

LRRK2 in Parkinson's



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Invercase hotel Dundee

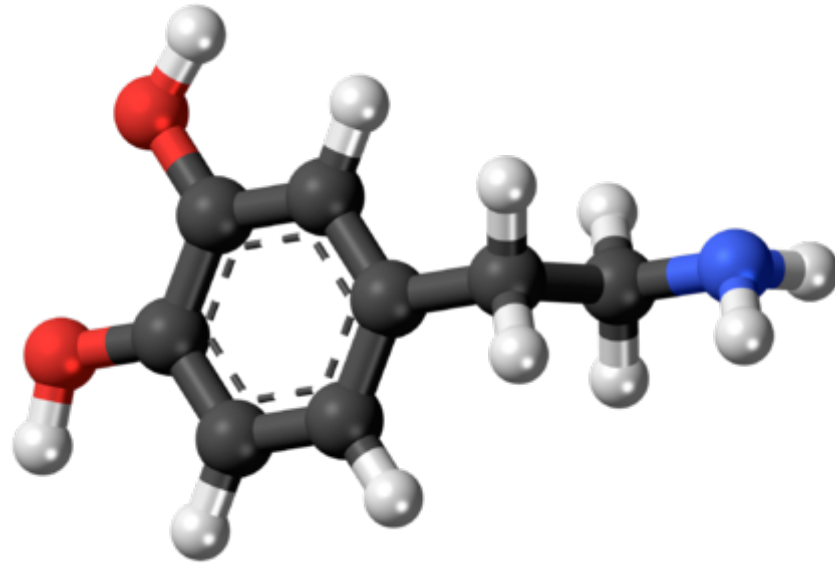
MRC Protein Phosphorylation & Ubiquitylation Unit



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

Understanding what goes wrong in Parkinson's

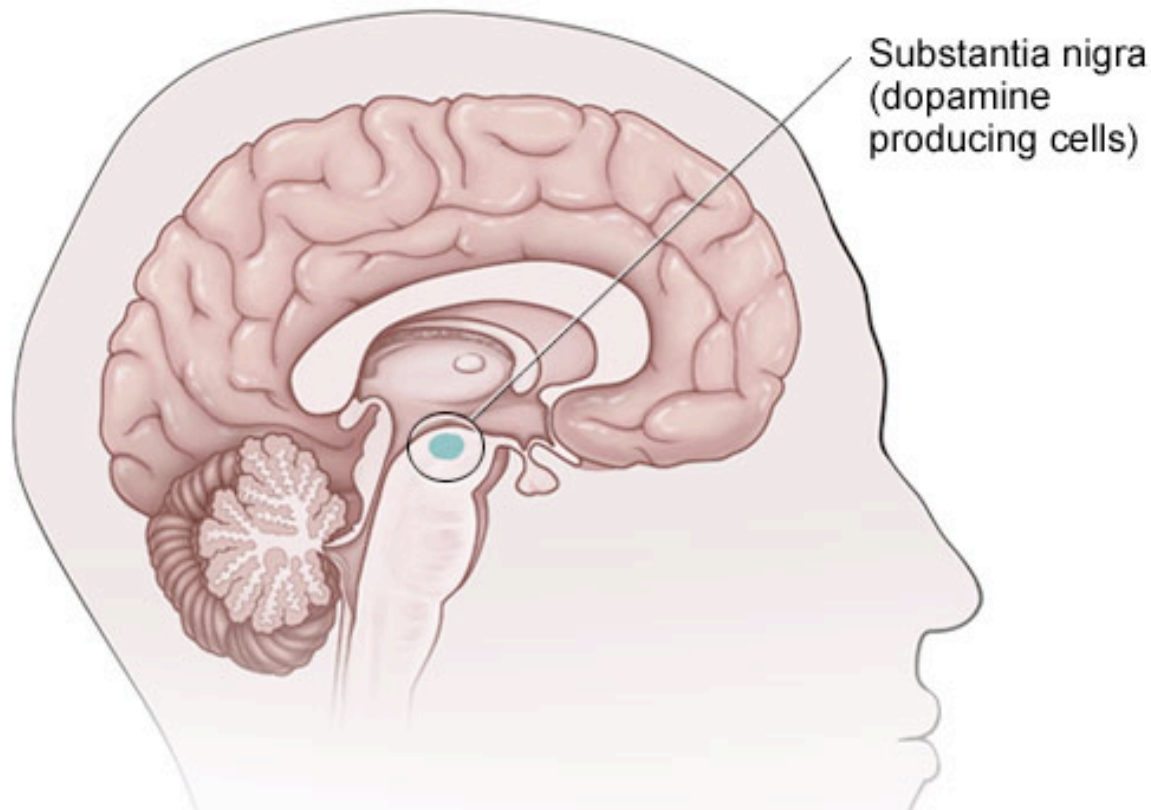
- Parkinson's disease is primarily caused by low and falling dopamine levels.
- Dopamine is responsible for relaying messages that plan and control body movement



Dopamine

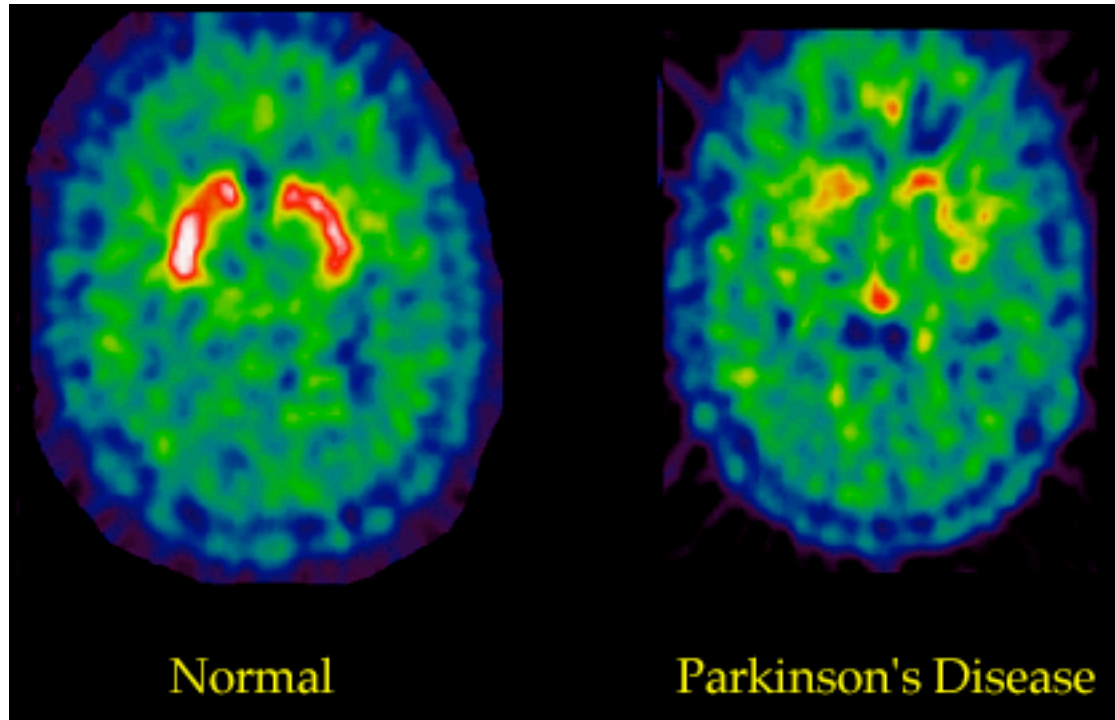
Understanding what goes wrong in Parkinson's

- Dopamine-generating cells, known as dopaminergic neurons (types of nerve cells) are located in part of the brain known as the substantia nigra



Understanding what goes wrong in Parkinson's

- A person with Parkinson's has abnormally low dopamine levels.



Understanding what goes wrong in Parkinson's

- A person with Parkinson's has abnormally low dopamine levels because dopamine producing cells in the substantia nigra brain region die
- We do not understand the reason for why dopamine producing cells in the substantia nigra brain region die in patients with Parkinson's disease
- This is our central research question

Understanding what goes wrong in Parkinson's

- There are likely to be many different reasons for a patient developing Parkinson's
- These include genetic as well as environmental factors such as exposure to toxic chemicals during the course of a lifetime
- Striking recent evidence is suggesting that increased immune responses can also result in the immune system attacking brain cells leading to a process known as “neuro-inflammation”. This may also contribute to Parkinson's and other neurodegenerative conditions

Understanding what goes wrong in Parkinson's

- Although side many symptoms of Parkinson's can be treated with drugs we do not have have any treatment that slows down or even halts the progression of Parkinson's
- We believe that understanding how disruption of biology caused by genetics or other factors such as environment lead to Parkinson's, will lead to new strategies to better treat and diagnose this condition in the future

Causes of Parkinson's - Genetic vs Unknown



~10% of cases familial that are caused by DNA mutations within genes

~90% of cases “sporadic” which means cause is unknown

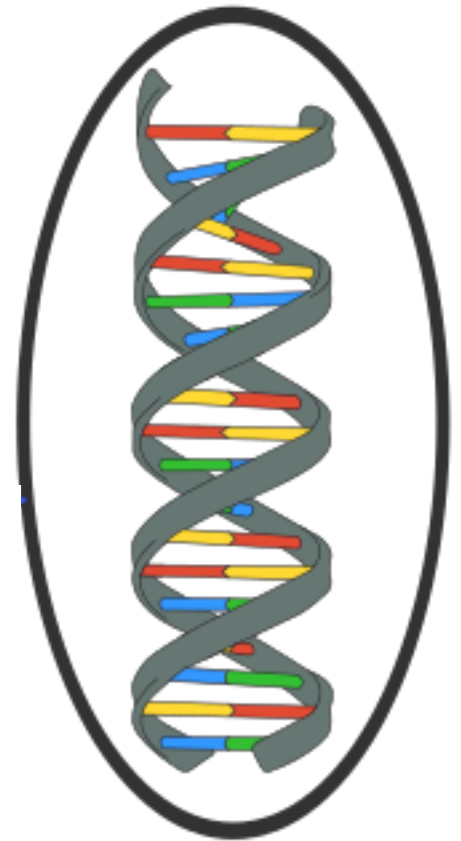
Environmental toxin ?

Infectious agent ?

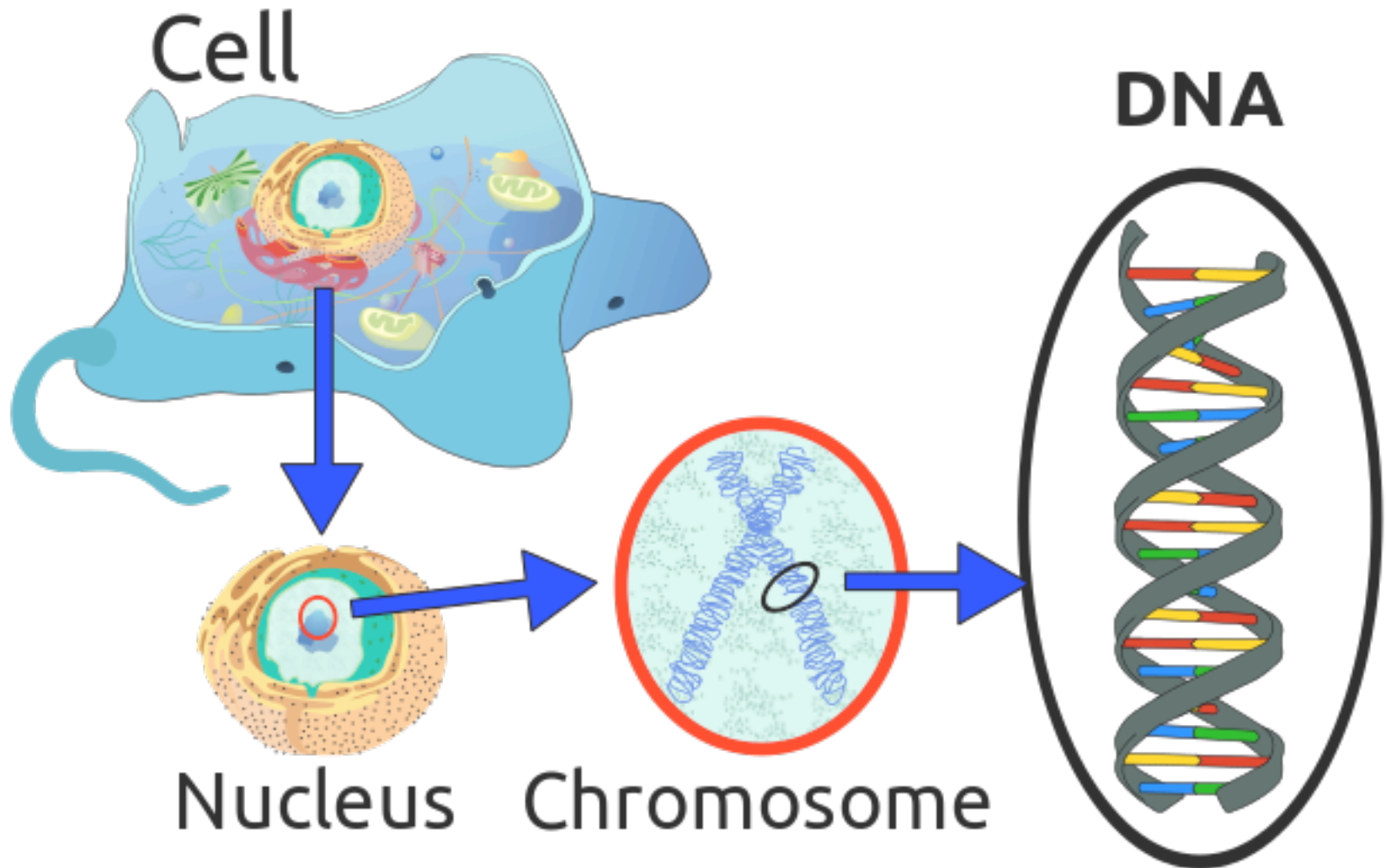
Age-dependent damage ?

