TRIPLET EXPANSION AND DISEASE

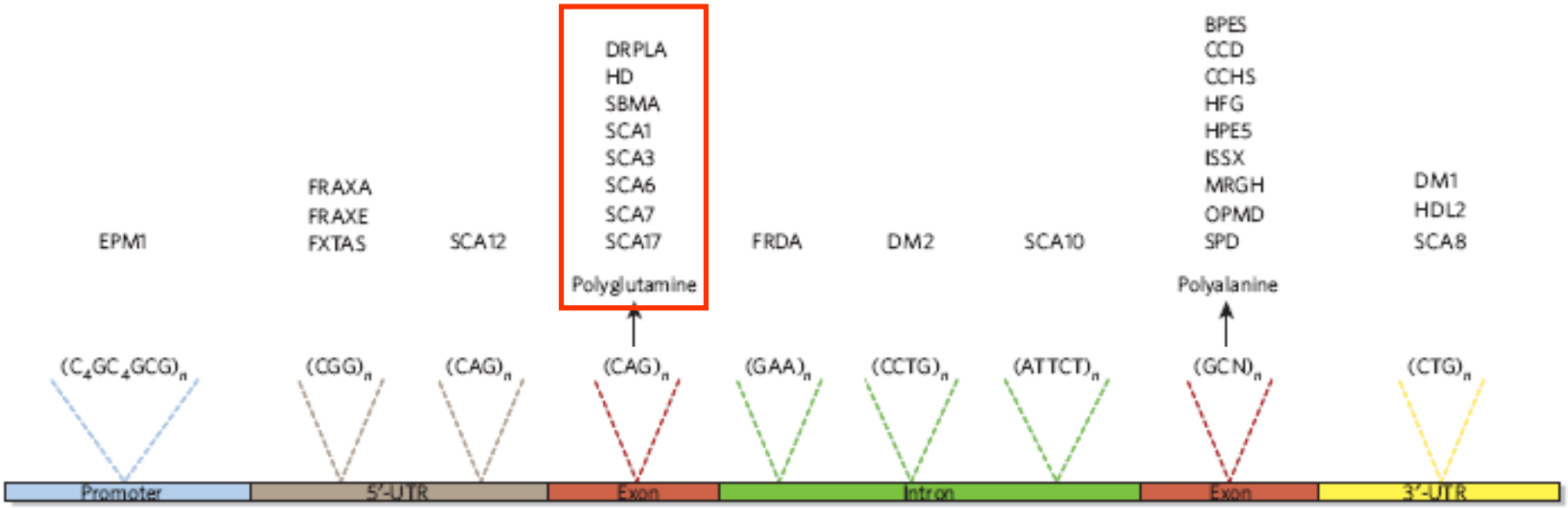
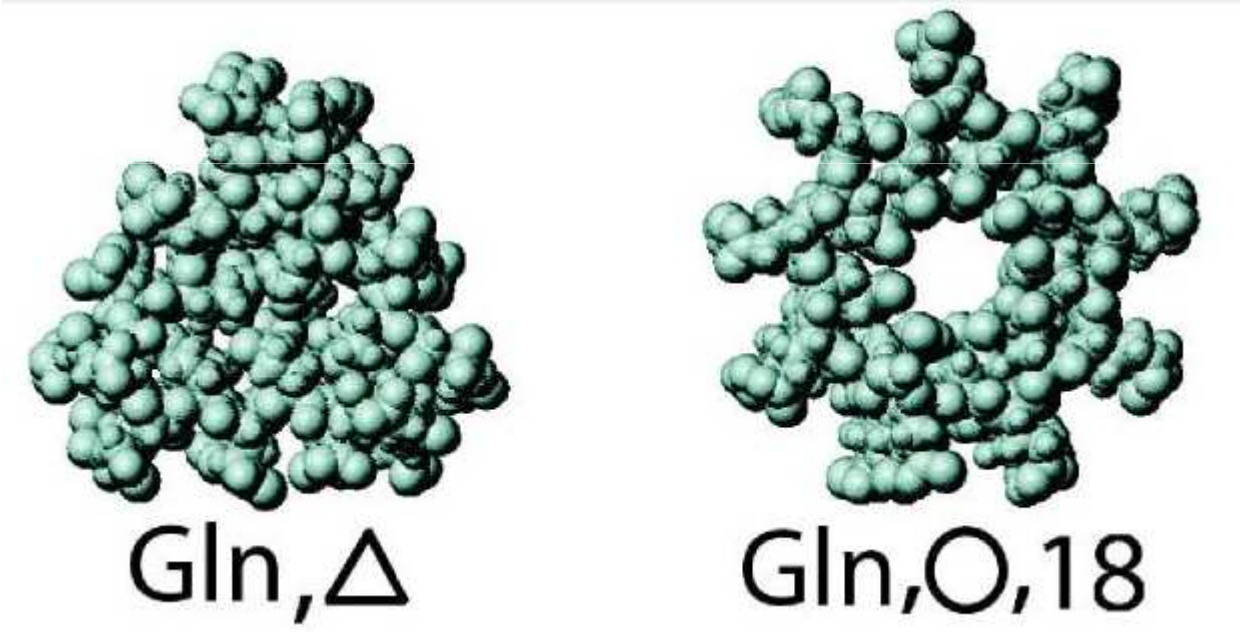


Figure 1 | Location of expandable repeats responsible for human diseases. The sequence and location within a generic gene of expandable repeats that cause human diseases are shown, and the associated diseases are listed. BPES, blepharophimosis, ptosis and epicanthus inversus; CCD, cleidocranial dysplasia; CCHS, congenital central hypoventilation syndrome; DM, myotonic dystrophy; DRPLA, dentatorubral–pallidoluysian atrophy; EPM1, progressive myoclonic epilepsy 1; FRAXA, fragile X syndrome; FRAXE, fragile X mental retardation associated with *FRAXE* site; FRDA, Friedreich's ataxia; FXTAS, fragile X tremor and ataxia syndrome; HD, Huntington's disease; HDL2, Huntington's-disease-like 2; HFG, hand–foot–genital syndrome; HPE5, holoprosencephaly 5; ISSX, X-linked infantile spasm syndrome; MRGH, mental retardation with isolated growth hormone deficiency; OPMD, oculopharyngeal muscular dystrophy; SBMA, spinal and bulbar muscular atrophy; SCA, spinocerebellar ataxia; SPD, synpolydactyly.

Poly Gln



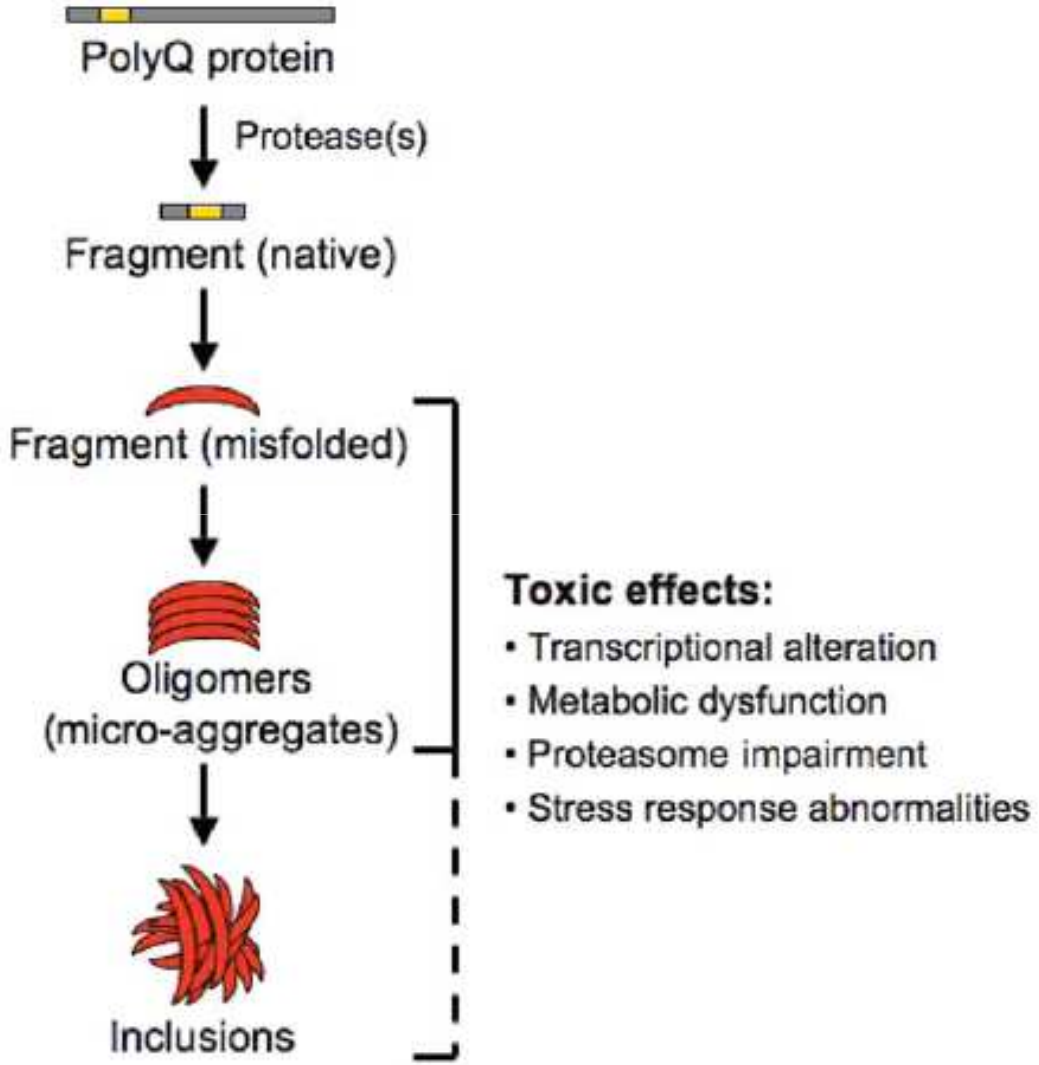
Space-filling models of various three-coiled β-helices viewed along their axes

