DISEASES CAUSED BY TRINUCLEOTIDE-REPEAT MUTATIONS

1991: ***discovery of expanding trinucleotide repeats as a cause of fragile-X syndrome,*** **a landmark in human genetics**

General principles that apply to these diseases:

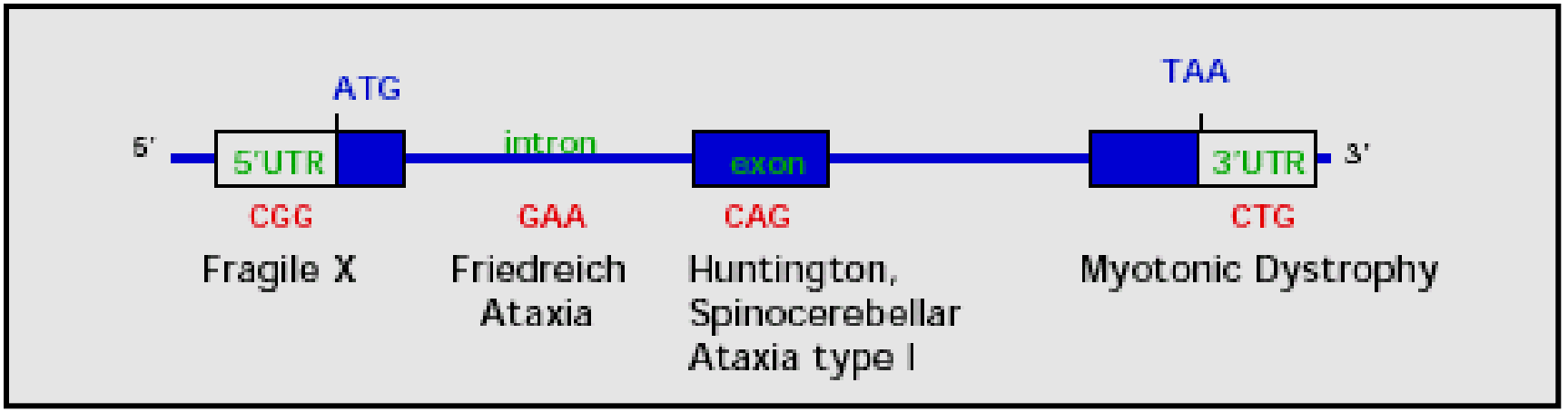
1. **Causative mutations are associated with the expansion of a stretch of trinucleotides that usually share the nucleotides G and C. Repeat length increases at every generation (dynamic mutation). Trinucleotide repeat expansions cause unusual forms of inheritance, including anticipation**

2. In all cases the **DNA is unstable**, and an **expansion of the repeats above a certain threshold impairs gene function** in various ways

3. **The proclivity to expand depends strongly on the sex of the transmitting parent**
   - in the fragile-X syndrome, expansions occur during oogenesis
   - in Huntington disease they occur during spermatogenesis

4. **Mutations can be divided into two groups**:
   I. the repeat expansions occur in noncoding regions (fragile-X syndrome and myotonic dystrophy)
   II. expansions occur in the coding regions (Huntington disease)

The pathogenetic mechanisms underlying disorders caused by expansions that affect coding regions seem to be distinct from those in which the expansions affect non coding regions



CAG → Gln → poly-Gln diseases, all presenting with CNS lesions

CGG → Arg (X-fragile)

I. Expansions affecting coding regions → gain of function (Huntington disease)

*usually involve **CAG repeats** coding for **polyglutamine tracts** in the corresponding proteins*

- **Polyglutamine diseases**, characterized by progressive neurodegeneration, typically striking in midlife

- Polyglutamine expansions lead to toxic gain of function, whereby the abnormal protein interferes with the function of the normal protein

The precise mechanisms by which expanded polyglutamine proteins cause disease is not fully understood

However, some general features have emerged:

In most cases the **proteins are misfolded and tend to aggregate**:
- aggregates may suppress transcription of other genes
- aggregates cause mitochondrial dysfunction
- aggregates trigger the unfolded-protein stress response and apoptosis

- **accumulation of aggregated mutant proteins in large intranuclear inclusions**

II. When expansions affect noncoding regions → loss of function

(e.g Fragile X syndrome)

the resulting mutations are **loss-of-function** type

Typically, such disorders affect many systems

NB: many noncoding repeat disorders are characterized by intermediate-size expansions, or premutations, that expand to full mutations in germ cells

Dynamic mutations

Anticipation <u>a hallmark of trinucleotide repeat expansion</u>

- severity and rapidity of disease progression increase over several generations (variable expressivity in the same family)

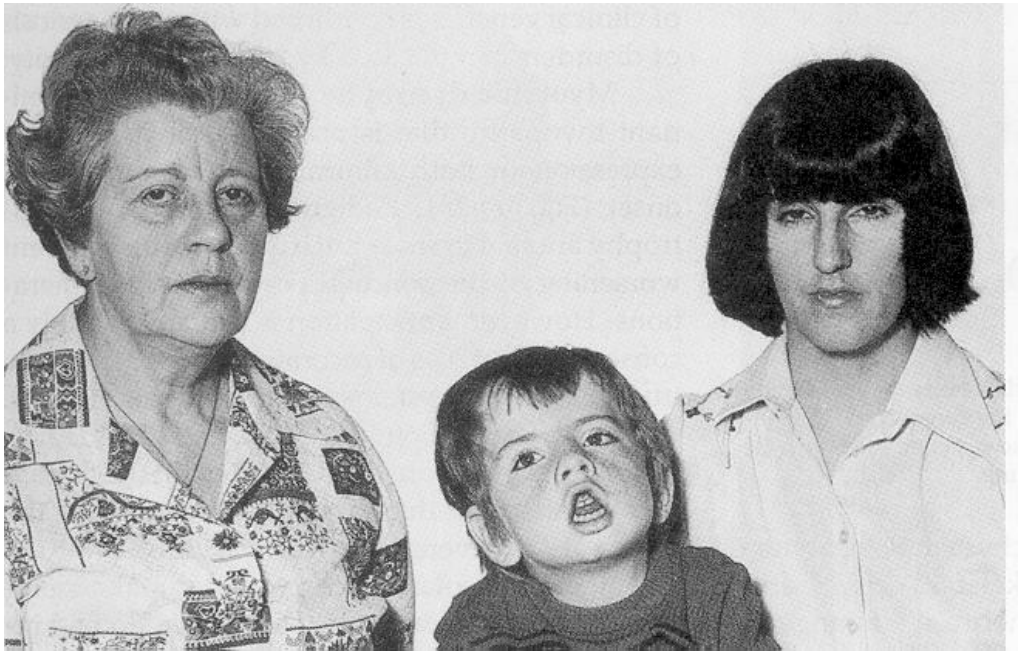
- **mild symptoms in the first generation to severe symptoms in later generations due to stepwise expansion of unstable triple repeats**

- **in the <u>normal population</u>, the length of the repeat is polymorphic, but <u>stable</u>**

- **the first step is the formation of a premutation that has a normal phenotype but is <u>unstable</u>**

- **the premutation then expands in a subsequent generation to a much greater length and further instability**

Anticipation in a family with myotonic dystrophy (DM)



Anticipation is shown in this family with **myotonic dystrophy** in which the grandmother has only late onset cataracts, her daughter has mild symptoms (drooping eyelids and muscular sagging and myotonia in the face), and the **grandson has a severe form of congenital myotonic dystrophy**

Fragile X syndrome

- X-linked

- occurs in 1/1,250 male births and is the second most common cause of mental retardation

- associated with moderate to severe mental retardation often with developmental delays and autistic behavior

- disease shows anticipation
  - increasing penetrance in succeeding generations
  - passage through female can increase risk to next generation (expansion during oogenesis)

- females with one affected chromosome and males with premutations can show mild cognitive defects and schizotypal symptoms

