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THE MICHAEL J. FOX FOUNDATION FOR PARKINSON'S RESEARCH

MRC Protein Phosphorylation and Ubiquitylation Unit

UNIVERSITY OF DUNDEE

Decoding Parkinson's disease

Dario Alessi
Director
MRC Protein Phosphorylation and Ubiquitylation Unit,
University of Dundee

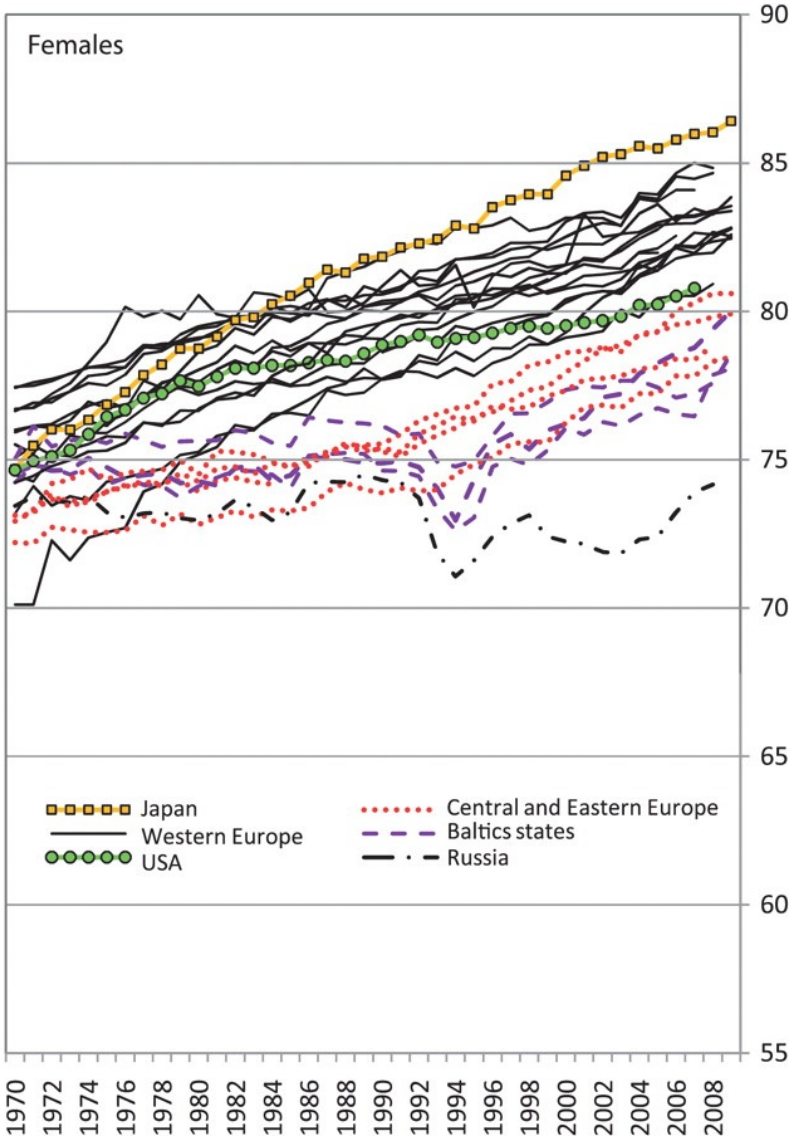
MRC Protein Phosphorylation & Ubiquitylation Unit

GOAL: To understanding the biological roles of phosphorylation and ubiquitylation and how disruption of these processes cause human diseases such as neurodegeneration, cancer, hypertension and immune disorders.

MRC Protein Phosphorylation and Ubiquitylation Unit

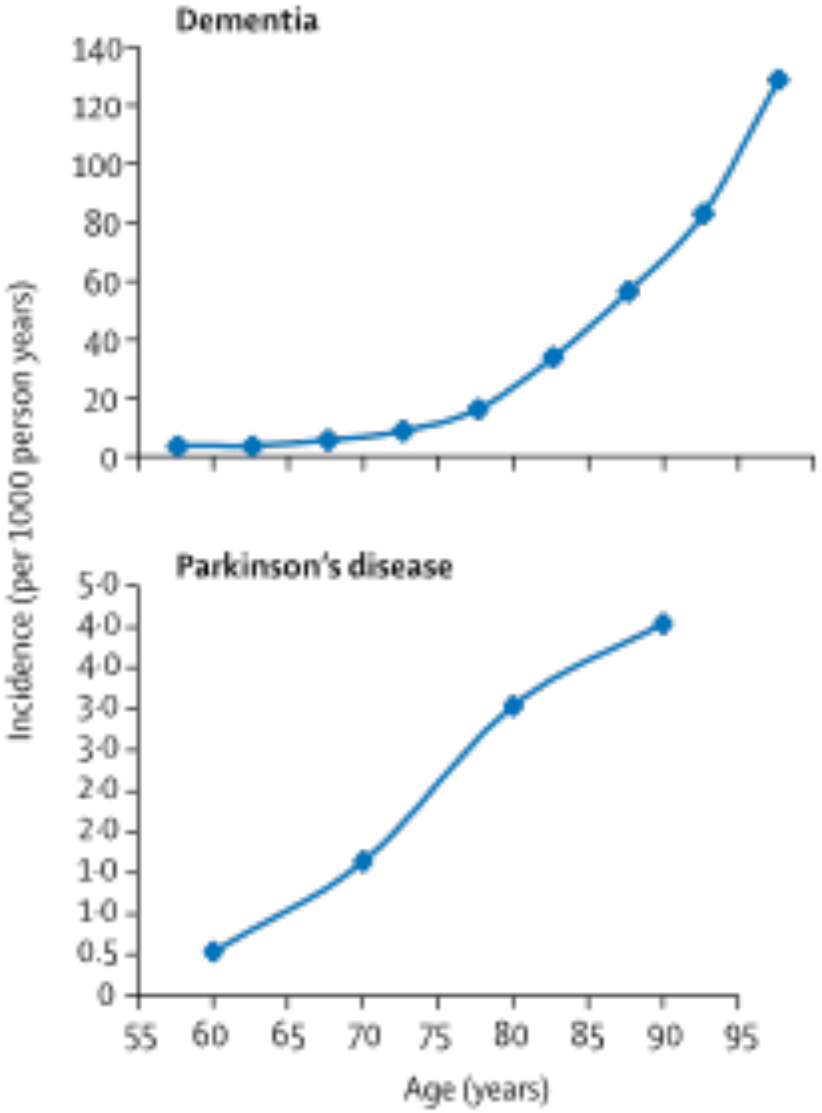
UNIVERSITY OF DUNDEE

Life Expectancy soaring



Leon, I J Epidemiol 2009

The rise and rise of degenerative brain disorders



Rotterdam study, Lancet Neuro 2006

History of Parkinson’s disease

James Parkinson’s described disease in 1817

James Parkinson (11 April 1755 – 21 December 1824) was an English surgeon, apothecary, geologist, palaeontologist, and political activist. He is most famous for his 1817 work, An Essay on the Shaking Palsy in which he was the first to describe "paralysis agitans", a condition that would later be renamed Parkinson's disease by Jean-Martin Charcot.

James Parkinson's described disease in 1817

AN

ESSAY

ON THE

SHAKING PALSY.

BY

JAMES PARKINSON,

MEMBER OF THE ROYAL COLLEGE OF SURGEONS.

LONDON:

PRINTED BY WHITTINGHAM AND ROWLAND,

Gaunt street,

FOR SHERWOOD, NEELY, AND JONES,

PATERNOSTER ROW.

1817.

James Parkinson: “shaking palsy”

Published “essay of the shaking palsy’ (paralysis agitans)

Recognised and defined clinical features based on 6 illustrated cases

3 patients treated in his Hoxton practice
2 individuals he casually met on the street and spoke to
1 individual observed across a busy square in Shoreditch

“involuntary tremulous motion, with lessened muscular power, in parts not in action and even when supported; with a propensity to bend the trunk forward, and to pass from a walking to a running pace: the senses and intellect being uninjured.”

James Parkinson: “Shaking palsy”

“Before concluding these pages, it may be proper to observe once more, that an important object proposed to be obtained by them is, the leading of the attention of those who humanely employ
anatomical examination in detecting the causes and nature of the diseases, particularly to this malady. By their benevolent labours, its real nature may be ascertained and appropriate modes of relief, or even of cure pointed out.”

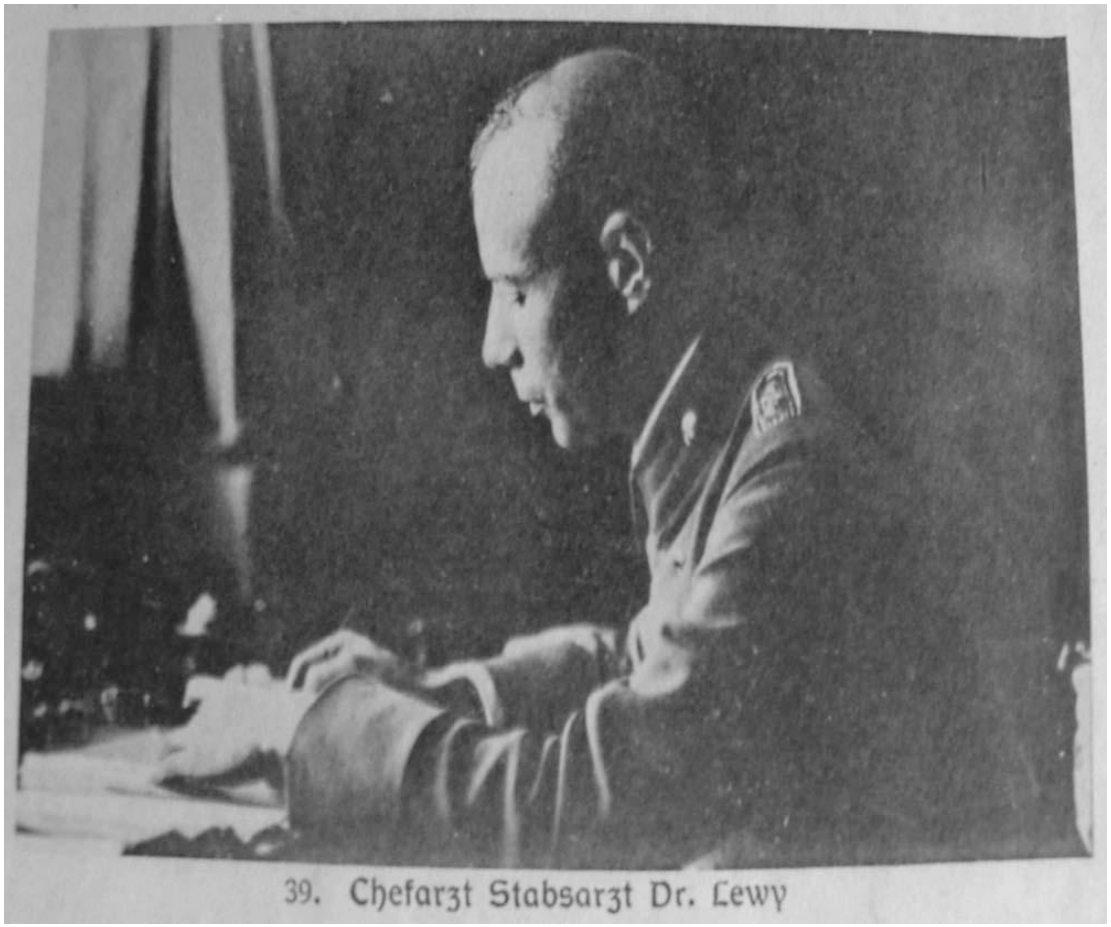
Charcot: “Malady de Parkinson”

Painting by Andre Brouillet

1872: Systematic re-evaluation of shaking palsy
Absence of true weakness
Delineation into akinetic and tremulous forms

Charcot J-M 1872. De la paralysie agitante. In Oeuvres Complètes (t 1) Leçons sur les maladies du système nerveux, pp. 155–188 A Delahaye, Paris

Pathological insights: Frederich Lewy 1912



Frederich Lewy Jewish German-born American neurologist.

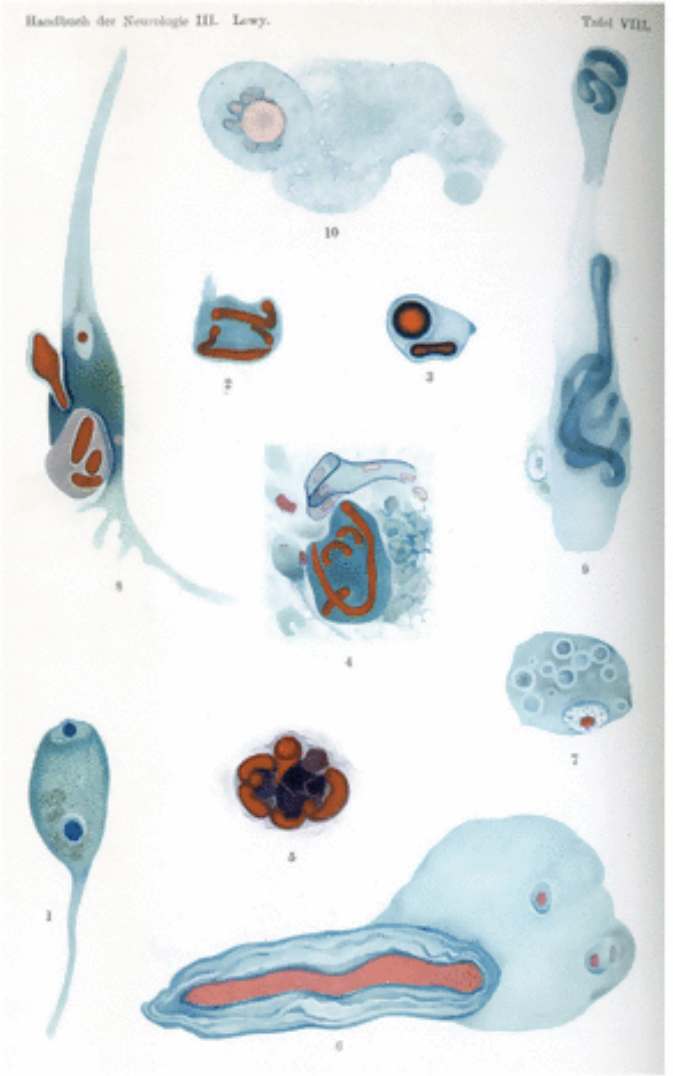
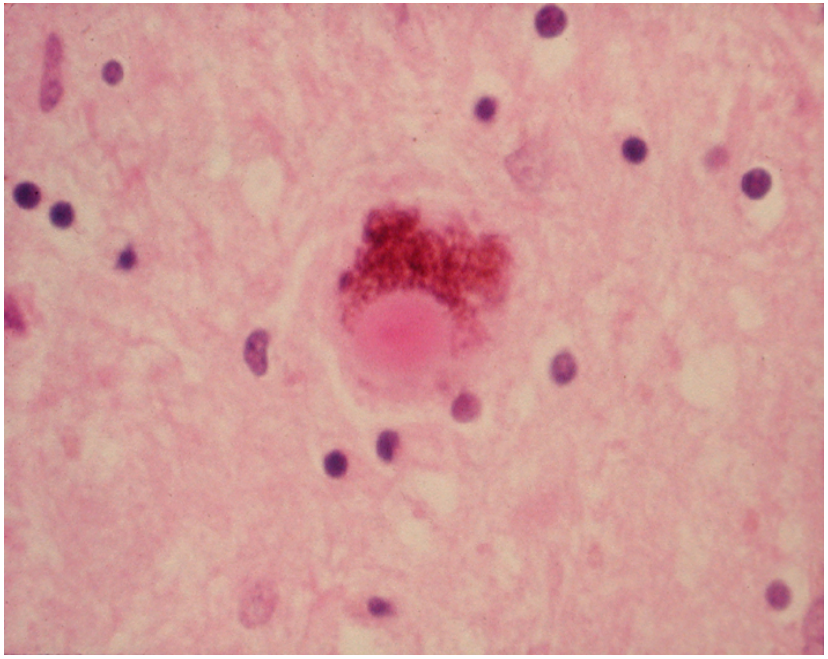
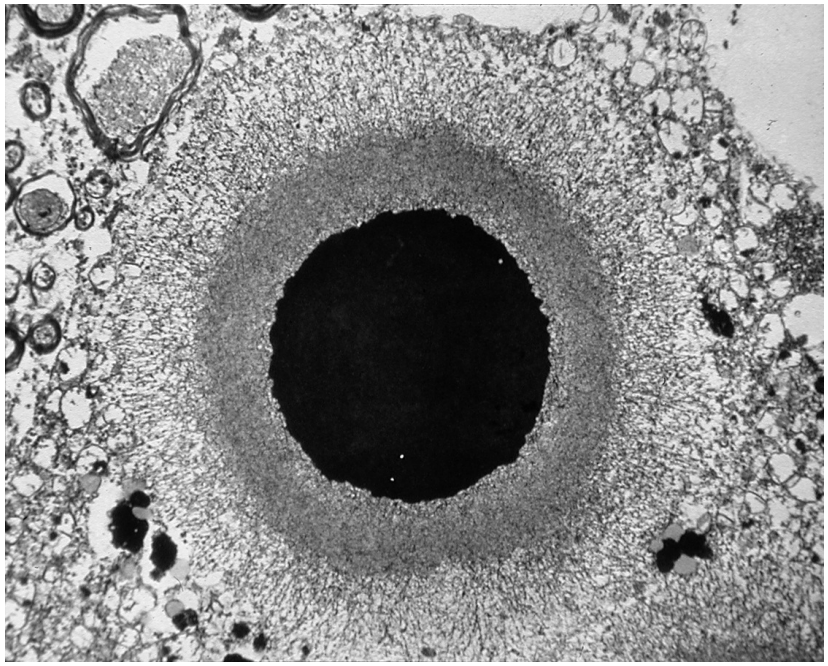


Fig. 1 Lewy-bodies 1912: Intracellular eosinophilic inclusion bodies. Cells 1–6 from dorsal nucleus nervus vagus (Mann-staining cells 1–6 + 8), cells 7–10 from nucleus paraventricularis and substantia innominata (Mallory-staining). (Lewy 1912)