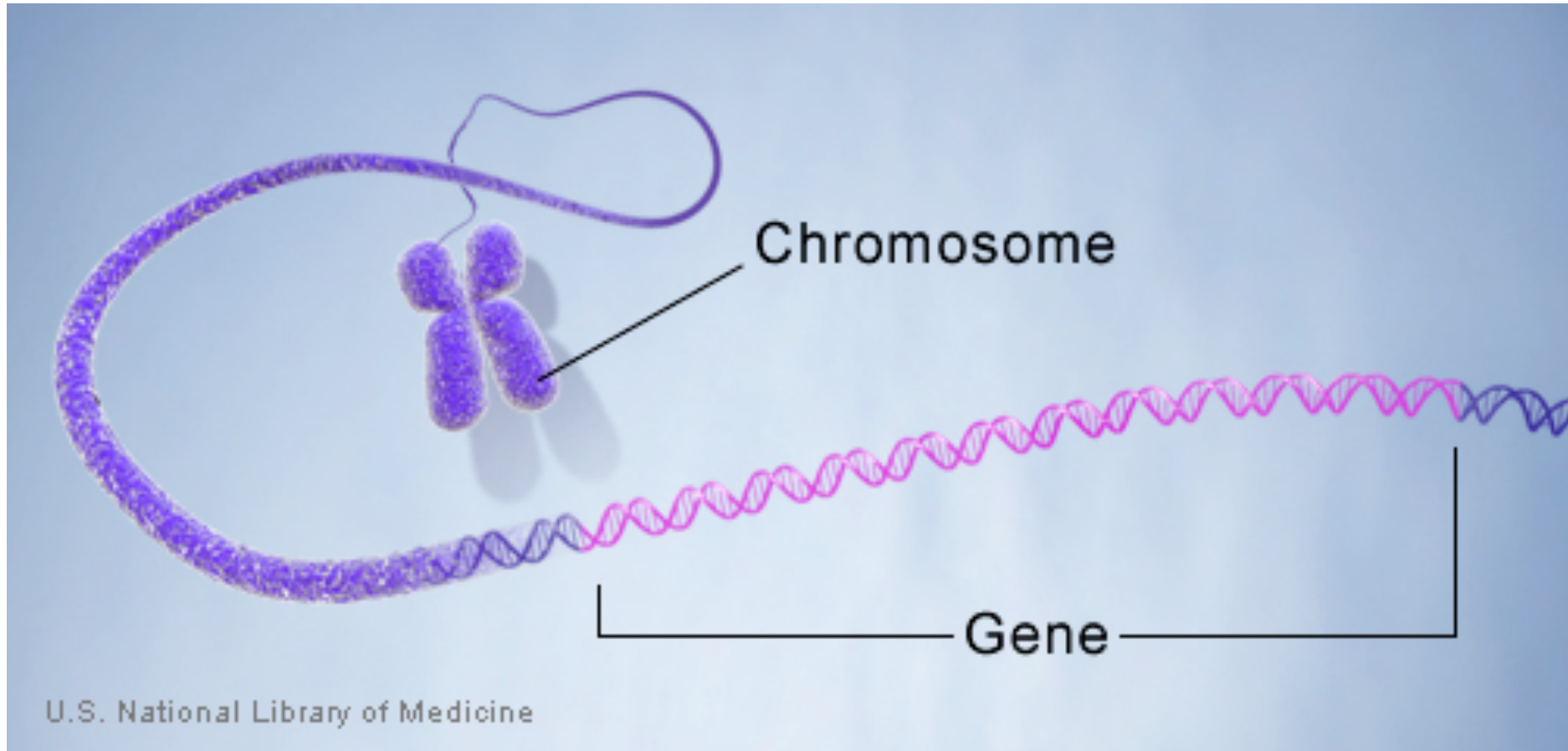
Mutations in DNA are the cause of inherited Parkinson's

DNA



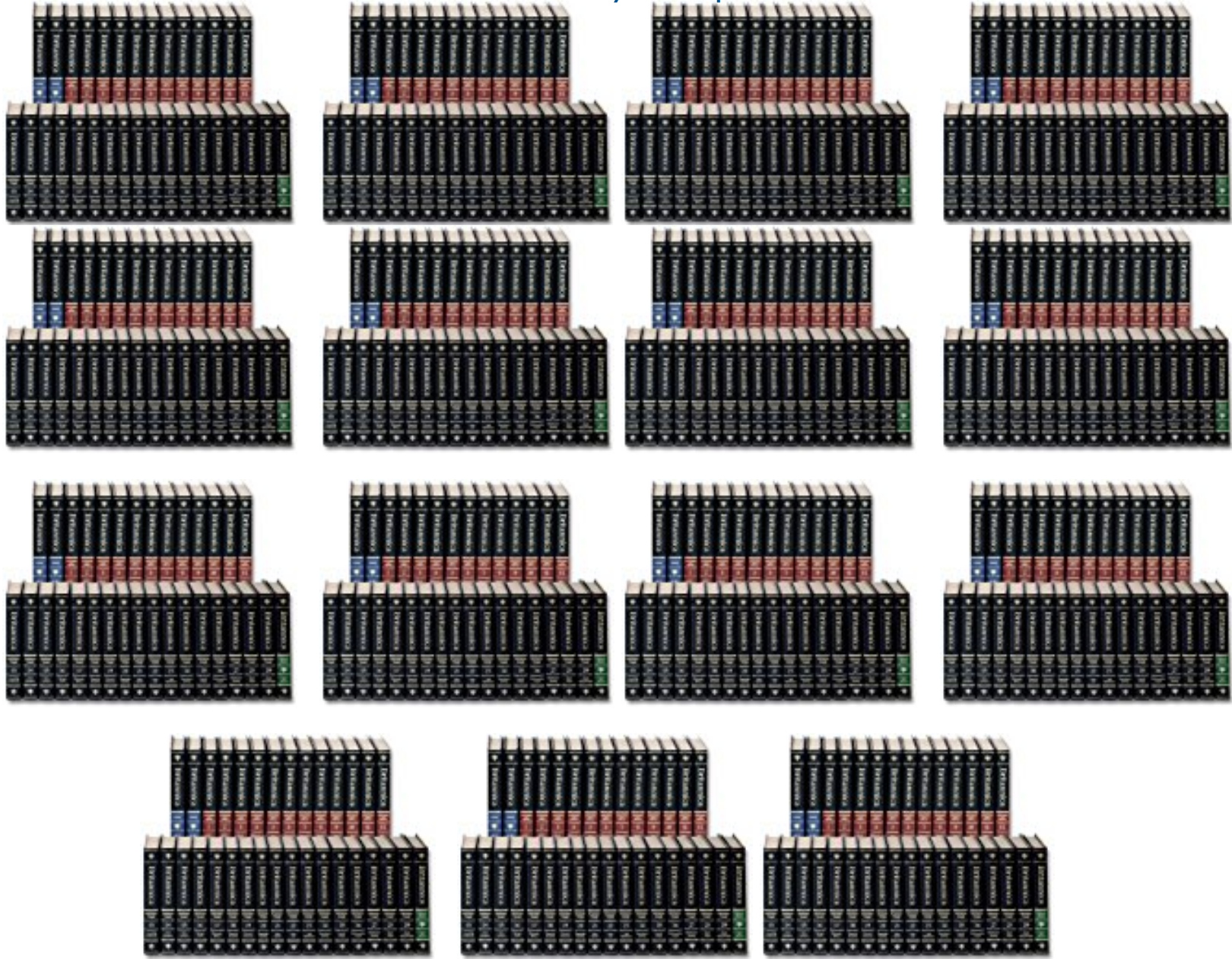
Mutations in DNA are the cause of inherited Parkinson's





A gene is a stretch of DNA that encodes for a protein that has a key function in biology. Each cell of our body has around 30,000 genes. The roles of the vast majority of these are unknown

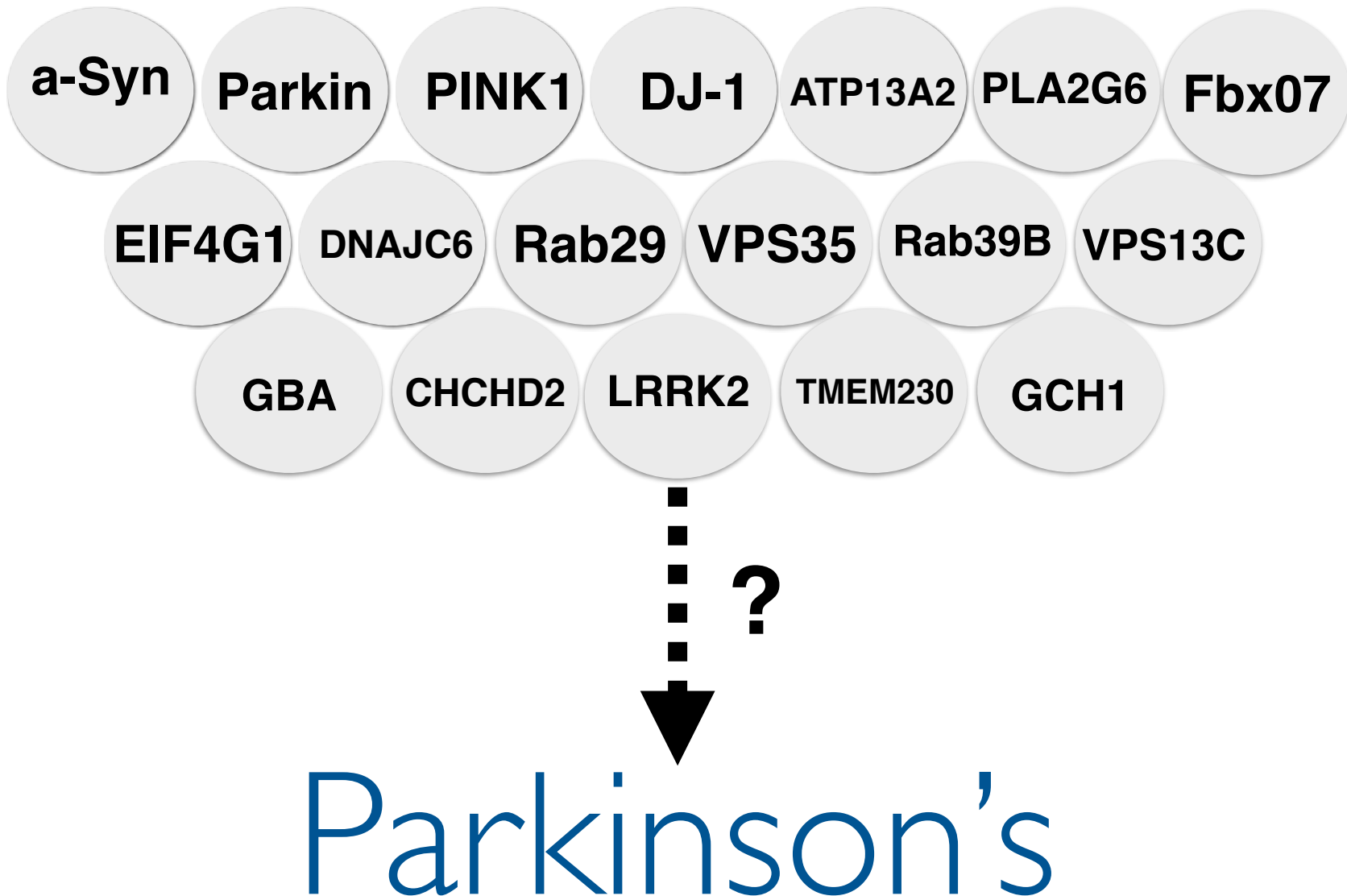
The information stored in our DNA is equivalent to 436 volumes of encyclopedia Britannica!



