Parkinson's Disease

6 million afflicted worldwide

Symptoms

Bradykinesia (slowed ability to start and continue movements)

Resting tremor

Postural rigidity

Well known individuals with Parkinson's

Michael J Fox

Muhammad Ali

Janet Reno

Linda Ronstadt

Mao Zedung

Eugene McCarthy

Billy Graham

Pope John Paul II

Most cases are idiopathic but inherited forms are known as well

7 genes known, including α-synuclein and parkin

In patients, large enclosures termed Lewy Bodies are seen in neurons. The most common protein in the LBs is α-syn, present as large aggregates

Studies with model organisms have shown that α-syn toxicity is associated with defects in vesicle transport, mitochondrial function, and lipid/sterol biosynthesis

7 genes associated with familial parkinsonism

First gene, α-synuclein, found in Italian kindred and three unrelated Greek families.

Missense mutation A53T

α-synuclein is a presynaptic protein. It is a major constituent of Lewy Bodies seen in idiopathic cases

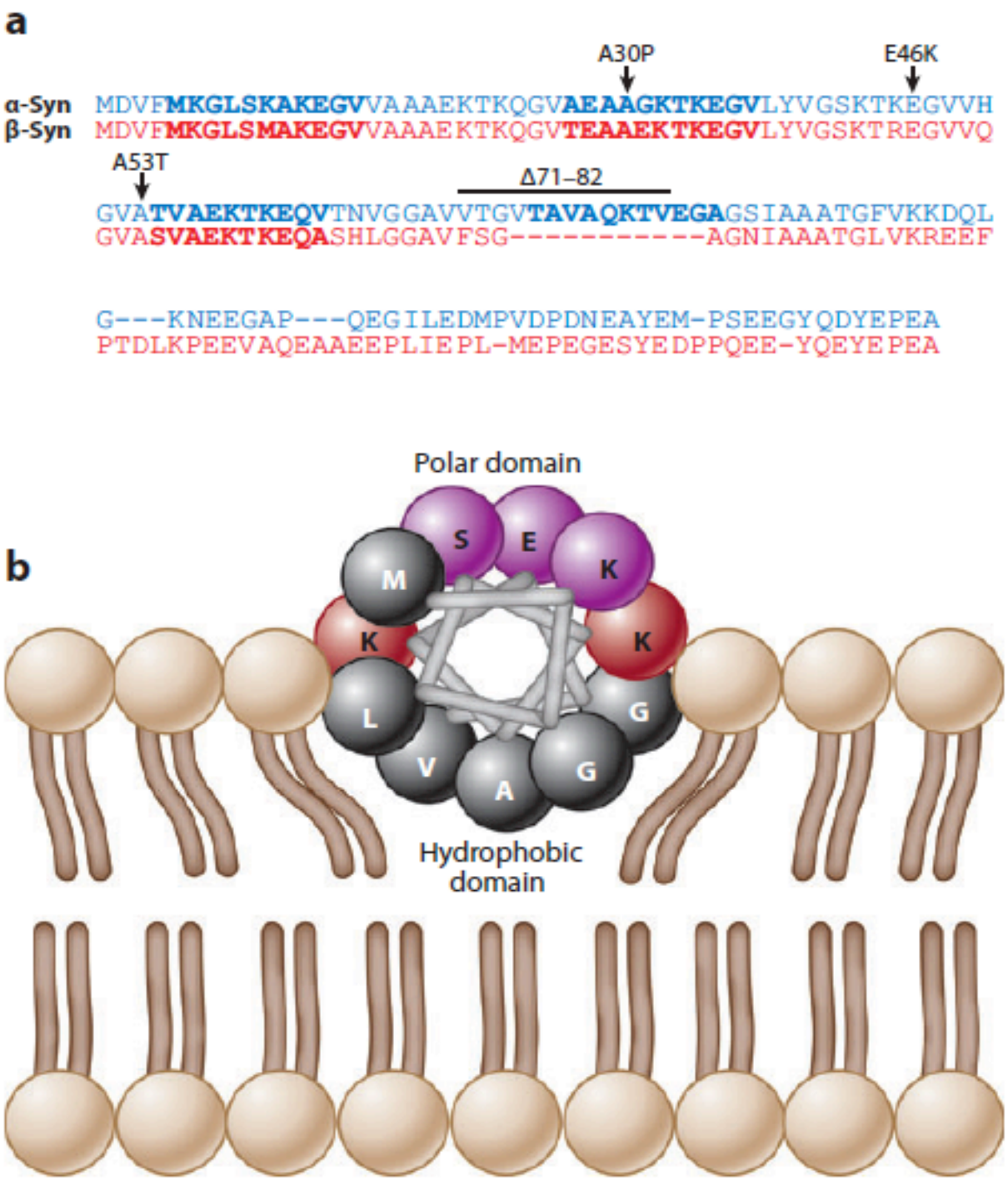
At least 6 other genes, including *Parkin* and *PINK1*

These two genes appear to be involved in sensing oxidative stress and regulating mitochondrial dynamics

α-syn associates with membranes and mutations alter its membrane properties

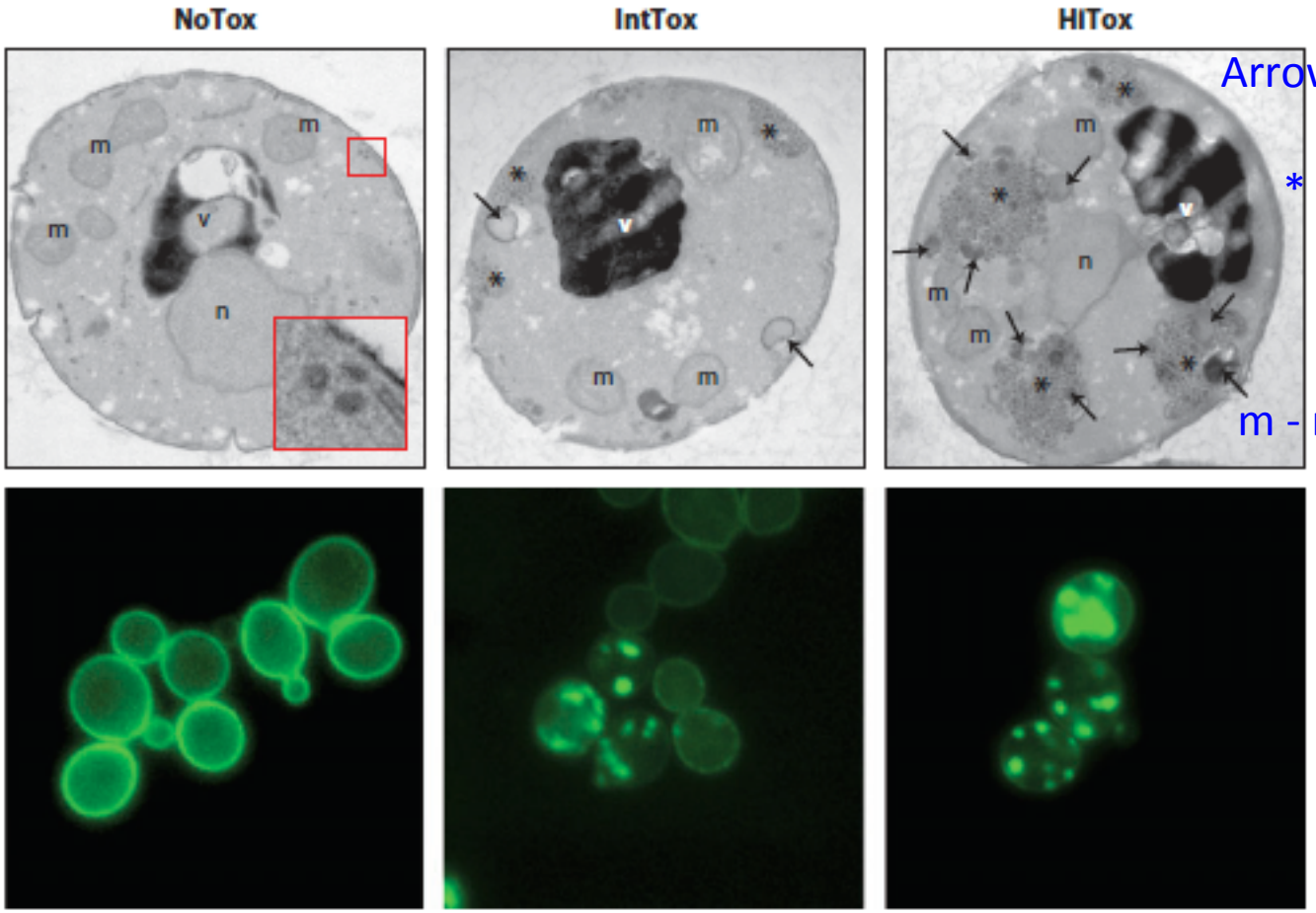
A53T A30P E46K

Part of the protein can form an amphipathic helix that allows membrane association; illustrated on next slide


a
A30P
E46K
α-Syn MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVH
β-Syn MDVFMKGLSMAKEGVVAAAEKTKQGVTEAAEKTKEGVLYVGSKTREGVVQ
A53T
Δ71–82
GVATVAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFVKKDQL
GVASVAEKTKEQASHLGGAVFSG----------AGNIAAATGLVKREEF
G---KNEEGAP---QEGILEDMPVDPDNEAYEM-PSEEGYQDYEPEA
PTDLKPEEVAQEAAEEPLIEPL-MEPEGESYEDPPQEE-YQEYEPEA
b
Polar domain
S
E
K
M
K
K
L
G
V
A
G
Hydrophobic domain