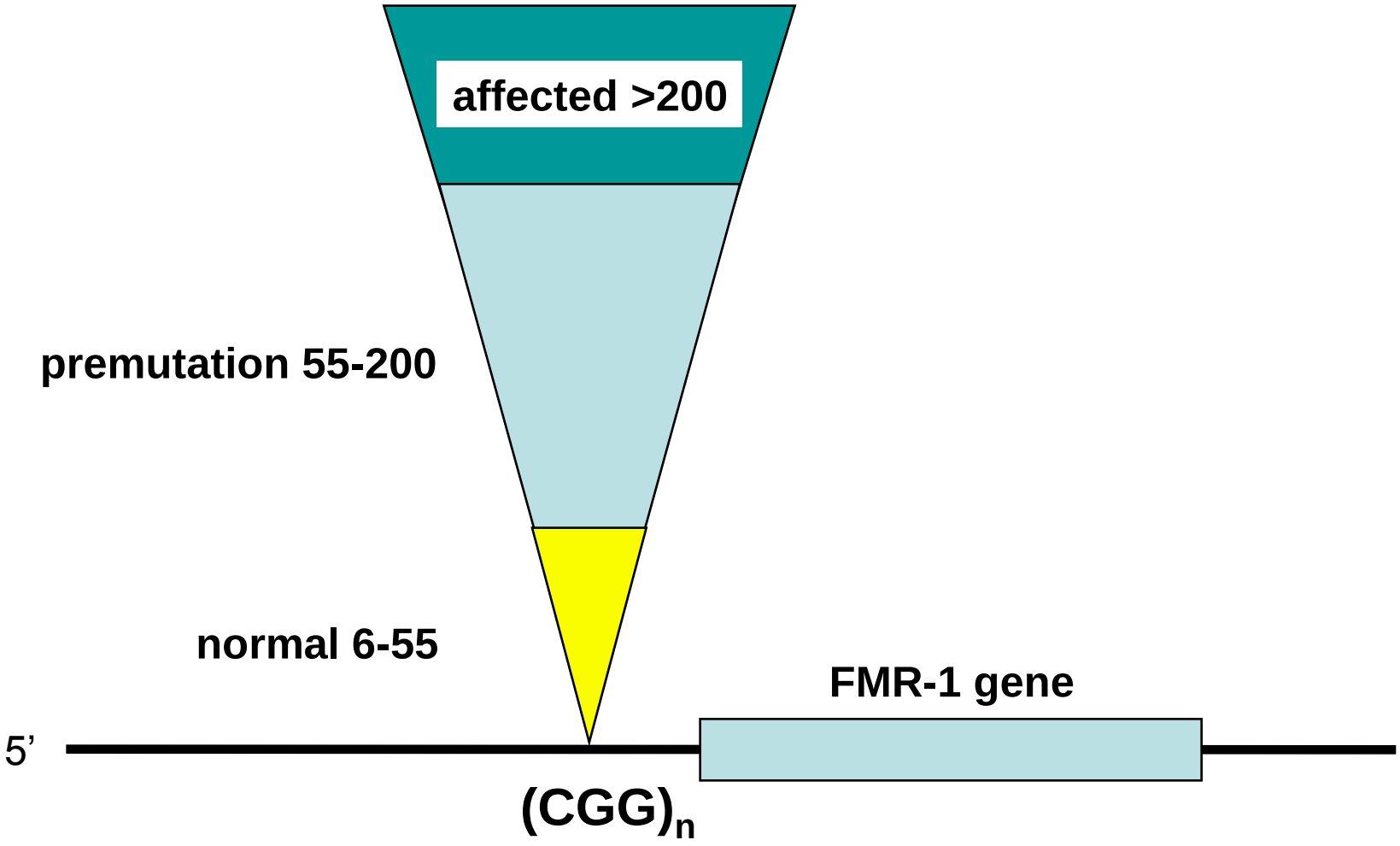
- caused by **mutations in the *FMR-1* *(familial mental retardation)* gene** which is expressed in brain and testes at highest levels, and widely in the embryo

- normal gene has a 5'-UTR **CGG repeat that is polymorphic in the population and that ranges from 6-55 repeats**

- **patients have expanded numbers of repeats, up to thousands**

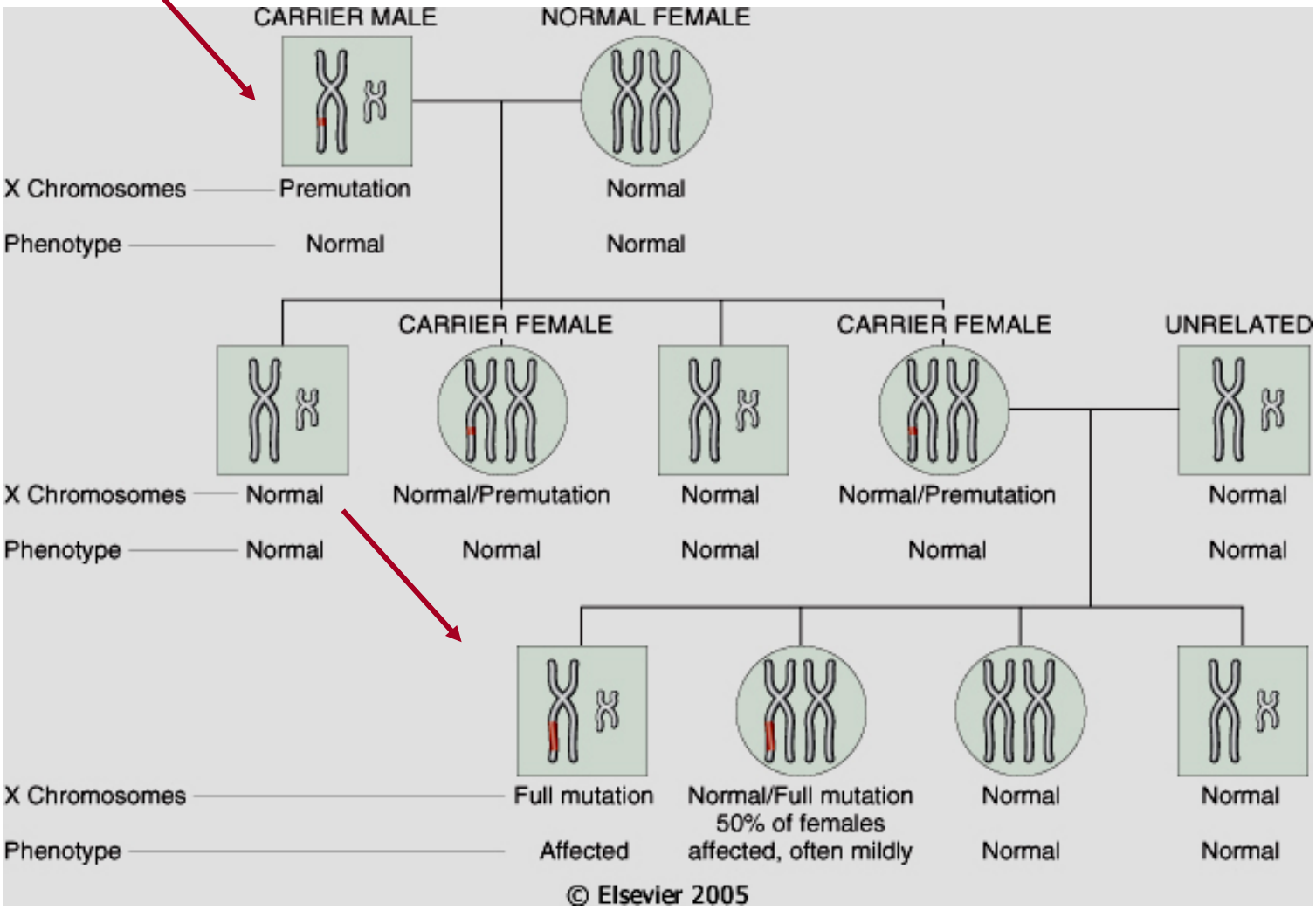
- results in transcriptional silencing (promoter hypermethylation)

