Lecture plan

1. Basal ganglia circuitry

2. Nigrostriatal degeneration and symptomatic treatments
   - Motor symptoms → dopamine replacement
   - Deep brain stimulation

3. Neuronal degeneration / Lewy body pathology
   - Selective vulnerability of neuronal subtypes
   - Spreading of the α-synuclein pathology

4. PD etiology: organelle quality control
   - Recessive forms: parkin, PINK1 and mitochondrial turnover

Parkinson's disease pathology: causes of selective neuronal vulnerability

Ventral midbrain dopamine neurons:

NB: the degeneration of dopamine neurons is the cause of the most important motor symptoms.
Other types of neurons are also affected in Parkinson's disease.

Neurotransmitter

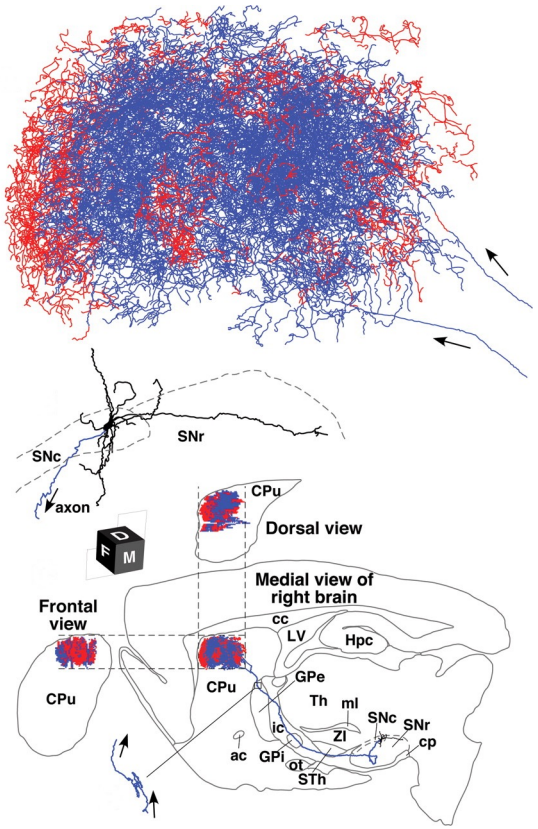
- Presence of **DOPAMINE** is a stress factor.
- Accumulation of DA degradation products in neuromelanin ⇒ risk factor?
- DA metabolism produces ROS ⇒ sequestration into vesicles is crucial.

Structure

- Long, unmyelinated axons, high energy demand
- Neuron function depends largely on axonal transport
- >1'000'000 presynaptic terminals/neuron in humans
- Massive dendritic arborization, cell body <<1% total cell volume

Activity

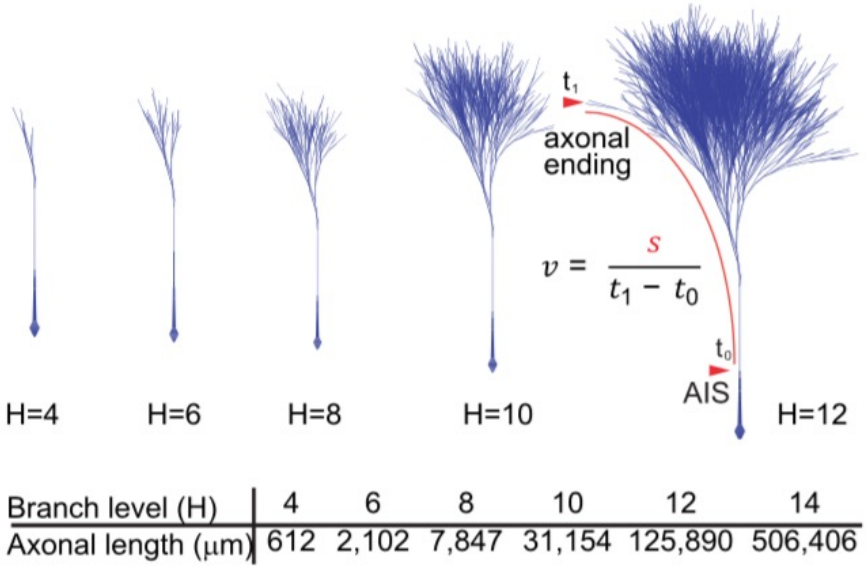
- Autonomously active, slow pacemaking firing activity (2-4Hz).
- Ca_v 1.3 channels: calcium-related stress.



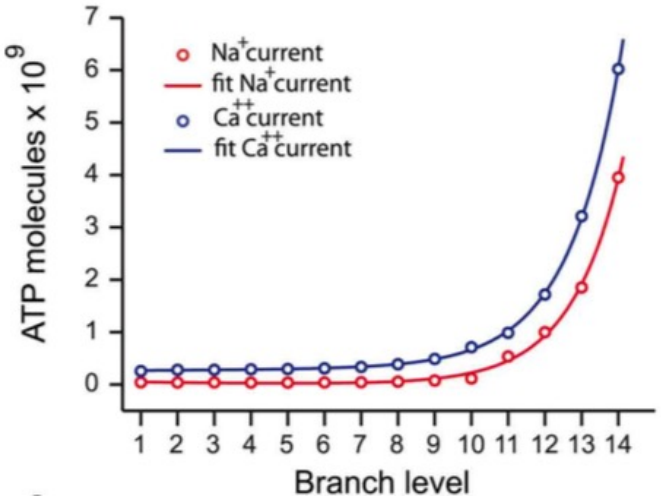
Chan CS et al., TINS 32(5), 2009
Matsuda W et al., J. Neurosci 29(2), 2009

Parkinson's disease pathology: selective neuronal vulnerability

ATP demand increases exponentially with increasing levels of axonal branching



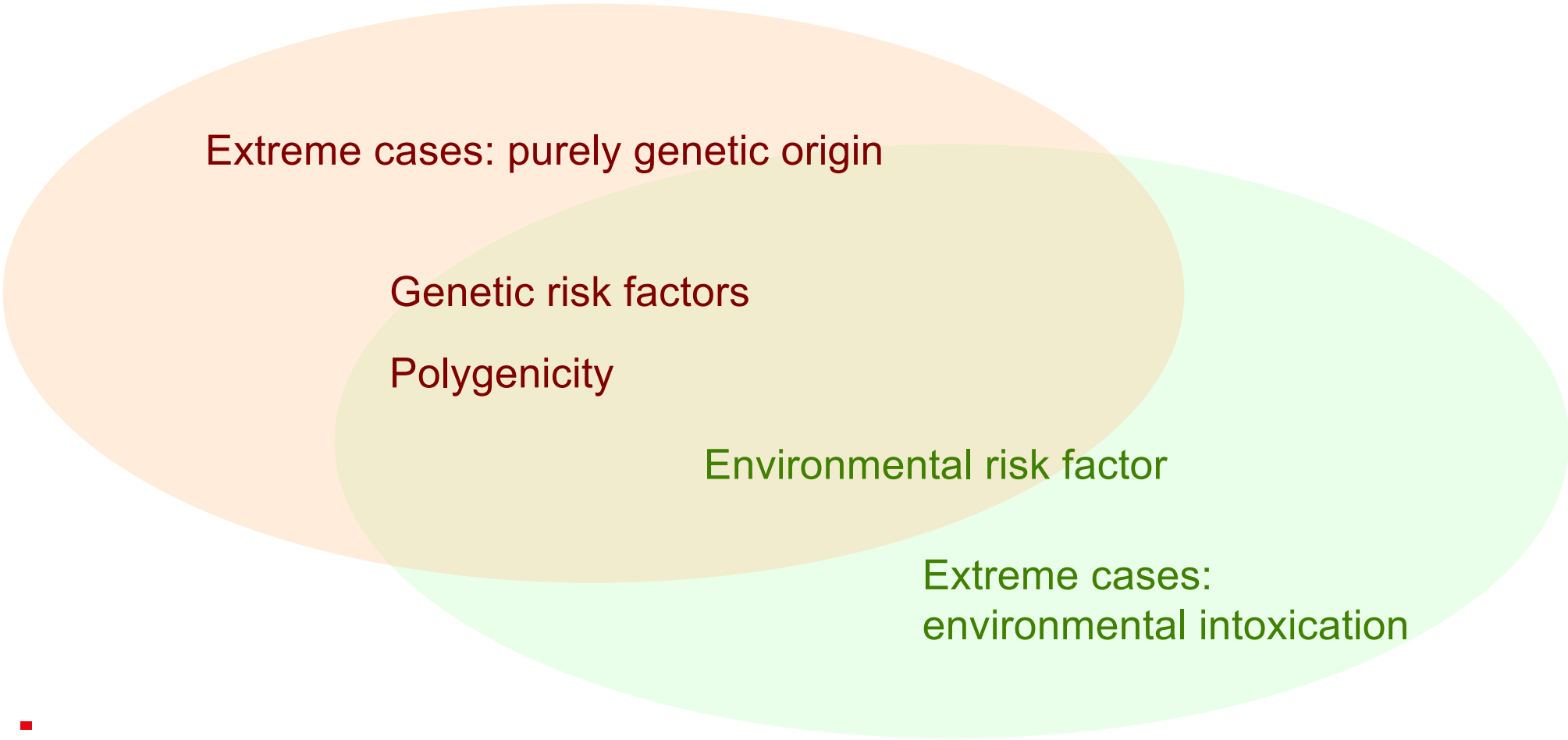
Branch level (H)	4	6	8	10	12	14
Axonal length (μm)	612	2,102	7,847	31,154	125,890	506,406



- Pissadaki E.K. & Bolam P, Front Comput Neurosci. 2013; 7: 13

Parkinson's disease : genetic and environmental causes

Parkinson's disease: complex etiology !



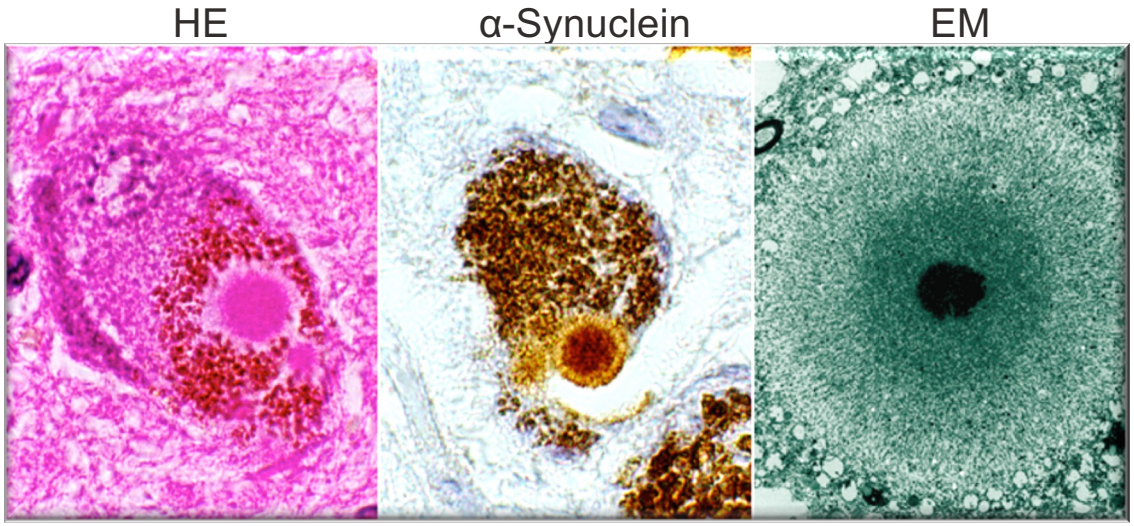
Parkinson's disease etiology : familial forms

Locus	Protein	Mode of inheritance	Atypical PD features	Lewy bodies	Frequency
PARK1/4	**α-synuclein (SNCA)**	**AD**	**Early onset, rapid progression**	**+**	**<1%**
PARK8	LRRK2	AD	-	+	1-7% of PD patients
PARK5	UCH-L1	AD	?	?	rare
PARK17	VPS35	AD	?	?	0.13% of PD patients
PARK2	Parkin	AR	Early onset, dyskinesias, slow progression	(-)	10-25% of early-onset PD
PARK6	PINK1	AR	Early onset, slow progression	(+)	1-8% of early-onset PD
PARK9	ATP13A2	AR	Juvenile onset, dementia	?	rare
PARK7	DJ-1	AR	Early onset, slow progression, psychiatric symptoms	?	1-2% of early-onset PD
PARK14	PLA2G6	AR	Early onset, dystonia	?	rare

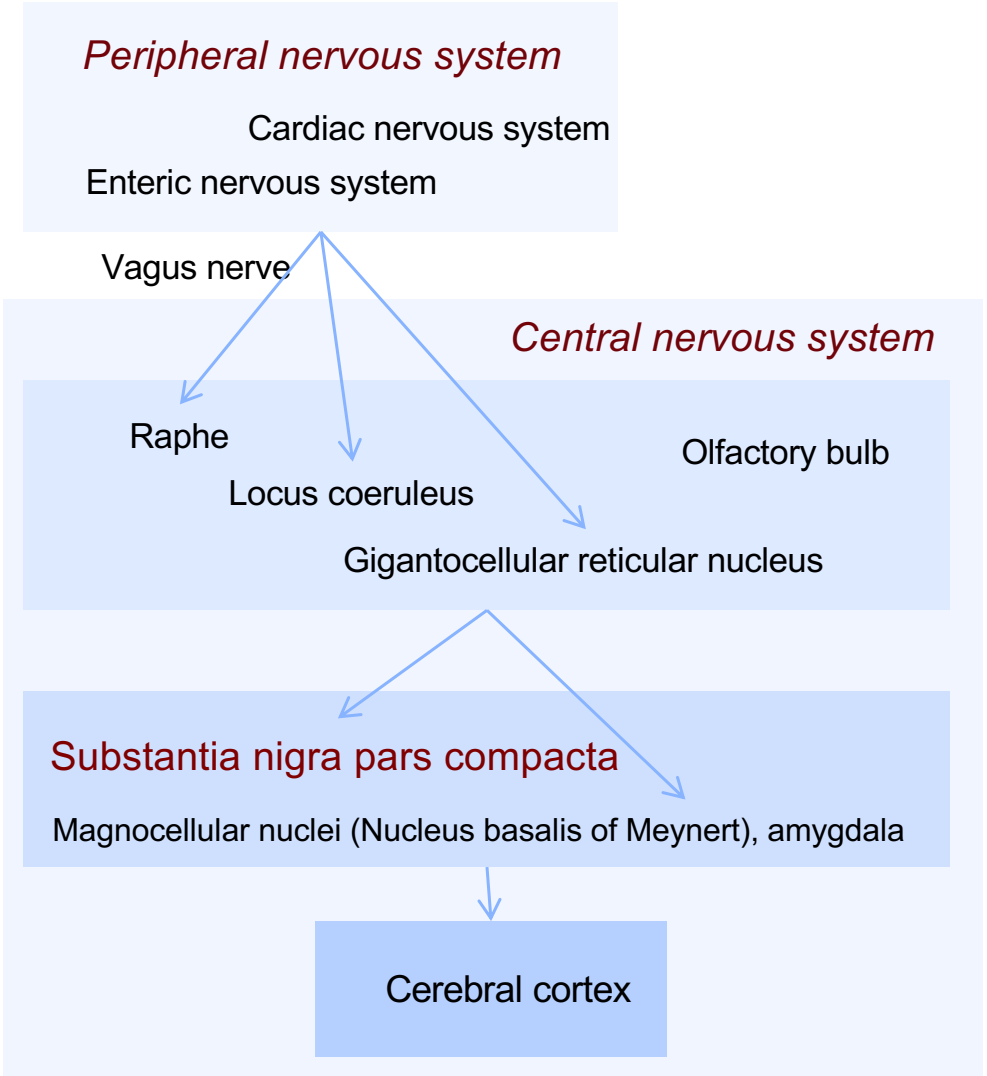
Parkinson's disease pathology: Lewy bodies

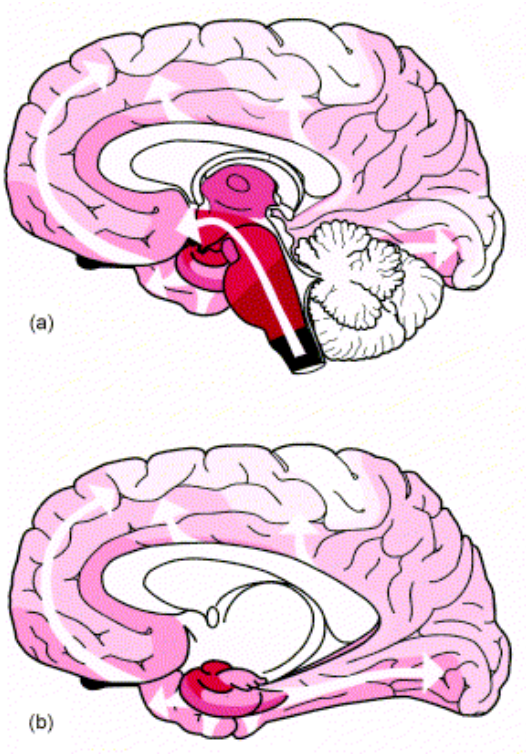
Pathological hallmark: **Lewy body** aggregates

- First described in 1912 by Friedrich Lewy
- Hallmark of synucleinopathies
- Aggregates, composed mainly of proteins, and some lipids
- The protein **alpha-synuclein** is the main constituent
- Cytoplasmic
- Mostly neuronal
- In the cell soma (Lewy bodies)
- In the neurites (Lewy neurites)



Widespread and progressive Lewy body pathology in Parkinson's disease

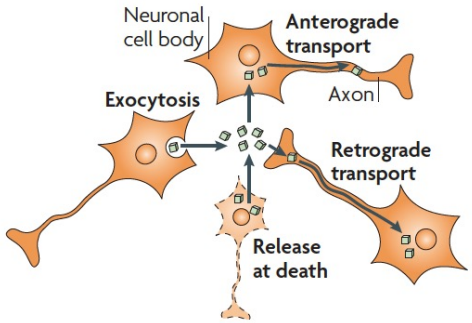




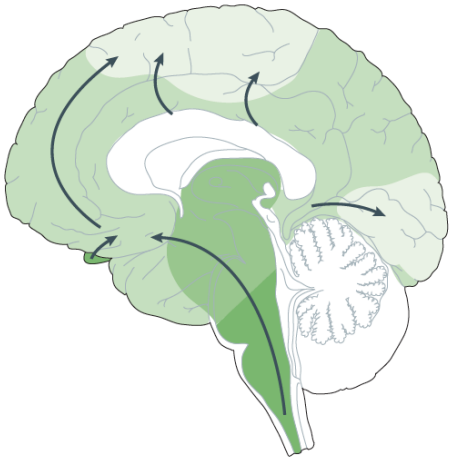
Braak H. et al.,
Neurobiology of Aging 2003, 24: 197-211

Neurodegeneration: spreading pathologies

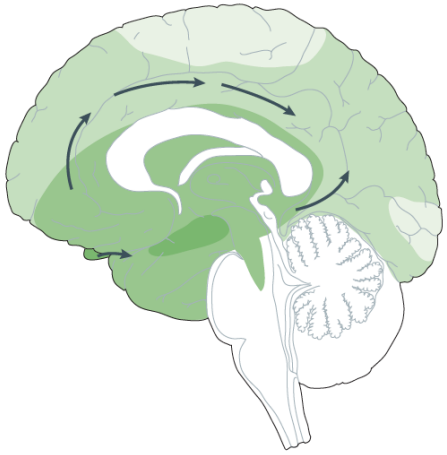
Propagation patterns of neurodegenerative proteopathies