Model organisms used to investigate α-syn biology and toxicity

In yeast, put α-syn-GFP under control of the *GAL* promoter



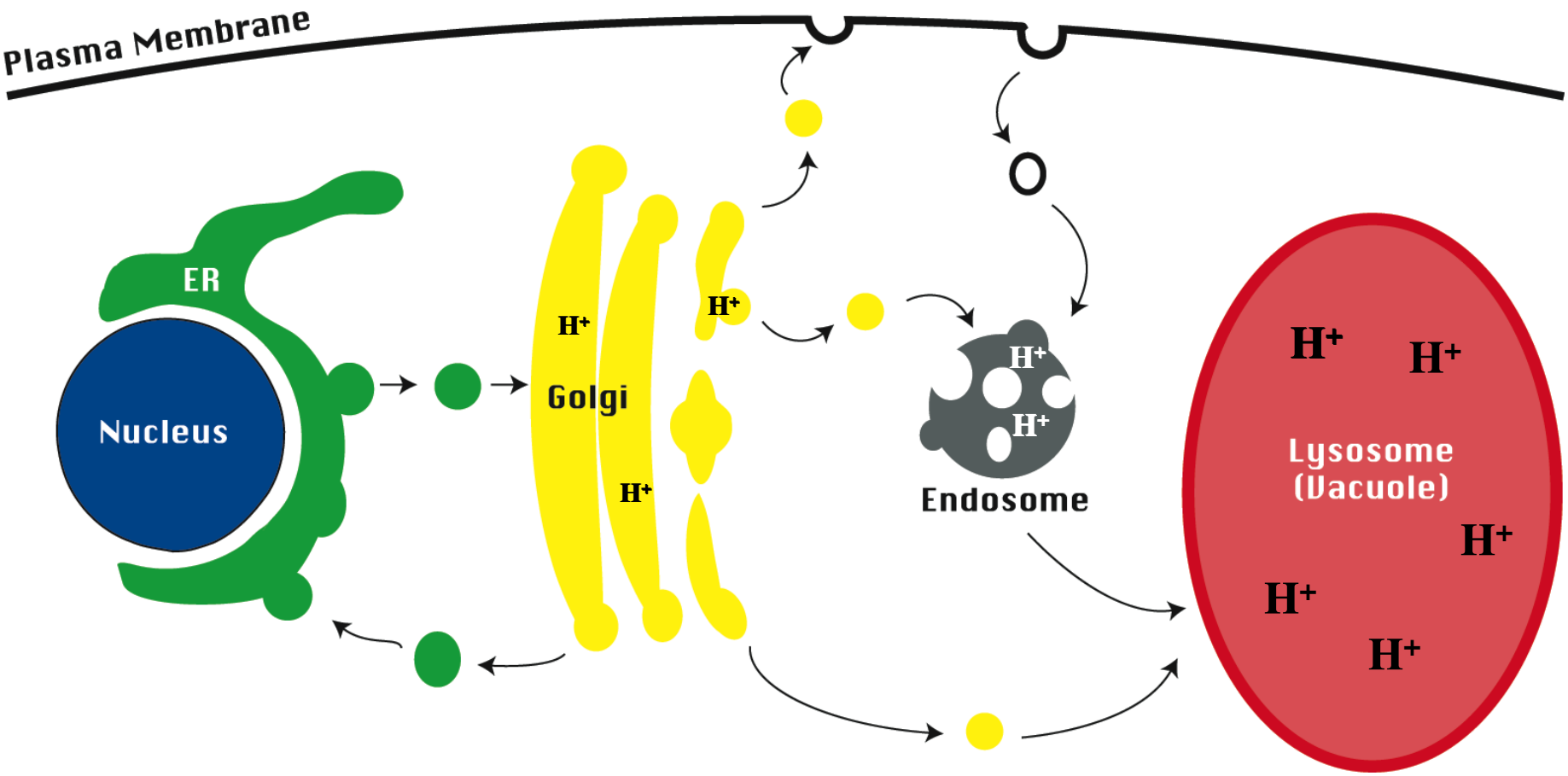
Arrow - lipid droplet

* -stalled vesicle

n - nucleus

m - mitochondrion

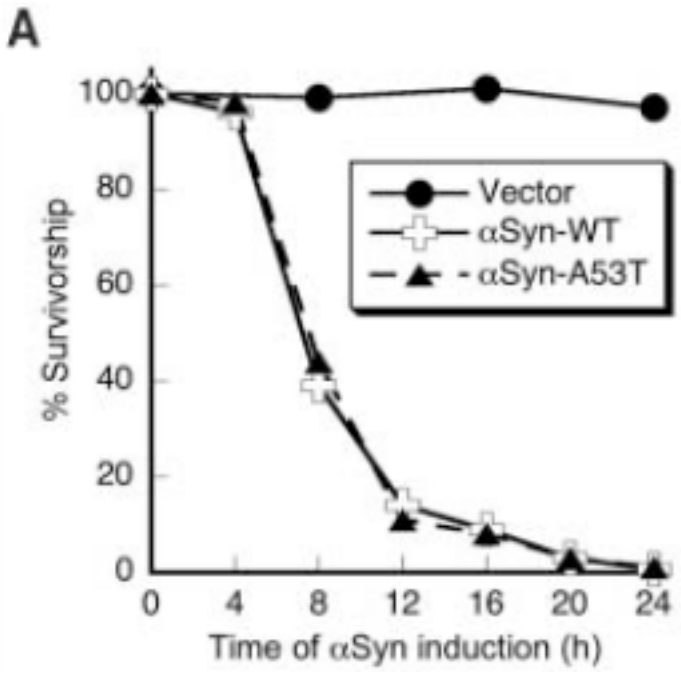
Secretory Pathway in Yeast

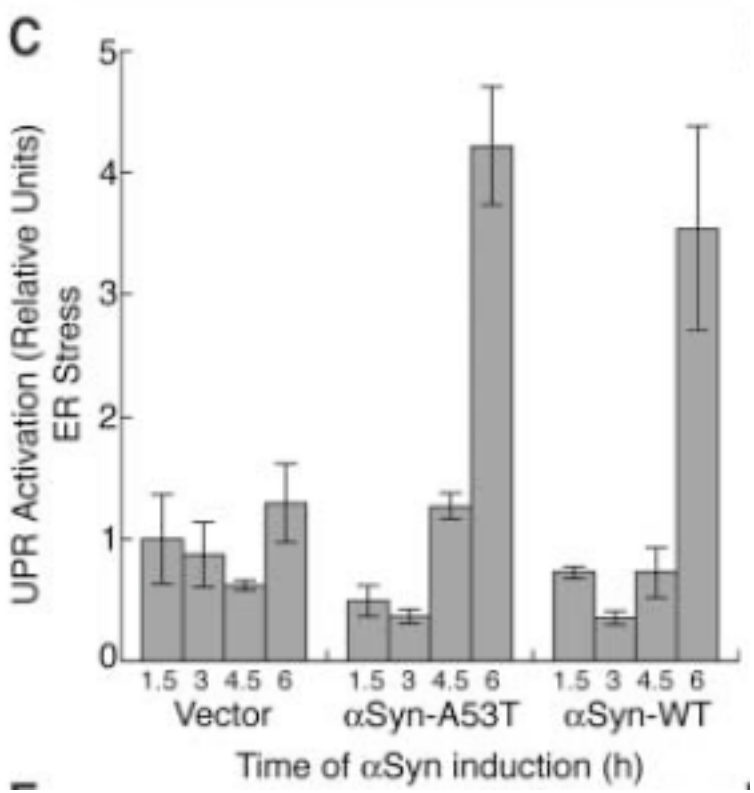


Summary of phenotypes

	NoTox	IntTox	HiTox
α-Syn localization	Membraneous	Membraneous Small foci	Large foci
Growth rate	Normal	Decreased	No growth
Vesicle accumulation	Mild	Moderate	High
ER-to-Golgi complex trafficking defect	Absent	Present	Present
Mitochondrial defects	None	Low	High
Lipid droplet accumulation	Absent	Rare	Present

α-syn expression induces toxicity, ER stress, and vesicle trafficking defects in yeast

