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Role of protein misfolding in neurodegenerative disorders

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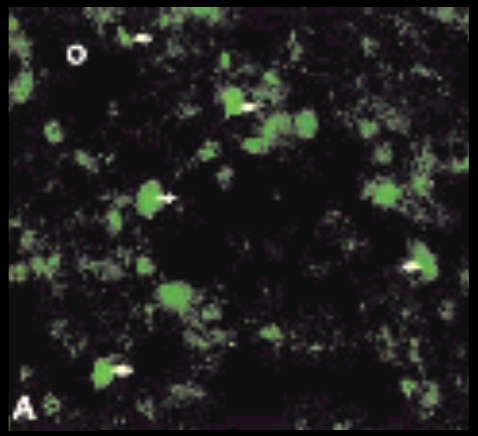
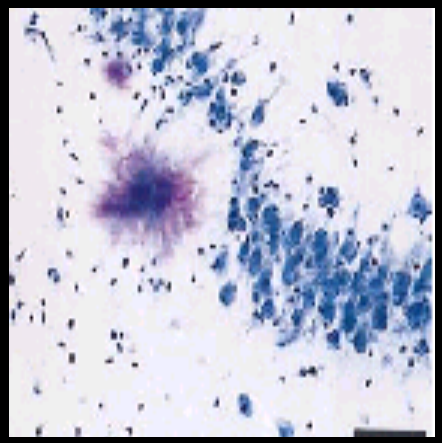
Protein aggregates in neurodegenerative diseases

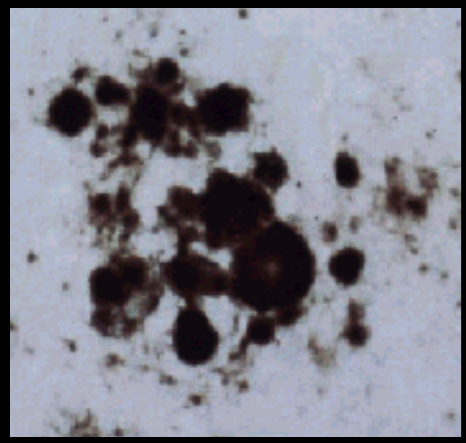
Parkinson



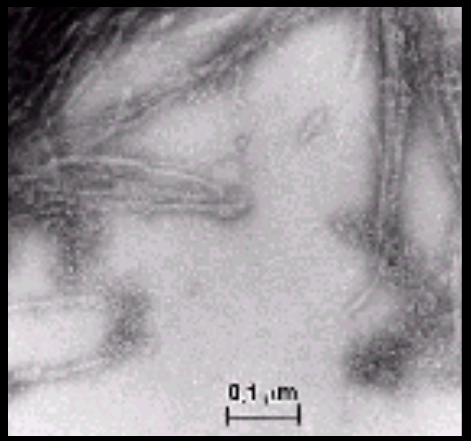
Alzheimer



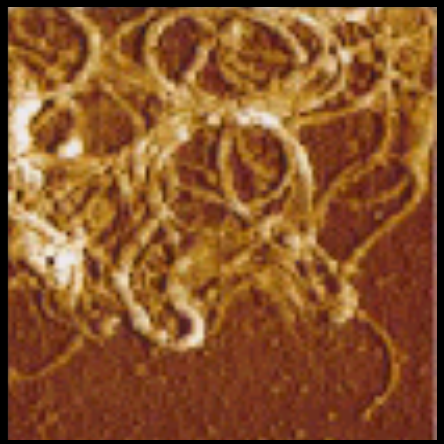
Prion Disorders



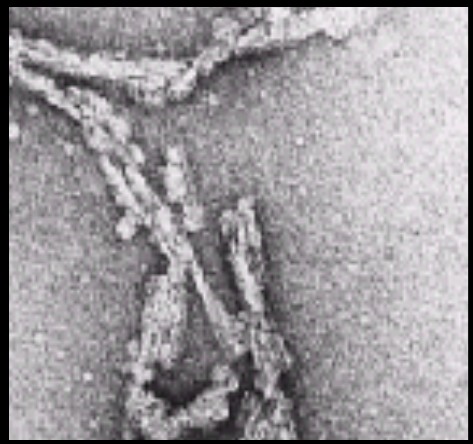
α-synuclein



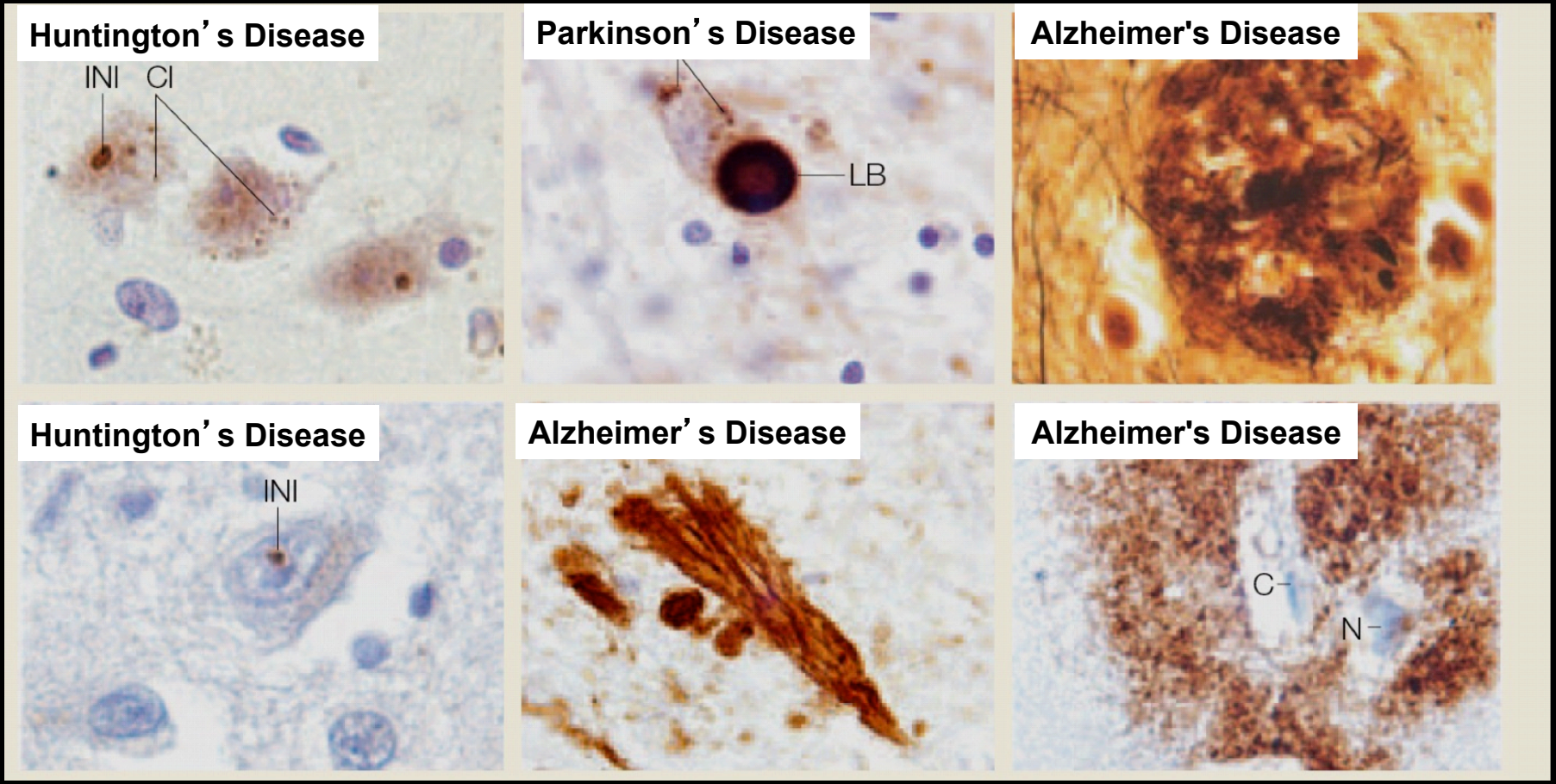
Amyloid β



PrP^{SC}

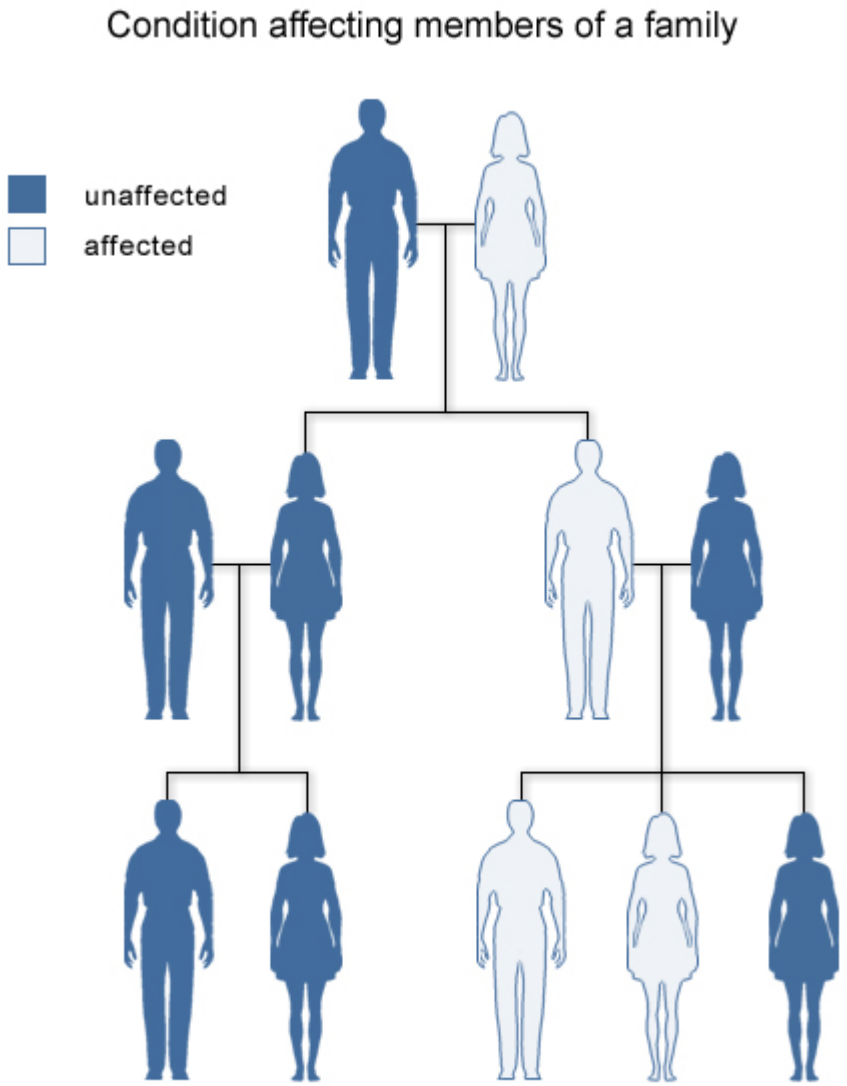


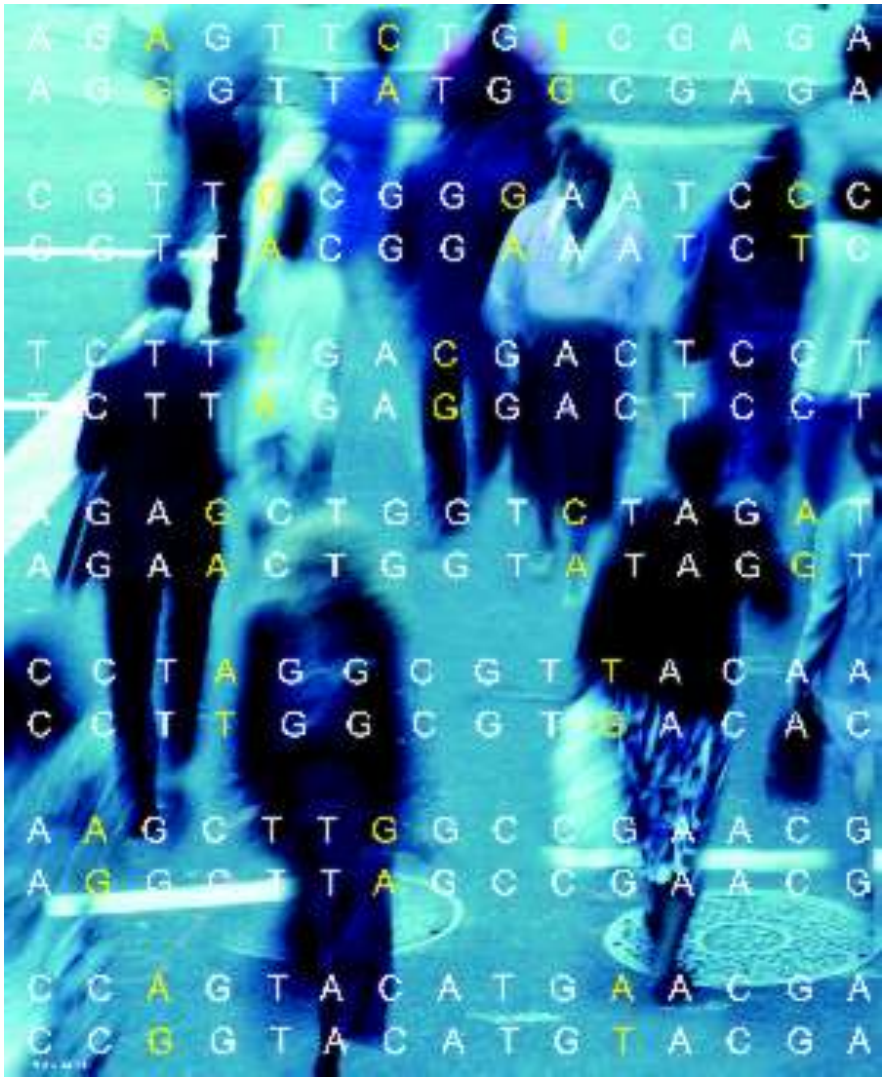
Protein aggregates in neurodegenerative diseases



Ross and Poirier (2005) *Nat Rev Mol Cell Biol*

Hereditary Diseases





U.S. National Library of Medicine

Protein structure: from shape to function

M.C. Escher's "Rind"
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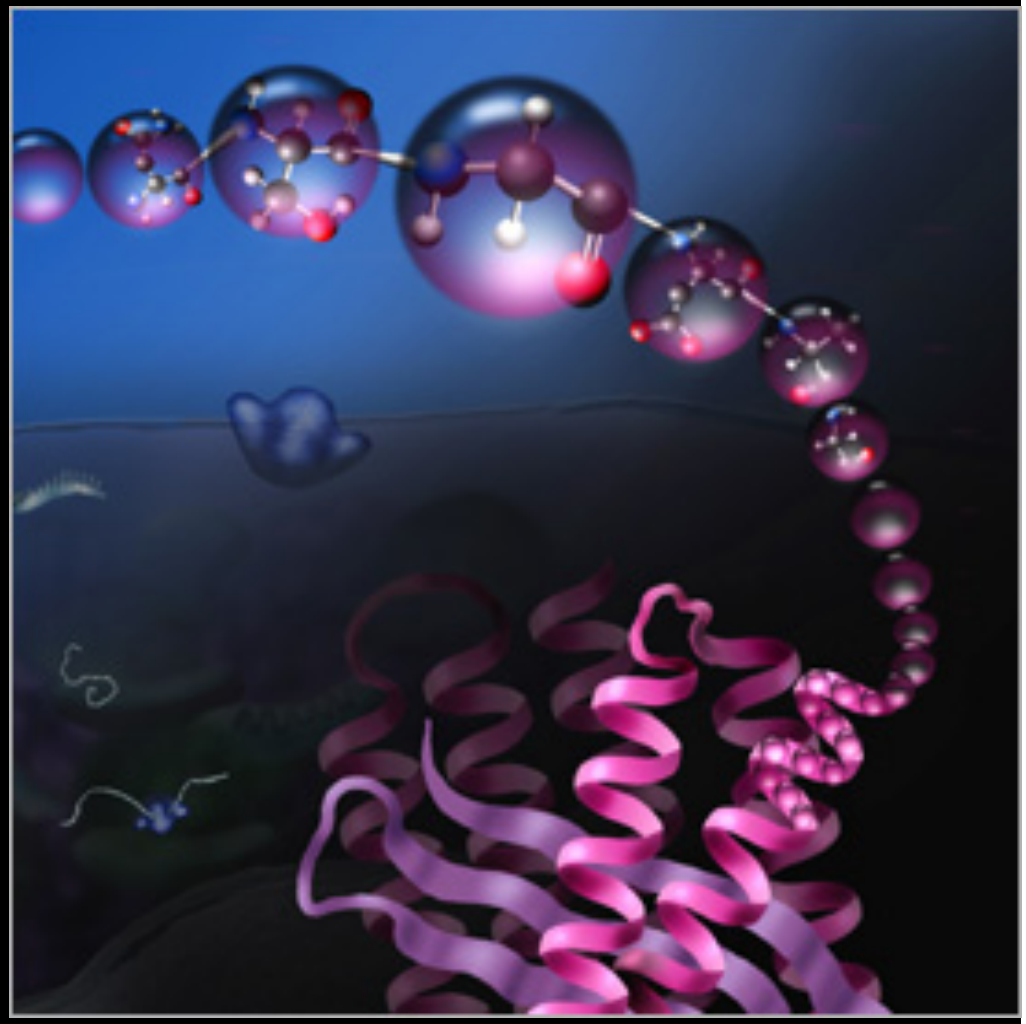
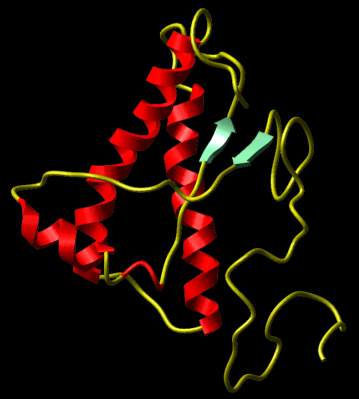
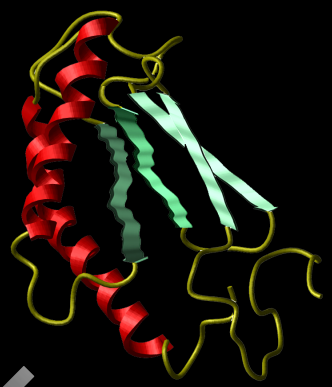