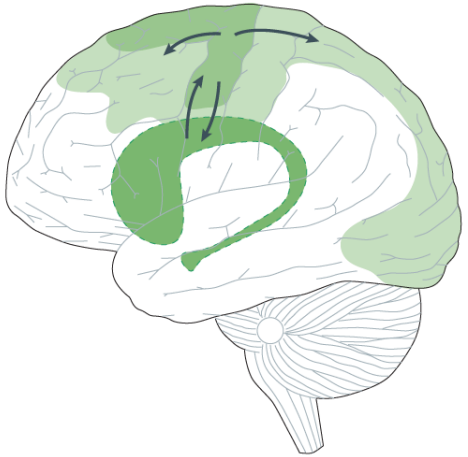
Parkinson's disease (α-synuclein)



Alzheimer's disease (tau)



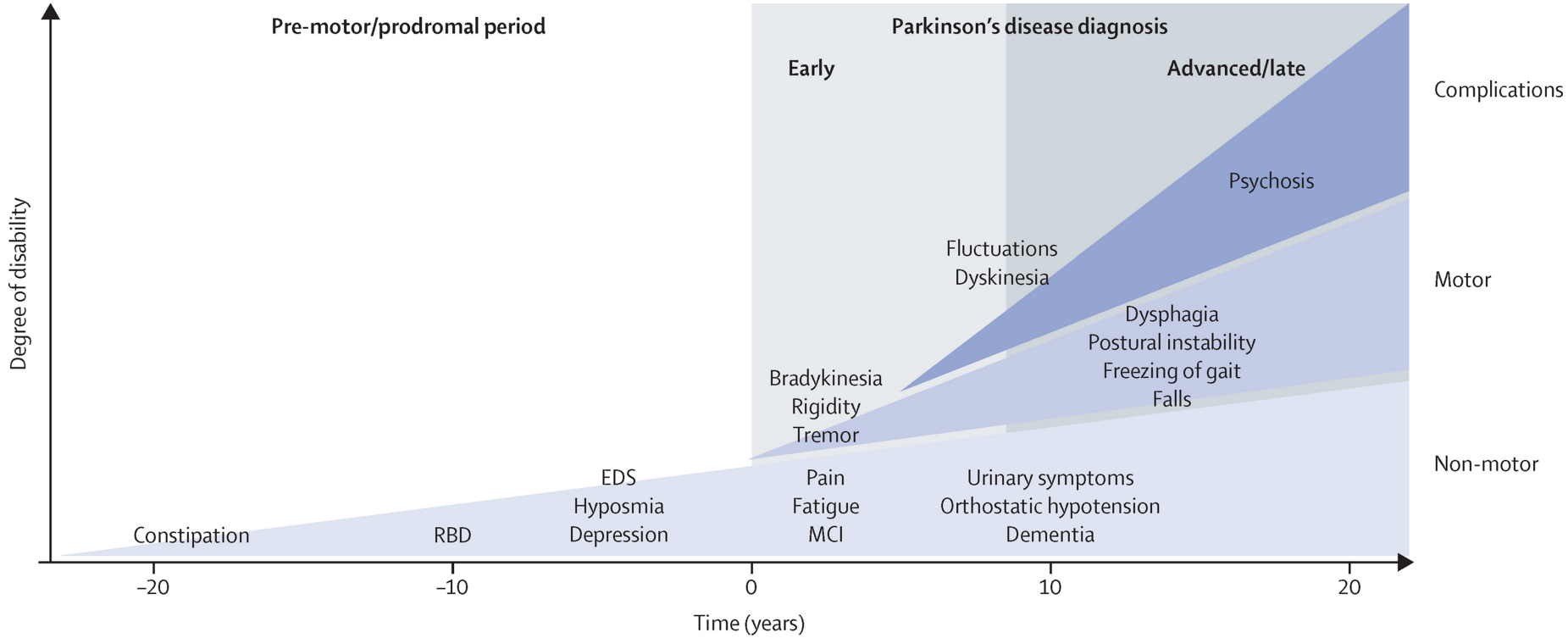
Huntington's disease (huntingtin)



Lewy body pathology in Parkinson's disease: correlation with early symptoms

Evolution of Parkinson's disease

(many symptoms poorly respond to dopamine treatment)



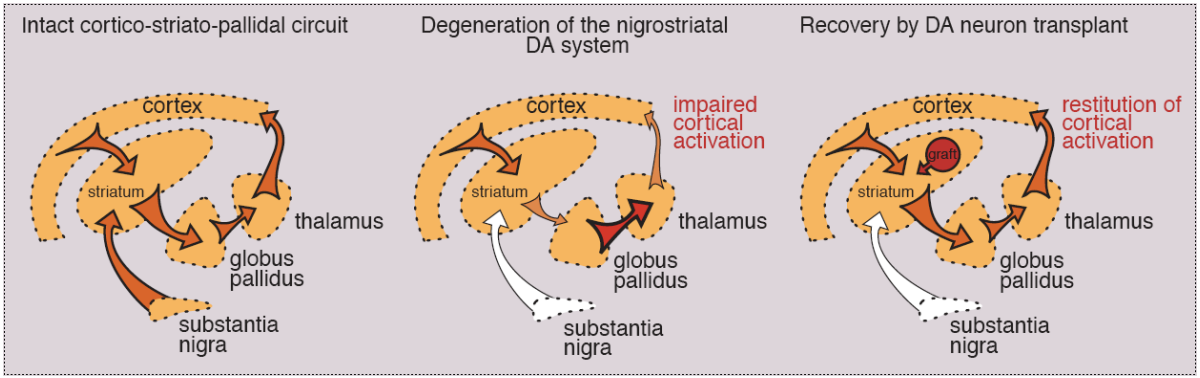
Kalia LV et al, the Lancet 2015

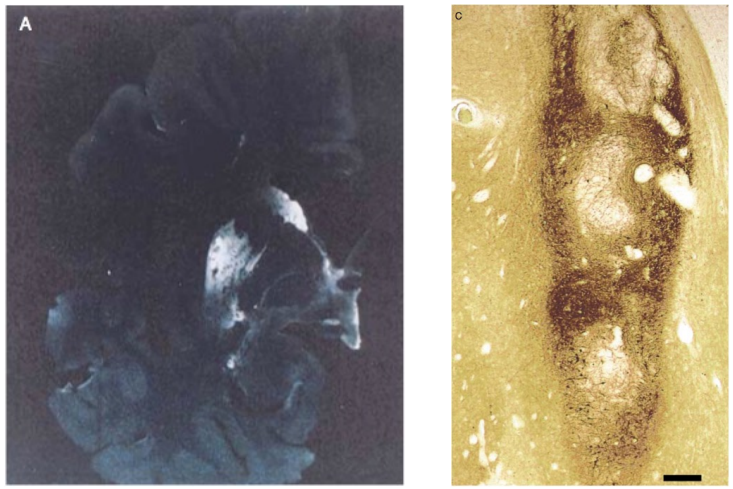
RBD: REM (rapid eye movement) Sleep Behavior Disorder
EDS: Excessive daytime sleepiness

PD pathology: evidence for spreading

Cell therapy: implantation in the striatum of fetal dopamine neurons

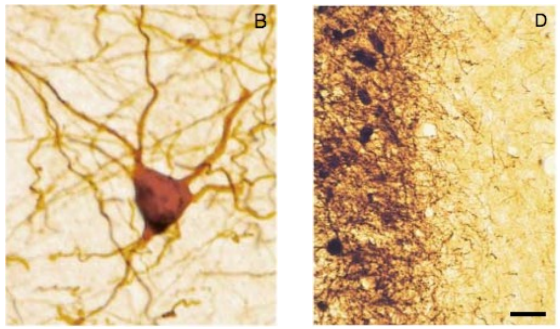
Cell therapy
for dopamine replacement
in the striatum (ectopic
implantation)





Survival / engraftment

Fetal graft (7 embryos, 6½-9 wks)
18 months post transplantation
Tyrosine hydroxylase staining



Connectivity with host striatum

Kordower et al., NEJM 332 (1995), Björklund & Lindvall, Nature Neuroscience 3 (2000)

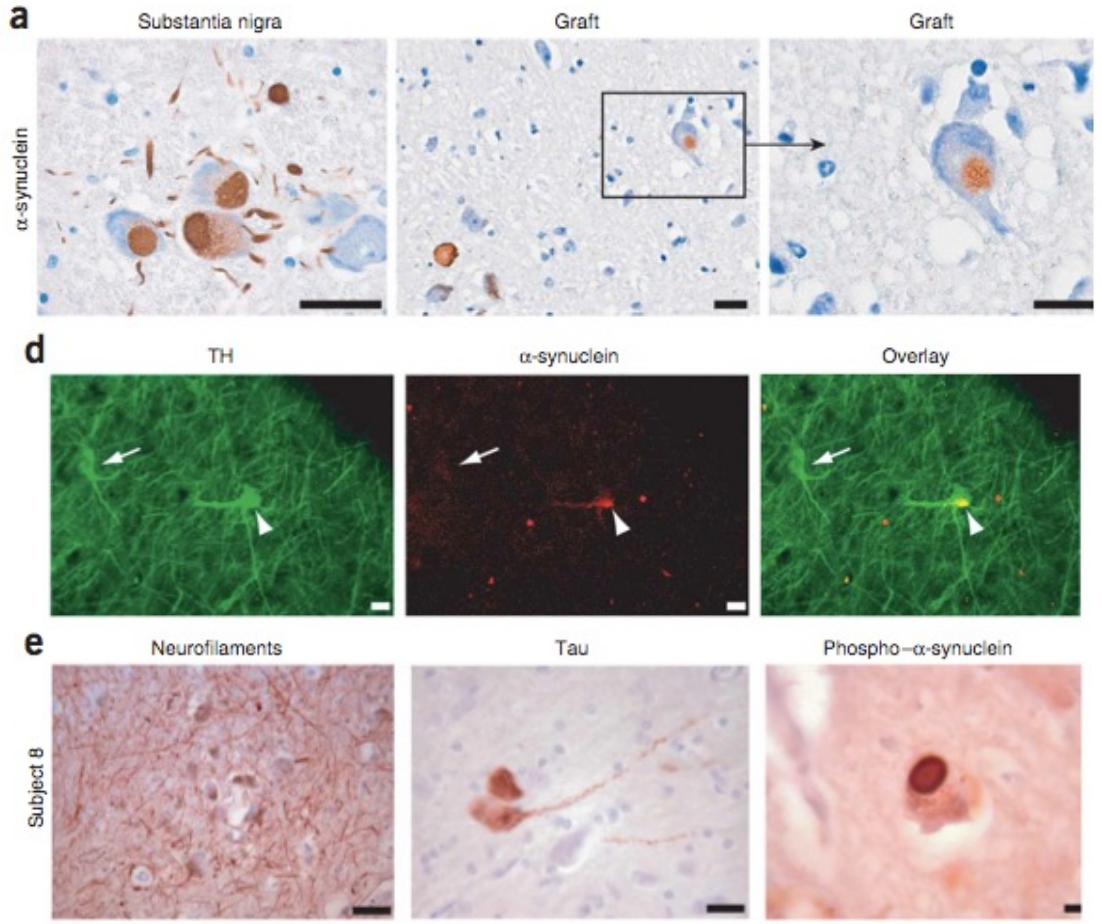
PD pathology: evidence for spreading

Post-mortem analysis of long-term grafted patients: 11-16 yrs post surgery

Lewy-body pathology in some grafted neurons !

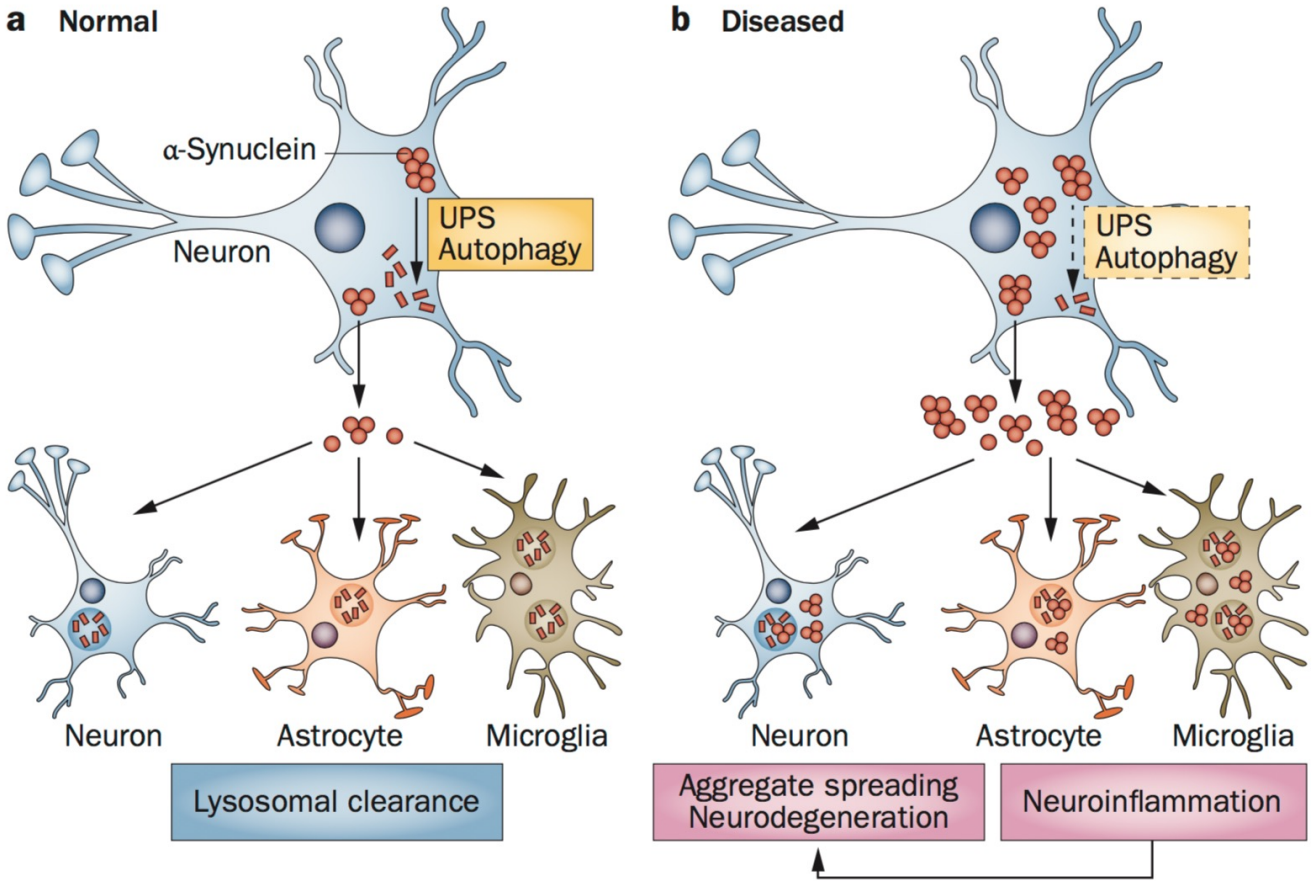
« Prion-like » disease transmission ?

Graft exposed to a noxious microenvironment (microglia, lack of trophic support) ??



Li et al., Nature Medicine 2008
Kordower et al., Nature Medicine 2008
Mendez et al., Nature Medicine 2008

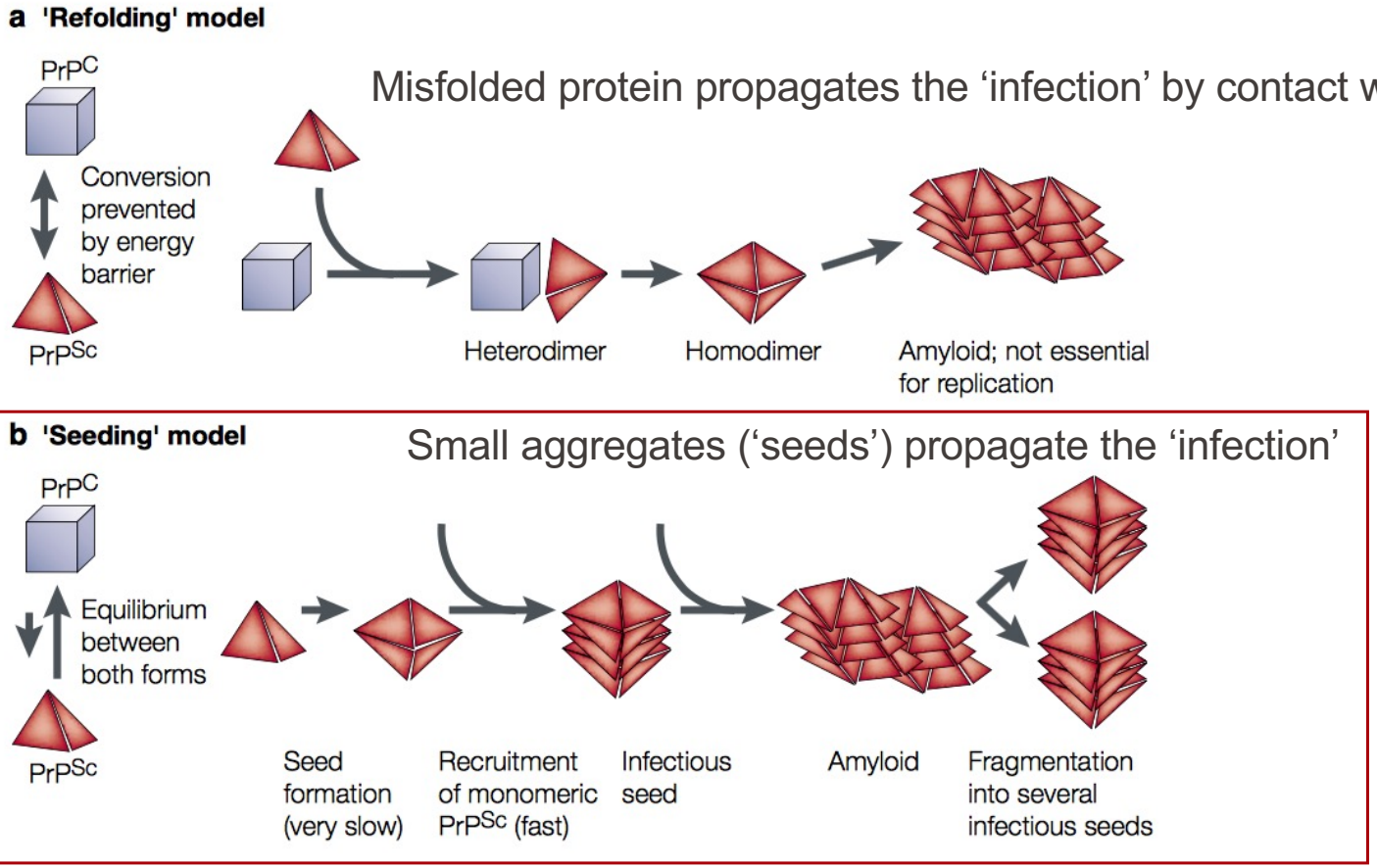
PD pathology: evidence for spreading



- *Lee HJ et al, Nat Rev Neurol 2014*

Spreading of PD pathology: a prion-like mechanism ?