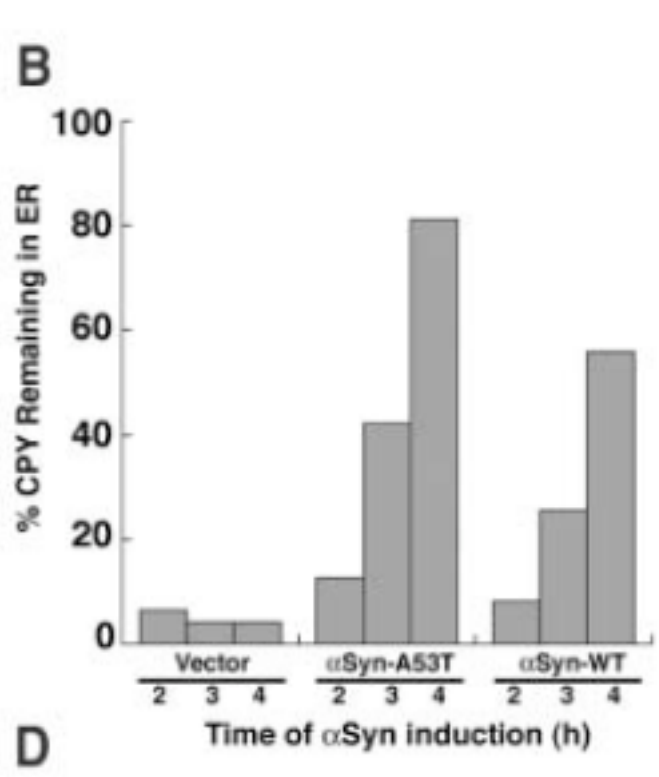


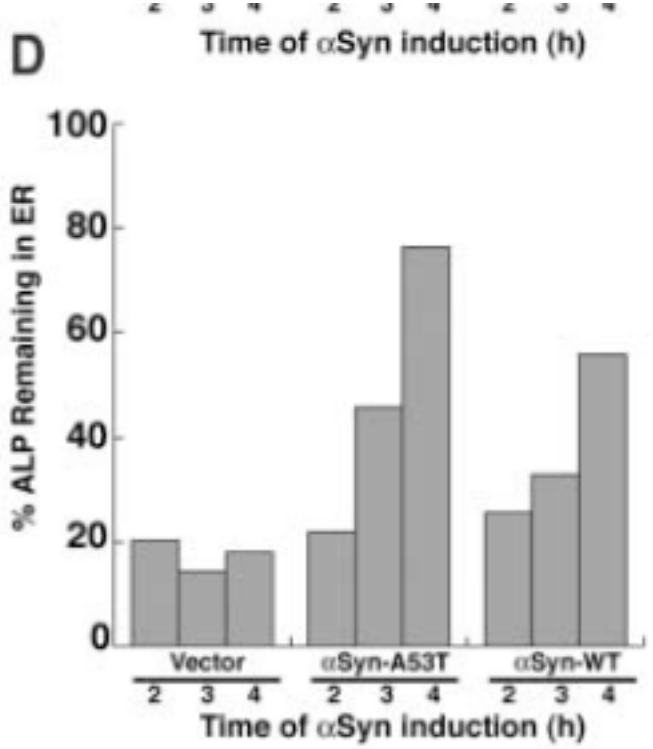


toxicity

ER stress

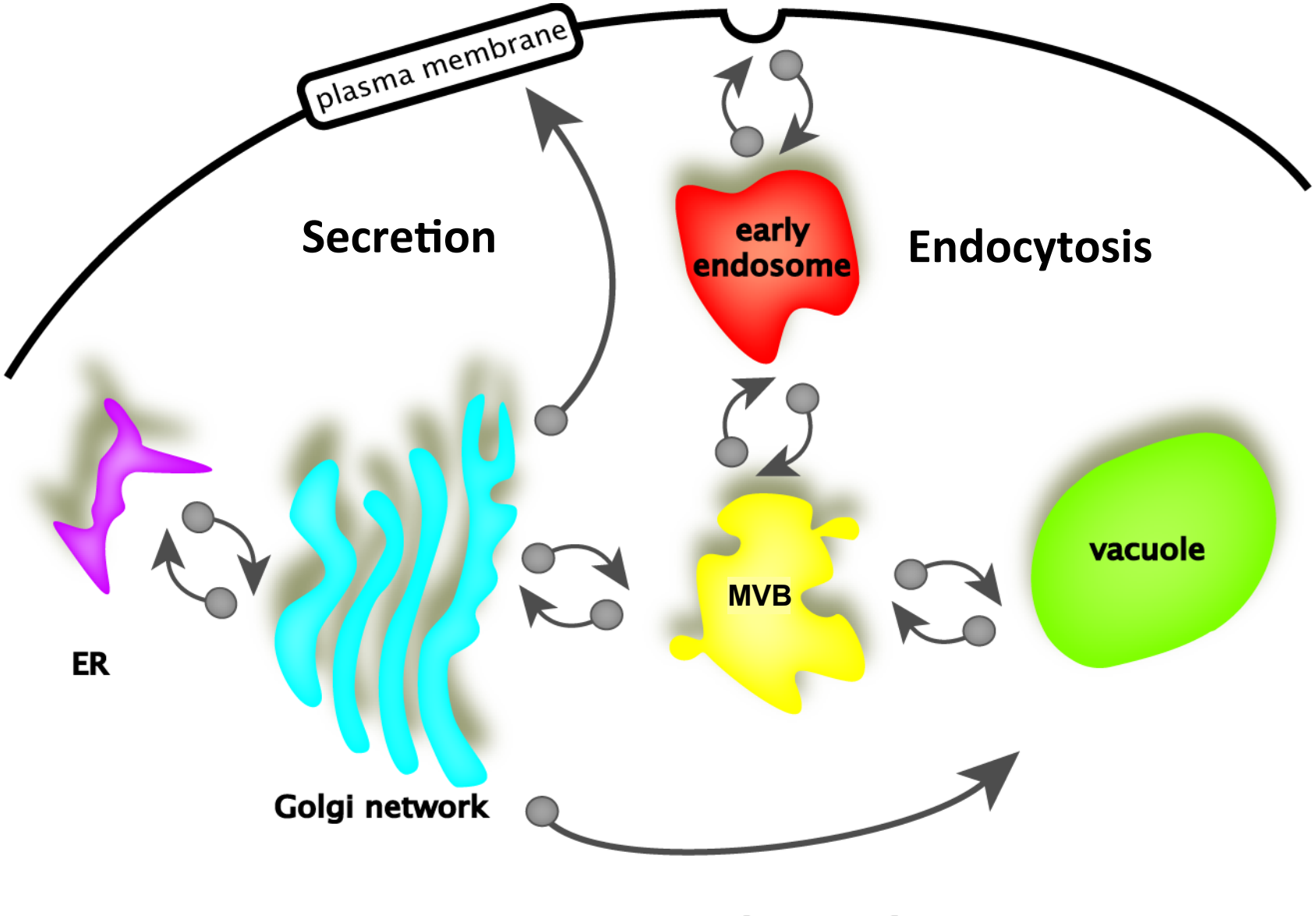
Vesicle trafficking defects



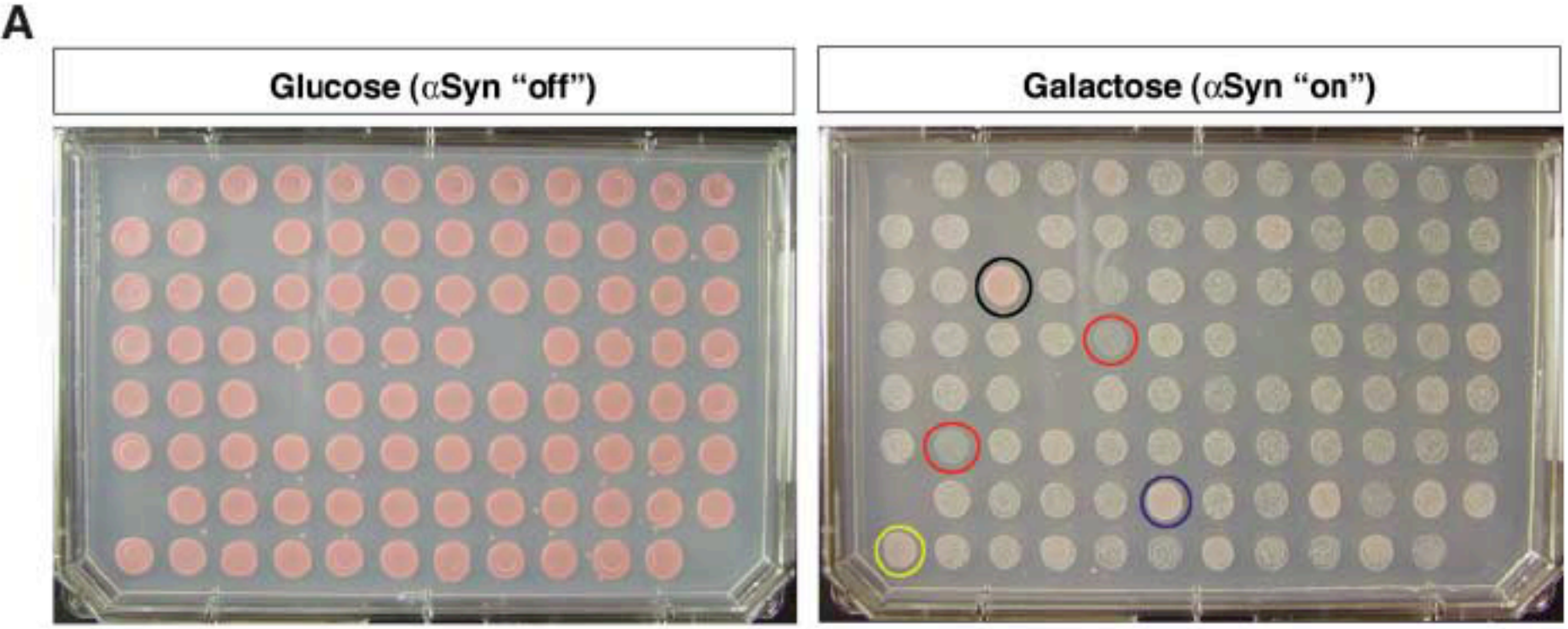


CPY and ALP are vacuolar proteins

Secretory Pathway in Yeast



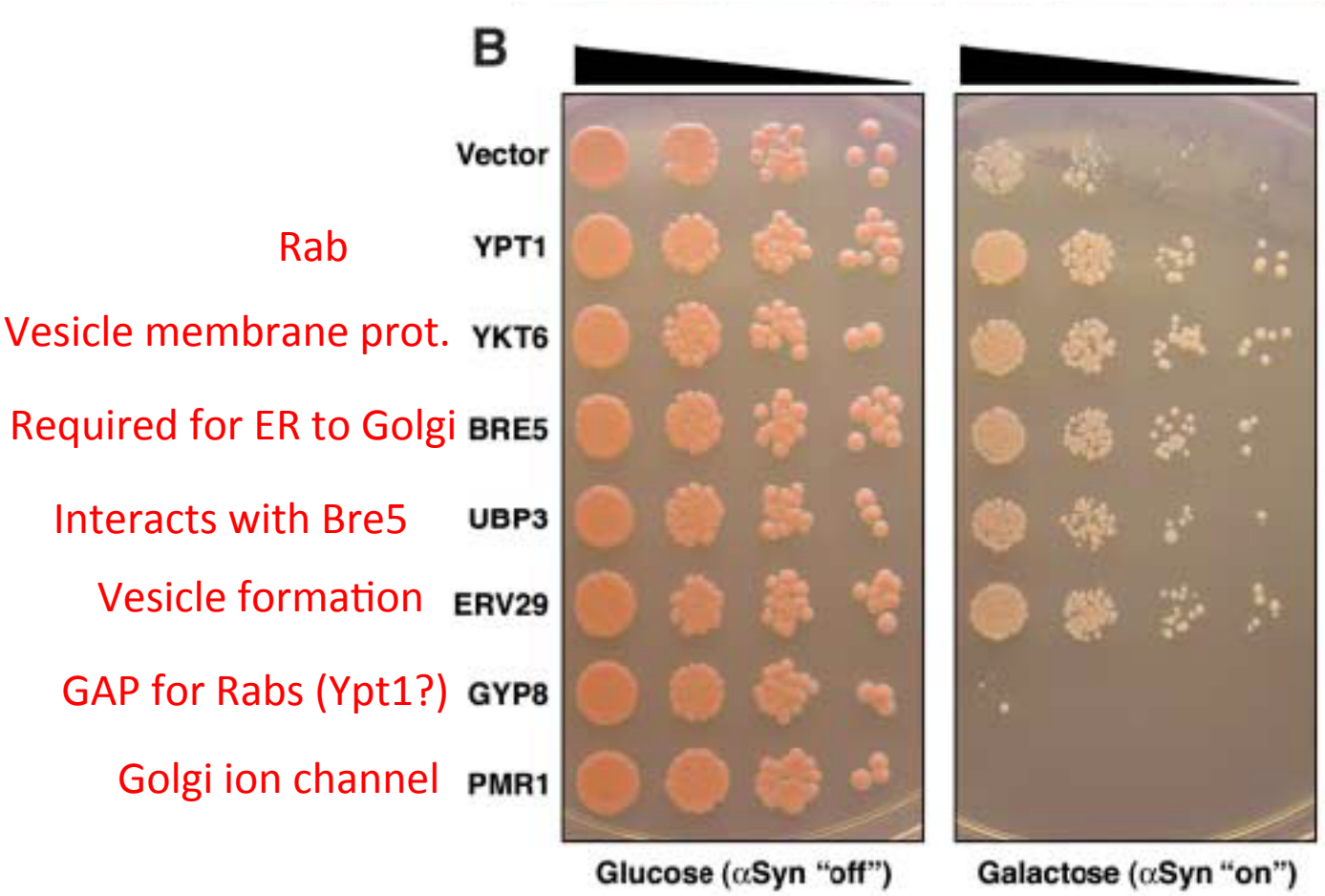
Screen for genes that overcome α-syn toxicity



Black circles – suppressors of toxicity

Red circles – enhancers of toxicity

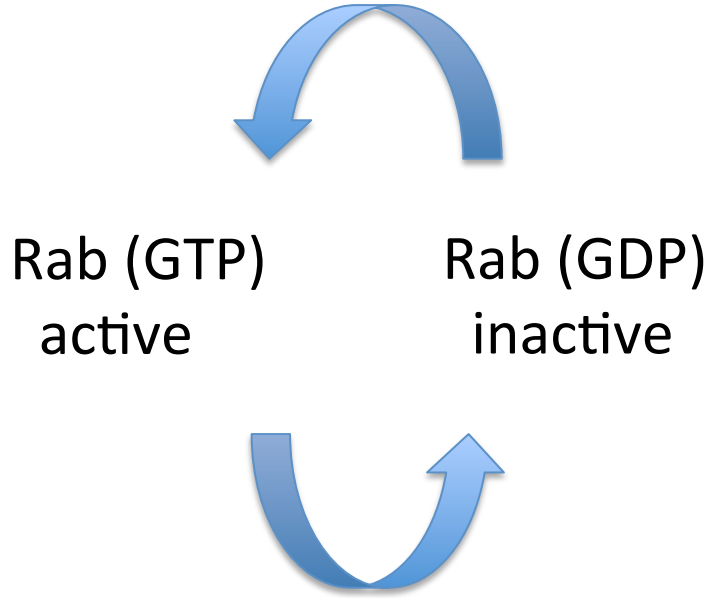
Overexpression of genes involved in ER to Golgi transport suppresses toxicity



Rabs are members of the Ras super family of p21 GTPases

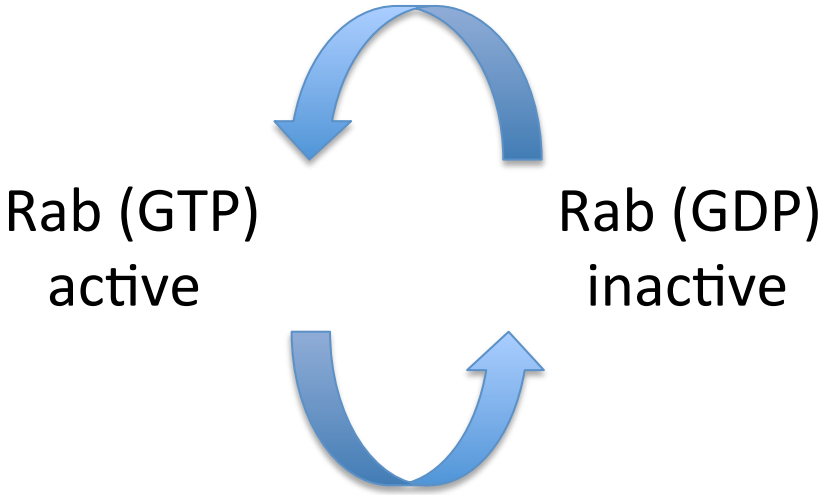
Molecular switches

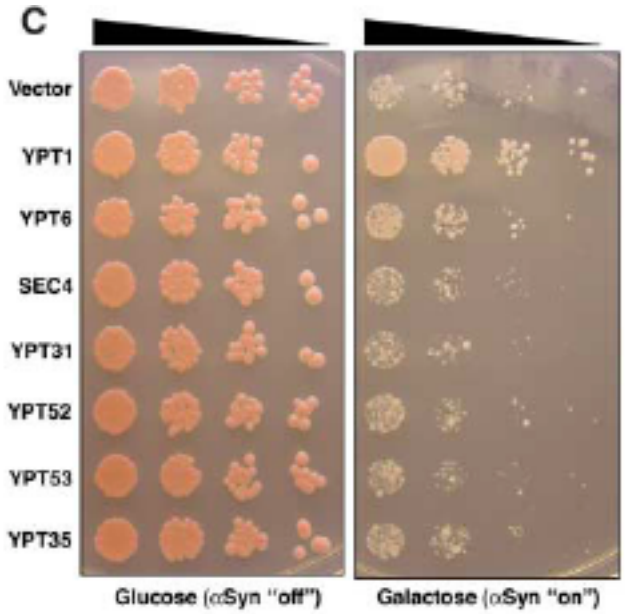
Exchange catalyzed by Guanine Nucleotide Exchange Factor (GEF)



