA, Mahadevan MS, Narang M, Lee DSC, Surh LC, et al. 1993. Effect of the myotonic dystrophy (DM) mutation on mRNA levels of the DM gene. *Nat. Genet.* 4:233–38
225. Sakamoto N, Chastain PD, Parniewski P, Ohshima K, Pandolfo M, et al. 1999. Sticky DNA: self-association properties of long GAA.TTC repeats in R.R.Y triplex structures from Friedreich's ataxia. *Mol. Cell.* 3:465–75
226. Sanchez I, Xu CJ, Juo P, Kakizaka A, Blenis J, et al. 1999. Caspase-8 is required for cell death induced by expanded polyglutamine repeats. *Neuron* 22:623–33
227. Sanpei K, Takano H, Igarashi S, Sato T, Oyake M, et al. 1996. Identification of the spinocerebellar ataxia type 2 gene using a direct identification of repeat expansion and cloning technique, DIRECT. *Nat. Genet.* 14:277–84
228. Sathasivam K, Hobbs C, Turmaine M, Mangiarini L, Mahal A, et al. 1999. Formation of polyglutamine inclusions in non-CNS tissue. *Hum. Mol. Genet.* 8:813–22
229. Sato T, Oyake M, Nakamura K, Nakao K, Fukusima Y, et al. 1999. Transgenic mice harboring a full-length human mutant DRPLA gene exhibit age-dependent intergenerational and somatic instabilities of CAG repeats comparable with those in DRPLA patients. *Hum. Mol. Genet.* 8:99–106
230. Sato T, Yamada M, Oyake M, Nakao K, Nakamura K, et al. 1999. Transgenic mice harboring a full-length human DRPLA gene with highly expanded CAG repeats exhibit severe disease phenotype. *Am. J. Hum. Genet.* 65(Suppl.):A30
231. Saudou F, Finkbeiner S, Devys D, Greenberg ME. 1998. Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. *Cell* 95:55–66
232. Schalling M, Hudson TJ, Buetow KH, Housman DE. 1993. Direct detection of novel expanded trinucleotide repeats in the human genome. *Nat. Genet.* 4: 135–39
233. Scherzinger E, Lurz R, Turmaine M, Mangiarini L, Hollenbach B, et al. 1997. Huntingtin-encoded polyglutamine expansions form amyloid-like protein aggregates in vitro and in vivo. *Cell* 90: 549–58
234. Schilling G, Becher MW, Sharp AH, Jinnah HA, Duan K, et al. 1999. Intranuclear inclusions and neuritic aggregates in transgenic mice expressing a mutant N-terminal fragment of huntingtin. *Hum. Mol. Genet.* 8:397–407
235. Schols L, Kruger R, Amoiridis G, Przuntek H, Epplen JT, et al. 1998. Spinocerebellar ataxia type 6:genotype and phenotype in German kindreds. *J. Neurol. Neurosurg. Psychiatry* 64:67–73
236. Schwemmle S, de Graaff E, Deissler H, Glaser D, Wohrle D, et al. 1997. Characterization of FMR1 promoter elements by in vivo-footprinting analysis. *Am. J. Hum. Genet.* 60:1354–62
237. Servadio A, Koshy B, Armstrong D, Antalffy B, Orr HT, et al. 1995. Expression analysis of the ataxin-1 protein in tissues from normal and spinocerebellar ataxia type 1 individuals. *Nat. Genet.* 10:94–98
238. Sharp AH, Loev SJ, Schilling G, Li S-H, Bao J, et al. 1995. Widespread expression of Huntington's disease gene (IT15) protein product. *Neuron* 14:1065–74
239. Shaw DJ, McCurrach M, Rundle SA, Harley HG, Crow SR, et al. 1993. Genomic organization and transcriptional units at the myotonic dystrophy locus. *Genomics* 18:673–79
240. Shelbourne PF, Killeen N, Hevner RF, Johnston HM, Tecott L, et al. 1999. A

Huntington's disease CAG expansion at the murine Hdh locus is unstable and associated with behavioural abnormalities in mice. *Hum. Mol. Genet.* 8:763–74

241. Sherman S. 1996. Epidemiology. In *Epidemiology*, ed. RJ Hagerman, A Cronister, pp. 165–92. Baltimore: Johns Hopkins Univ. Press