Infectious proteins: proposed models

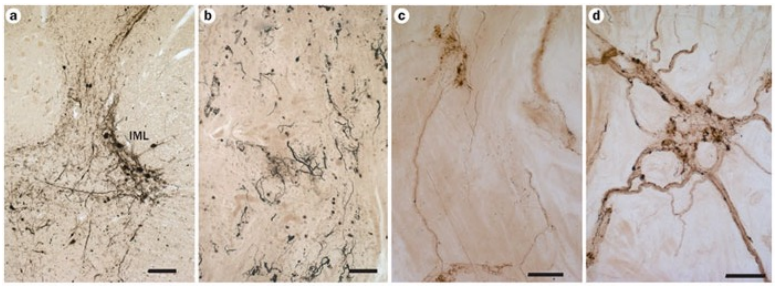


Most likely model for Parkinson's disease and α-synuclein

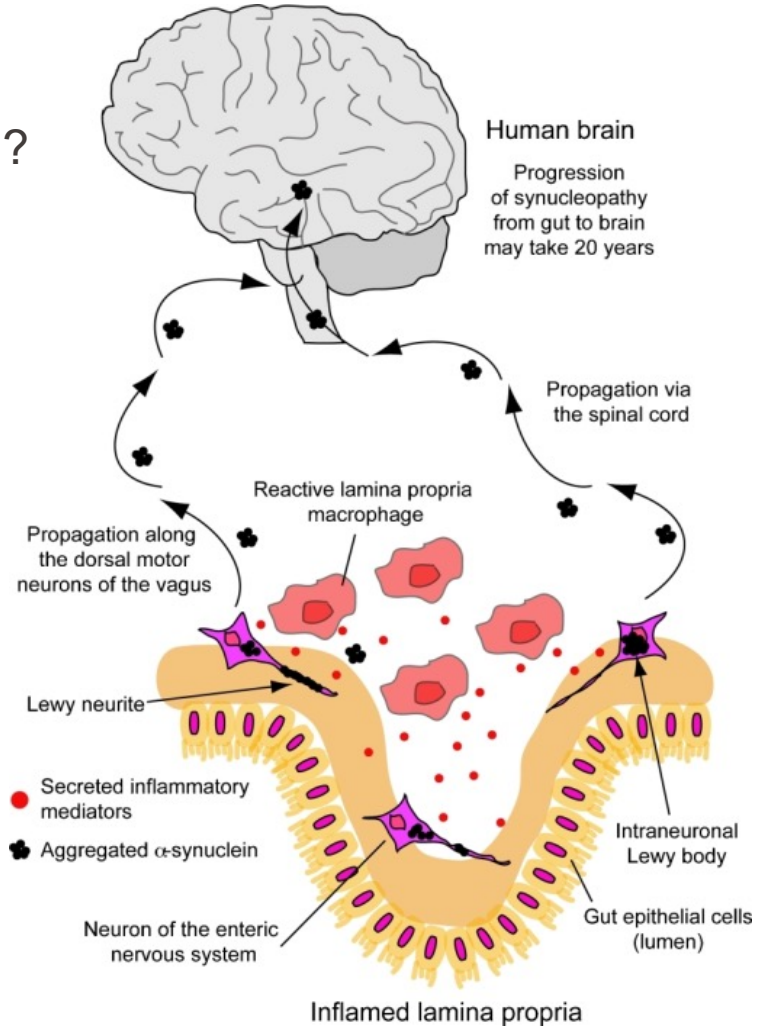
- *Aguzzi A et al, Nat Rev Mol Cell Biol 2, 118-126 (2001) | doi:10.1038/35052063*

PD pathology: evidence for spreading

Is there a propagation of the α-synuclein pathology from the periphery towards the brain ?

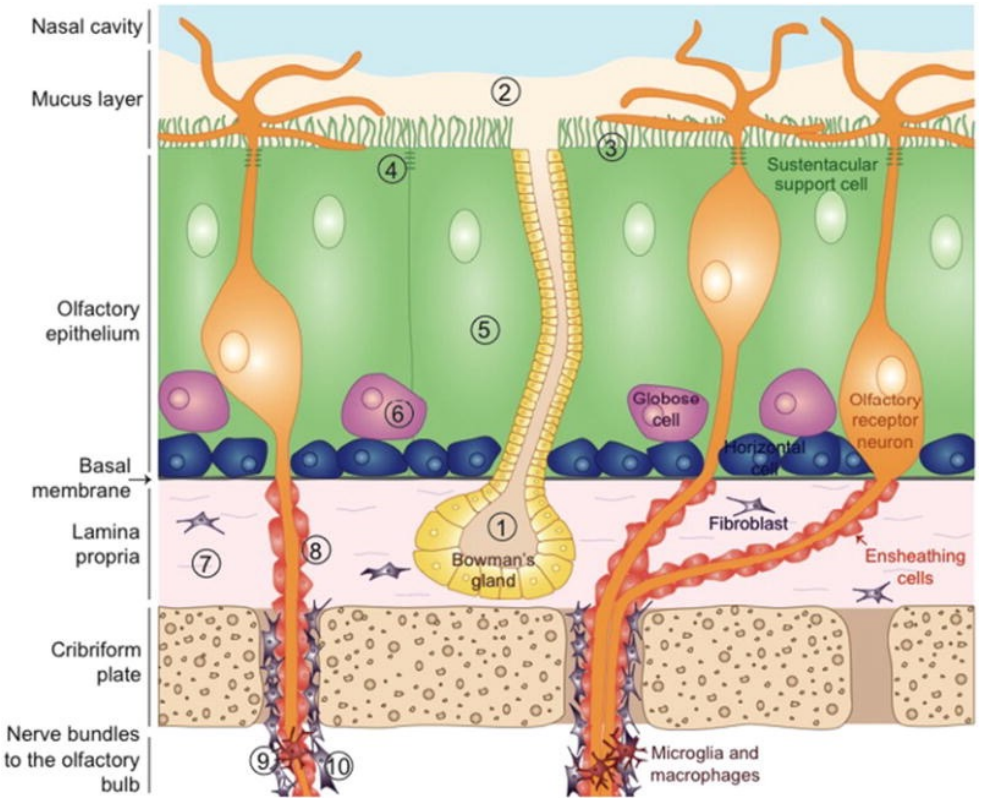


Synuclein-immunoreactive Lewy pathology in the PD spinal cord,
coeliac ganglion and gastrointestinal tract

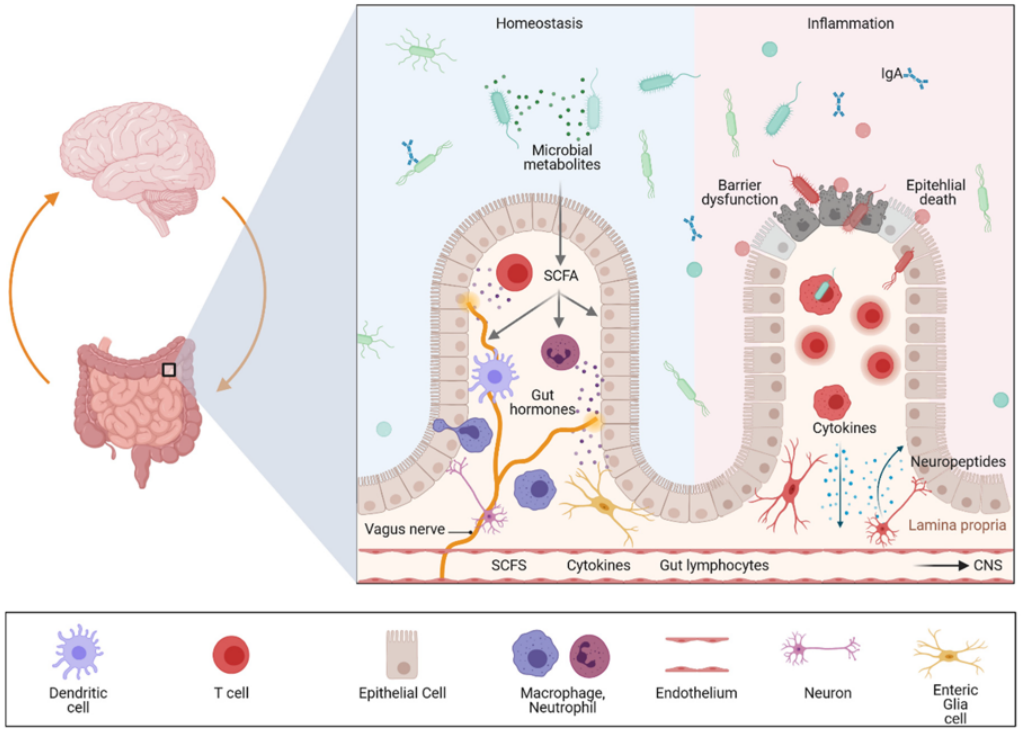


Are the olfactory epithelium and the gut epithelium starting points for spreading of the α-synuclein pathology to the central nervous system ?

Olfactory epithelium



Gut epithelium



Parkinson's disease: question 6

Alpha-synuclein pathology propagates across defined pathways in the CNS. What are the implications of this observation ?

Select all the correct statements:

A. Similar to prion, α-synuclein misfolding is an infectious mechanism

B. This shows that the protein may be accessible to therapeutic intervention outside cells

C. This mechanism may be either pathological or physiological

D. This mechanism has no therapeutic implication

Pathology: α-synuclein

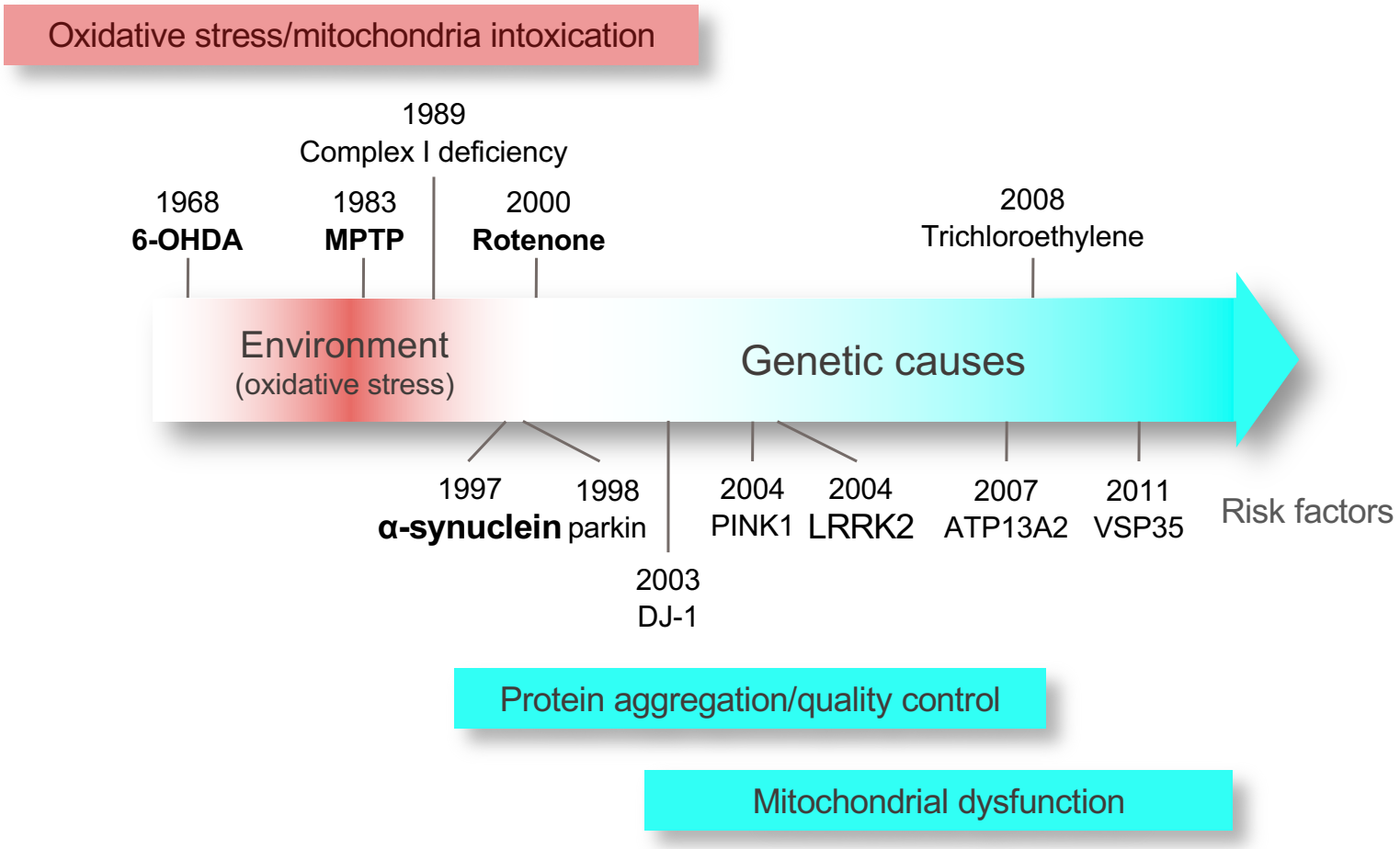
Parkinson's disease: a cerebral proteopathy ?

	Aggregated protein	Pathological lesion	Proteopathy
Alzheimer's disease	Amyloid β	Amyloid β Plaques	Amyloidosis
	Tau	Neurofibrillary tangles	Tauopathy
Pick's disease (FTLD)	Tau	Pick bodies	Tauopathy
Parkinson/Lewy Body Disease	αSynuclein	Lewy bodies/neurites	Synucleinopathy
Creutzfeldt-Jakob Disease	Prion	Prion plaques	Prionopathy

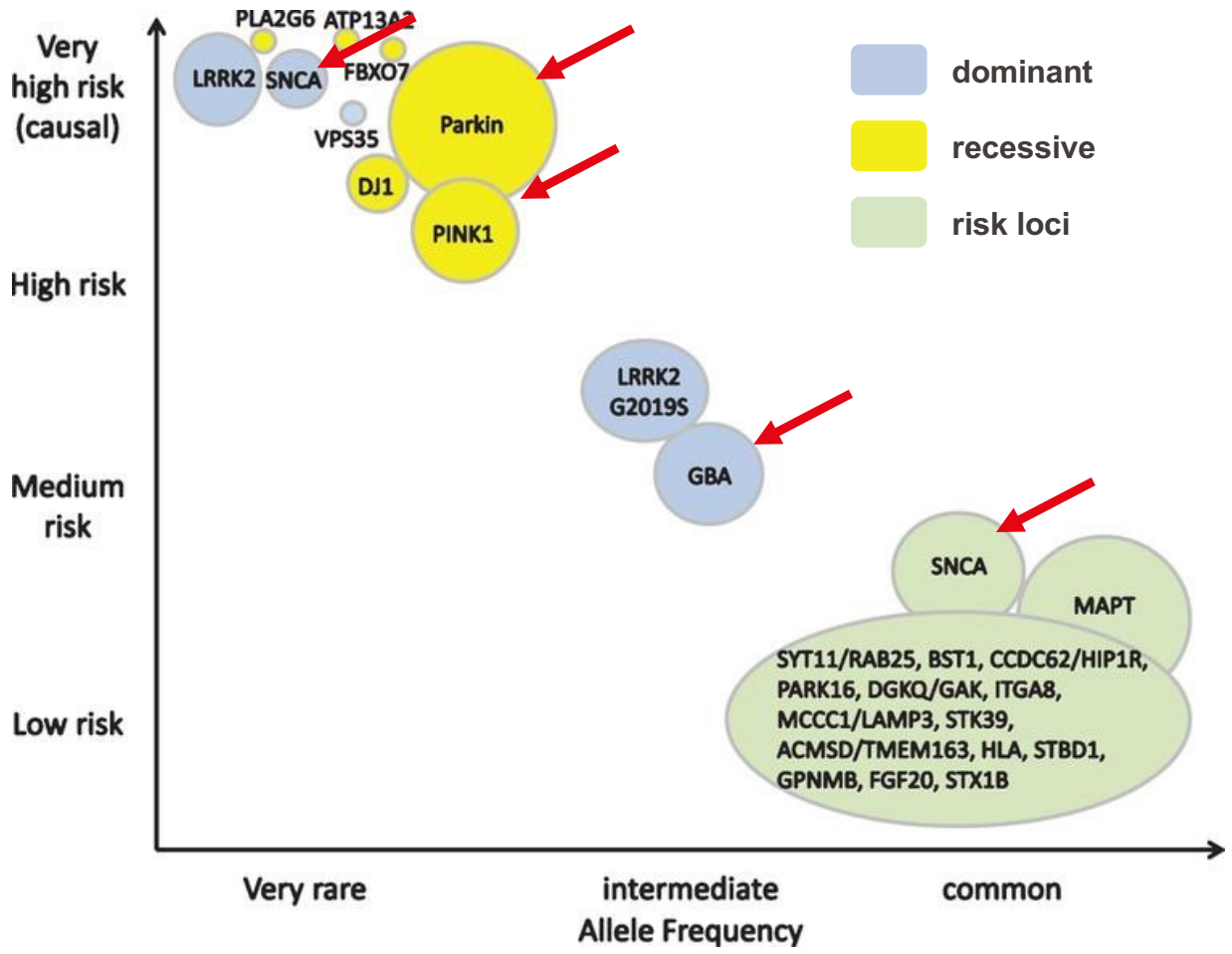
"Prionoids"?

Parkinson's disease etiology : genetic factors

Parkinson's disease: a shift in the paradigm to understand causality.