Lewy bodies



H&E

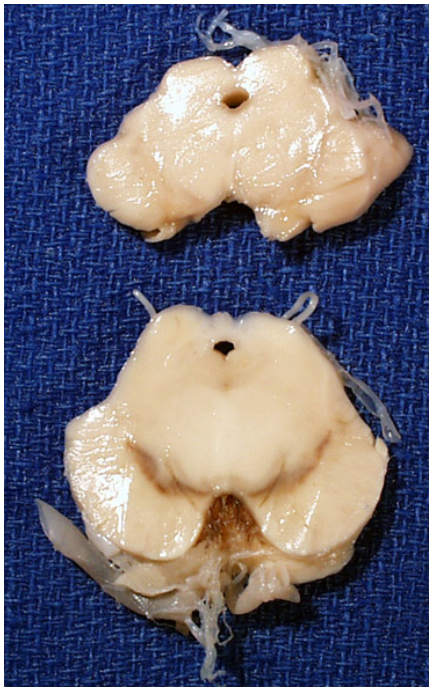


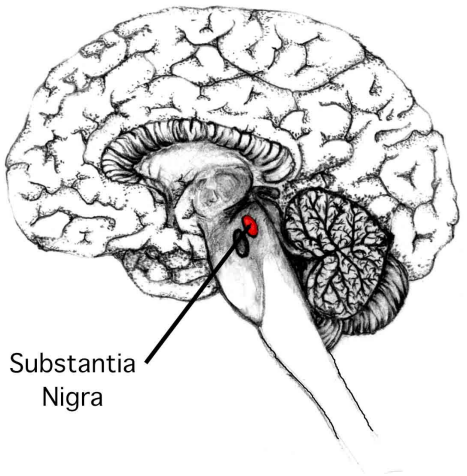
EM

Courtesy of Queen Square Brain Bank, National Hospital for Neurology and Neurosurgery

Pathological insights: Konstantin Trettiakof 1919

Konstantin Tretiakoff



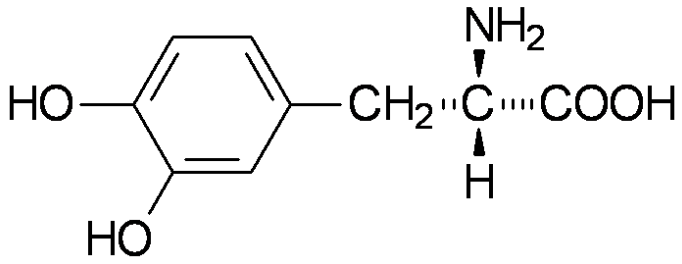


Tretiakof was first to recognise degeneration within substantia nigra and therefore link this anatomic structure with parkinsonism

Neuro-chemical basis to Parkinson's: dopamine

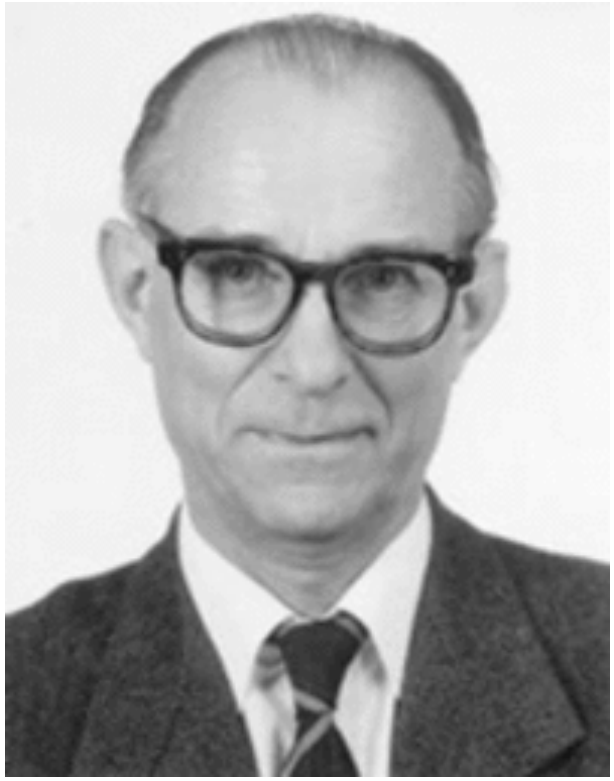
Arvid Carlsson

Nobel Prize in physiology and Medicine 2000

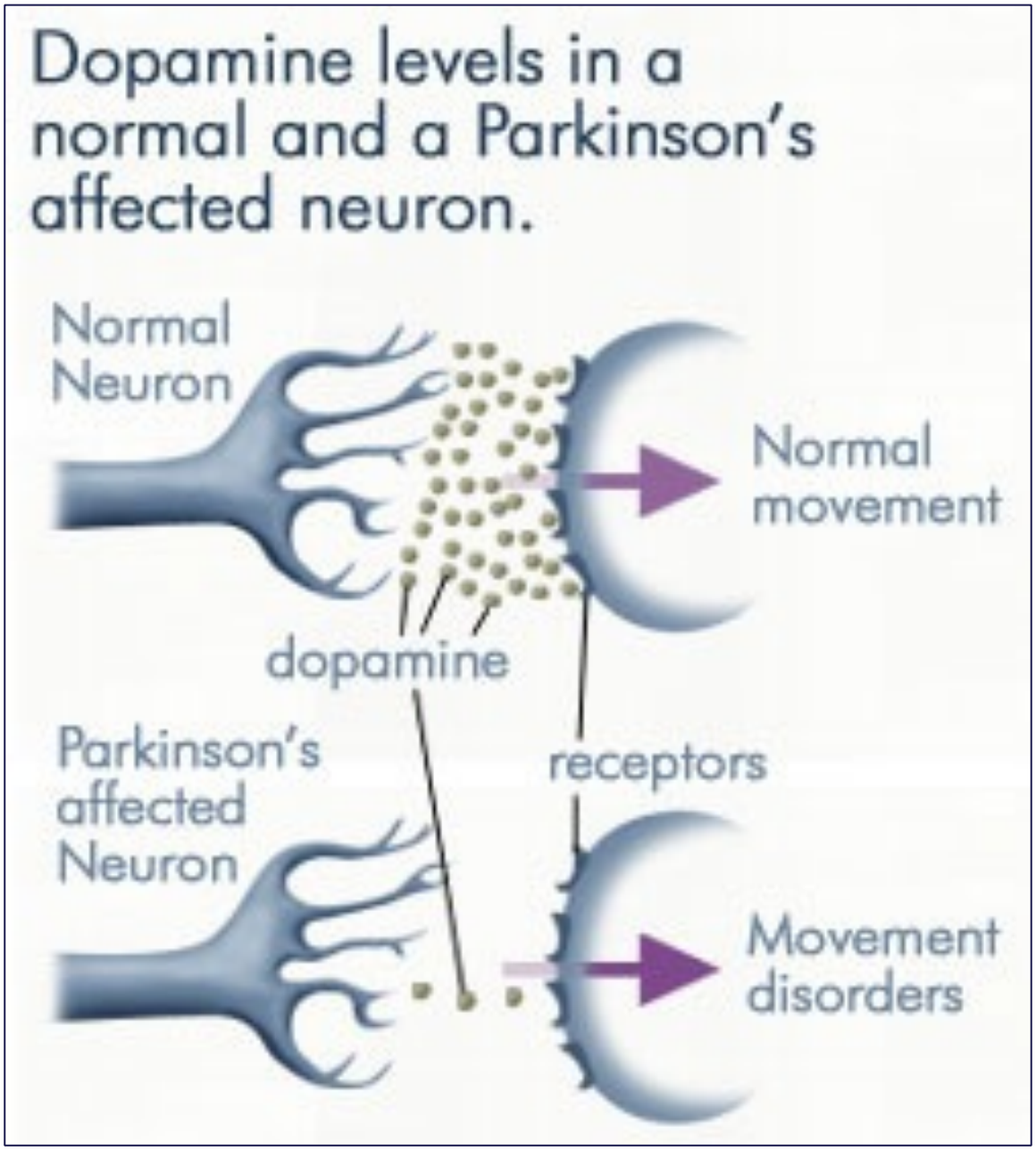


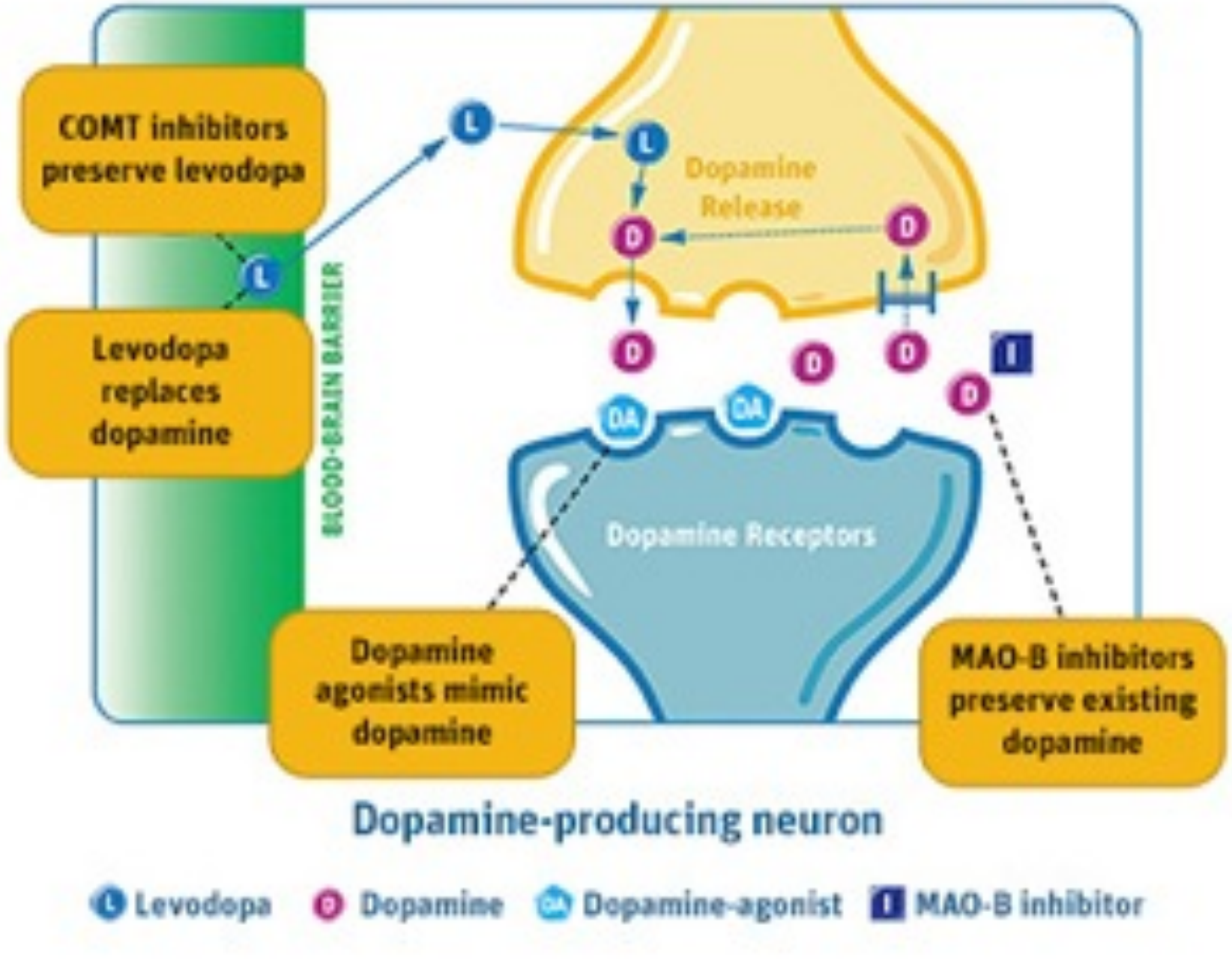
Chemical structure of 3-(3',4'-dihydroxyphenyl)-L-alanine (L-DOPA).

Discovery of dopamine deficiency: development of L-Dopa

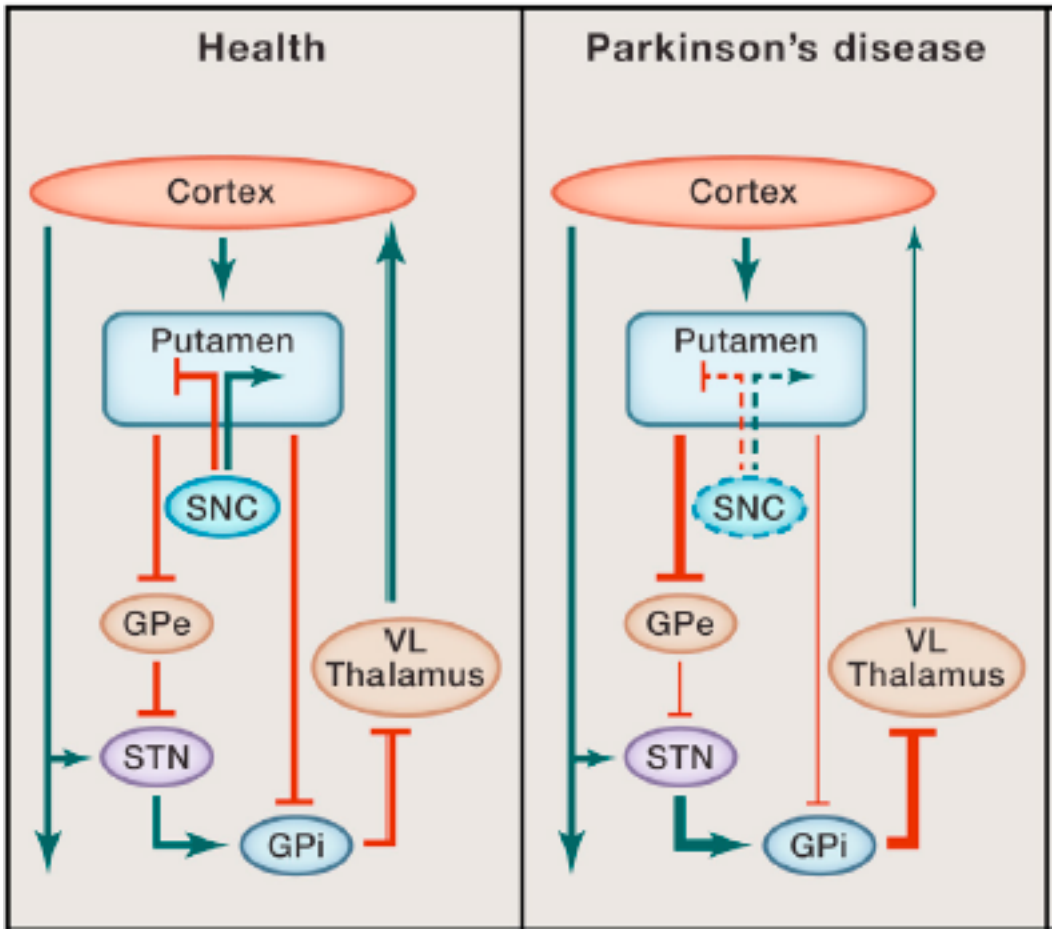


Oleh Hornykiewicz


Dopamine levels in a normal and a Parkinson's affected neuron.
Normal Neuron
Normal movement
dopamine
receptors
Parkinson's affected Neuron
Movement disorders


COMT inhibitors preserve levodopa
Levodopa replaces dopamine
BLOOD-BRAIN BARRIER
Dopamine Release
Dopamine Receptors
Dopamine agonists mimic dopamine
MAO-B inhibitors preserve existing dopamine
Dopamine-producing neuron
Levodopa
Dopamine
Dopamine-agonist
MAO-B inhibitor

Development of Deep Brain Stimulation for the treatment of Parkinson's disease



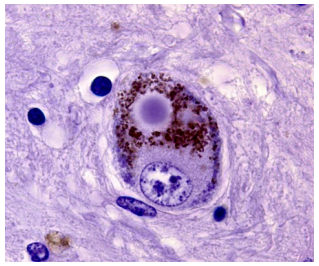
Adapted: Bergman, Wichmann, De Long. Science 1990 249: 1436-1438.