Structure and inheritance of CGG (Arg) repeats in fragile X syndrome

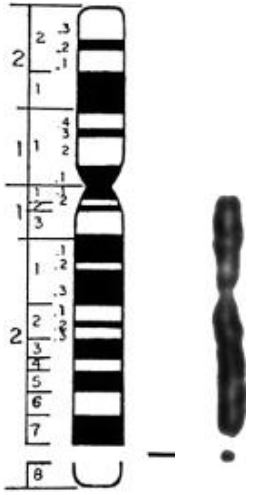


Fragile-X pedigree

CGG expansion

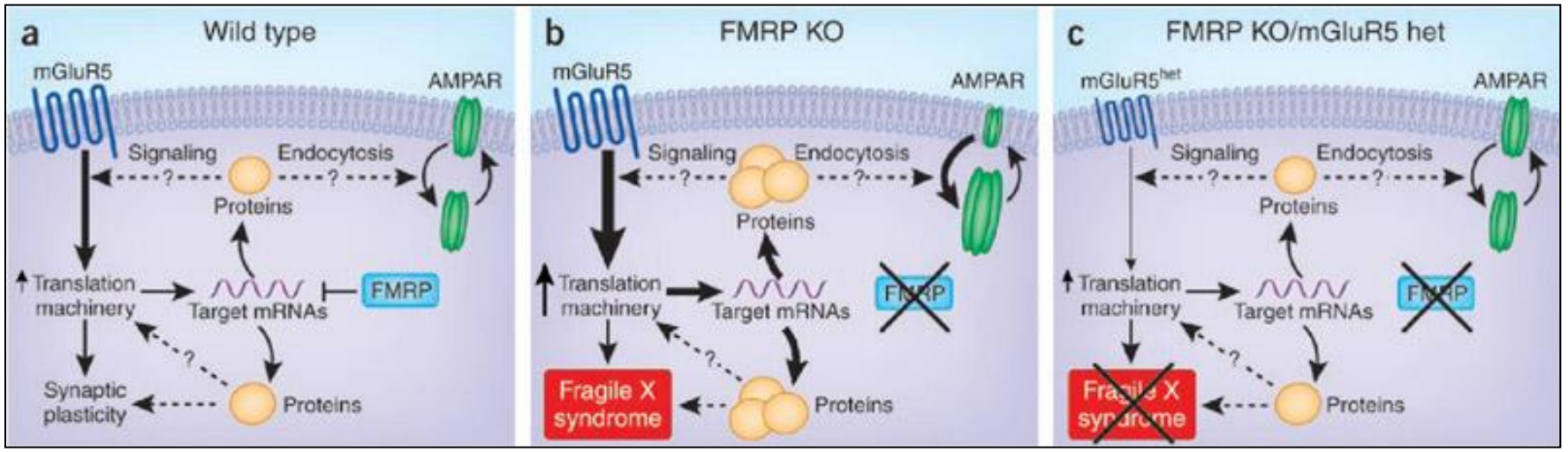


Fragile-X seen as discontinuity of staining

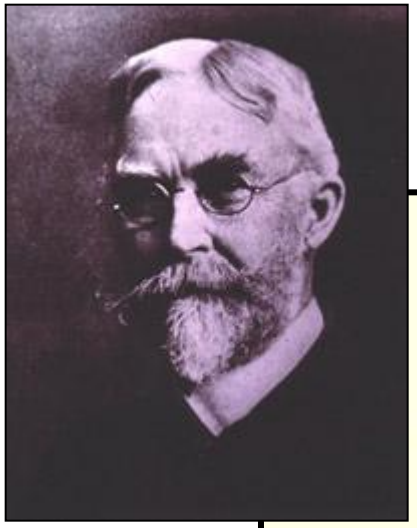


Expansion during oogenesis

Absence of FMRP results in constitutive translation

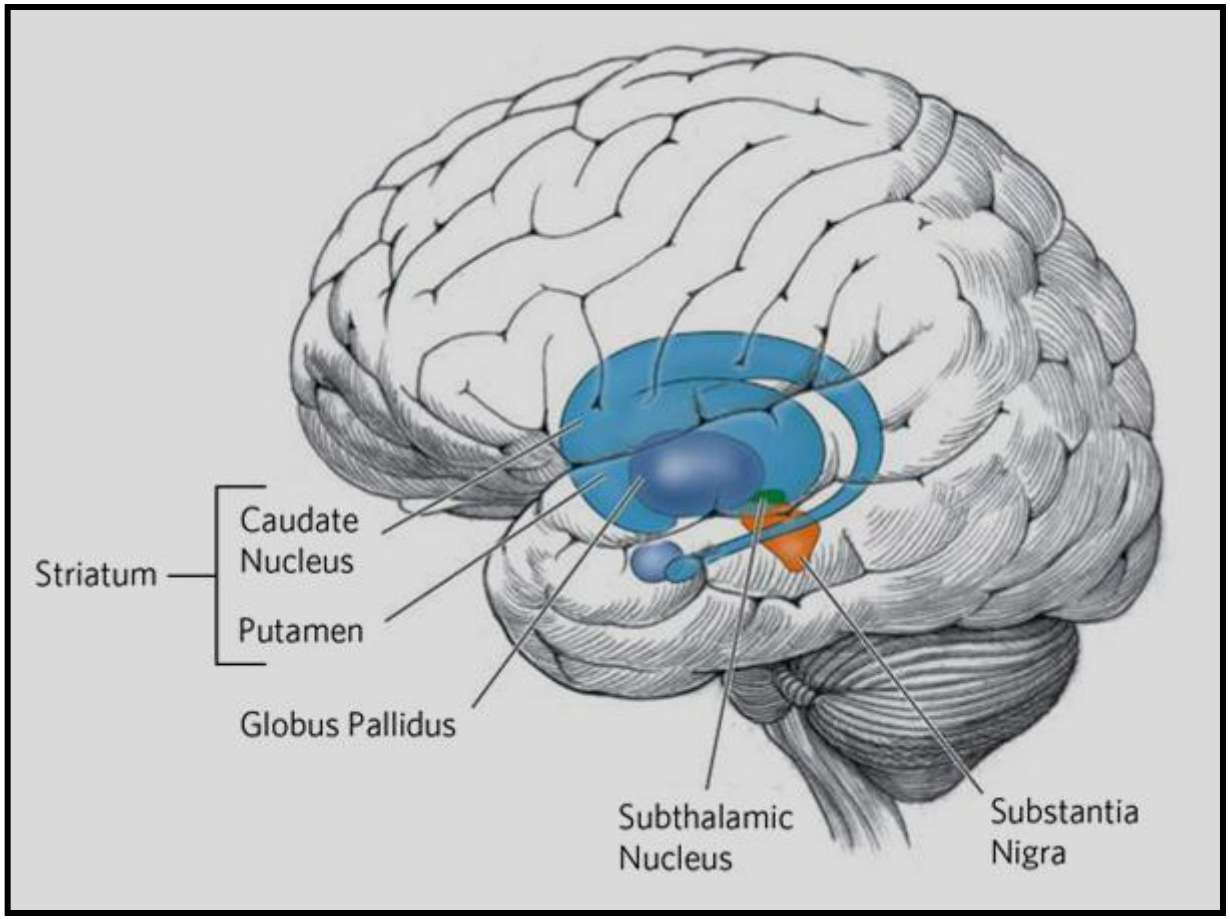


(**a**) mGluR5 signaling in wild-type mice activates the translation machinery and induces specific protein synthesis–dependent forms of synaptic plasticity. Some of the mGluR5-regulated mRNAs are translationally suppressed by fragile X mental retardation protein (FMRP). (**b**) In FMRP knockout mice (FMRP KO), FMRP target mRNAs are translated excessively and mGluR5 signaling is exaggerated. (**c**) Dolen *et al*.[1] now show that genetic reduction of mGluR5 signaling in mGluR5 heterozygous mice (mGluR5 het) restores translation rates and rescues FXS phenotypes in FMRP KO. Putative functions of the proteins encoded by FMRP target mRNAs might include control of -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) endocytosis, cell signaling or translation (Bassel and Gross, 2008).



Huntington Disease

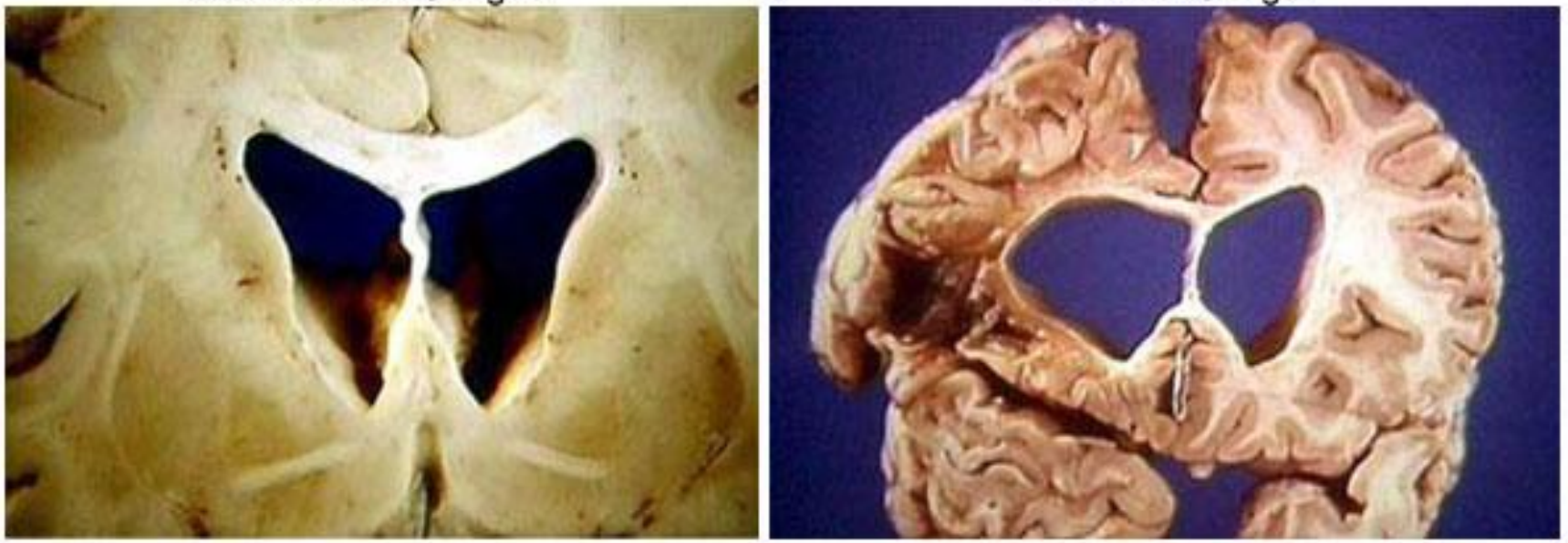
- HD gene, 'huntington', short arm of chromosome #4
  - Protein produced by the gene has no homology with other genes
  - Accumulates inside neuronal nuclei (forming inclusions) and inside nerve cell processes
  - Autosomal dominant
- ❖Fatal, progressive degeneration and atrophy of the striatum (caudate nucleus and putamen) and frontal cortex with neuronal depletion and gliosis
  - Especially cholinergic and GABA-ergic neurons
    - **GABA – CNS neurotransmitter; aa; inhibitory nerve impulses**
- Clinical
  - Delay of clinical abnormalities until age 30-40
    - **Age of onset is related to length of the HD gene's nucleotide repeat sequence (longer length is earlier onset)**
    - **Through generations (paternal transmission) the sequence tends to increase**