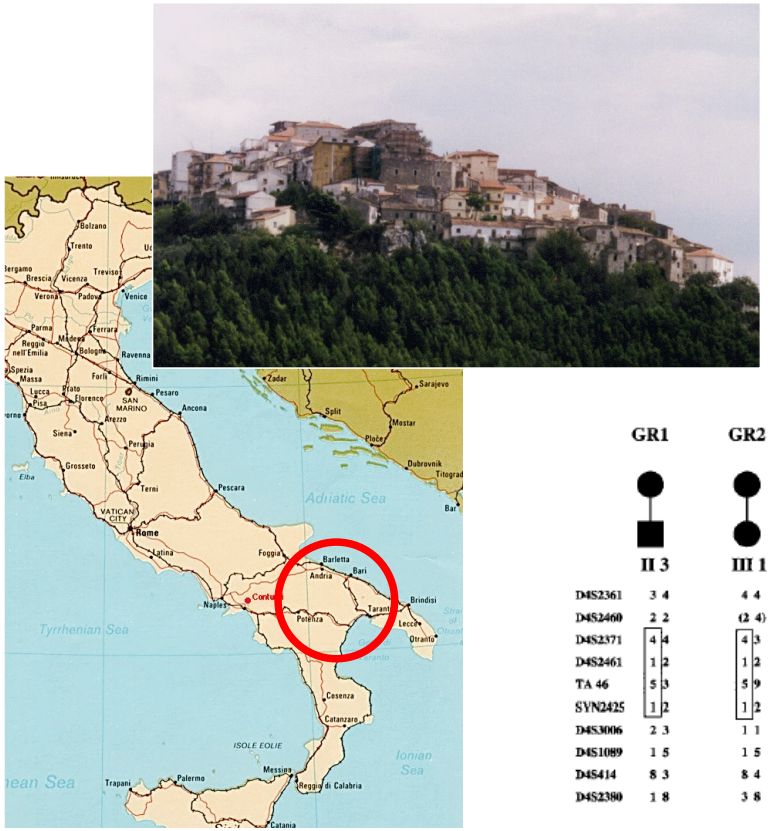
Classification of the genes linked to Parkinson's disease

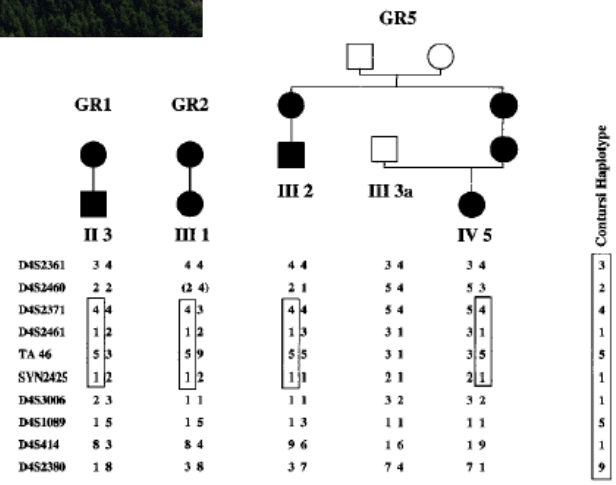


- Gasser T, Journal of Parkinson's Disease, vol. 5, no. 2, pp. 209-215, 2015

Genetic factors: α-synuclein (SNCA)

Contursi kindred (α-synuclein A53T mutation)





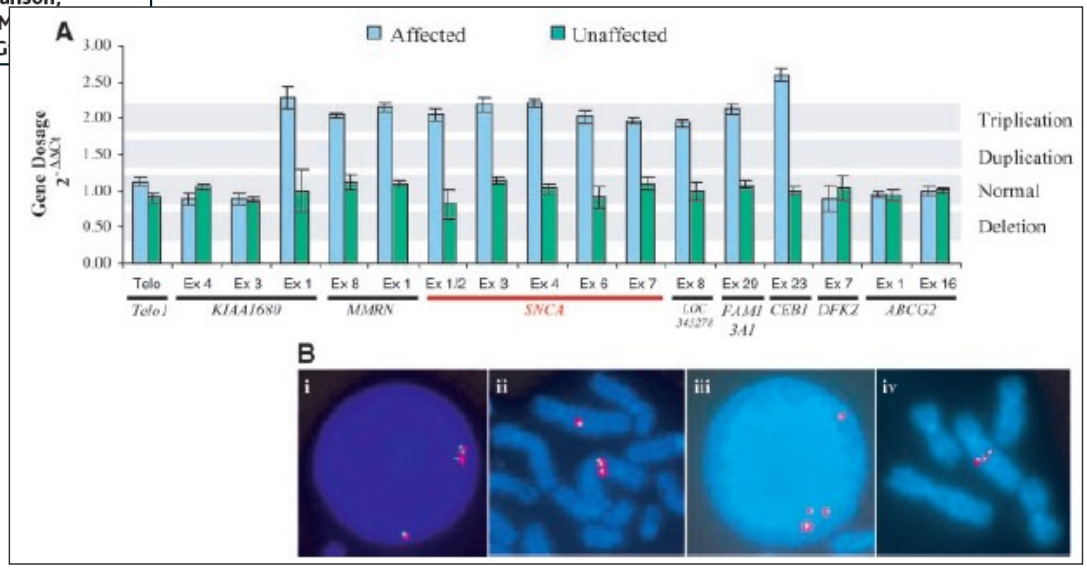
Autosomal dominant inheritance

Genetic factors: α-synuclein (SNCA)

α-Synuclein: importance of gene dosage

α-Synuclein Locus Triplication Causes Parkinson's Disease

A. B. Singleton,[1]*† M. Farrer,[4]† J. Johnson,[1] A. Singleton,[2] S. Hague,[1] J. Kachergus,[4] M. Hulihan,[4] T. Peuralinna,[1] A. Dutra,[3] R. Nussbaum,[2] S. Lincoln,[4] A. Crawley,[2] M. Hanson,[1] D. Maraganore,[5] C. Adler,[6] M. R. Cookson,[1] M. M. Baptista,[1] D. Miller,[1] J. Blancato,[7] J. Hardy,[1] K. G



α-synuclein locus duplication as a cause of familial Parkinson's disease

Marie-Christine Chartier-Harlin, Jennifer Kachergus, Christophe Roumier, Lydie Larvor, Joris Andrieux, Mary Hulihan, Nawal Waucquier, Luc Defeb

Causal relation between α-synuclein gene duplication and familial Parkinson's disease

Lancet 2004

P Ibáñez, A-M Bonnet, B Débarges, E Lohmann, F Tison, P Pollak, Y Agid, A Dürr, A Brice, French Parkinson's Disease Genetics Study Group*

Genetic factors: α-synuclein (SNCA)

α-Synuclein: importance of gene dosage

	Fold increase in expression	Syndrom	Age of onset	Mode of inheritance
Promoter polymorphism	?	Risk factor for Parkinson	?	?
Duplication	1.5 X	Parkinsonism	48 yrs (39-65)	Dominant (incomplete penetrance)
Triplication	2 X	Parkinsonism Dementia	34 yrs	Dominant

Parkinson's disease: question 7

You have discovered that a triplication of a genomic locus containing the α-synuclein gene can lead to autosomal dominant Parkinson's disease (PD), which is characterized by the deposition of Lewy bodies.

Which one(s) of the following statements can you conclude from this finding?

A. PD can be caused by an overabundance of α-synuclein.

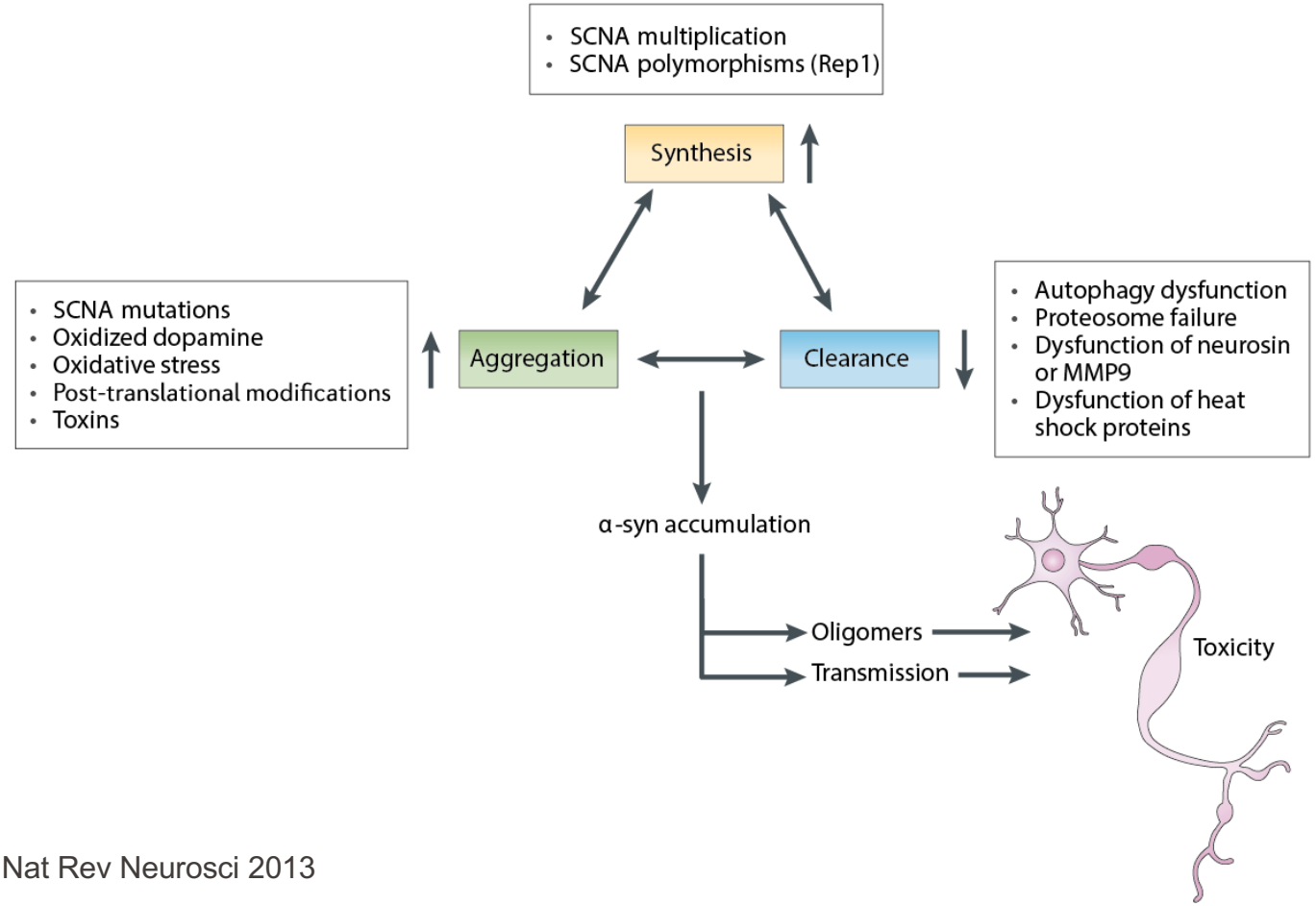
B. PD is not caused by the loss of the α-synuclein physiological function.

C. The locus does not contain only the α-synuclein gene. There could be another gene involved in this case. (it was verified that this is not the case)

D. When overabundant, α-synuclein leads to the formation of Lewy bodies and these aggregates are toxic to the neurons.

Genetic factors: α-synuclein (SNCA)

Alpha-synuclein – dysregulation

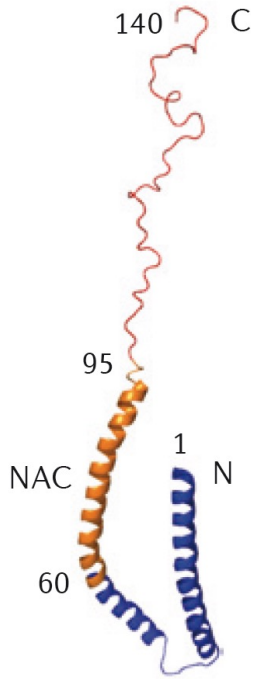


- Lashuel HA et al, Nat Rev Neurosci 2013

Genetic factors: α-synuclein (SNCA)

α-Synuclein protein

Chromosom 4q
140 a.a., cytoplasmic, MW ≈ 19 kDa
Mainly neuronal

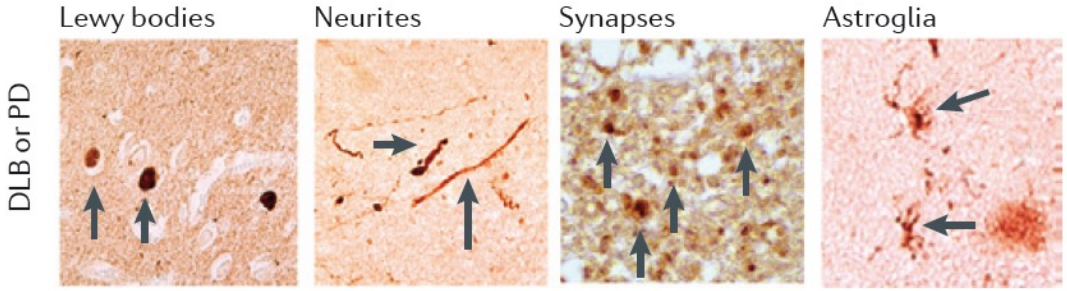


Three regions:

N-terminal (a.a.1-60):
Repetitive, associates with membranes

NAC (a.a. 61-97):
hydrophobic
prone to aggregation

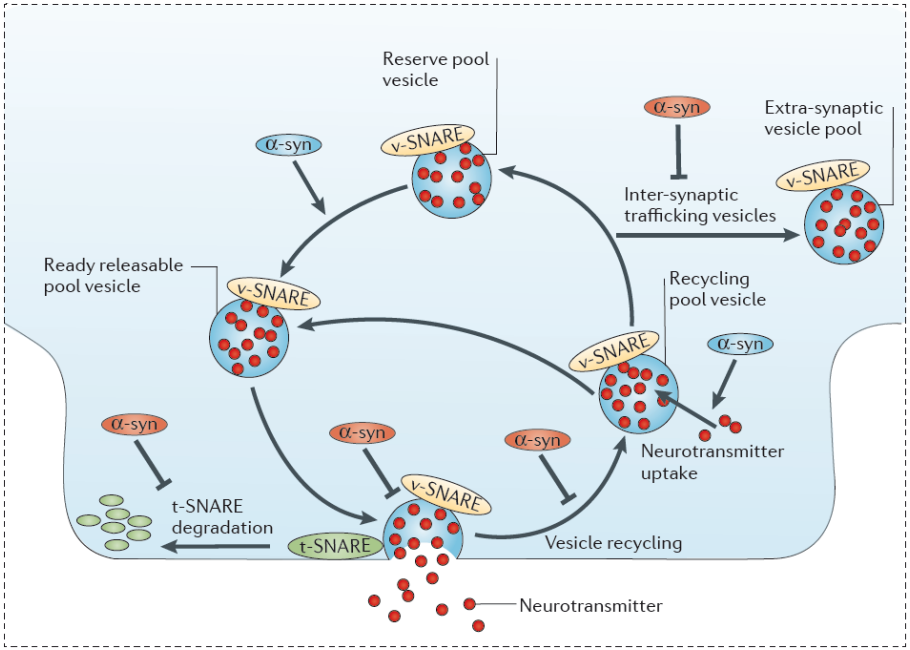
C-terminal (a.a. 98-140):
acidic



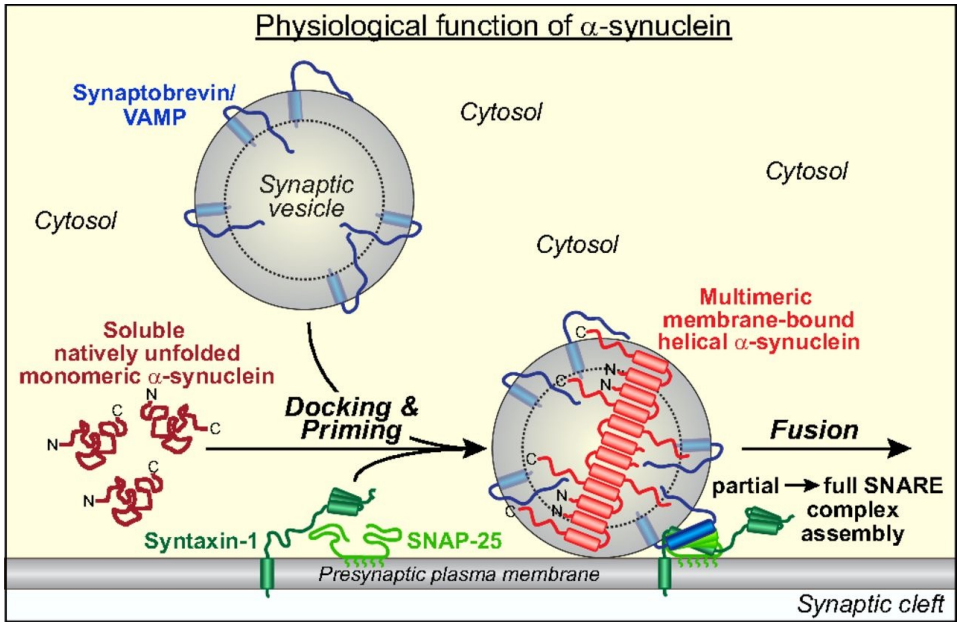
- Lashuel HA et al, Nat Rev Neurosci 2013

Genetic factors: α-synuclein (SNCA)

A physiological function for α-synuclein oligomers: regulation of presynaptic vesicle fusion

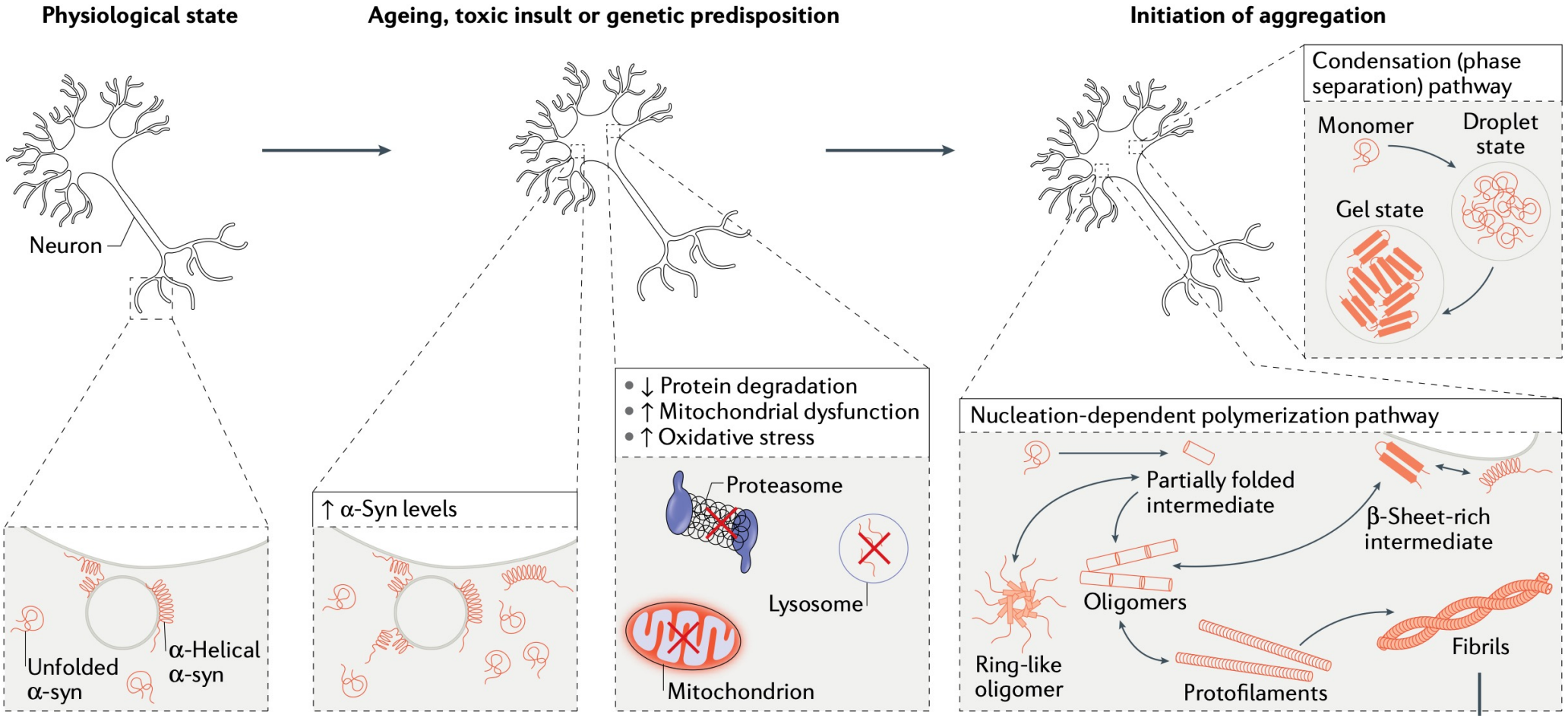


α-Synuclein forms multimers upon membrane binding to promote SNARE complex formation