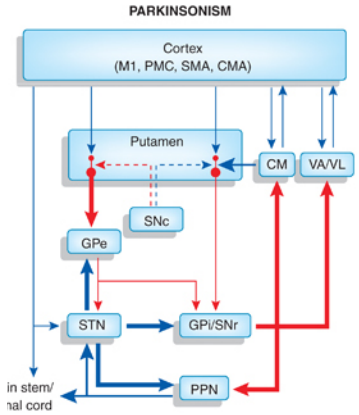
Mahon de Long

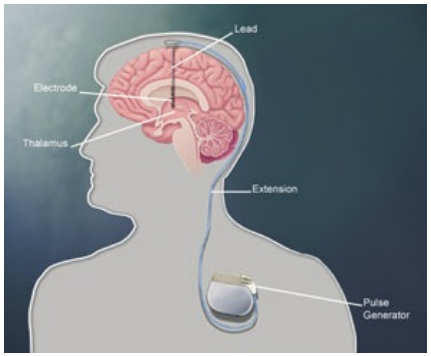
Alim Benabid

Lasker and Breakthrough prizes 2014

Decoding Parkinson's disease



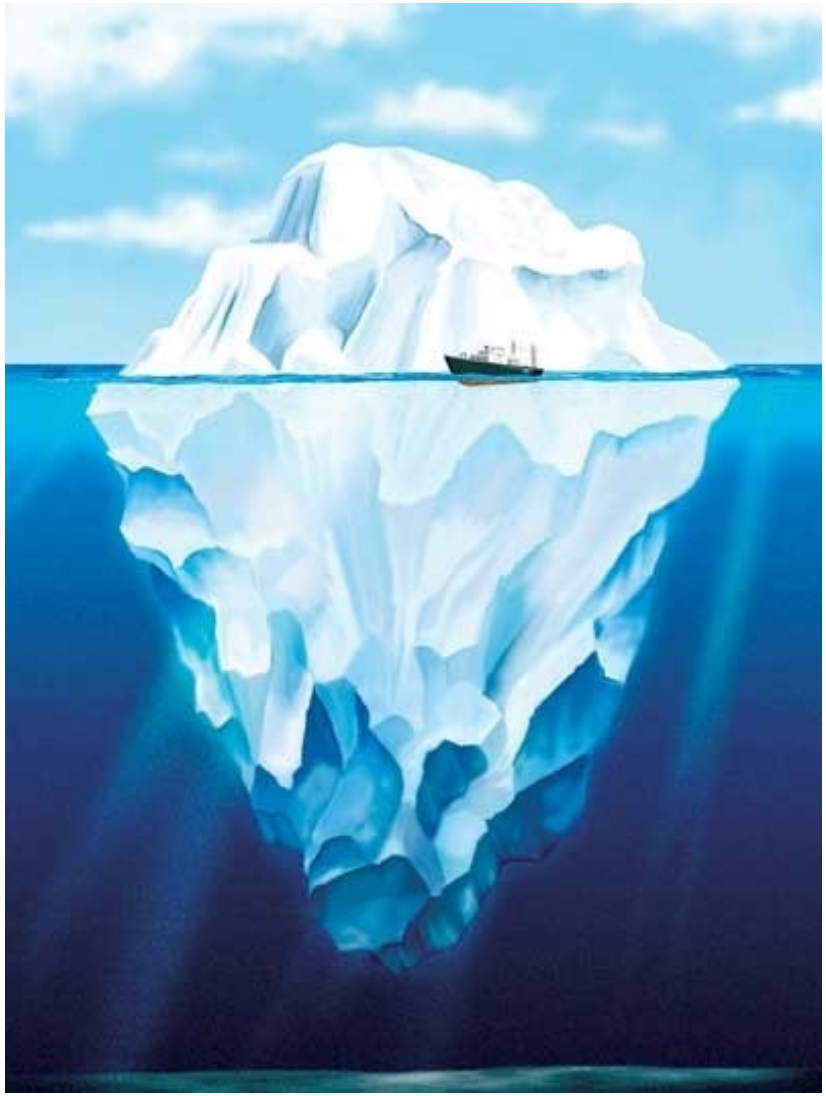




No Disease Modifying Therapy

Parkinson's Disease Overview

Elucidating the cause of Parkinson's disease – focusing on rare variants



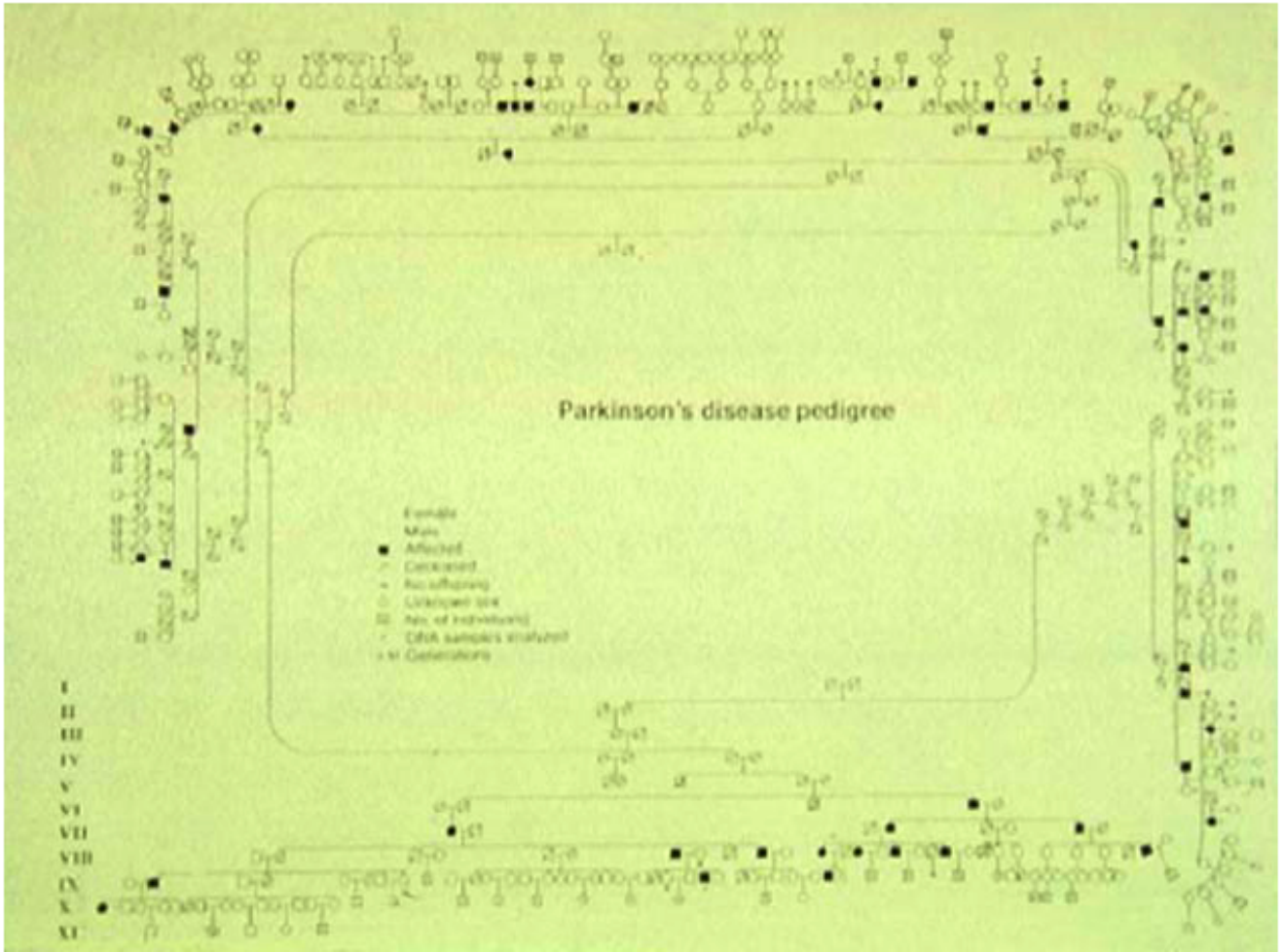
5% of cases familial

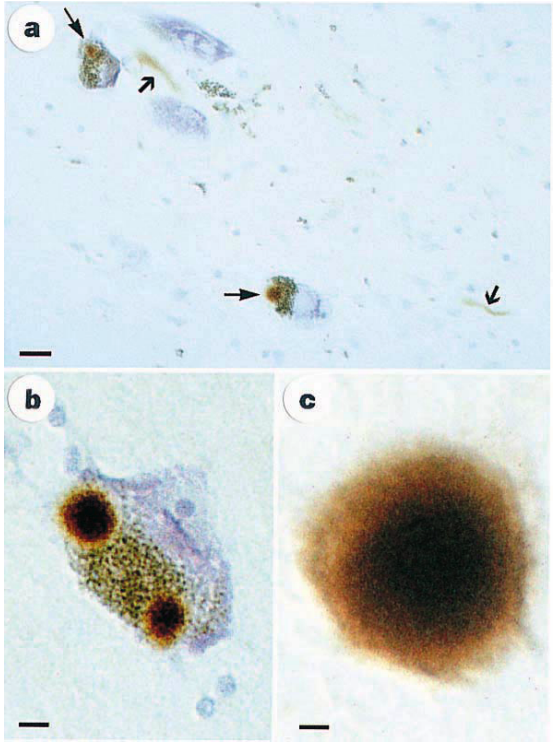
95% of cases sporadic

- Environmental toxin ?
- Infectious agent ?
- Age-dependent damage ?

Parkinson's disease genetic loci

LOCUS	MODE	TYPE	GENE	FUNCTION
PARK1/4	AD	LO/EOPD	α-synuclein	Unknown



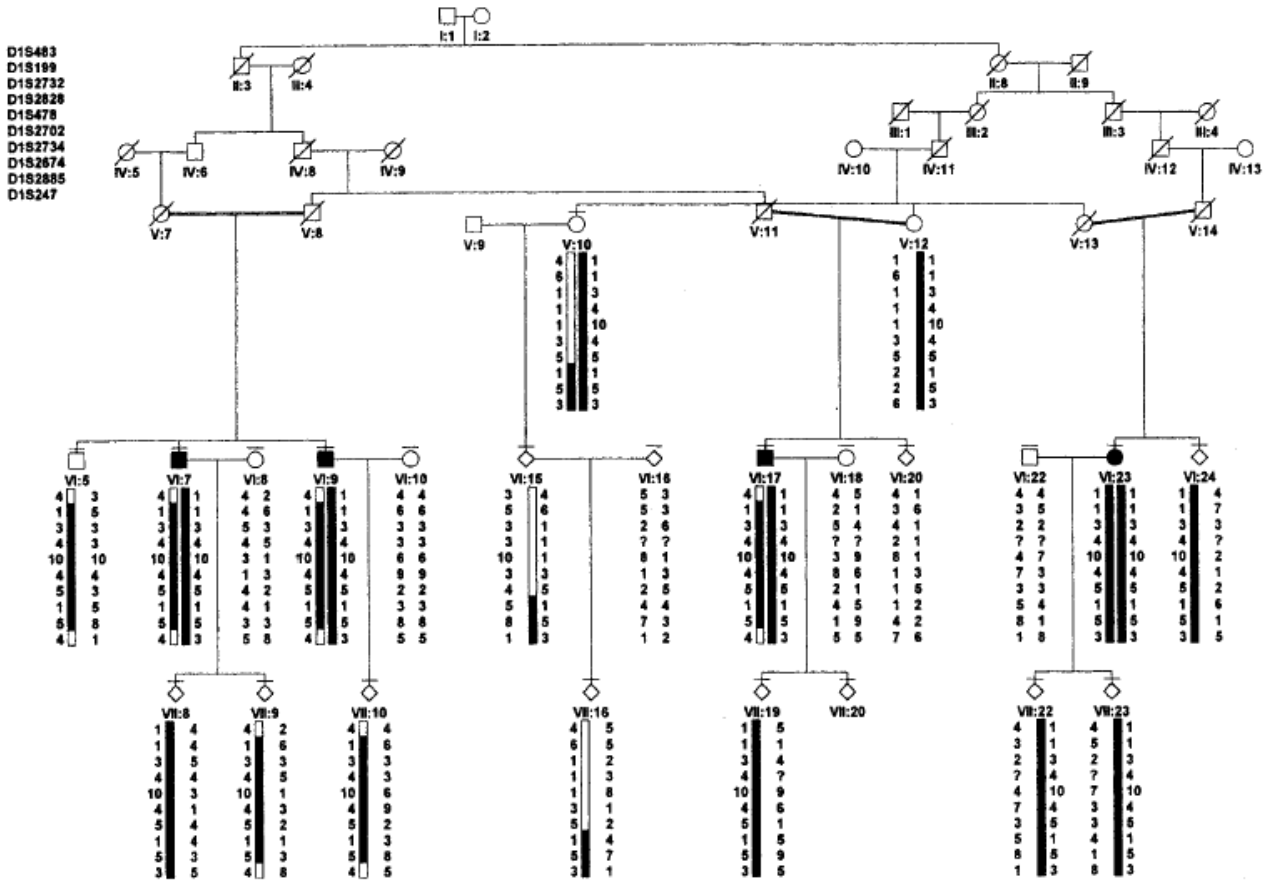


Parkinson's genes

LOCUS	MODE	TYPE	GENE	FUNCTION
PARK1/4	AD	LO/EOPD	α-synuclein	Unknown
PARK2	**AR**	**EOPD**	**Parkin**	**Ubiquitin ligase**
PARK3	AD	LOPD	Unknown	
PARK5	AD	LOPD	UCH-L1	DUB
PARK6	**AR**	**EOPD**	**PINK1**	**Kinase**
PARK7	AR	EOPD	DJ-1	Oxidative chaperone
PARK8	**AD**	**LOPD**	**LRRK2**	**Kinase**
PARK9	AR	EOPD*	ATP13A2	ATPase
PARK10	Complex	LOPD	Unknown	
PARK11	Complex	LOPD	*GIGYF2* (controversial)	
PARK12	X-linked	LOPD	Unknown	
PARK13	AD	LOPD	HtrA2/Omi	Serine protease
PARK14	AR	EOPD*	PLA2G6	Phospholipase A2
PARK15	AR	EOPD*	FBXO7	F Box protein
PARK16	Complex	LOPD	Unknown	
PARK17	Complex	LOPD	GAK	Kinase
PARK18	Complex	LOPD	HLA	immune recognition
PARK19	AD	LOPD	VPS35	endosomal-Golgi trafficking
PARK20	AD	LOPD	EIF4G1	mRNA translation-initiation

* Complex syndrome

“Marsala Kindred” genetically links to chromosome 1p35-36



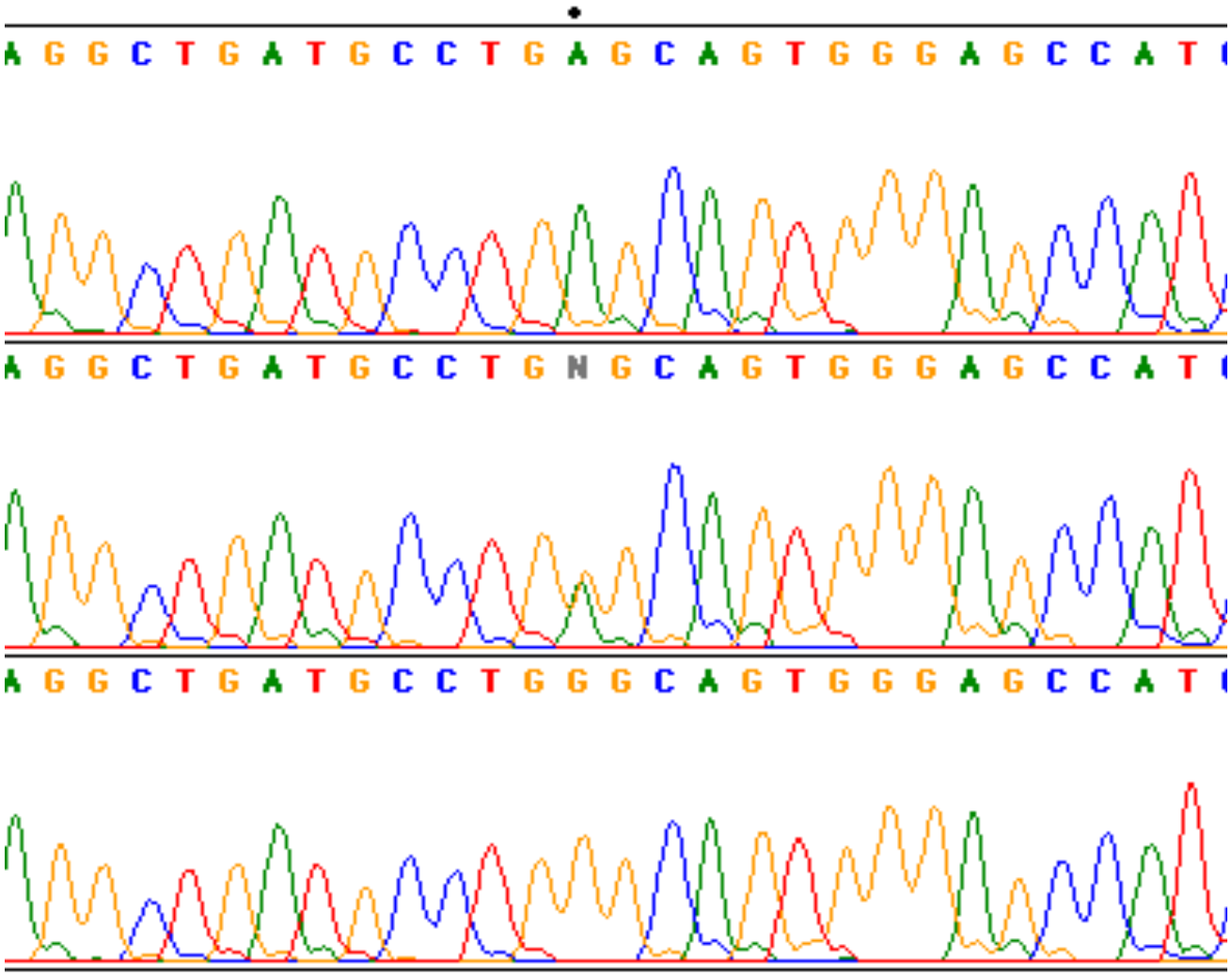
Autosomal recessive PD
Early-onset (age at onset 32-47)
Slowly progressive
Sustained response to Levodopa

Discovery in 2004 that mutations in PINK1 gene cause Parkinson's disease

Valente

Wood

Muqit

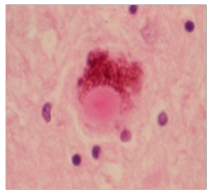


Clinical Era

1817

James Parkinson describes ‘shaking palsy’