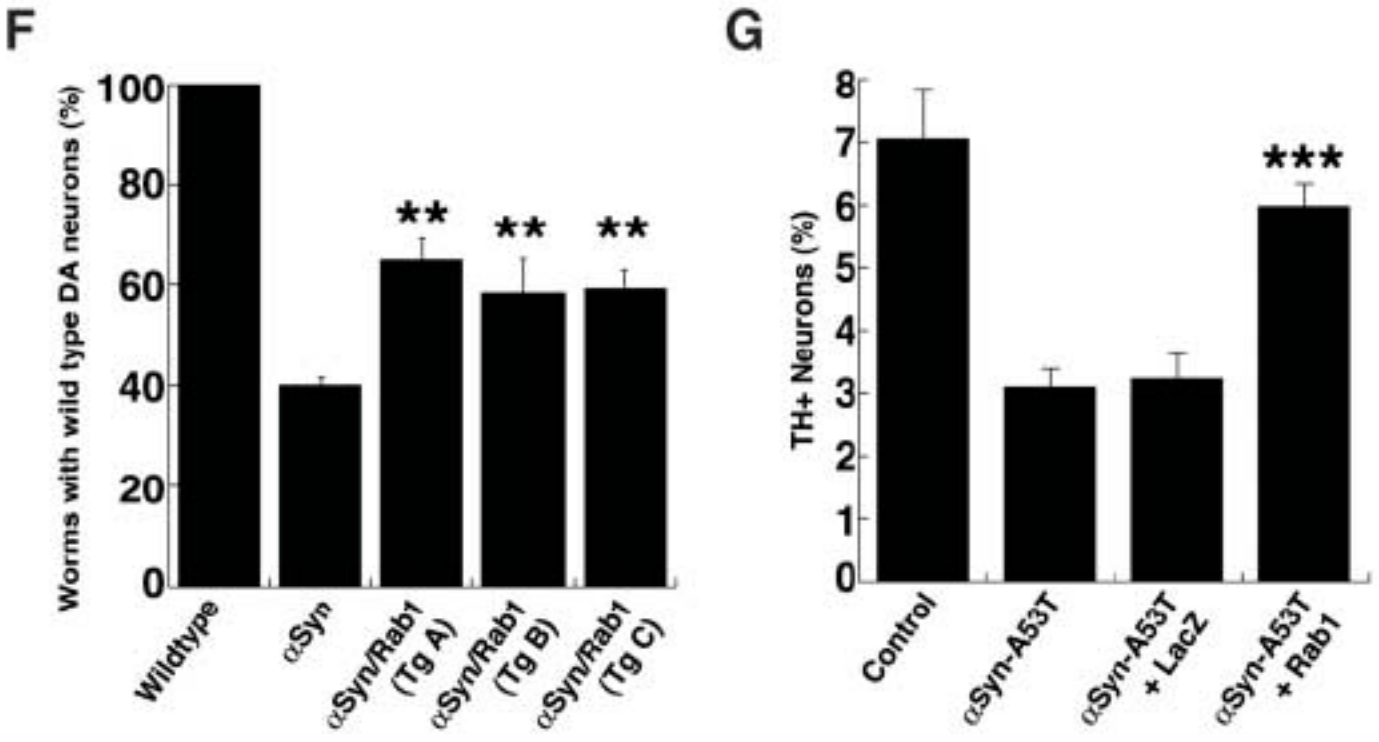
Activity stimulated by GTPase Activating Protein (GAP)

Among yeast Rabs, only YPT1, the ER to Golgi Rab, suppresses





Overexpression of Rab1 suppresses neuron defects in worms and mice

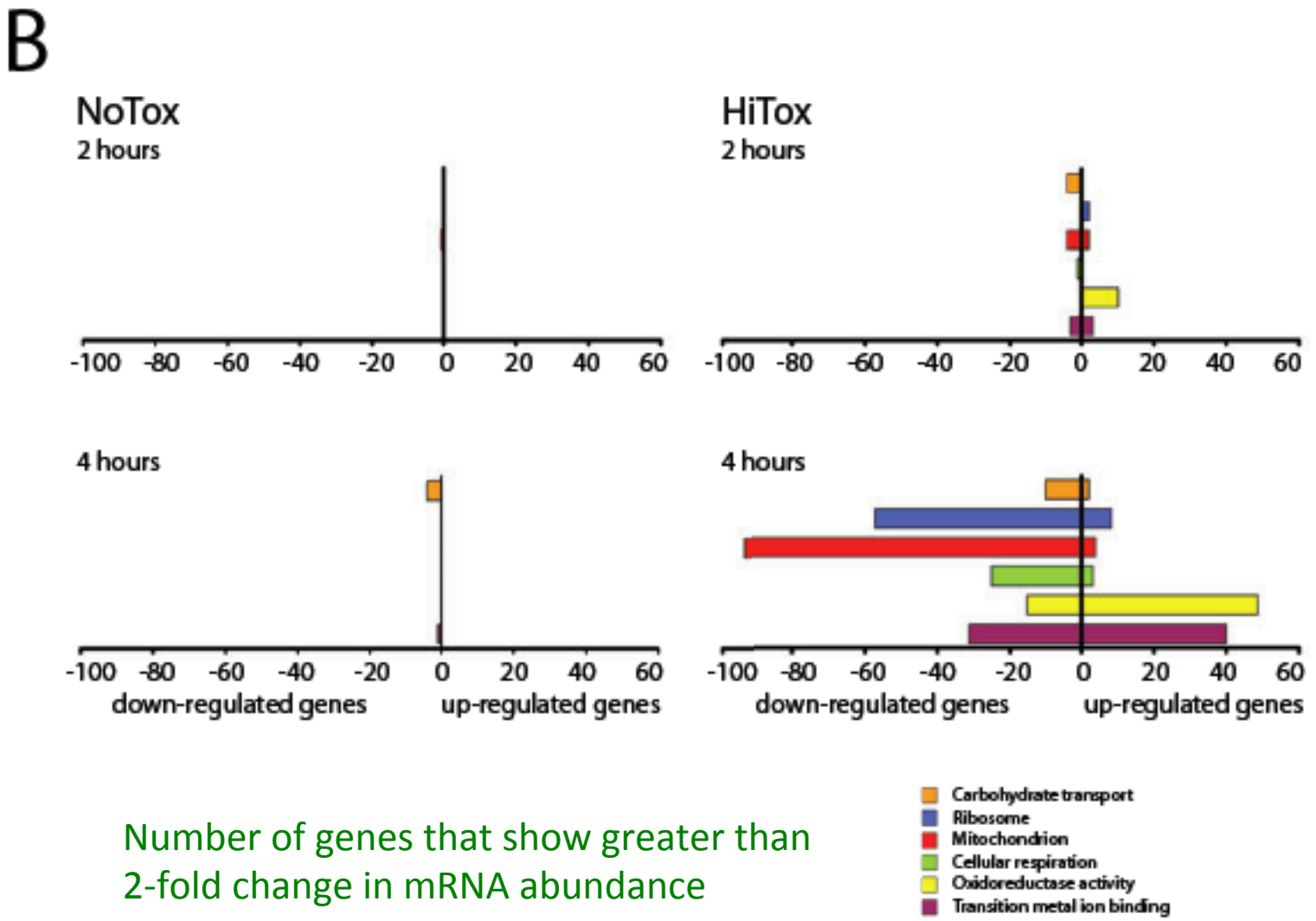


worms

mice

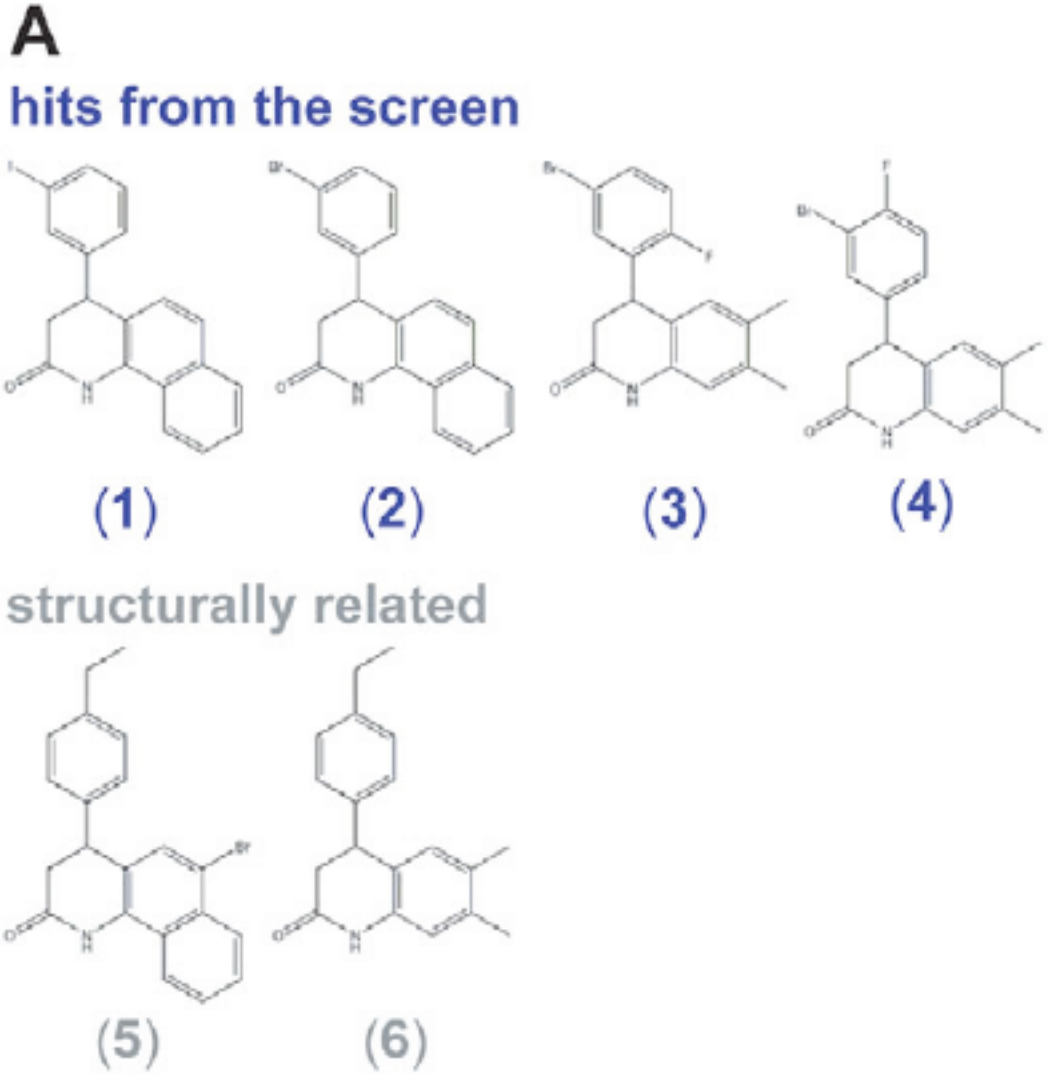
Express α-syn alone or with Rab1 in dopamine neurons