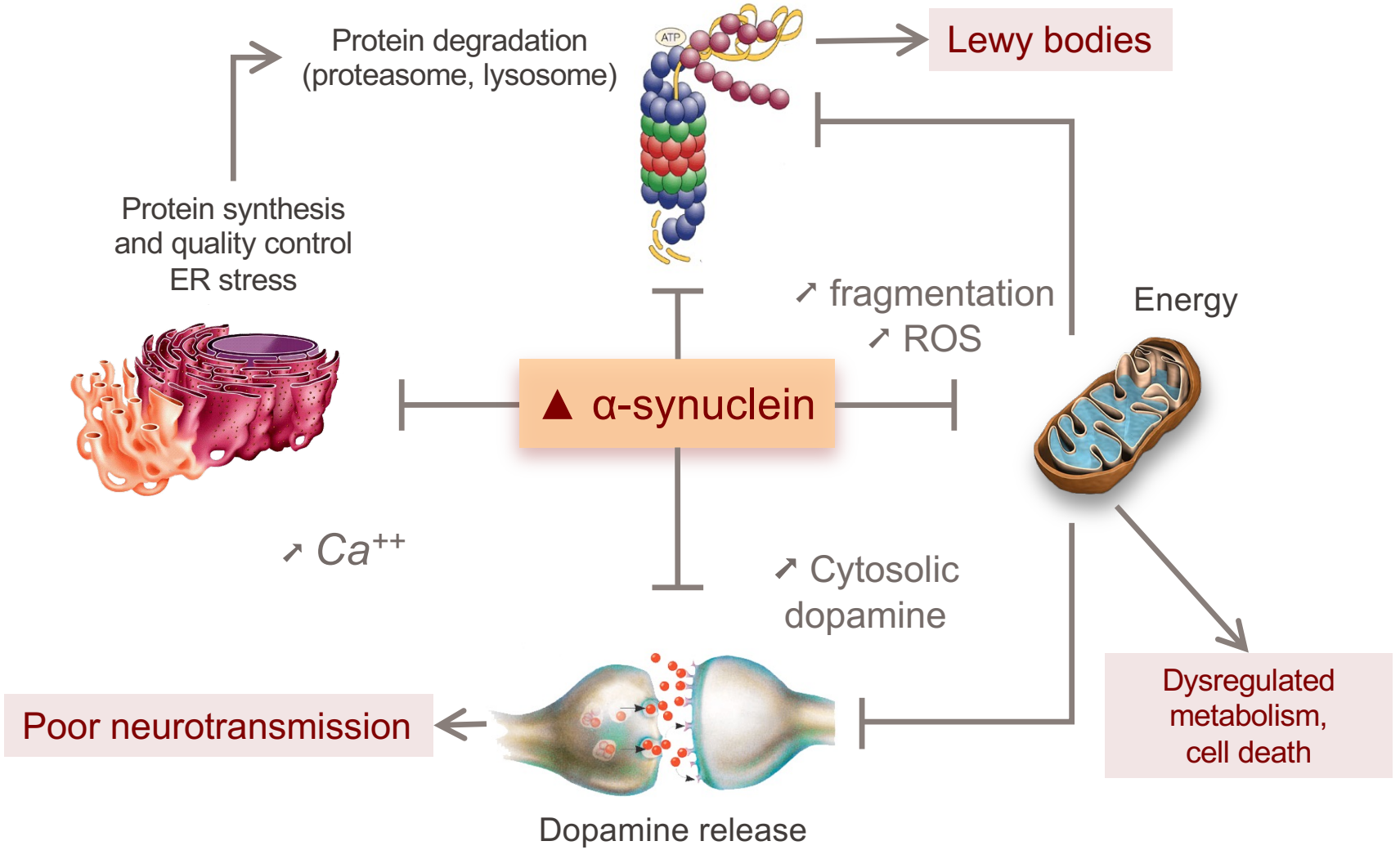
Lashuel HA et al, Nat Rev Neuroci 2013
Burré et al, PNAS 2014
Wang et al, Current Biol 2014

Alpha-synuclein: pathology



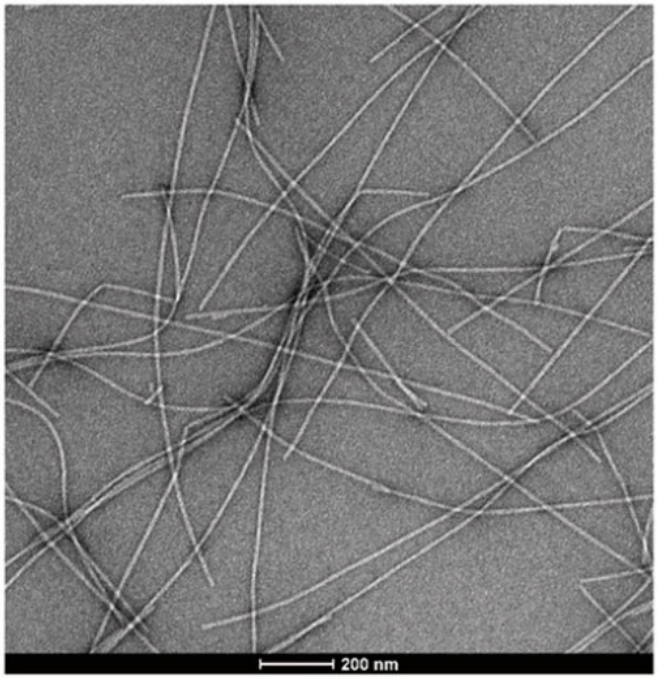
Fares MB et al, Nat Rev Neurosci 2021

Alpha-synuclein: cellular pathology

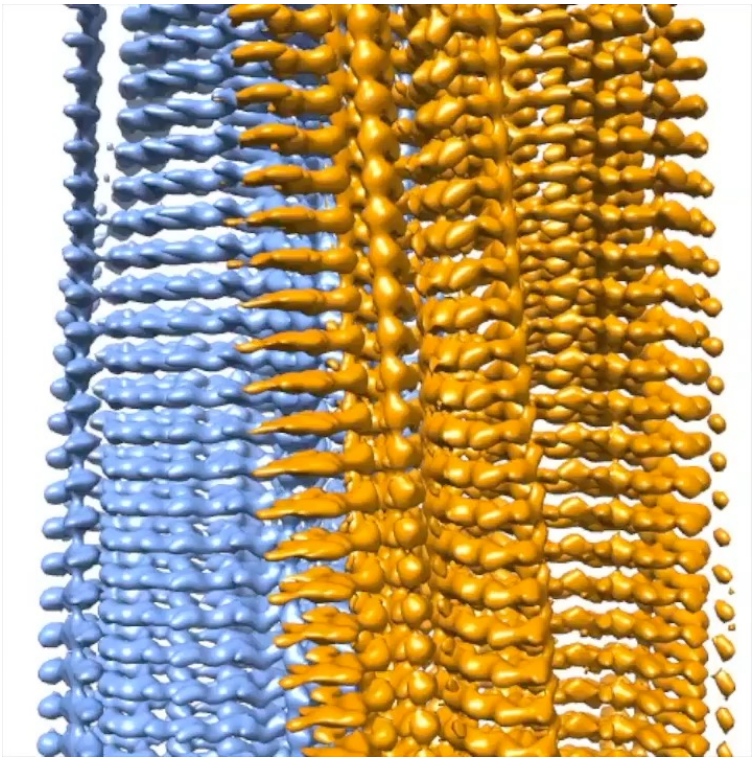


α-synuclein (SNCA): a protein which forms amyloid fibrils

α-synuclein fibrils

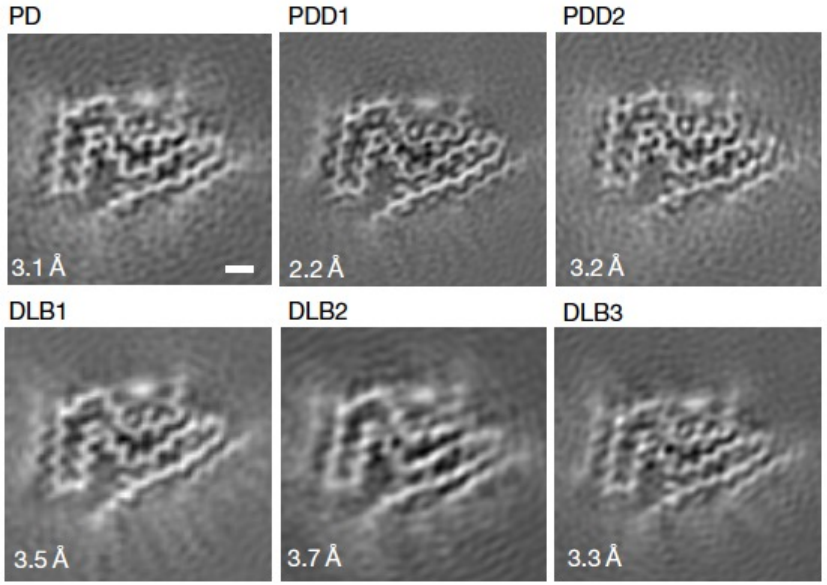


Cryo-EM structure analysis



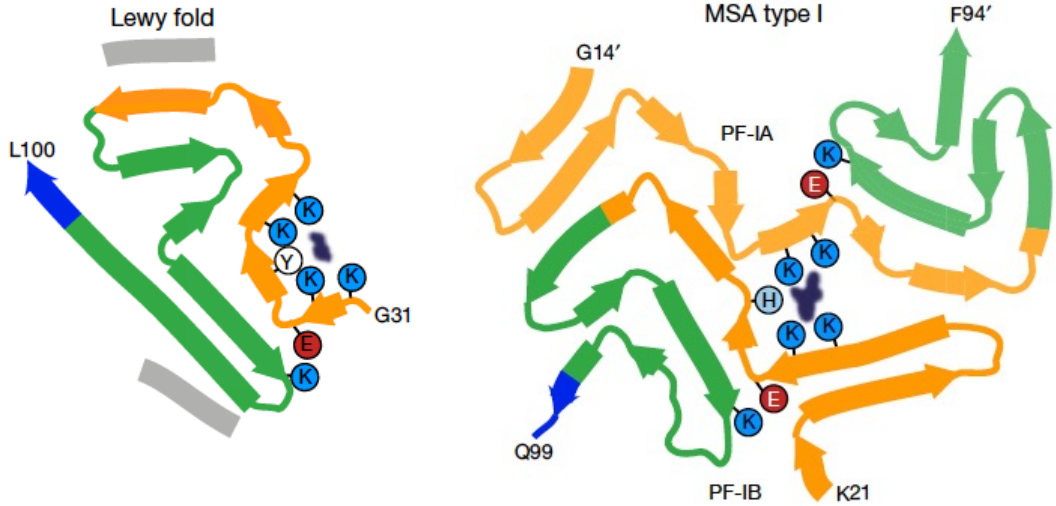
- Li Y et al, Cell Research 2018
  Guerrero-Ferreira G et al, eLife 2018

CryoEM structure of α-synuclein fibrils from PD, DLB and MSA patients

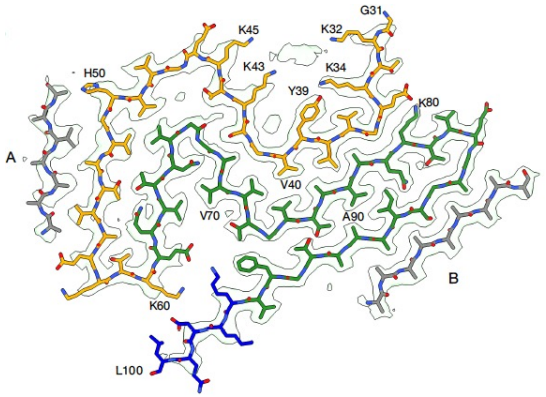


Cryo-EM cross-sections of α-synuclein filaments (Lewy fold) from the cingulate or frontal cortex

Amino acid sequence of human α-synuclein



Comparison of the Lewy and MSA α-synuclein filament folds.



Cryo-EM density map of the Lewy fold

Nature 610, 791–795 (2022). https://doi.org/10.1038/s41586-022-05319-3
Nature 2020, https://doi.org/10.1038/s41586-020-2317-6

Parkinson's disease: question 8

Why is it important to explore the structure of alpha-synuclein fibrils?
Rank the following statements from "correct" to "possible" to "wrong" ?

A. The shape of the fibrils will tell use what is the structure of the α-synuclein protein in normal conditions.

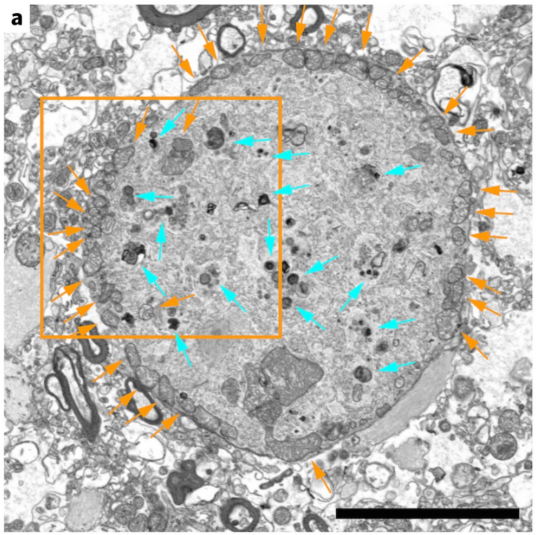
B. It will guide the design of therapeutic antibodies targeting alpha-synuclein pathology.

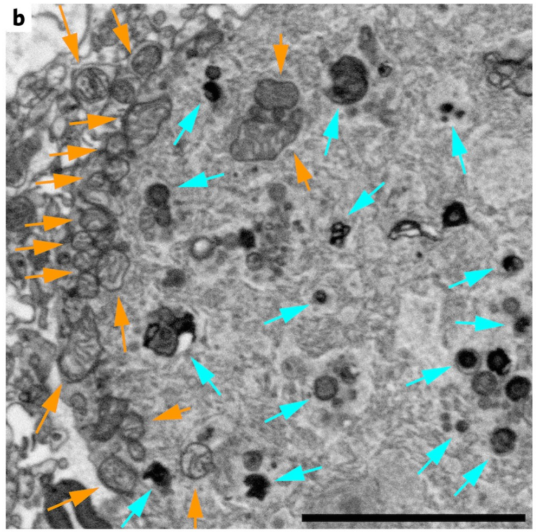
C. Because different types of fibrils may by associated with different diseases (e.g. PD or DLB).

D. We could try to identify therapeutic molecules to disassemble these fibrils. (possible, but likely not therapeutic as it may generate oligomers)

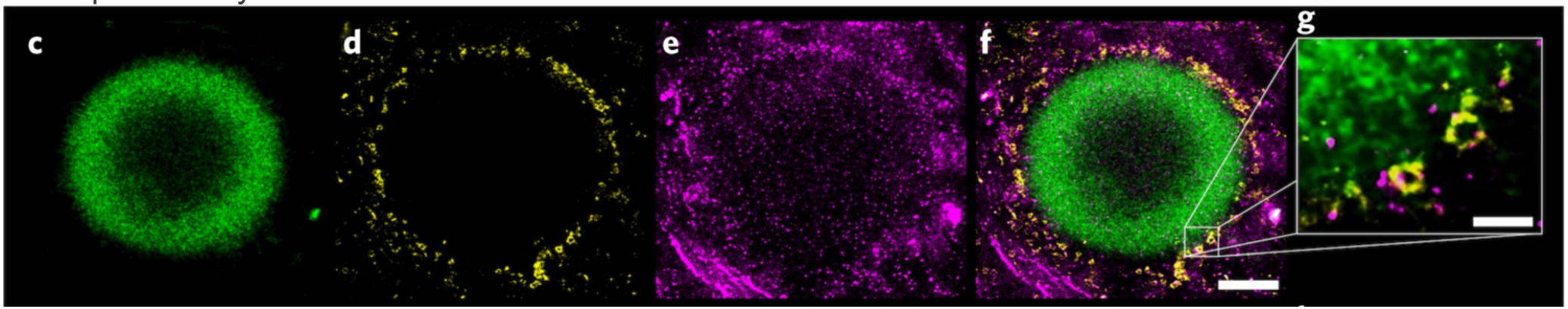
Lewy bodies: accumulation of (defective?) organelles

Lewy bodies contain mitochondria and lysosomal structures



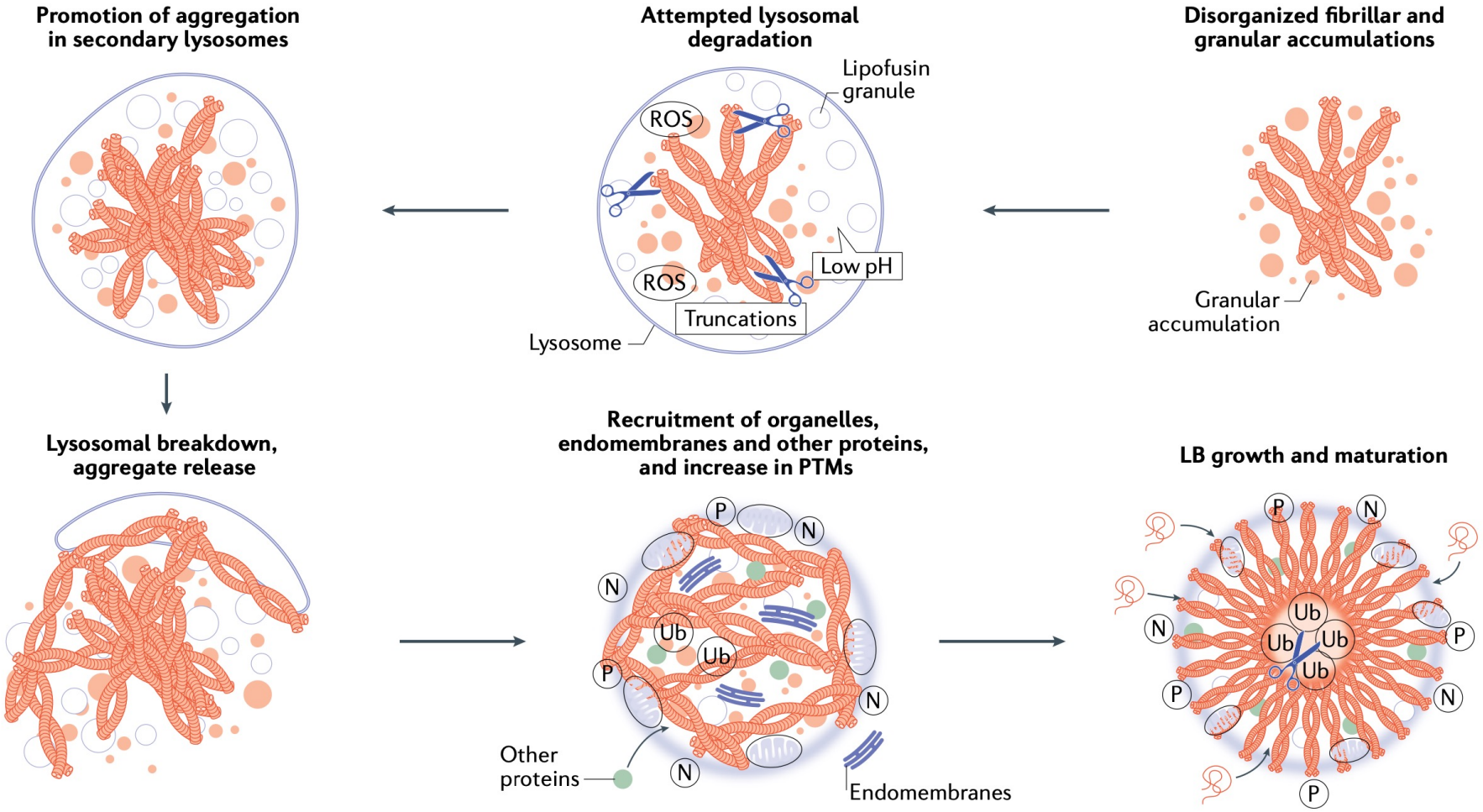


pS129 α-syn VDAC1 LAMP1



- Shahmoradian, S.H. et al. Nat Neurosci 22, 1099–1109 (2019)

From α-synuclein fibrils to Lewy bodies



- Fares MB et al, Nat Rev Neurosci 2021

Parkinson's disease: question 9

The presence of Lewy bodies (LB) is considered as a pathological signature of Parkinson's disease. Rank the following statements from "correct" to "possible" to "wrong" ?