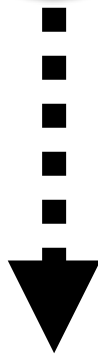
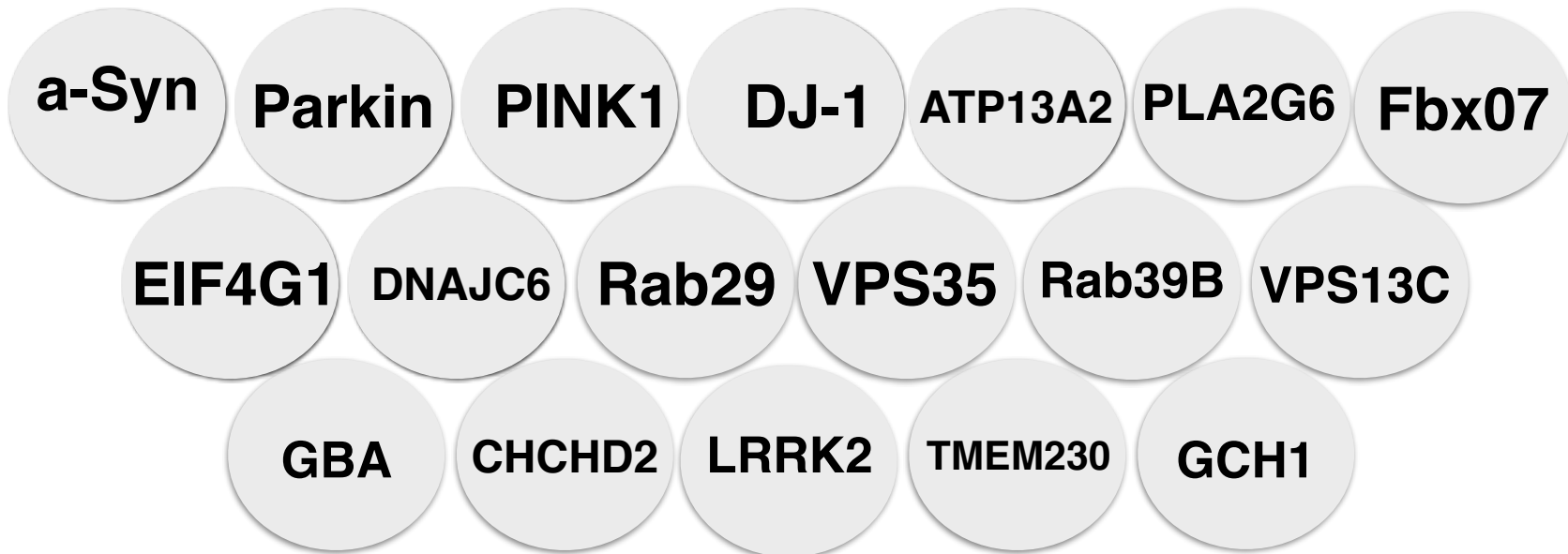
One Spelling mistake in the 436 volumes of the encyclopaedia
Britannica is sufficient to cause Parkinson's disease



Mutations within ~18 known genes are known to lead to Parkinson's in humans



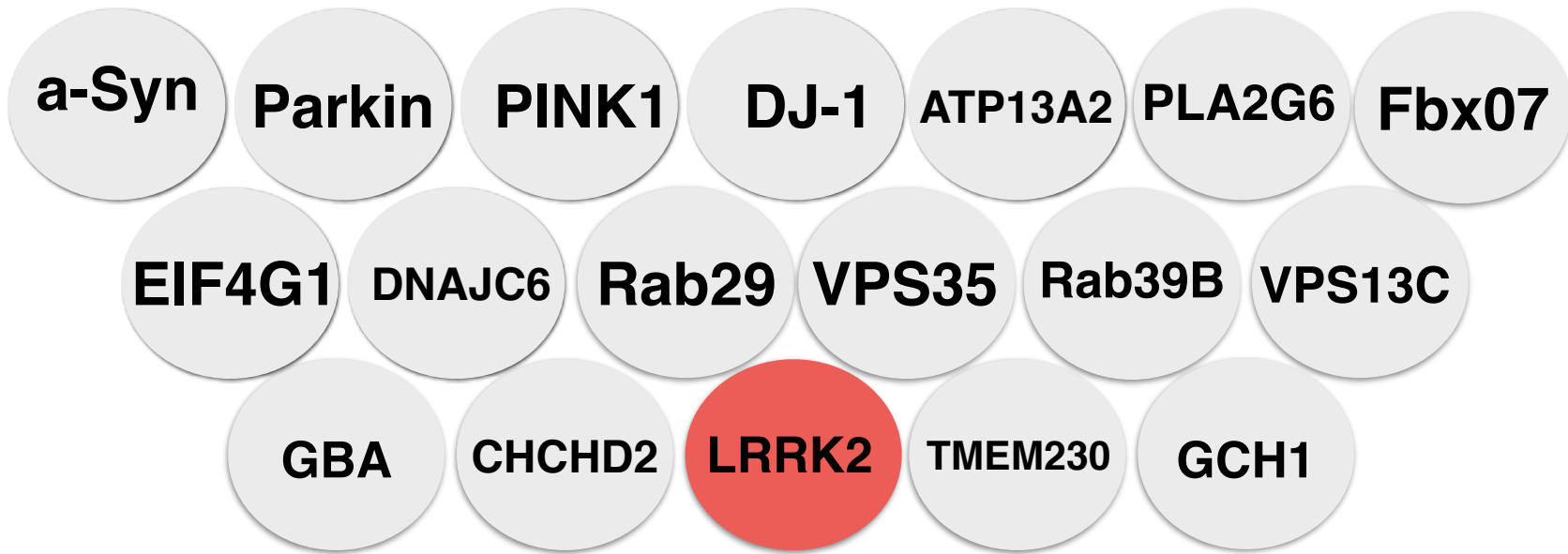
Mutations within ~18 known genes are known to lead to Parkinson's in humans



Environmental factors likely interact with genetics in complex ways

Parkinson's

Mutations within ~18 known genes are known to lead to Parkinson's in humans



Parkinson's

DISCOVERY OF LRRK2 IN 2004

Neuron, Vol. 44, 601–607, November 18, 2004, Copyright ©2004 by Cell Press

Mutations in *LRRK2* Cause Autosomal-Dominant Parkinsonism with Pleomorphic Pathology

Alexander Zimprich,^{1,2,11} Saskia Biskup,^{3,11}
Petra Leitner,¹ Peter Lichtner,³ Matthew Farrer,⁴
Sarah Lincoln,⁴ Jennifer Kachergus,⁴ Mary Hulihan,⁴
Ryan J. Uitti,⁵ Donald B. Calne,⁶ A. Jon Stoessel,⁶
Ronald F. Pfeiffer,⁷ Nadja Patenge,¹
Iria Carballo Carbajal,¹ Peter Vieregge,⁸
Friedrich Asmus,¹ Bertram Müller-Myhsok,⁹
Dennis W. Dickson,⁴ Thomas Meitinger,^{3,10,*}
Tim M. Strom,^{3,10} Zbigniew K. Wszolek,^{5,*}
and Thomas Gasser^{1,*}



**Thomas
Gasser
(Tübingen)**

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Cloning of the Gene Containing Mutations that Cause *PARK8*-Linked Parkinson's Disease

Coro Paisán-Ruiz,^{1,11} Shushant Jain,^{4,3,11}
E. Whitney Evans,⁴ William P. Gilks,³ Javier Simón,¹
Marcel van der Brug,⁵ Adolfo López de Munain,^{6,7}
Silvia Aparicio,¹ Angel Martínez Gil,⁸
Naheed Khan,³ Janel Johnson,⁴
Javier Ruiz Martinez,⁹ David Nicholl,¹⁰
Itxaso Marti Carrera,⁷ Amets Saénz Peña,⁶
Rohan de Silva,³ Andrew Lees,³
José Félix Martí-Massó,⁷ Jordi Pérez-Tur,^{1,*}
Nick W. Wood,^{2,*} and Andrew B. Singleton^{4,*}

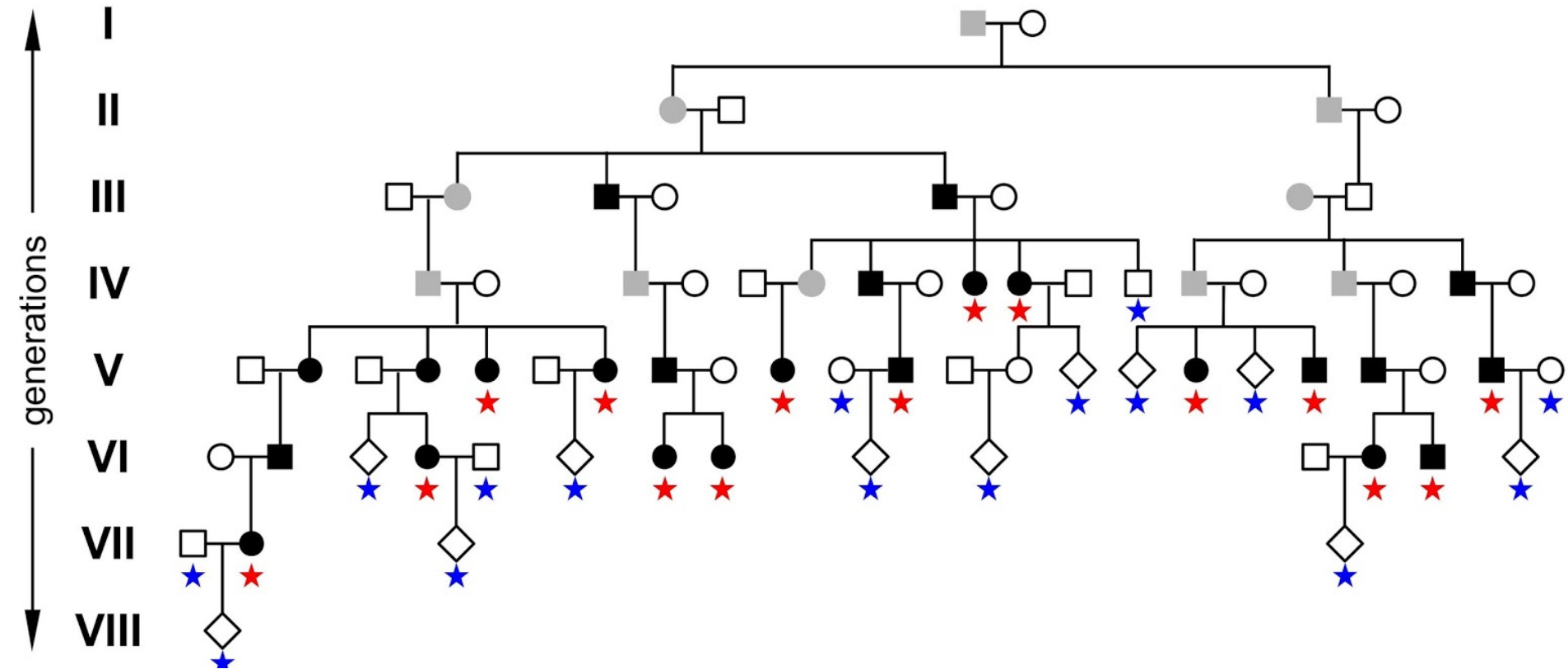


**Andrew
Singleton
(NIH Washington)**

How was LRRK2 Discovered?

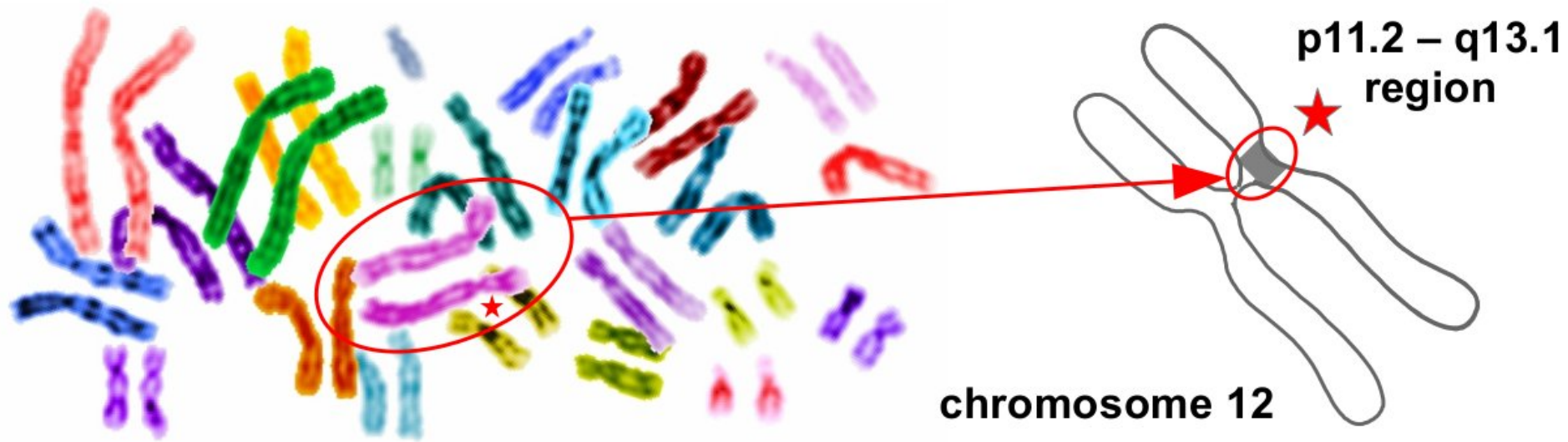
Finding LRRK2, the gene for LRRK2, was an international effort involving scientists from Japan, Germany, Spain, the United Kingdom and the United States that started in 2000. Just as important were the 52 families from these same countries with familial Parkinson's that formed the numerous study groups. LRRK2 took a lot of work and was uncovered in 2004 in two paper but in the end it turned out to be very important as it is the most frequently encountered causative PD gene.