Transcription profiling reveals changes in mitochondrial function

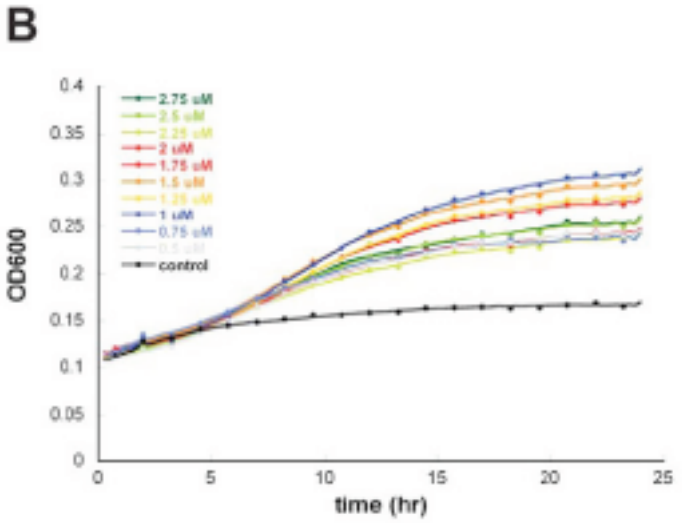


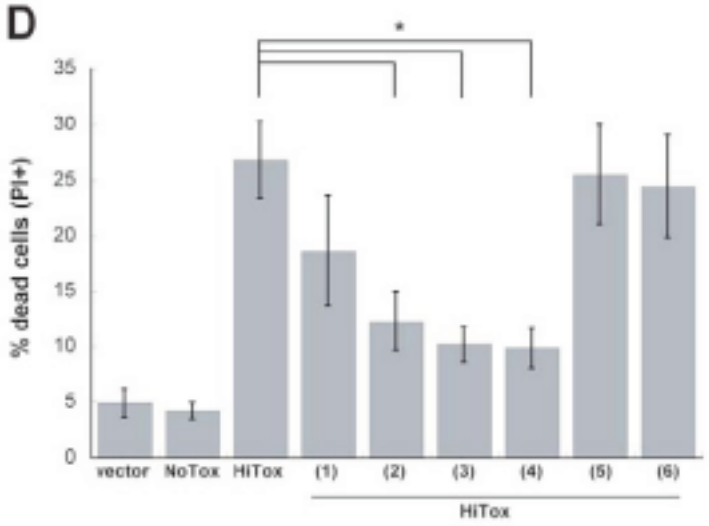
Number of genes that show greater than 2-fold change in mRNA abundance

High-throughput chemical screen for suppressors of α-syn toxicity (115,000 compounds)



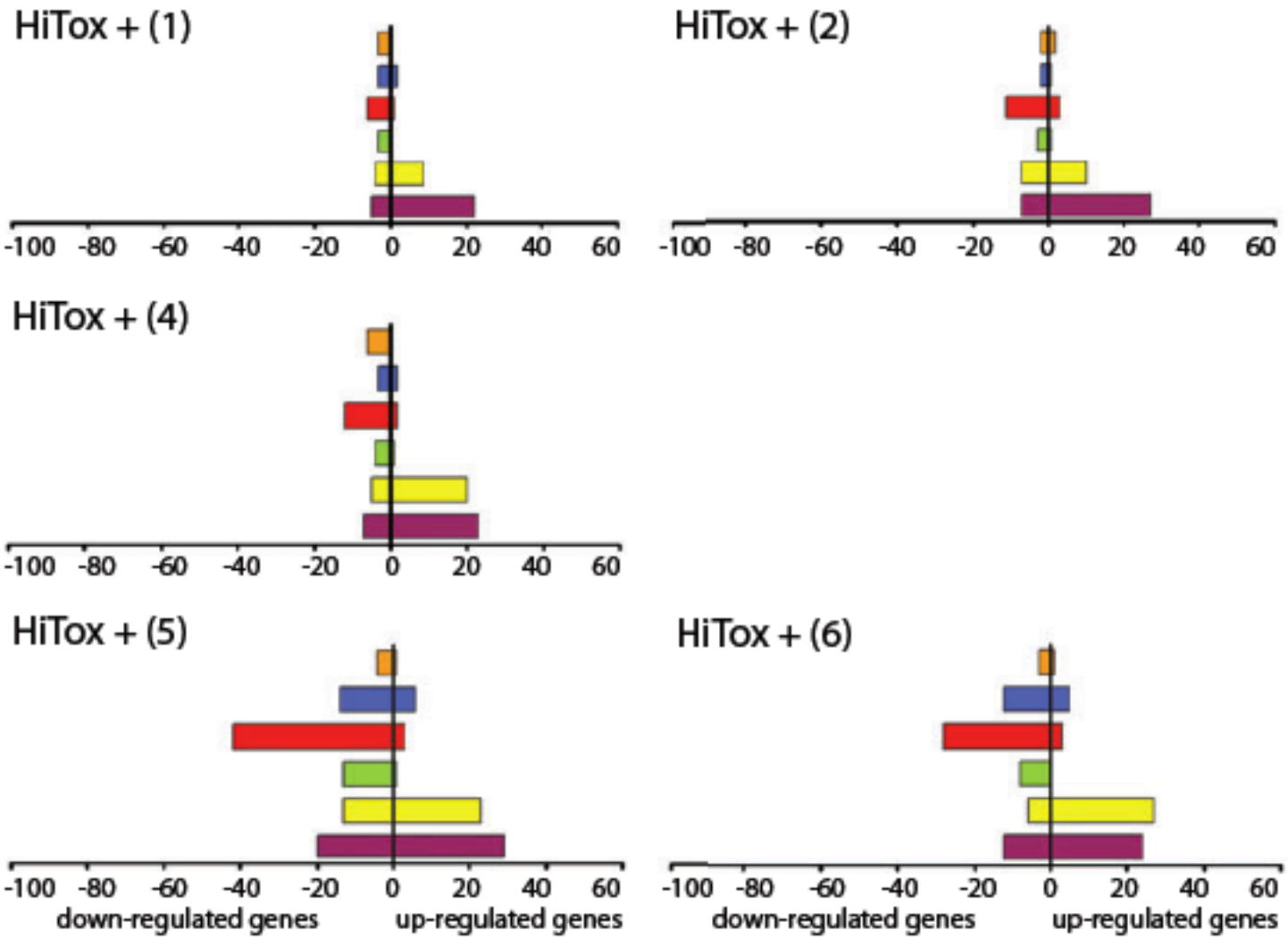
The chemicals restore growth and viability



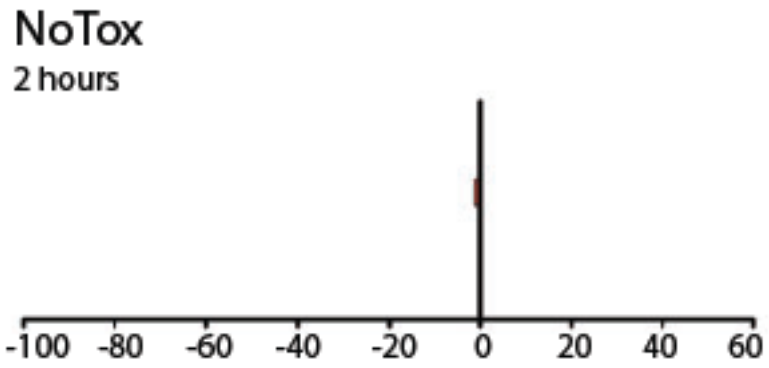


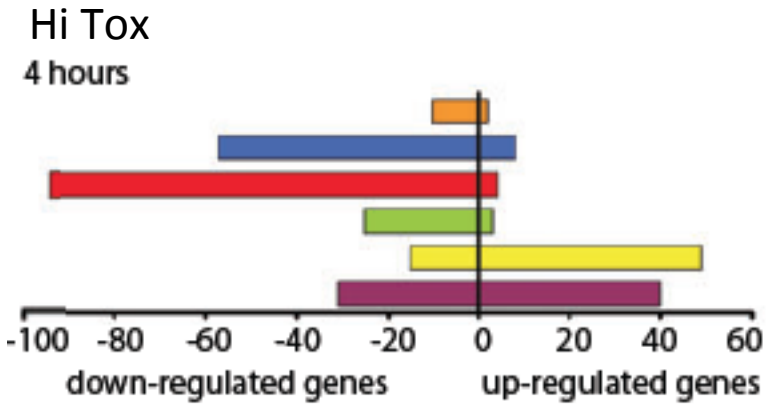
The chemicals ameliorate transcription changes

