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From single genes to gene networks:

High-Throughput, High-Content Screening for neurological Disease

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vrije Universiteit *amsterdam*

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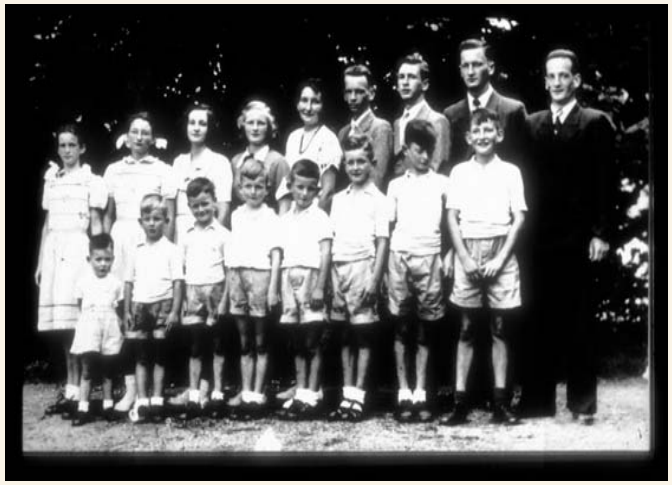
Mendelian genetics: a success story

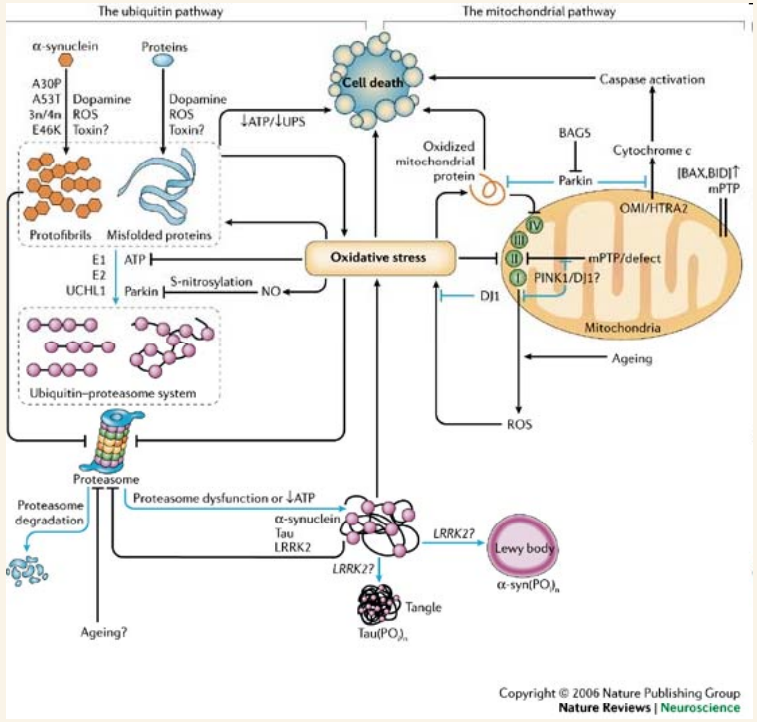


families

+

Genetic Markers

=



models and function

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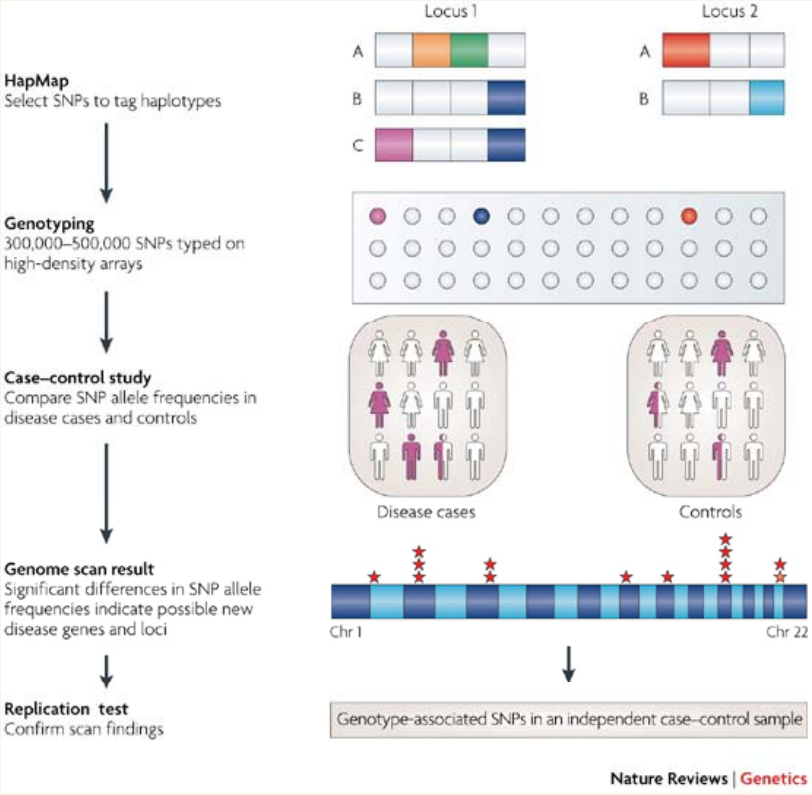
Genome wide association studies