LRRK2 was discovered in families of Parkinson's Patients

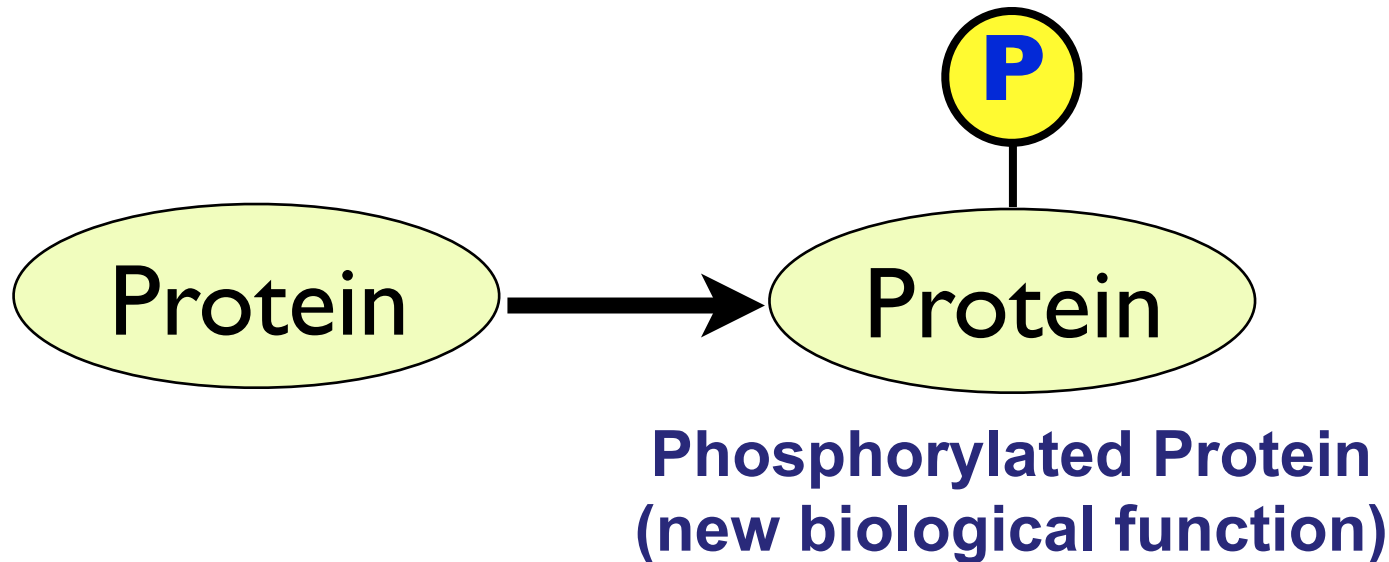


The high frequency of PD observed in this family is typical for a dominant trait. This means that a single copy was enough to cause Parkinson's

The LRRK2 gene is located on chromosome 12 in the p11.2 – q13.1 region. The red star shows the approximate location of LRRK2

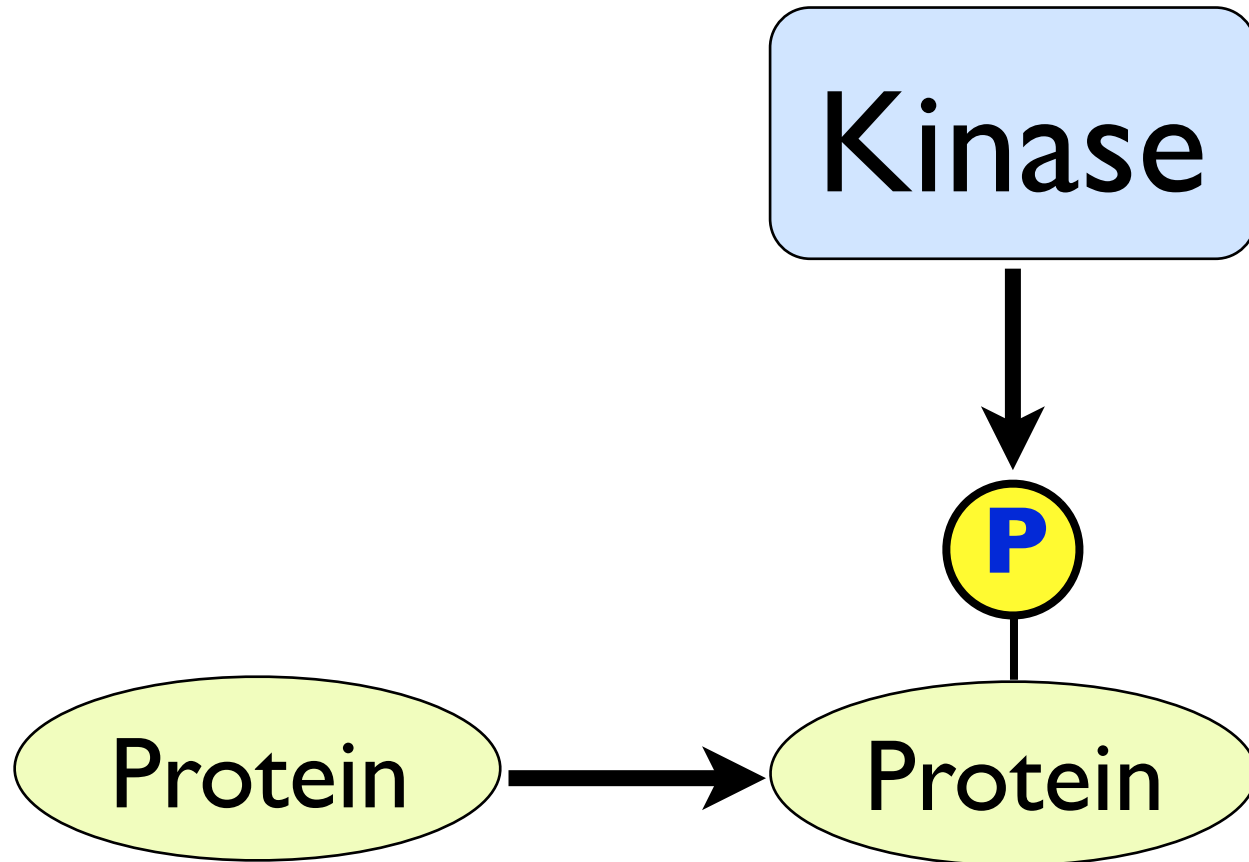


Biology is controlled by a physiological pathway known as “Protein Phosphorylation” in which the chemical termed phosphate is attached to proteins

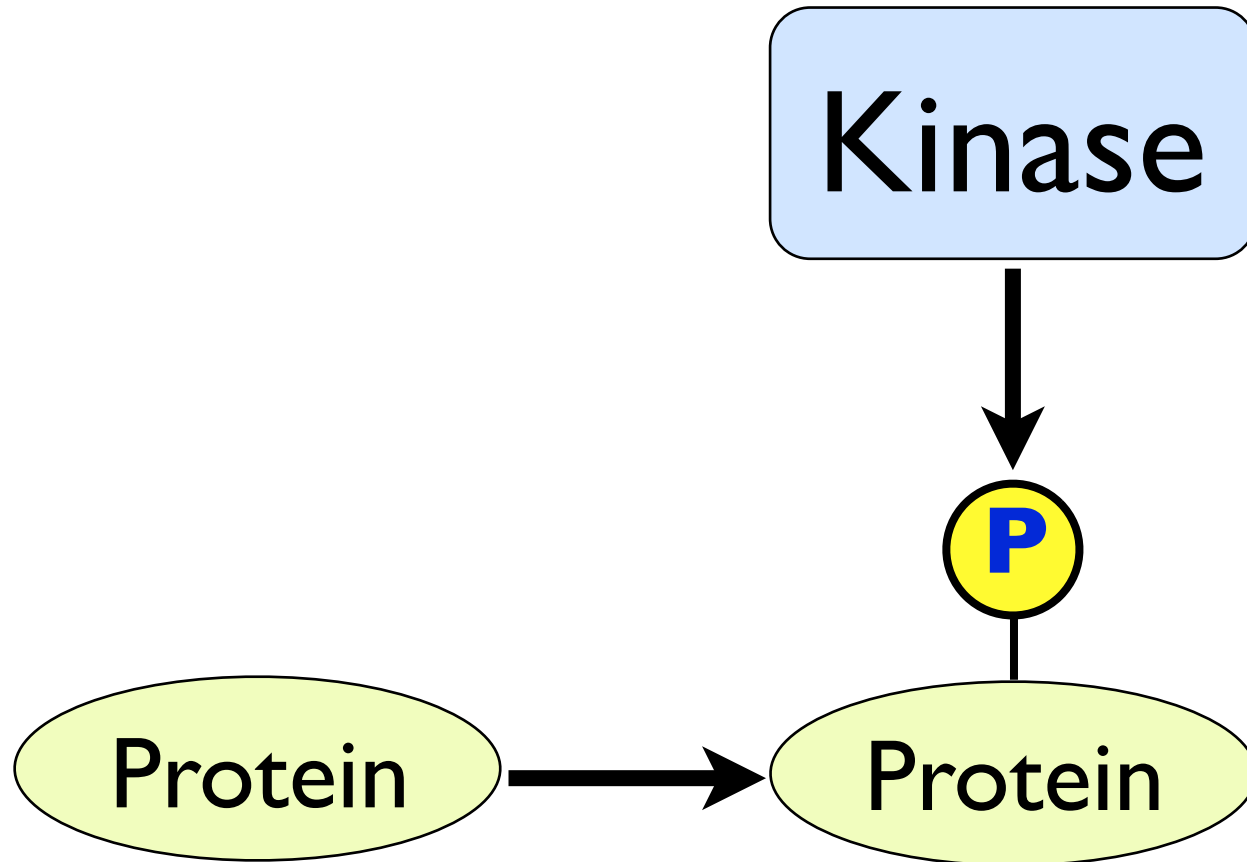


Many human disorders including diabetes, cancer, inflammatory conditions, hypertension, Alzheimer's and Parkinson's are caused by disruption in Protein Phosphorylation

Phosphate molecules are attached to proteins by a class of enzyme termed “Kinase”. These enzymes are the master controllers of biology

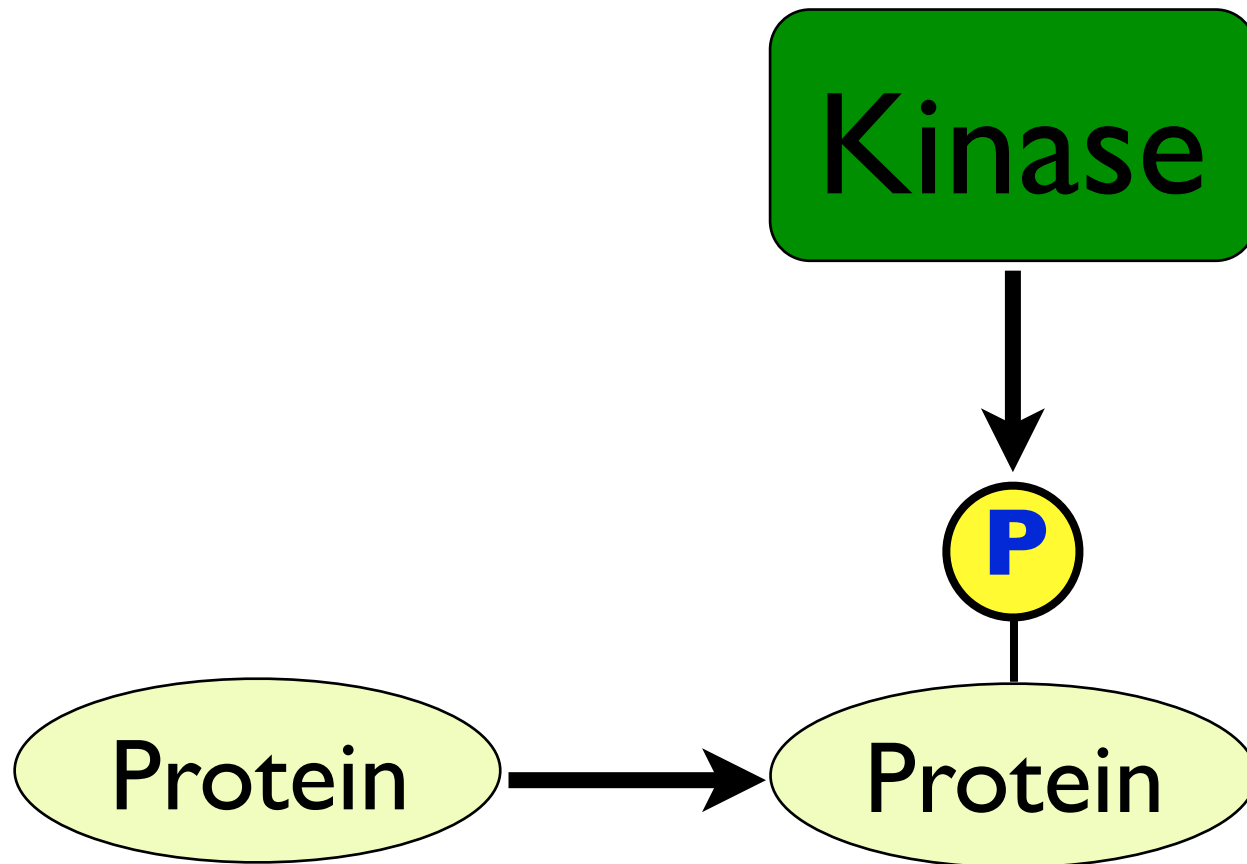


Phosphate molecules are attached to proteins by a class of enzyme termed “Kinase”. These enzymes are the master controllers of biology



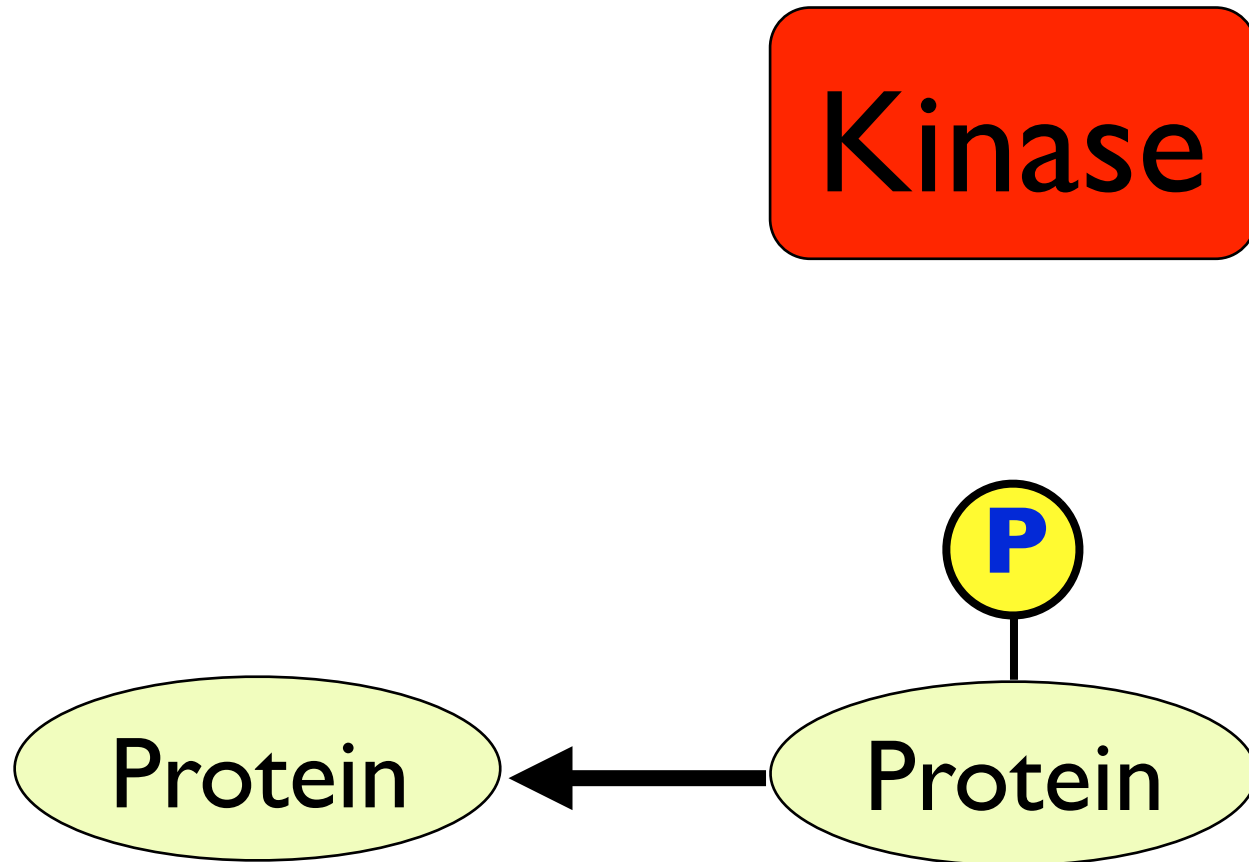
The activity of protein kinases can be switched on and off.