A. Neurons containing Lewy bodies are usually in the process of dying. - 3

B. The presence of Lewy bodies is an indicator of α-synuclein misfolding. - 1

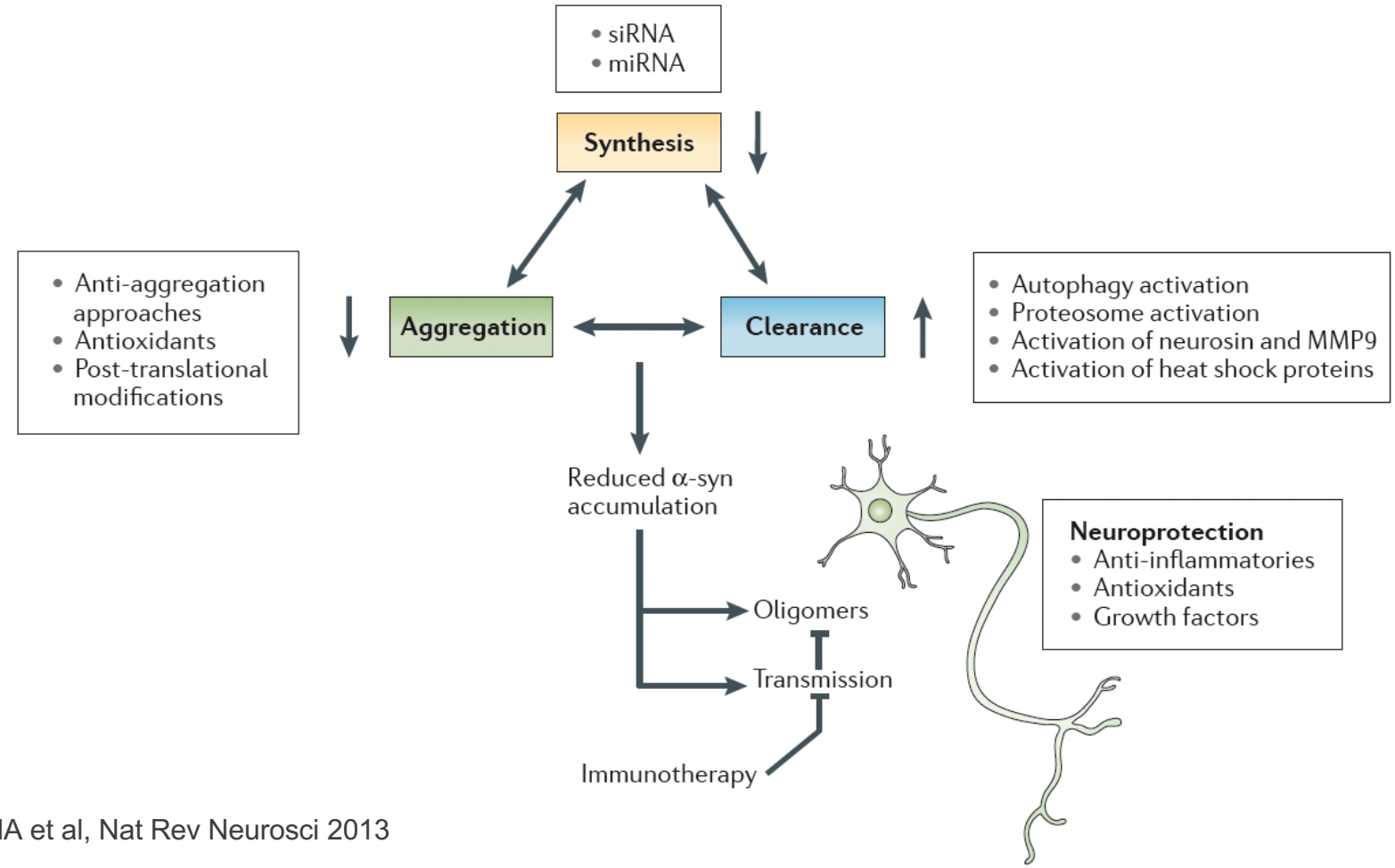
C. The presence of Lewy bodies is a sign of brain aging and not necessarily of the disease. - 5

D. α-Synuclein accumulation in Lewy bodies is a protective mechanism in neurons. - 2

E. Therapeutic strategies that protect neurons in model systems and enhance the formation of Lewy bodies are unlikely to succeed. - 4

Genetic factors: α-synuclein (SNCA)

Alpha-synuclein – therapeutic approaches



- Lashuel HA et al, Nat Rev Neurosci 2013

Lecture plan

1. Basal ganglia circuitry

2. Nigrostriatal degeneration and symptomatic treatments
   - Motor symptoms → dopamine replacement
   - Deep brain stimulation

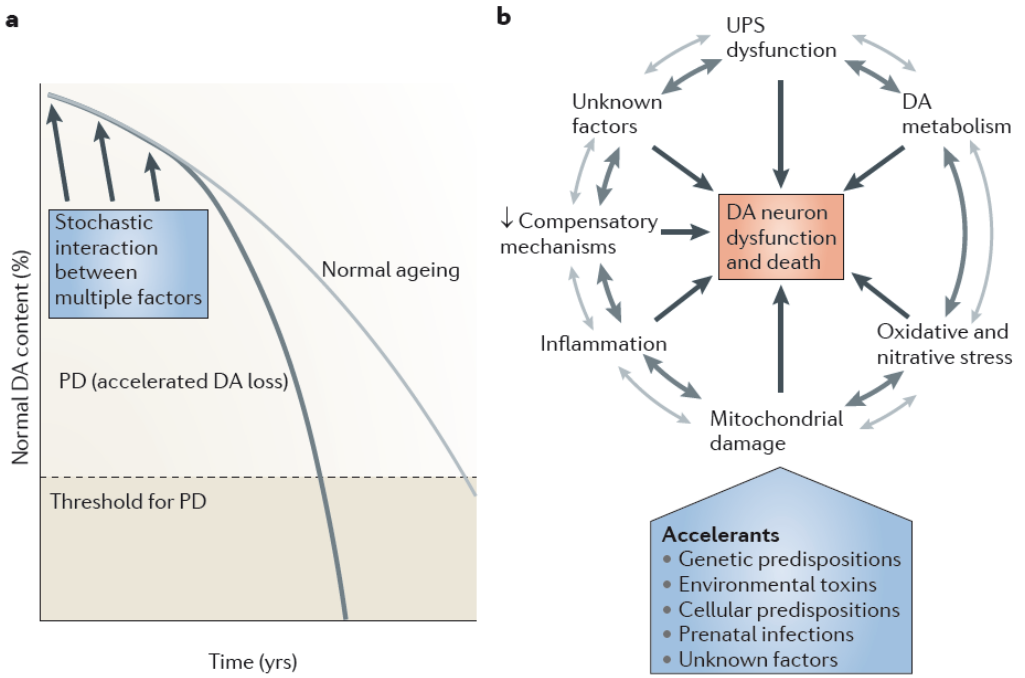
3. Neuronal degeneration / Lewy body pathology
   - Selective vulnerability of neuronal subtypes
   - α-synuclein (physiology, pathology, spreading)

4. PD etiology: organelle quality control
   - Aging, environmental factors, PD risk genes
   - Recessive forms: parkin, PINK1 and mitochondrial turnover

Risk factors: Parkinson's and Gaucher disease

Risk factors for Parkinson's disease: multiple hits?

- In most cases, α-synuclein is not the only factor implicated in Parkinson's disease.
- **Environmental factors** may trigger α-synuclein pathology.
- Interaction with **other genetic predisposition factors** is important (e.g. GBA1).



Parkinson's disease : genetic and environmental causes

Parkinson's disease etiology

Genetic factors

- **α-synuclein**
- LRRK2
- Parkin
- PINK1
- DJ-1
- ATP13A2
- …

Environmental factors

Standardized Odds ratio:

- Pesticides: 1.76
- Herbicides: 1.33 (paraquat: 2.2)
- Insecticides: 1.53
- MPP+

Aging

Gender

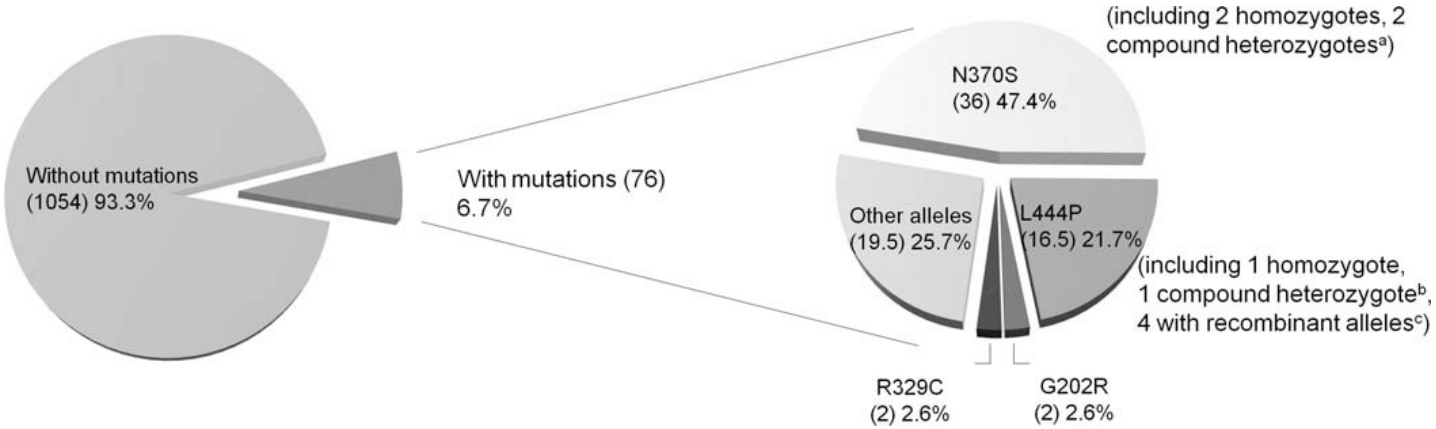
Age-standardized M:F ratio = 1.57

A genetic risk factor in Parkinson's and Gaucher diseases

Glucocerebrosidase (**GBA1**) mutations as a risk factor for Parkinson's disease

- Patients with Gaucher disease (autosomal recessive) have relatives heterozygous for GBA1 mutations who develop parkinsonism.
- Gaucher disease is a **Lysosomal storage disorder.**
- Patients with parkinsonism have an increased incidence of GBA1 mutations.
- **GBA1 is the most common known genetic risk factor for PD** (GBA mutations increase the risk of PD by >20-fold).

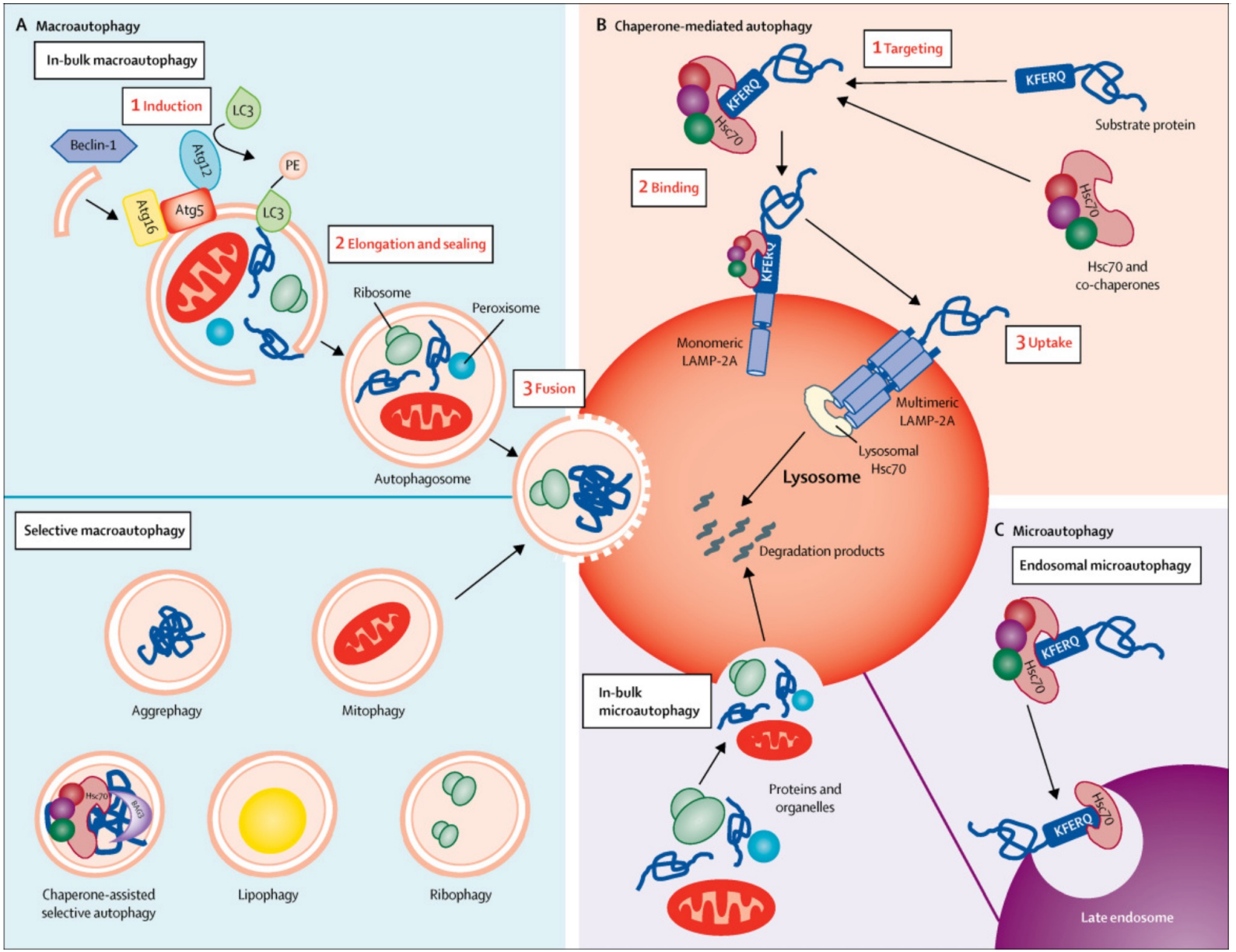
GBA mutations in Parkinson's disease patients



Lesage S et al., Hum Mol Genet, 2011, Vol. 20, No. 1 (French Parkinson's Disease Genetics Study Group)

The role of the **lysosome**:

autophagic pathways in mammalian cells



The Lancet
https://doi.org/10.1016/S1474-4422(18)30238-2

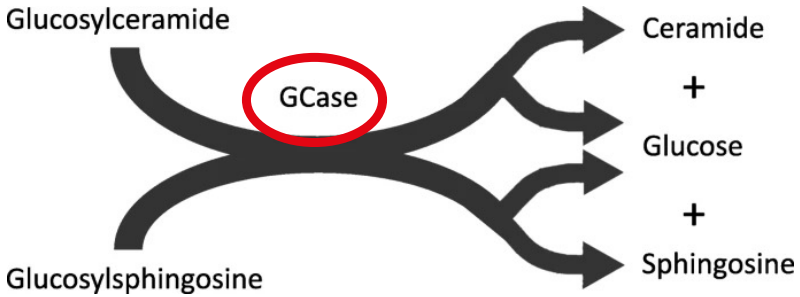
Risk factors: glucocerebrosidase and α-synuclein

- Gaucher disease is a **Lysosomal storage disorder** caused by the accumulation of glucocerebrosides (defective lipid metabolism)
- Due to mutations in the GBA1 gene, which encodes the enzyme **glucocerebrosidase** (GCase).

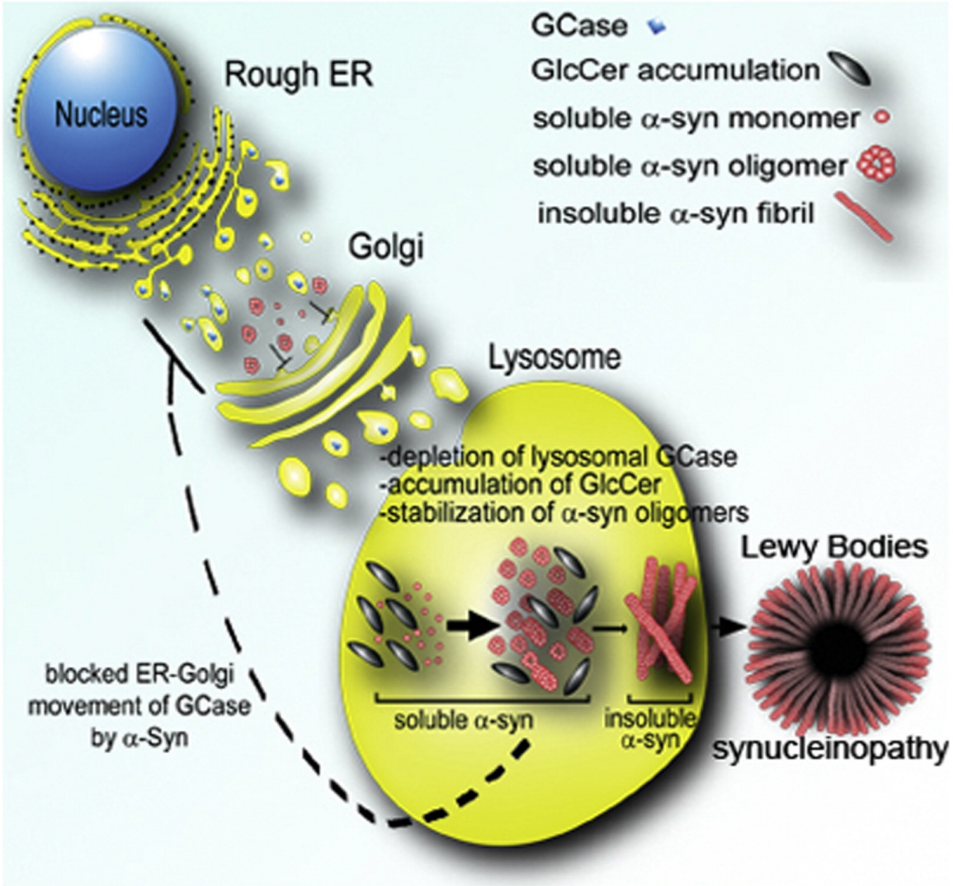
Normal activity:

GCase — Lysosomal degradation ⊣ **α-synuclein accumulation**

Glucocerebrosides



Accumulation of GlcCer impairs lysosomal activity.