New concept: Protein Conformational Disorders

The hallmark event is the misfolding of a natural protein



Folded functional protein

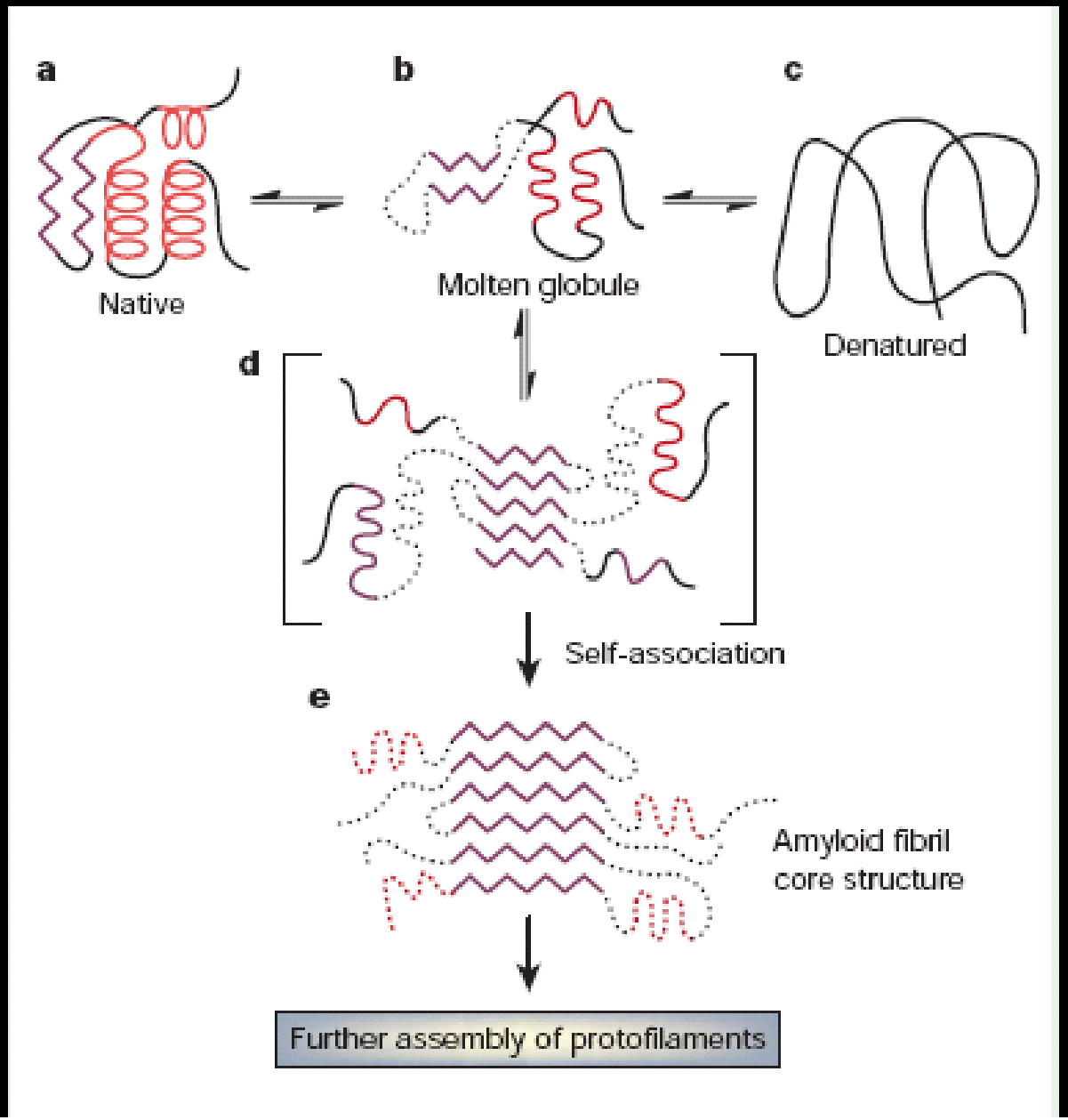


Disease-associated abnormal protein

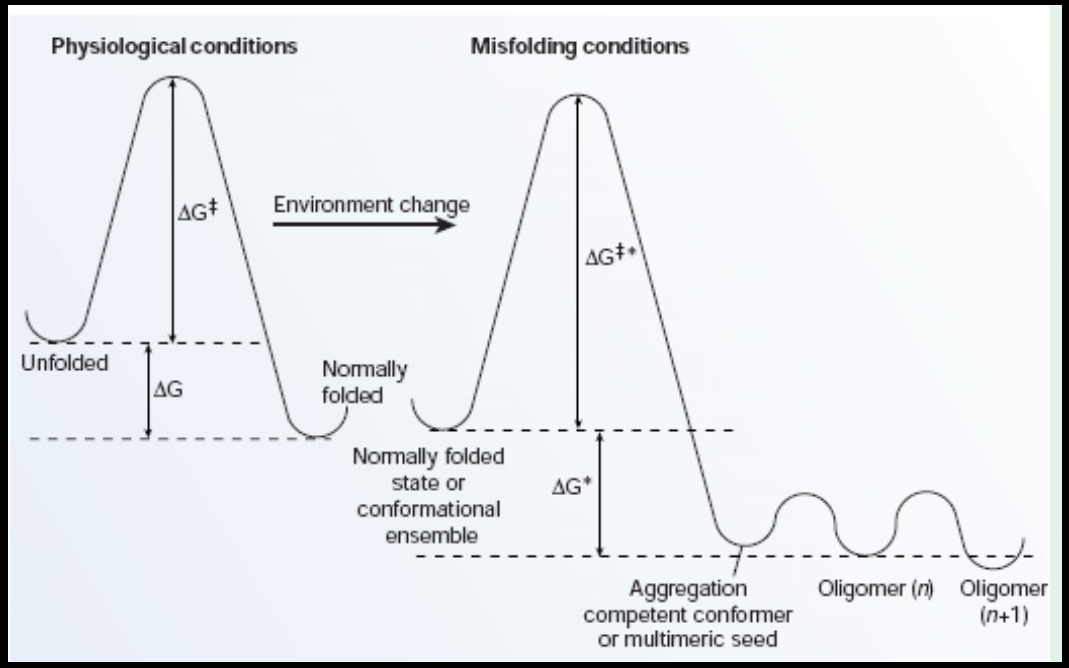
Gain of Toxic Activity?

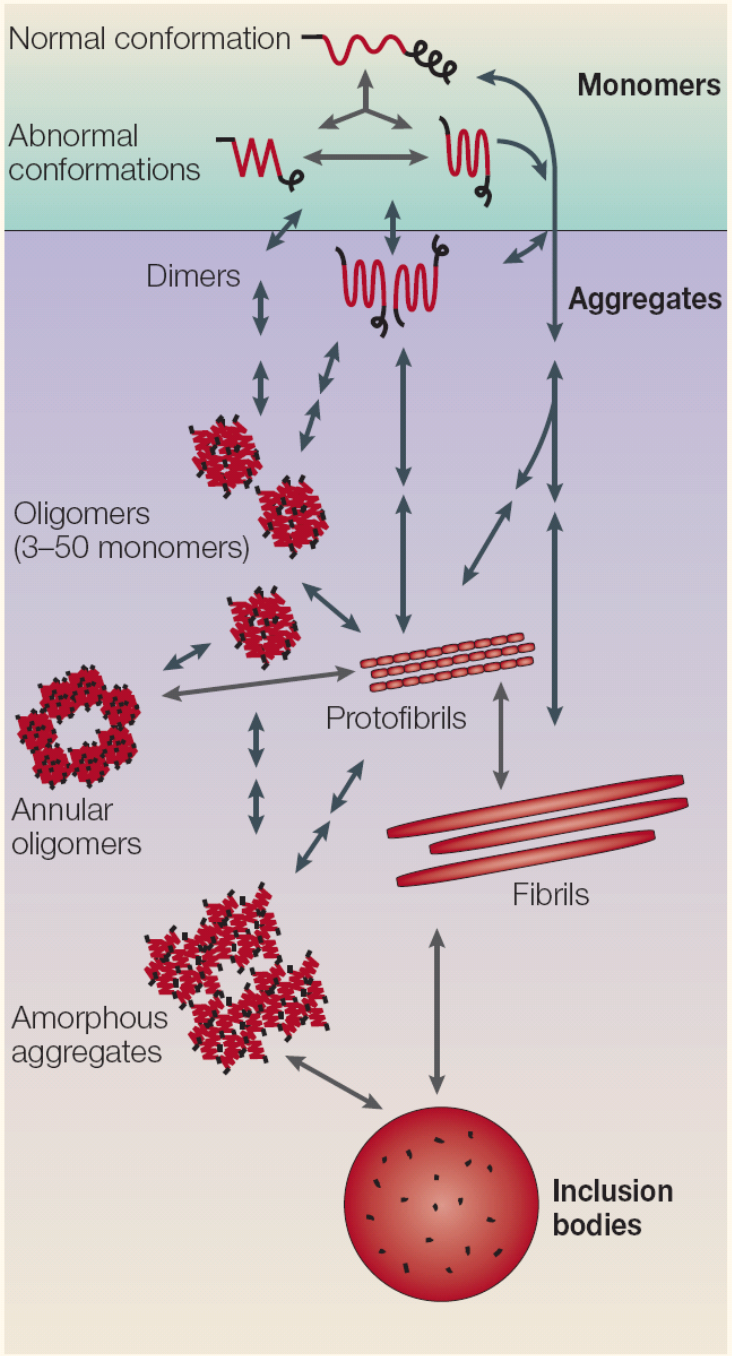
Loss of physiological function?

Protein Conformational Disorders



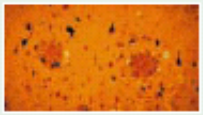
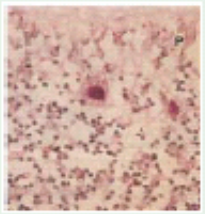
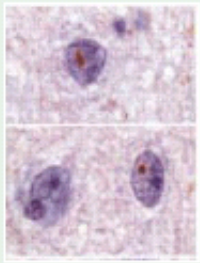
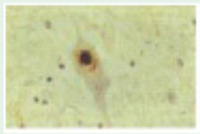
Protein Conformational Disorders





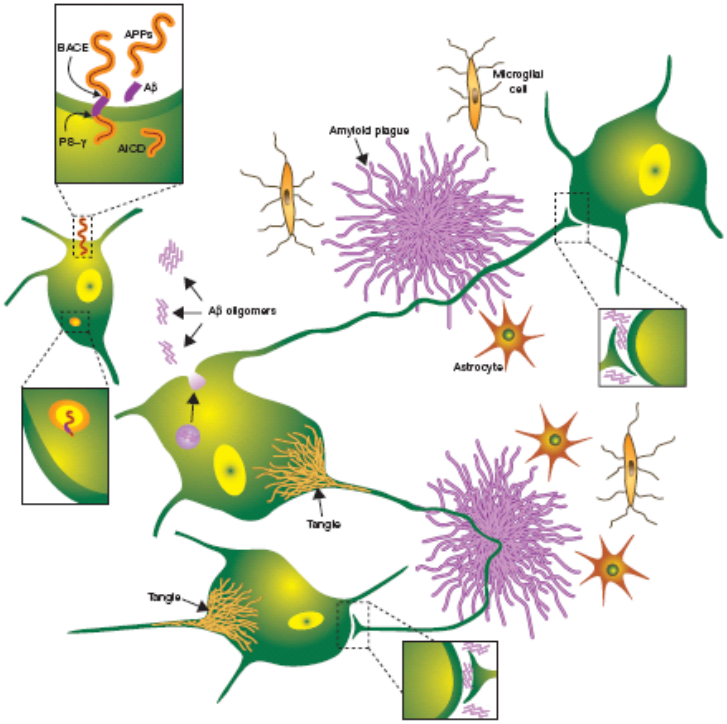
Protein Conformational Disorders

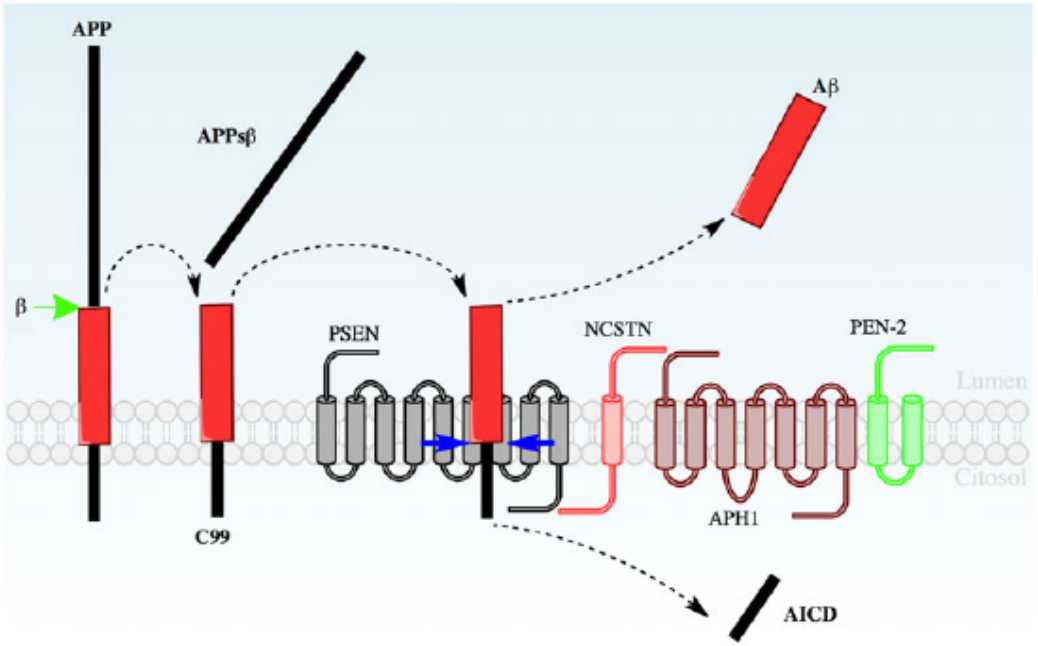
Table 3 **Some human brain diseases characterized by progressive misfolding and aggregation of proteins**

Disease	Protein	Locus	
Alzheimer's disease	Amyloid β-protein Tau	Extracellular plaques Tangles in neuronal cytoplasm	
Frontotemporal dementia with parkinsonism	Tau	Tangles in neuronal cytoplasm	
Parkinson's disease; dementia with Lewy bodies	α-Synuclein	Neuronal cytoplasm	
Creutzfeldt–Jakob disease; 'mad cow disease'*	Prion protein (PrP^{Sc})	Extracellular plaques Oligomers, inside and outside neurons	
Polyglutamine expansion diseases (Huntington's disease, spinocerebellar ataxias, and so on)*	Long glutamine stretches within certain proteins	Neuronal nuclei and cytoplasm	
Amyotrophic lateral sclerosis*	Superoxide dismutase	Neuronal cytoplasm	

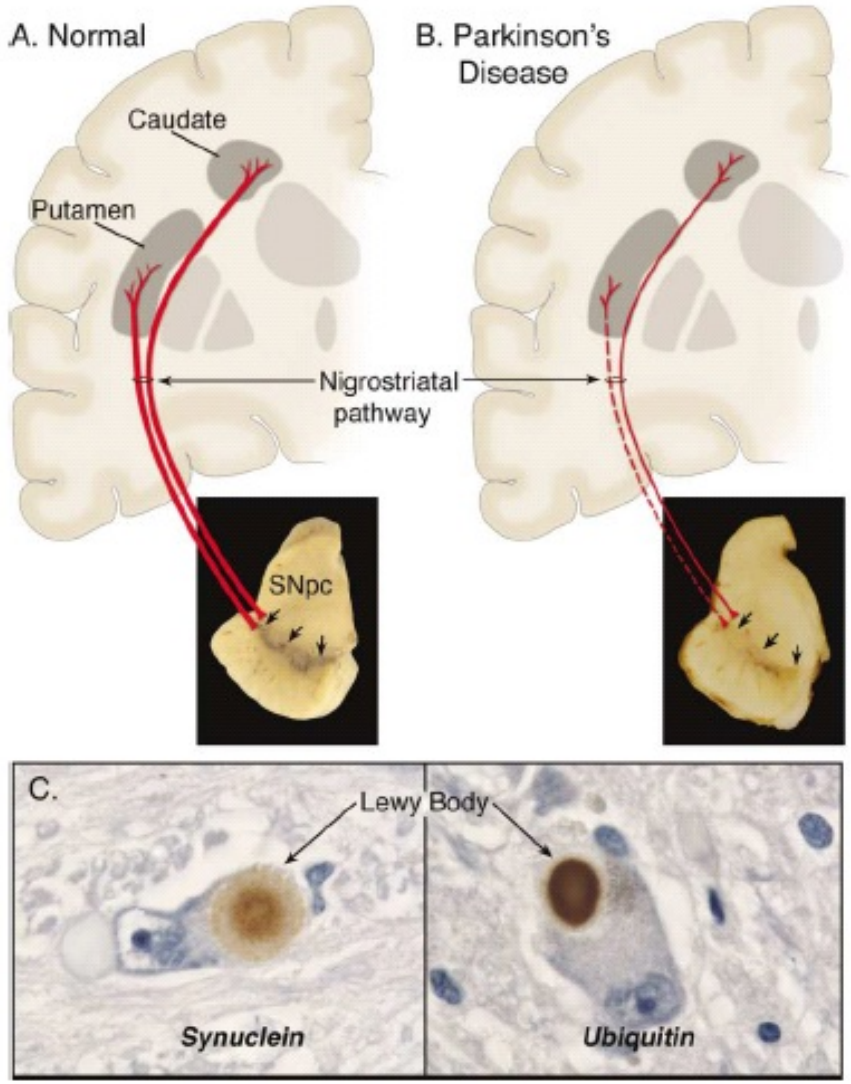
*Figures reproduced from *Greenfield's Neuropathology* (copyright Hodder Arnold)

Alzheimer's disease



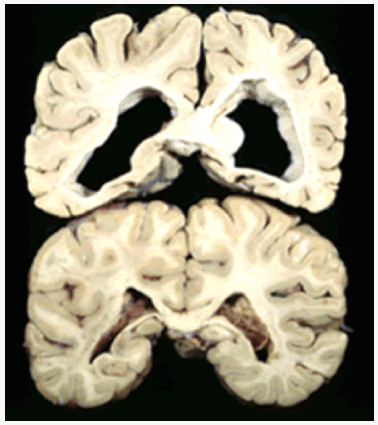


Parkinson's disease



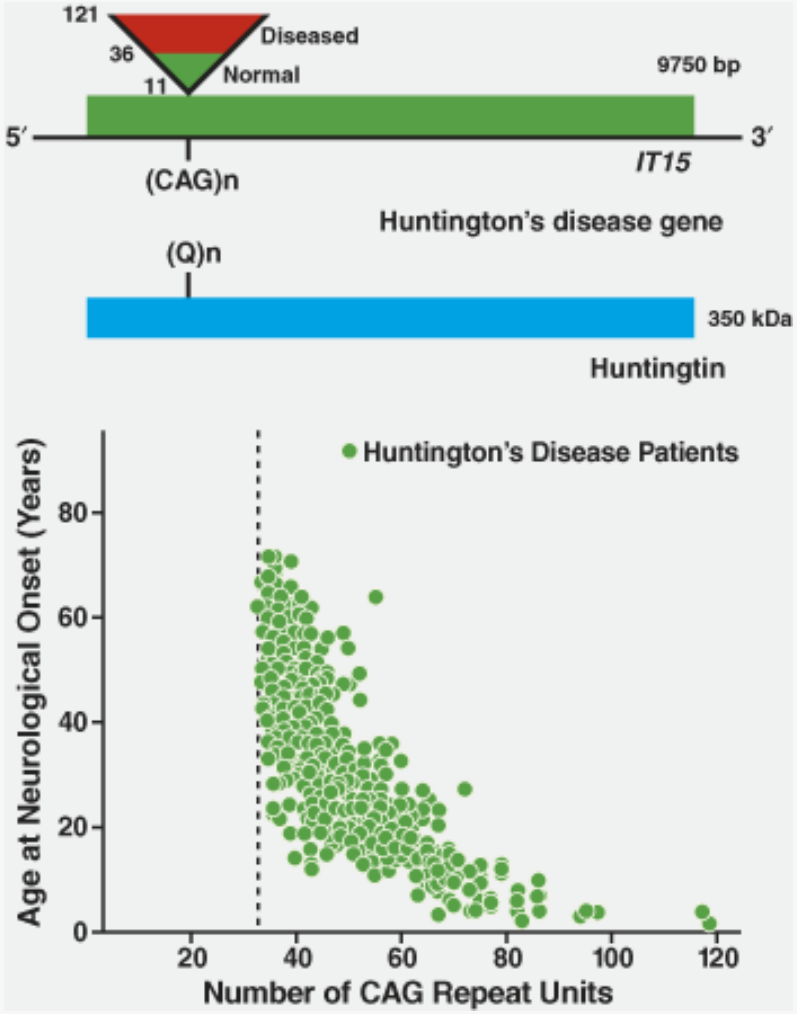
Huntington's disease

Onset & Symptoms:
- Personality changes
- Involuntary movements
- Middle adulthood, progresses over 15-20 years
- Loss of motor control and intellectual function