When a kinase is switched on- its substrate proteins are phosphorylated

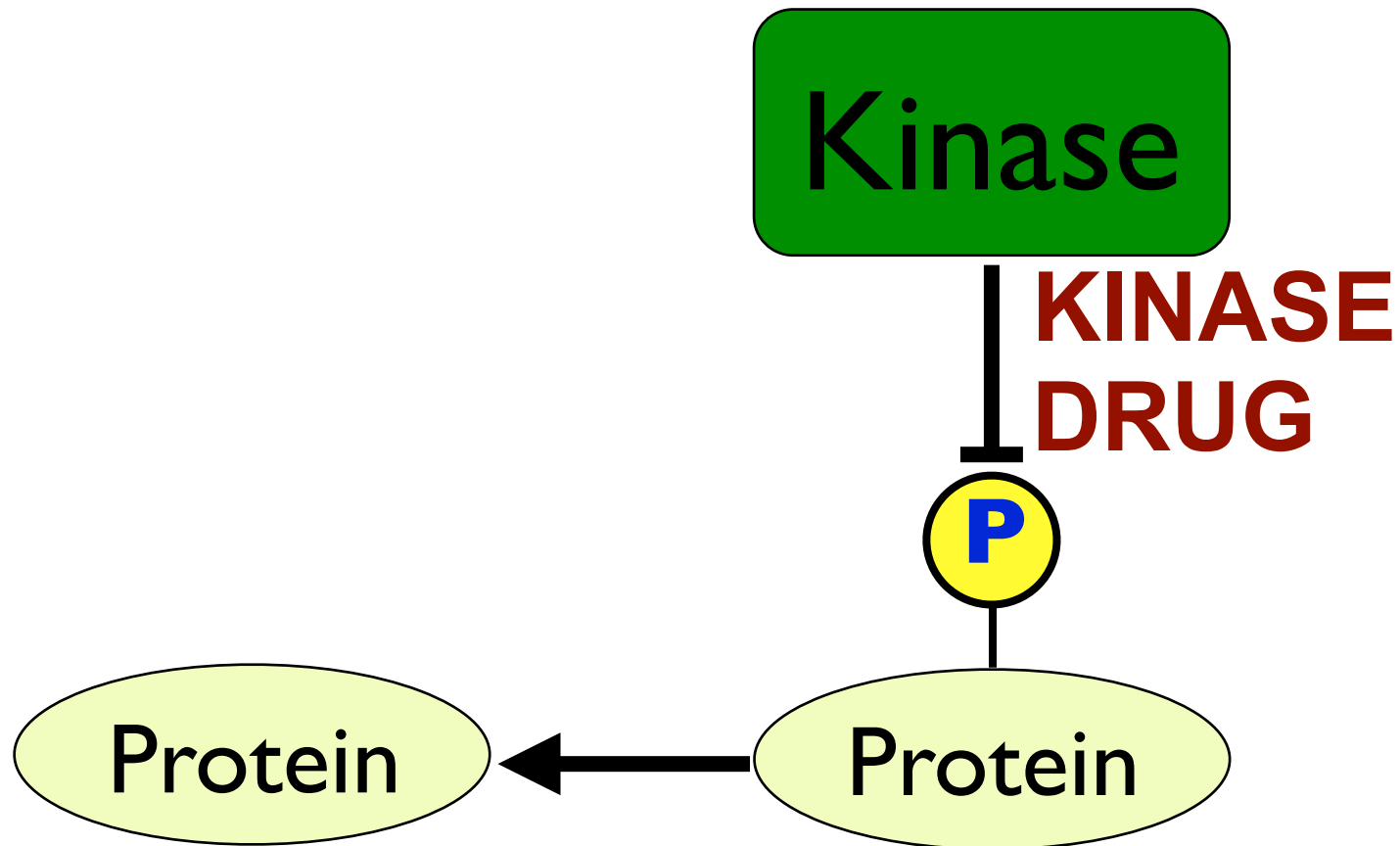


The activity of protein kinases can be switched on and off.

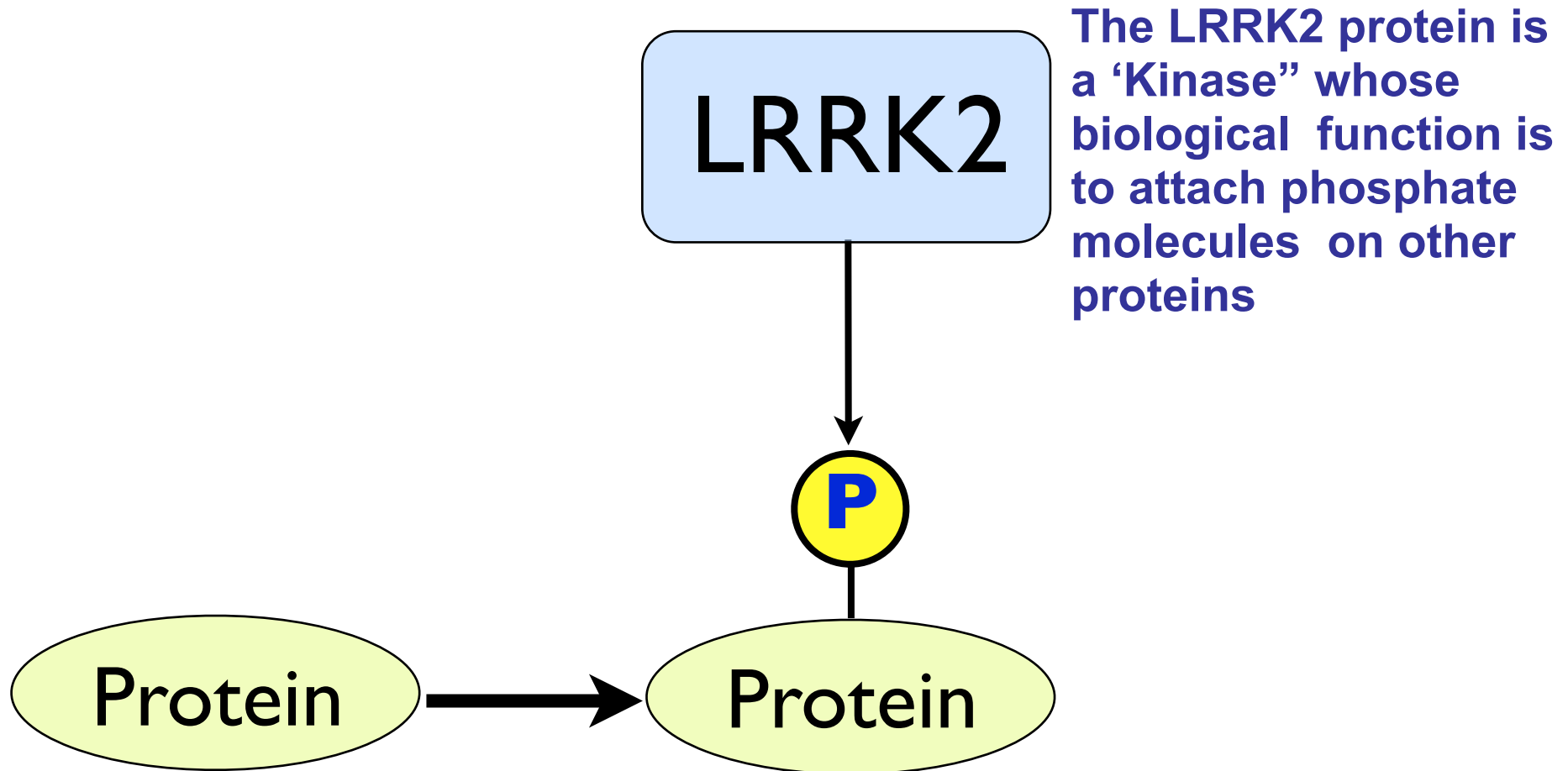
When a kinase is switched off- its substrate proteins are not phosphorylated



Kinases are major drug targets.
Over 40 different drugs that target
kinases have been approved mostly
in the field of cancer



The LRRK2 gene Encodes a Enzyme termed Kinase



LRRK2 and Parkinson's

- Mutations in LRRK2 can cause 1-2% of all Parkinson's making it one of the most commonly mutated gene known to be linked to this condition
- LRRK2 mutations cause Late onset Parkinson's that is similar to sporadic non-genetic Parkinson's. Not every patient with LRRK2 mutation will get Parkinson's (not 100% penetrant)
- There is increasing evidence that disruption of biology controlled by LRRK2 is relevant to understanding sporadic Parkinson's and not just the genetic form

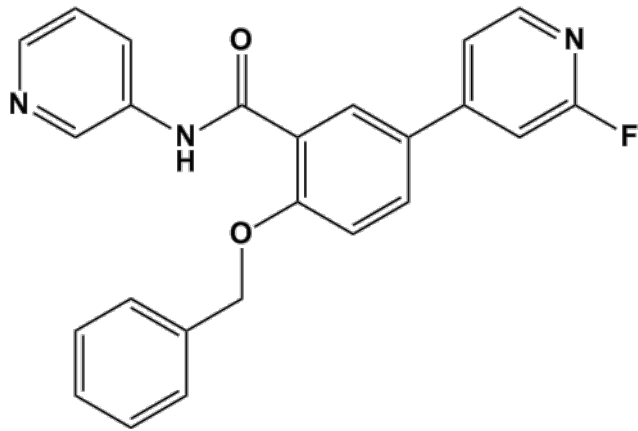
LRRK2 and Parkinson's

- Most importantly all inherited mutations in the LRRK2 gene increases kinase activity
- This suggests that a drug that targeted and inhibited (switch off) LRRK2 may offer therapeutic benefit for treatment of Parkinson's.

LRRK2 and Parkinson's

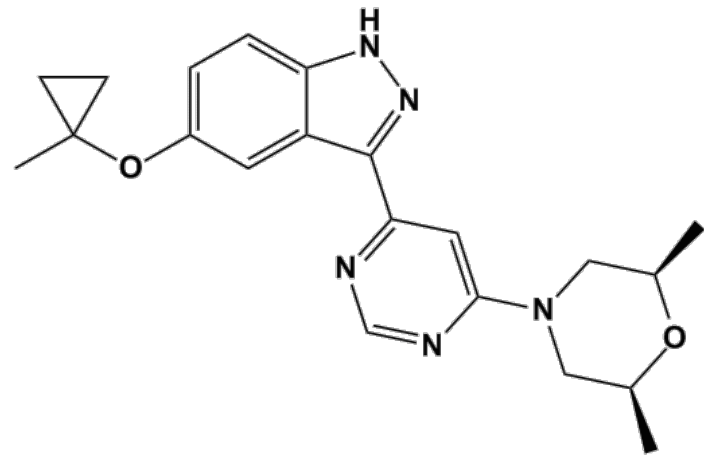
- Because mutations activate LRRK2 many Pharmaceutical companies have embarked on developing drugs that target LRRK2 for the treatment of Parkinson's
- One company termed Denali have initiated clinical trials with two LRRK2 inhibitors termed DNL201 or DNL151
- Many other companies including Merck, GlaxoSmithKline, Biogen, Lundbeck, Sanofi, Vernalis and several others are believed to be very close to entering clinical trails

LRRK2 Inhibitors are being developed by the Pharmaceutical Industry for the treatment of Parkinson's