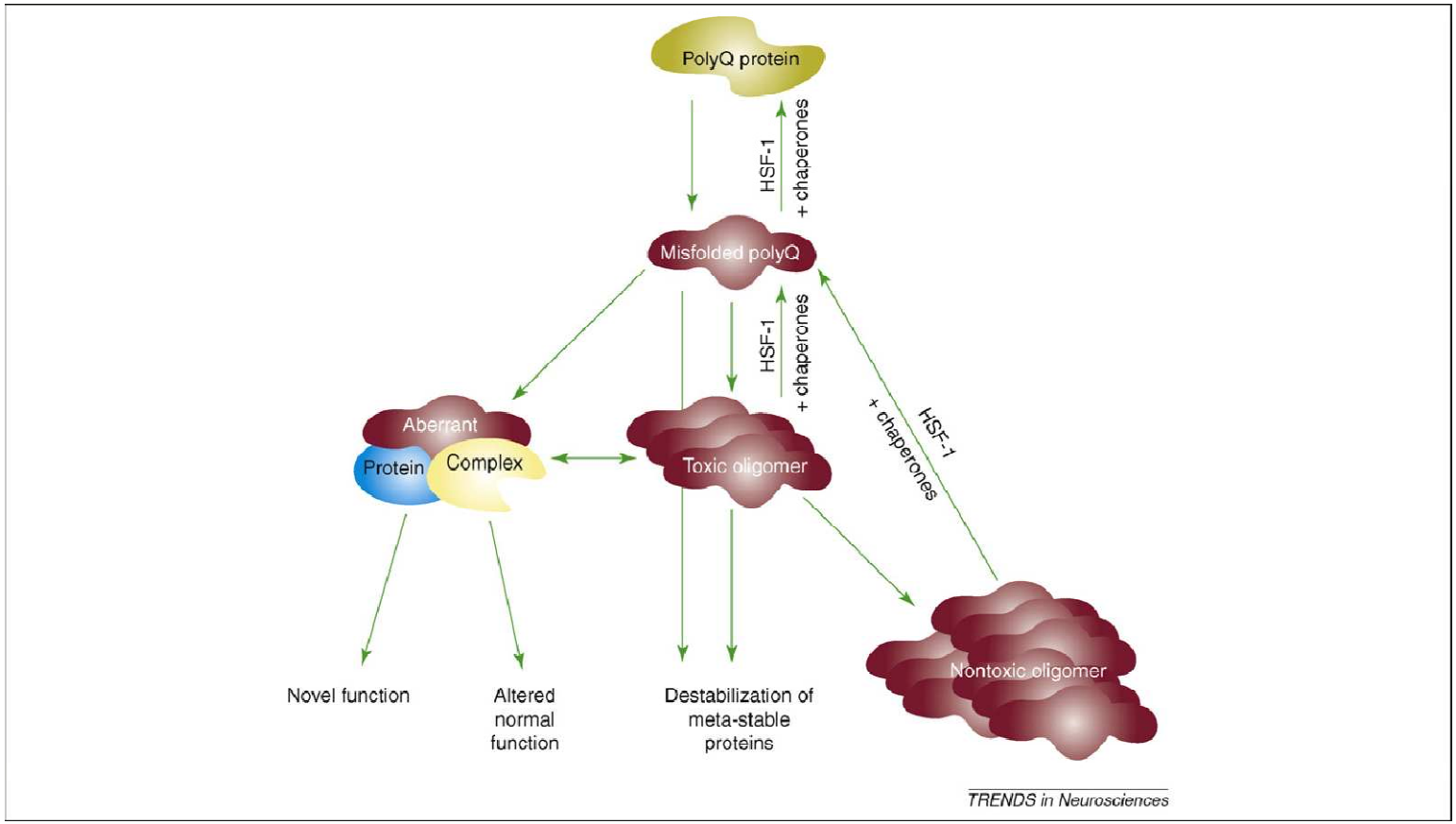
Table 1. Polyglutamine diseases: emerginig concepts in pathogenesis and therapy

Disease	Protein	Repeat	Normal repeat length	Pathogenic repeat length	Inclusions	Brain regions most affected
Typical polyglutamine diseases (gain of function)						
HD	Huntingtin	CAG	6–34	36–121	Nucleus and cytoplasm	Striatum, cerebral cortex
SBMA	Androgen receptor	CAG	9–36	38–62	Nucleus and cytoplasm	Anterior horn and bulbar neurons, dorsal root ganglia
DRPLA	Atrophin 1	CAG	7–34	49–88	Nucleus	Cerebellum, cerebral cortex, basal ganglia, Luys body
SCA1	Ataxin 1	CAG	6–39	40–82	Nucleus	Cerebellar Purkinje cells, dentate nucleus, brainstem
SCA2	Ataxin 2	CAG	15–24	32–200	Nucleus	Cerebellar Purkinje cells, brain stem, frontotemporal lobes
SCA3	Ataxin 3	CAG	13–36	61–84	Nucleus	Cerebellar dentate neurons, basal ganglia, brain stem, spinal cord
SCA7	Ataxin 7	CAG	4–35	37–306	Nucleus	Cerebellum, brain stem, macula, visual cortex
SCA17	TATA box binding protein	CAG	25–42	47–63	Nucleus	Cerebelllar Purkinje cells, inferior olive
Atypical polyglutamine disease (mimicked by missense mutation)						
SCA6	α1a voltage-dependent calcium channel subunit	CAG	4–20	20–29	Cytoplasm	Cerebellar Purkinje cells, dentate nucleus, inferior olive
Atypical polyglutamine disease (reverse transcription of CTG repeats)						
SCA8	Unknown	CTG	16–34	>74	Nucleus	Cerebellar Purkinje cells, granule cells, inferior olive

Table 1. Polyglutamine disease proteins and their functions

Disease	Protein name	Protein size, polyQ position and disease repeat range	Protein function
SBMA	Androgen receptor		Testosterone-activated steroid receptor
HD	Huntingtin		Possible scaffolding protein linked to diverse cellular pathways
DRPLA	Atrophin-1		Possible transcriptional corepressor
SCA1	Ataxin-1		Transcriptional corepressor involved in transcription regulation, cell specification and synaptic activity
SCA2	Ataxin-2		Component of RNA processing and translational regulation pathways
SCA3	Ataxin-3		Deubiquitinating enzyme involved in protein quality control
SCA6	P/Q-type calcium-channel subunit α 1A		Voltage-sensitive calcium-channel subunit
SCA7	Ataxin-7		Component of histone acetyltransferase complex (TFTC/STAGA) and transcriptional regulation pathways
SCA17	TATA-box-binding protein		Component of core transcriptional complex TRID


PolyQ protein
Protease(s)
Fragment (native)
Fragment (misfolded)
Oligomers
(micro-aggregates)
Inclusions
Toxic effects:
• Transcriptional alteration
• Metabolic dysfunction
• Proteasome impairment
• Stress response abnormalities


PolyQ protein
HSF-1
+ chaperones
Misfolded polyQ
HSF-1
+ chaperones
HSF-1
+ chaperones
Aberrant
Protein
Complex
Toxic oligomer
Nontoxic oligomer
Novel function
Altered
normal
function
Destabilization of
meta-stable
proteins
TRENDS in Neurosciences

Modelli animali

Table 2. Genetic modifiers of polyglutamine toxicity in animal models

Name	Class	Function	Effect on polyQ toxicity	Animal model
Hsp70	Chaperone	Binds unfolded proteins, ATP hydrolysis	Suppressor	Fly, worm
Hsp60/TRiC/CCT	Chaperone	Binds unfolded proteins, ATP hydrolysis	Suppressor	Worm
Hsp40	Chaperone	Binds unfolded proteins, cochaperone for Hsp70	Suppressor	Fly, worm
αB-Crystallin	Chaperone	Small heat-shock protein	Suppressor	Fly
VCP	Chaperone, ERAD	Translocation of substrates from ER to cytosol, ATP hydrolysis	Suppressor	Fly
CHIP	Chaperone UPS	Binds chaperones, ubiquitin ligase	Suppressor	Fly, mouse
E6-AP	Chaperone UPS	Ubiquitin ligase, might functionally interact with Hsp70	Suppressor	Mouse
E4B	UPS	Ubiquitin ligase	Suppressor	Fly
Ubiquitin	UPS	Targets proteins for degradation, various cellular processes	Suppressor	Fly, worm
Uba	UPS	Ubiquitin-activating enzyme	Suppressor	Worm
Ubc-E2H	UPS	Ubiquitin-conjugating enzyme	Suppressor	Fly
Usp9X/fat facets	UPS	Deubiquitinating enzyme	Suppressor	Fly
Proteasome core subunits	UPS	Protein degradation	Suppressor	Worm
Proteasome cap subunits	UPS	Regulation of proteasome activity	Variable (depends on specific cap subunit)	Fly, worm
Atg proteins (atg6, atg7, atg12, atg18)	Autophagy	Components of autophagic cycle	Suppressors	Fly, worm
HDAC6	Autophagy	Histone deacetylase	Suppressor	Fly
14–3-3	Signal transduction	Binds phosphorylated proteins	Enhancer	Fly
Akt	Signal transduction	Serine/threonine kinase	Variable (depends on polyQ disease)	Fly
RhoGAP	Signal transduction	Regulates GTPases	Enhancer	Fly
α- and β-tubulin	Cytoskeleton	Vesicle trafficking, cell structure	Suppressor	Worm
Exportin-1	Nuclear export	Binds and transports proteins	Suppressor	Fly
HSF-1	Transcription factor	Binds DNA, regulates expression of chaperones	Suppressor	Worm
Ribosomal proteins	Protein synthesis	Protein synthesis, binds mRNA	Suppressor	Worm