C

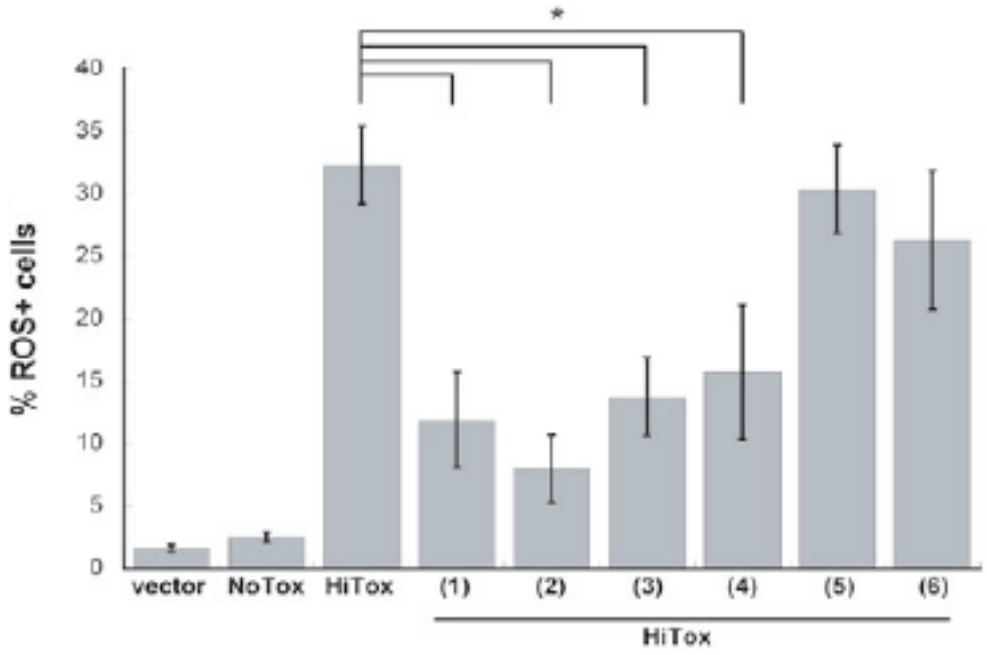


Just to remind you

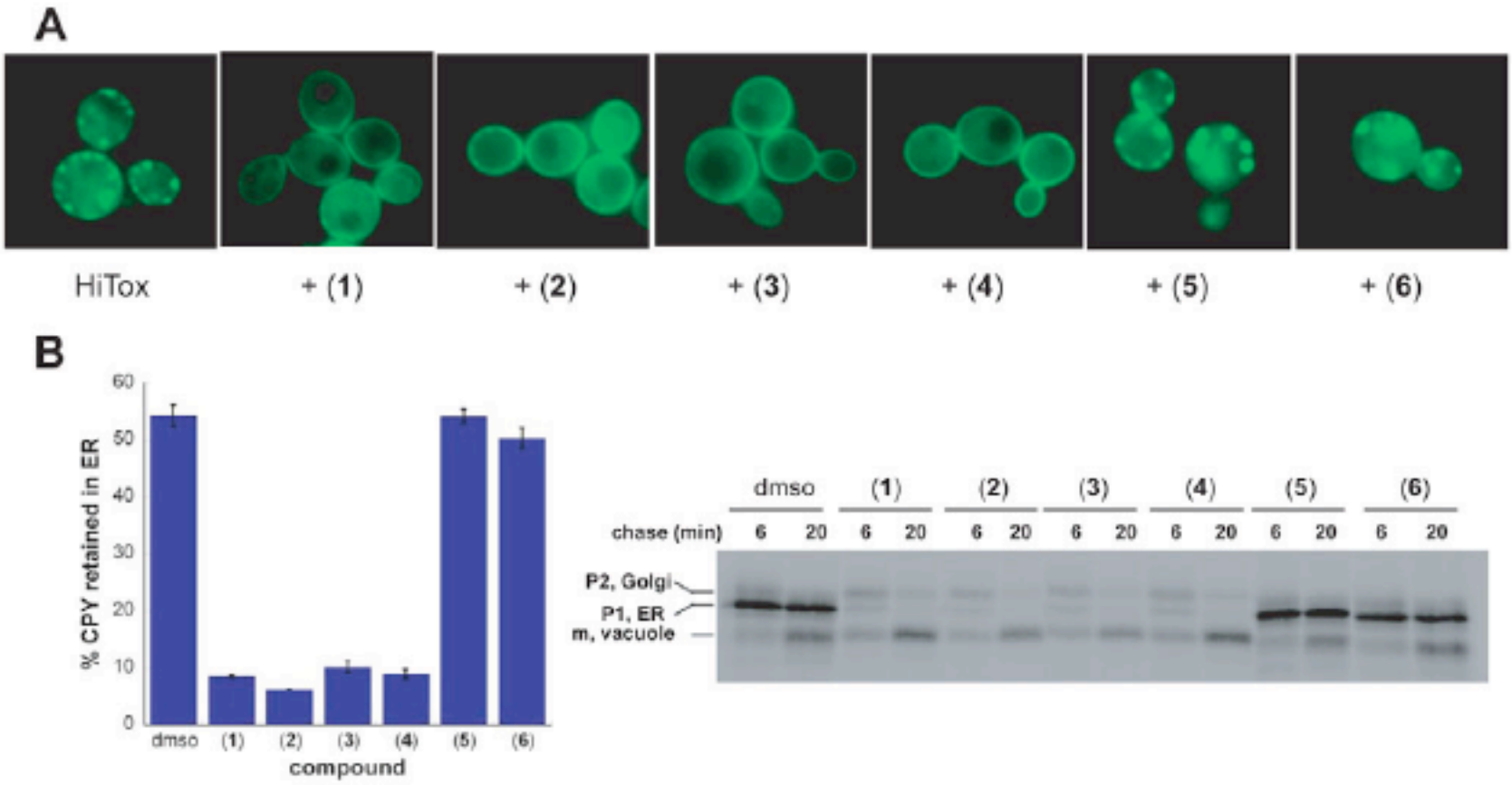




And mitochondrial function

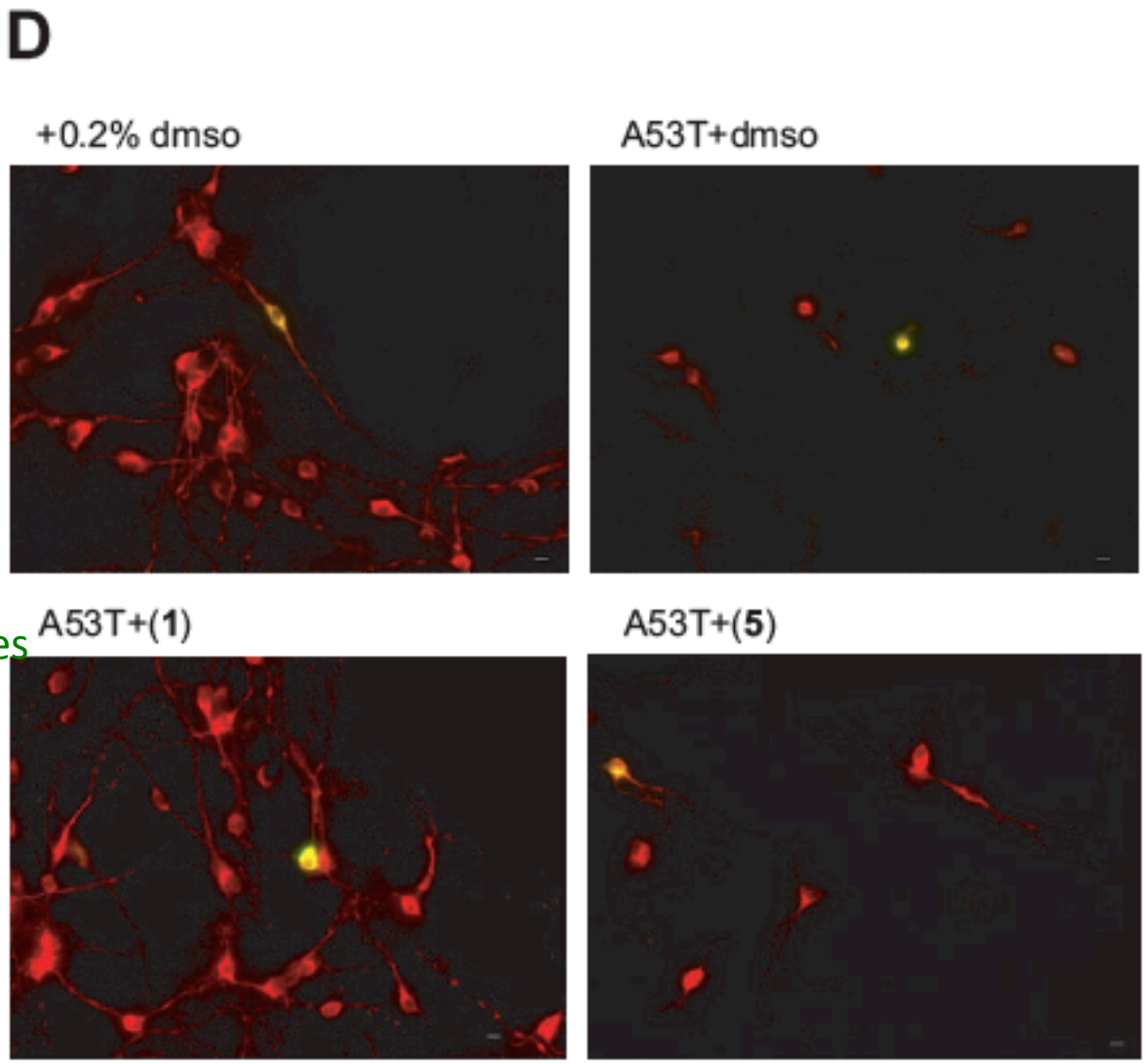


And ER-to-Golgi trafficking and α-syn membrane localization

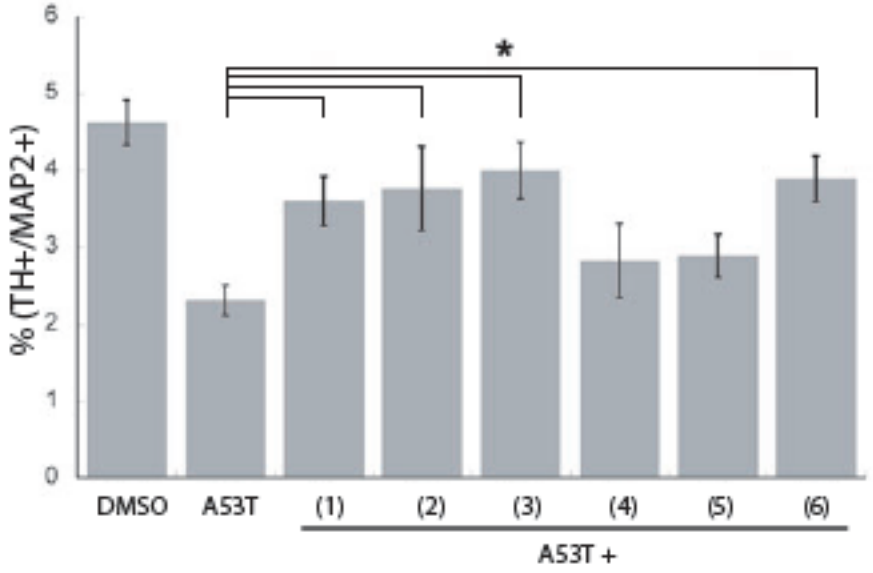


The compounds also protect rat midbrain neurons from damage by α-syn A53T

Primary neurons retract processes and are reduced in number when A53T is introduced by viral infection; compound 1 restores normal morphology



Quantitation



What have we learned?

Major problems include vesicle trafficking and mitochondrial dysfunction. These problems manifest at low levels of toxicity and well before aggregate formation and Lewy Bodies are detected

Hence, in terms of treatment, efforts to target aggregate formation may not be fruitful

Chemical genetics identified some lead compounds that ameliorate α-syn toxicity in a variety of species

Huntington's disease

Symptoms

Hyperkinetic movements

Psychosis

Cognitive dysfunction

Inheritance

Autosomal dominant trait

Cause

Triplet repeat (CAG) expansion leads to expanded polyQ in the N-terminal region of huntingtin. These forms of huntingtin are toxic and form aggregates

Cause

Triplet repeat (CAG) expansion leads to expanded poly(Q) in the N-terminal region of huntingtin. These forms of huntingtin are toxic and form aggregates

In normal individuals, the poly(Q) tract is ~25 residues. A tract longer than ~35 can initiate disease. The longer the tract length, the more severe the disease

Individuals with Huntington's Disease

Woody Guthrie

Popular folk singer in '30s and 40's

Son, Arlo, folk singer in '60s and '70s; apparently free of the disease

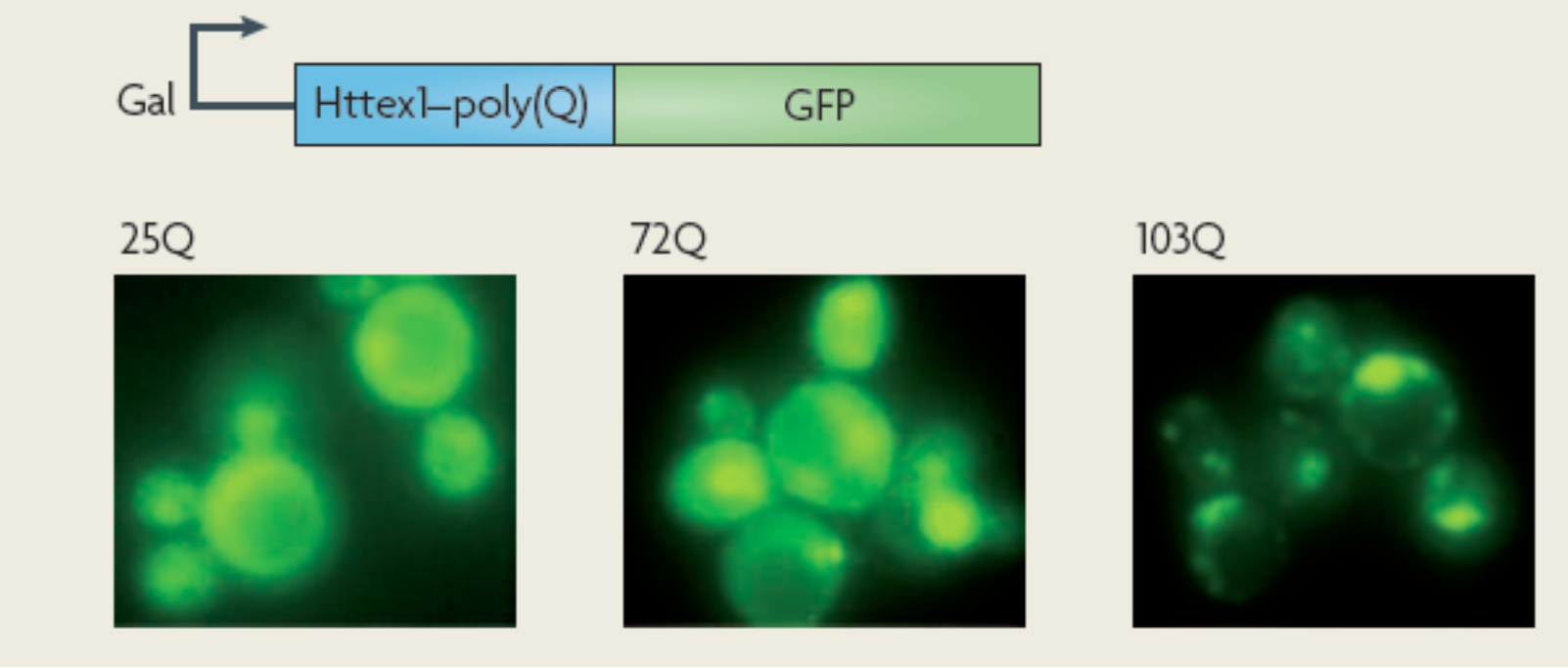
Two daughters contracted HD

Nancy Wexler

Geneticist who identified the HD gene. Incredibly difficult task because gene is in recombination "cold" spot