- HapMap and 1000 Genomes projects provided us with high resolution LD map
- December 2010: 1212 published loci at $p \leq 5 \times 10^{-8}$ for 210 traits



How do we move from association to causation?

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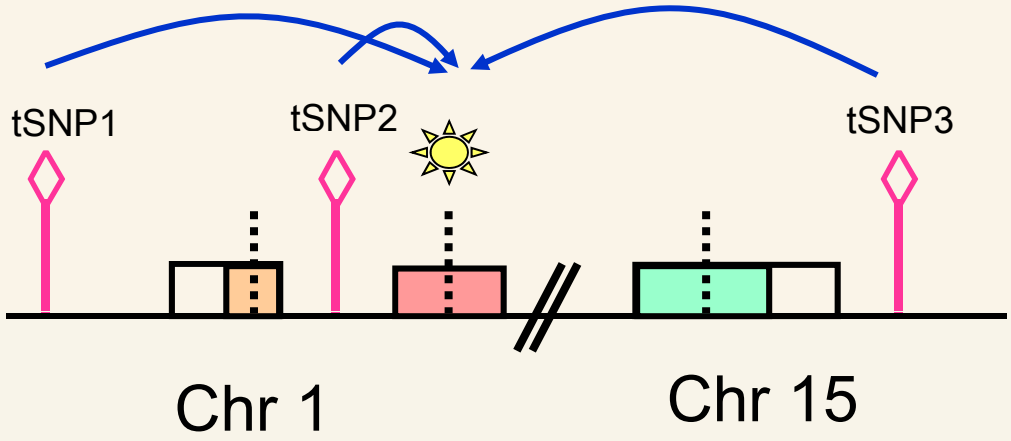
Improving our analysis

- GWAS studies use an overly simplistic genetic model that detects the effect of single genomic variants while from biology it is obvious that the extensive phenotypic variations can not be explained by the single effects of ~25.000 protein encoding genes. (epistatic effects or gene-gene interactions).

- Problem: Testing all possible SNP-SNP combinations would require extremely large sample size (multiple testing)

- Pre-select *physically unlinked* pairs that show strong LD, these are more likely to be involved in functional interactions