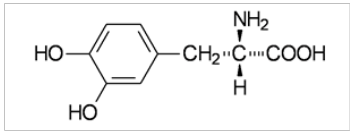
Pathology Era



1912

Lewy discovers inclusions

Treatment Era



1967

L-Dopa introduced into practice

1980s

Deep Brain Stimulation surgery

Genetic Era

1997

Discovery of gene mutations in families with Parkinson’s

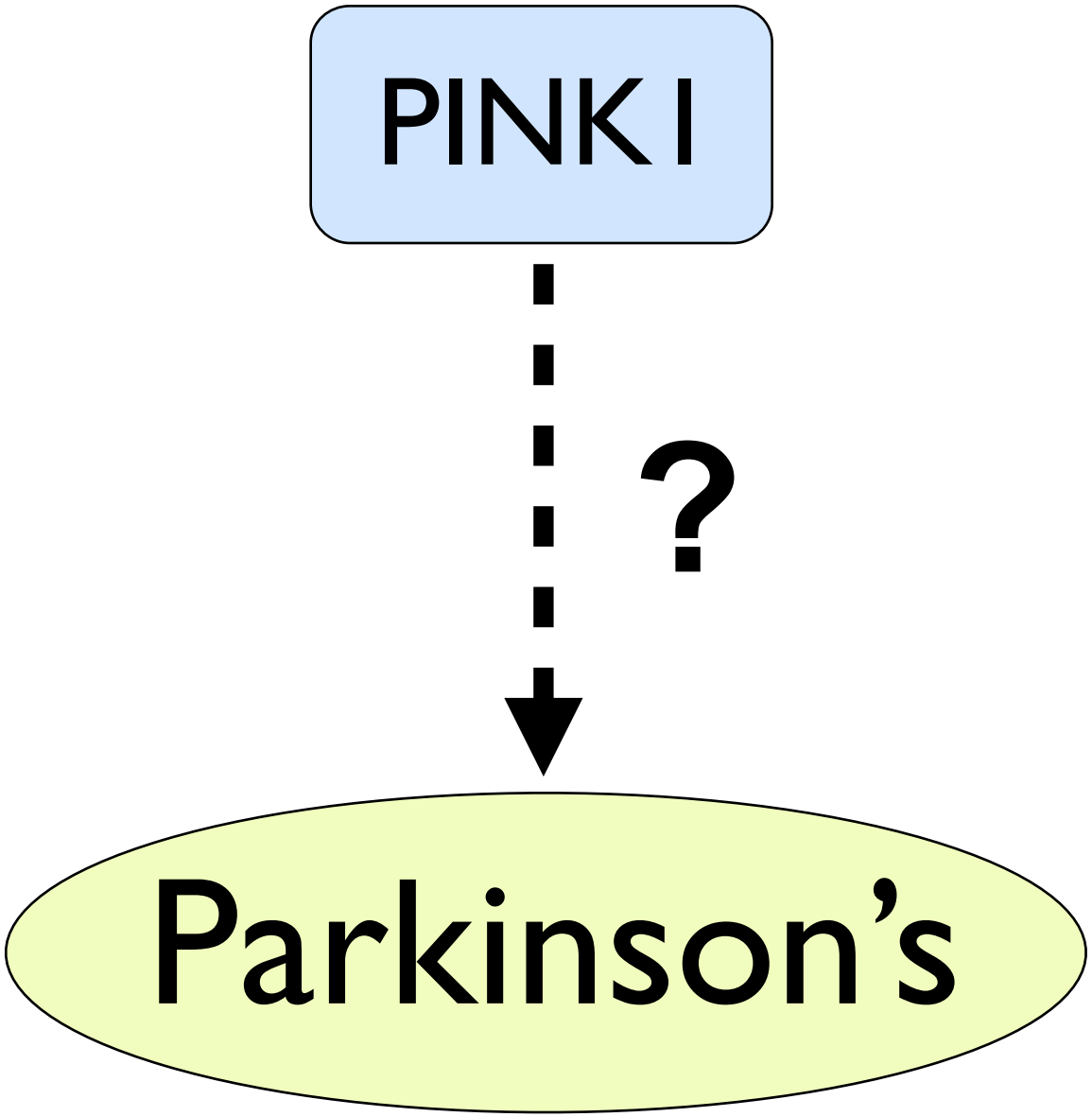
2004

Biochemical Era

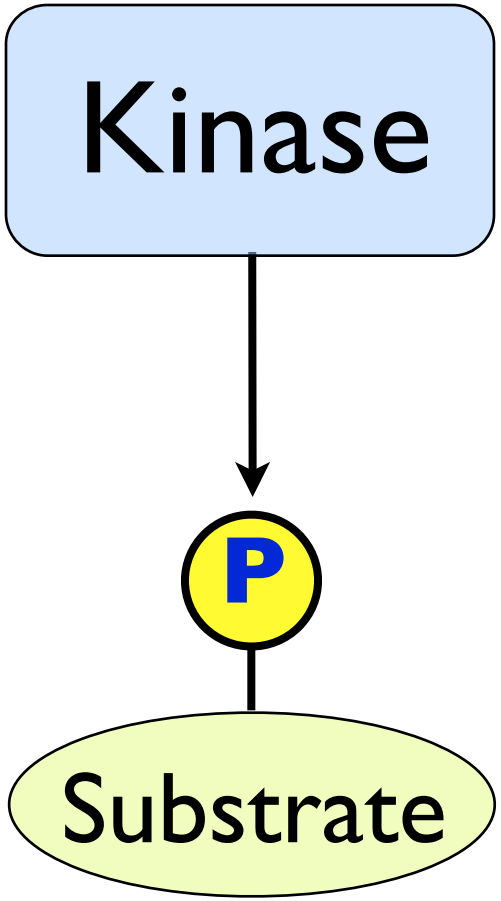
Present day

Understanding function of genes

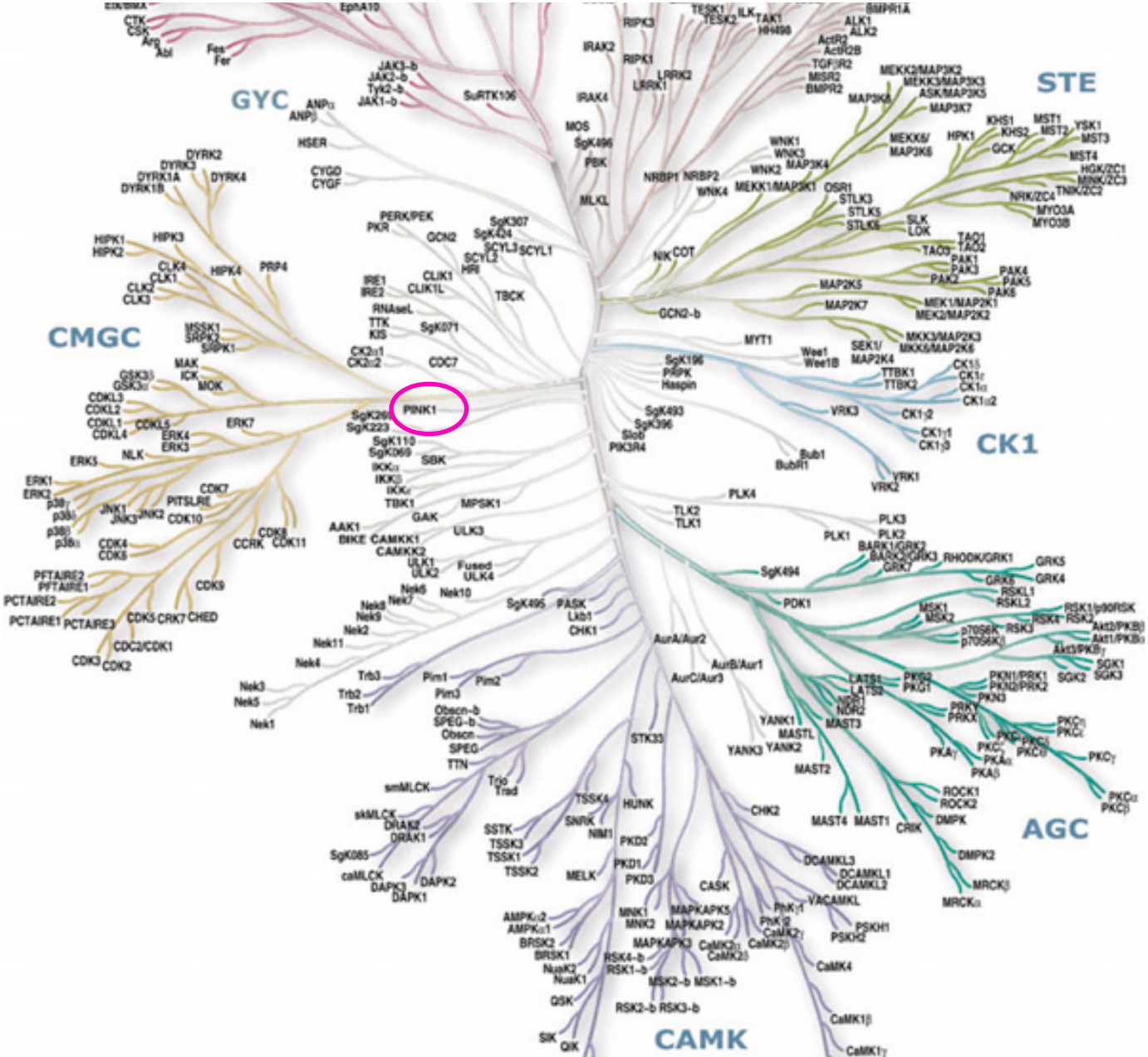
PINK1 Research Question



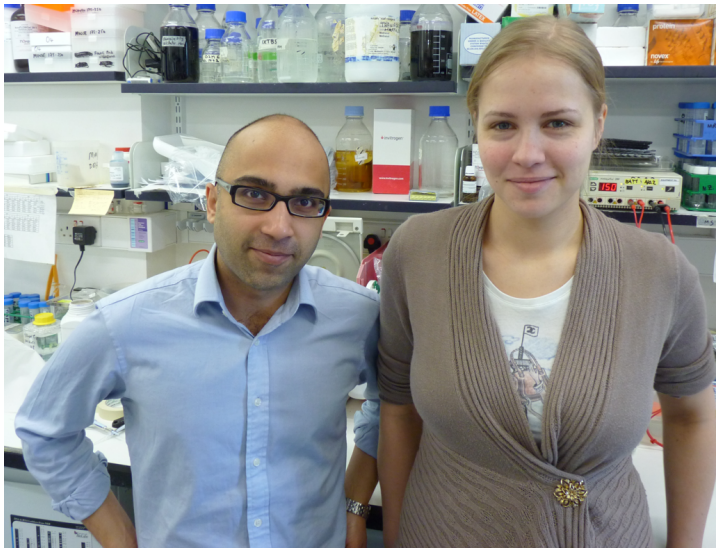
Protein Phosphorylation



Human kinases in the genome



PINK1



Miratul Muqit

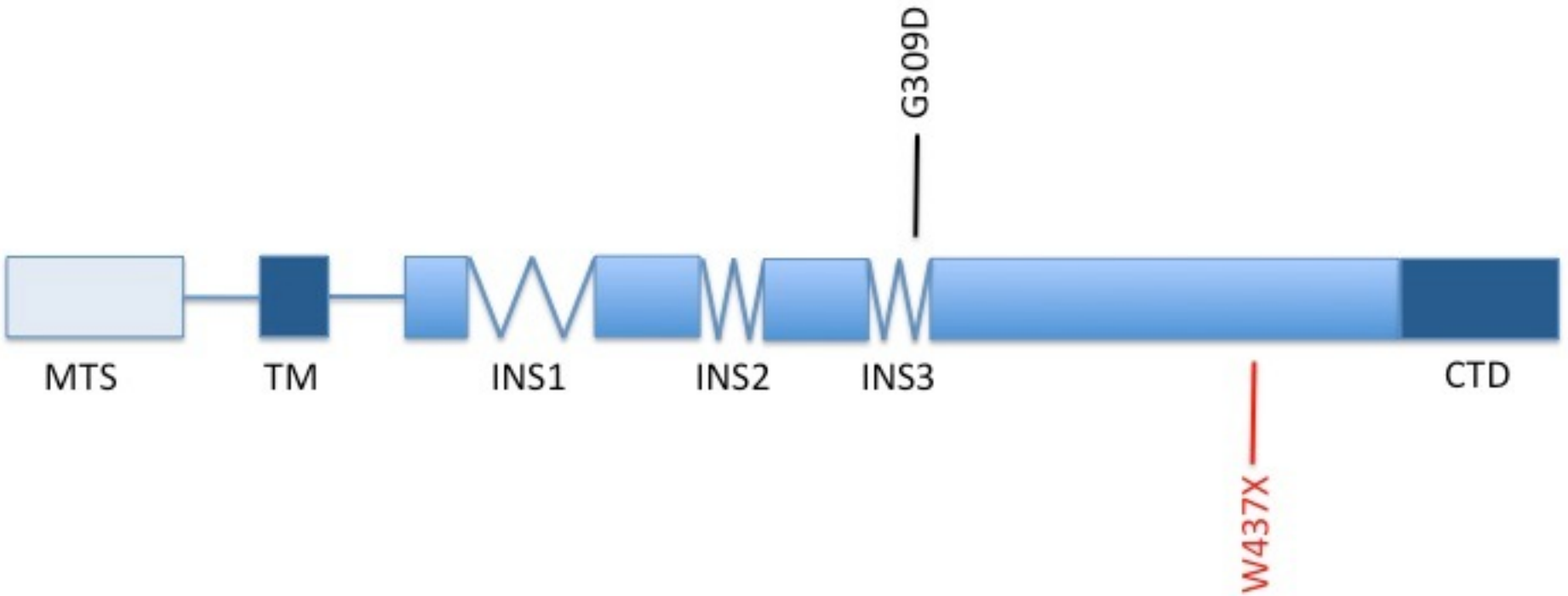
Agne Kazlauskaite

Chandana Kondapalli

Matthias Trost

Axel Knebel

Human Mutations in the PINK1 kinase



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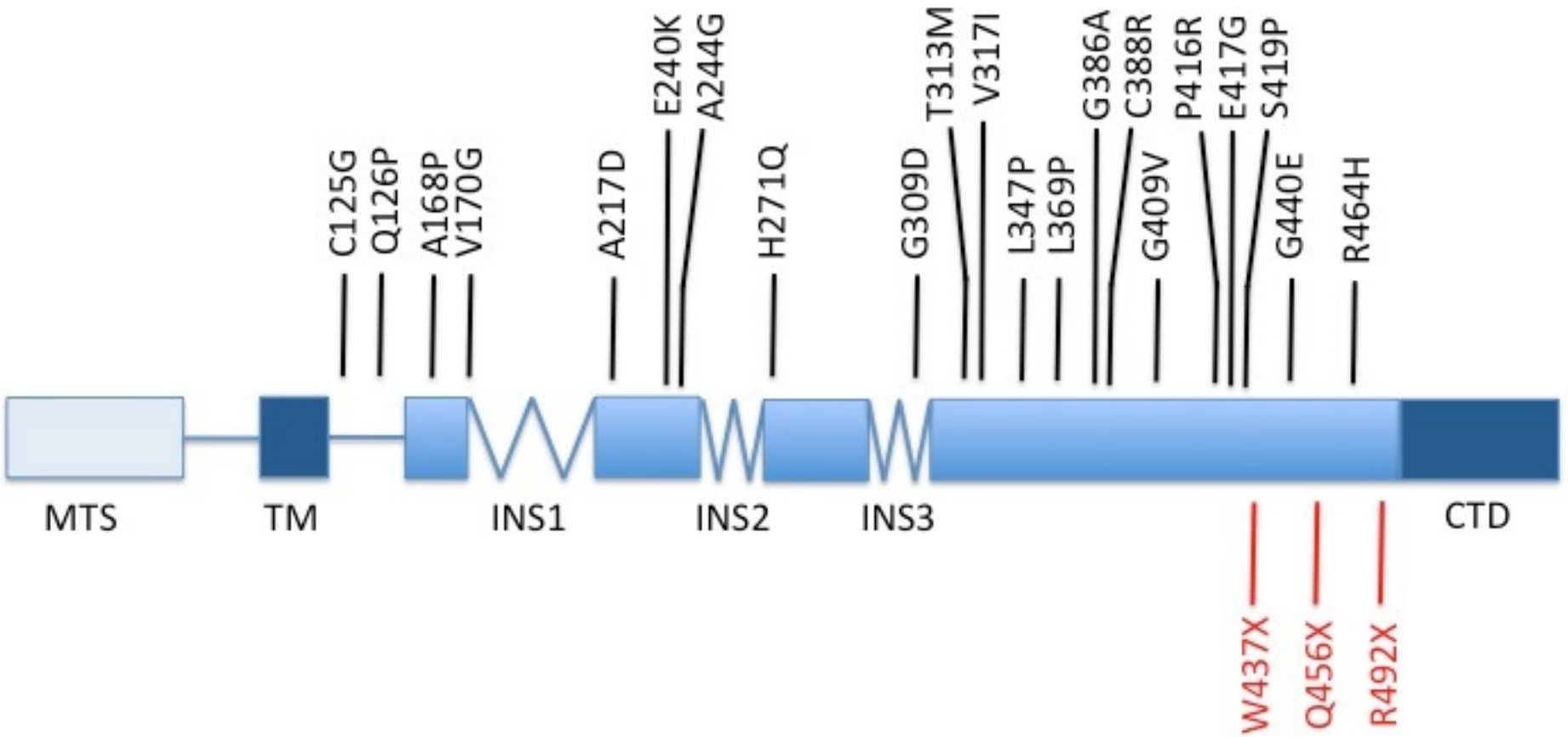
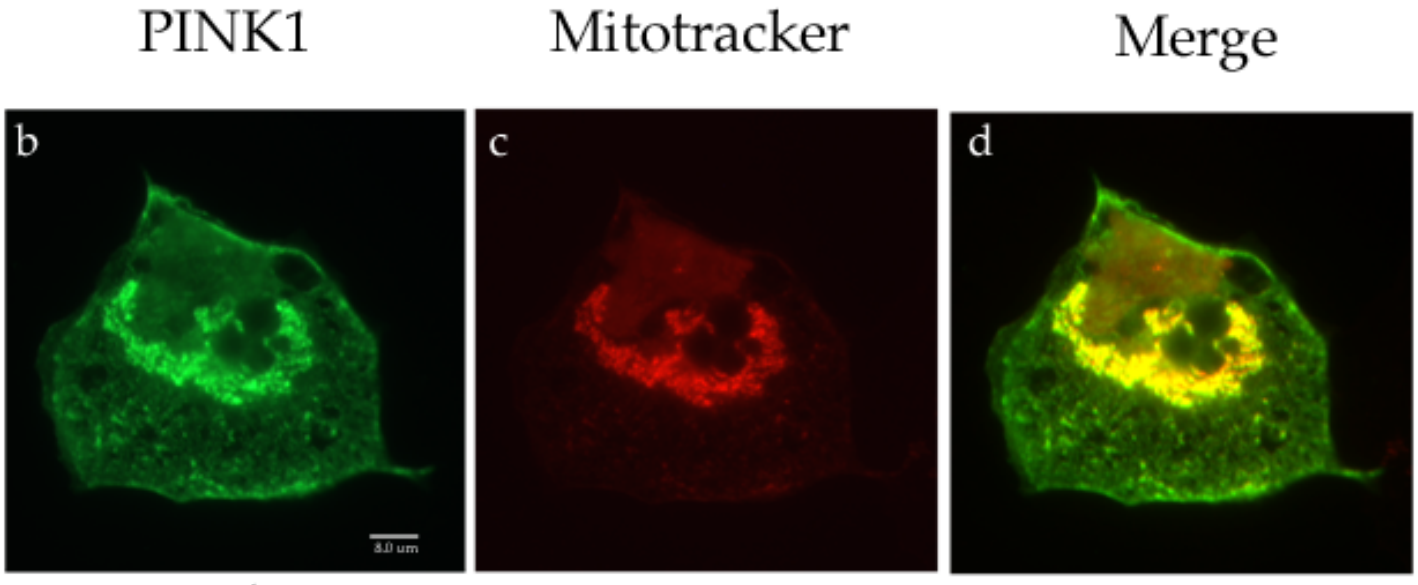
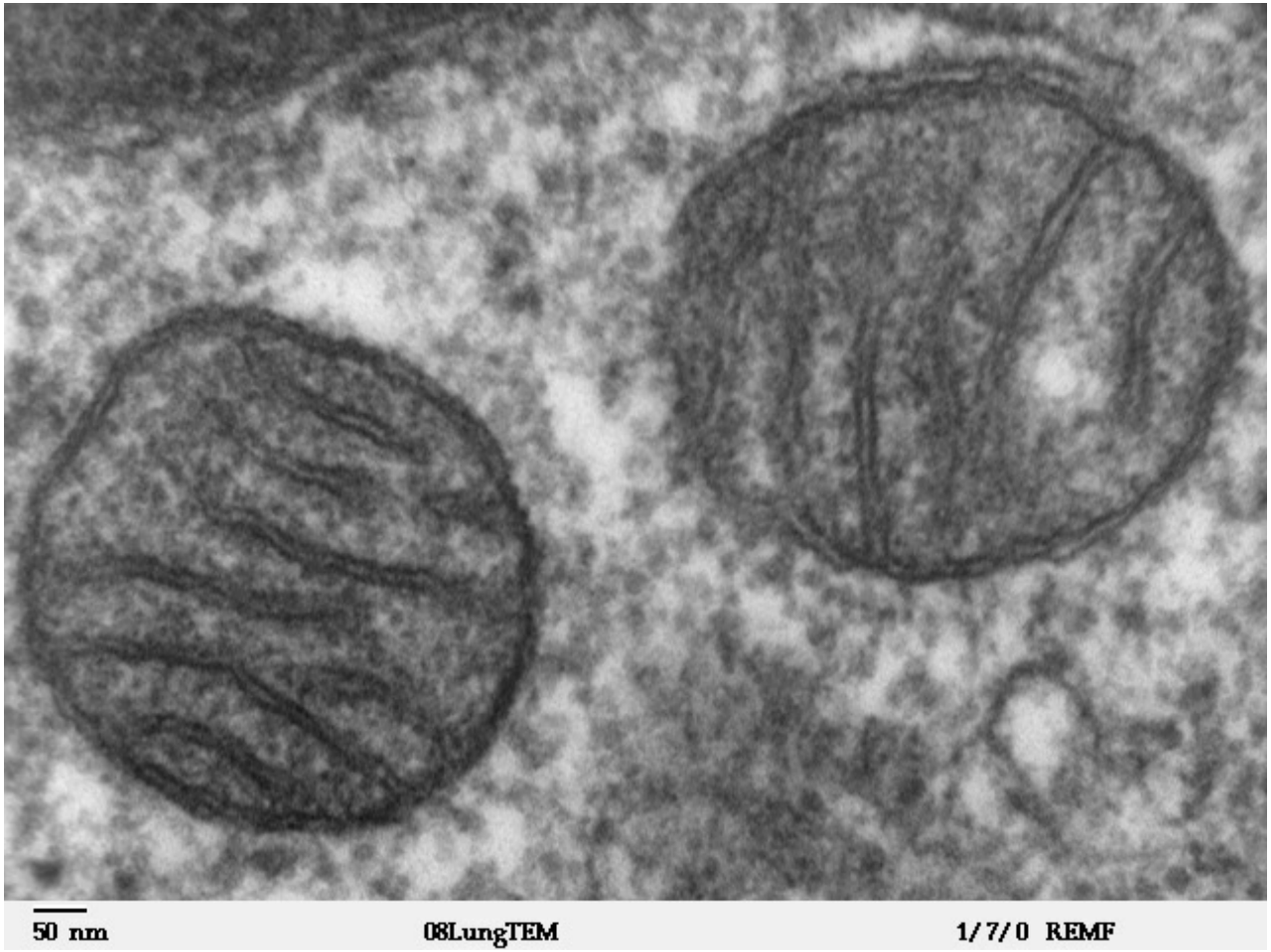