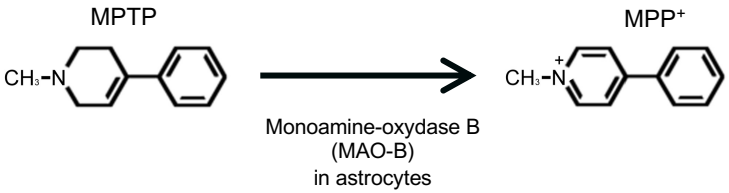
GlcCer = Glucosylceramide (GCase substrate)

- Mazzulli JR, et al. Cell. 2011 Jul 8;146(1):37-52, https://doi.org/10.1016/j.cell.2011.06.001

Parkinson's disease etiology : mitochondrial toxins

« Frozen addicts » (early 1980s) :
intoxication with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)

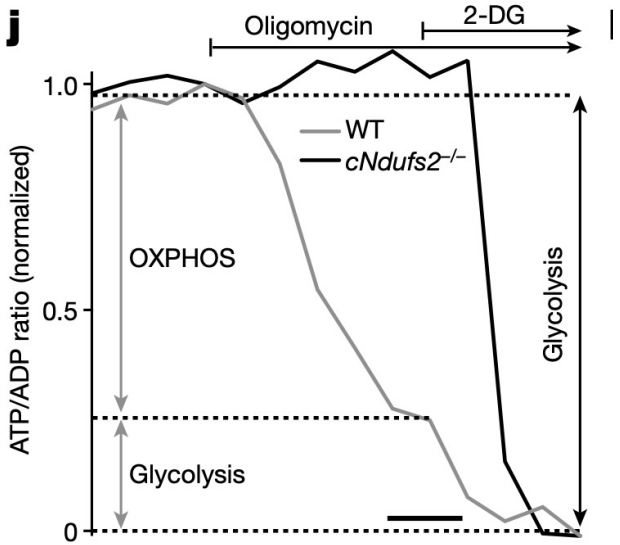
- **MPTP** produced accidentally instead of MPPP, a synthetic opioid (effects similar to heroin)
- Induces Parkinsonism in mice and primates (not in rats)
- Crosses the blood-brain barrier

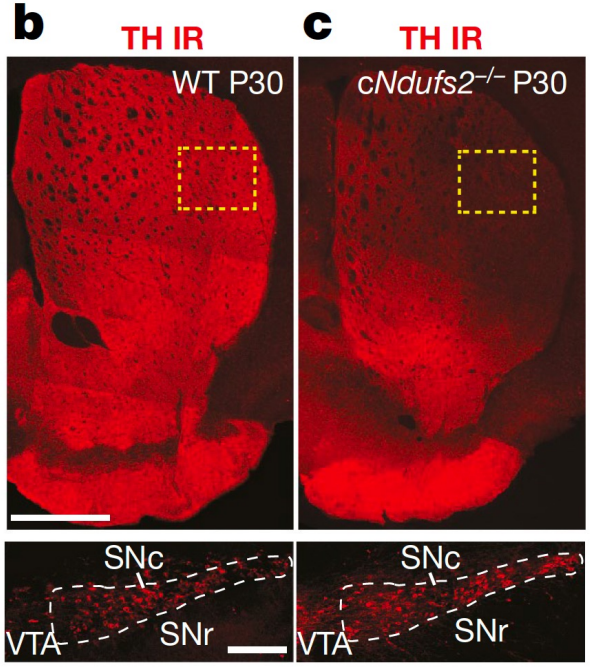


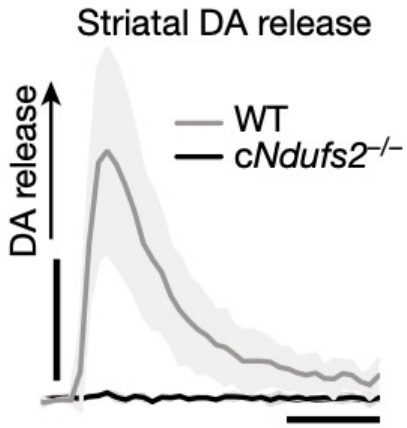
- **MPP+** toxin is preferentially uptaken by the DAT transporter
- Inhibits NADH--CoQ1 (Complex I) of mitochondrial respiratory chain
- ATP production falls

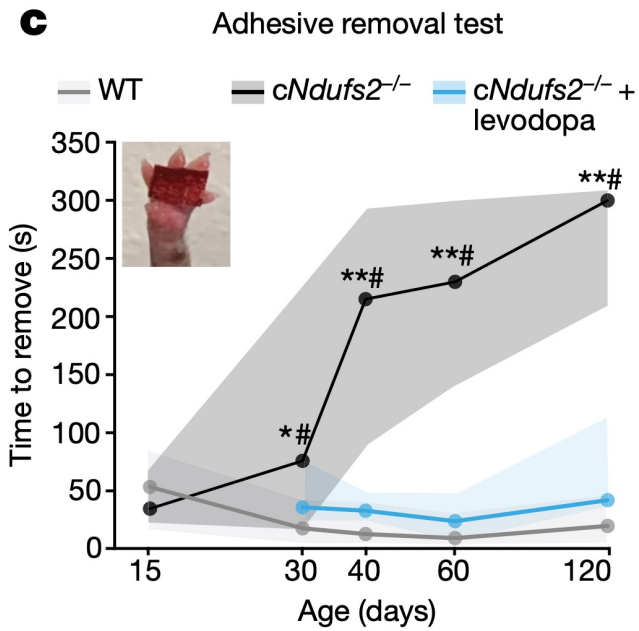
Complex I dysfunction induces Parkinsonism

- Ndufs2 is a core unit essential for the catalytic activity of **mitochondrial complex I**.
- Selective Ndufs2 KO in dopaminergic neurons disrupts mitochondrial complex I and induces progressive Parkinsonism.





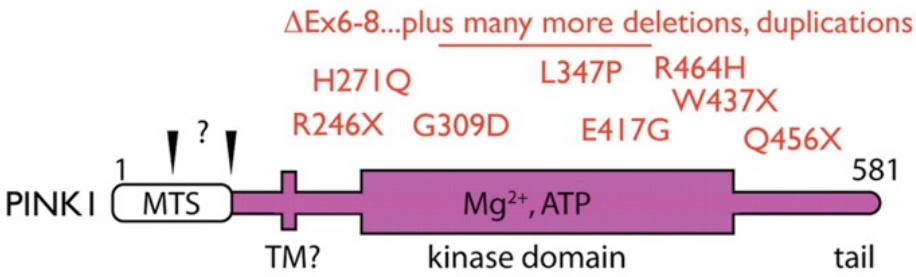




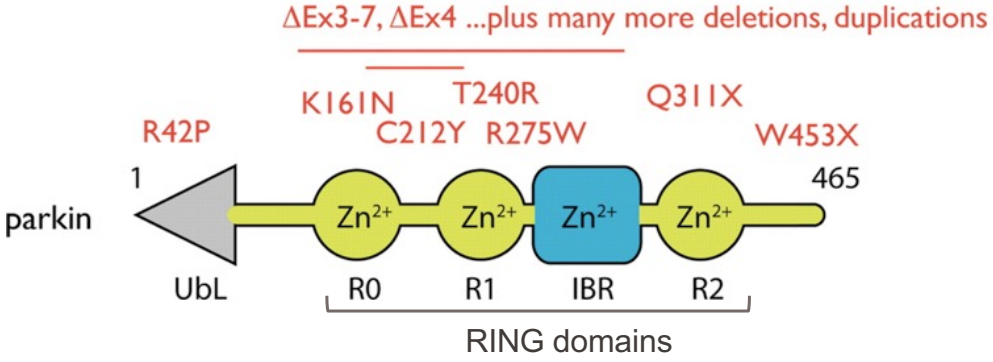
- *Nature 2021, https://doi.org/10.1038/s41586-021-04059-0*

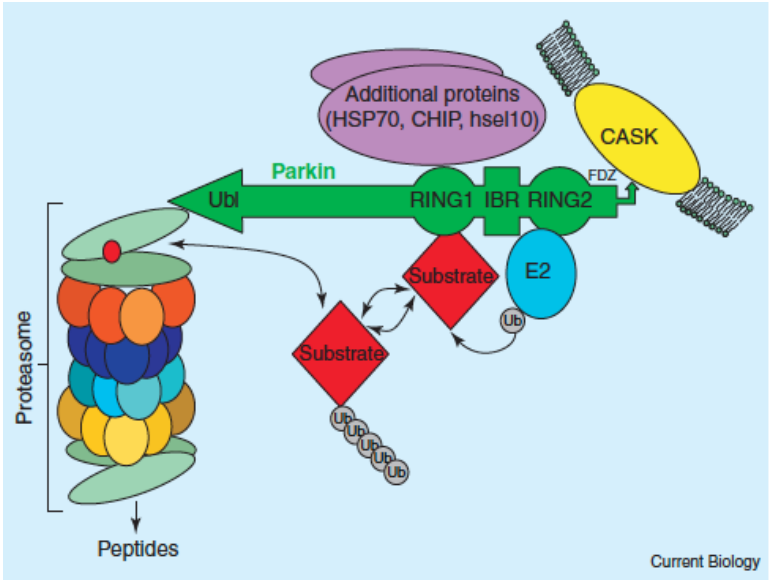
Genetic factors: Parkin and PINK1

PINK1: serine/threonin kinase



Parkin: E3 ubiquitin ligase





Parkinson's disease: question 10

Parkinson's patients with recessive mutations in the Parkin gene **have in most cases no Lewy bodies**.

What would you conclude from this observation?

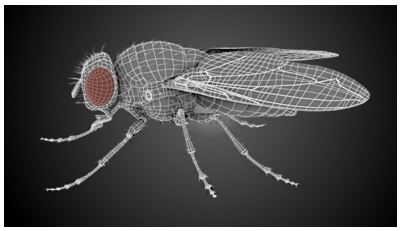
A. This is a different form of Parkinson's disease, with mechanisms different from the typical disease.

B. α-synuclein deposition may require Parkin activity.

C. Parkin-mediated ubiquitination of α-synuclein is required for Lewy body formation.

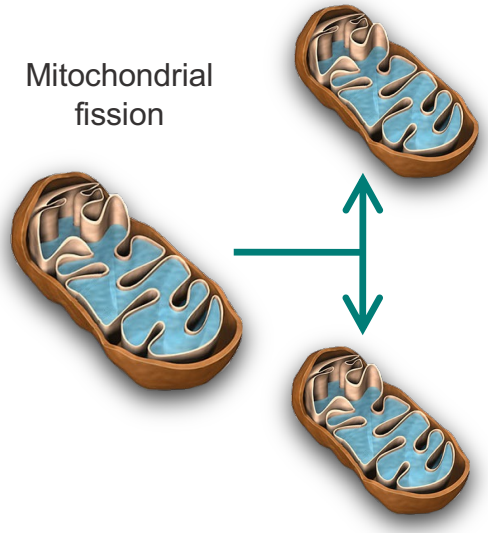
D. As Parkin mutations lead to early-onset juvenile Parkinsonism, Lewy bodies do not have time to develop.

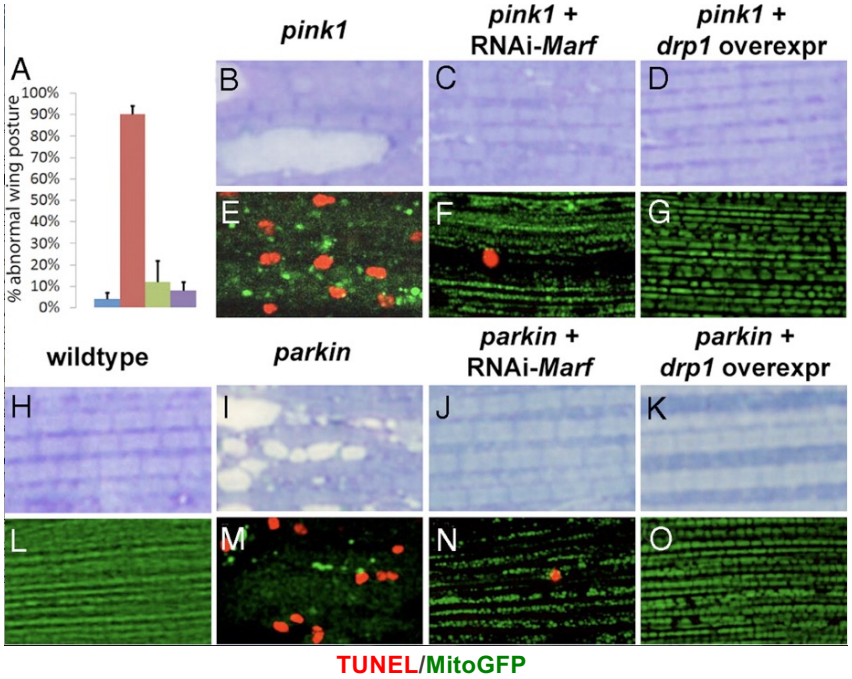
Genetic factors: Parkin and PINK1



Parkin/PINK1:
pathway regulating mitochondrial morphology
(*Drosophila*)

PINK1 → Parkin →





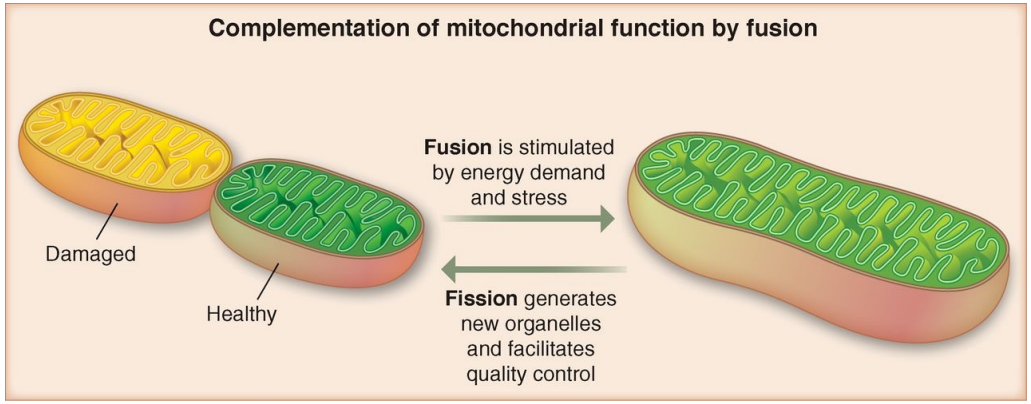
TUNEL/MitoGFP

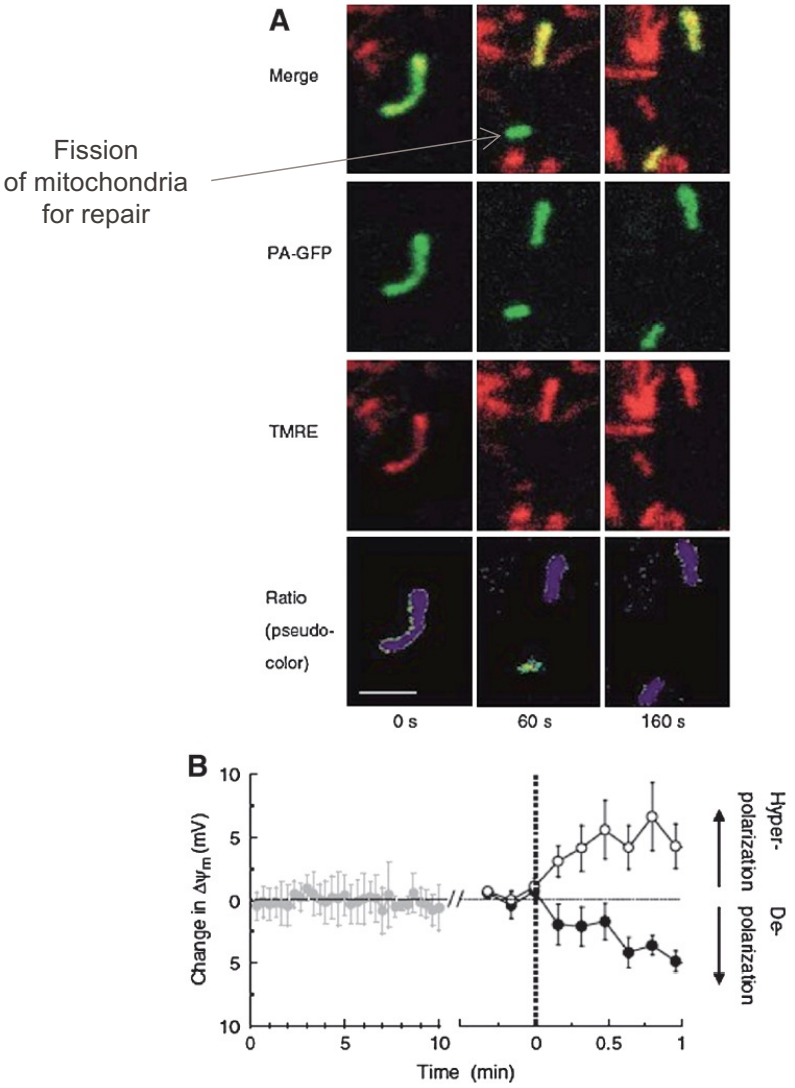
Drosophila melanogaster
Muscle

Deng H et al., PNAS 105(38) 2008
Poole AC et al., PNAS 105(5) 2008
Clark IE et al, Nature 441(7097) 2006
Park J et al., Nature 441(7097) 2006
Clark IE et al., Nature 441 2006

Genetic factors: Parkin and PINK1

Parkin/PINK1 in mammalian cells:
Fission as part of quality control of mitochondria





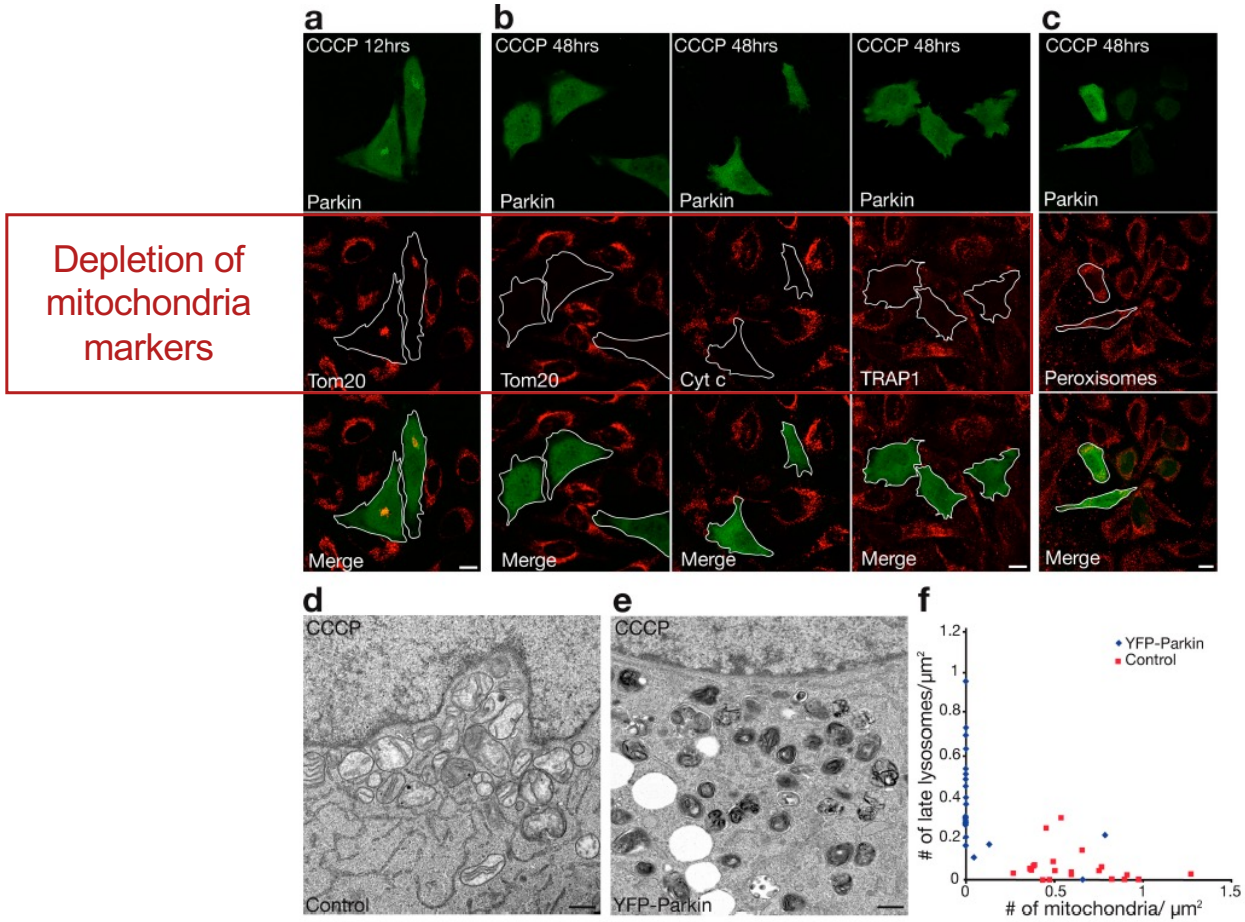
Genetic factors: Parkin and PINK1

Parkin in mammalian cells: a role in the autophagy of depolarized mitochondria

- Parkin promotes the selective elimination of depolarized mitochondria in CCCP-treated **HeLa cells.**
  [CCCP induces mitochondrial depolarization].