UPS- ubiquitinproteasome system

ERAD- endoplasmic reticulum-associated degradation

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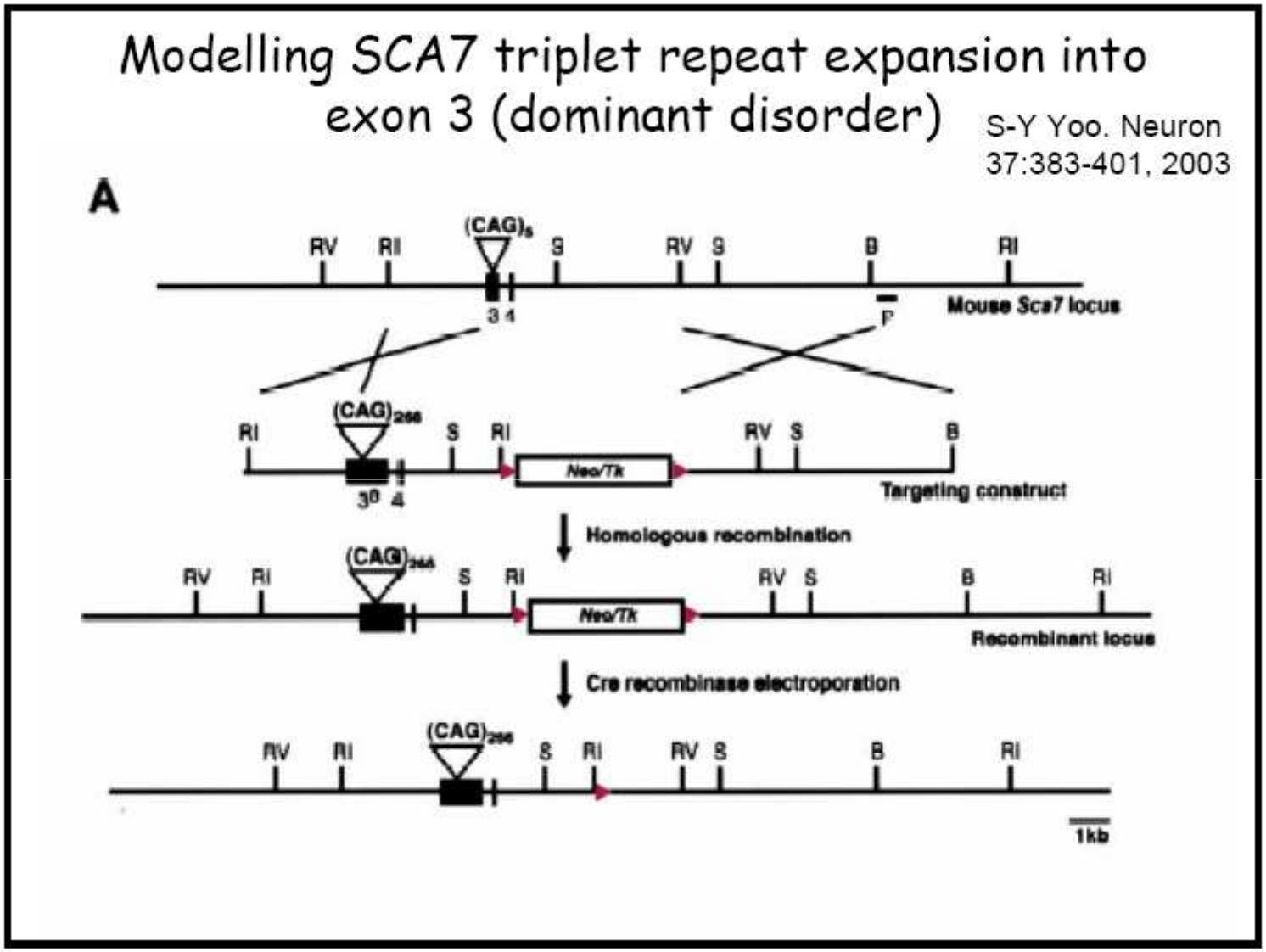
Atassia SpinoCerebellare

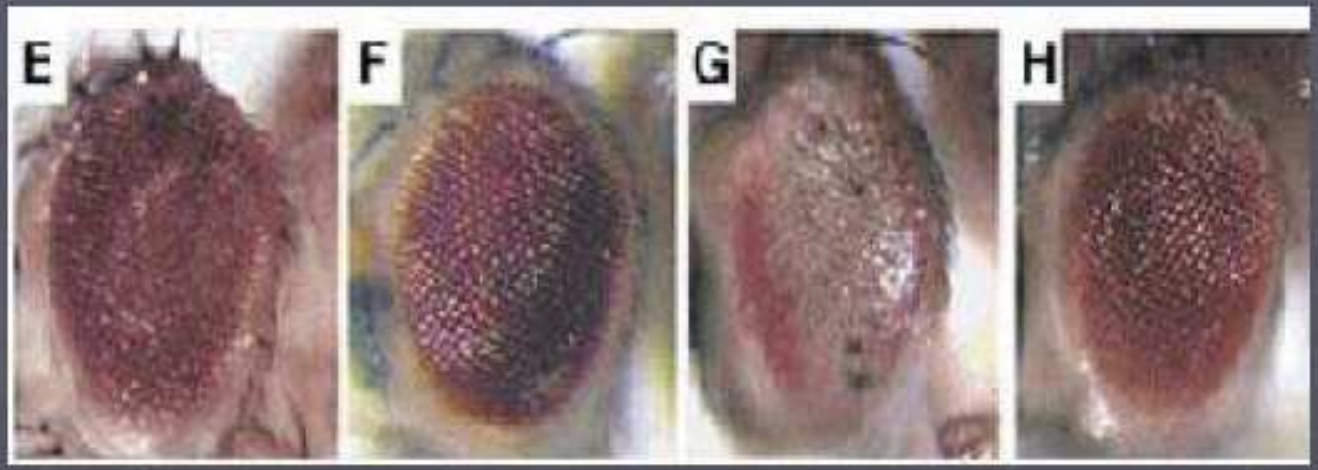


SCA7 knockin mice model human SCA7 and reveal gradual accumulation of mutant ataxin-7 in neurons

- We targeted 266 CAG repeats (a number that causes infantile-onset disease) into the mouse Sca7 locus to generate an authentic model of spinocerebellar ataxia type 7 (SCA7).
- These mice reproduced features of infantile SCA7 (ataxia, visual impairments, and premature death) and showed impaired short-term synaptic potentiation; downregulation of photoreceptor-specific genes, led to shortening of photoreceptor outer segments.
- Neurons that appeared most vulnerable had relatively high levels of mutant ataxin-7; marked dysfunction occurred in these neurons weeks prior to the appearance of nuclear inclusions.
- These data demonstrate that glutamine expansion stabilizes mutant ataxin-7 and provide an explanation for selective neuronal vulnerability.

Rescue from polyglutamine toxicity in Drosophila

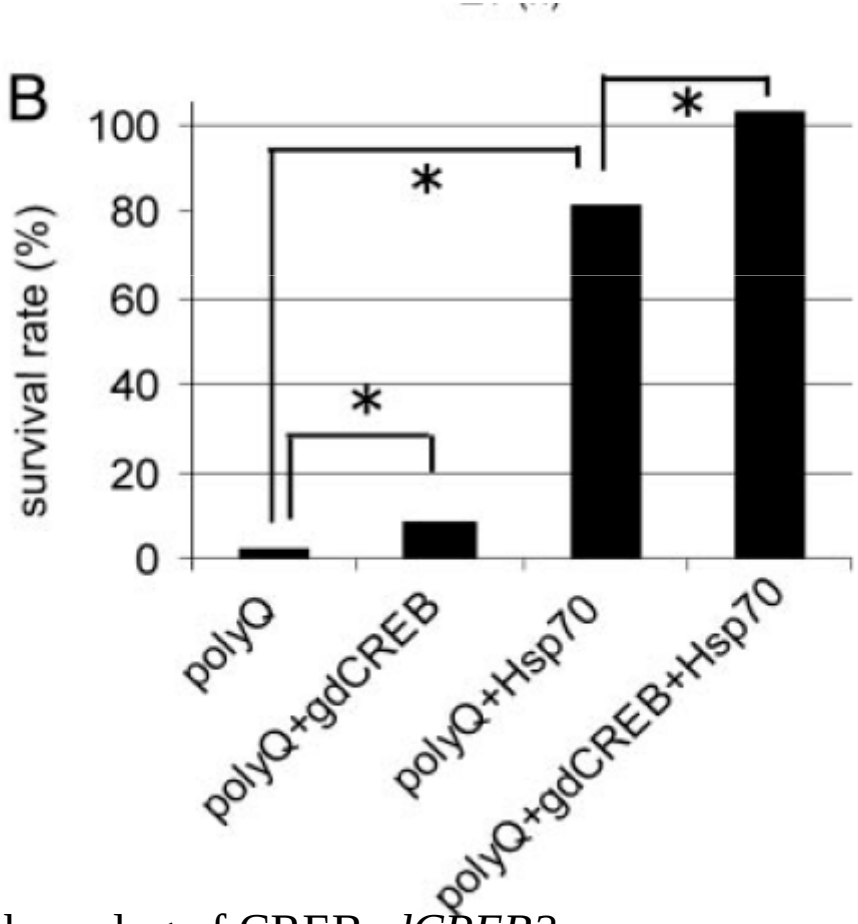
Normal Hsp70 PolyGlu Hsp70 and PolyGlu



cAMP-response element-binding protein and heat-shock protein 70 additively suppress polyglutamine-mediatedtoxicity in Drosophila

Kanae Iijima-Ando†, Priscilla Wu, Eric A. Drier‡, Koichi Iijima, and Jerry C. P. Yin§