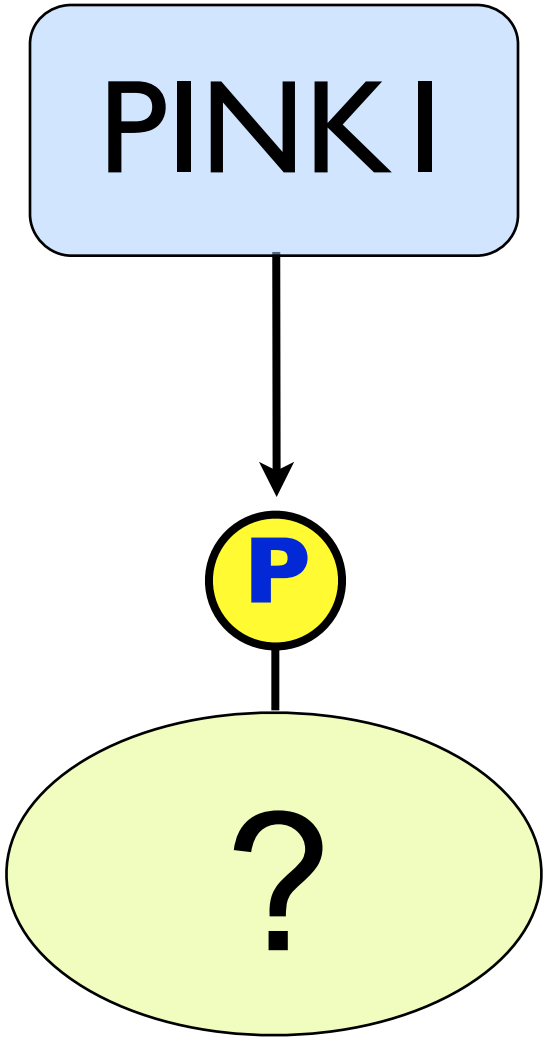
PINK1 is present in cellular power generators called mitochondria



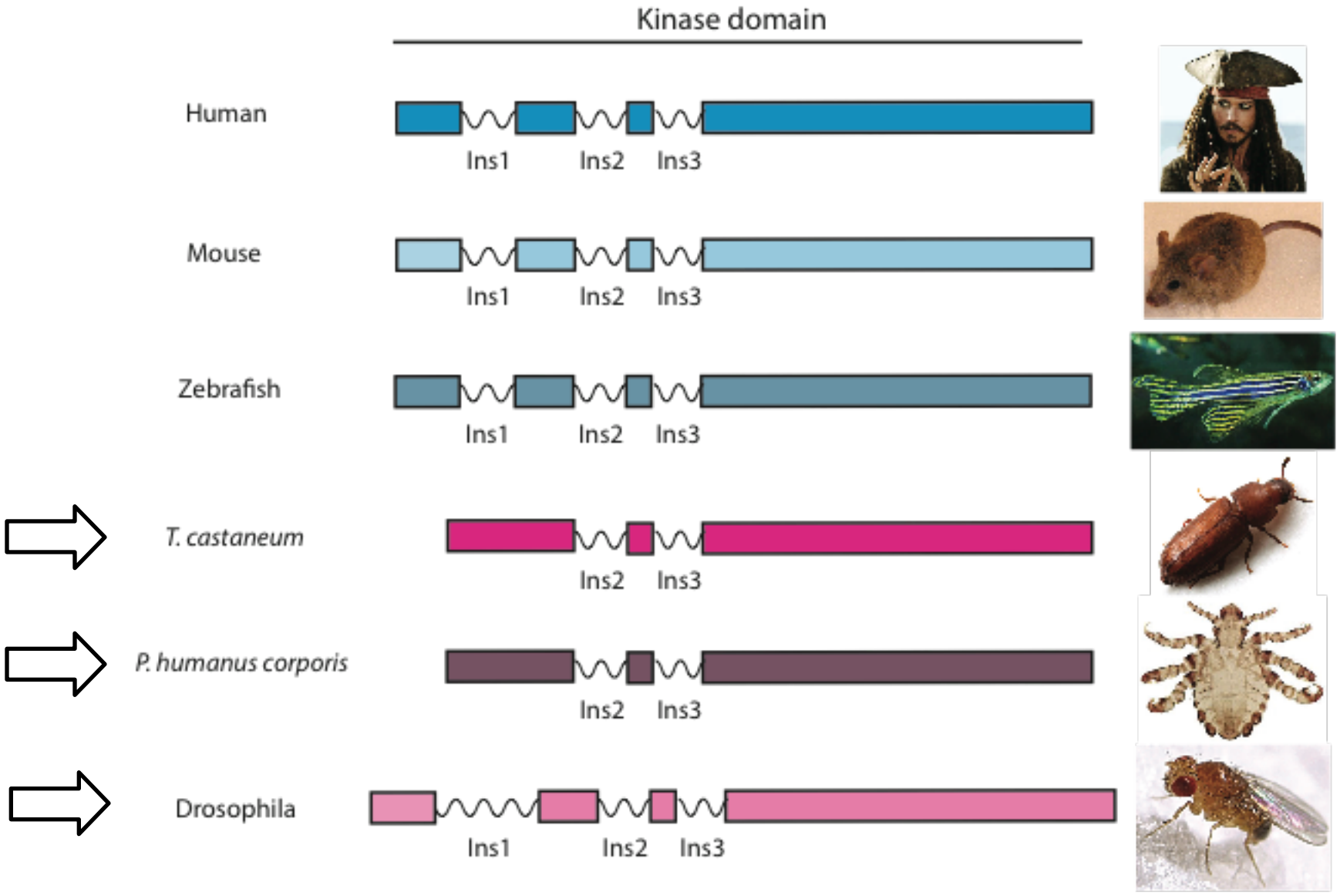
Mitochondria



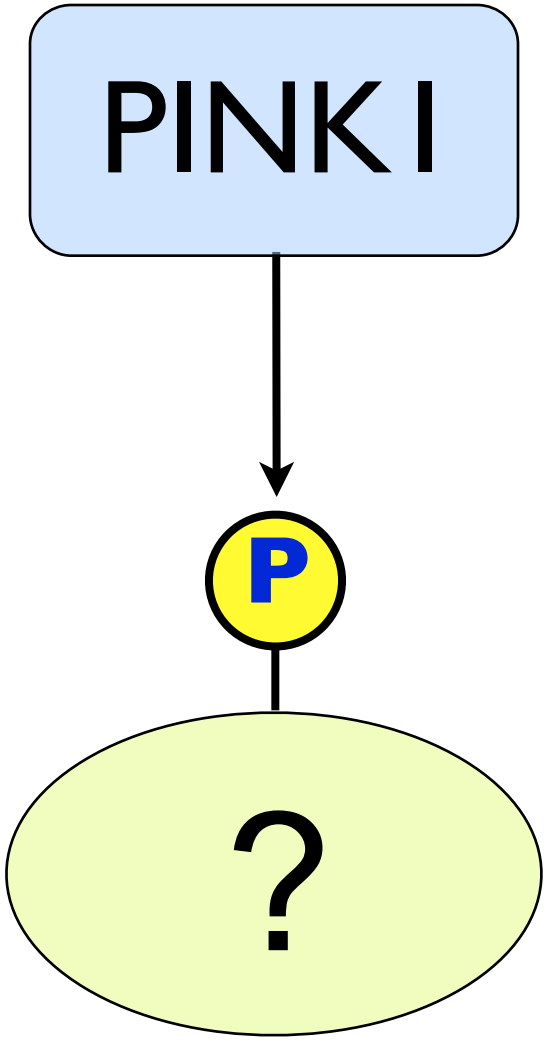
PINK1 is a Kinase: what does it act on?



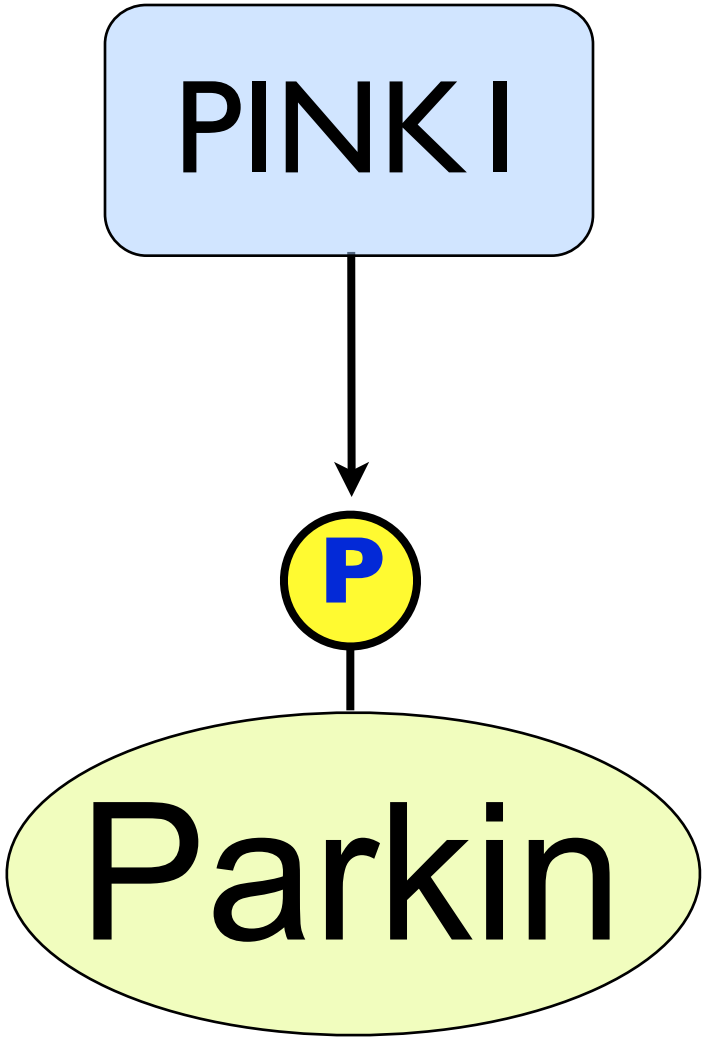
PINK1 orthologues



PINK1 is a Kinase: what does it act on?



2012 breakthrough we discovered that PINK1 acts on Parkin



Parkinson's genes

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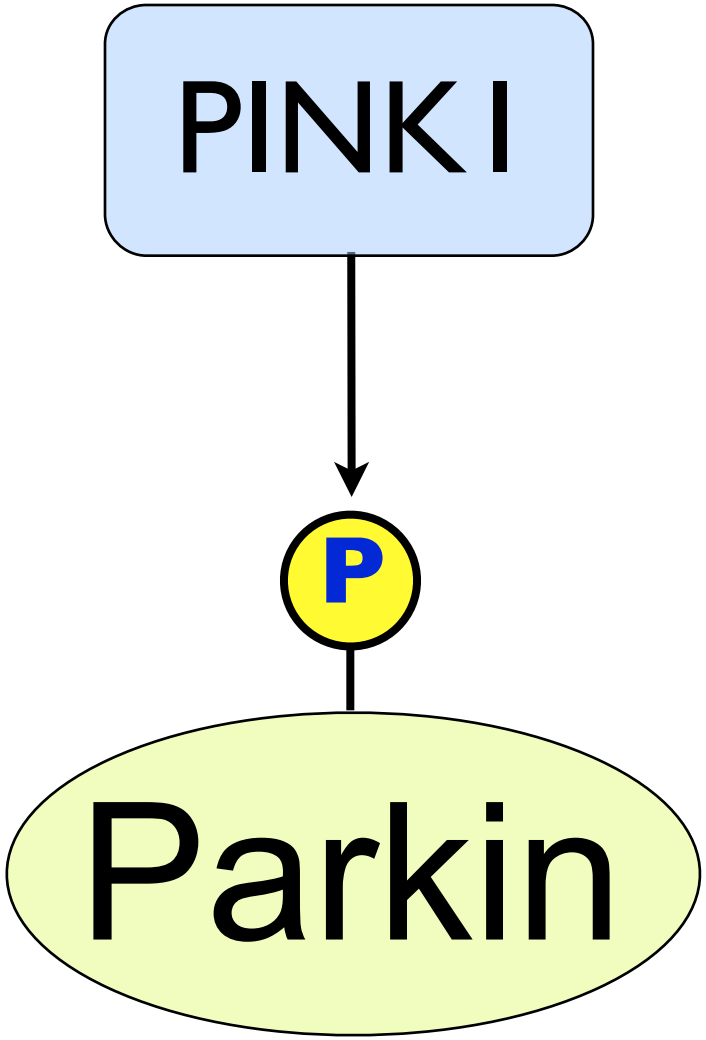
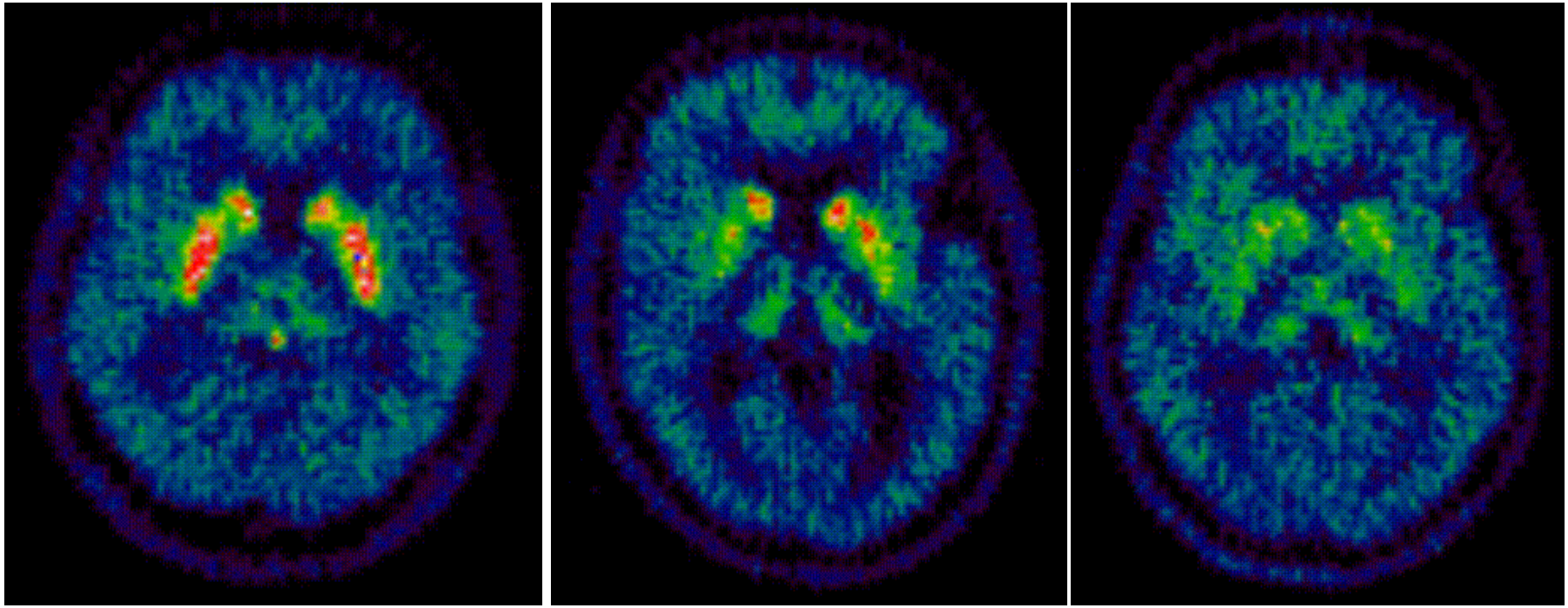
* Complex syndrome

PINK1 is a Kinase: what does it act on?