HD

Normal



Prion-related disorders

Table 1. The prion diseases

Disease	Host	Mechanism of pathogenesis
Kuru	Fore people	Infection through ritualistic cannibalism
iCJD	Humans	Infection from prion-contaminated HGH, dura mater grafts, etc.
vCJD	Humans	Infection from bovine prions?
fCJD	Humans	Germ-line mutations in PrP gene
GSS	Humans	Germ-line mutations in PrP gene
FFI	Humans	Germ-line mutation in PrP gene (D178N, M129)
sCJD	Humans	Somatic mutation or spontaneous conversion of PrP^{C} into PrP^{Sc}?
FSI	Humans	Somatic mutation or spontaneous conversion of PrP^{C} into PrP^{Sc}?
Scrapie	Sheep	Infection in genetically susceptible sheep
BSE	Cattle	Infection with prion-contaminated MBM
TME	Mink	Infection with prions from sheep or cattle
CWD	Mule deer, elk	Unknown
FSE	Cats	Infection with prion-contaminated bovine tissues or MBM
Exotic ungulate encephalopathy	Greater kudu, nyala, oryx	Infection with prion-contaminated MBM

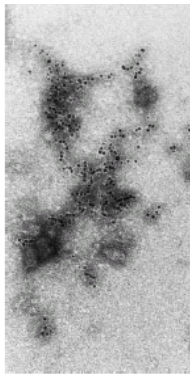
iCJD, iatrogenic CJD; vCJD, variant CJD; fCJD, familial CJD; sCJD, sporadic CJD; GSS, Gerstmann–Sträussler–Sheinker disease; FFI, fatal familial insomnia; FSI, fatal sporadic insomnia; BSE, bovine spongiform encephalopathy; TME, transmissible mink encephalopathy; CWD, chronic wasting disease; FSE, feline spongiform encephalopathy; HGH, human growth hormone; MBM, meat and bone meal.

TSE: *The role of Prion (PrP^C) misfolding*

A

Normal

(i)



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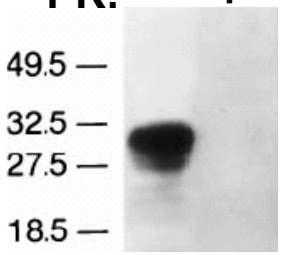

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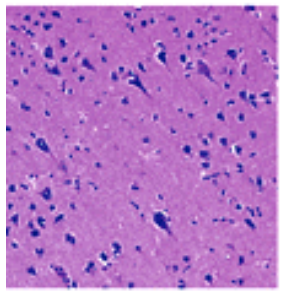

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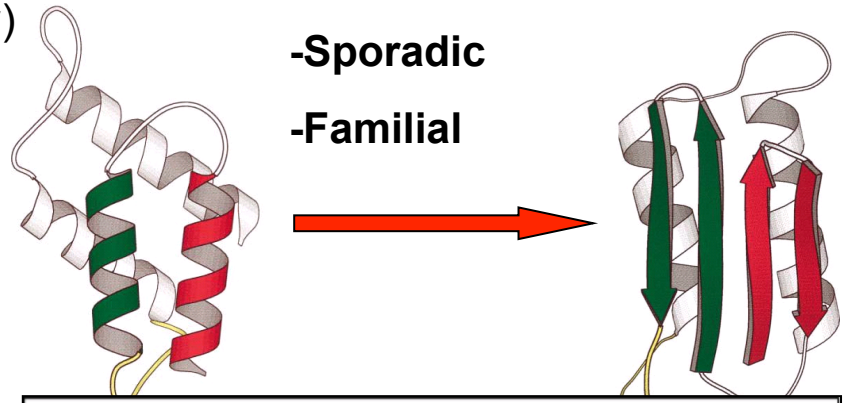
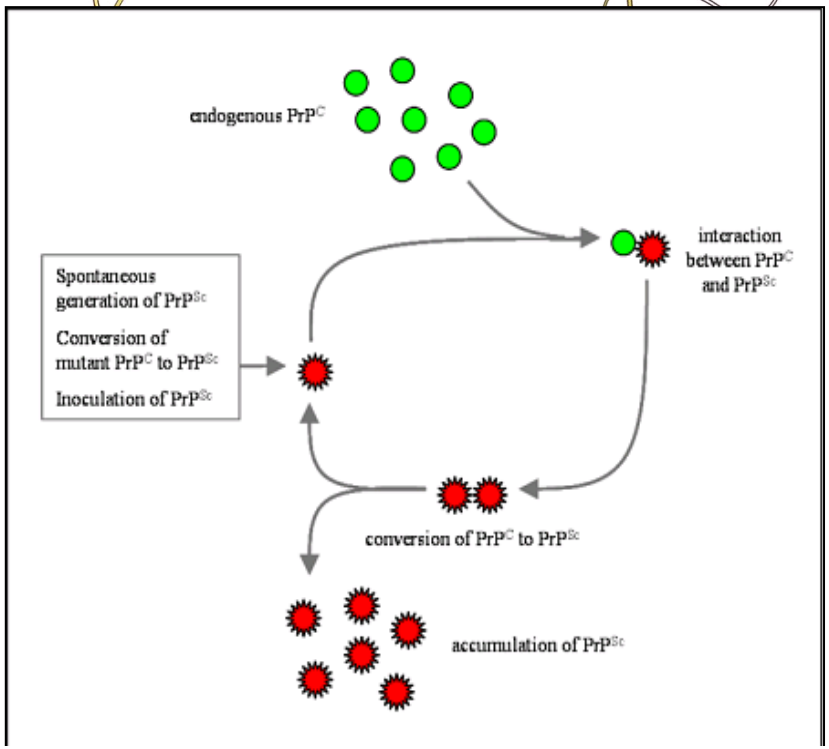
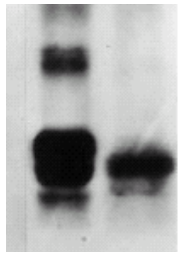


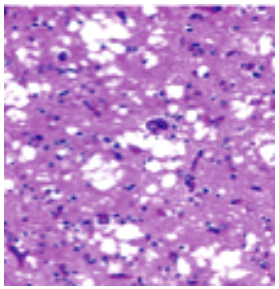
Disease origin:
-infectious
-Sporadic
-Familial



Disease

PK: - +





Mad Cow Disease and Creutzfeldt-Jacob

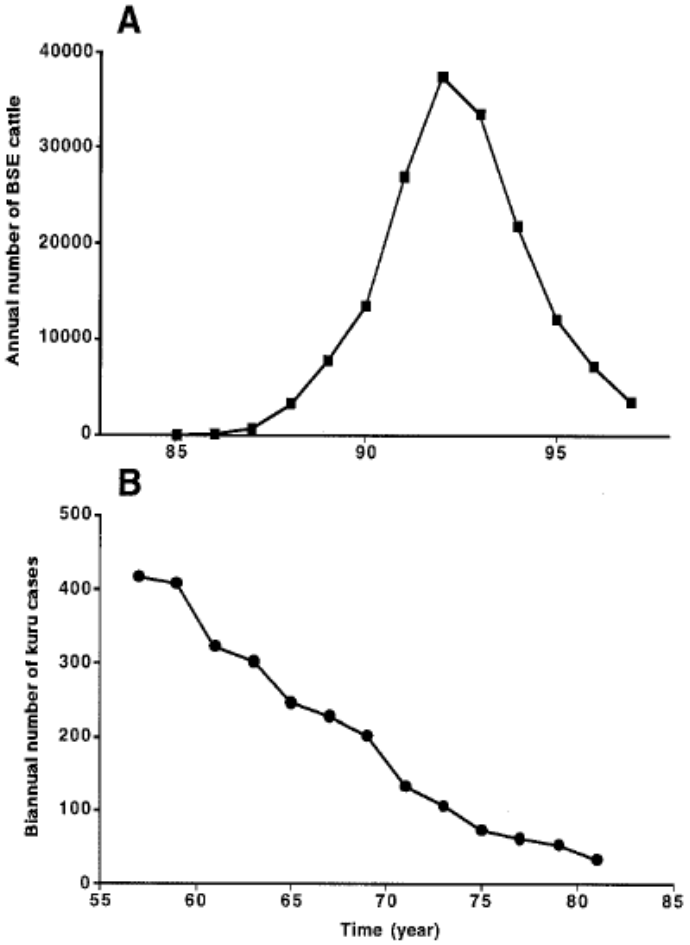
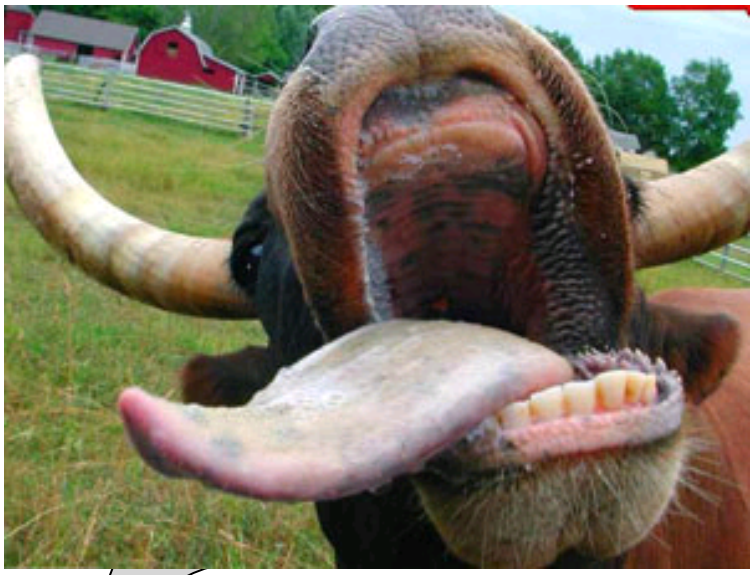


FIG. 8. Disappearance of the kuru and BSE epidemics. (*A*) Annual number of cases of BSE in cattle in Great Britain. (*B*) Biannual number of cases of kuru in Papua New Guinea. Data were compiled for BSE by John Wilesmith and for kuru by Michael Alpers.



Novel concept: Prion-like propagation of disease

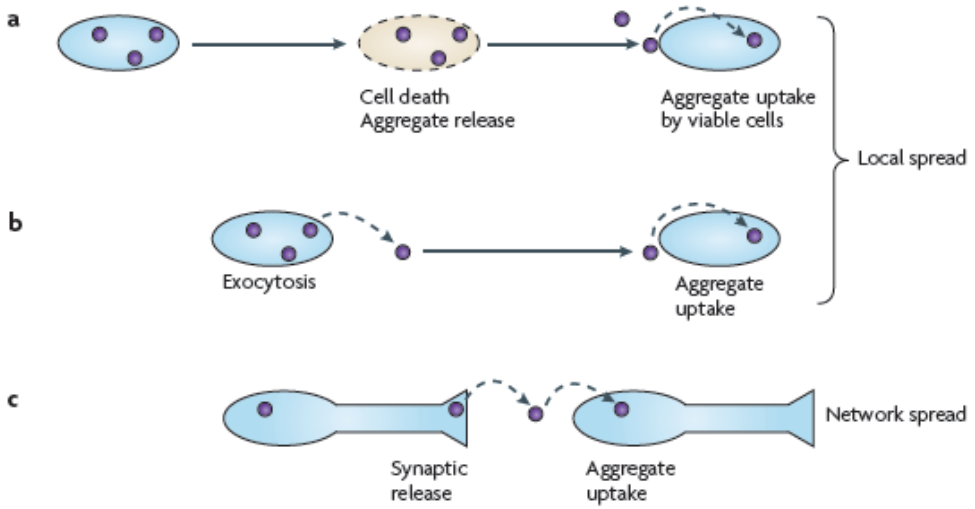


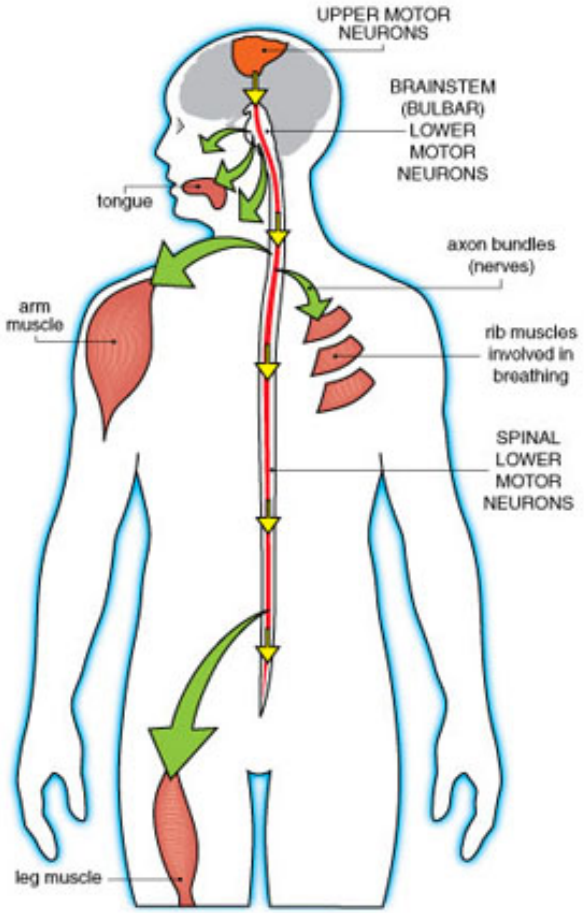
Table 1. Potential Candidate Disease-Associated Transmissible Proteins

Disease	Protein (Location)	Experimental Transmission	Natural Transmission	References[a]
Prion diseases	PrP^{Sc} (extracellular)	infectious in diverse animal species by various routes	infectious in diverse species by various routes	(Prusiner, 1998; Aguzzi and Calella, 2009)
Alzheimer's disease	Aβ (extracellular)	induction of pathology in transgenic mice by intracerebral and intraperitoneal inoculation	not shown	(Kane et al., 2000; Meyer-Luehmann et al., 2006; Eisele et al., 2010; Morales et al., 2011)
Parkinson's disease	α-synuclein (cytoplasmatic)	cell-to-cell and host-to-graft spreading in animal models and transmission by intracerebral inoculation	host-to-graft spreading in humans	(Desplats et al., 2009; Luk et al., 2009; Volpicelli-Daley et al., 2011; Mougenot et al., 2012; Hansen et al., 2011; Luk et al., 2012)
Huntington's disease	Huntingtin (nuclear)	cell-to-cell spreading in culture	not shown	(Ren et al., 2009)
Tauopathies	Tau (cytoplasmatic)	cell-to-cell spreading in culture and transmission in transgenic mice by intracerebral inoculation	not shown	(Clavaguera et al., 2009; Frost et al., 2009; Nonaka et al., 2010; Guo and Lee, 2011; de Calignon et al., 2012; Liu et al., 2012)
Secondary amyloidosis	Amyloid-A (extracellular)	acceleration of pathology in mice by various routes of administration	possible transmission to captive cheetah	(Lundmark et al., 2002; Zhang et al., 2008)
Mouse senile amyloidosis	Apolipoprotein A (extracellular)	acceleration of pathology in mice by various routes of administration	transmission to mice in the same cage by feces consumption	(Xing et al., 2001; Korenaga et al., 2006)

Soto (2012) *Cell*

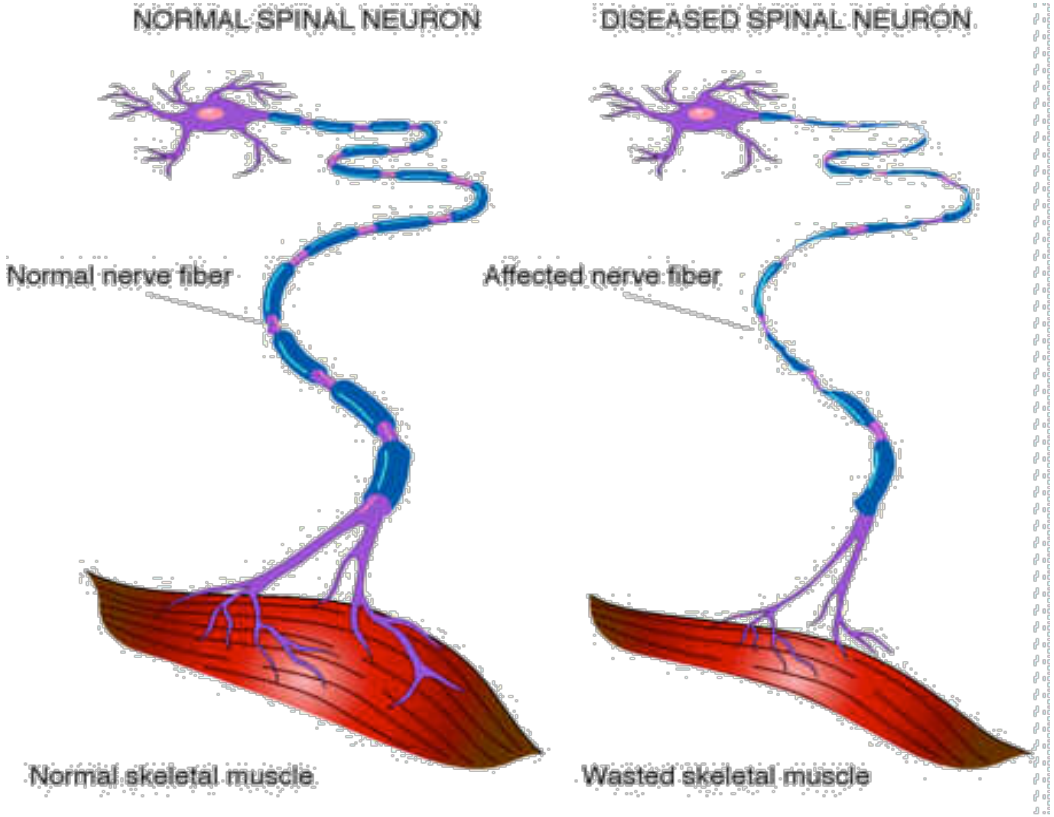
Amyotrophic Lateral Sclerosis (ALS)

Amyotrophic Lateral Sclerosis (ALS)