PNAS **July 19, 2005** vol. 102 no. 29 **10261–10266**



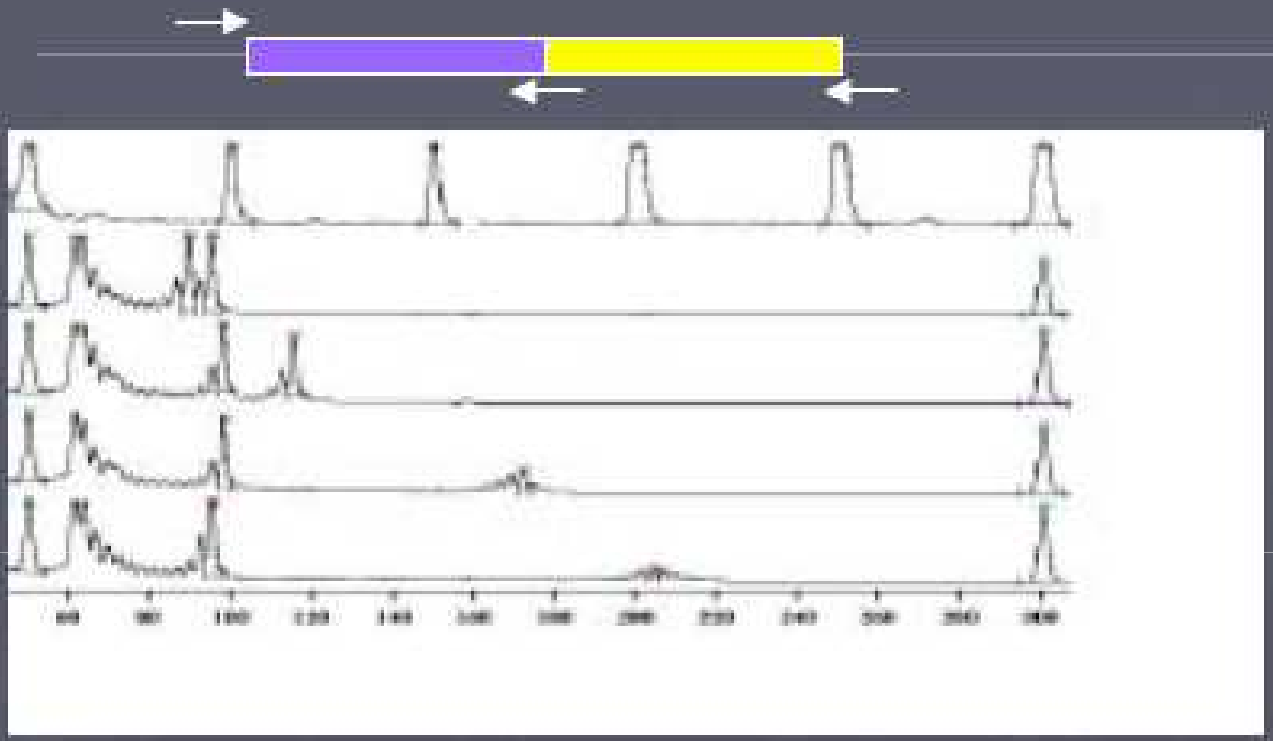
mutation in the *Drosophila* homolog of CREB, *dCREB2*

The protective effect of Hsp70 and dCREB2 against Q108-mediated lethality are additive.

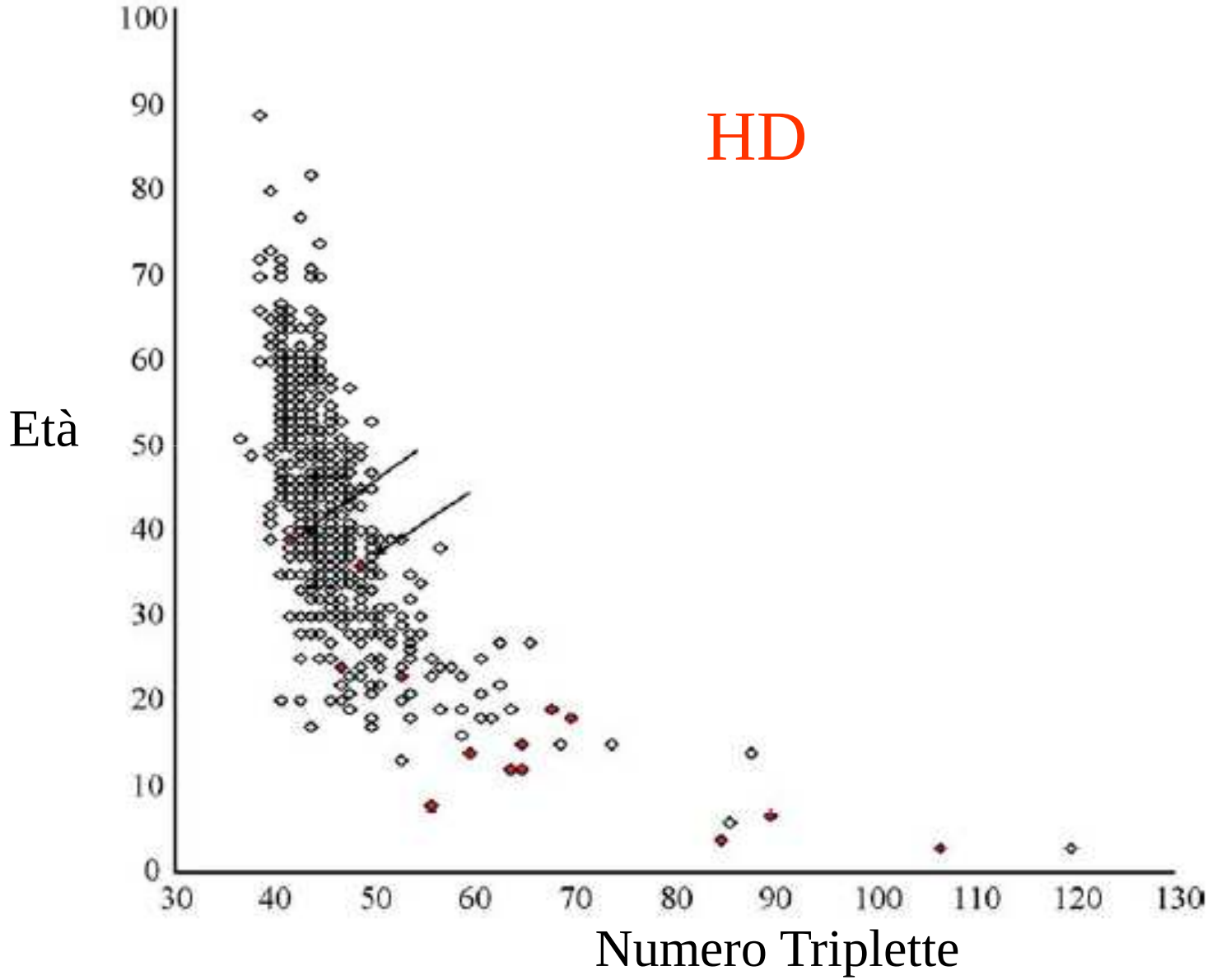
The survival rates for the flies with polyQ only, polyQ plus an extracopy of dCREB2 (polyQgdCREB), polyQ plus Hsp70 (polyQHsp70), and polyQ plus both gdCREB and Hsp70 (polyQgdCREBHsp70) are shown.

There is a significant difference between polyQ and polyQgdCREB, polyQ and polyQHsp70, and polyQHsp70 and polyQgdCREBHsp70

Huntington Disease PCR



CAG repeats	*Allele description*
≤ 26	*Normal allele*
27 - 35	*Mutable normal allele*
36 - 39	*HD allele with reduced penetrance*
≥ 40	*HD allele*


HD
Età
Numero Triplette
100
90
80
70
60
50
40
30
20
10
0
30
40
50
60
70
80
90
100
110
120
130

Huntingtin

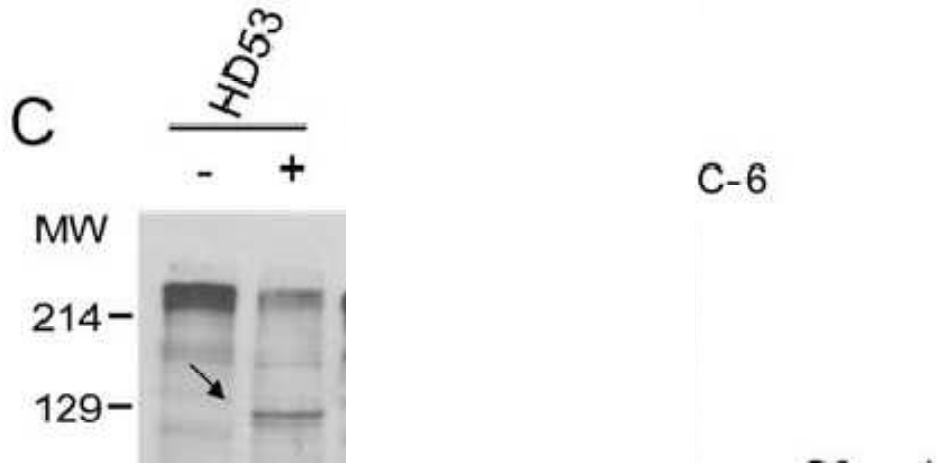
- A polyglutamine expansion in the N terminus of htt (N-htt) causes neurodegeneration in Huntington disease (HD) and accumulation of htt in neurons

Cleavage fragments of huntingtin (htt) represents an early pathological change in brains of Huntington's disease (HD) patients

Altered regulation of transcription and apoptosis in HD

Caspases cleave htt

Caspase-6 (+) cleaves mutant htt selectively in HD53 (mouse model of HD 120 CAG repeats), murine brain, resulting in a 115 kDa fragment (arrow)

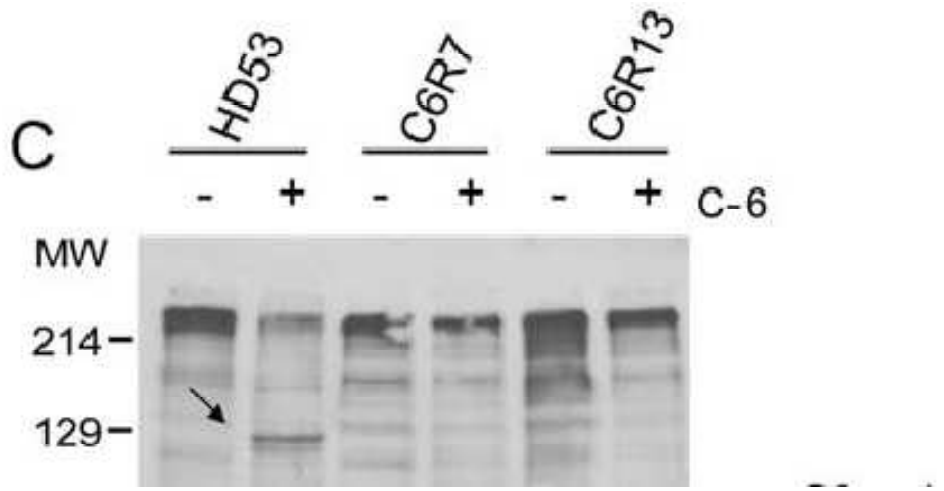


- To determine whether caspase cleavage of htt is a key event in the neuronal dysfunction and selective neurodegeneration in HD, we generated mice expressing caspase-3- and caspase-6-resistant mutant htt.