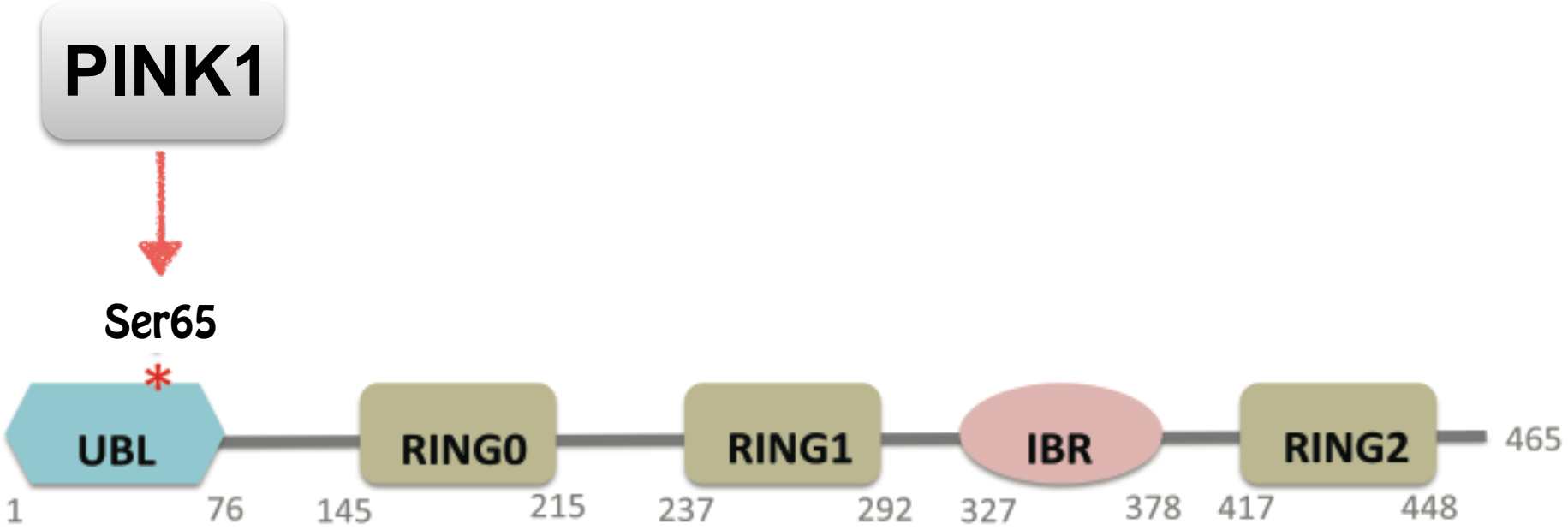
Parkin and PINK1 patients clinically similar



Normal | Sporadic PD | YOPD

Patients with Parkin and PINK1 mutations have similar phenotype suggesting that they might function in a similar pathway

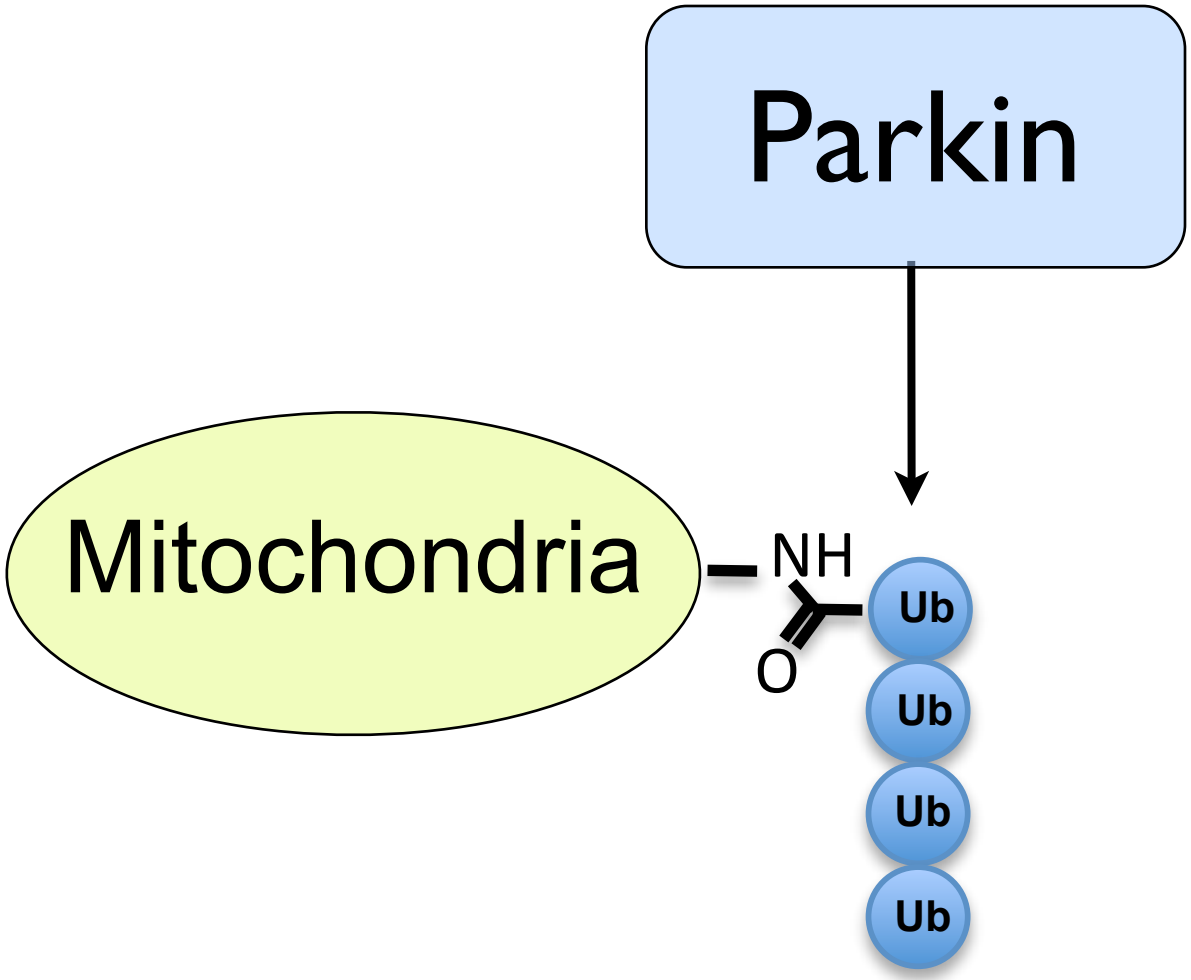
PINK1 activates Parkin by phosphorylating Parkin at Ser^{65} a highly conserved residue in the Ubl domain



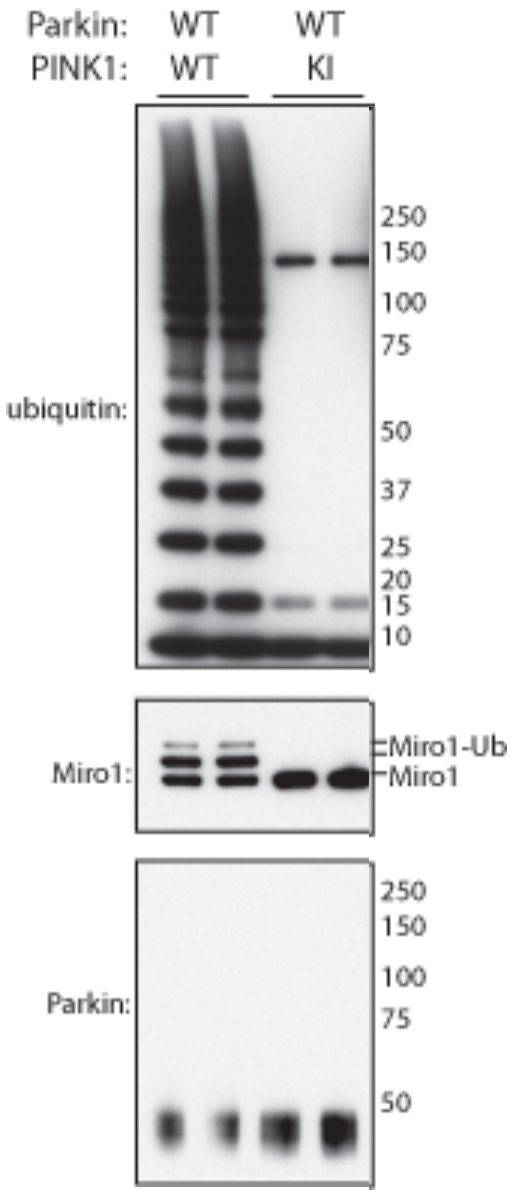
Miratul Muqit, Chanda Kondapalli, Agne

Parkin is regulator of protein ubiquitylation

Protein Ubiquitylation



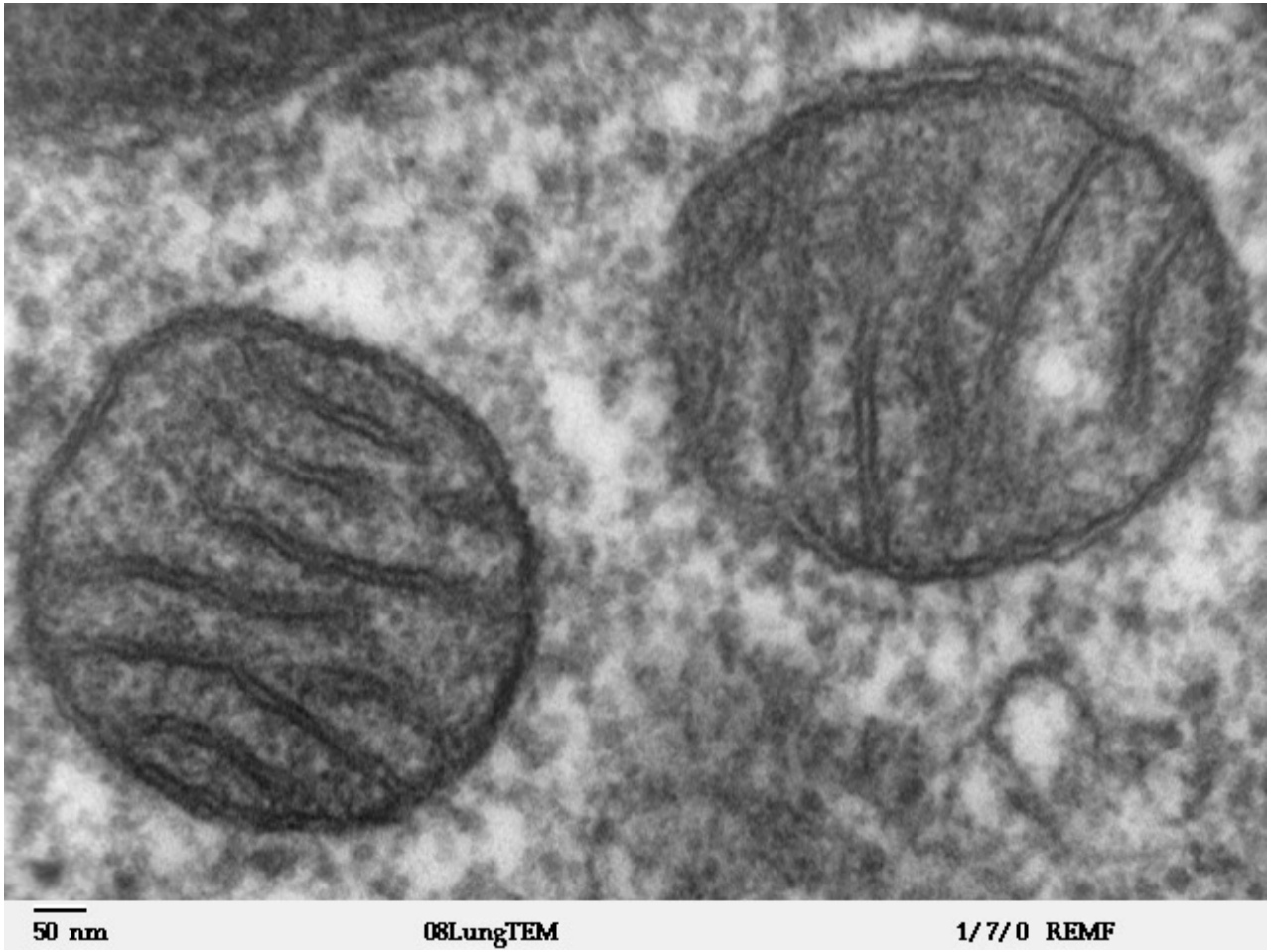
PINK1 activates Parkin



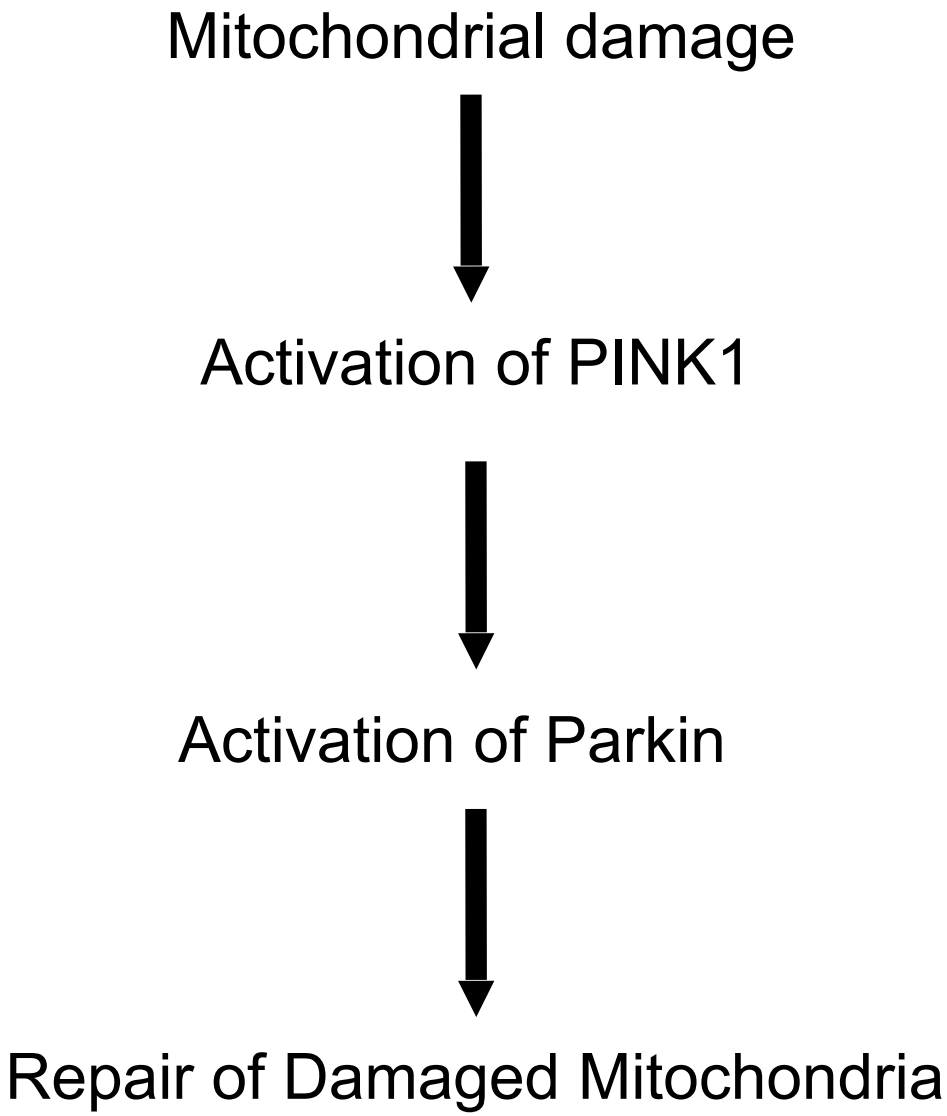
Miratul Muqit

Agne Kazlauskaite

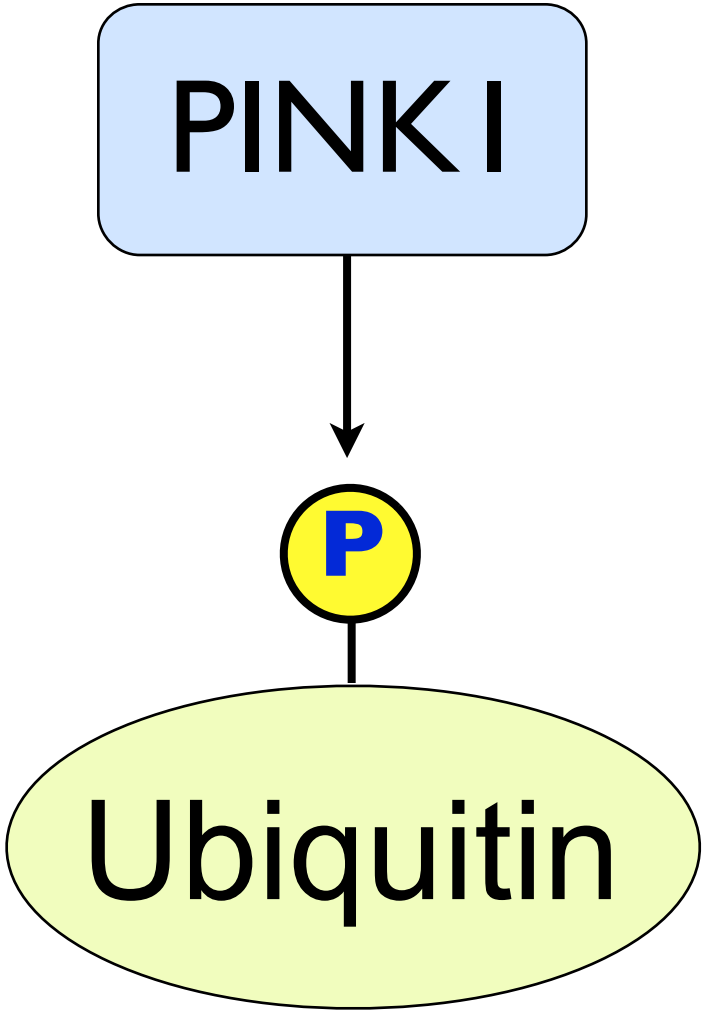
Key role of the PINK1 Parkin pathway is to keep cells healthy by removing damaged mitochondria