GSK2578215A

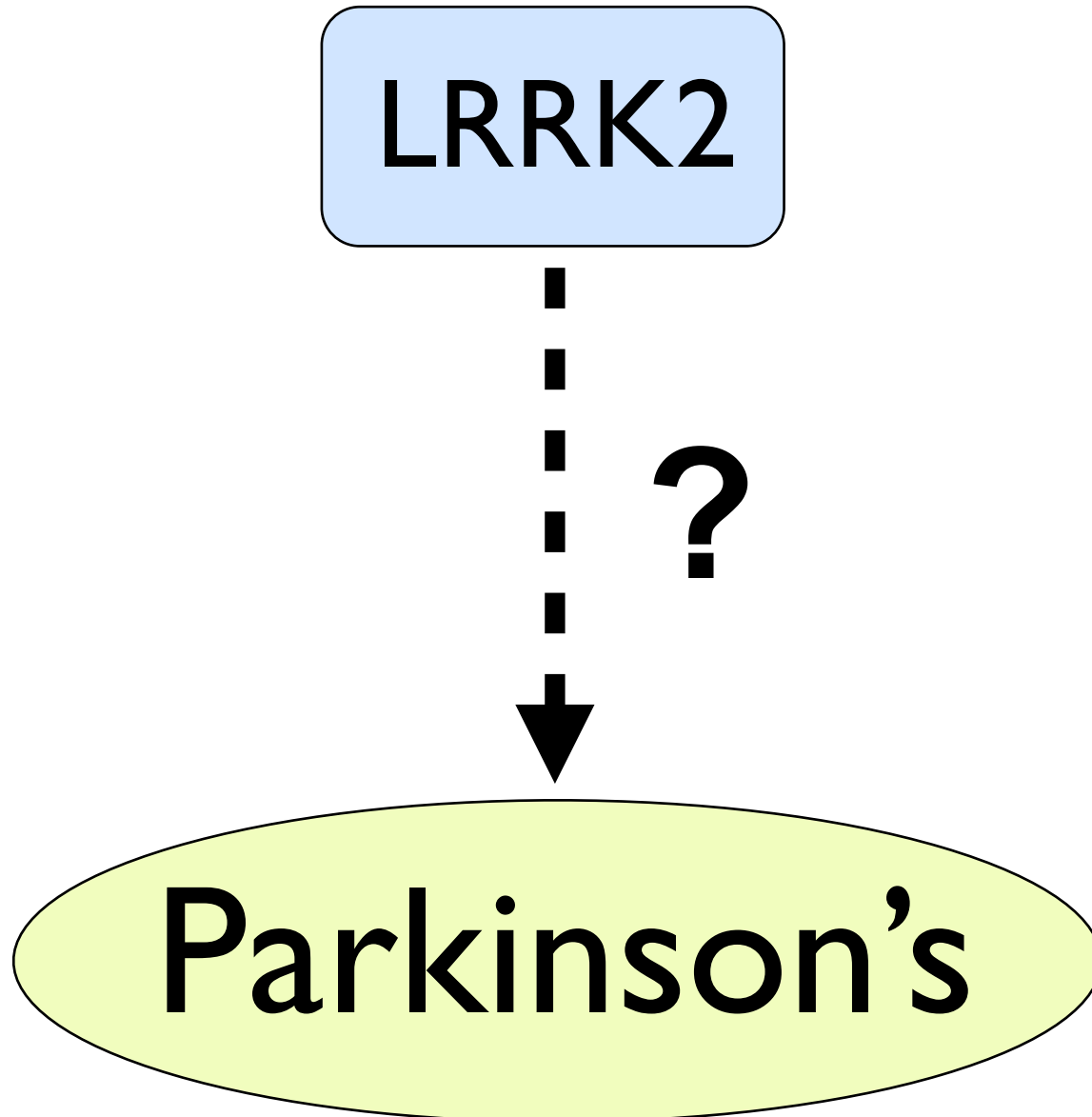
GlaxoSmithKline



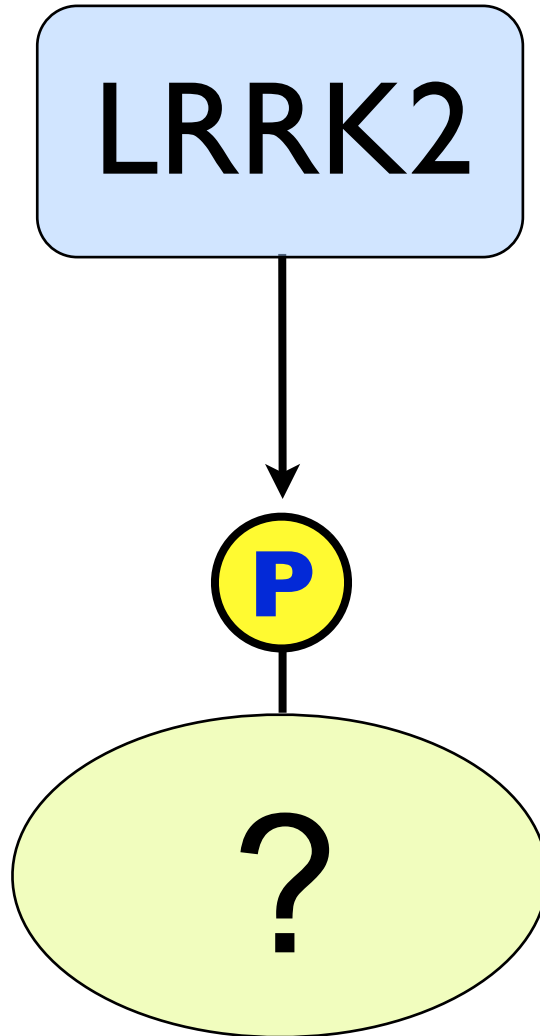
MLI-2

Merck

LRRK2 Research Question



LRRK2 is a Kinase: what does it act on?



The LRRK2 Substrate Team

Team Trost

Matthias Trost – Group Leader

Dimethyl labeled approach to phospho and total proteome analysis

Team Mann

Matthias Mann – Group Leader

SILAC and Label free approach to phospho and total proteome analysis

Team Marto

Jarrold Marto – Group Leader

Global and targeted approach to phospho proteome analysis

Team Alessi

Dario Alessi – Substrate Team Coordinating PI

Validating and characterization of the hits that emerge from the mass spectrometry screens

GlaxoSmithKline

Pharmaceuticals R&D

Alastair Reith (Neurosciences TAU), Graham Duddy (Molecular Discovery Research) and Stephen Wilson (RD Platform Technology & Science)

Genetic LRRK2 mouse models, tool compound, patient-derived cell lines & bioinformatics expertise

Michael J Fox Foundation

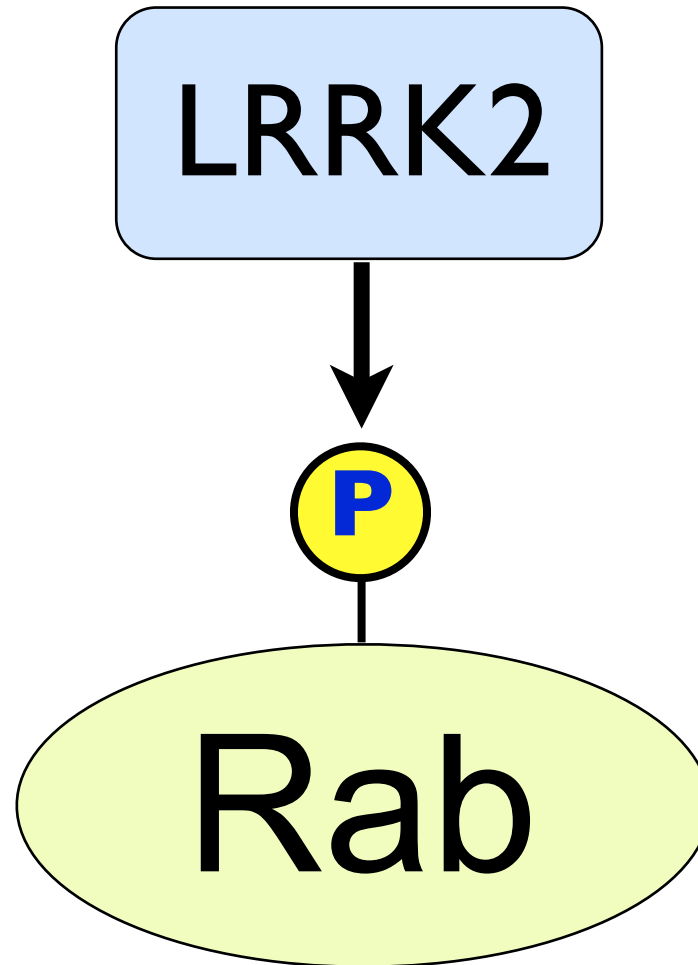
Marco Baptista
Brian Fisk

Merck Research Laboratories

John Morrow
Matthew Kennedy
Matthew Fell

MLi-2 LRRK2 Inhibitor

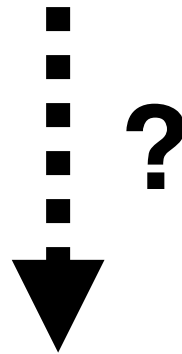
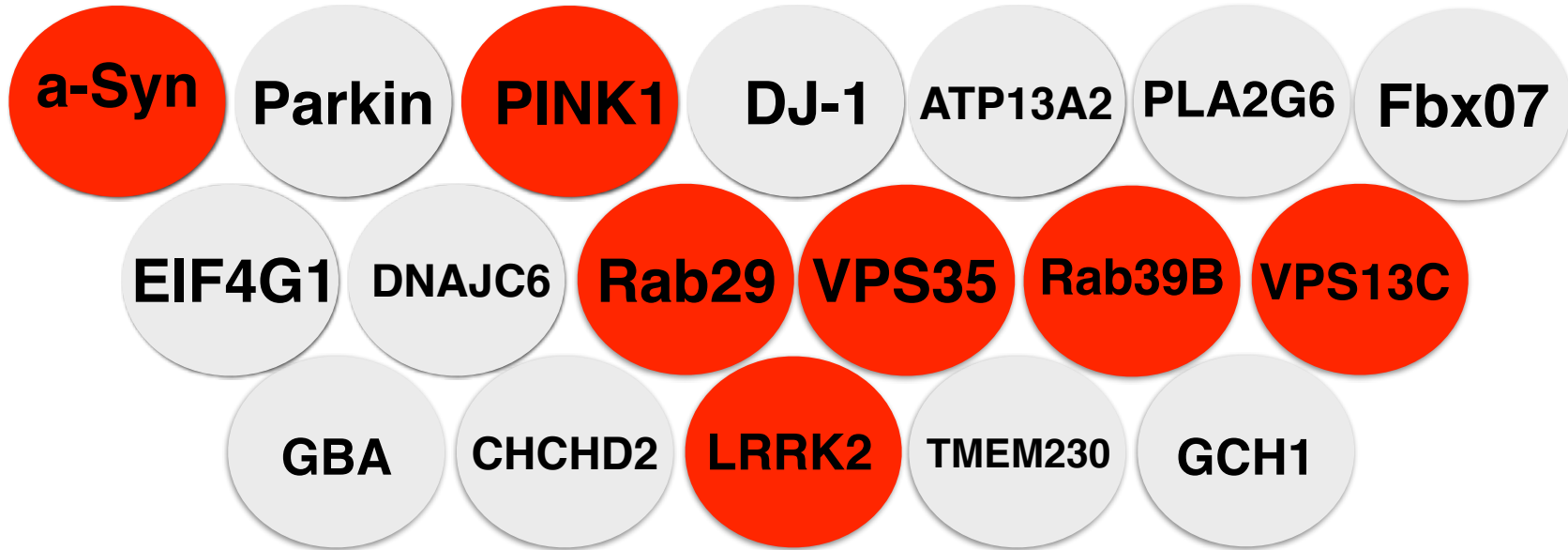
2016 breakthrough we discovered that LRRK2 acts on a class of enzyme called Rabs



Rab proteins are master regulators of cell biology

Previous work has implicated Rab proteins in Parkinson's disease.

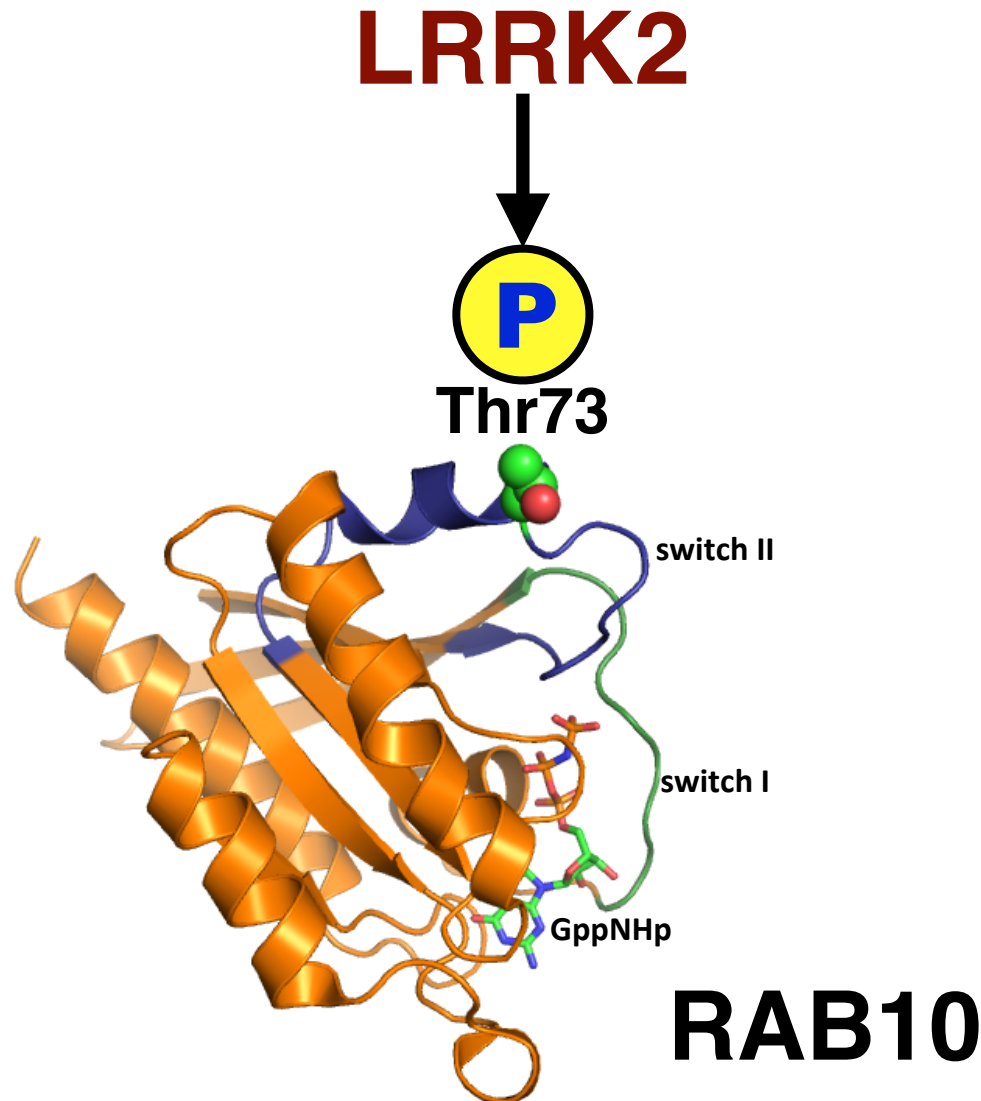
At least 7 Parkinson's genes are involved in Rab biology