Htt exon 13 encodes aa 584 to 624 and contains the active caspase-6 site at position 586 (583IVLD586)
Mutation at residue 586 generates C6Resistant htt

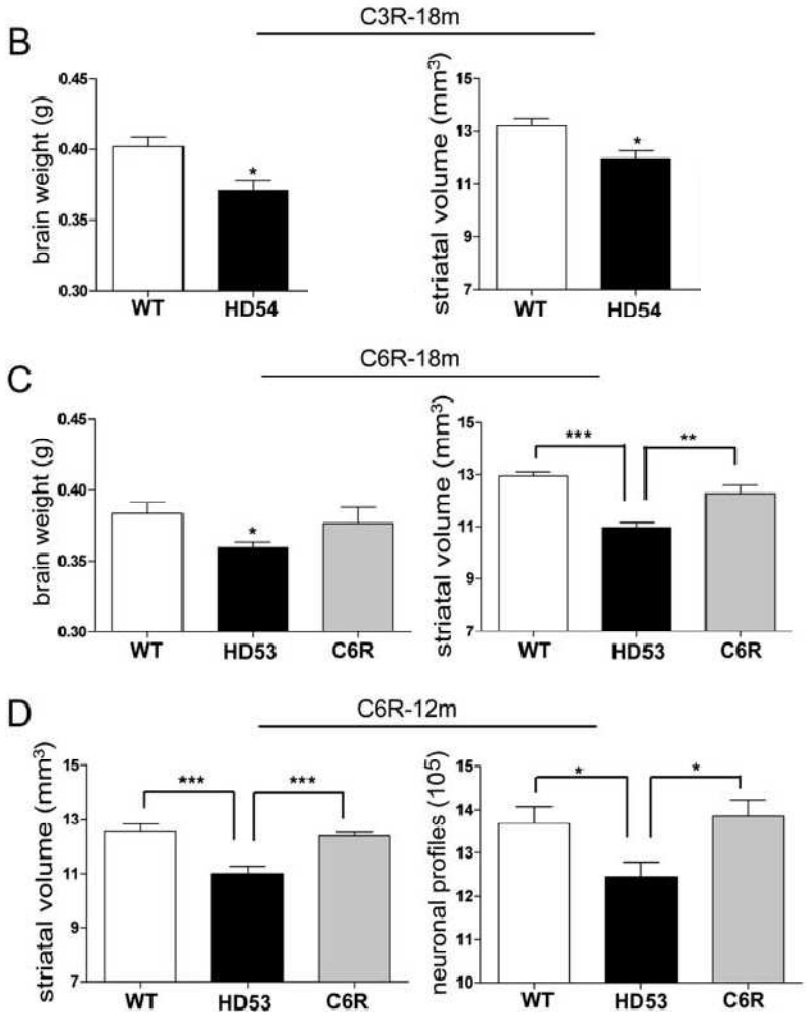
Caspase-6 (+) cleaves mutant htt selectively in HD53 (mouse model of HD 120 CAG repeats), murine brain, resulting in a 115 kDa fragment (arrow)

<u>BUT NOT C6R7 and C6R13 (htt with caspase 6 cleavage site inactivated and 133 CAG repeats),</u>



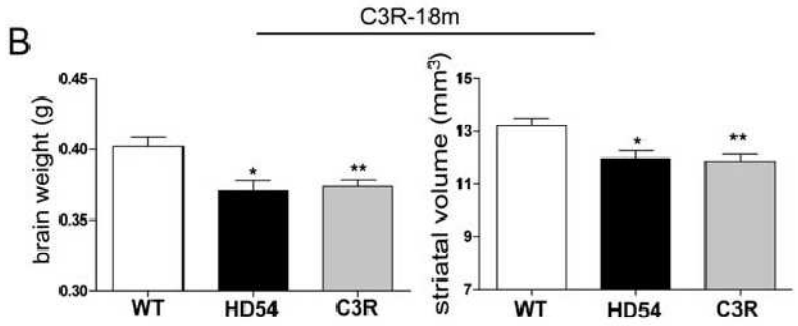
- Mice expressing mutant htt, resistant to cleavage by caspase-6 maintain normal neuronal function and do not develop striatal neurodegeneration.

Inhibition of Caspase 6 Cleavage of Mutant Huntingtin Prevents Neurodegeneration In Vivo



- Mice expressing mutant htt, resistant to cleavage by caspase-6 **but not caspase-3,** maintain normal neuronal function and do not develop striatal neurodegeneration.

Inhibition of Caspase 6 Cleavage of Mutant Huntingtin Prevents Neurodegeneration In Vivo



TRIPLET EXPANSION AND DISEASE

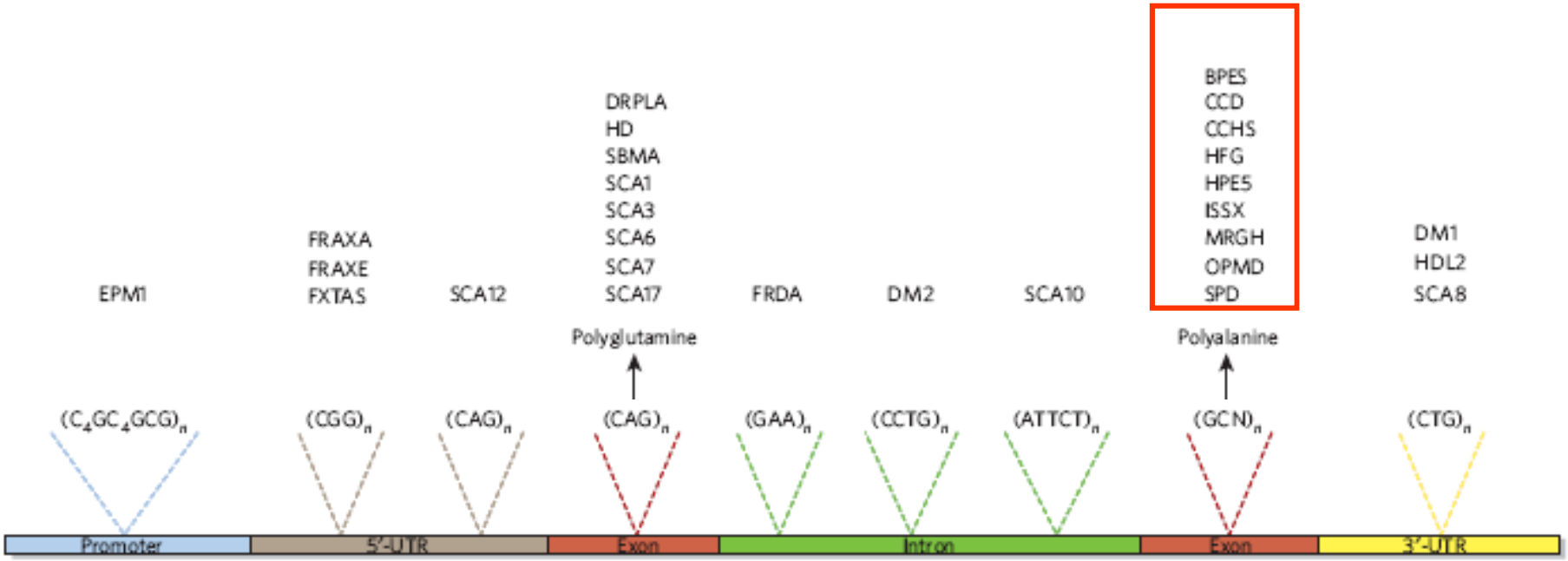
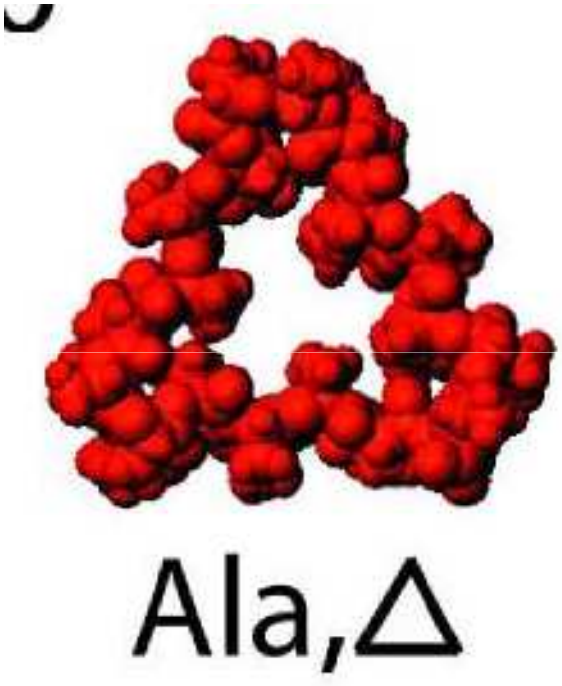