PINK1/Parkin signaling pathway

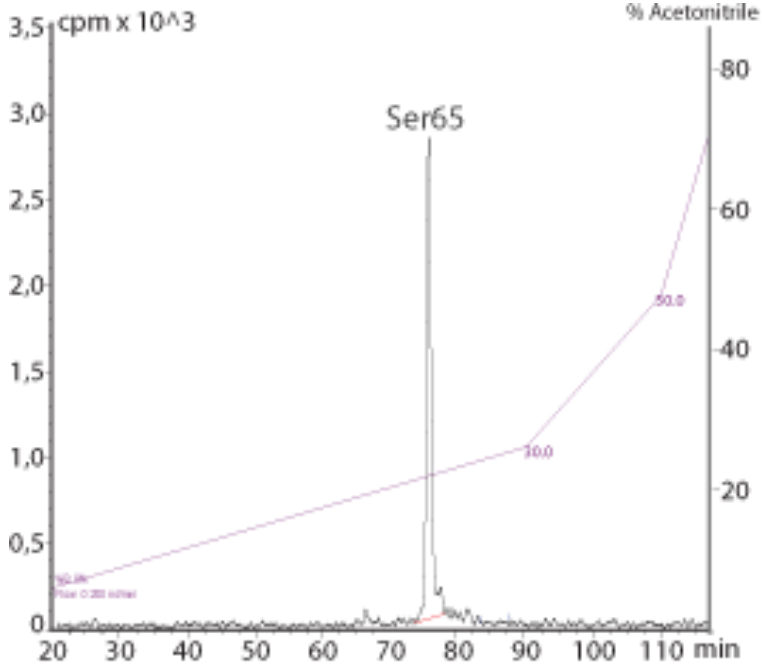


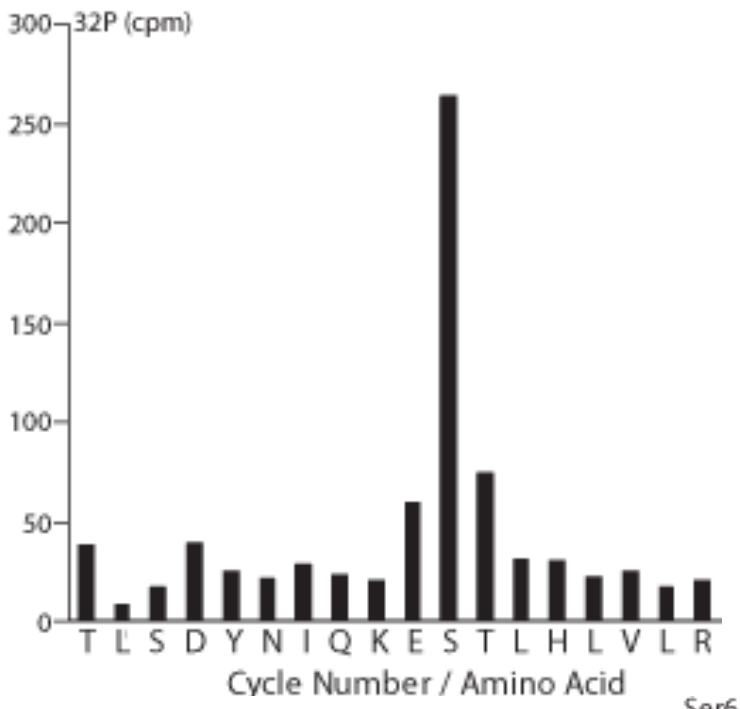
2014 we made a major breakthrough finding that PINK1 also generated a new Parkinson's chemical called phospho-ubiquitin

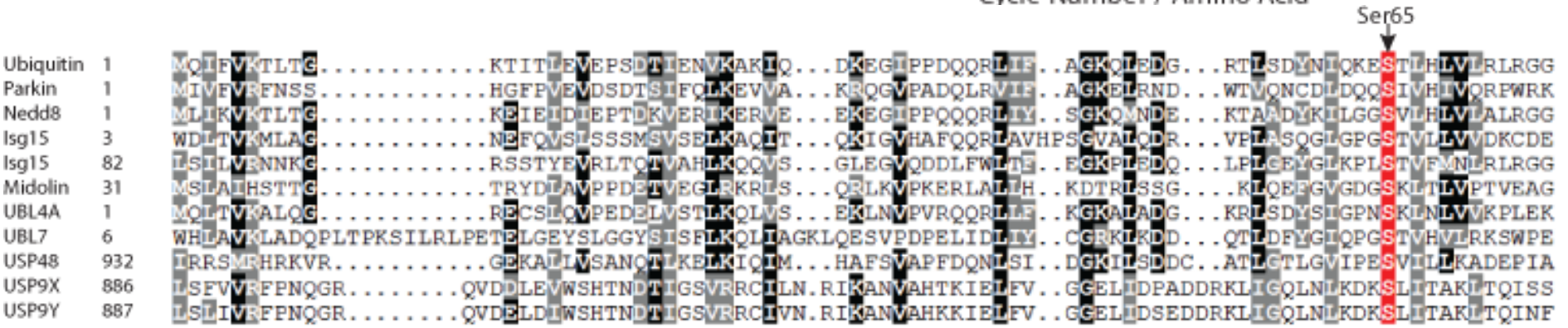


phospho-ubiquitin

PINK1 phosphorylates Ubiquitin specifically at Ser65

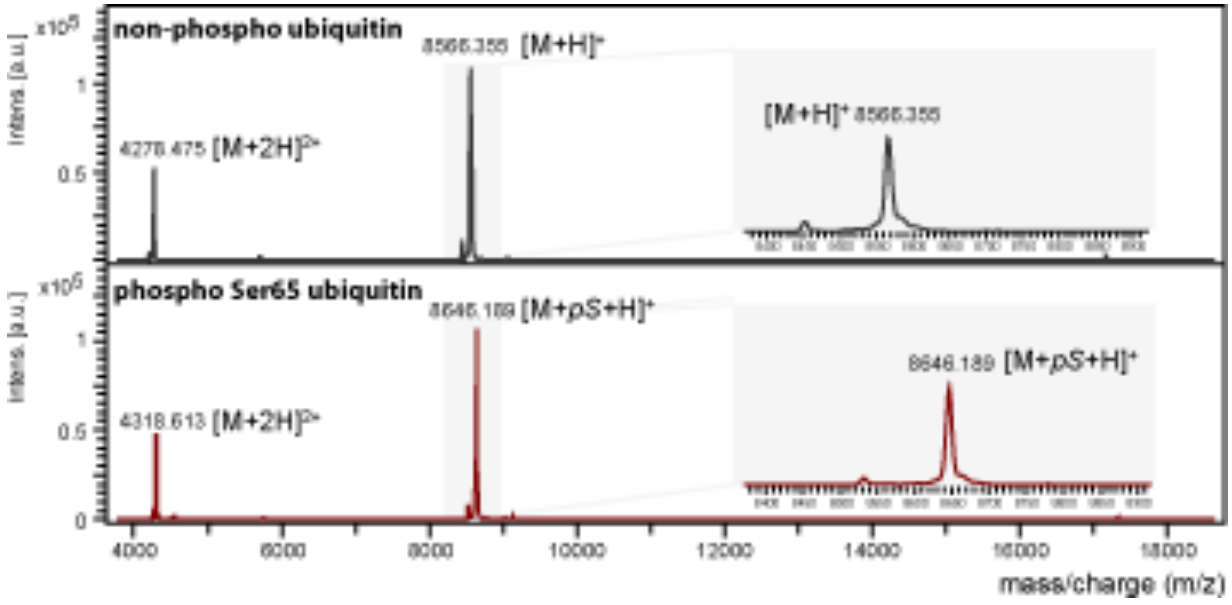


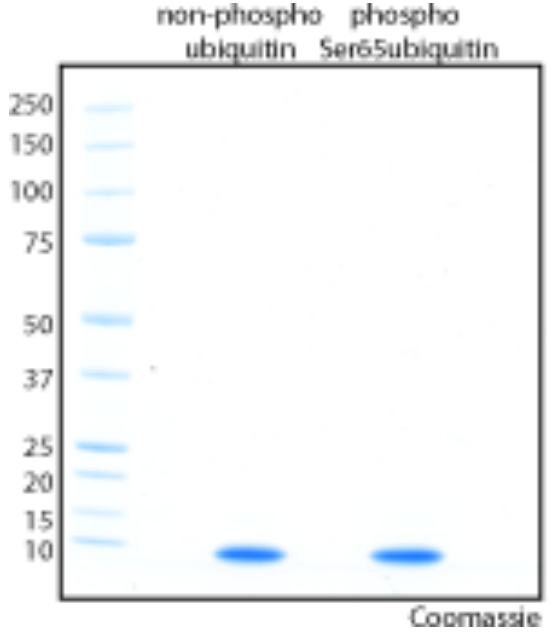




Agne Kazlauskaite, Miratul Muqit, David Campbell and Kay Hofmann

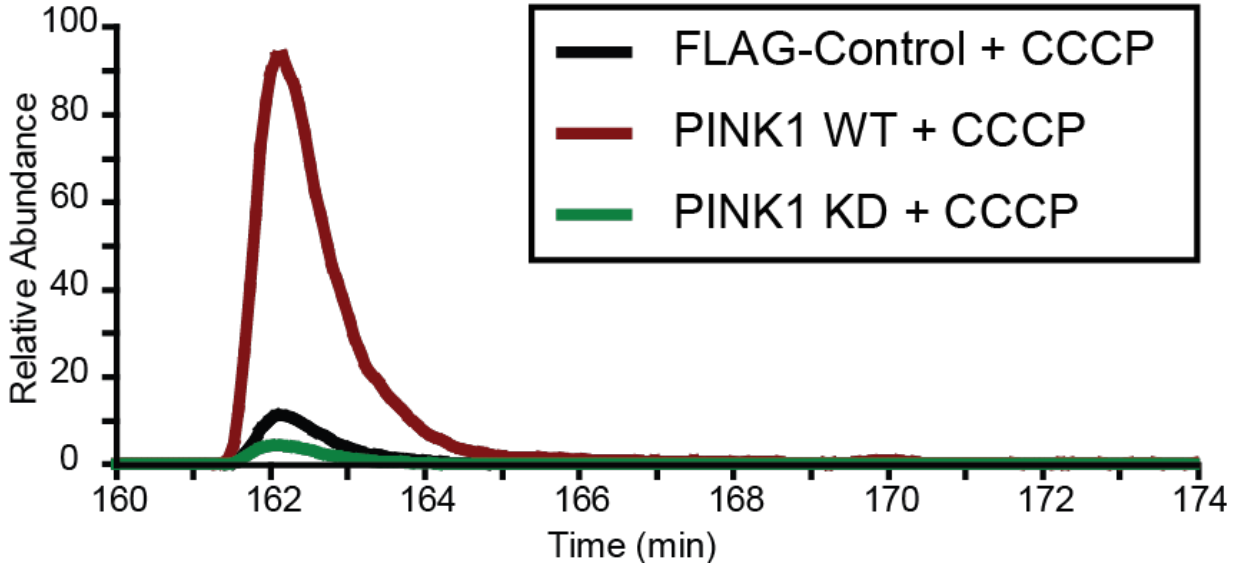
Generation of homogeneous stoichiometrically phosphorylated Ser65 phosphorylated ubiquitin





Axel Knebel

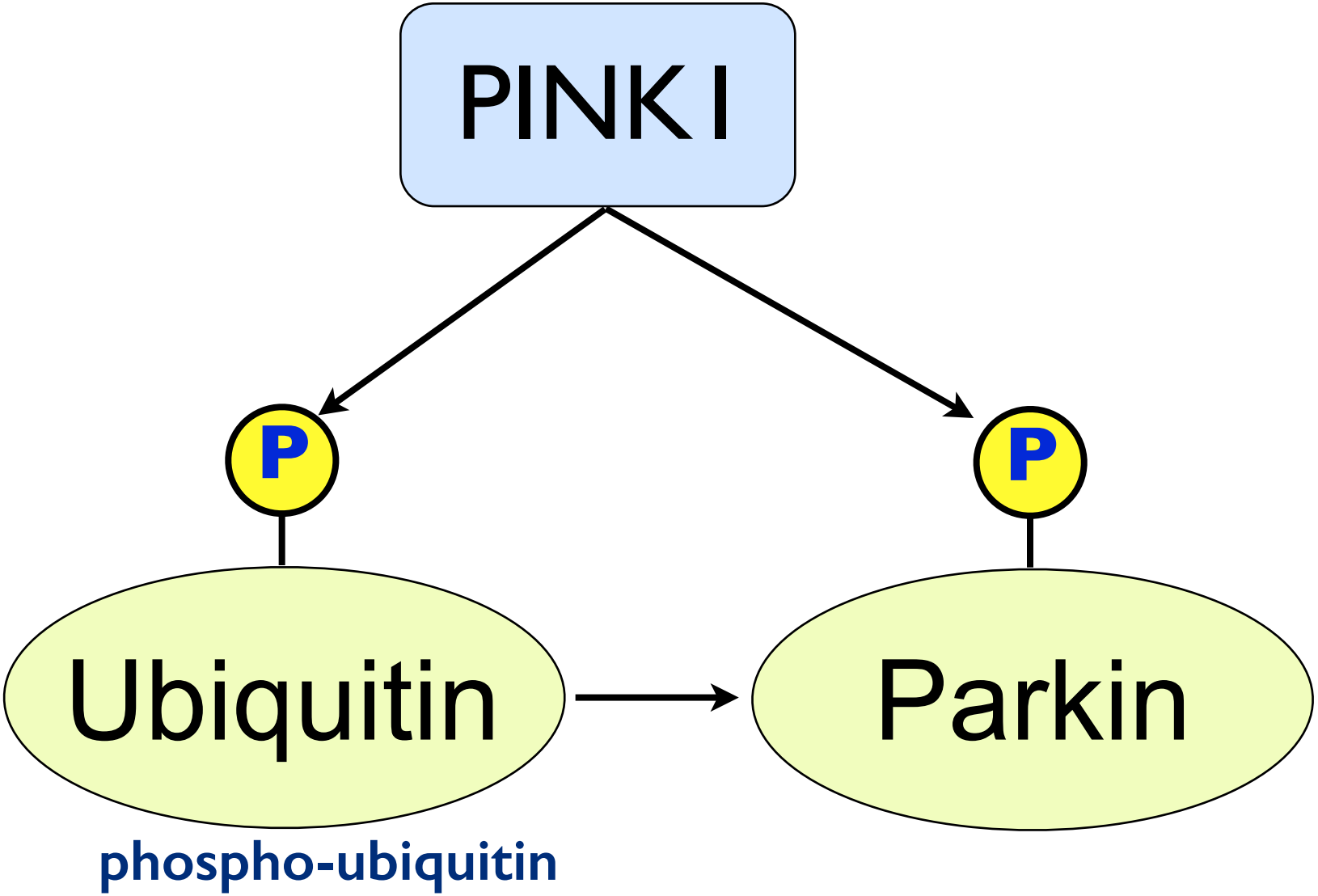
Overexpression of wild type PINK1 induces a 14-fold increase of Ser65 phosphorylated Ubiquitin in the mitochondria following treatment with the mitochondrial uncoupler CCCP



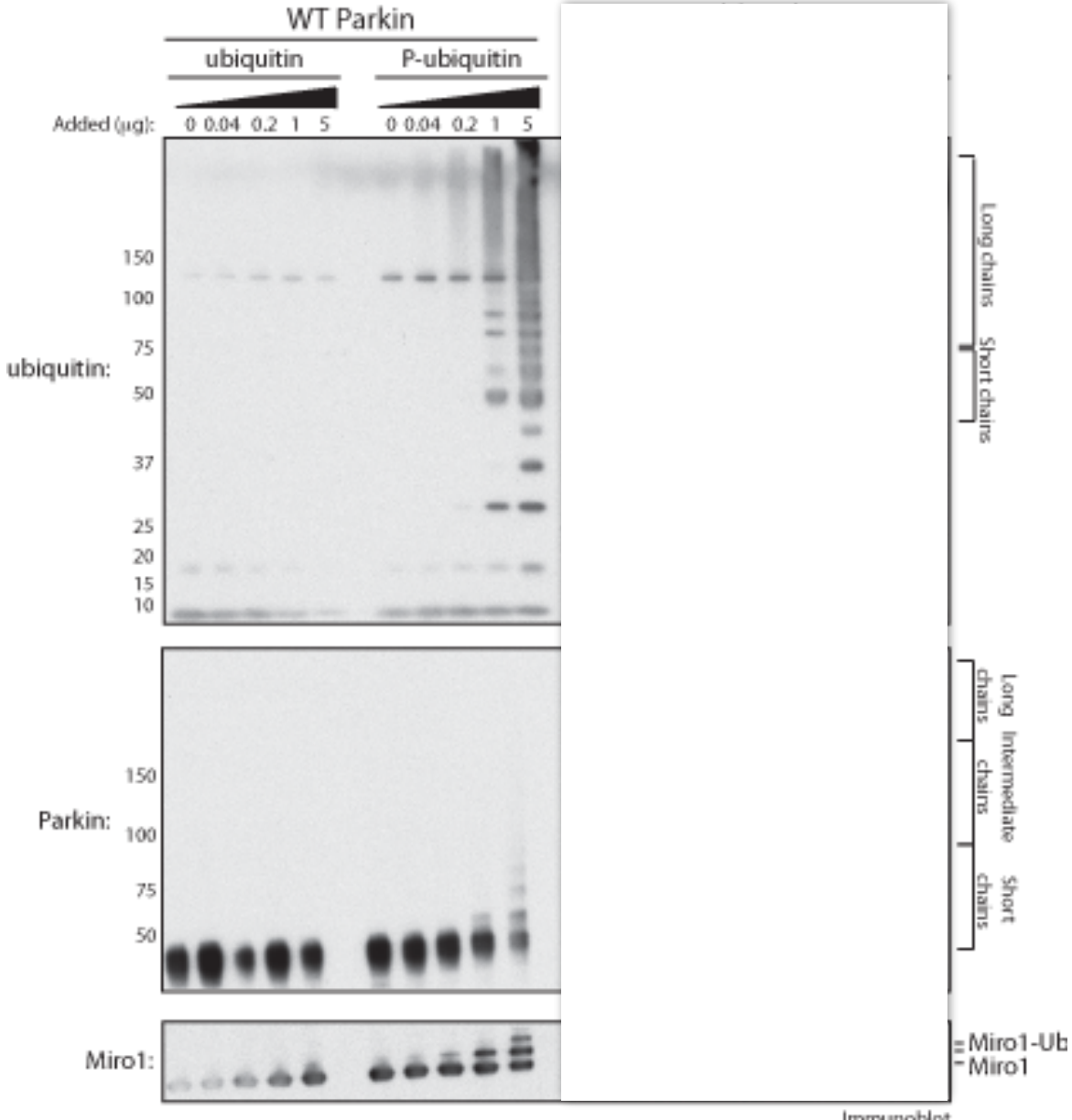
Ubiquitin	*H. sapiens*	56	L	S	D	Y	N	I	Q	K	E	S	T	L	H	L	V	L	R	72
Ubiquitin	*M. musculus*	56	L	S	D	Y	N	I	Q	K	E	S	T	L	H	L	V	L	R	72
Ubiquitin	*D. melanogaster*	56	L	S	D	Y	N	I	Q	K	E	S	T	L	H	L	V	L	R	72
Ubiquitin	*C. elegans*	56	L	S	D	Y	N	I	Q	K	E	S	T	L	H	L	V	L	R	72
Ubiquitin	*S. cerevisiae*	56	L	S	D	Y	N	I	Q	K	E	S	T	L	H	L	V	L	R	72
PARK2	*H. sapiens*	56	V	Q	N	C	D	L	D	Q	Q	S	I	V	H	I	V	Q	R	72
			:	.	:		:	:	:	:	:	*		:	*	:	*		*	