Parkinson's

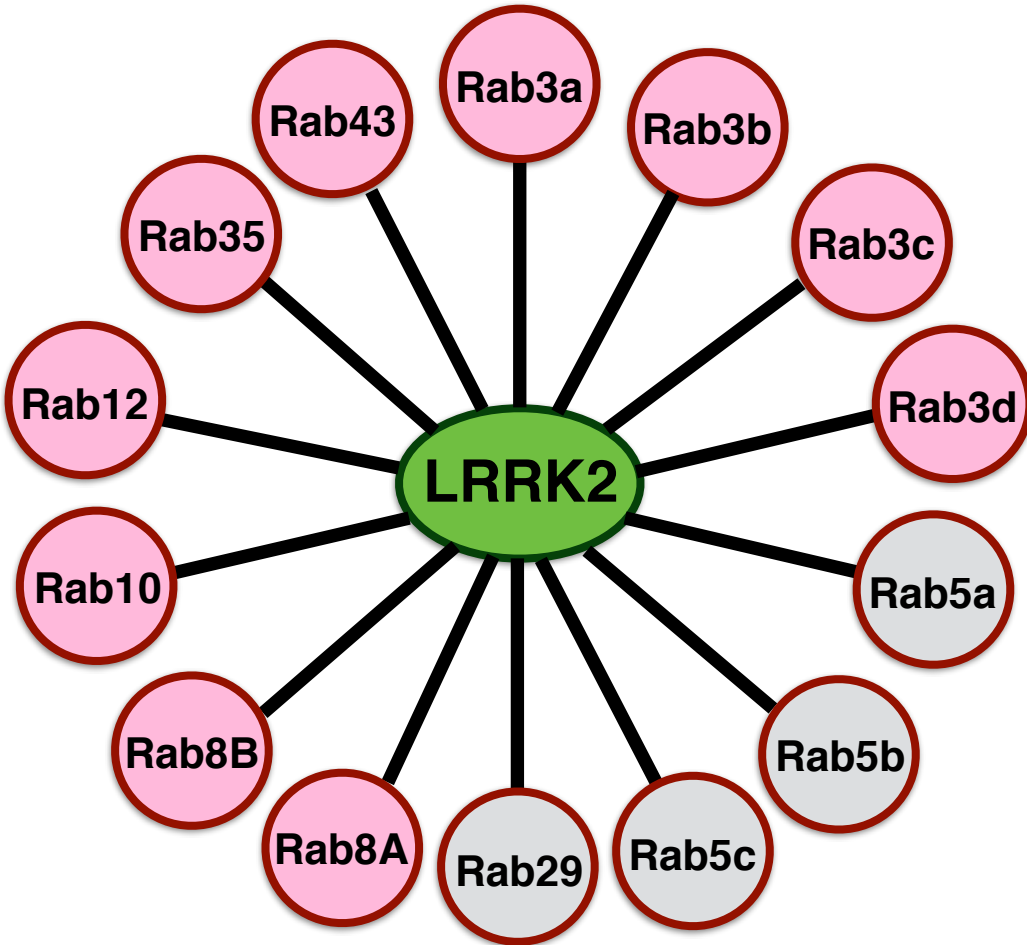
Disruption of Rab biology may lie at
the heart of understanding
Parkinson's Disease

LRRK2 has a major role a regulating Rab proteins



LRRK2 PHOSPHORYLATES UP TO 14 RAB PROTEINS

Definitely phosphorylated

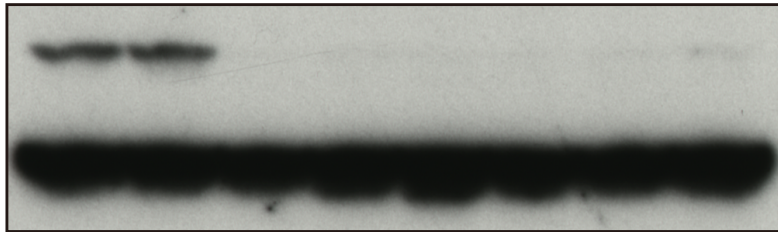


Possibly phosphorylated

New tests to Assess LRRK2 Dugs and Biology

LRRK2 DRUG: - GSK ROCHE MERCK

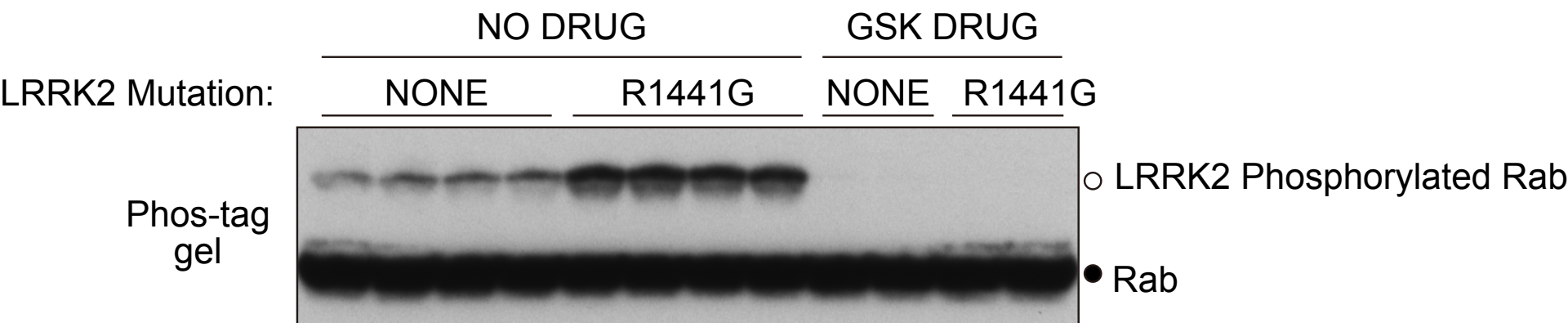
Phos-tag
gel



○ LRRK2 Phosphorylated Rab

● Rab

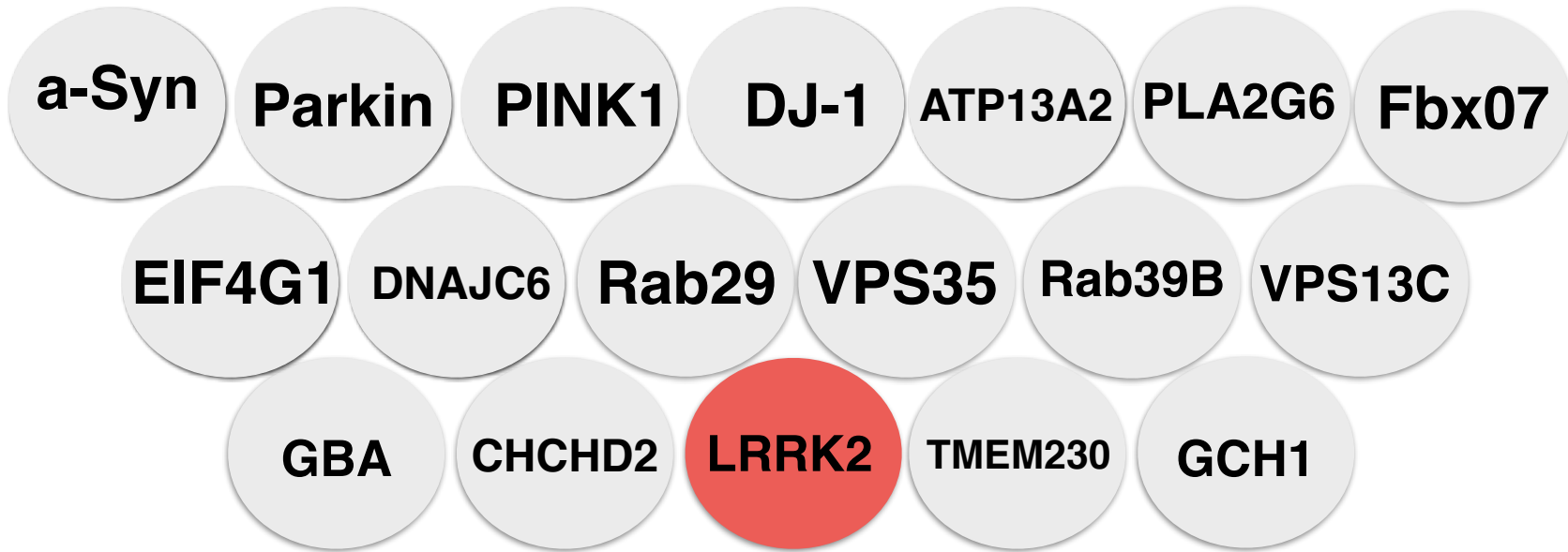
New tests to Assess LRRK2 mutations and whether LRRK2 is switched on in patient cells



Esther Sammler will talk more about new sophisticated LRRK2 tests that allow LRRK2 pathway to be interrogated in humans for the first time

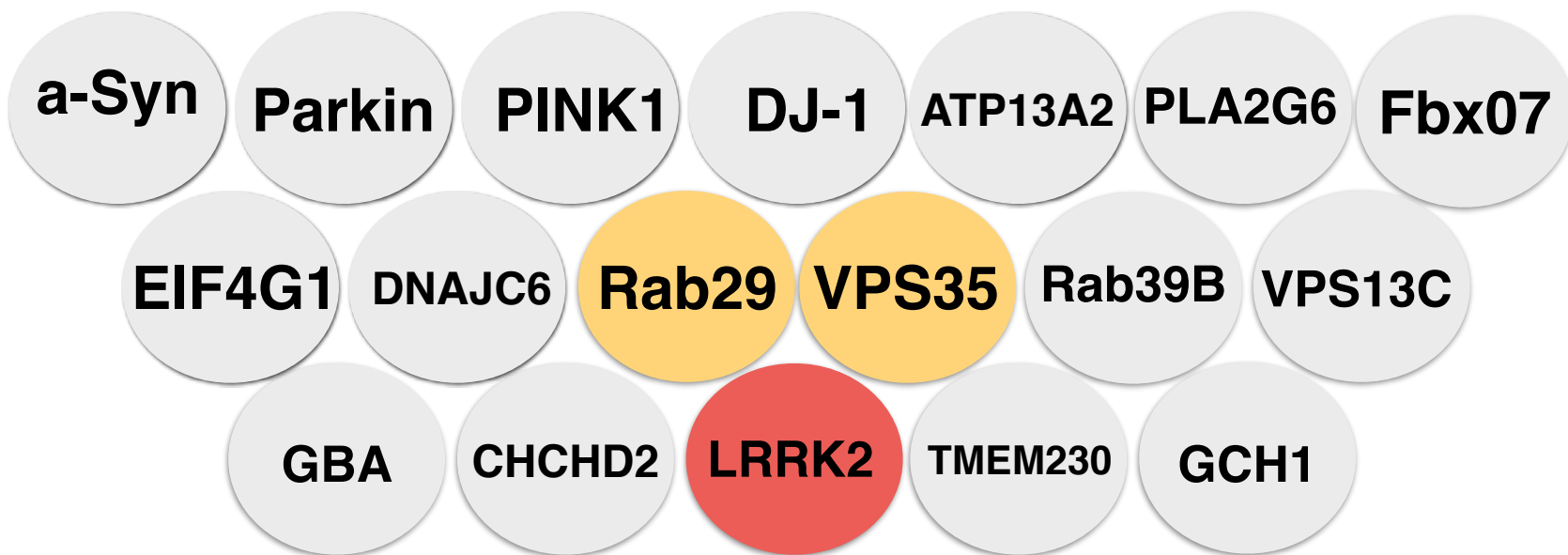
It will be important to identify patients with elevated LRRK2 pathway as these might benefit most from future LRRK2 therapy

Could other Parkinson's genes be connected to LRRK2?