- Fatal neurodegenerative disease.
- Selective lost of motoneurons in the primary cortex, brainstem and spinal cord.
- Symptoms: muscle weakness, spasms, lack of coordination, paralysis, respiratory insufficiency and death.
- Symptoms start in adulthood (40-70 years).
- And also, in some cases, behavioral and cognitive impairment, related to of **frontotemporal dementia**

Amyotrophic Lateral Sclerosis (ALS)



Stephen Hawkings

Panel 3: **Amyotrophic lateral sclerosis functional rating scale, revised[106]**

1 Speech
- 4 Normal speech
- 3 Detectable disturbance
- 2 Intelligible without repeating
- 1 Speech with non-verbal communication
- 0 Loss of useful speech

2 Salivation
- 4 Normal
- 3 Slight, but definite excess of saliva
- 2 Moderate excessive saliva, minimum drooling
- 1 Marked excessive of saliva, some drooling
- 0 Marked drooling, needs constant tissue

3 Swallowing
- 4 Normal eating habits
- 3 Early eating problems, occasional choking
- 2 Dietary consistency changes
- 1 Needs supplemental tube feeding
- 0 Nil orally

4 Handwriting
- 4 Normal
- 3 Slow or sloppy, all words legible
- 2 Not all words legible
- 1 Able to grip pen, but cannot write
- 0 Unable to grip pen

5 Cutting food and handling utensils
- 4 Normal
- 3 Slow and clumsy, but no help needed
- 2 Can cut most foods, although clumsy and needs some help
- 1 Food must be cut by someone else
- 0 Needs to be fed

6 Dressing and hygiene
- 4 Normal
- 3 Independent, but decreased efficiency
- 2 Some help with closures and fasteners
- 1 Provides minimum assistance to caregiver
- 0 Unable to perform any task

7 Turning in bed
- 4 Normal
- 3 Slow and clumsy
- 2 Can turn alone with difficulty
- 1 Can initiate but cannot turn or adjust sheets
- 0 Total dependence

8 Walking
- 4 Normal
- 3 Early ambulation difficulties
- 2 Walks with assistance
- 1 Non-ambulatory, functional movement
- 0 No purposeful leg movement

9 Climbing stairs
- 4 Normal
- 3 Slow
- 2 Mild unsteadiness or fatigue
- 1 Needs assistance
- 0 Cannot do

10 Dyspnoea
- 4 None
- 3 Occurs when walking
- 2 Occurs when eating, bathing, or dressing
- 1 Occurs at rest
- 0 Considerable difficulty

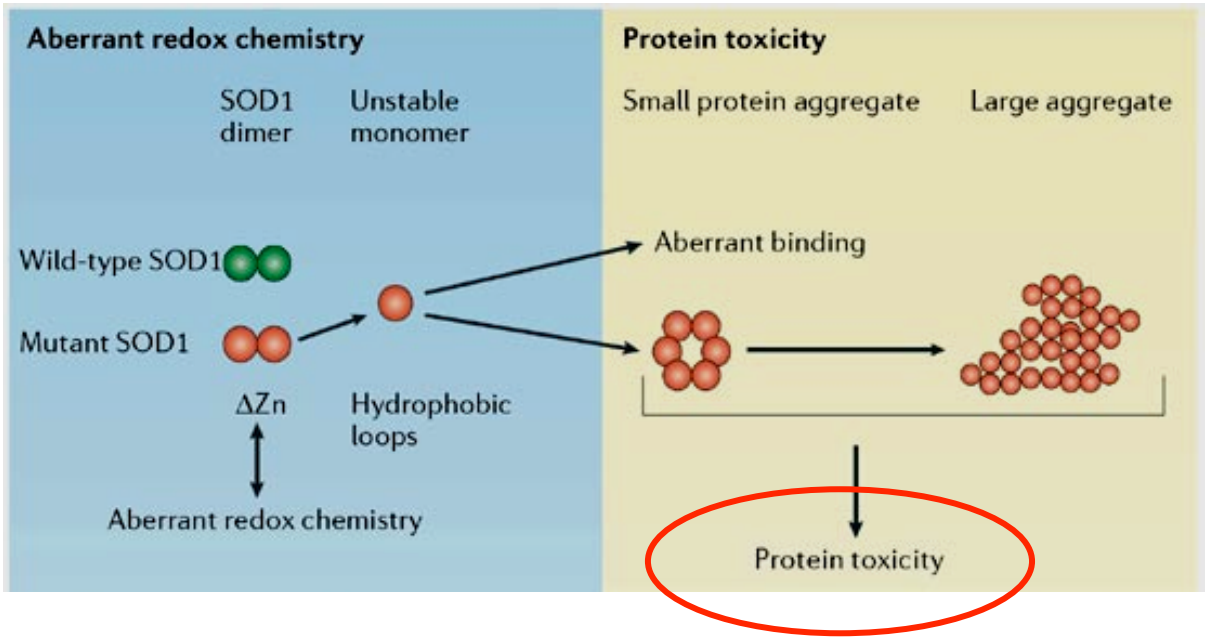
11 Orthopnoea
- 4 None
- 3 Some difficulty, does not routinely use more than two pillows
- 2 Needs extra pillows to sleep
- 1 Only sleeps sitting up
- 0 Unable to sleep

12 Respiratory insufficiency
- 4 None
- 3 Intermittent use of non-invasive ventilation
- 2 Continuous use of non-invasive ventilation at night
- 1 Continuous use of non-invasive ventilation, day and night
- 0 Mechanical ventilation via tracheostomy

Amyotrophic Lateral Sclerosis (ALS)

- **ALS is the most common adult-onset paralytic disease (4-6 cases per 100,000 individuals). Estimated 400 total cases in Chile each year.**

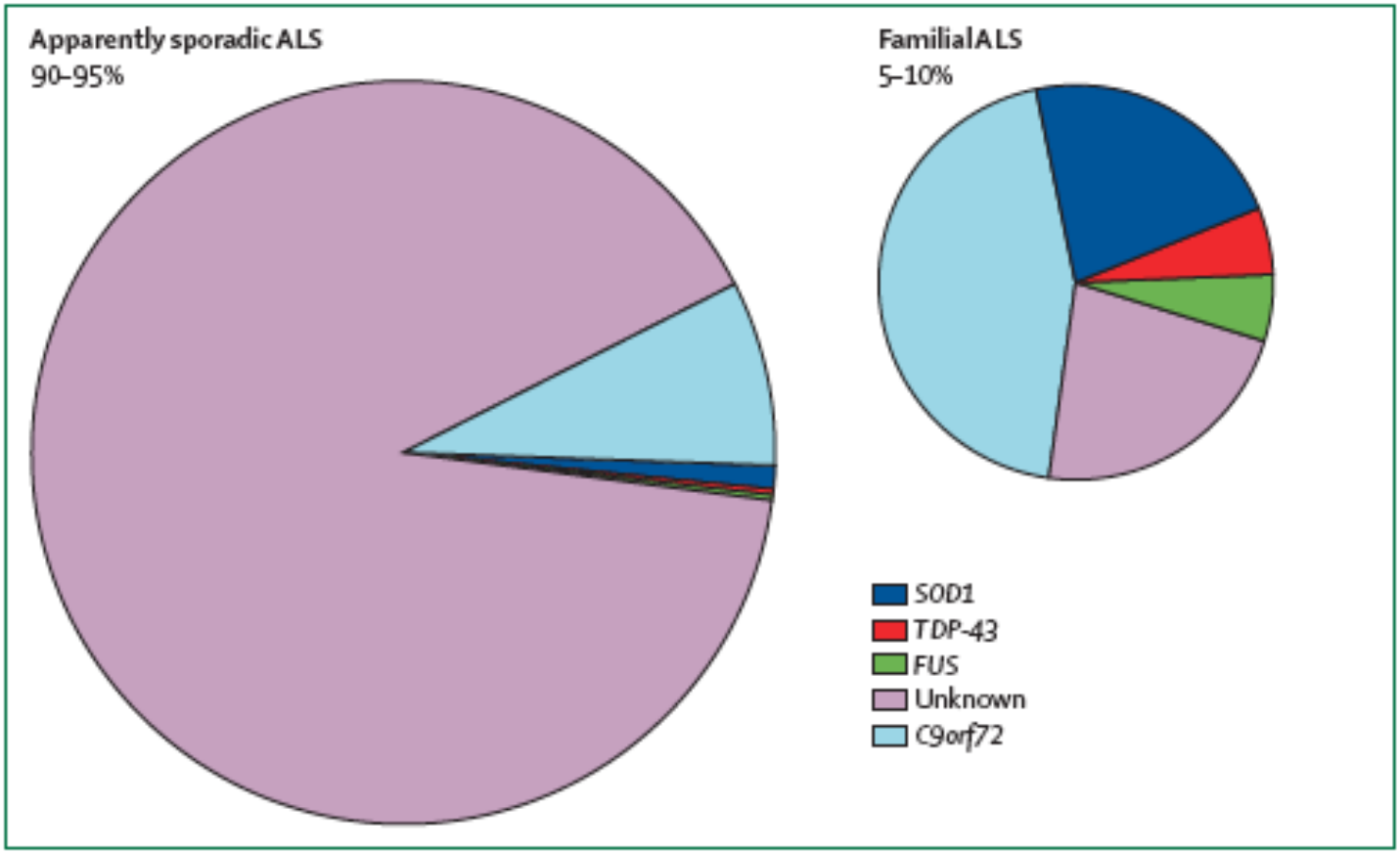
- **10% of ALS cases are familial. Dominant mutations in the gene for superoxide dismutase-1 (SOD1) cause familial ALS.**



Pascinelli and Brown (2006) *Nat Rev Neurosci*

ALS etiology

- Mutations in four genes (*C9ORF72*, *SOD1*, *TARDBP*, and *FUS/TLS*) account for most familial ALS cases.



Lancet Neurol 2013; 12: 310–22

Genetic of ALS

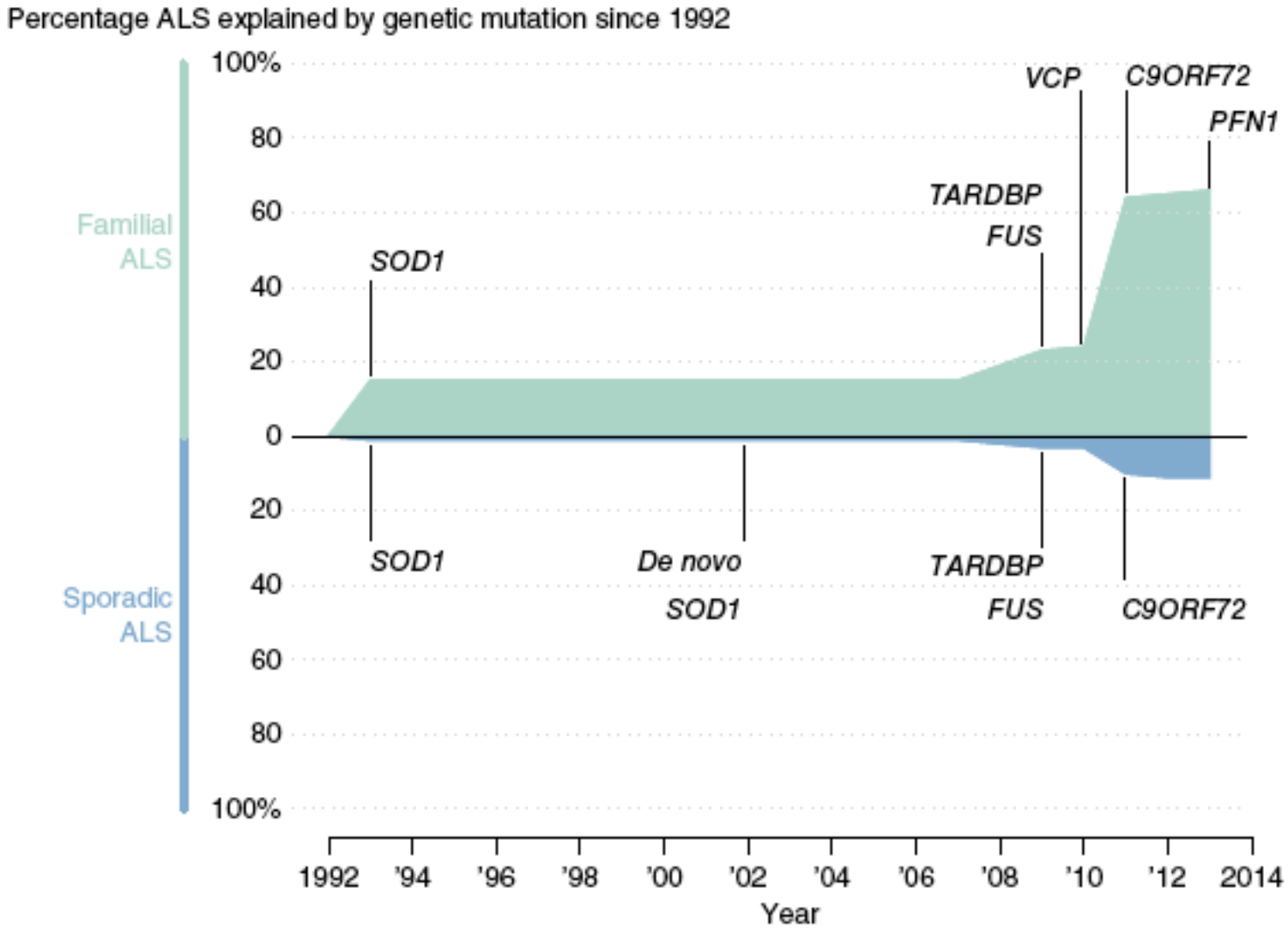
	Clinical involvement	Neuropathological protein aggregates
SOD1	LMN, UMN	Cytoplasmic inclusions of SOD1, or neurofilaments (eg, I113T); generally no TDP-43 aggregates
VAPB	LMN, UMN	Probable TDP-43 aggregates
ANG	LMN, UMN, FTD	TDP-43 aggregates
TARDBP	LMN, UMN, FTD	TDP-43 aggregates
FIG4	UMN	Not known
FUS	LMN, UMN, FTD	FUS aggregates, no TDP-43 aggregates
OPTN	LMN, UMN, FTD	TDP-43 aggregation (Glu478Gly)
C9orf72	LMN, UMN, FTD	TDP-43 aggregates, but also pathognomonic TDP-43-negative aggregates in cerebellar and hippocampal neurons (molecule currently unknown)
UBQLN2	LMN, UMN, FTD	Ubiquilin-2, also TDP-43-positive and FUS-positive

LMN=lower motor neurons. UMN=upper motor neurons. FTD=frontotemporal dementia.

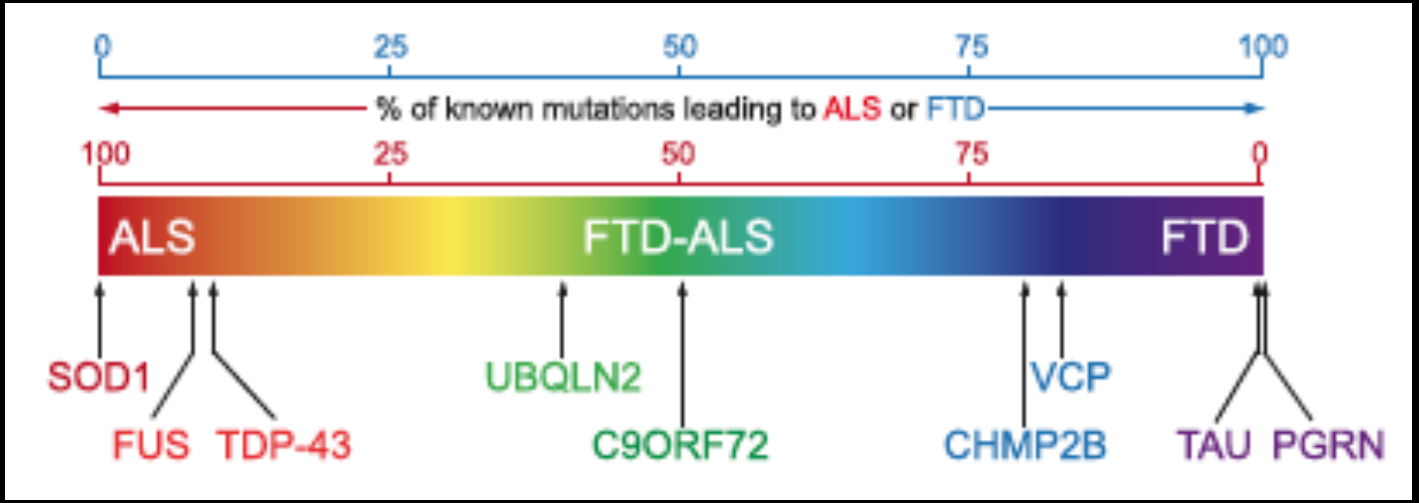
Table 1: **Genes (in order of discovery) associated with dominant mutations linked to adult-onset motor-predominant syndromes, showing the range of clinical phenotypes and principal neuropathological protein aggregates**

Turner et al (2013) ***Neurology***

Genetic of ALS



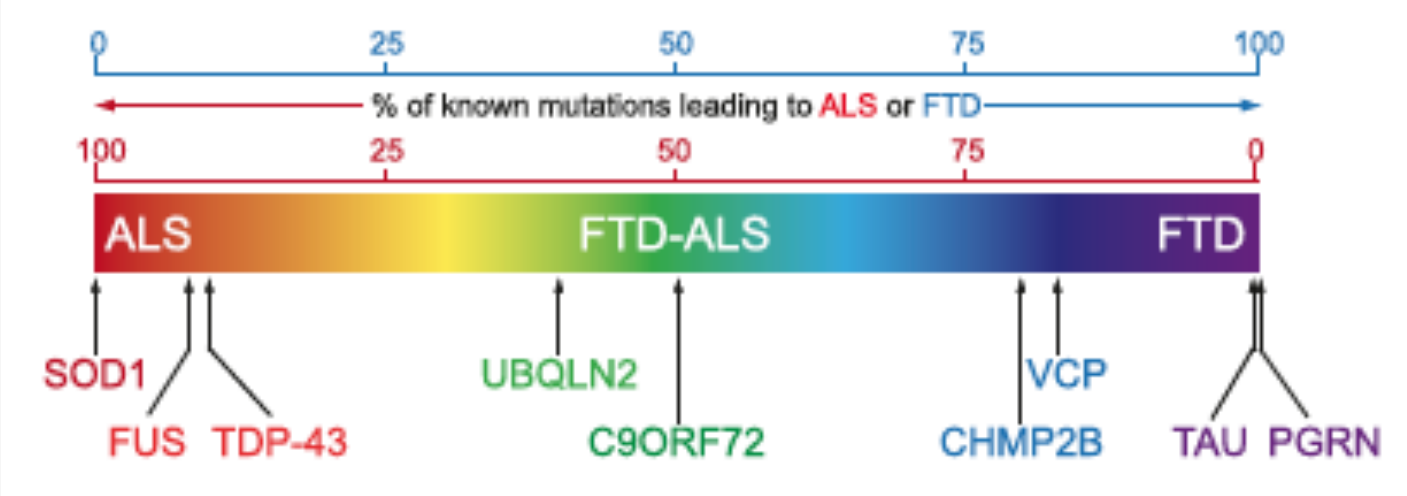
ALS and FTD


0
25
50
75
100
% of known mutations leading to ALS or FTD
100
25
50
75
0
ALS
FTD-ALS
FTD
SOD1
FUS
TDP-43
UBQLN2
C9ORF72
VCP
CHMP2B
TAU
PGRN