Bochdanovits et al. PloS One 2008

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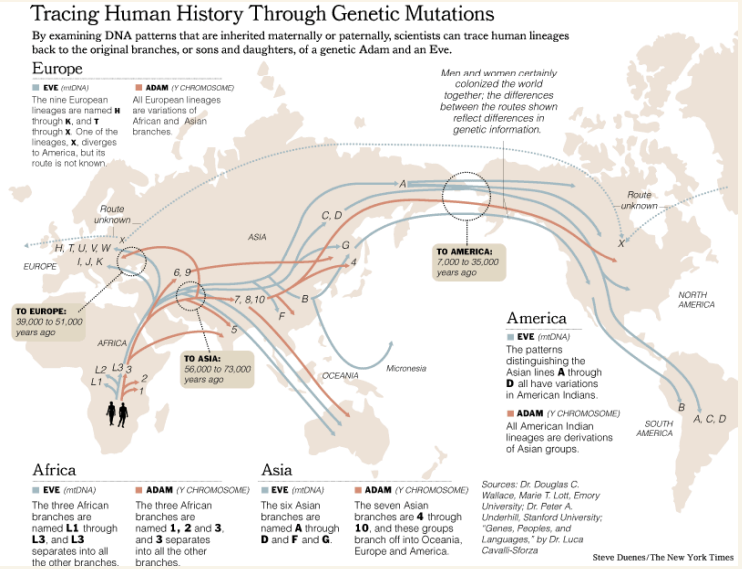
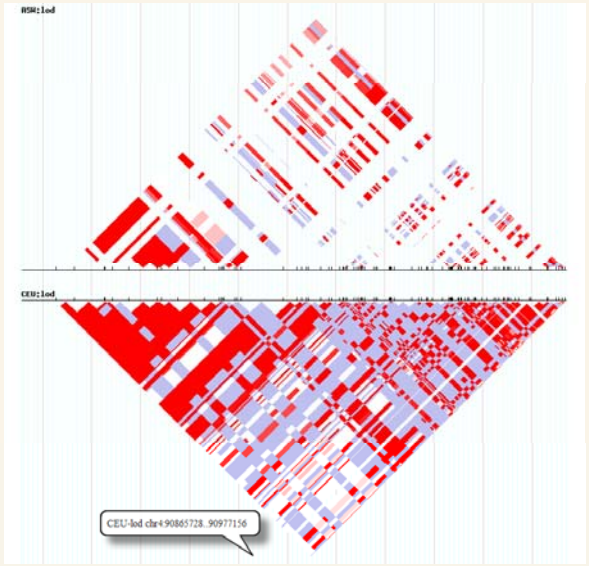
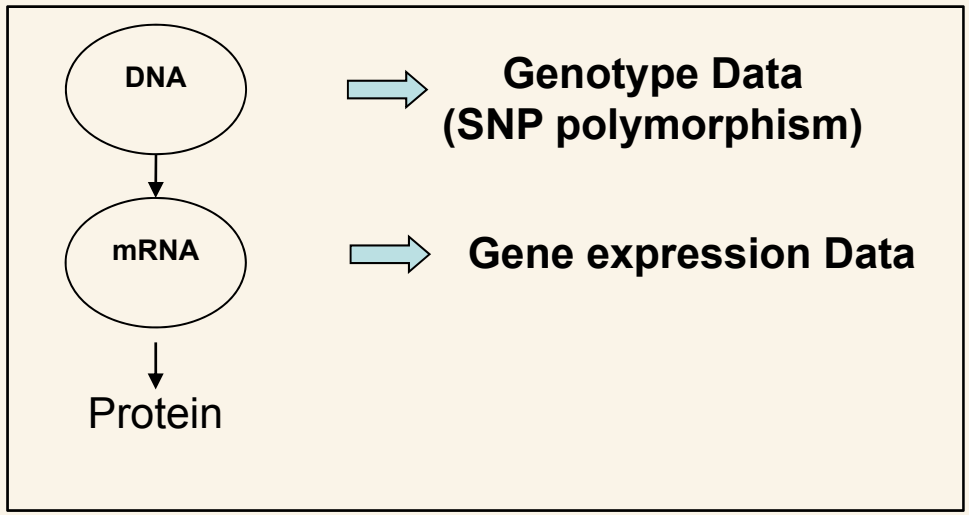
Association fine mapping and causality

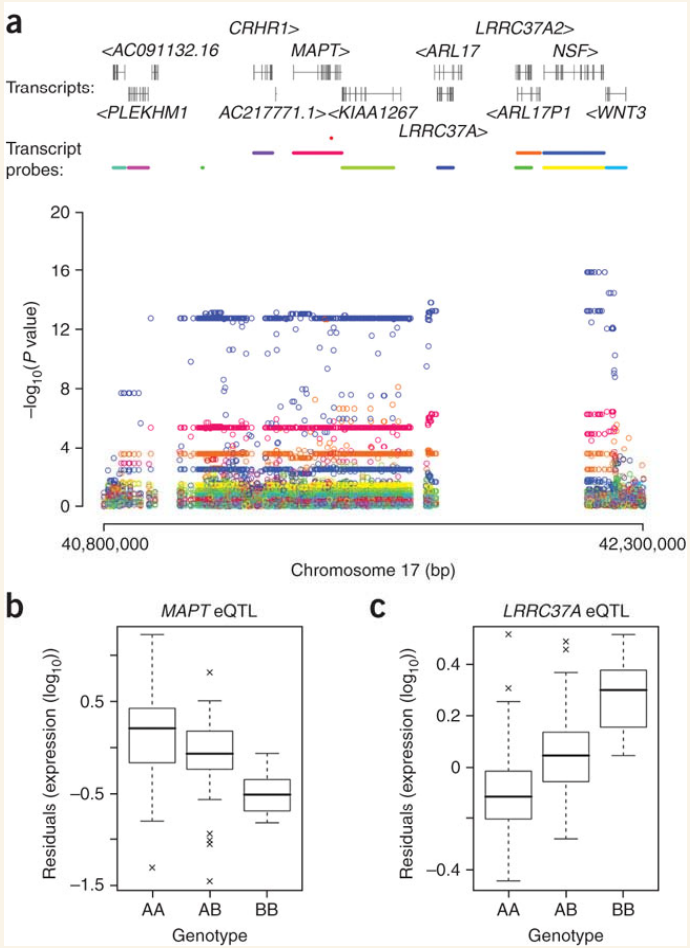
- Linkage disequilibrium (LD) mapping by SNP tagging widely used for gene localization
- Existence of LD limits the resolution of fine mapping
- Population specific LD patterns can help reduce the critical region
- Resequencing to identify all variants (100s to 1000s)
- Which is the causal (often non-coding) variant?