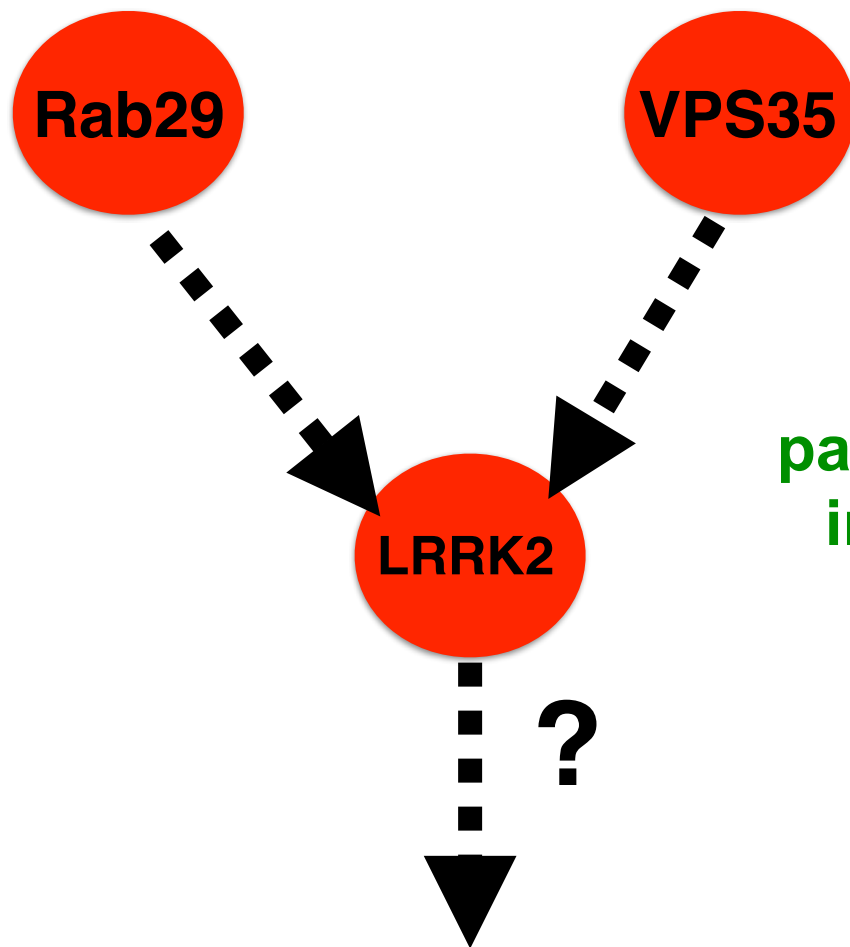
Parkinson's

In recent exciting work we have found that two other Parkinson's genes namely Rab29 and VPS35 are connected to the LRRK2 pathway



Parkinson's

In recent exciting work we have found that two other Parkinson's genes namely Rab29 and VPS35 are connected to the LRRK2 pathway

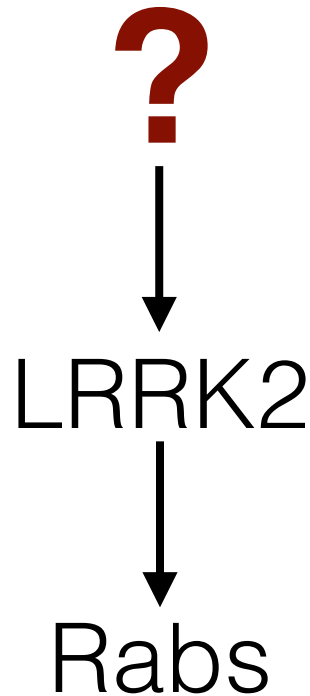


This suggests that patients with mutations in VPS35 and Rab29 might benefit from LRRK2 inhibitors

Parkinson's

Outstanding questions that we are
working hard to address

What Switches LRRK2 on ?



We are obtaining striking preliminary data that in immune cells infect with pathogenic bacteria or yeast may activate LRRK2. This may point to a role of the LRRK2 enzyme in regulating immune response and perhaps neuro-inflammation

What Biology does Rabs Control?

LRRK2

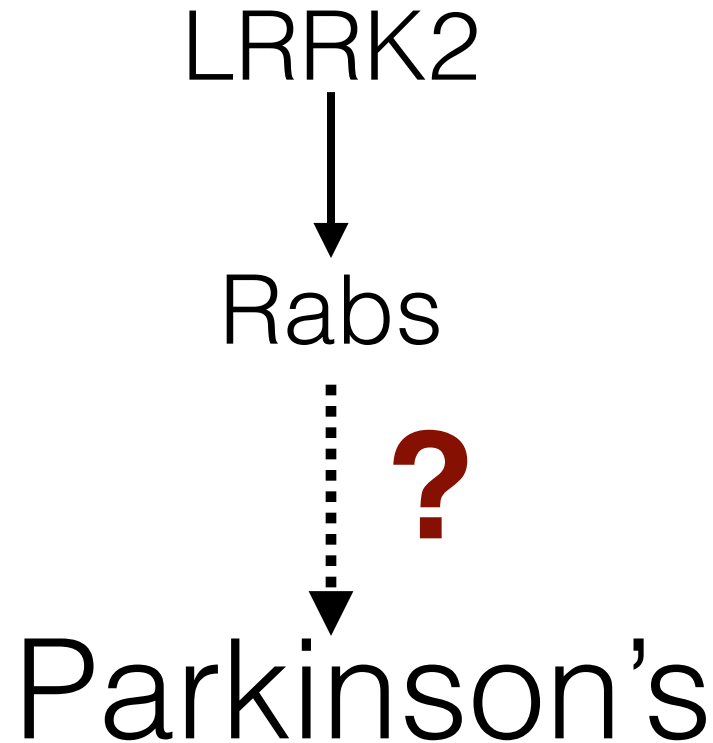


Rabs

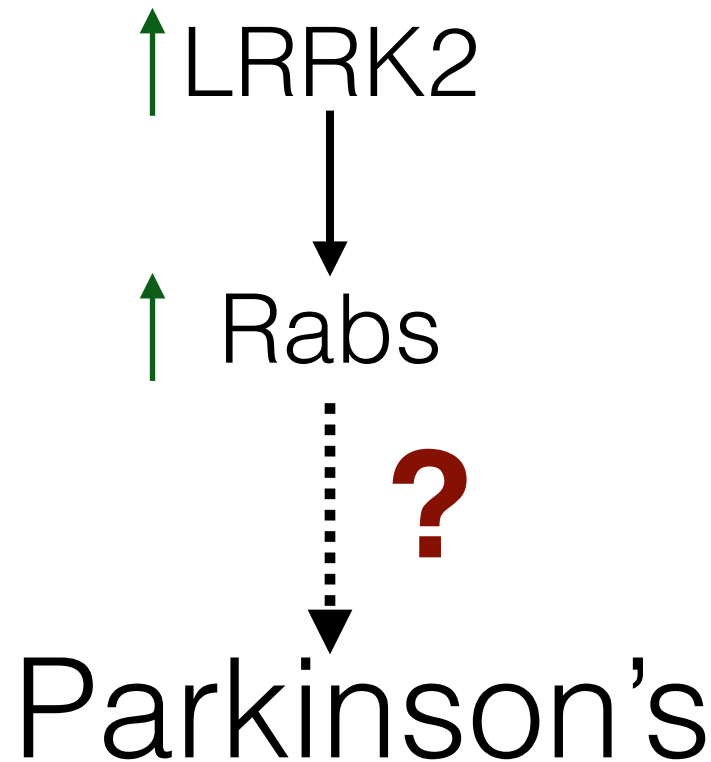


We are identifying novel proteins that are controlled by LRRK2 phosphorylated Rab proteins such as “RILPL1 and RILPL2”

How does LRRK2 disrupt RAB Biology to Cause Parkinson's?

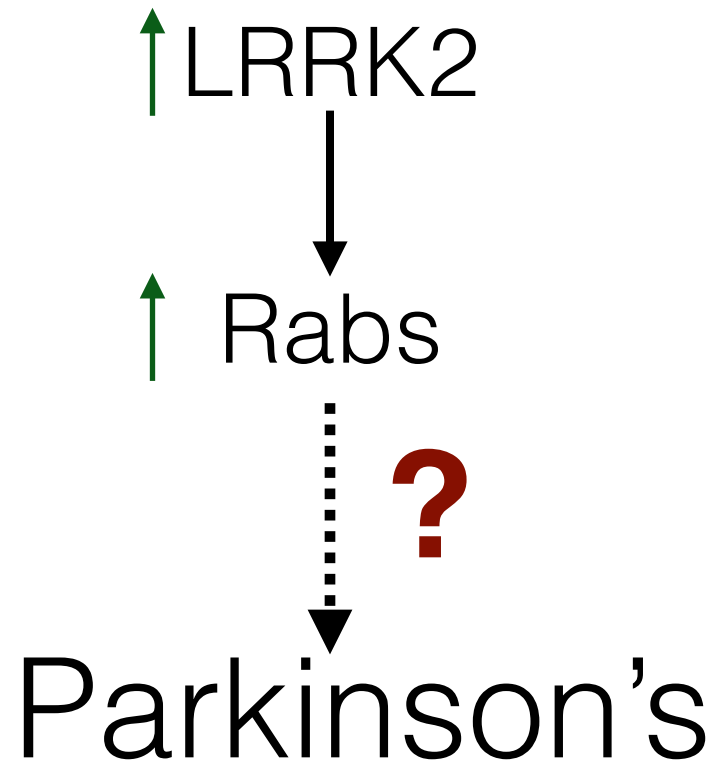


Is LRRK2 and Rab biology disrupted in patients with non-genetic (sporadic) Parkinson's ?



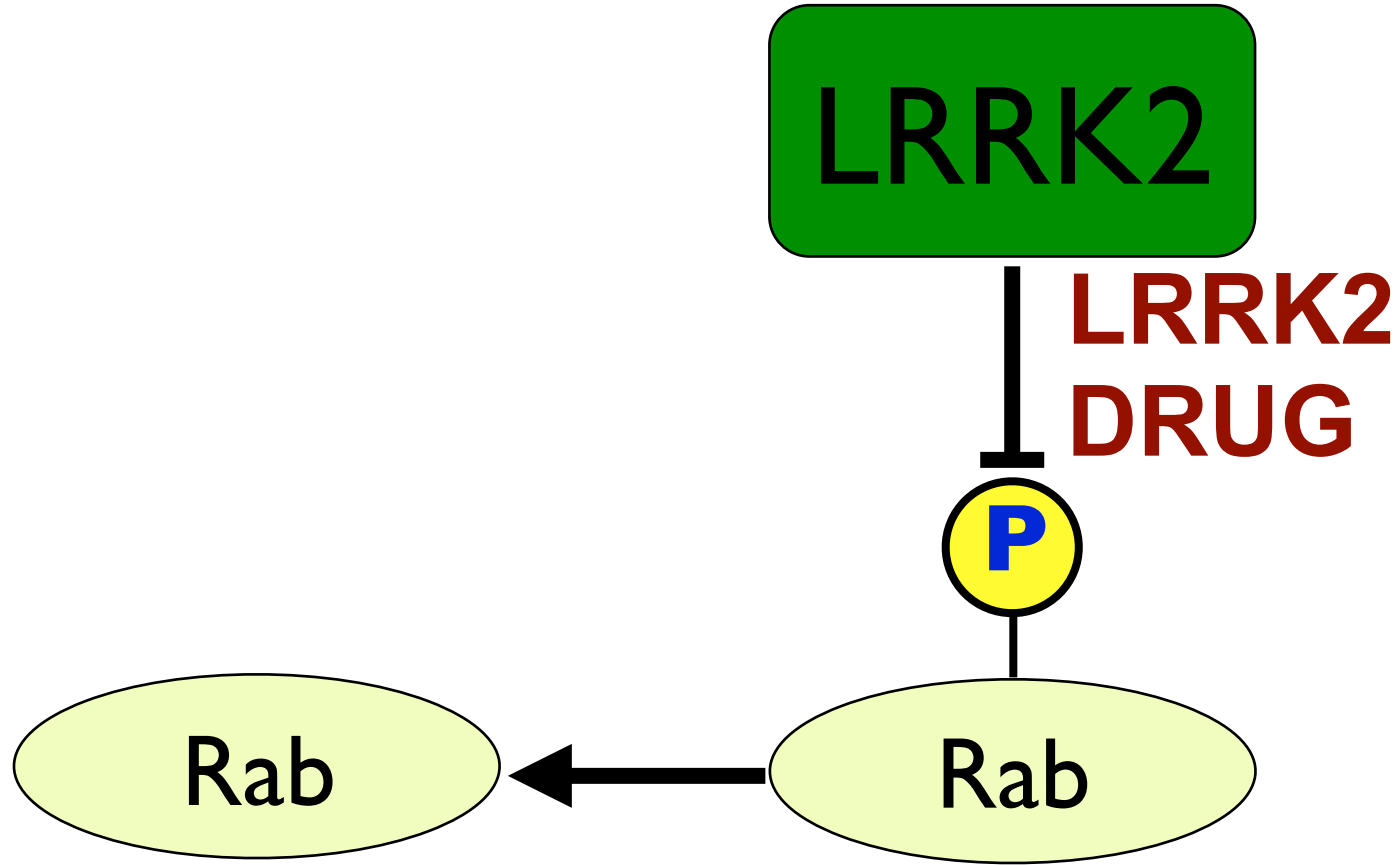
Vital question as it will determine whether or not patients with sporadic Parkinson's might benefit from future LRRK2 therapies

Is LRRK2 and Rab biology disrupted in patients with non-genetic (sporadic) Parkinson's ?



Vital question as it will determine whether or not patients with sporadic Parkinson's might benefit from future LRRK2 therapies

Would LRRK2 kinase inhibitors slow down or even halt progression of Parkinson's ?



LRRK2 inhibitors have the potential to also prevent the onset of Parkinson's. More work is needed to pre-diagnose the patients that are likely to develop Parkinson's before key symptoms emerge



ACKNOWLEDGEMENTS

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Brian Fiske

Max Plank

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Martin Steger
Esben Lorentzen
Stefanie Wachter
Melanie Vetter

Merck

Matthew J. Fell
John A. Morrow

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FOX PARKINSON'S
RESEARCH



All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals



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