HD affects the whole brain, but certain areas are more vulnerable than others. Pictured above are the basal ganglia - a group of nerves cell clusters, called nuclei. These nuclei play a key role in movement and behavior control and are the parts of the brain most prominently affected in early HD (http://hdsa.org/what-is-hd/?gclid=CjwKEAjwl4q-).

Figure D-4: Effect of HD on the Basal Ganglia

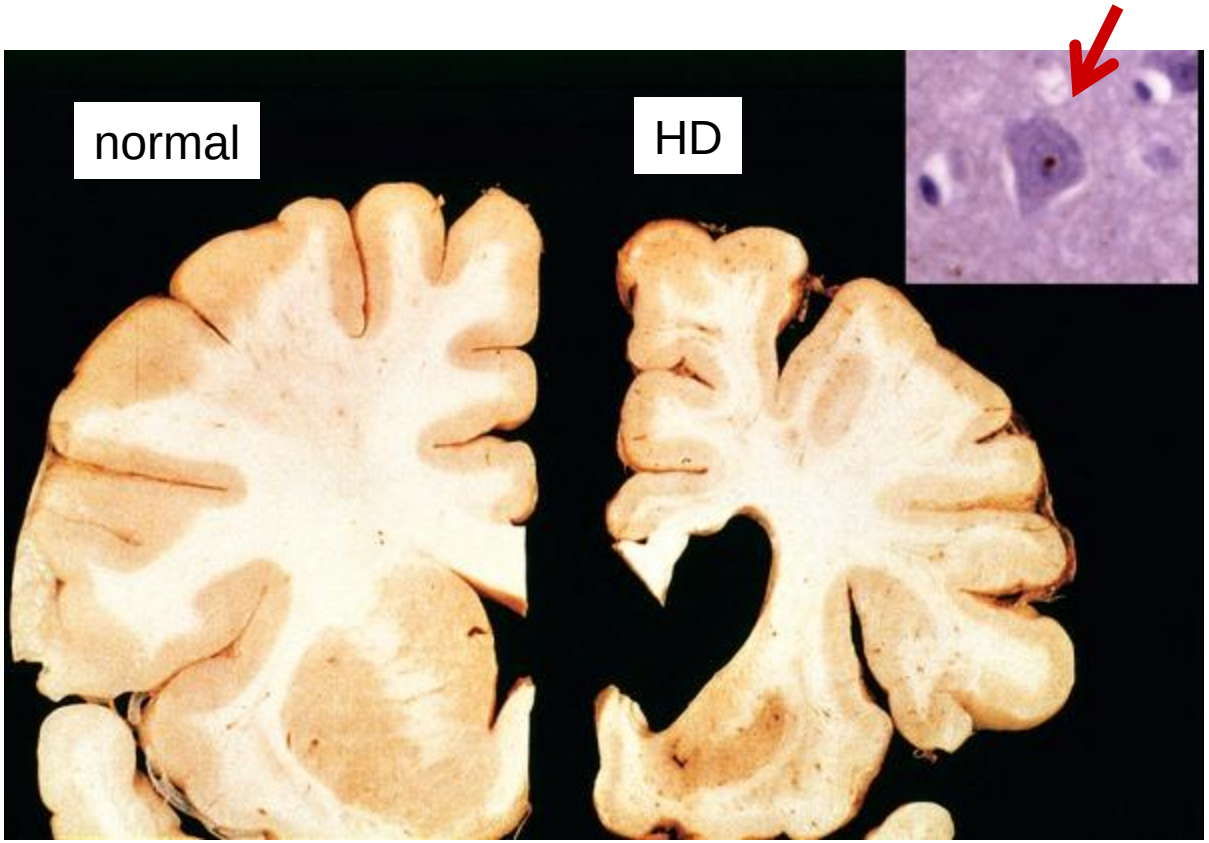
Normal Basal Ganglia vs. HD Basal Ganglia



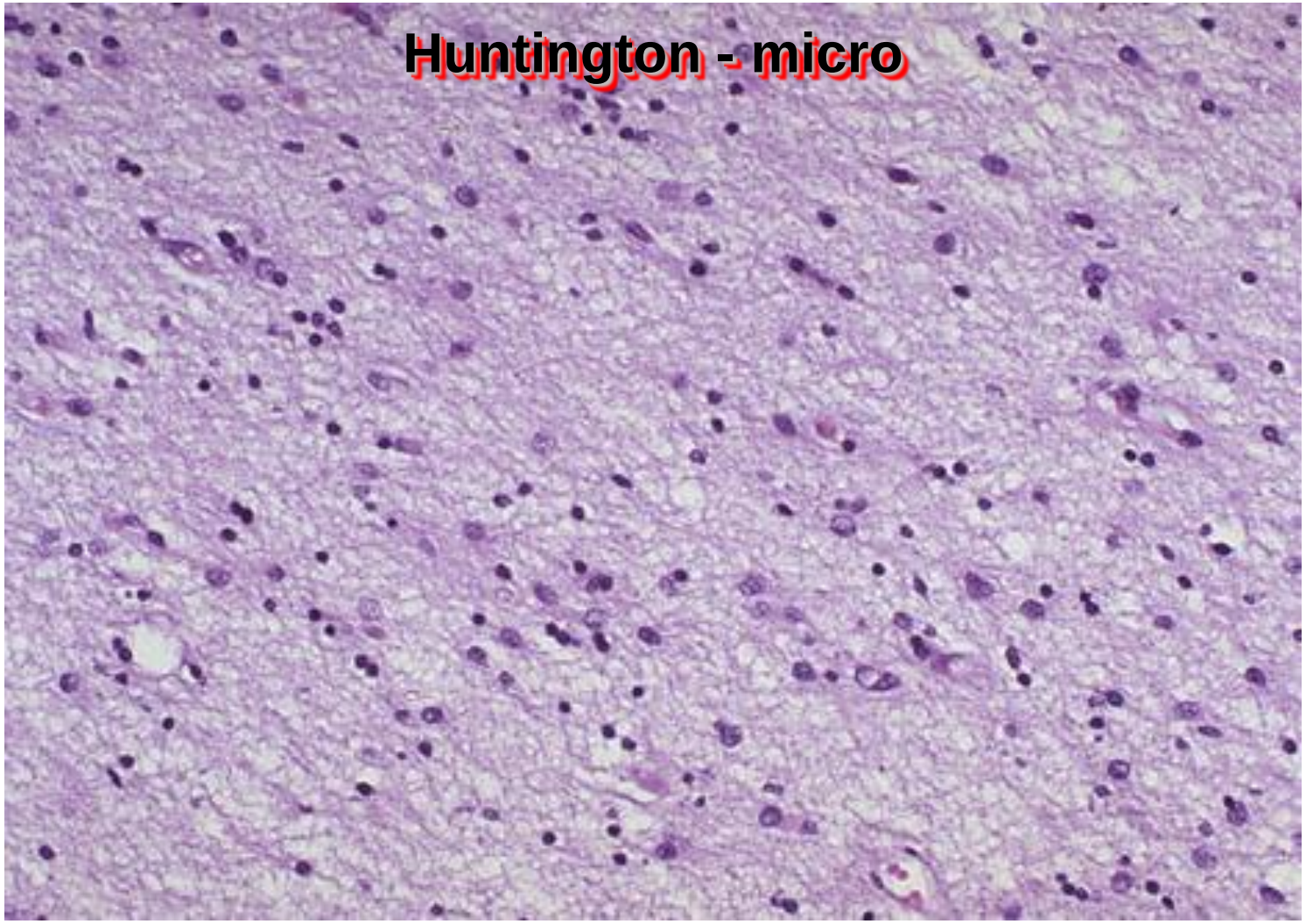
The basal ganglia of the human brain, showing the impact of HD on brain structure in this region. Note especially that the brain of a person with HD has bigger openings due to the death of nerve cells in that region.