Non-coding variants- eQTL





- Expression profiles from large tissue series are related with SNPs across the genome through their correlated variations.

- Until now based on microarray data
  (limited to protein coding genes)

Símon-Sánchez et al. Nat. Genet 2009
Int. Parkinson Disease Genomics Consortium; Lancet 2011

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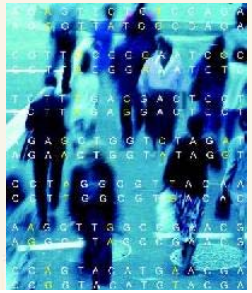
The case of missing causality

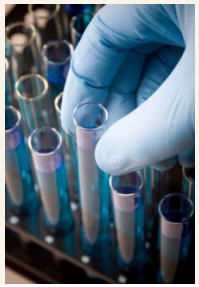
- Association studies point to a genomic locus but do not identify the causal variant(s).
- Gene-gene and gene-environment interactions are not systematically tested
- Majority of GWAS signals points to noncoding regions in our genome
- The combined effects of many (small) risk factors are responsible for disease

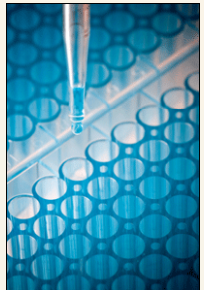
Major obstacles for demonstrating causality are

1) the very limited functional annotation of our genome

2) the lack of appropriate biological validation tools for (combinations of) risk factors with small effect size and gene-gene interactions.







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How to prove causality?

Mendelian disorders:

- co-segregation of variant and phenotype in family
- Variant rare in general population

- *Functional confirmation*

(Evolutionary conservation; cellular or animal model; complementation)

Multifactorial disease:

- Individual risk factors are neither necessary nor sufficient
- Variants common, no co-segregation with phenotype in families

- *Majority of identified variants are non-coding and biologically plausibility is often not apparent.*
- *How do we model risk factors with OR 1,1-1,3?*
- *Different effects can be predicted, no single test for effect of mutation.*

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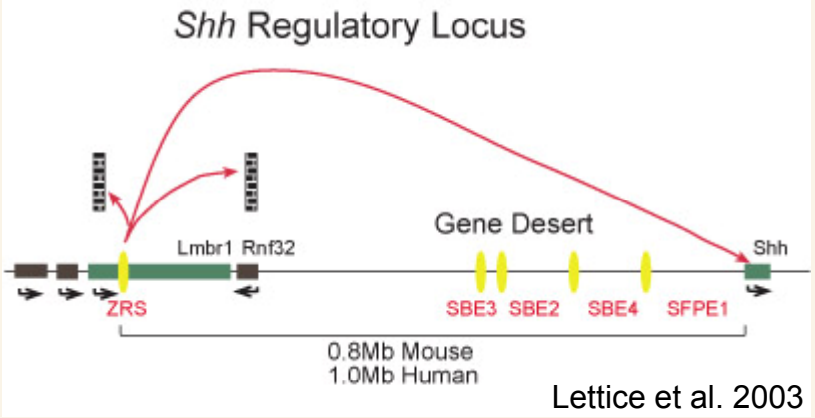
Functional annotation of the human genome

- **Our definition of a gene has changed;**
- Only 1-2% of the genome consists of protein encoding genes but ~80% of the genome is transcribed into RNA
- Most protein encoding genes also show: antisense transcription, alternative start sites, alternatively splicing
- Regulatory elements can be located as far as 1Mb away and skip over genes



Lettice et al. 2003

- ENCODE was performed on 1% of genome using well established cell lines.