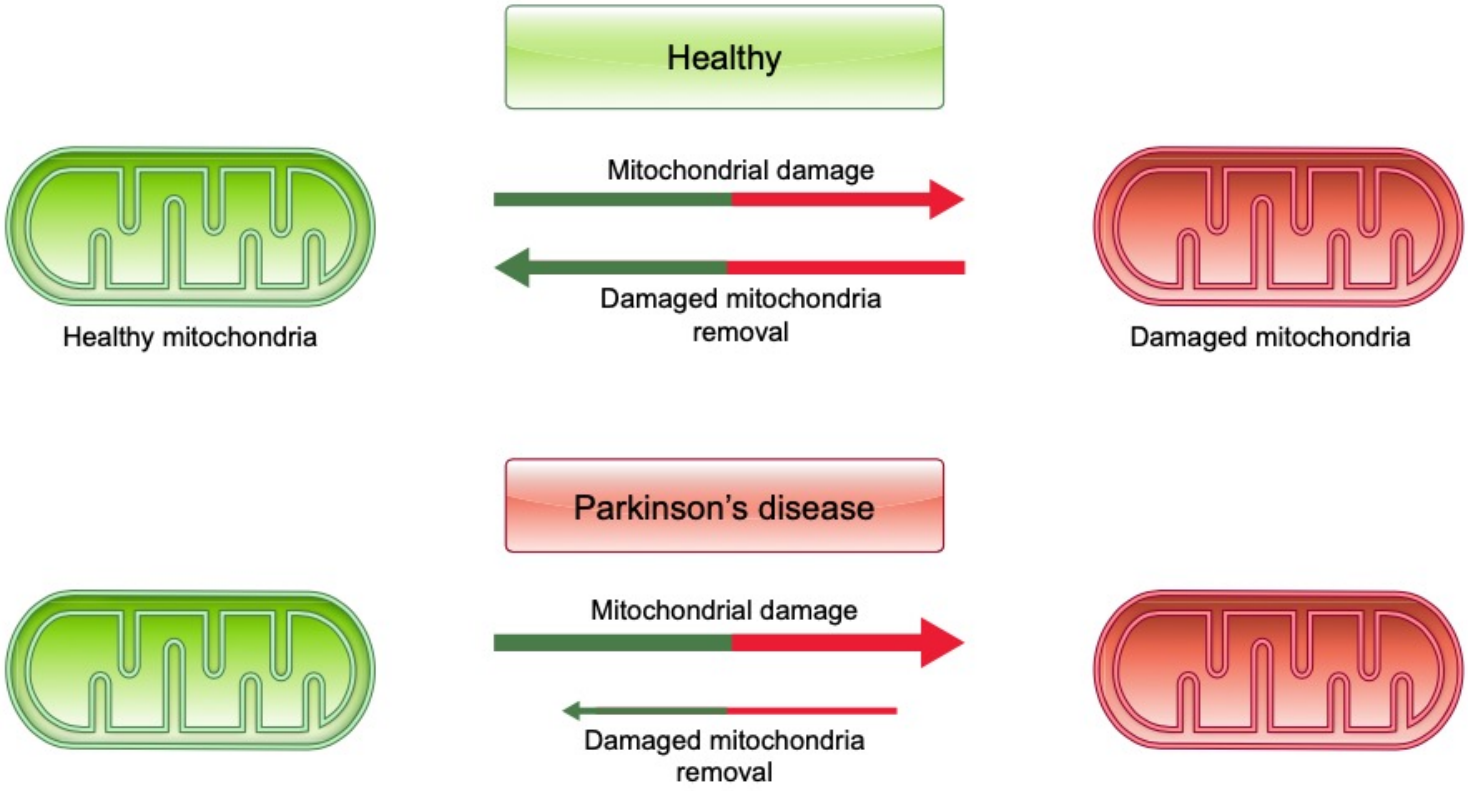
- **Cells overexpressing Parkin deplete mitochondria** and accumulate lysosomes following CCCP exposure.

  - Narendra D et al., JCB 2008



Genetic factors: Parkin and PINK1

Mitochondria quality control

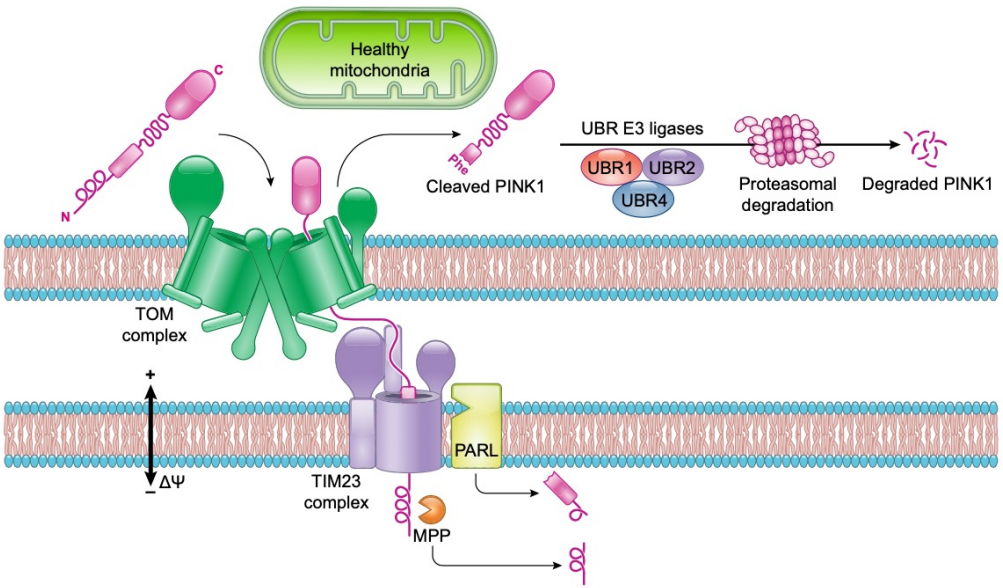


- ELDEEB ET AL., 2022, Physiol Rev102: 1721–1755
  https://doi.org/10.1152/physrev.00041.2021

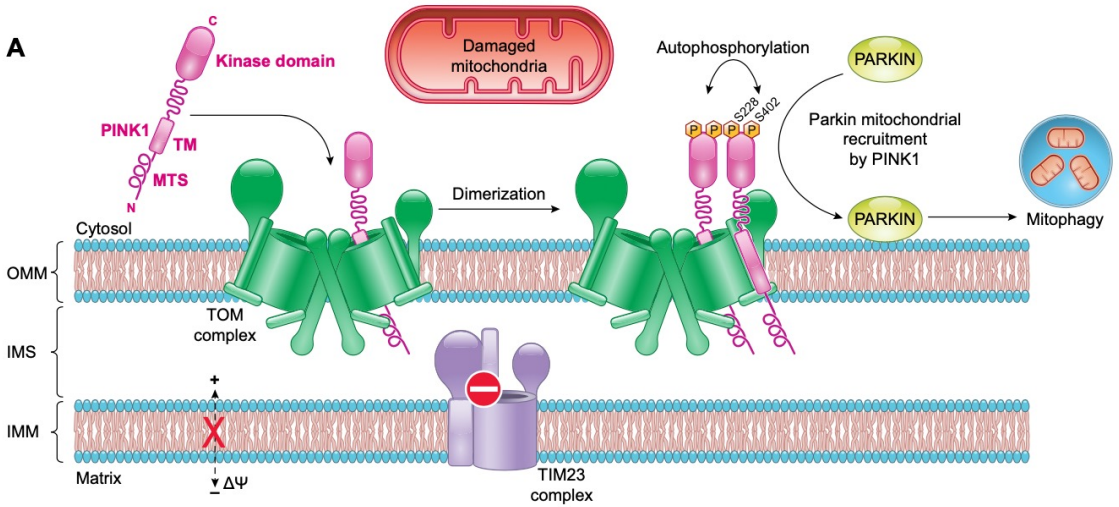
Genetic factors: Parkin and PINK1

Mitochondria quality control: PINK1-mediated recruitment of Parkin

Rapid turnover of PINK1 in healthy mitochondria



Mitochondrial damage: **PINK1 ⇒ Parkin activation**

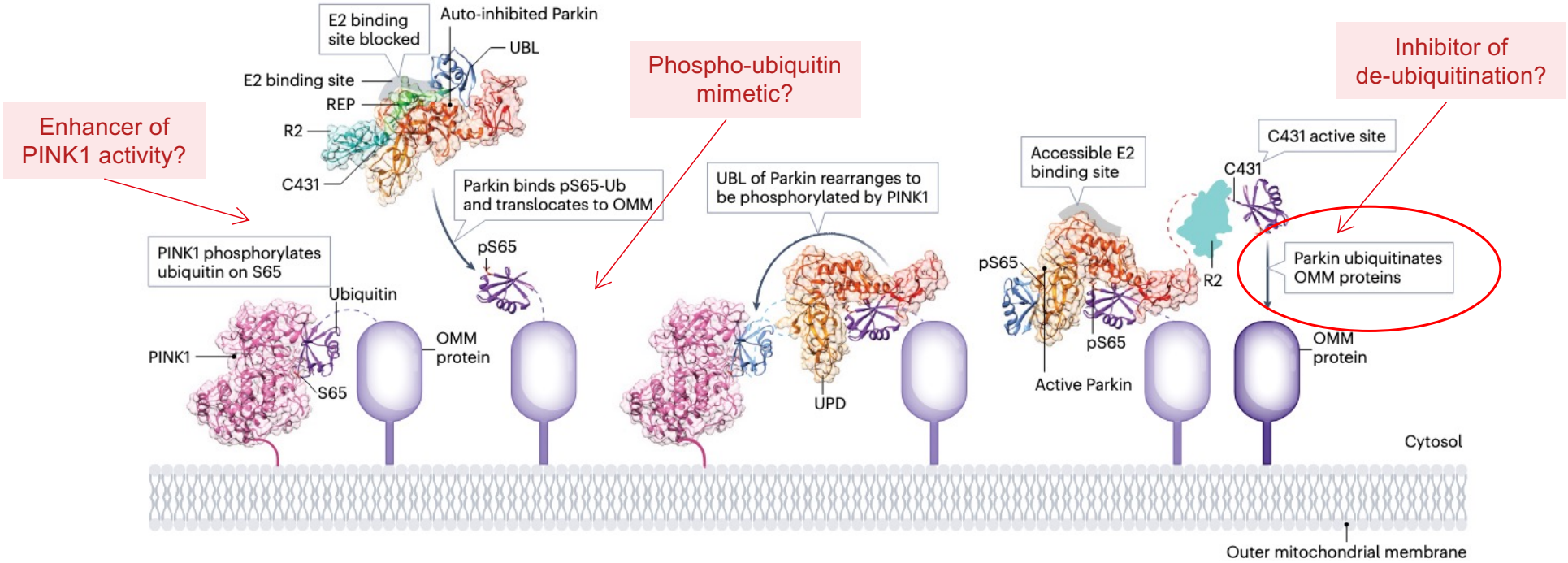


- ELDEEB ET AL., 2022, Physiol Rev102: 1721–1755
  https://doi.org/10.1152/physrev.00041.2021

Genetic factors: Parkin and PINK1

Parkin ubiquitinates proteins on the mitochondrial outer membrane (OMM)

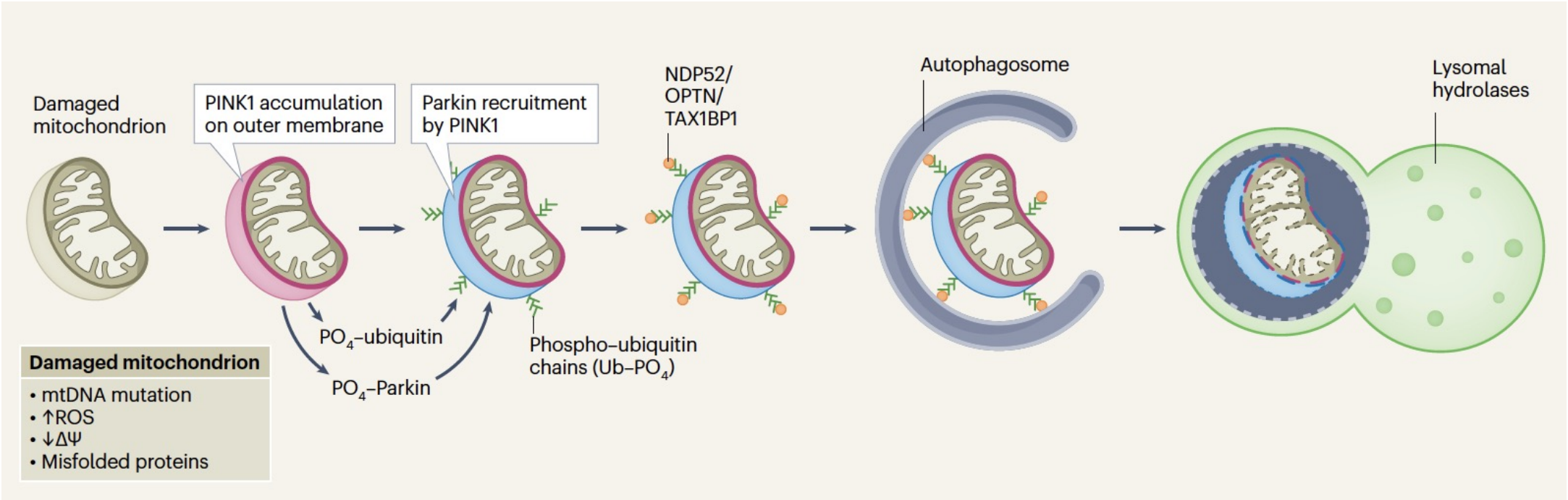
(In red: possible pharmacological approaches to enhance this pathway)



- *https://doi.org/10.1038/s41556-024-01513-9*

Genetic factors: Parkin and PINK1

Molecular pathway leading to the formation of autophagosome for **mitophagy**

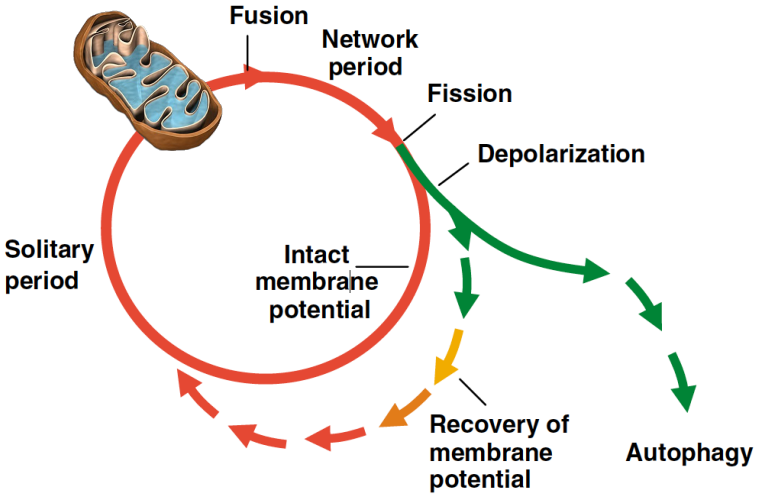


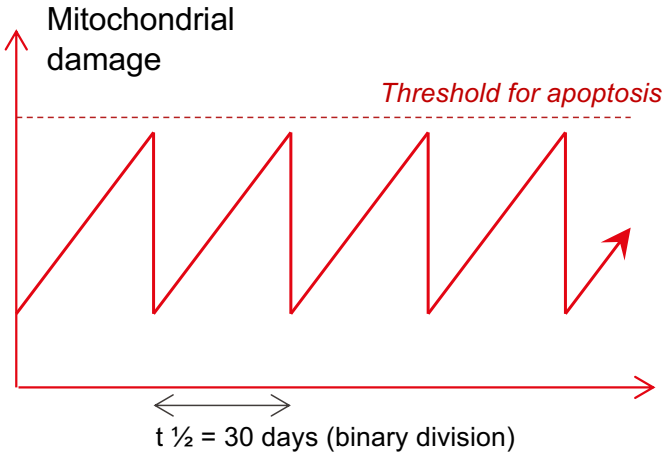
- *https://doi.org/10.1038/s41556-024-01513-9*

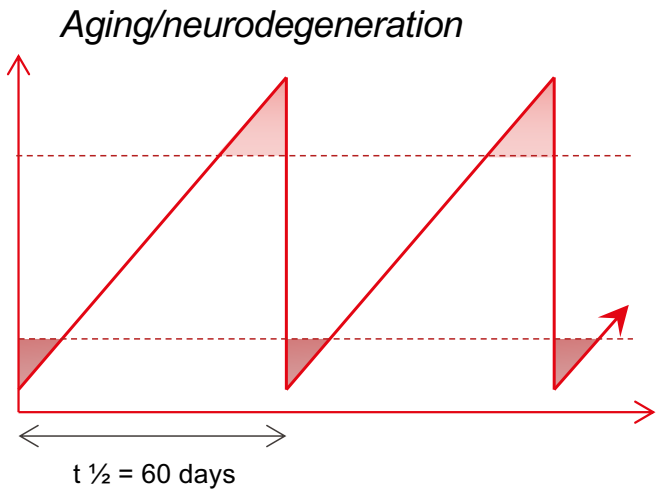
Parkinson's disease: mitochondria turnover

Mitochondrial turnover: an essential component of PD ?

- PD is associated with a reduction of **ETC complex I activity**
- PD toxins affect ETC complex I
- Parkin/PINK1 implicated in **mitophagy**
- Other genetic factors directly involved in **mitochondria/lysosomal function** (incl. α-synuclein)







- *Twig G et al, EMBO J, 27 (2008)*
  Navarro A, Boveris A, Front Aging Neurosci 2010

Parkinson's disease: question 11

There is ample evidence that **mitochondrial impairment** has a critical role in Parkinson's disease. Why are all neurons not equally affected by the disease? Among these statements, which ones are correct?

A. Mitochondrial activity is important to the survival and function of neurons producing dopamine.

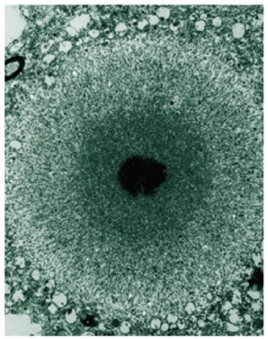
B. Only the neurons that express α-synuclein are sensitive.

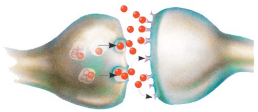
C. Different types of neurons have various mitochondrial content.

D. Mitochondrial function is critical in neurons with long axons.

E. Mitochondrial pathology depends on calcium signals.

Overview of Parkinson's disease





Dopamine

Mitochondrial activity

Alpha-synuclein aggregation and spreading

Parkin, PINK1 deficiency

Mitochondria quality control and turnover

Autophagic activity

Low GBA1 activity

Selective vulnerability

Neuro-degeneration