Source: Singer, Jonathan. Huntington's Disease. Online. Available at: http://ist-socrates.berkeley.edu/~jmp/HD.html

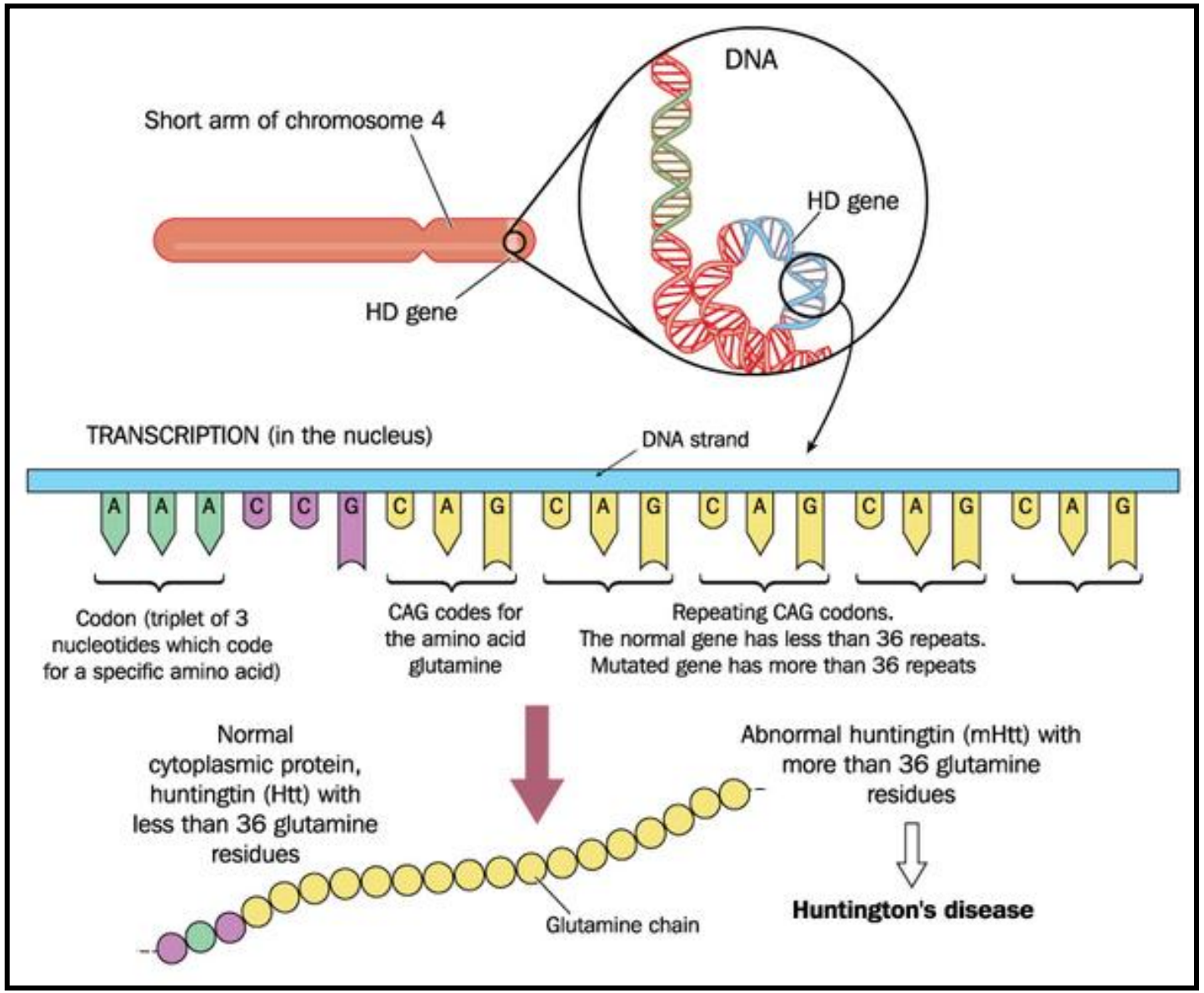
Huntington - macro



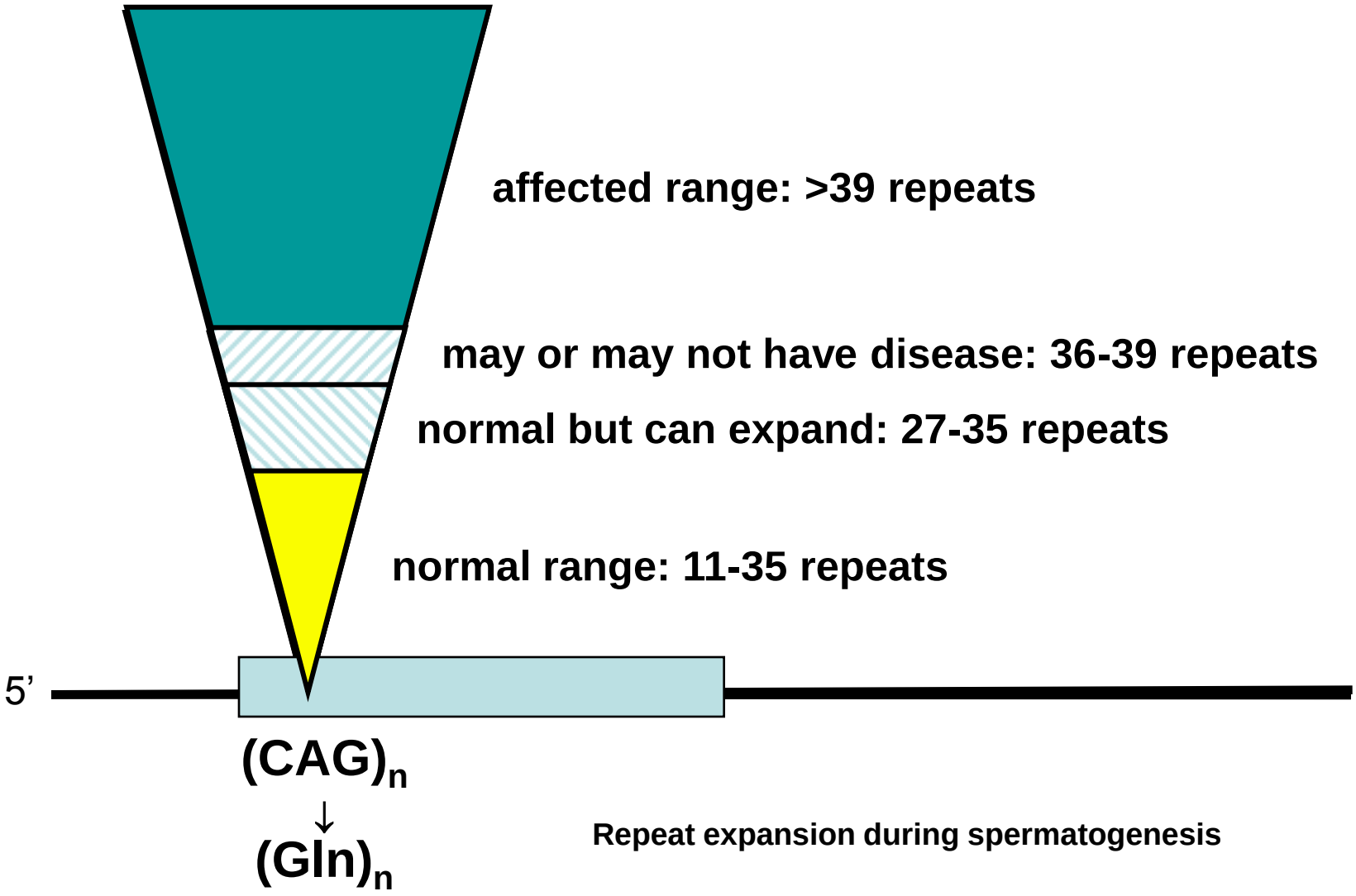
Normal hemisphere on the left compared with the hemisphere with HD on the right showing atrophy of the striatum and ventricular dilation.
Inset: intranuclear inclusions in neurons are highlighted by immunohistochemistry against ubiquitin.