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2010-2013

FANTOM 5

Aims:

- map of human promoters of primary cells and tissues!
- models of transcriptional regulatory network models of each cellular state.

Methods:

- deepCAGE sequencing on the Heliscope on RNA isolated from
- every major human organ,
- >200 cancer cell lines,
- 30 time courses of cellular differentiation,
- mouse developmental time courses
- >200 primary cell types.

We are the main provider of human brain tissues and primary cells and participate in data analysis.

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FANTOM5

Functional annotation

- 15 brain regions; selected cell types; neonatal, adult and aged and brains
- Correlate gene expression (CAGE) in human post-mortem brain with genomic variation (SNPs/CNVs), epigenetic changes (methylation) and DNA binding (ChIP-seq)
- Compare brain expression and conservation of promoter sequence between human and other primates (Macaque; marmoset)
- Improved annotation will generate testable hypothesis for identified associations
- Data will be available in public domain (ZENBU and UCSC browsers)

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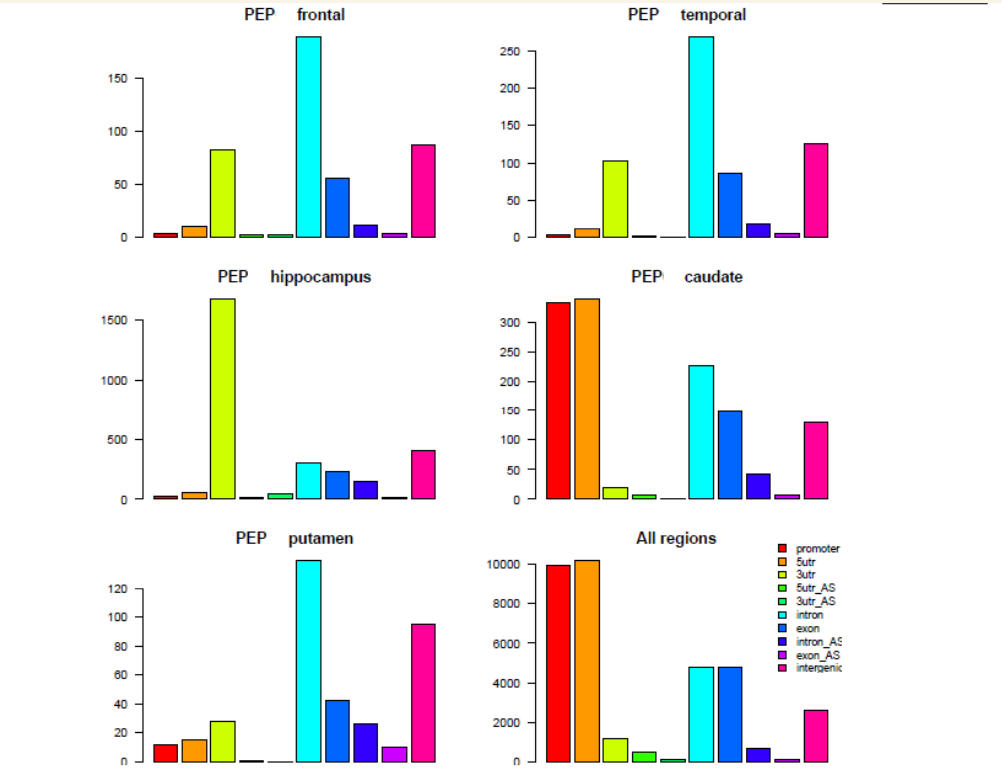
Pilot study; 5 aged individuals, 5 brain regions

- 80M tags sequenced: caudate, frontal and temporal lobe, hippocampus and putamen
- 70% TSS in 5' region of annotated genes
- 25% promoters intergenic
- 5% >500 kb from annotated gene
- Many alternative promoters and antisense transcription
- 20% preferentially expressed in one brain region