ALS and FTD

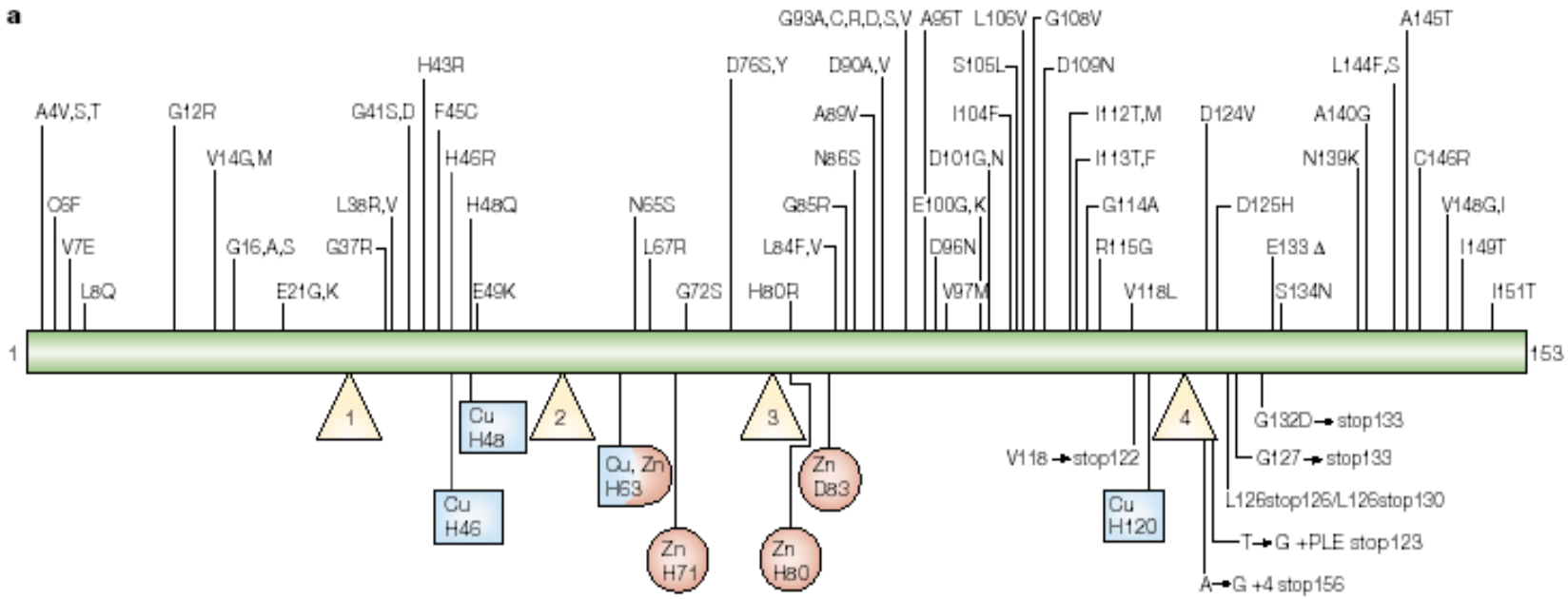
ALS usually first affects the limbs (spinal onset). Then brainstem and upper motor neurons in the cortex are affected in ALS. 15% of ALS patients develop behavioral or cognitive impairment.

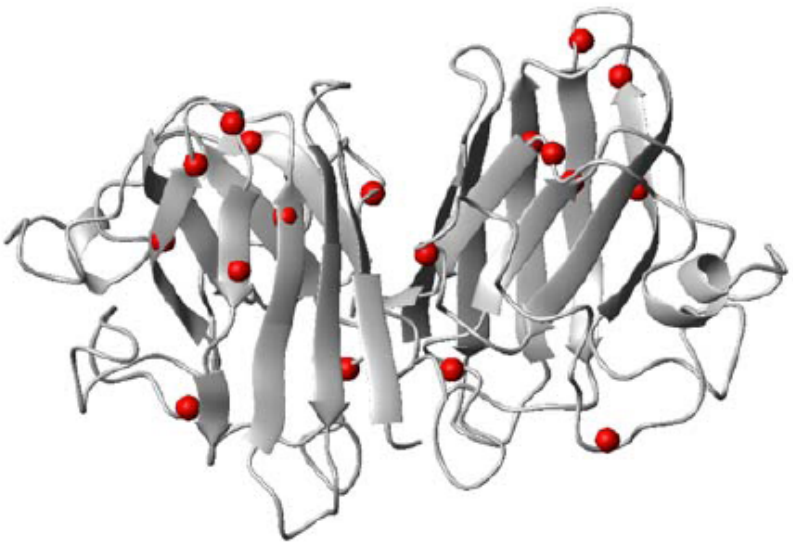
Fronto-temporal lobe degeneration (FTLD) is characterized by abnormalities of behavior in a large proportion of patients. 15% of FTD patients develop symptoms that fit ALS disease.



Ling et al (2013) Neuron

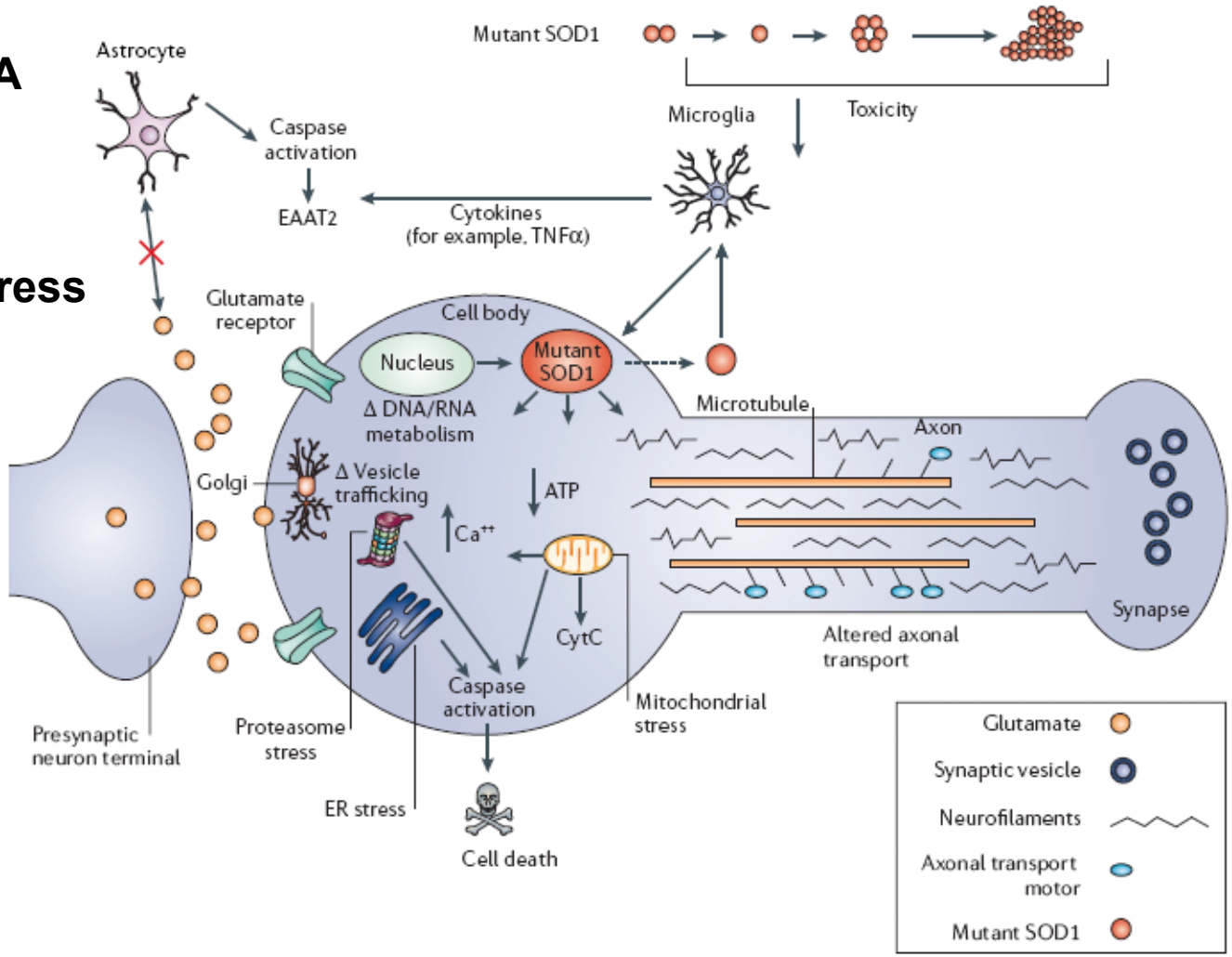
Mutant SOD1 and ALS





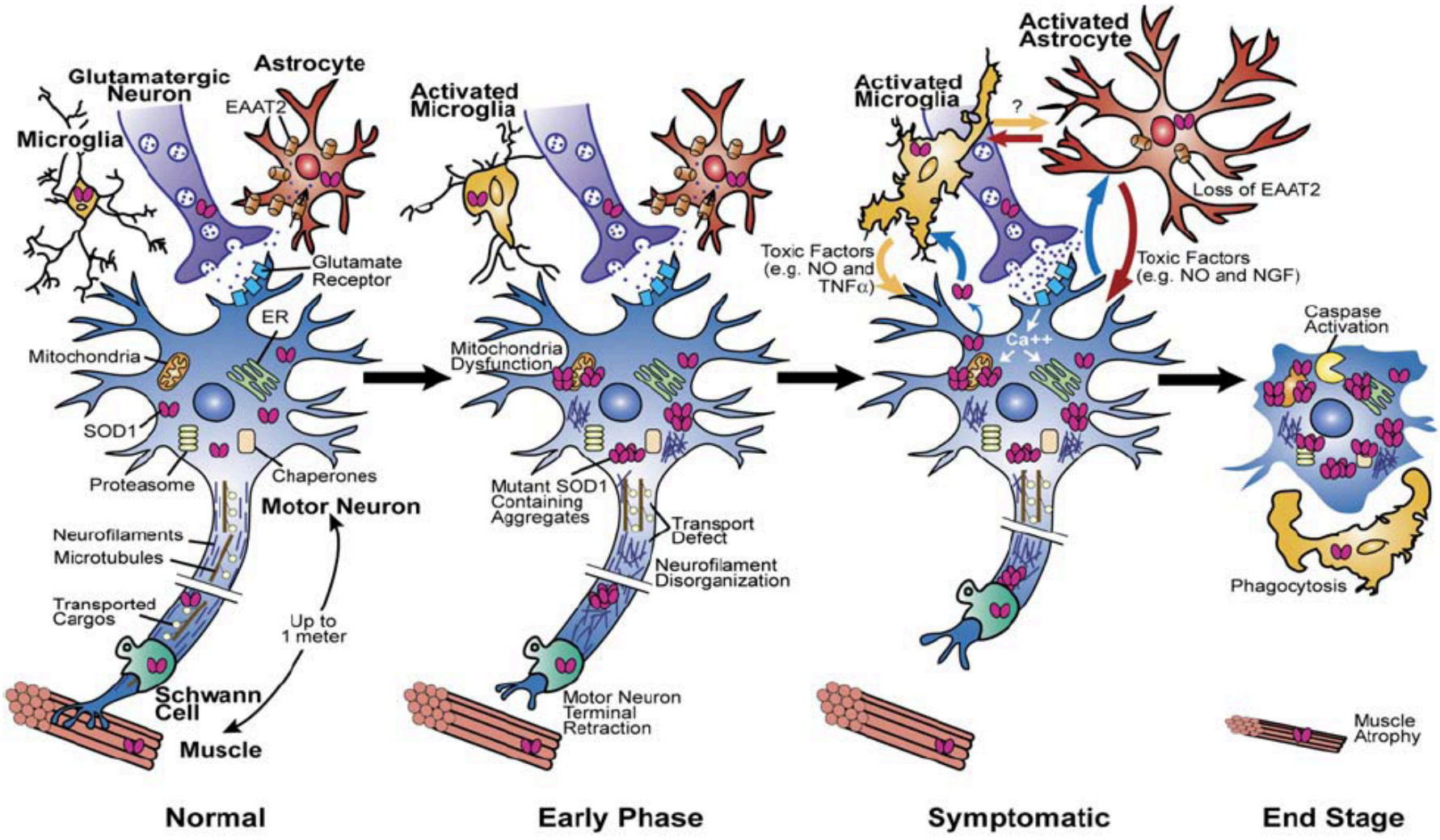
Cellular events triggered by SOD1

- Axonal transport
- Neurotoxicity -NMDA
- Inflammation
- Oxidative and ER stress
- Apoptosis



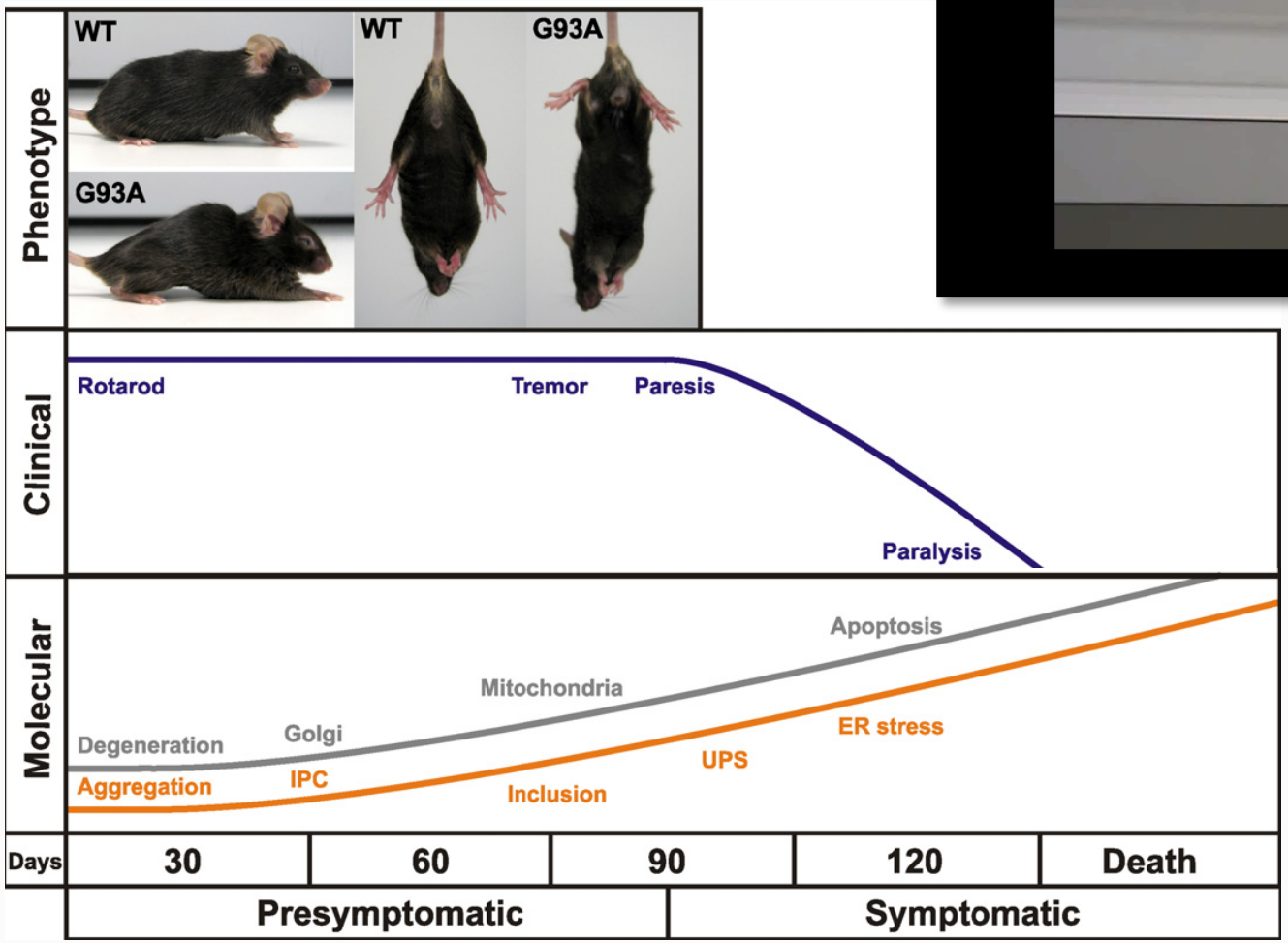
Pascinelli and Brown (2006) *Nat Rev Neurosci*