Caudate nucleus in Huntington's disease**: loss of neurons along with fibrillary gliosis** that is more extensive than in the usual reaction to neuronal loss. There is a direct relationship between the degree of degeneration in the striatum and the severity of clinical symptoms. Protein aggregates containing huntingtin can be found in neurons in the striatum and cerebral cortex


DNA
Short arm of chromosome 4
HD gene
HD gene
TRANSCRIPTION (in the nucleus)
DNA strand
A A A C C G C A G C A G C A G C A G C A G
Codon (triplet of 3 nucleotides which code for a specific amino acid)
CAG codes for the amino acid glutamine
Repeating CAG codons. The normal gene has less than 36 repeats. Mutated gene has more than 36 repeats
Normal cytoplasmic protein, huntingtin (Htt) with less than 36 glutamine residues
Abnormal huntingtin (mHtt) with more than 36 glutamine residues
Glutamine chain
Huntington's disease

CAG repeats in Huntington's disease



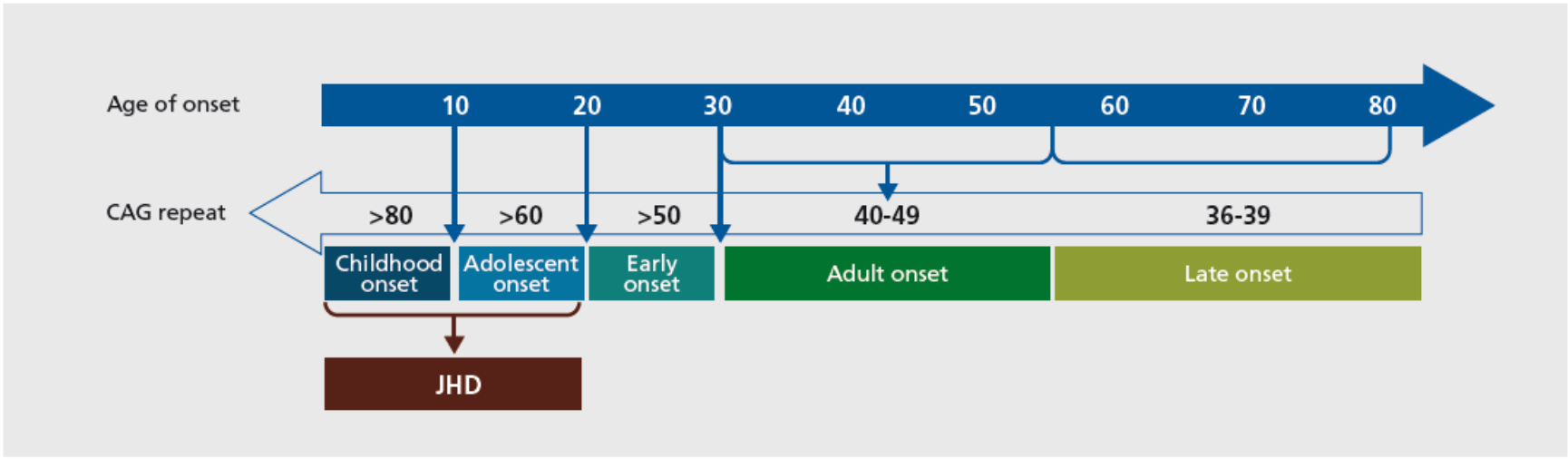


Figure 1. In Huntington disease, the length of CAG repeat is inversely related to age of onset. This relationship is shown with approximations of age of onset based on repeat length. JHD, juvenile Huntington disease

Nopoulos, 2016

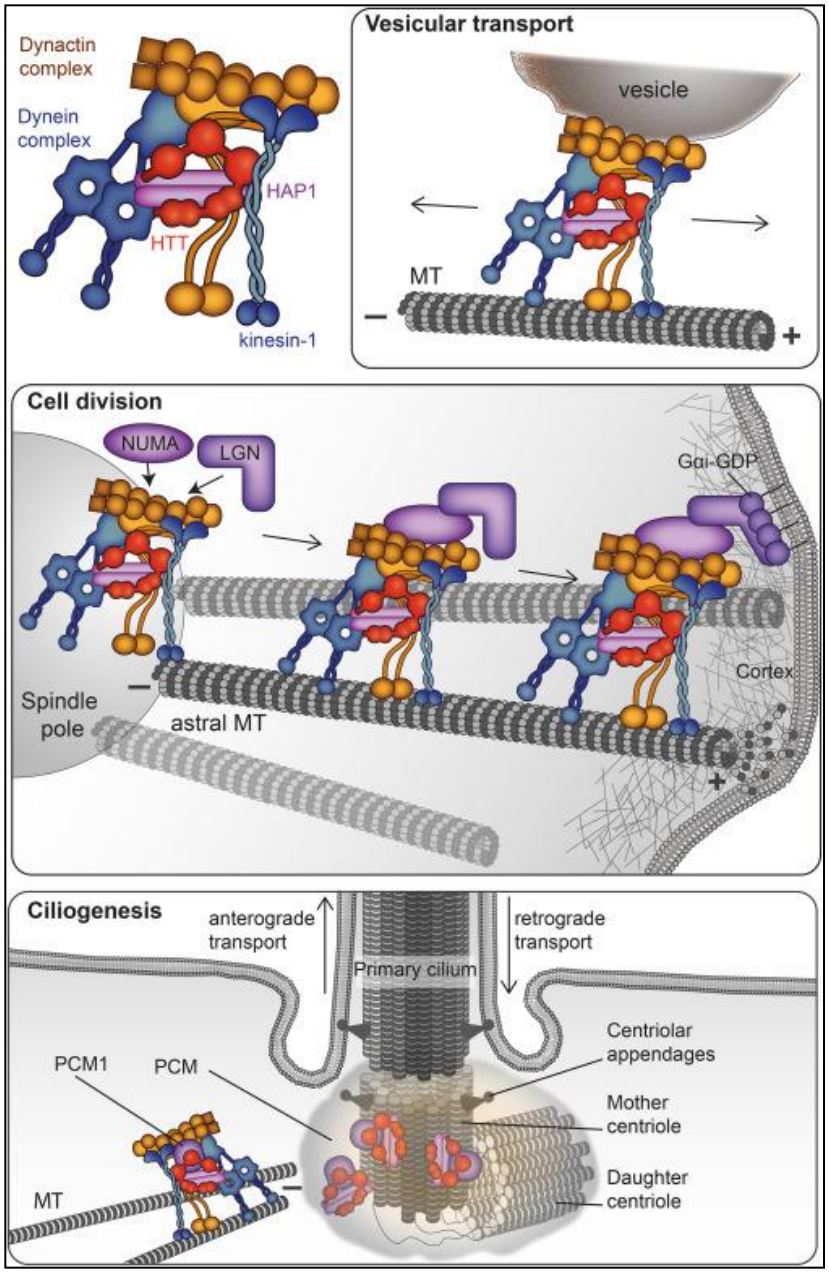


Figure 2. Huntingtin Scaffolds Dynein/Dynactin to Regulate Several Cellular Processes
Top: HTT controls the transport of organelles, in both anterograde and retrograde directions, and in axons and dendrites within neurons. Middle: During mitosis, HTT is important for spindle pole assembly and also regulates the kinesin 1-dependent trafficking of dynein/dynactin/NUMA/LGN to the cell cortex. Bottom: HTT mediates the dynein/dynactin/HAP1-dependent transport of proteins to the pericentriolar material, including PCM1 protein that is required for ciliogenesis. MT, microtubules; PCM, pericentriolar material (Saudou & Humbert, 2016).