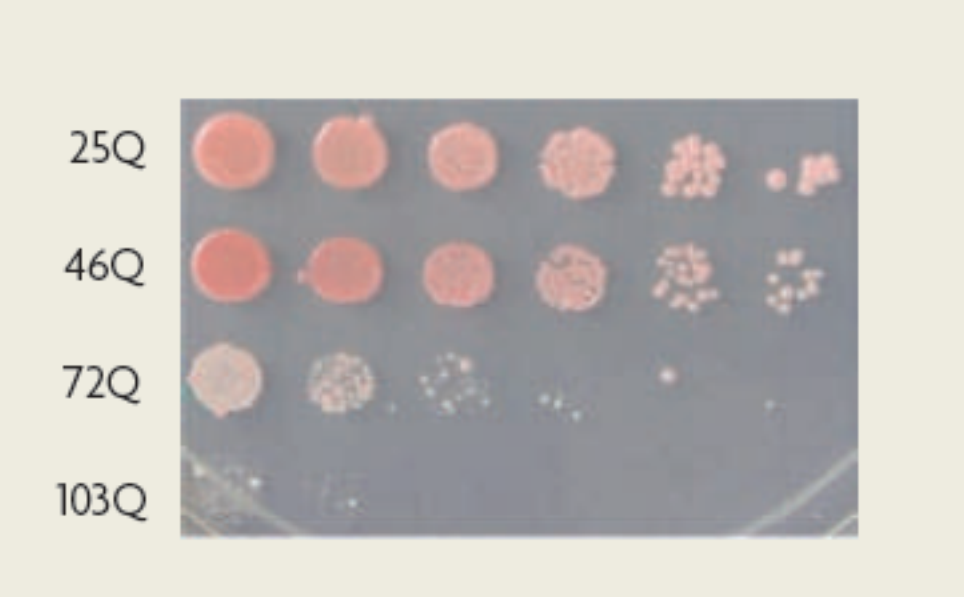
Has relatives with HD

The Huntingtin poly(Q) aggregates in and is toxic to yeast cells



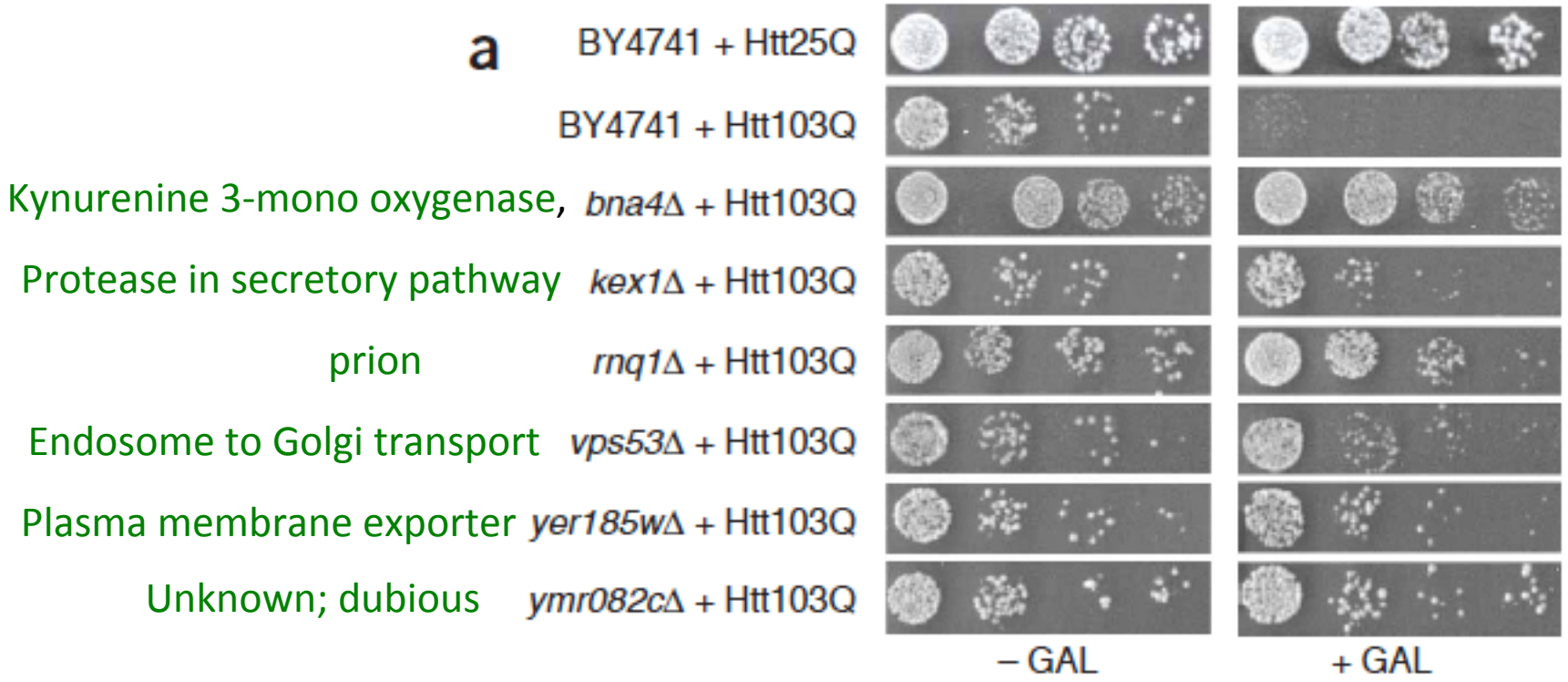
General cytoplasmic staining at low “Q” number;
Aggregates at high Q

High poly(Q) is toxic to yeast growth



Spots are 10-fold serial dilution of yeast cell suspension

Genetic studies in yeast implicate chaperones, vesicle transport, tryptophan degradation, and vacuolar degradation in huntingtin toxicity



The *BNA4* product may be a therapeutic target. The kynurenine pathway has been implicated in HD pathology in humans

Other ideas

Heat shock proteins

First discovered because expression induced upon heat shock

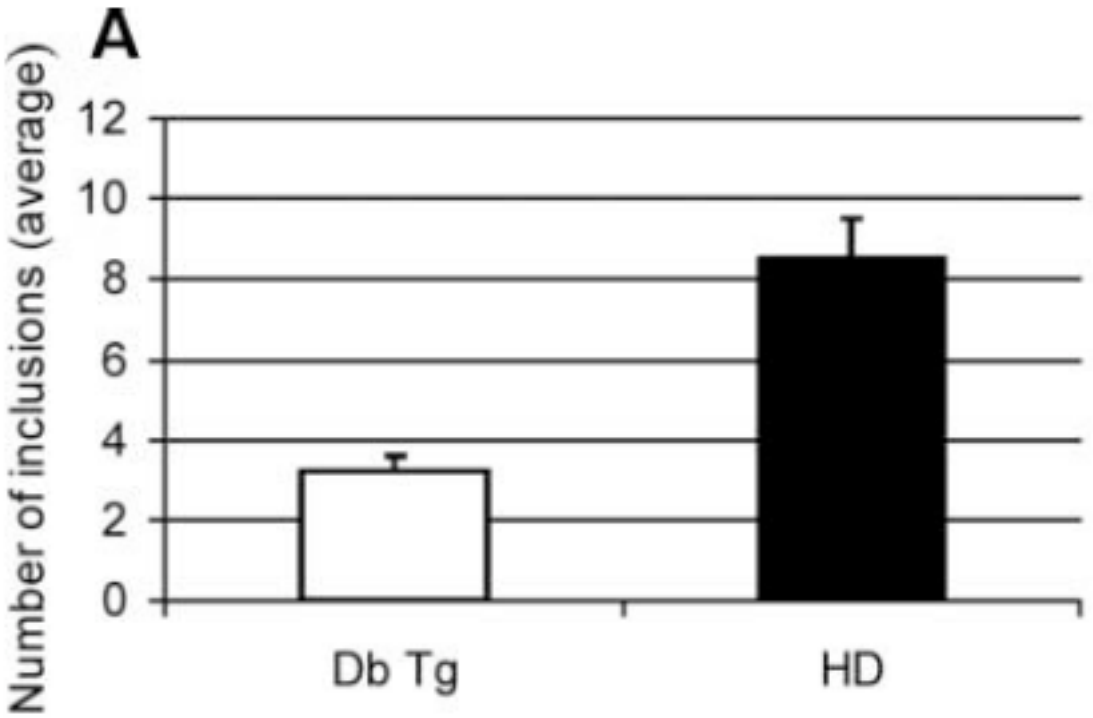
Many now recognized as molecular chaperones.
Help proteins fold and help refold misfolded proteins.
Some can help disaggregate protein aggregates

Overexpression of yeast Hsp104 in *C. elegans* reduces aggregation of poly(Q)-containing GFP

…. So, what about in mice?

Overexpression of yeast Hsp104 reduces polyglutamine aggregation and prolongs survival of a transgenic mouse model of Huntington's disease

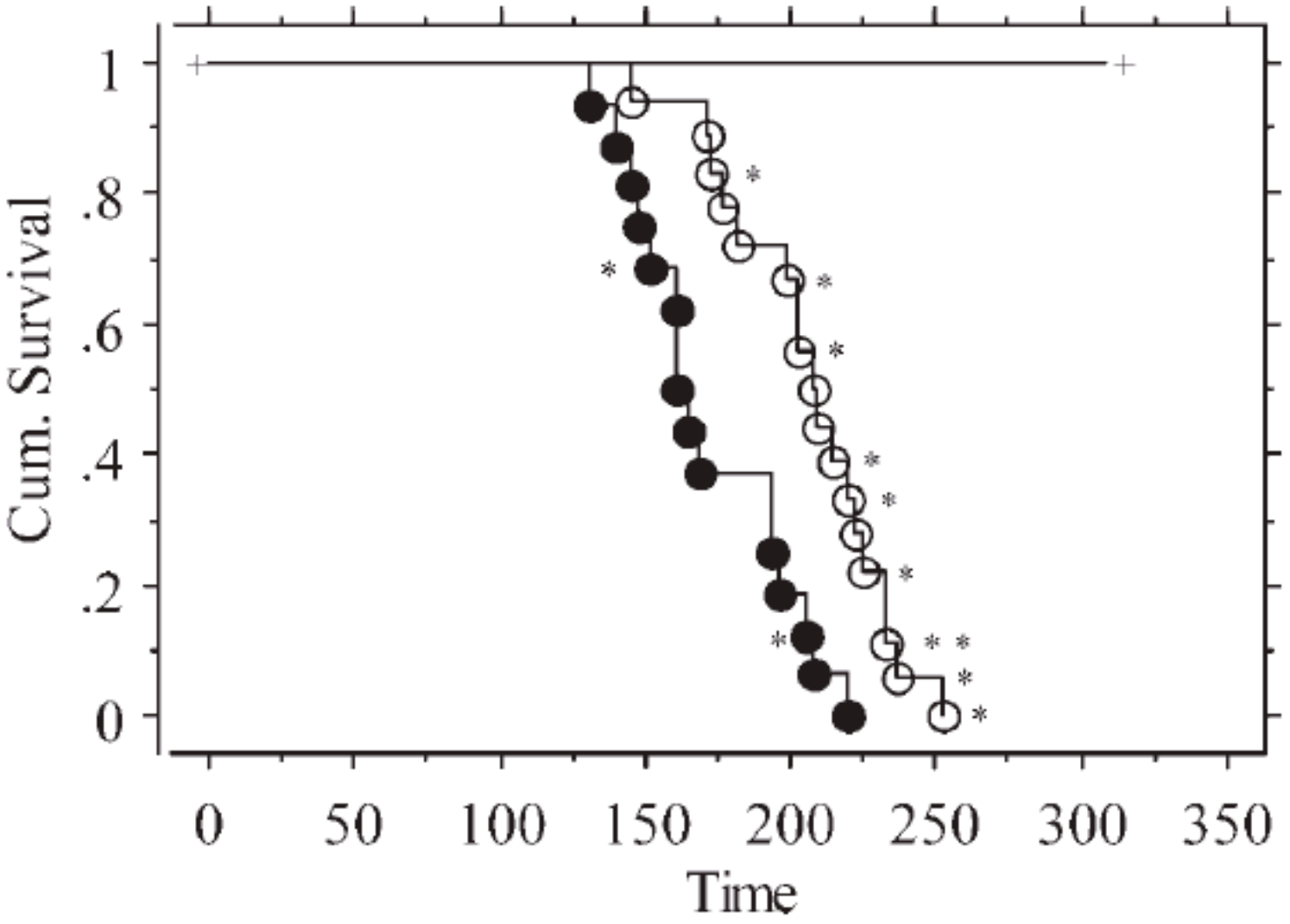
Number of aggregates in brain cortex



Hsp104 and hunt82Q

hunt82Q alone

Hsp104 enhances longevity



… but does not improve motor skills (not shown)