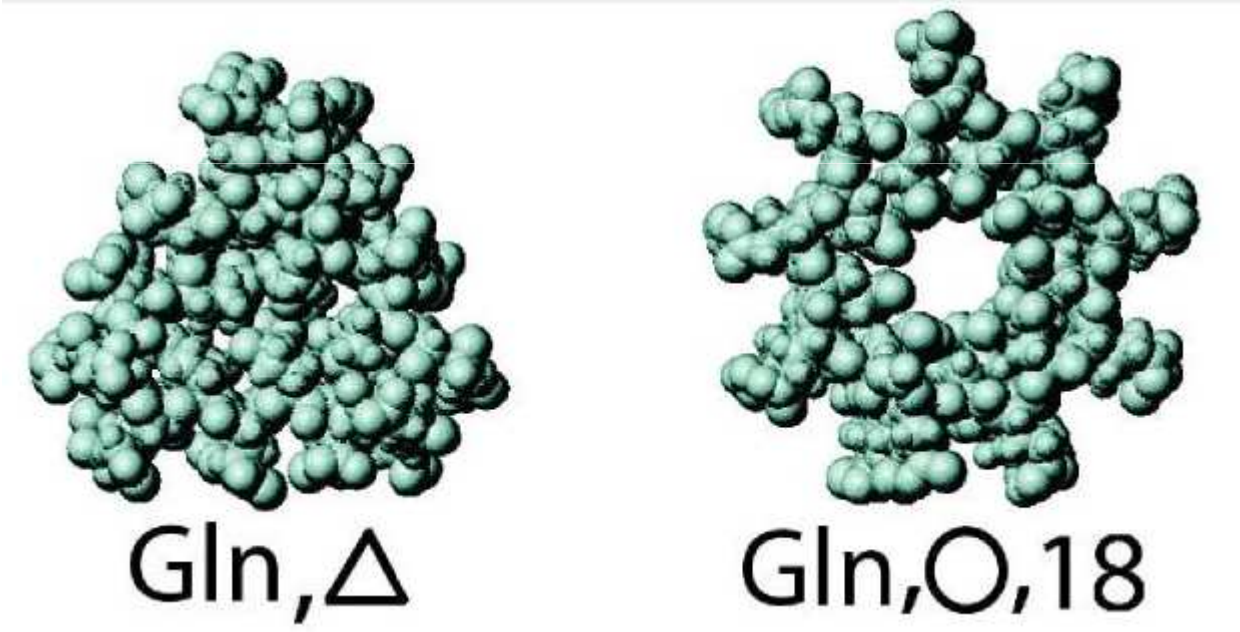
Figure 1 | Location of expandable repeats responsible for human diseases. The sequence and location within a generic gene of expandable repeats that cause human diseases are shown, and the associated diseases are listed. BPES, blepharophimosis, ptosis and epicanthus inversus; CCD, cleidocranial dysplasia; CCHS, congenital central hypoventilation syndrome; DM, myotonic dystrophy; DRPLA, dentatorubral–pallidoluysian atrophy; EPM1, progressive myoclonic epilepsy 1; FRAXA, fragile X syndrome; FRAXE, fragile X mental retardation associated with *FRAXE* site; FRDA, Friedreich's ataxia; FXTAS, fragile X tremor and ataxia syndrome; HD, Huntington's disease; HDL2, Huntington's-disease-like 2; HFG, hand–foot–genital syndrome; HPE5, holoprosencephaly 5; ISSX, X-linked infantile spasm syndrome; MRGH, mental retardation with isolated growth hormone deficiency; OPMD, oculopharyngeal muscular dystrophy; SBMA, spinal and bulbar muscular atrophy; SCA, spinocerebellar ataxia; SPD, synpolydactyly.

Poly Ala



Space-filling models of various three-coiled β-helices viewed along their axes

Poly Gln



Space-filling models of various three-coiled β-helices viewed along their axes

Table 1. Conditions and genes in which alanine tract expansion has occurred

Condition	OMIM number	Gene	Gene type	Expansion size	Proposed mutation mechanism	Protein dysfunction
Synpolydactyly type II	186000	*HOXD13*	Transcription factor	15A → 22–29A (an addition of 7–14)	Unequal recombination	Dominant negative
Cleidocranial dysplasia	119600	*RUNX2* (*CBFA1*)	Transcription factor	17A → 27A (an addition of 10)	Unequal recombination	Loss-of-function
Oculopharyngeal muscular dystrophy	164300	*PABPN1*	Polyadenylate-binding protein	10A → 11–17A (an addition of 1–7)	Replication slippage and/or unequal recombination	Toxic protein aggregates
Holoprosencephaly (HPE5)		*ZIC2*	Transcription factor	15A → 25A (an addition of 10)	Unequal recombination	Loss-of-function
Hand-foot-genital syndrome	140000	*HOXA13*	Transcription factor	18A → 24A or 26A (an addition of 6–8)	Unequal recombination	Unclear, might be dominant negative
Blepharophimosis, ptosis and epicanthus inversus	110100	*FOXL2*	Transcription factor	14A → 22–24A (an addition of 8–10)	Replication slippage	Partial loss-of-function
Mental retardation; X-linked, with isolated growth hormone deficiency	300123	*SOX3*	Transcription factor	15A → 26A (an addition of 11)	Unequal recombination	Unknown
Infantile spasm syndrome, X-linked; Partington syndrome; lissencephaly with ambiguous genitalia, X-linked; mental retardation X-linked 36 and 54	308350 309510 300215 300430 300419	*ARX*	Transcription factor	A-tract #1 (amino acids 100–115) 16A → 18 or 23A (an addition of 2 or 7) A-tract #2 (amino acids 144–155) 12A → 20A (an addition of 8)	Recombination and/or replication slippage	Partial loss-of-function
Congenital central hypoventilation syndrome/Ondine curse	209880	*PMX2B* (PHOX2B)	Transcription factor	20A → 25–29A (an addition of 5–9)	Unequal recombination	Loss-of-function

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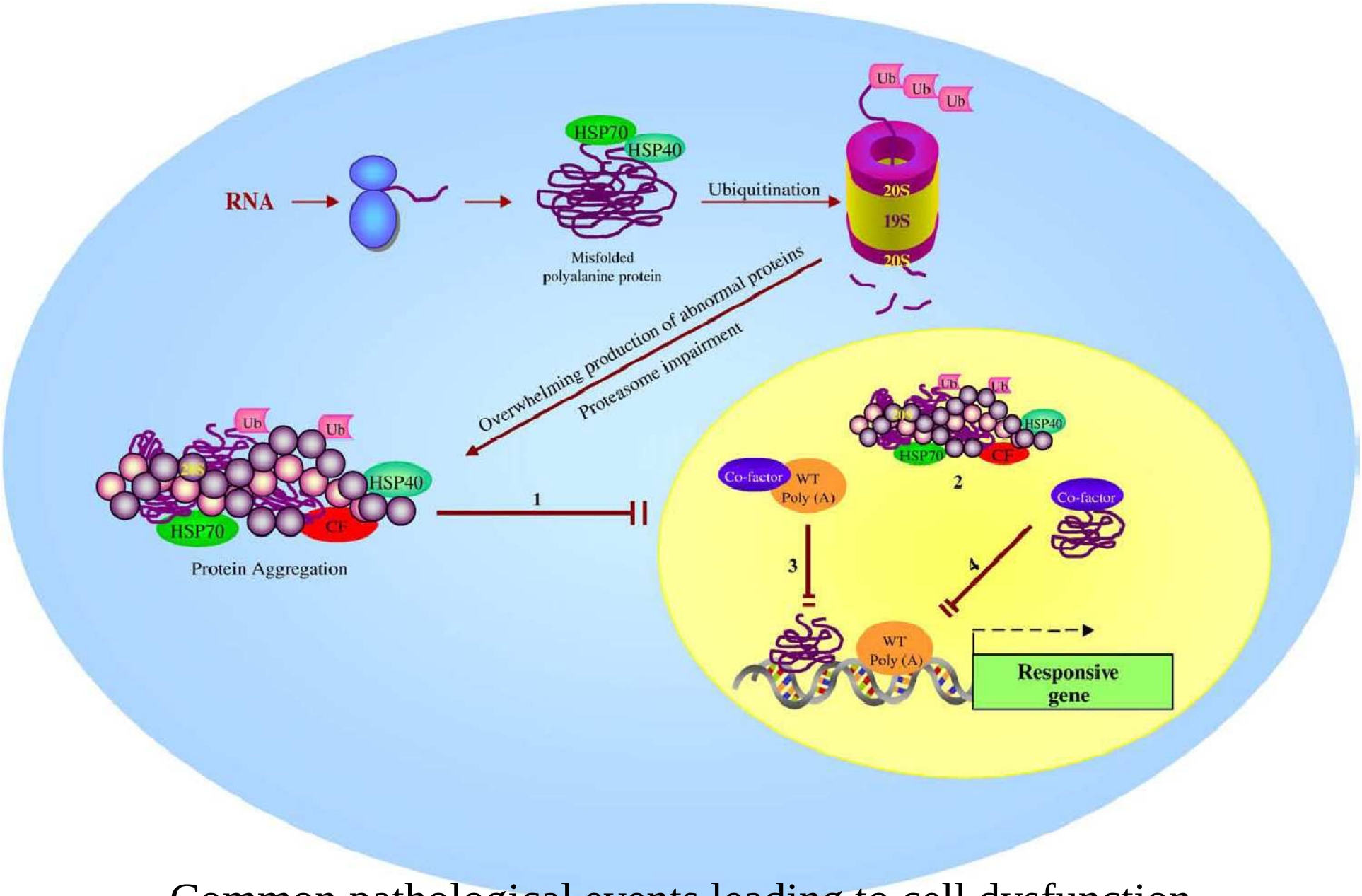
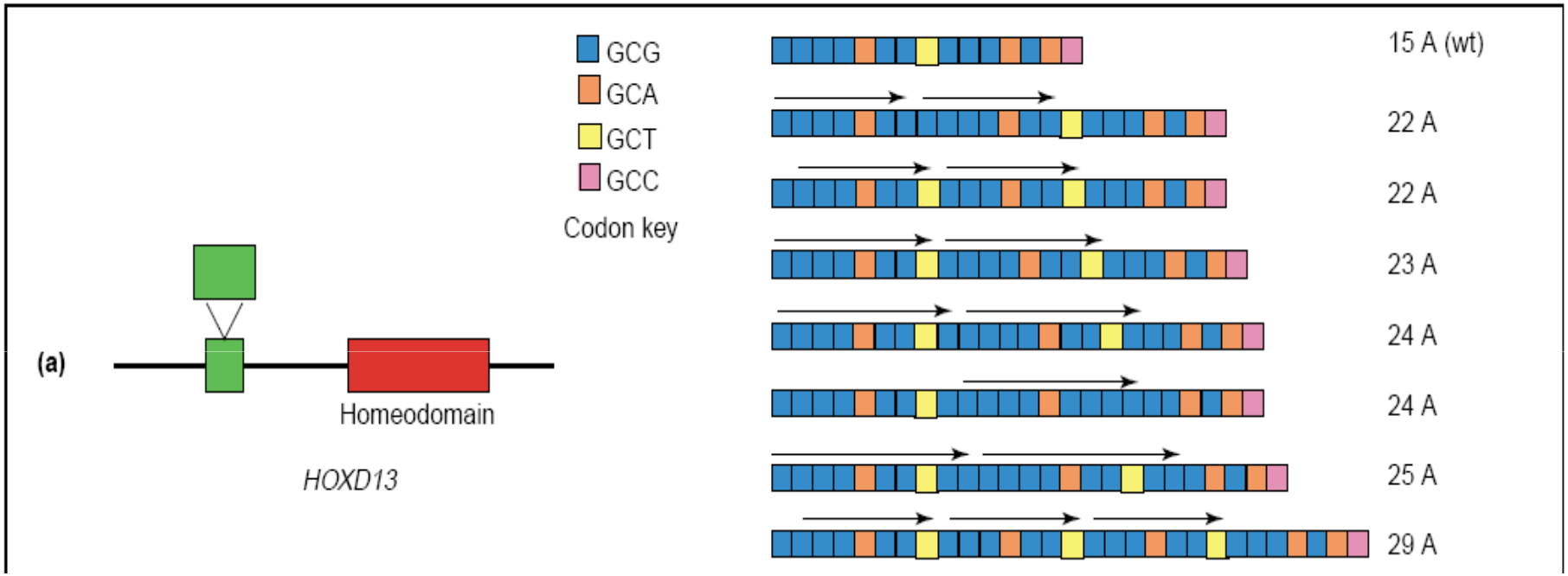
Table 1
Polyalanine expansion disorders.