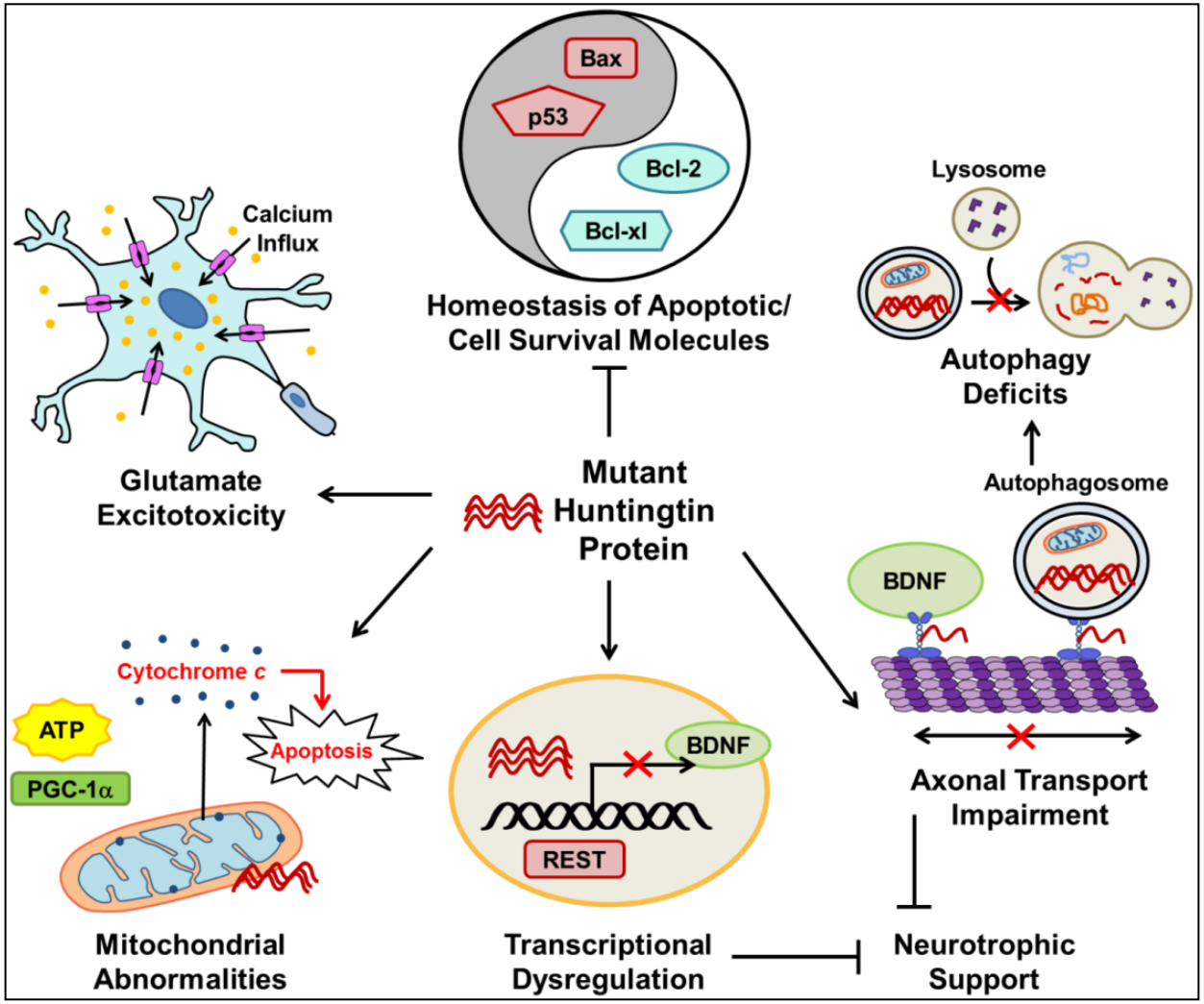


Figure 1. The major pathophysiological pathways in Huntington's disease (HD).
Mutant huntingtin (mHtt) protein disrupts many normal physiological processes and leads to unbalanced homeostasis of apoptotic molecules, deficits in autophagy, axonal transport impairment, transcriptional dysregulation, reduced cellular neurotrophic support, mitochondrial abnormalities, and glutamate excitotoxicity. mHtt disturbs the balance between pro-apoptotic (such as Bax and p53) and cell survival (such as Bcl-2 and Bcl-xl) molecules. Transcriptional regulation is disrupted in HD; as described in the text, mHtt allows REST translocation to the nucleus resulting in repression of genes including BDNF. As a result of decreased BDNF axonal transport and repression of gene transcription by REST, neurotrophic support of cells is diminished in HD. Impaired axonal transport of autophagosomes also increases autophagy deficits observed in HD. Mitochondrial abnormalities in HD include decreased ATP production and PGC-1a expression, as well as increased cytochrome *c release which leads to cell apoptosis. Glutamate excitotoxicity, caused by hyperactivation of* excitatory amino acid receptors that increase cell ion permeability and lead to intracellular calcium overload and ultimately cell death, is strongly implicated in HD. The pointed arrows indicate that mHtt increases the described physiological pathway; arrows with blocked ends indicate prevention of a physiological event (Scheuing et al., 2014).

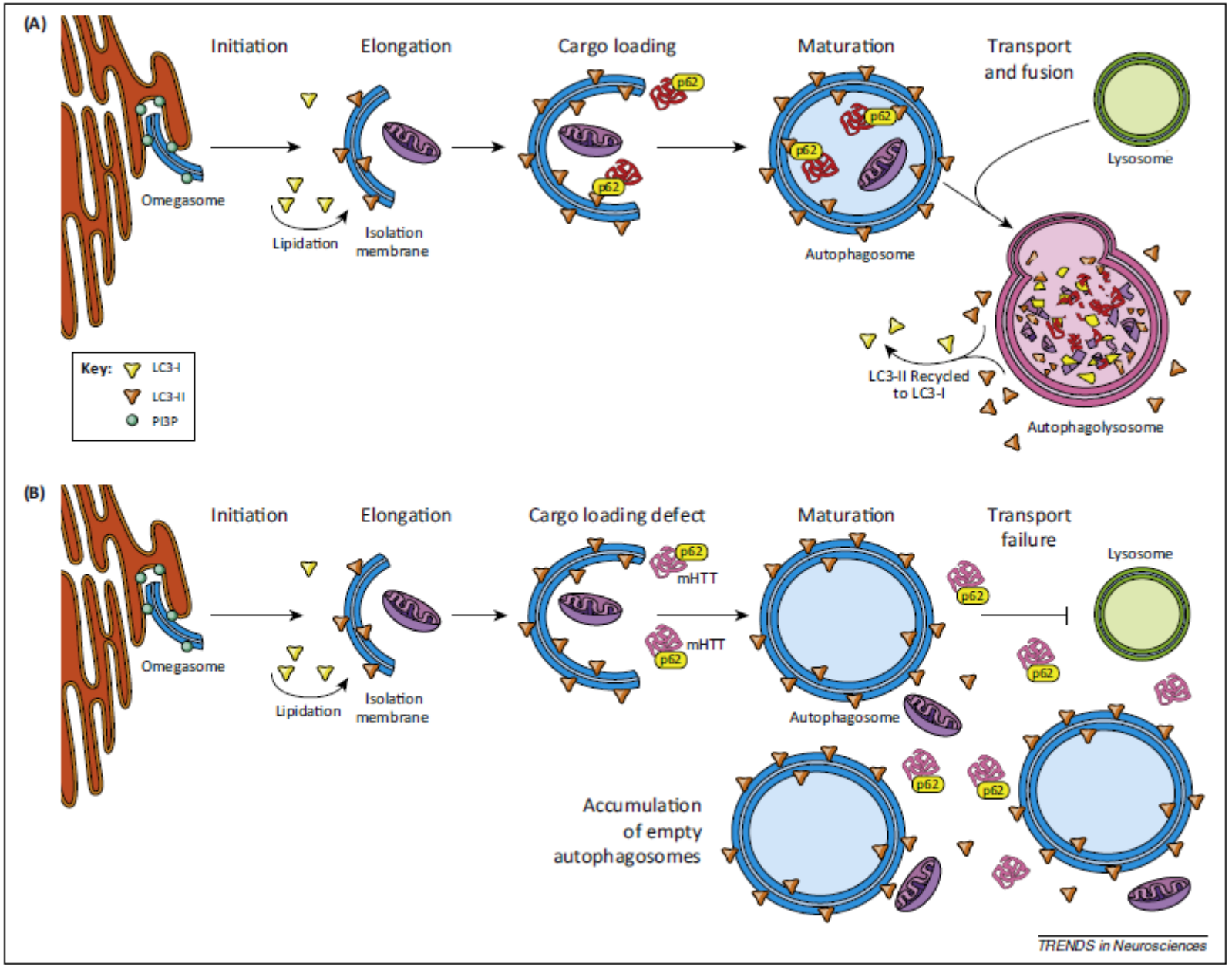


Figure 1. Autophagy is altered in Huntington disease (HD). **(A)** Autophagy involves the formation of double-membraned vesicles that incorporate damaged organelles and toxic or aggregated proteins, and fuse with the lysosome for degradation. **(B)** In HD it has been shown that autophagy is affected at several steps including a defect in cargo loading, trafficking of autophagosomes, and decreased fusion between autophagosomes and lysosomes leading to a build-up of toxic materials in the cytoplasm and empty autophagosomes.