Alzheimer's

Sporadic and familial cases

Familial cases are generally early onset and have revealed 3 culprit genes, *APP* (amyloid precursor protein), and *PSEN1* and *PSEN2* (presenilins 1 and 2)

APP can be processed into fragments, notably the Aβ peptide. Plaques containing the Aβ peptide are the pathological hallmark of Alzheimer's

Genome-wide association studies have identified a number of additional risk factors; for example, *PICAM*, *BIN1*, and *CD2AP*, all implicated in endocytosis

Well known individuals with Alzheimer's

Ronald Reagan

Aaron Copeland

Rita Hayworth

Dean Smith

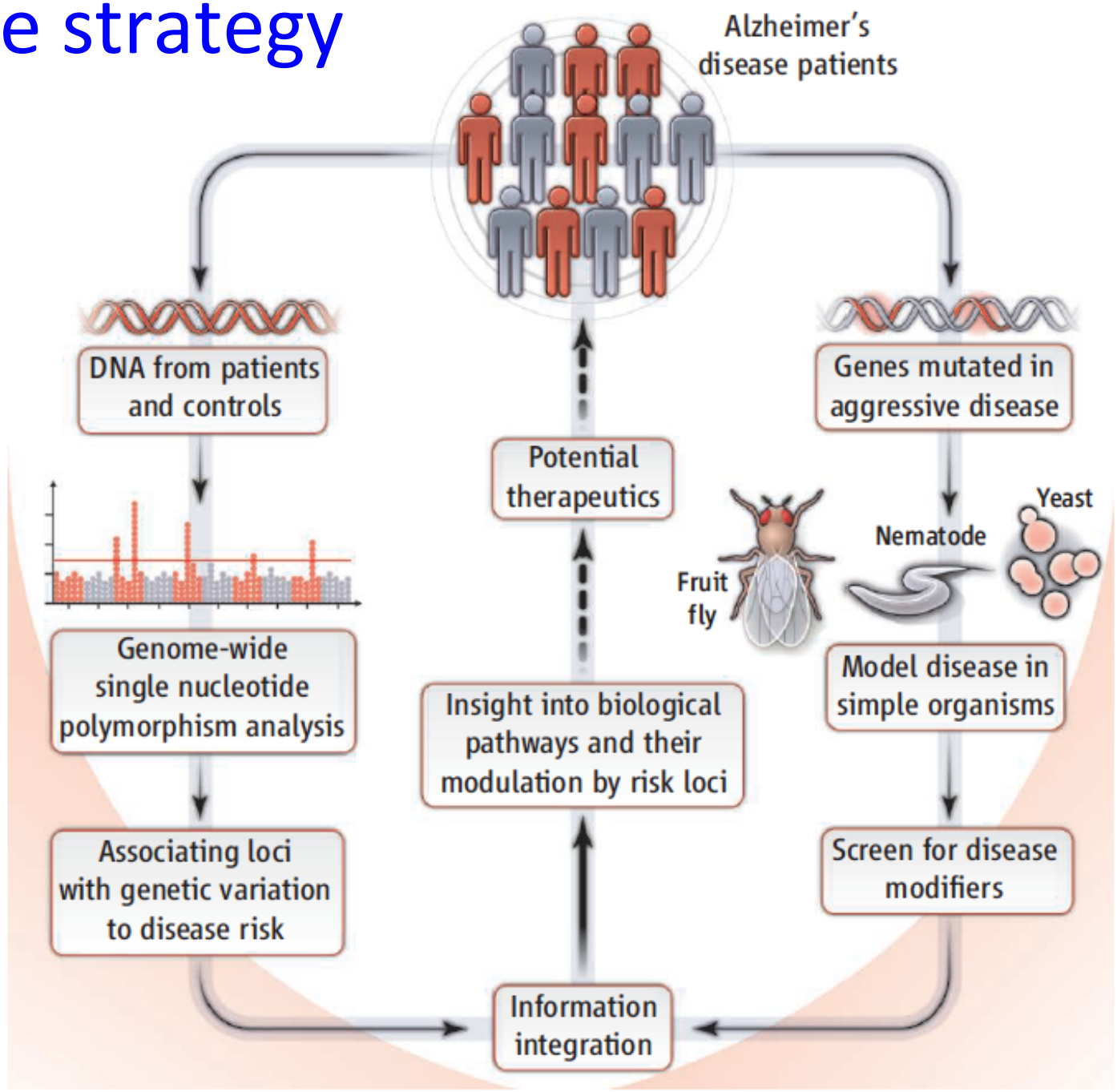
Jack Lord

Barry Goldwater

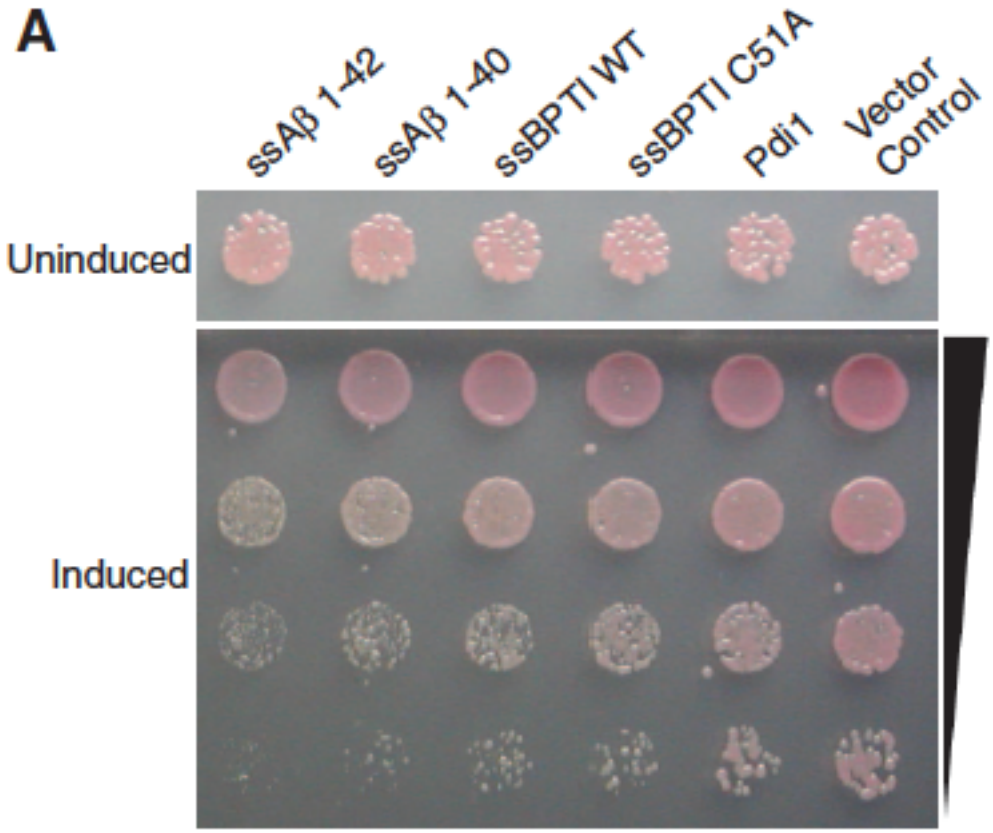
Willem DeKooning

Charles Bronsan

The strategy

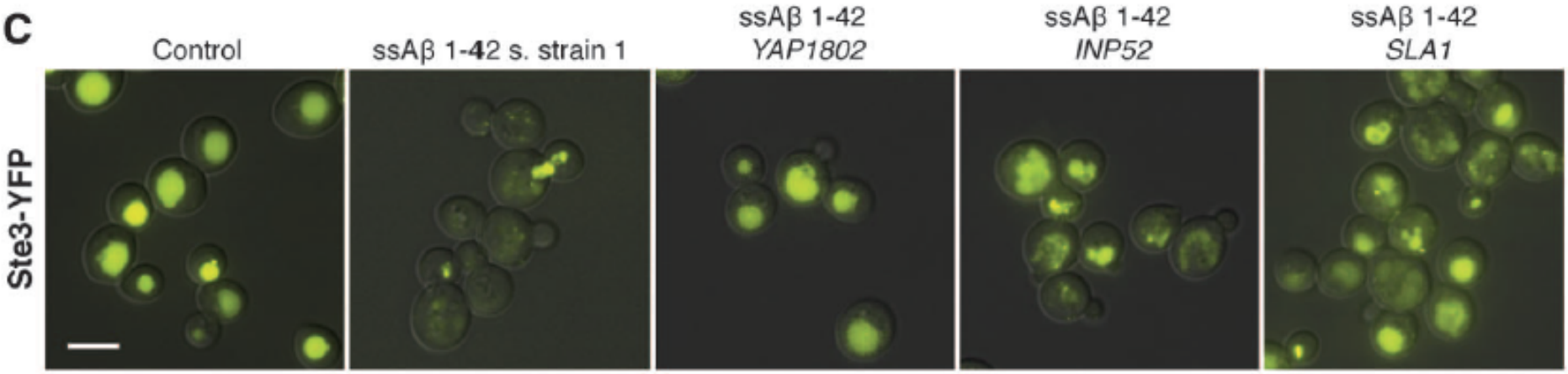


Expression of AB1-42, the most toxic AB peptide, is toxic to yeast cells



In humans, AB1-42 is produced in the secretory pathway; the version expressed in yeast was targeted to the ER via a signal sequence and distributes throughout the secretory pathway

Expression of Aβ in yeast interferes with endocytosis



Ste3 is cell surface receptor but it resides there only a few minutes before it is endocytosed and delivered to the vacuole. Hence, in the steady state Ste3 is located in the vacuole. Aβ prevents delivery of Ste3 to the surface (and hence to the vacuole); the suppressors restore delivery to the vacuole

Screen an overexpression library of 5500 yeast genes for alteration of toxicity

23 suppressors and 17 enhancers were identified

12 had clear human homologs and these were investigated further

3 were homologs of validated risk factors, providing proof of principle and heightening the interest in the other identified modifiers

The identified genes were distinct from those found in a similar screen based on α-syn toxicity (Parkinson's)

List of suppressors and enhancers

Yeast Aβ suppressors	Cellular function	*C. elegans* homolog	Human homolog	Connection of human homologs to AD risk
YAP1802	Endocytosis	*unc-11**	*PICALM*	Validated risk factor†‡
INP52	Endocytosis	*unc-26**	*SYNJ1*	Interacts with validated risk factor *BIN1* (*28*)
SLA1	Endocytosis	*Y44E3A.4**	*SH3KBP1*	Interacts with validated risk factor *CD2AP* (*29*)
RTS1	Phosphatase regulation	*pptr-2**	*PPP2R5C*	
ADE12	Adenylosuccinate synthesis	*C37H5.6b**	*ADSSL1*	Potential risk factor, this study§
CRM1	Nuclear protein export	*xpo-1**	*XPO1*	Potential risk factor, this study‡
GRR1	Ubiquitination	*C02F5.7*	*FBXL2*	
VPS9	Vesicle transport	*rabx-5*	*RABGEF1*	Potential risk factor, this study§
Yeast Aβ enhancers				
PBS2	Osmotic stress response	*mkk-4**	*MAP2K4*	Activated by Aβ oligomers in cortical neurons (*37*)
KEM1	RNA processing	*xrn-1*	*XRN1*	
MVP1	Vacuolar sorting	*lst-4*	*SNX8*	
PMT2	Mannosylation	–	*POMT2*	

Hits from the yeast screen ameliorate Aβ1-42 toxicity to glutamanergic neurons in *C. elegans*