242. Sherman SL, Jacobs PA, Morton NE, Froster-Iskenius U, Howard-Peebles PN, et al. 1985. Further segregation analysis of the fragile X syndrome with special reference to transmitting. *Hum. Genet.* 69:289–99
243. Sherman SL, Morton NE, Jacobs PA, Turner G. 1984. The marker (X) syndrome: a cytogenetic and genetic analysis. *Hum. Genet.* 48:21–37
244. Simeoni S, Mancini MA, Stenoien DL, Marcelli M, Weigel NL, et al. 2000. Motoneuronal cell death is not correlated with aggregate formation of androgen receptors containing an elongated polyglutamine tract. *Hum. Mol. Genet.* 9:133–44
245. Siomi H, Choi M, Siomi MC, Nussbaum RL, Dreyfuss G. 1994. Essential role for KH domains in RNA binding: impaired RNA binding by a mutation in the KH domain of FMR1 that causes fragile X syndrome. *Cell* 77:33–39
246. Siomi MC, Siomi H, Sauer WH, Srinivasan S, Nussbaum RL, Dreyfuss G. 1995. FXR1, an autosomal homolog of the fragile X mental retardation gene. *EMBO J.* 14:2401–8
247. Sittler A, Walter S, Wedemeyer N, Hasenbank R, Scherzinger E, et al. 1998. SH3GL3 associates with the Huntingtin exon 1 protein and promotes the formation of polygln-containing protein aggregates. *Mol. Cell* 2:427–36
248. Skinner PJ, Koshy B, Cummings C, Klement IA, Helin K, et al. 1997. Ataxin-1 with extra glutamines induces alterations in nuclear matrix–associated structures. *Nature* 389:971–74
249. Smith JK. 1975. Dentatorubropallidoluysian atrophy. In *Dentatorubropallidoluysian atrophy*, ed. PJ Vinken, GW Bruyn, pp. 519–34. Amsterdam: North Holland
250. Sobue G, Hashizume Y, Mukai E, Hirayama M, Mitsuma T, et al. 1989. X-linked recessive bulbospinal neuronopathy: a clinicopathological study. *Brain* 112:209–32
251. Stenoien DL, Cummings CJ, Adams HP, Mancini MG, Patel K, et al. 1999. Polyglutamine-expanded androgen receptors form aggregates that sequester heat shock proteins, proteasome components and SRC-1, and are suppressed by the HDJ-2 chaperone. *Hum. Mol. Genet.* 8:731–41
252. Stevanin G, Giunti P, Belal GDS, Durr A, Ruberg M, et al. 1998. De novo expansion of intermediate alleles in spinocerebellar ataxia 7. *Hum. Mol. Genet.* 7: 1809–13
253. Stott K, Blackburn JM, Butler PJG, Perutz M. 1995. Incorporation of glutamine repeats makes protein oligomerize: implications for neurodegenerative diseases. *Proc. Natl. Acad. Sci. USA* 92:6509–13
254. Sutcliffe JS, Nelson DL, Zhang F, Pieretti M, Caskey CT, et al. 1992. DNA methylation represses FMR-1 transcription in fragile X syndrome. *Hum. Mol. Genet.* 1:397–400
255. Tait D, Riccio M, Sittler A, Scherzinger E, Santi S, et al. 1998. Ataxin-3 is transported into the nucleus and associates with the nuclear matrix. *Hum. Mol. Genet.* 7:991–97
256. Takahashi H, Ohama E, Naito H, Takeda S, Nakashima S, et al. 1988. Hereditary dentatorubral-pallidoluysian atrophy: clinical and pathologic variants in a family. *Neurology* 38:1065–70
257. Takiyama Y, Oyanagi S, Kawashima S, Sakamoto H, Saito K, et al. 1994. A clinical and pathologic study of a large Japanese family with Machado-Joseph disease tightly linked to the DNA

markers on chromosome 14q. *Neurology* 44:1302–8
258. Tamanini F, Willemsen R, van Unen L, Bontekoe C, Galjaard H, et al. 1997. Differential expression of FMR1, FXR1 and FXR2 proteins in human brain and testis. *Hum. Mol. Genet.* 6:1315–22
259. Taneja KL, McCurrach M, Schalling M, Housman D, Singer RH. 1995. Foci of trinucleotide repeat transcripts in nuclei of myotonic dystrophy cells and tissues. *J. Cell Biol.* 128:995–1002