Polyalanine diseases	Disease phenotype	Gene	Protein function	Expansion size and position	Protein dysfunction
Synpolydactyly type II (SPD)	Limb malformation	*HOXD13*	Genitorurinary tract and limb development	Exon 1: (15A→22-29A)	Dominant negative effect
Cleidocranial dysplasia (CCD)	Skeletal dysplasia	*RUNX2*	Osteoblast differentiation and skeletal development	Exon3: (17A→27A)	Gain-of-function or dominant negative effect?
Oculopharyngeal muscular dystrophy (OPMD)	Ptosis, dysphagia and limb weakness	*PABPN1*	mRNA nucleocytoplasmic export, polyadenylation, and muscle differentiation	Exon 1: (10A→17A)	Gain-of-function or loss-of-function
Holoprosencephaly (HPE)	Central nervous system (CNS) developmental malformation	*ZIC2*	Central nervous system development	Exon 3: (15A→25A)	Partial loss-of-function
Hand–foot–genital syndrome (HFGS)	Skeletal anomalies and urogenital malformations	*HOXA13*	Genitorurinary tract and limb development	Exon 1: Tract# 1 (14A→22A) Tract# 2 (12A→18A) Tract# 3 (18A→24-30A)	Loss-of-function or dominant negative effect?
Blepharophimosis, ptosis and epicanthus inversus (BPES)	Eyelid abnormalities and Premature ovarian failure (POF)	*FOXL2*	Ovarian maintenance and eyelid development	Single exon: (14A→A19, 22 and 24A)	Partial loss-of-function
X-linked hypopituitarism (XH)	Short stature and growth hormone deficiency	*SOX3*	Neural development and the correct function of the hypothalamic–pituitary axis	Single exon: (15A→22, 26A)	Partial loss-of-function
Syndromic and non-syndromic X-linked mental retardation (XLMR)	MR associated with infantile seizures related to West syndrome or dystonic movements of the hands and dysarthria related to Partington syndrome	*ARX*	Cerebral development and patterning	Exon 2: Tract# 1 (16A→23A) Tract# 2 (12A→20A)	Gain-of-function
Congenital central hypoventilation syndrome (CCHS)	Autonomic nervous system abnormalities	*PHOXB2*	Regulation of the autonomic nervous system (ANS)	Exon 3: (20A→ 25-33A)	Gain-of-function or Loss-of-function

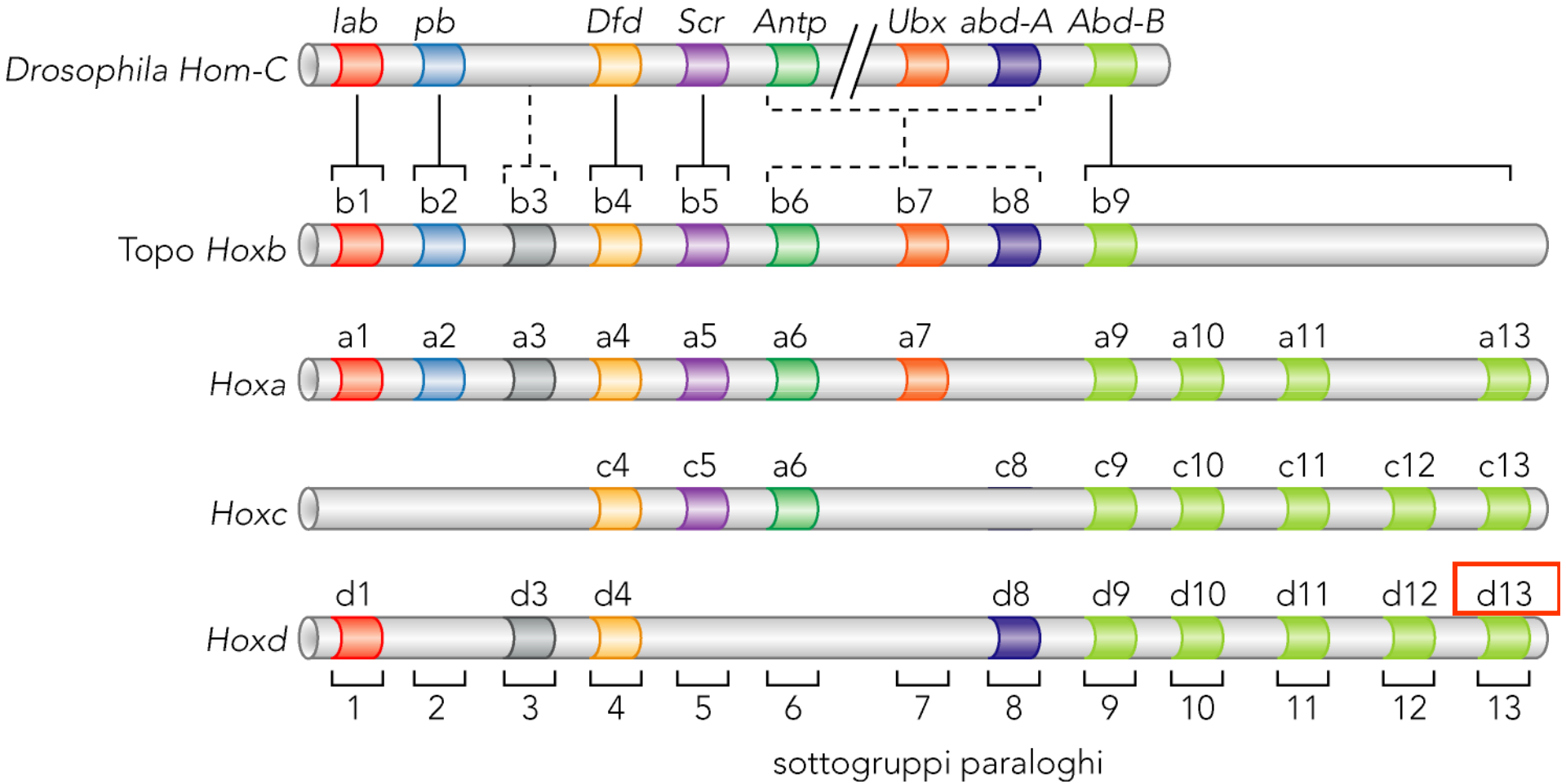
Please cite this article as: Messaed, C., Rouleau, G.A., Molecular mechanisms underlying polyalanine diseases, Neurobiol. Dis. (2009),



Common pathological events leading to cell dysfunction or death in polyalanine diseases