Temporal events in ALS



Boille et al., (2006) *Neuron*

Mouse models of ALS

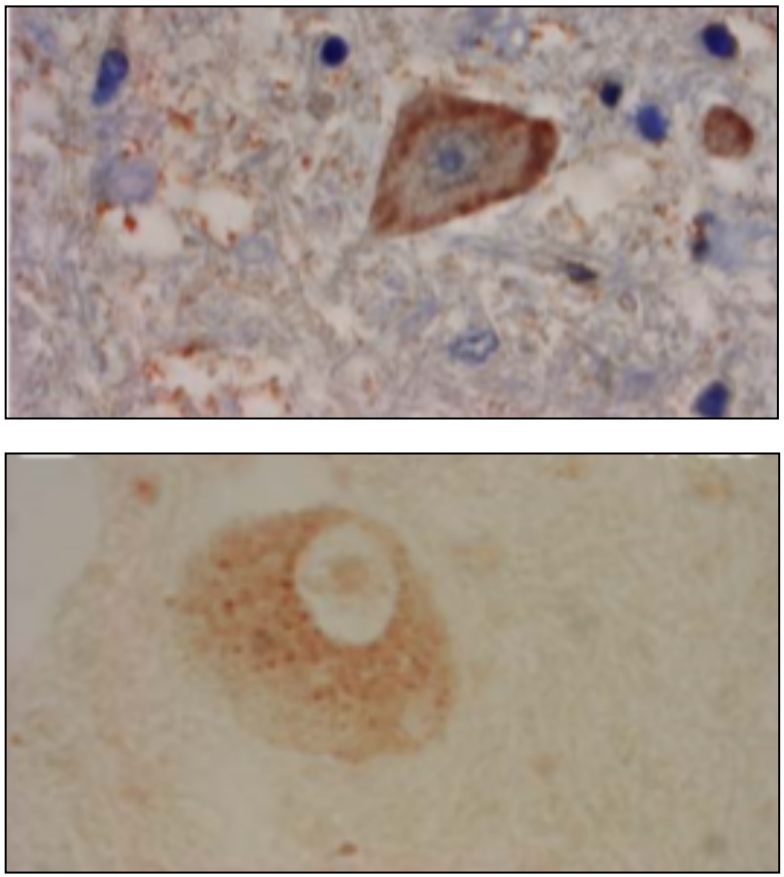


Turner y Tabot, Progress in Neurobiology 2008

Mouse models of ALS

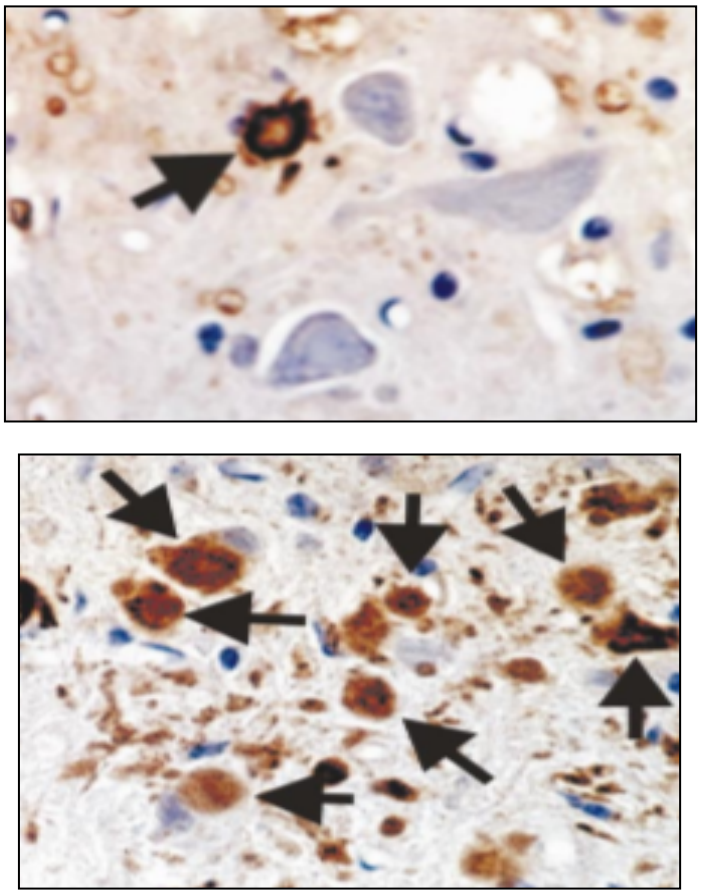
Anti-SOD1 staining

Spinal cord ALS patients



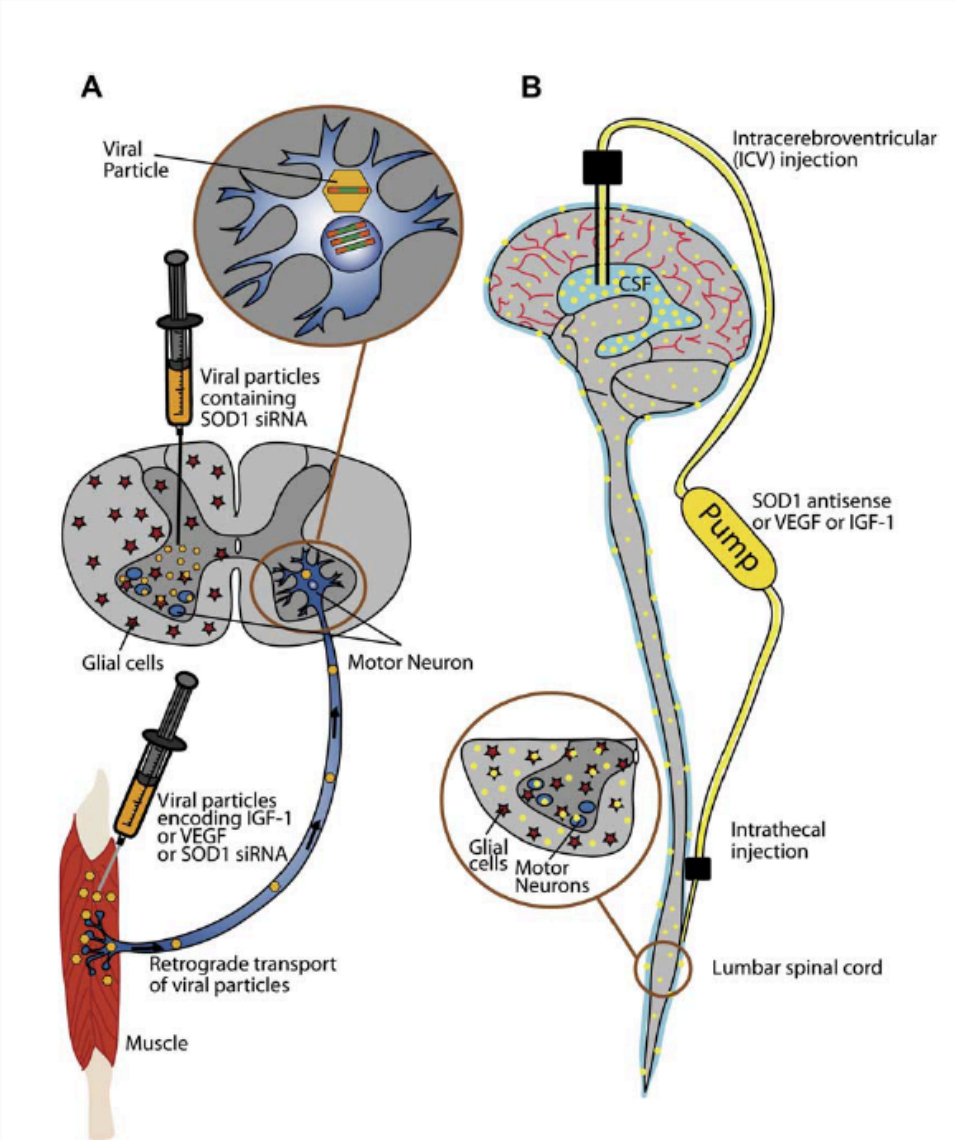
Forsberg et al. PLoS One, 2010

Mutant SOD1 transgenic mice



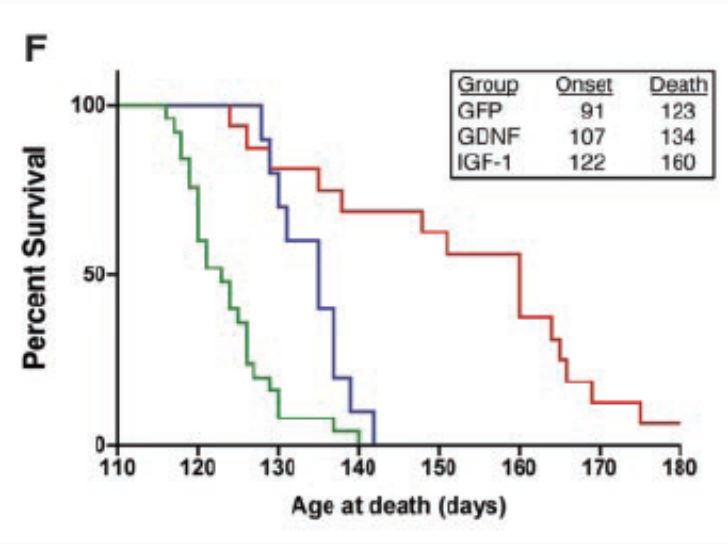
Watanabe et al. Neurobiol Dis, 2010

Gene therapy in ALS: Prosurvival factor

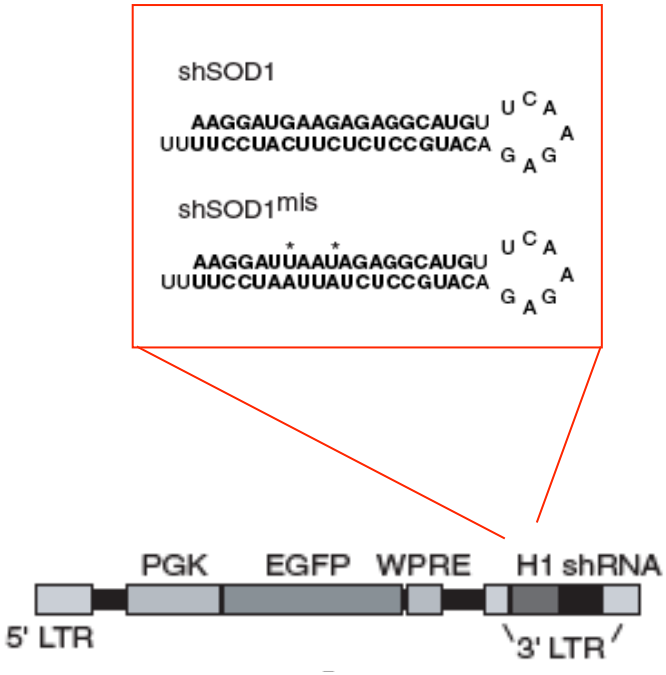


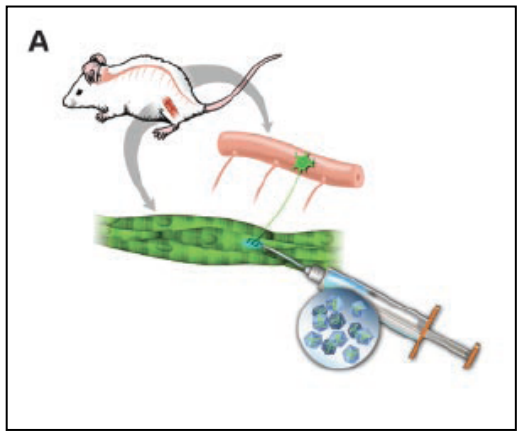
Retrograde Viral Delivery of IGF-1 Prolongs Survival in a Mouse ALS Model

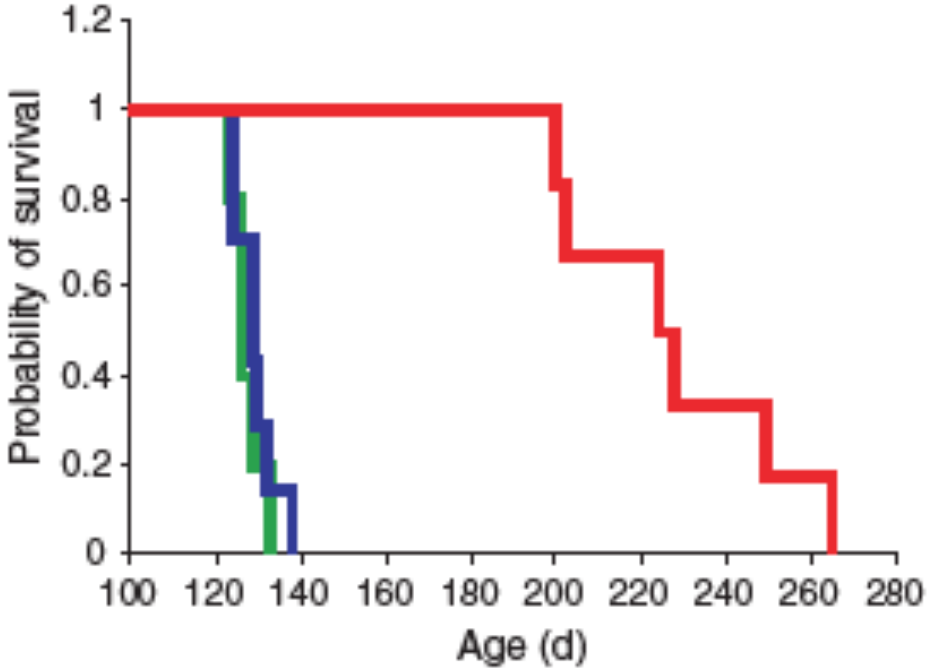
Brian K. Kaspar,[1] Jerònia Lladó,[2]* Nushin Sherkat,[1] Jeffrey D. Rothstein,[2] Fred H. Gage[1]†



Gene therapy to target mutant SOD1



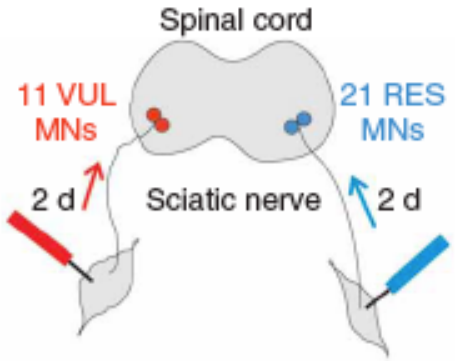




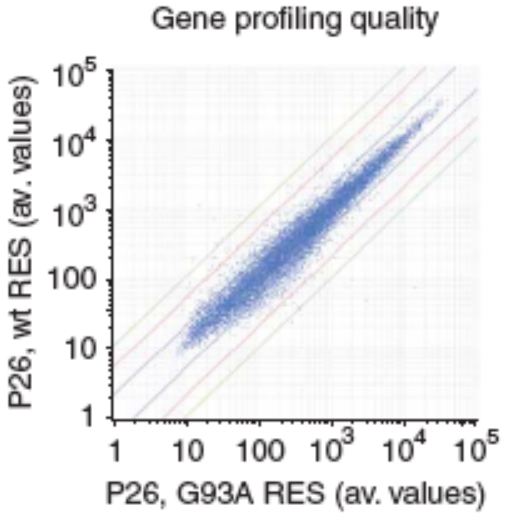
Ralph et al., (2005) *Nat Med*

Raoul al., (2005) *Nat Med*

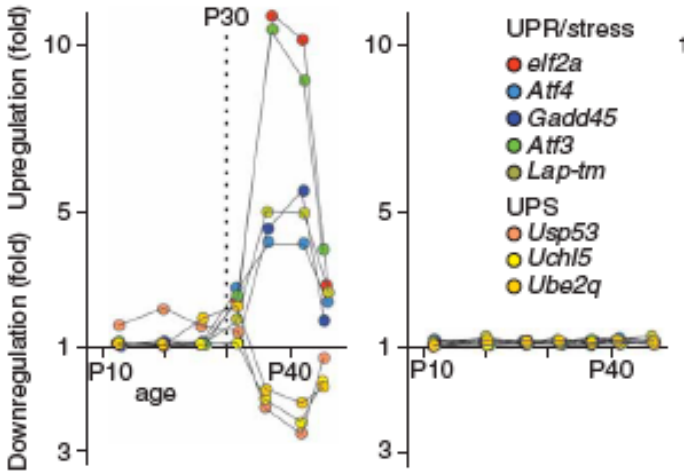
Gene expression profile in ALS



Laser capture



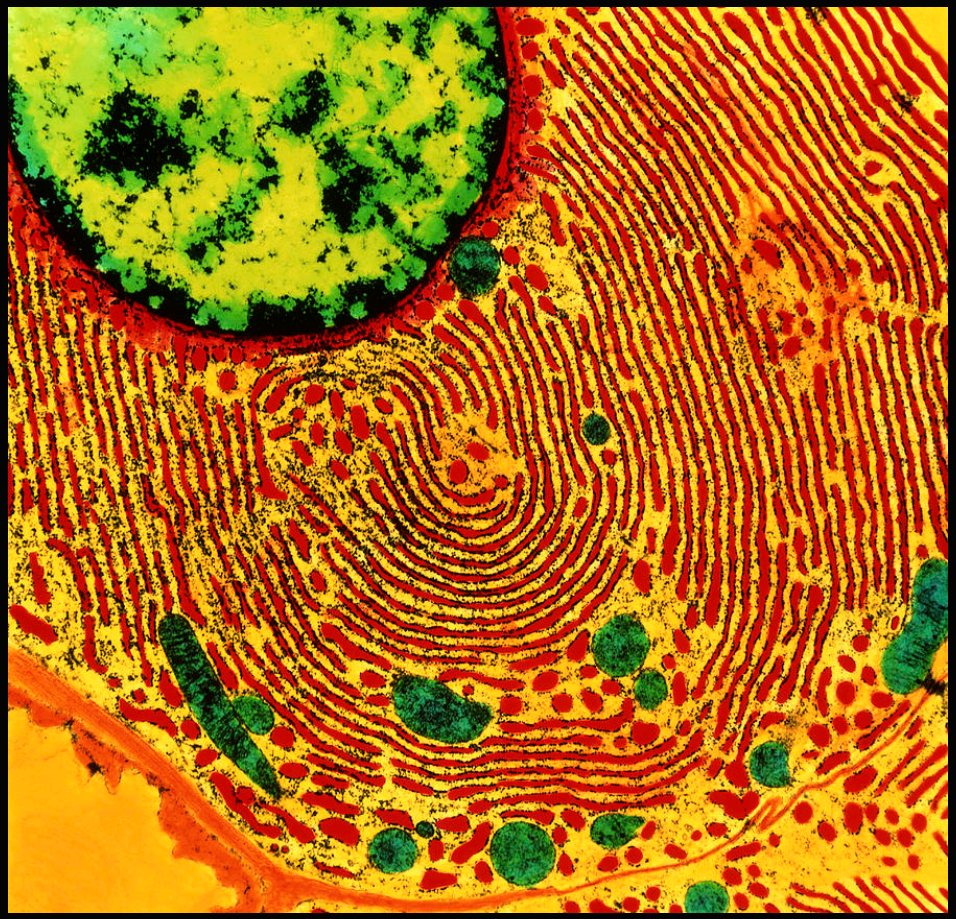
Vulnerable **Resistant**



Saxena et al., (2009) *Nat Neurosci*

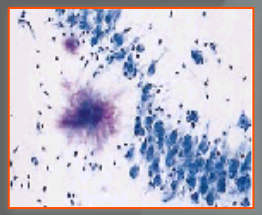
Biomedical research preclinical models of ALS

A new target:
Protein folding stress at the endoplasmic reticulum (ER)

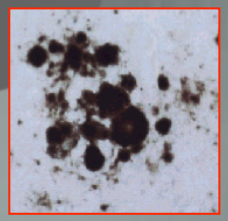


Protein misfolding and ER stress

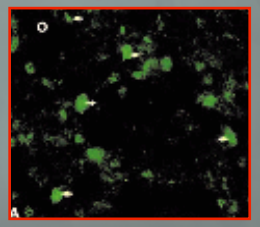
Amyloid β Deposition



Prion aggregation



α-Synuclein



ER STRESS

Accumulation of misfolded Proteins

UPR target genes

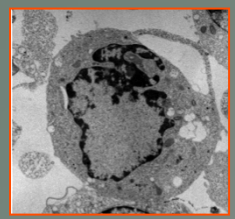
Autophagy

Folding

Protein quality control

Irreversible or chronic ER stress

APOPTOSIS



ER stress in neurodegenerative diseases

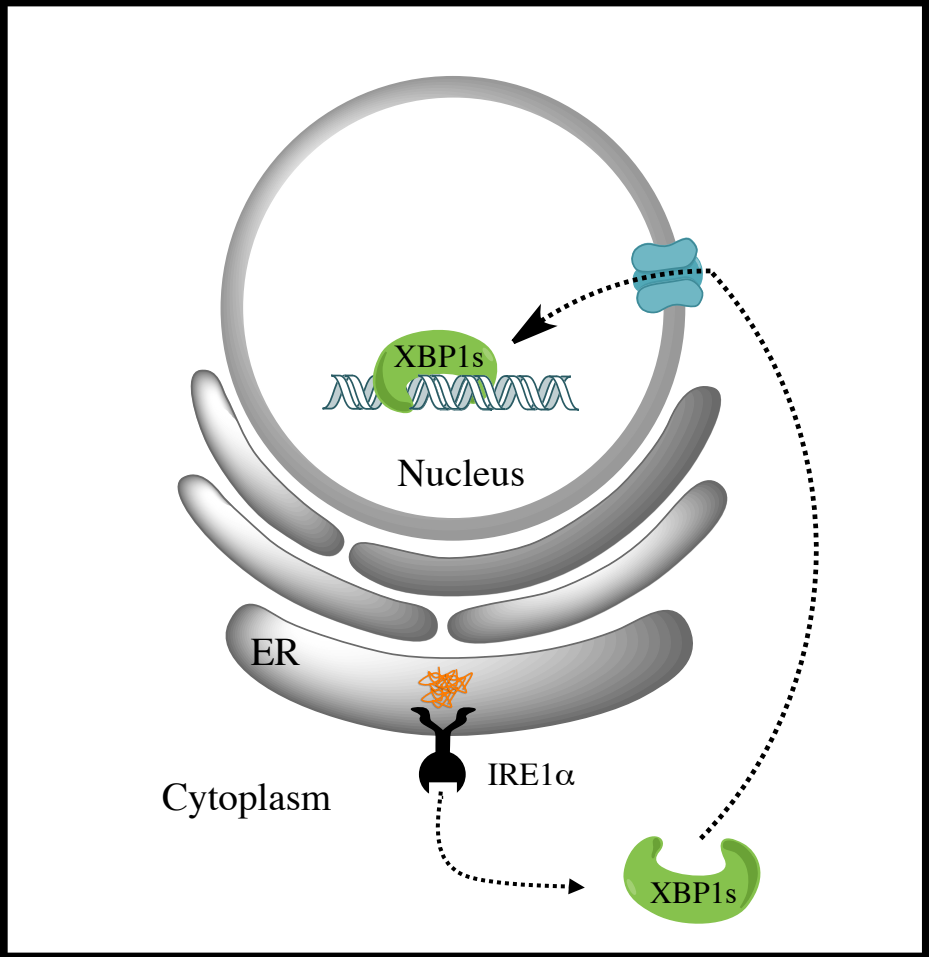
Disease	Animal models	Human studies (post-mortem)
Alzheimer	✓	✓
Parkinson	✓	✓
Amyotrophic lateral sclerosis	✓	✓
Creutzfeldt-Jacob (Prion)	✓	✓
Multiple sclerosis	✓	
Huntington	✓	✓
Spinocerebellar Ataxia	✓	
Spinal cord injury	✓	
Ischemia	✓	

NATURE REVIEWS | NEUROSCIENCE

Hetz and Mollereau (2014) *Nature Rev Neurosci*

The Unfolded Protein Response (UPR)

Is a cellular response triggered by conditions that alters the function of the ER (ER stress) and aims restoring organelle homeostasis.