260. Thornton CA, Johnson K, Moxley RT, 3rd. 1994. Myotonic dystrophy patients have larger CTG expansions in skeletal muscle than in leukocytes. *Ann. Neurol.* 35:104–7
261. Thornton CA, Wymer JP, Simmons Z, McClain C, Moxley RT, 3rd. 1997. Expansion of the myotonic dystrophy CTG repeat reduces expression of the flanking DMAHP gene. *Nat. Genet.* 16:407–9
262. Timchenko LT, Caskey CT. 1999. Triplet repeat disorders: discussion of molecular mechanisms. *Cell. Mol. Life Sci.* 55:1432–47
263. Timchenko LT, Miller JW, Timchenko NA, DeVore DR, Datar KV, et al. 1996. Identification of a (CUG)n triplet repeat RNA-binding protein and its expression in myotonic dystrophy. *Nucleic Acids Res.* 24:4407–14
264. Timchenko LT, Timchenko NA, Caskey CT, Roberts R. 1996. Novel proteins with binding specificity for DNA CTG repeats and RNA CUG repeats: implications for myotonic dystrophy. *Hum. Mol. Genet.* 5:115–21
265. Tran PB, Miller RJ. 1999. Aggregates in neurodegenerative disease: crowds and power? *Trends Neurosci.* 22:194–97
266. Trottier Y, Devys D, Imbert G, Saudou F, An I, et al. 1995. Cellular localization of the Huntington's disease protein and discrimination of the normal and mutated form. *Nat. Genet.* 10:104–10
267. Trottier Y, Imbert G, Poustka A, Fryns JP, Mandel JL. 1994. Male with typical fragile X phenotype is deleted for part of the FMR1 gene and for about 100 kb of upstream region. *Am. J. Med. Genet.* 51:454–57
268. Trottier Y, Lutz Y, Stevanin G, Imbert G, Devys D, et al. 1995. Polyglutamine expansion as a pathological epitope in Huntington's disease and four dominant cerebellar ataxias. *Nature* 378:403–6
269. Tsilfidis C, MacKenzie AE, Mettler G, Barcel J, Korneluk RG. 1992. Correlation between CTG trinucleotide repeat length and frequency of severe. *Nat. Genet.* 1:192–95
270. Turner G, Webb T, Wake S, Robinson H. 1996. Prevalence of fragile X syndrome. *Am. J. Med. Genet.* 64:196–97
271. Usdin MT, Shelbourne PF, Myers RM, Madison DV. 1999. Impaired synaptic plasticity in mice carrying the Huntington's disease mutation. *Hum. Mol. Genet.* 8:839–46
272. Verheij C, Bakker CE, de Graaff E, Keulemans J, Willemsen R, et al. 1993. Characterization and localization of the FMR-1 gene product associated with fragile X. *Nature* 363:722–24
273. Verkerk AJMH, Pieretti M, Sutcliffe JS, Fu Y-H, Kuhl DPA, et al. 1991. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* 65:905–14
274. Vonsattel JP, DiFiglia M. 1998. Huntington disease. *J. Neuropathol. Exp. Neurol.* 57:369–84
275. Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, et al. 1985. Neuropathological classification of Huntington's disease. *J. Neuropathol. Exp. Neurol.* 44:559
276. Wang J, Pegoraro E, Menegazzo E, Gennarelli M, Hoop RC, et al. 1995. Myotonic dystrophy: evidence for a possible dominant-negative RNA mutation. *Hum. Mol. Genet.* 4:599–606

277. Wang YH, Gellibolian R, Shimizu M, Wells RD, Griffith J. 1996. Long CCG triplet repeat blocks exclude nucleosomes: a possible mechanism for the nature of fragile sites in chromosomes. *J. Mol. Biol.* 263:511–16
278. Wang YH, Griffith J. 1996. Methylation of expanded CCG triplet repeat DNA from fragile X syndrome patients enhances nucleosome exclusion. *J. Biol. Chem.* 271:22937–40
279. Waragai M, Lammers CH, Takeuchi S, Imafuku I, Udagawa Y, et al. 1999. PQBP-1, a novel polyglutamine tract-binding protein, inhibits transcription activation by Brn-2 and affects cell survival. *Hum. Mol. Genet.* 8:977–87