Martin et al., 2015

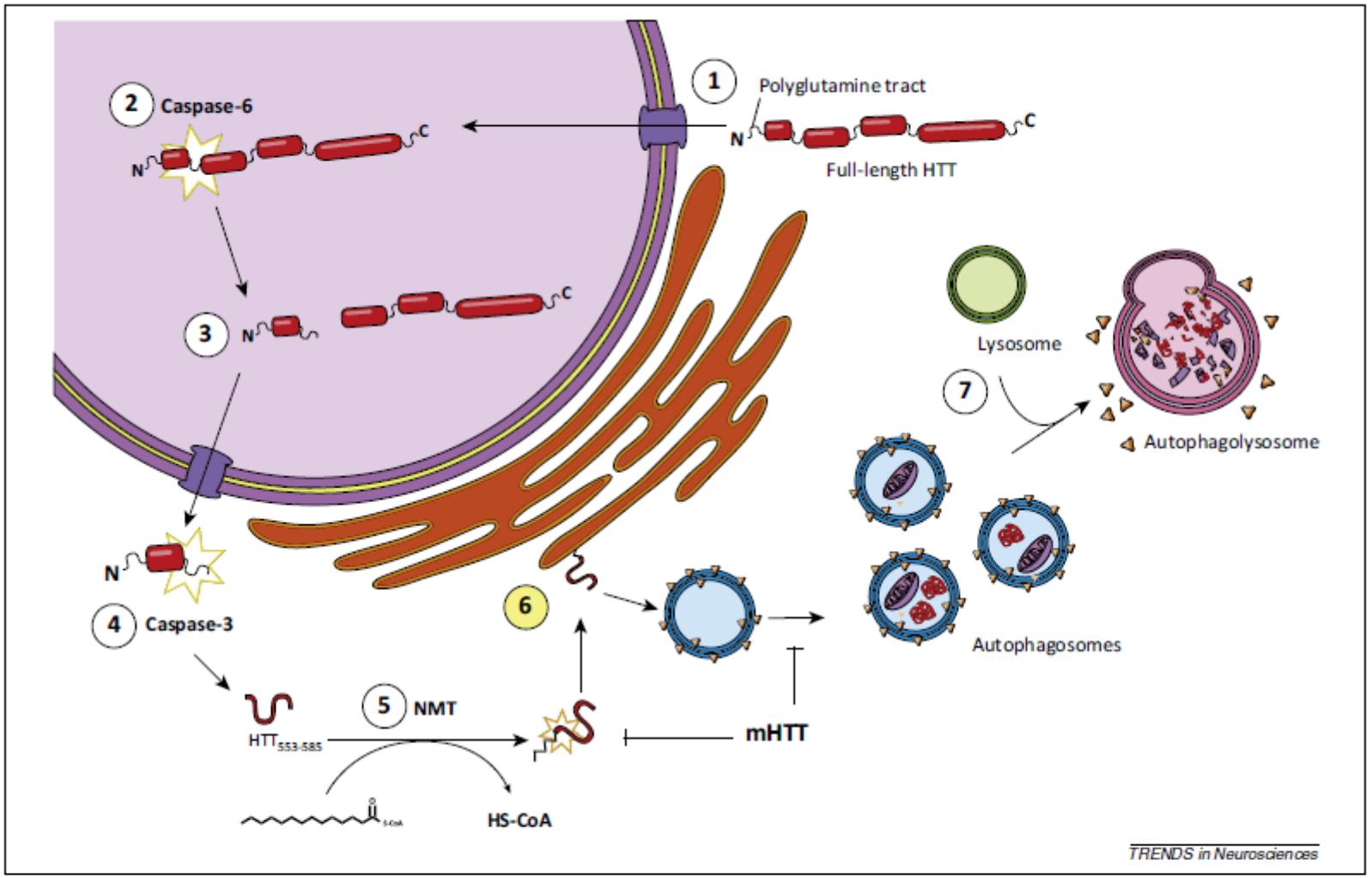


Figure 2. An autophagy-inducing domain within huntingtin (HTT) is released by caspase-cleavage and is dependent on post-translational myristoylation. HTT (1) has been shown to be cleaved at D586 by caspase-6 in the nucleus (2). Subsequent processing by caspase-2 or -3 at D552 exposes an N-terminal glycine at position 553 (3,4), which is post-translationally myristoylated (5) by *N*-myristoyltransferase (NMT). Myristoylation was shown to direct $HTT_{553-586}$–EGFP to the endoplasmic reticulum (ER) (6) and to promote the formation of autophagosomes which ultimately fuse with the lysosome (7). Myristoylation at G553 is decreased in mutant (m)HTT. The myristoylated HTT peptide is predicted to detect the highly curved membranes of the endoplasmic reticulum (ER) where it inserts the myristate moiety into the membrane (6). As the peptide accumulates, it may increase membrane curvature to promote the formation of autophagosomes. Inhibiting autophagosome–lysosome fusion did not increase levels of myristoylated-$HTT_{553-586}$–EGFP, suggesting that it does not accumulate within the autophagosome.

Martin et al., 2015

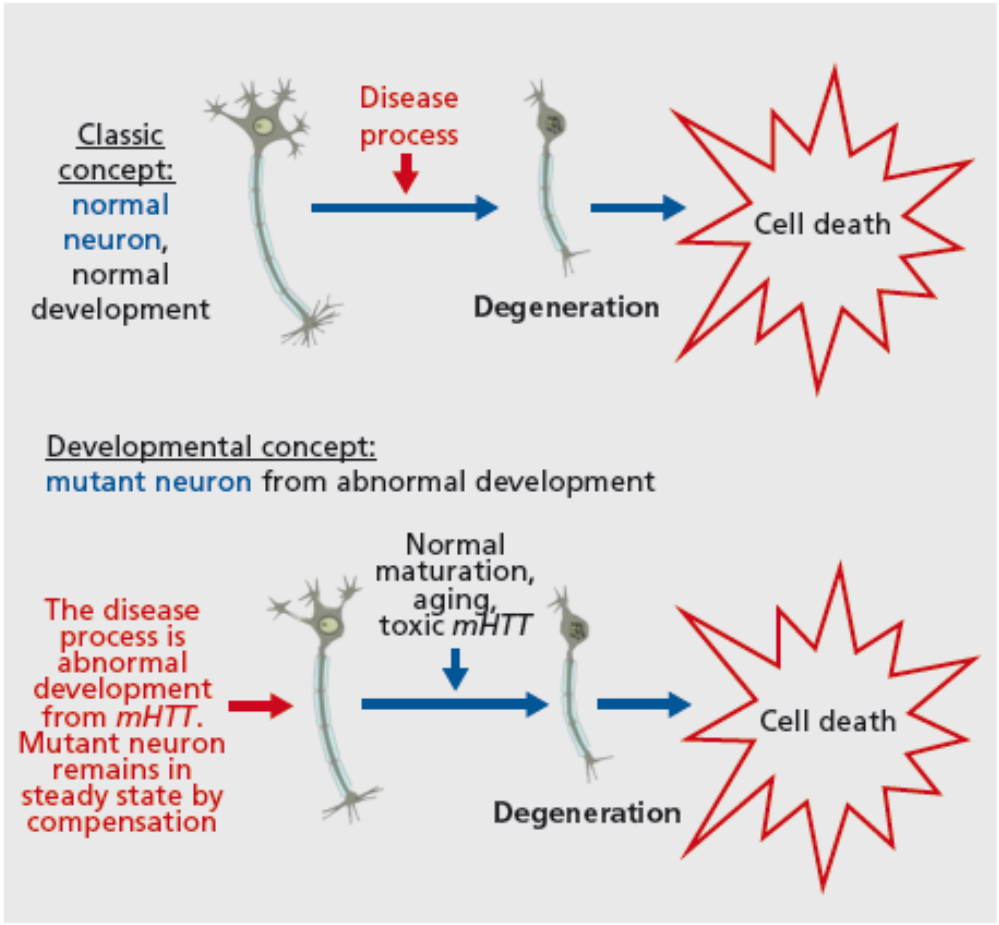


Figure 2. Models of degeneration. **(Top)** The classic concept of the etiology of degeneration, where a normal neuron is attacked by the disease process (effect of the toxic mutant gene) and dies. **(Bottom)** The etiology based on aberrant development, where the disease process begins with abnormal neuronal development, compensation leads to a mutant steady state, and degeneration occurs due to stress caused by factors such as maturation, aging, or toxic gene effects. mHTT, mutant Huntingtin gene

Nopoulos, 2016