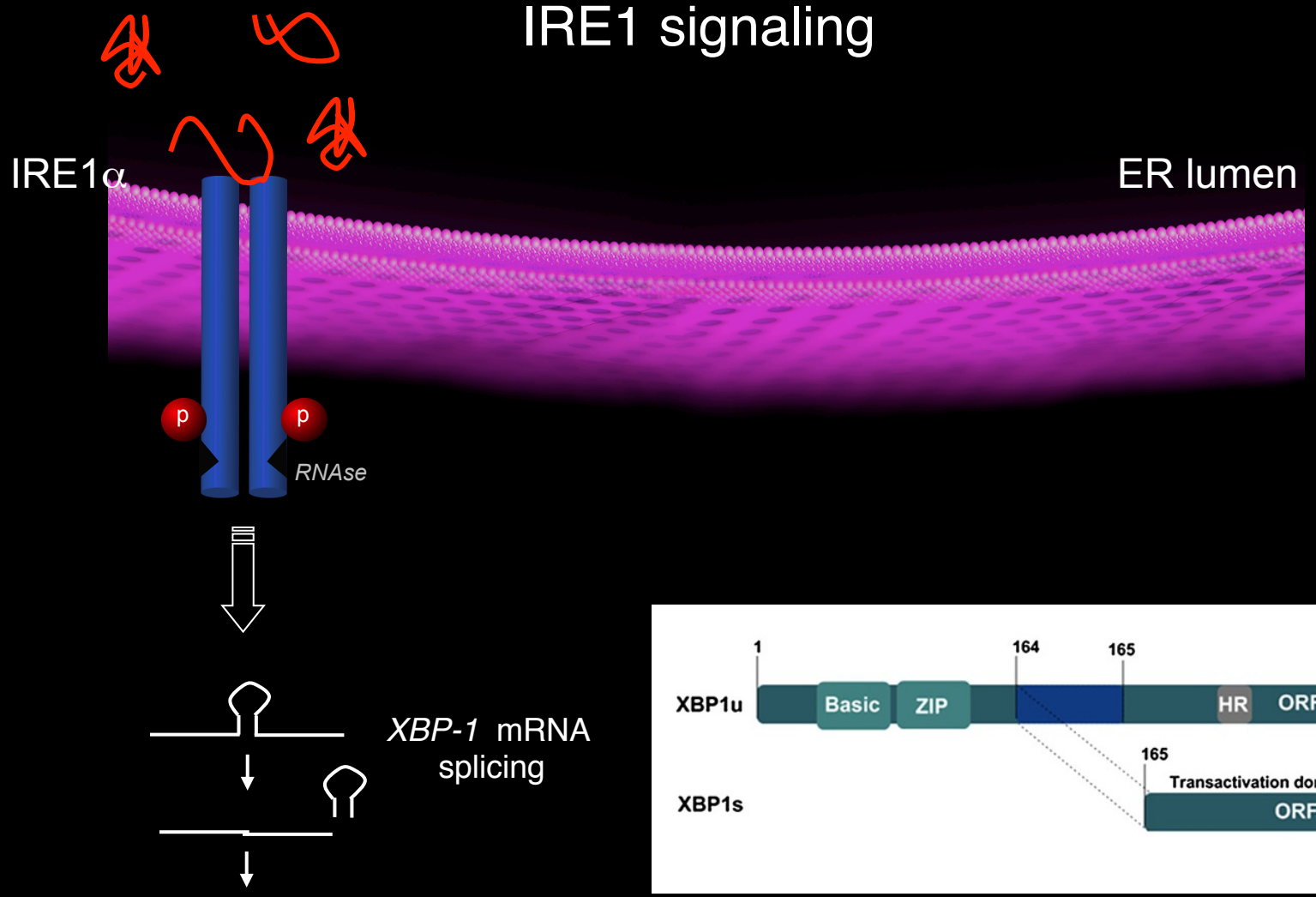


Effecter genes:
Adaptation to stress

Transcription factor

Stress sensor

IRE1 signaling



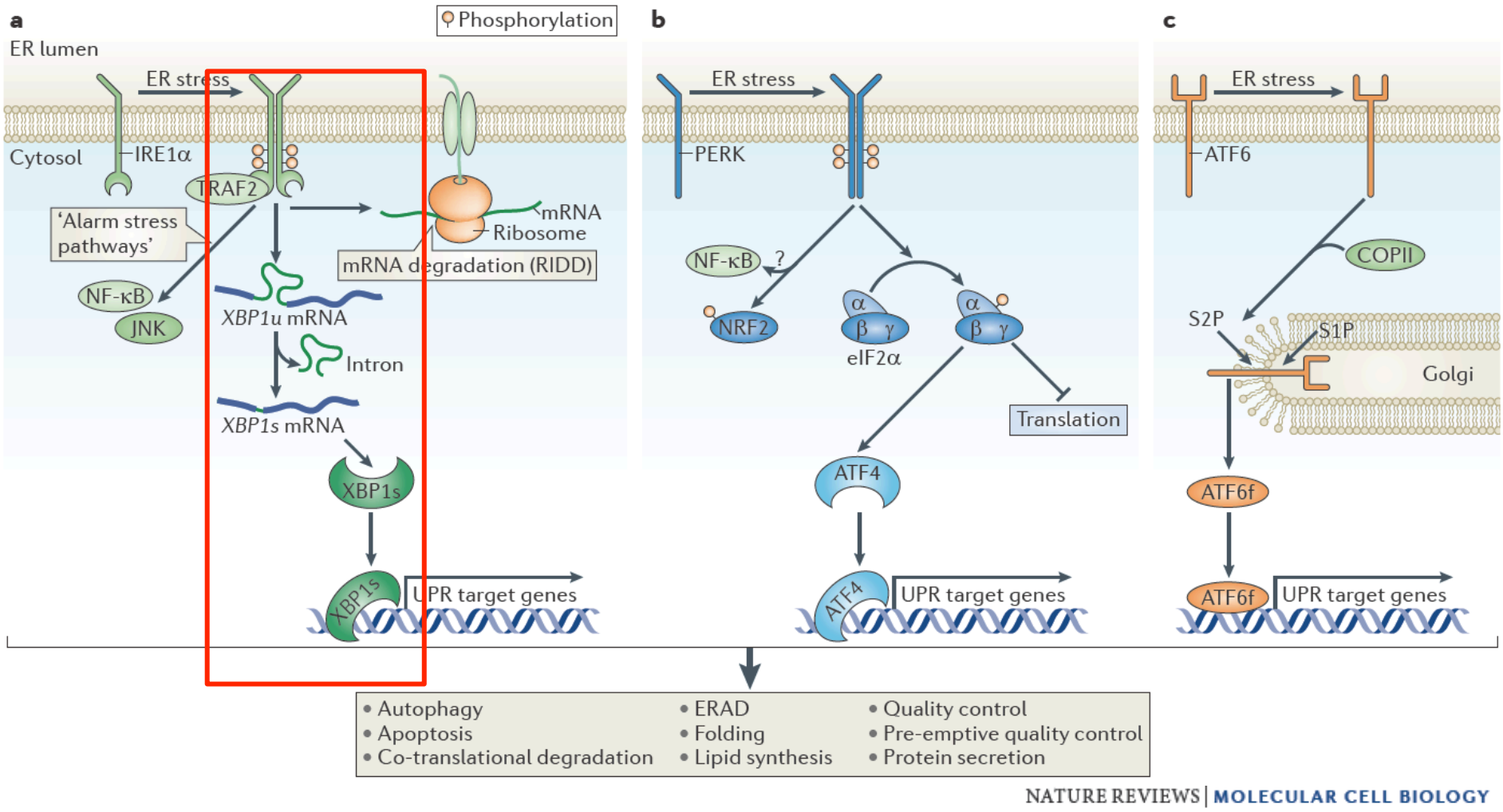
XBP-1s → *UPR genes*

Nucleus

ERAD
Chaperones
Quality control

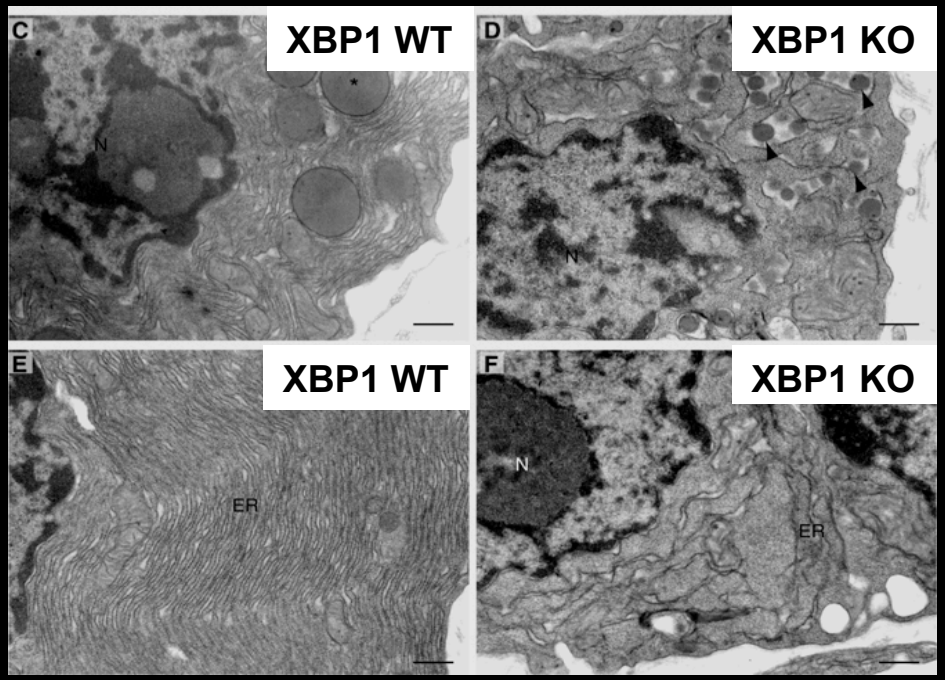
Calfon et al. (2002). *Nature*
Yoshida et al., (2001). *Cell*
Lee et al (2002). *Genes & Dev*

ER stress signalling

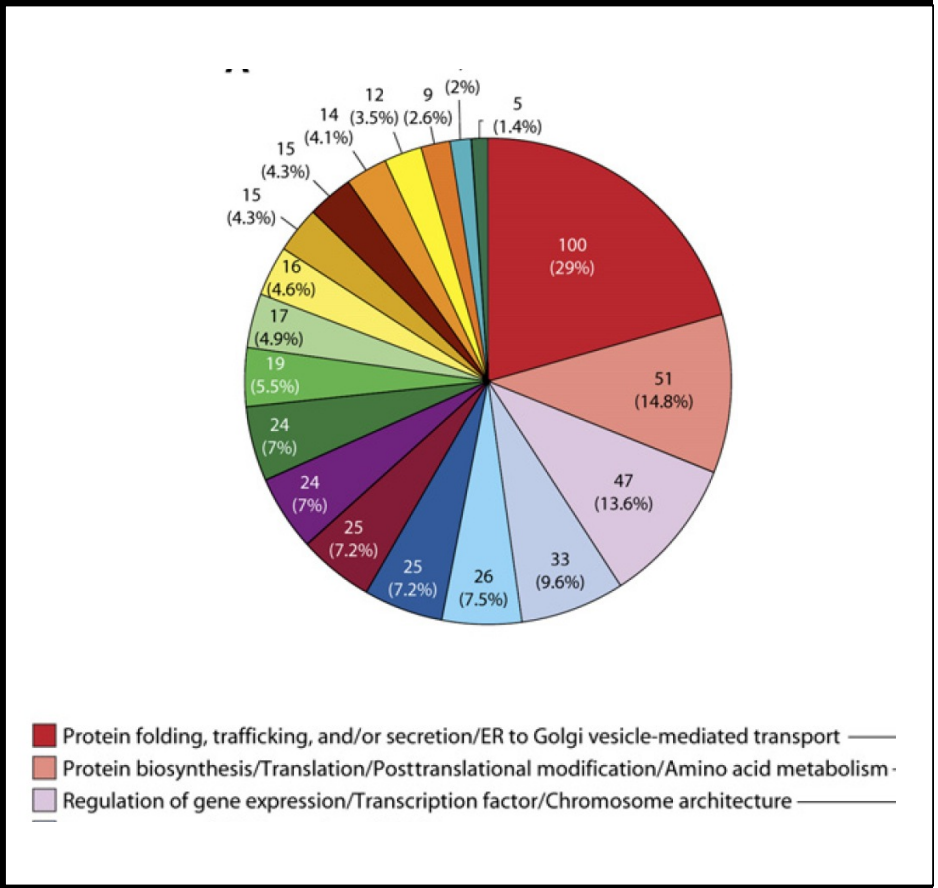


NATURE REVIEWS | MOLECULAR CELL BIOLOGY

The UPR is essential for secretory cell function

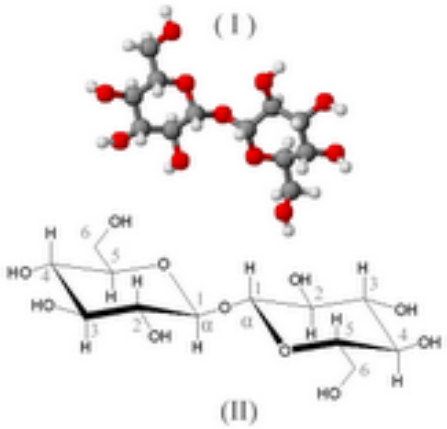


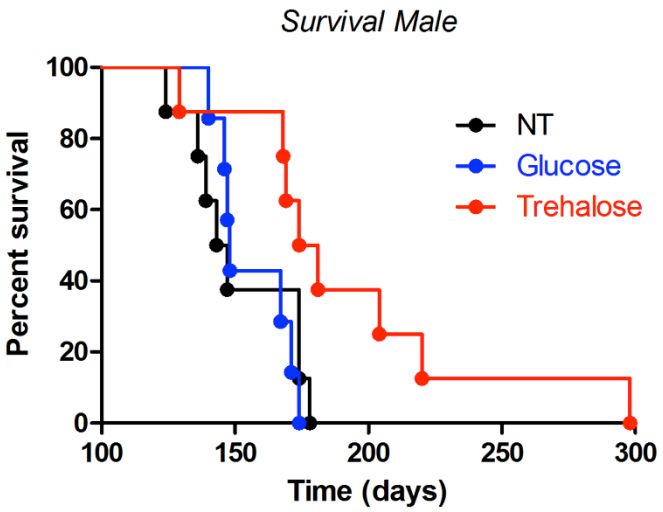
Lee et al., (2005) *EMBO J*

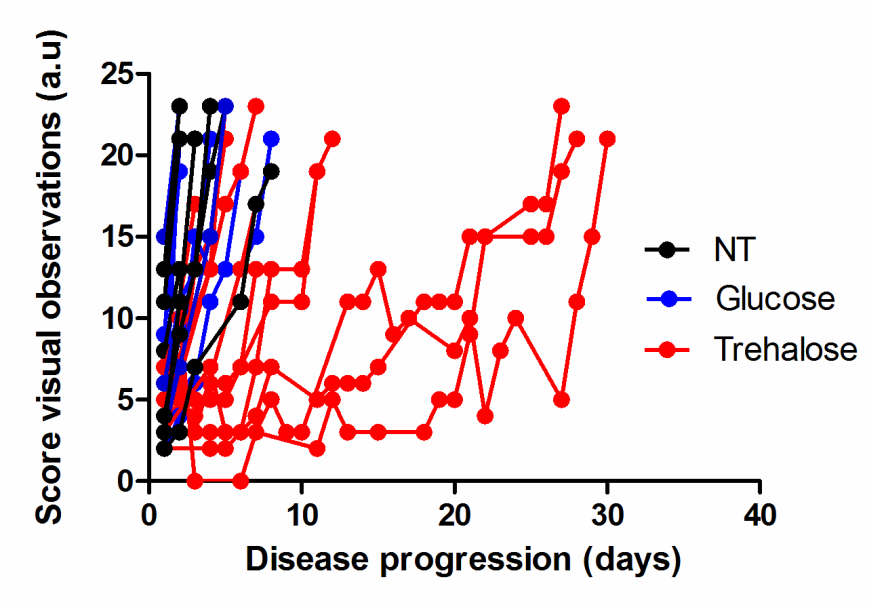


Acosta-Alvear et al., (2007) *Mol Cell*

A neuroprotective compound:
Trehalose, alleviates neuronal stress







Castillo et al., (2013) *Autophagy*

Gene therapy

Collaboration Donna Armentano and Geoffrey Parsons

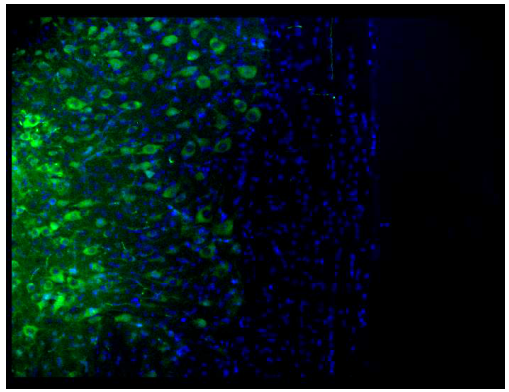
AAVs: Adeno-associated viruses

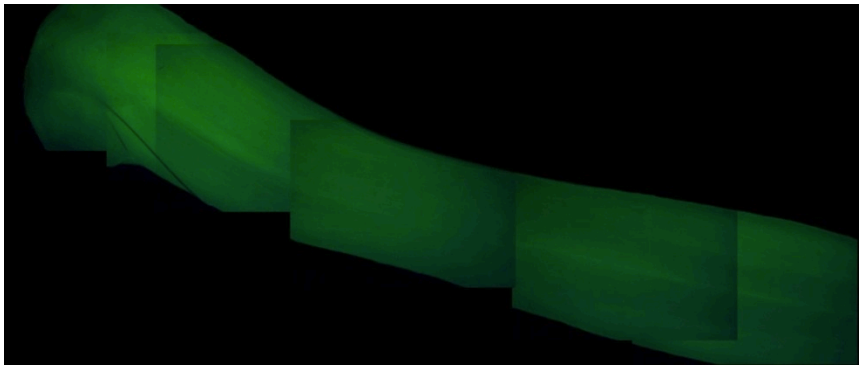
- Small viruses. No diseases associated with AAV infections.
- AAVs causes minimal immune responses.
- Can infect dividing and non dividing cells.
- The vector is maintained on an extra-chromosomal state without integrating into the genome of the host cell.
- Can be produces on a large scale.
- Recent clinical trials in humans show safety properties and therapeutic benefits in Leber´s Congenital Amaurosis