280. Warner TT, Williams L, Harding AE. 1994. DRPLA in Europe. *Nat. Genet.* 6:225
281. Warrick JM, Chan HY, Gray-Board GL, Chai Y, Paulson HL, Bonini NM. 1999. Suppression of polyglutamine-mediated neurodegeneration in Drosophila by the molecular chaperone HSP70. *Nat. Genet.* 23:425–28
282. Warrick JM, Paulson HL, Gray-Board GL, Bui QT, Fischbeck KH, et al. 1998. Expanded polyglutamine protein forms nuclear inclusions and causes neural degeneration in Drosophila. *Cell* 93:939–49
283. Wellington CL, Ellerby LM, Hackam AS, Margolis RL, Trifiro MA, et al. 1998. Caspase cleavage of gene products associated with triplet expansion disorders generates truncated fragments containing the polyglutamine tract. *J. Biol. Chem.* 273:9158–67
284. Wells RD. 1996. Molecular basis of genetic instability of triplet repeats. *J. Biol. Chem.* 271:2875–78
285. Wexler NS, Young AB, Tanzi RE, Travers H, Starosta-Rubinstein S, et al. 1987. Homozygotes for Huntington's disease. *Nature* 326:194–97
286. White JK, Auerbach W, Duyao MP, Vonsattel JP, Gusella JF, et al. 1997. Huntingtin is required for neurogenesis and is not impaired by the Huntington's disease CAG expansion. *Nat. Genet.* 17:404–10
287. Wilson RB, Roof DM. 1997. Respiratory deficiency due to loss of mitochondrial DNA in yeast lacking the frataxin homologue. *Nat. Genet.* 16:352–57
288. Wong A, Yang J, Cavadini P, Gellera C, Lonnerdal B, et al. 1999. The Friedreich's ataxia mutation confers cellular sensitivity to oxidant stress which is rescued by chelators of iron and calcium and inhibitors of apoptosis. *Hum. Mol. Genet.* 8:425–30
289. Wood JD, Yuan J, Margolis RL, Colomer V, Duan K, et al. 1998. Atrophin-1, the DRPLA gene product, interacts with two families of WW domain-containing proteins. *Mol. Cell. Neurosci.* 11:149–60
290. Woods BT, Schaumburg HH. 1972. Nigro-spino-dentatal degeneration with nuclear ophthalmoplegia: a unique and partially treatable clinico-pathological entity. *J. Neurol. Sci.* 17:149–66
291. Yazawa I, Nukina N, Hashida H, Goto J, Yamada M, Kanazawa I. 1995. Abnormal gene product identified in hereditary dentatorubral-pallidoluysian atrophy (DRPLA) brain. *Nat. Genet.* 10:99–103
292. Zeitlin S, Liu J-P, Chapman DL, Papaioannou VE, Efstratiadis A. 1995. Increased apoptosis and early embryonic lethality in mice nullizygous for the Huntington's disease gene homologue. *Nat. Genet.* 11:155–63
293. Zhang JF, Randall AD, Ellinor PT, Horne WA, Sather WA, et al. 1993. Distinctive pharmacology and kinetics of cloned neuronal Ca2+ channels and their possible counterparts in mammalian CNS neurons. *Neuropharmacology* 32:1075–88
294. Zhang Y, O'Connor JP, Siomi MC, Srinivasan S, Dutra A, et al. 1995. The fragile X mental retardation syndrome protein interacts with novel homologs FXR1 and FXR2. *EMBO J.* 14:5358–66
295. Zhou ZX, Wong CI, Sar M, Wilson

EM. 1994. The androgen receptor: an overview. *Recent Prog. Horm. Res.* 49:249–74

296. Zhuchenko O, Bailey J, Bonnen P, Ashizawa T, Stockton DW, et al. 1997. Autosomal dominant cerebellar ataxia (SCA6) associated with small polyglutamine expansions in the α1A-voltage-dependent calcium channel. *Nat. Genet.* 15:62–69
297. Zoppi S, Wilson CM, Harbison MD, Griffin JE, Wilson JD, et al. 1993. Complete testicular feminization caused by an amino-terminal truncation of the androgen receptor with downstream initiation. *J. Clin. Invest.* 91:1105–12