Matthias Trost, Miratul Muqit, Chanda Kondapalli

2014 we made a major breakthrough finding that PINK1 also generated a new Parkinson's chemical called phospho-ubiquitin



Ser65 phosphorylated ubiquitin activates Parkin in the absence of PINK1



Miratul Muqit
Agne Kazlauskaite

Implications of our research for new Parkinson's therapies

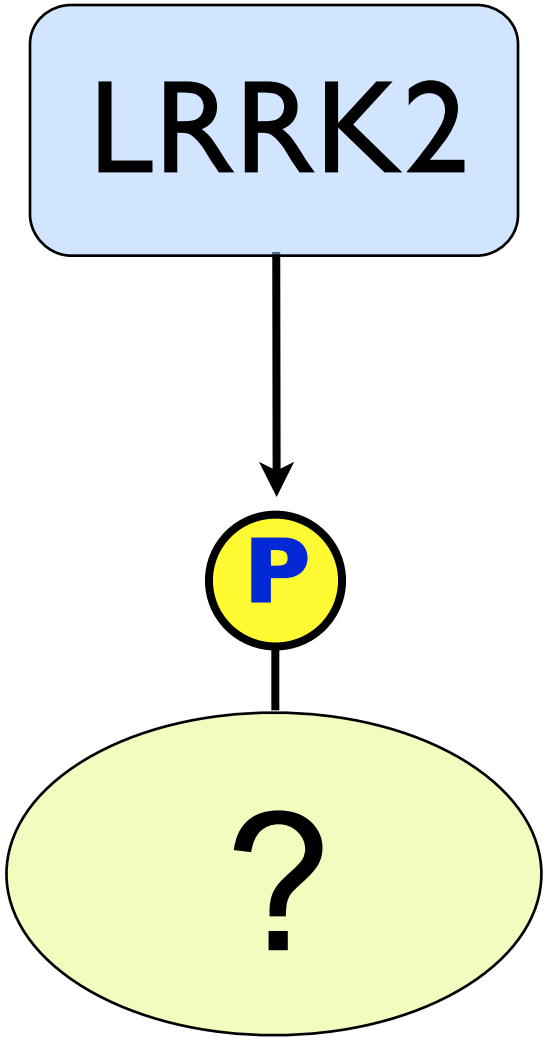
- Use Knowledge and Technology to develop compounds that mimic phospho-ubiquitin to activate Parkin and we are working with leading pharmaceutical companies and clinicians to achieve this

Parkinson's genes

LOCUS	MODE	TYPE	GENE	FUNCTION
PARK1/4	AD	LO/EOPD	α-synuclein	Unknown
PARK2	**AR**	**EOPD**	**Parkin**	**Ubiquitin ligase**
PARK3	AD	LOPD	Unknown	
PARK5	AD	LOPD	UCH-L1	DUB
PARK6	**AR**	**EOPD**	**PINK1**	**Kinase**
PARK7	AR	EOPD	DJ-1	Oxidative chaperone
PARK8	**AD**	**LOPD**	**LRRK2**	**Kinase**
PARK9	AR	EOPD*	ATP13A2	ATPase
PARK10	Complex	LOPD	Unknown	
PARK11	Complex	LOPD	*GIGYF2* (controversial)	
PARK12	X-linked	LOPD	Unknown	
PARK13	AD	LOPD	HtrA2/Omi	Serine protease
PARK14	AR	EOPD*	PLA2G6	Phospholipase A2
PARK15	AR	EOPD*	FBXO7	F Box protein
PARK16	Complex	LOPD	Unknown	
PARK17	Complex	LOPD	GAK	Kinase
PARK18	Complex	LOPD	HLA	immune recognition
PARK19	AD	LOPD	VPS35	endosomal-Golgi trafficking
PARK20	AD	LOPD	EIF4G1	mRNA translation-initiation

* Complex syndrome

LRRK2 is also a Kinase

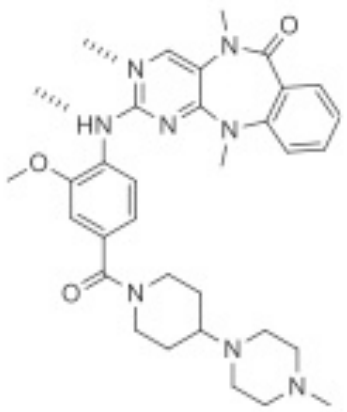


LRRK2

- LRRK2 is one of the most frequently mutated gene known to cause Parkinson's
- Importantly the most common mutation (G2019S) triggers activation of the LRRK2 enzyme
- This suggests that drugs that inhibit LRRK2 could be of benefit to Parkinson's patients

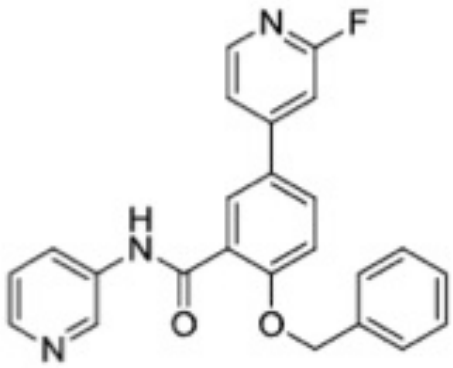
LRRK2 Specific Inhibitors

LRRK2-IN-1



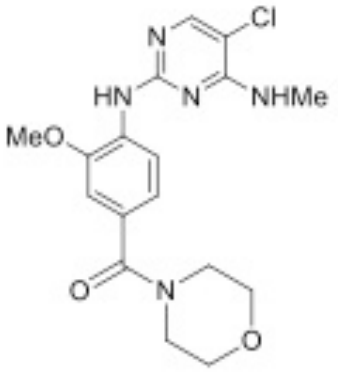
Formula: $C_{31}H_{38}N_8O_3$
MW: 570.69 g/mol

GSK2578215A



Formula: $C_{24}H_{18}F_2N_3O_2$
MW: 399.42 g/mol

HG-10-102-01



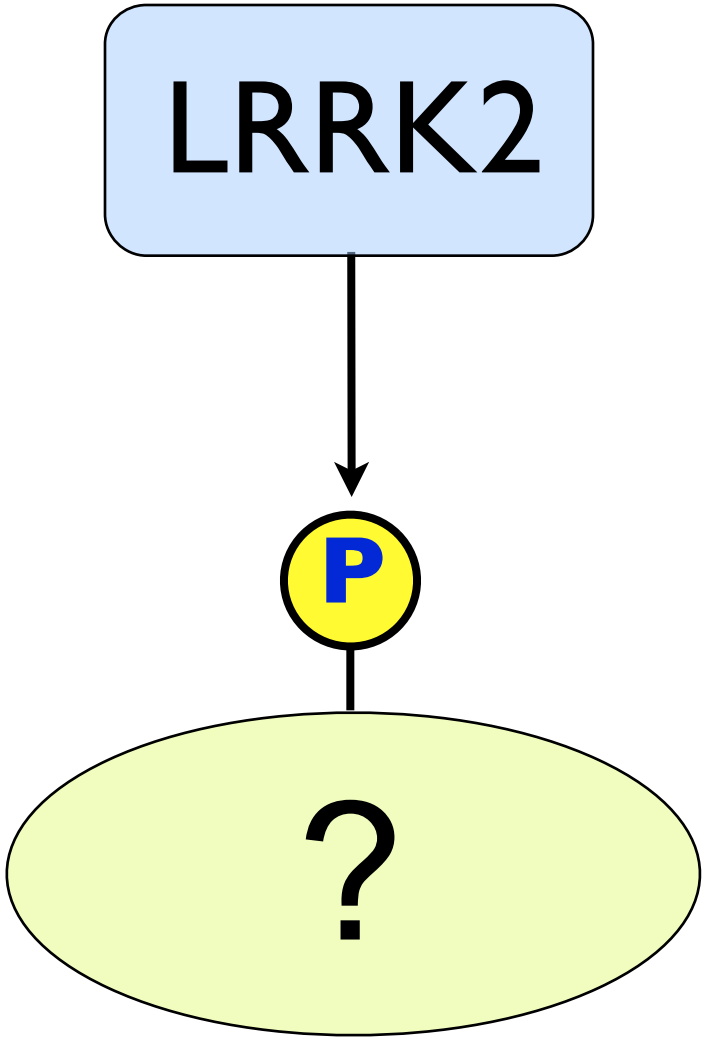
HG-10-102-01

Formula: $C_{22}H_{25}FN_6O_4S$
MW: 377.13 g/mol

IC50's (nM)	LRRK2-IN-1	GSK2578215A	HG-10-102-01
Wild type LRRK2	12.7	10.9	20.3
LRRK2 [G2019S]	6.3	8.9	3.2
LRRK2 [A2016T]	2408	81.1	153.7
LRRK2 [G2019S + A2016T]	4126	61.3	95.9

LRRK2-IN-1 *(Nature chemical biology* 2011, *7*, 203-205) Collaboration with Nathanael Gray Harvard
GSK2578215A (*Bioorganic & medicinal chemistry letters, 2012,* 22(17), 5625-5629) Collaboration with Alastair Reith & Colleagues GlaxoSmithKline and Nathanael Gray Harvard
HG-10-102-01*(ACS Medicinal Chemistry Letters. 2012, 3* (8), 658–666)) Collaboration with Nathanael Gray Harvard

LRRK2 is a Kinase: what does it act on?



Key Considerations for PD Targets at the MRC-PPU

- Genetics
- Druggability
- Structural/Biochemical Analyses
- Cellular Readouts

Amenable to blocking/enhancement of function?

LRRK2

PINK1

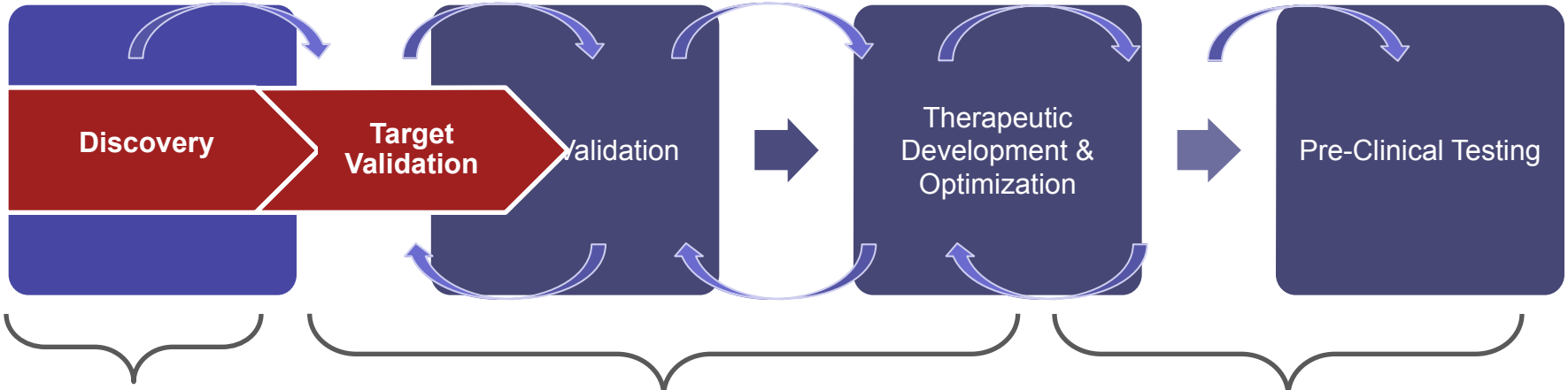
Parkin

Fbxo7

+ Industry Partnerships

Disease Modifying Therapies

Progress requires translation of discoveries through the pipeline

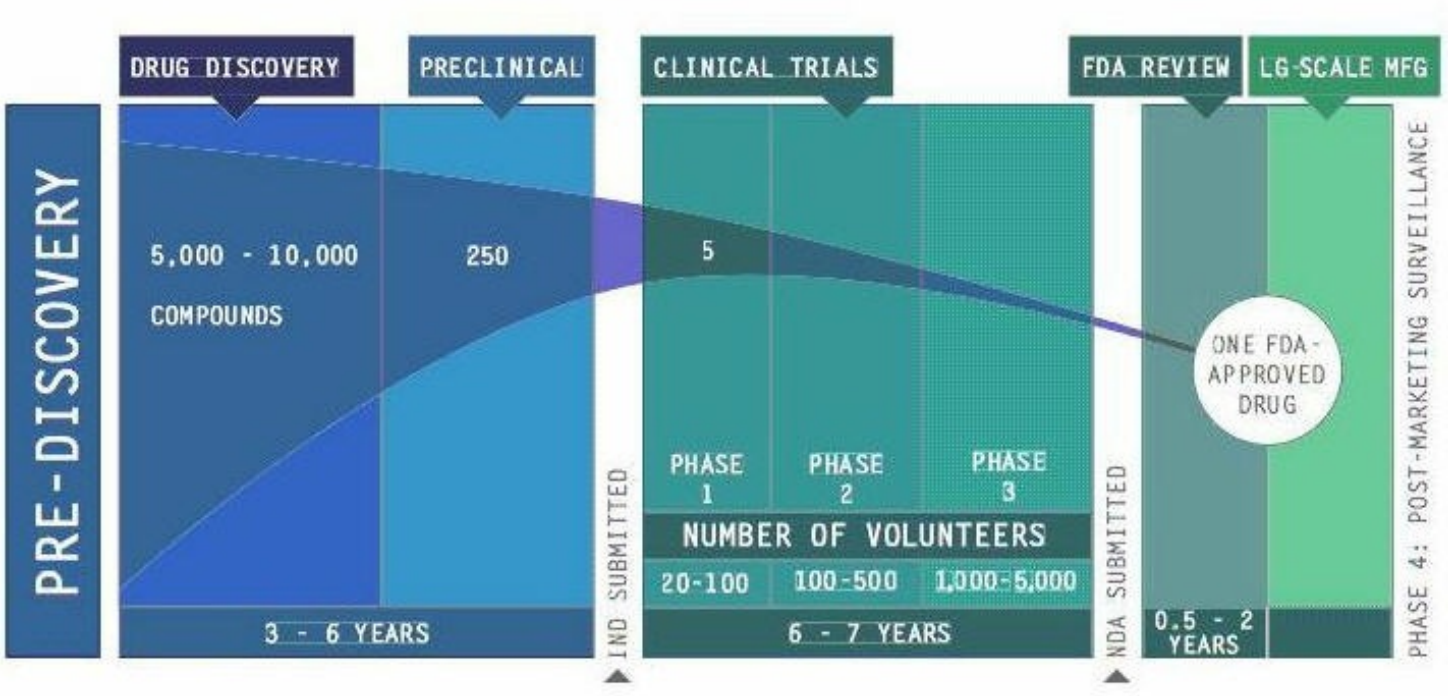


Basic Discovery
Understanding disease mechanisms

Preclinical
Convert biology into therapies
Partly done in academic and biotech laboratories

Clinical
Determine safety and efficacy in patients
Mostly done by large pharma

Attrition in Drug Development



- **Drugs to Treat CNS Diseases Take 35% Longer to Develop than Other Drugs**
  - **CNS drugs take as long as 18 years from preclinical work to launch**
- **Trial failures in CNS tend to occur later in the clinical development process, when resource demands and costs are at their highest.**
- **The costs associated with development of 1 drug are upwards of $1 Billion**

Tufts Center for the Study of Drug Development, 2012

Acknowledgments

Agne Kazlauskaite
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Charles Williams
Jevgenia Tamjar

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Axel Knebel and team
Mark Peggie and team
James Hastie and team
Mass Spectrometry team
DNA sequencing team
Kirsten Airey and Tisssue Culture

MRC Protein Phosphorylation and Ubiquitylation Unit

wellcome trust Fellow

Funded by PARKINSON'S UK CHANGE ATTITUDES. FIND A CURE. JOIN US.

THE MICHAEL J. FOX FOUNDATION FOR PARKINSON'S RESEARCH

Boehringer Ingelheim

MERCK SERONO

gsk GlaxoSmithKline

Pfizer

AstraZeneca UNITED KINGDOM

janssen PHARMACEUTICAL COMPANIES OF Johnson&Johnson

LRRK2- Rationale

- Mutations account for >30% affected PD individuals in of N.African/ Ashkenazi Jewish descent
  - Penetrance varies in patients
- Encodes a kinase and
  - Several PD-linked mutations result in increased kinase function
  - Pharma considers this a highly druggable target
- Identification of substrates and/or interacting proteins in unique cell populations may point to means by which disease progression occurs

PINK1 - Rationale

- Mutations linked to autosomal recessive forms of PD
  - There is a strong interplay with other PD genes (eg. Parkin)
- Mitochondrial Protein Kinase
  - Data from models indicate that PINK1 is required to protect against mitochondrial damage
  - There are several lines of evidence that mitochondrial dysfunction occurs in PD
- Substrates
  - Parkin
  - Ubiquitin

Parkin - Rationale

- First linked to autosomal-recessive juvenile PD
  - Numerous mutations of the gene have been linked to early onset PD
- Parkin is auto-inhibited under basal conditions – confirmed by recent structural analysis
- Recent demonstration that phospho-ubiquitin is capable of activating Parkin points to a unique mechanism to enhance ligase function that could have therapeutic implications

Fbxo7 - Rationale

- Autosomal recessive inheritance
  - Associated with young-onset
- F-box protein can form a complex with SCF (Skp1-Cullin1-Rbx1-F-box E3 ligases and are implicated in the ubiquitin proteosomal system
- Literature suggestions that the signalling pathways of FBXO7, PARKIN and PINK1 interact with each other

The Path to Therapies

PATIENT CARE

ADVOCACY

MONUMENT DISCOVERY PROJECT

GDNF TRIAL

RESEARCH SUPPORT

PPMI

Alpha-Synuclein

Calcium Channels

Urate

Trophic Factors

DBS

30 ongoing clinical studies/interventional trials in the United Kingdom