Poli Ala Simpolidattilia


lab
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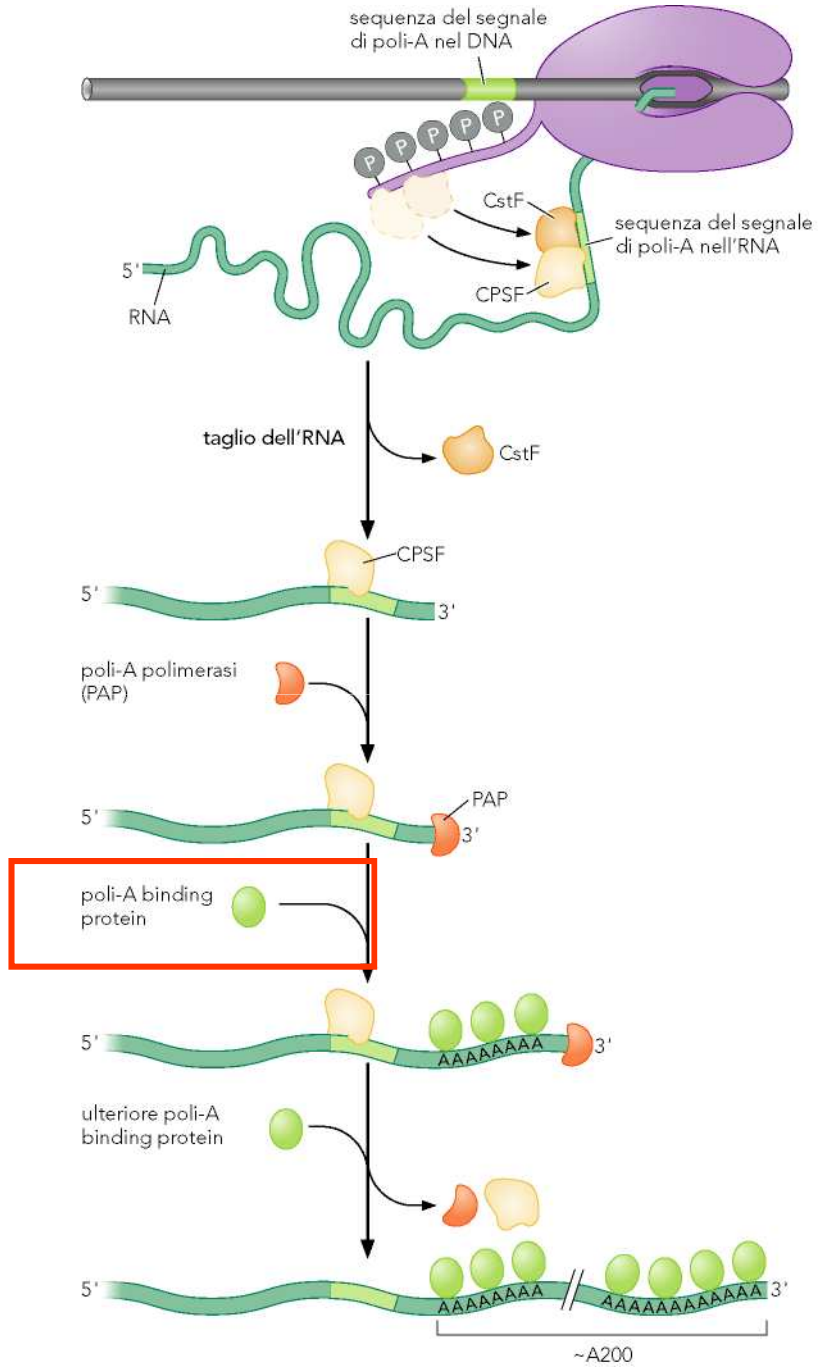
Hand–foot–genital syndrome (HFGS)	Skeletal anomalies and urogenital malformations	*HOXA13*	Genitorurinary tract and limb development	Exon 1: Tract# 1 (14A→22A) Tract# 2 (12A→18A) Tract# 3 (18A→24-30A)	Loss-of-function or dominant negative effect?
Blepharophimosis, ptosis and epicanthus inversus (BPES)	Eyelid abnormalities and Premature ovarian failure (POF)	*FOXL2*	Ovarian maintenance and eyelid development	Single exon: (14A→A19, 22 and 24A)	Partial loss-of-function
X-linked hypopituitarism (XH)	Short stature and growth hormone deficiency	*SOX3*	Neural development and the correct function of the hypothalamic–pituitary axis	Single exon: (15A→22, 26A)	Partial loss-of-function
Syndromic and non-syndromic X-linked mental retardation (XLMR)	MR associated with infantile seizures related to West syndrome or dystonic movements of the hands and dysarthria related to Partington syndrome	*ARX*	Cerebral development and patterning	Exon 2: Tract# 1 (16A→23A) Tract# 2 (12A→20A)	Gain-of-function
Congenital central hypoventilation syndrome (CCHS)	Autonomic nervous system abnormalities	*PHOXB2*	Regulation of the autonomic nervous system (ANS)	Exon 3: (20A→ 25-33A)	Gain-of-function or Loss-of-function

Please cite this article as: Messaed, C., Rouleau, G.A., Molecular mechanisms underlying polyalanine diseases, Neurobiol. Dis. (2009),

PABN1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P
CstF
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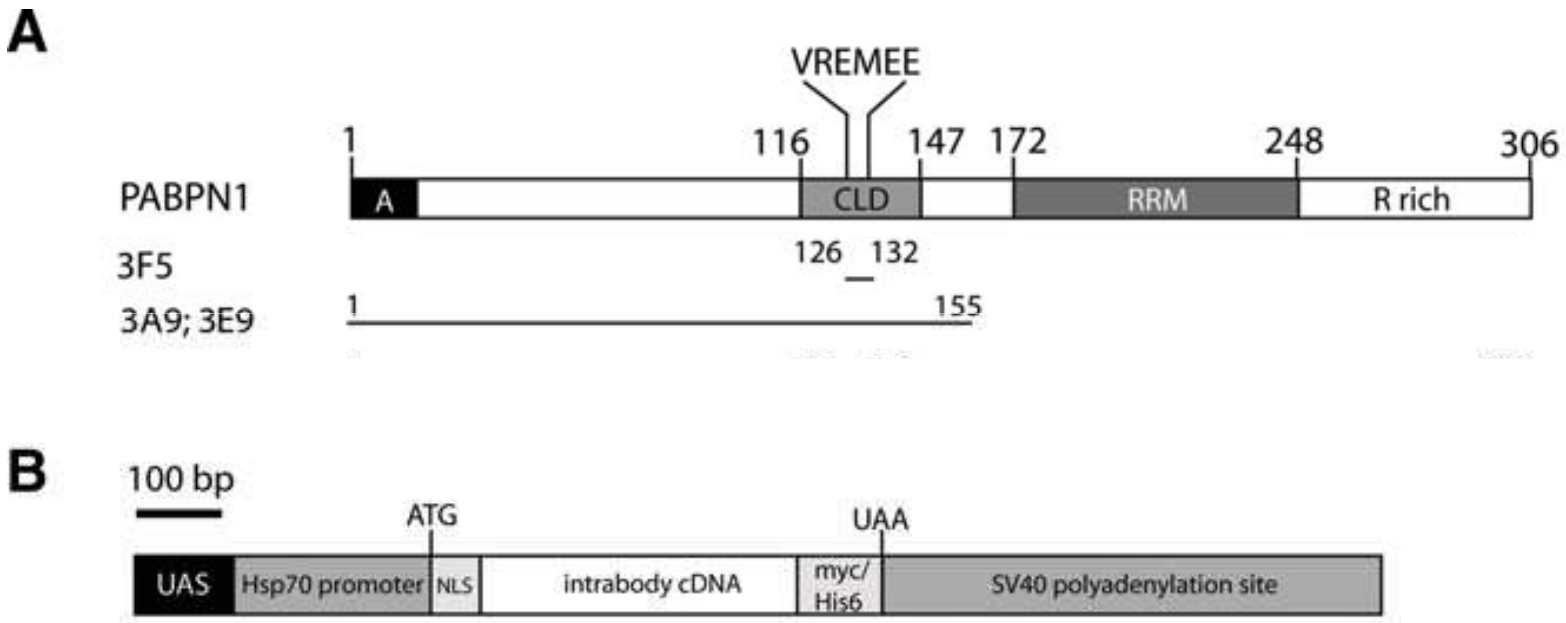
5'
RNA
CPSF
taglio dell'RNA
CstF
CPSF
5'
3'
poli-A polimerasi
(PAP)
PAP
poli-A binding
protein
AAAAAAAA
ulteriore poli-A
binding protein
~A200

OPMD phenotype in Drosophila PABPN1-17ala

% of flies with abnormal wing posture	
day 6	day 11
87%	93%

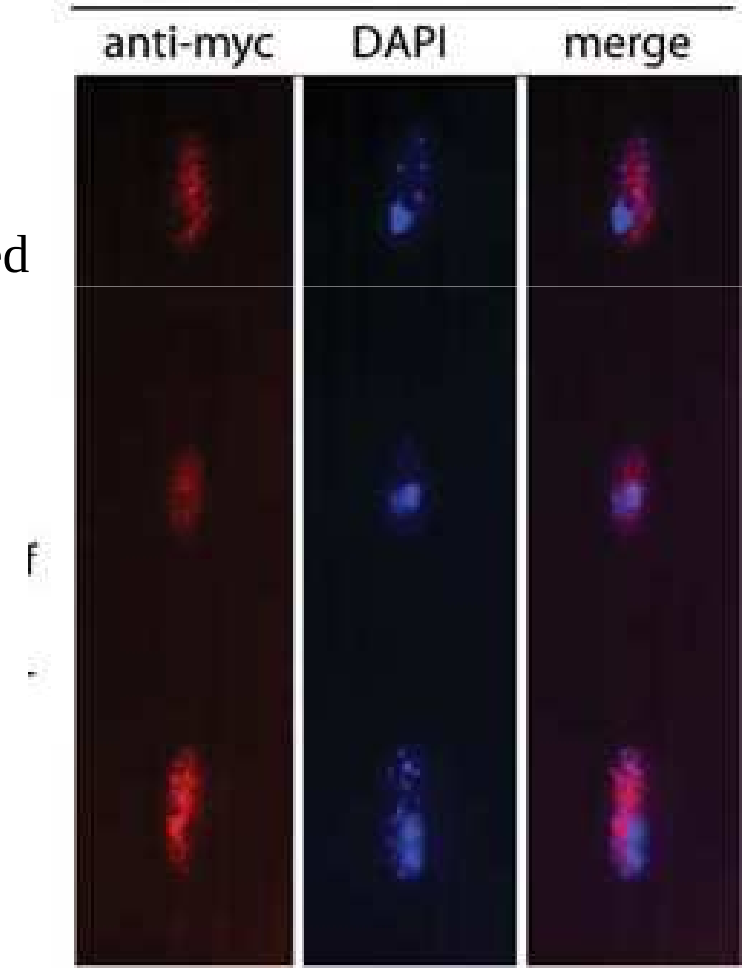
Suppression of OPMD phenotypes in Drosophila by intramuscular expression of anti-PABPN1 single-chain antibodies



Suppression of OPMD phenotypes in Drosophila by intramuscular expression of anti-PABPN1 single-chain antibodies

Immunostaining of adult thoracic muscles showing the nuclear accumulation of the 3F5 intrabody.

The intrabody was detected using anti-myc antibody
DNA was revealed with DAPI.



Suppression of OPMD phenotypes in Drosophila PABPN1-17ala by intramuscular expression of anti-PABPN1 single-chain antibodies

Intrabody clone	% of flies with abnormal wing posture		suppressor activity	number of lines
	day 6	day 11		
-	87%	93%	-	-
3F5	3 to 32%	3 to 46%	+++	4
3E9	31 to 41%	39 to 44%	++	2
3A9	44 to 62%	51 to 64%	+	5

Post-translational modification of polyQ proteins.