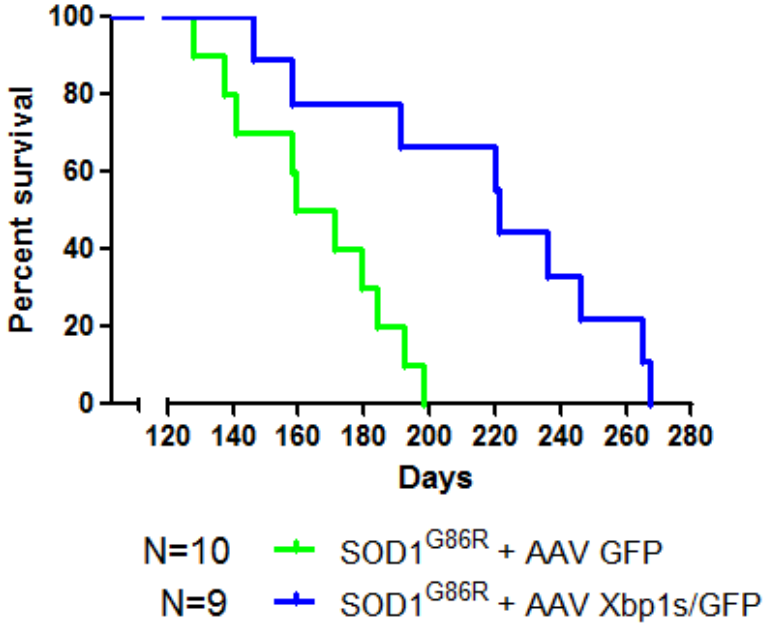
AAV-XBP1s delivery protects against ALS

In progress…

SOD1^{G86R} Tg mice

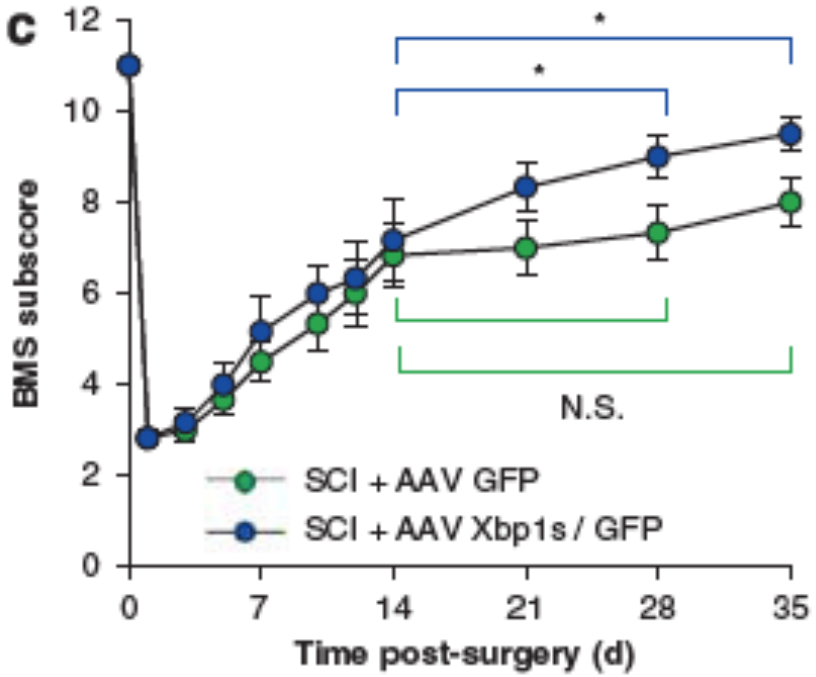


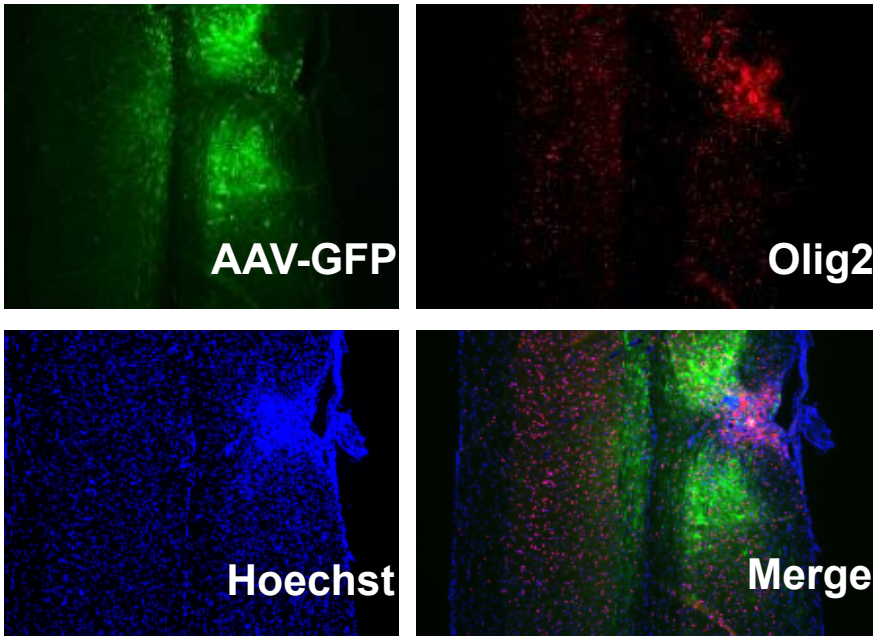




Castillo et al., (2013) *Cell Death Dis*

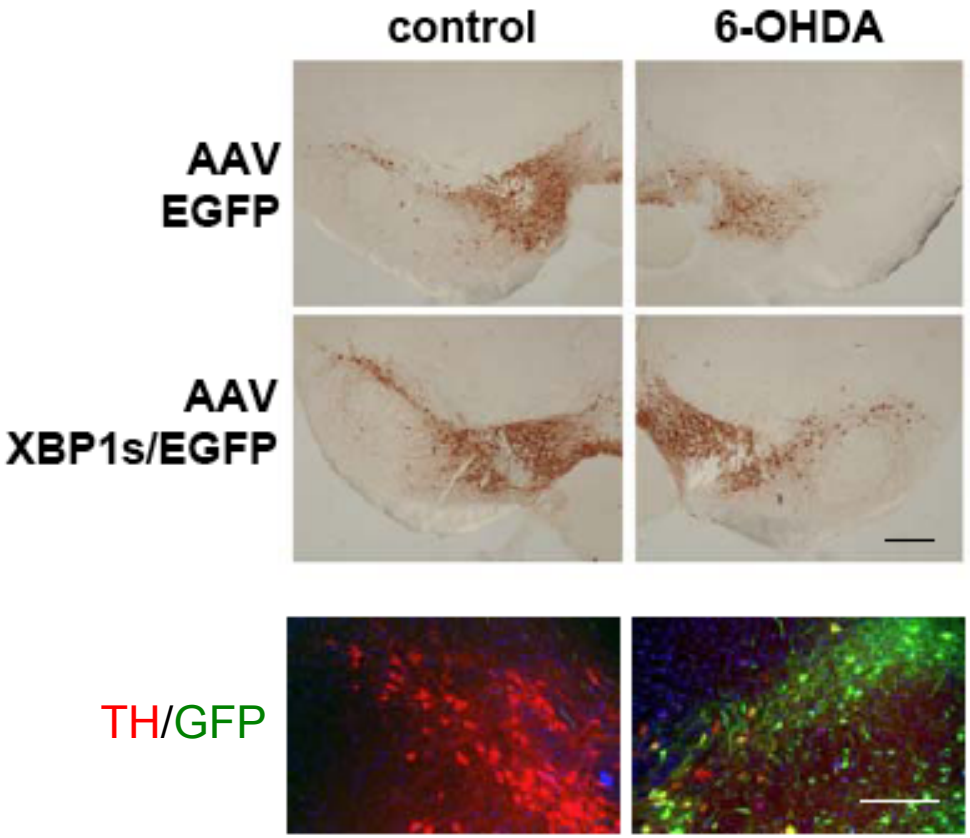
Gene-therapy in Spinal cord injury

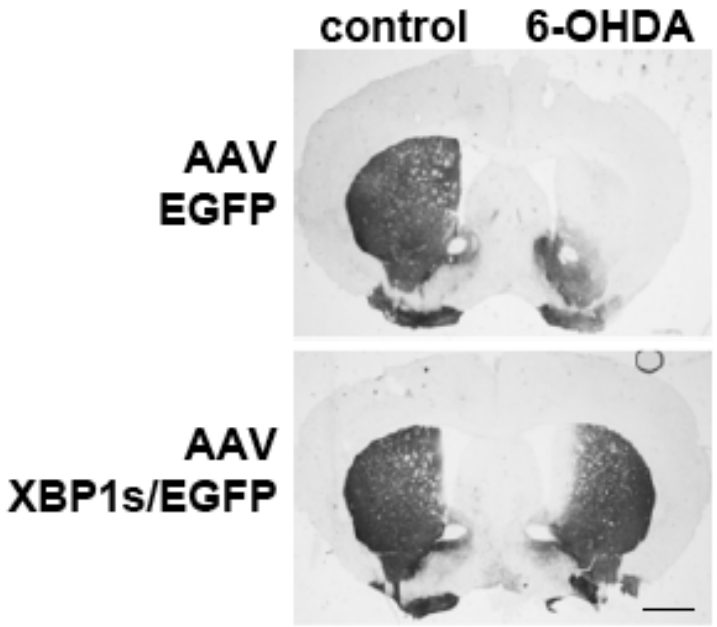




Valenzuela et al., 2012 *Cell Death Dis*

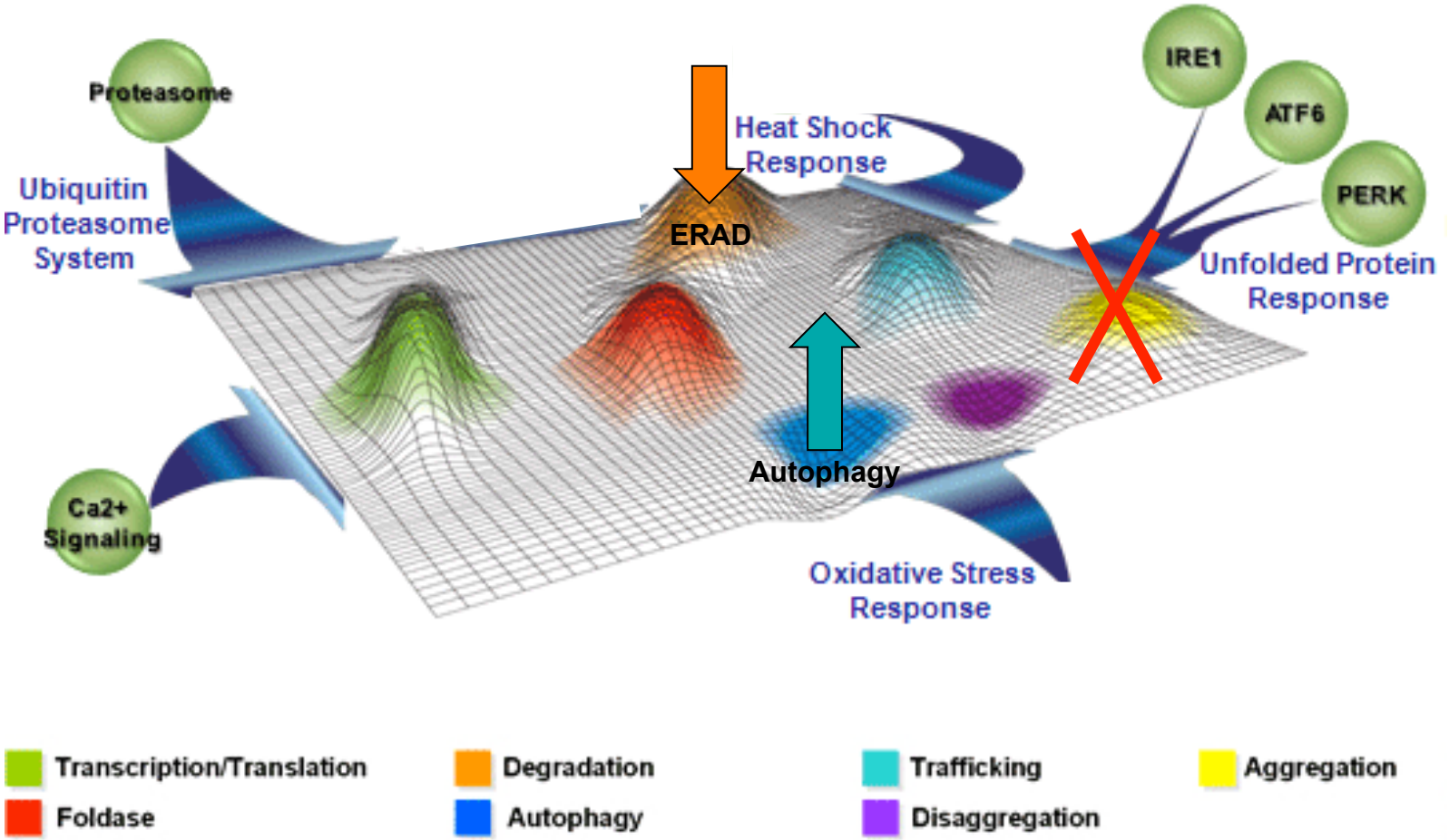
AAV-XBP1s delivery protects against Parkinson





Valdez, Mercado et al (2014). *PNAS*

Shifting the *protein homeostasis* network to protect against protein misfolding



Therapeutic strategies to target ER stress

Table 1 | **Examples of therapeutic effects of targeting the UPR in disease models *in vivo***

Organ	Disease	Agent	Effects	Refs
Pharmacological approaches				
CNS	Amyotrophic lateral sclerosis	Salubrinal	Extends lifespan of mutant *SOD1* transgenic mice	147
	Parkinson's disease	Salubrinal	Extends lifespan and increases neuronal survival of α-synuclein transgenic mice	146
	Ischaemia	BIX	Reduces infarct volume	161,162
	Excitotoxicity	Salubrinal	Improves neuronal survival	145
	Parkinson's disease	Tunicamycin	Protects neurons against 6-hydroxydopamine-induced toxicity	167
Gene therapy				
CNS	Retinitis pigmentosa	AAV-BiP	Restores visual function in mutant rhodopsin transgenic rats	169
	Retinal degeneration	AAV-XBP1s	Reduces axonal degeneration	170
	Glaucoma	AAV-XBP1s	Increases neuronal survival	170
	Spinal cord injury	AAV-XBP1s	Improves locomotor recovery	58
	Huntington's disease	AAV-XBP1s	Reduces aggregation of mutant huntingtin	171
	Parkinson's disease	AAV-BiP	Reduces toxicity and aggregation of α-synuclein	172
	Parkinson's disease	AV-XBP1s	Protects neurons against MPTP-induced toxicity	173
	Prion-related disorder	LV-GADD34	Reduces neuronal degeneration	60
Heart	Ischaemia	AV-PDIA1	Improves survival of myocardial cells	174
Liver	Diabetes	AAV-XBP1s	Improves glucose metabolism and insulin resistance	78
	Obesity	AAV-BiP	Reduces liver steatosis in obese mice	175

Hetz et al (2013). *Nature Rev Drug Discovery*

Hetz·Lab

Laboratory of Cellular Stress and Biomedicine

www.hetzlab.cl

Distinct contribution of the UPR to brain diseases

Table 2 | **Functional impact of distinct UPR signalling components in brain diseases**

Disease	Model	UPR manipulation	Phenotype	Refs
Amyotrophic lateral sclerosis	Mutant SOD1 Tg mice	PERK heterozygous	Disease exacerbation, increased SOD1 aggregation	40
		Salubrinal	Extended lifespan	39
		ATF4 knockout	Partial embryonic lethality, protection against disease progression	41
		XBP1 CNS-specific knockout	Neuroprotection, extended lifespan, decreased SOD1 aggregation	46
Parkinson's disease	α-synuclein Tg mice	Salubrinal	Neuroprotection	36
	Neurotoxins	AV XBP1s	Increased dopaminergic neuron survival	209
		ATF6 knockout	Increased neurodegeneration	33,34
		CHOP knockout	Neuroprotection	38
		AAV BiP	Dopaminergic neuron survival, decreased α-synuclein aggregation	35
Huntington's disease	Mutant HTT Tg mice	ATF4 knockout	No effects on mutant HTT aggregation	47
		XBP1 CNS-specific knockout	Neuroprotection, improved motor performance, reduced HTT levels	47
		AAV XBP1s	Decreased mutant HTT aggregation	210
Prion-related diseases	Scrapie prion	Salubrinal	Disease exacerbation	55
		XBP1 CNS-specific knockout	No effects on disease progression or prion replication	53
		Caspase 12 knockout	No effect on disease progression or prion replication	54
		LV GADD34	Global neuroprotection	55
		PERK inhibitor	Reduced neurodegeneration, delayed disease progression	56
Spinal cord injury	Mechanical injury	Salubrinal	Improved motor recovery and oligodendrocyte survival	67,211
		ATF4 knockout	Reduced motor recovery, increased oligodendrocyte apoptosis	66
		XBP1 CNS-specific knockout	Reduced locomotor recovery	66
		CHOP knockout	Increased locomotor recovery and oligodendrocyte survival	68,69
		AAV XBP1s	Improved motor recovery and oligodendrocyte survival	66
Alzheimer's disease	APP/PS1 Tg mice	JNK3 knockout	Reduced amyloid-β, neuronal loss and cognitive dysfunction	59
		PERK CNS-specific knockout	Improved learning, memory and LTP	61

NATURE REVIEWS | NEUROSCIENCE

Hetz and Mollereau (2014). *Nature Rev Neurosc*