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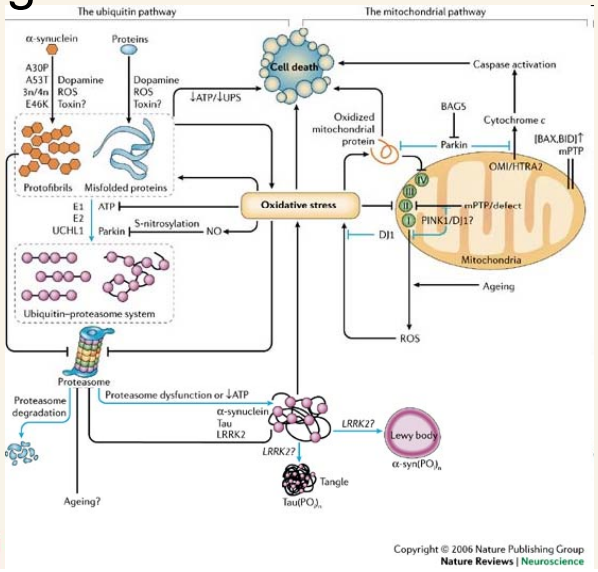
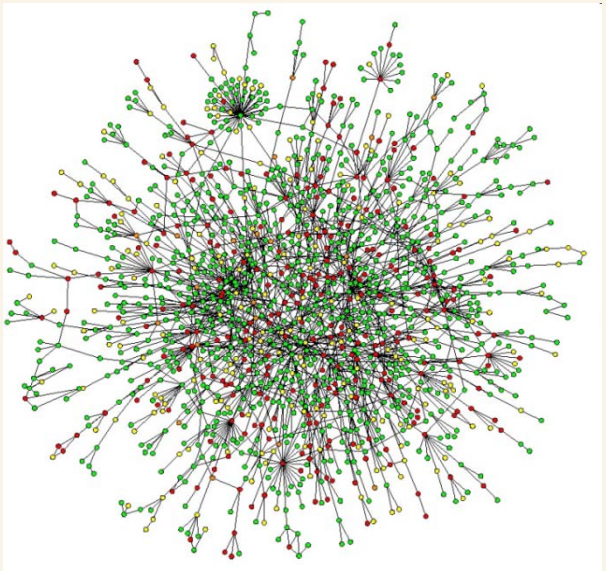
Functional pathways and gene networks

- For multifactorial disorders the combined and small effects of many different genomic variants in many different genes within a pathway result in disease
- Models for multifactorial disorders should integrate the effects of multiple gene variants.
- Improved annotation will help generate testable hypothesis for identified associations

- Are classical cellular and animal models feasible?
  - small effect size
  - large numbers of variations



Mendelian
vs.
Multifactorial



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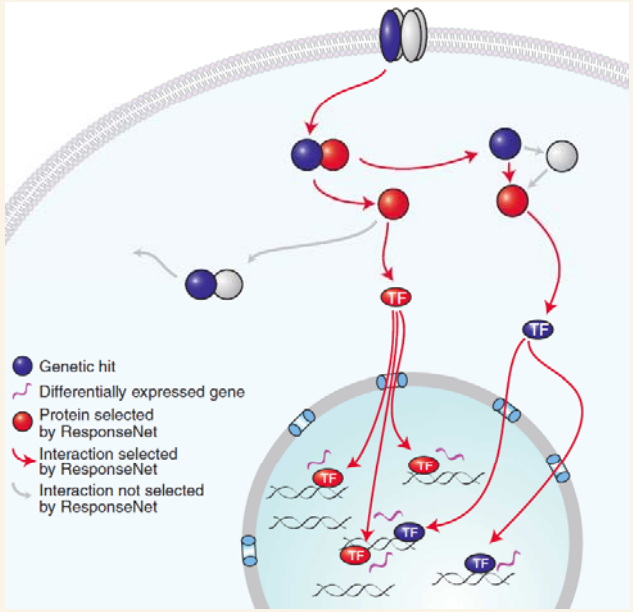
Integrating datasets to study functional networks

Examples in lower organisms:

Yeast: Yeger-Lotem et al. Bridging high-throughput genetic and transcriptional data reveals cellular responses to alpha-synuclein toxicity. Nature Genetics 2009.

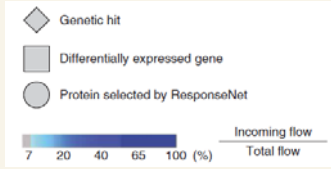
C.elegans: Kamath et al Systematic functional analysis of the Caenorhabditis elegans genome using RNAi *Nature 2002*

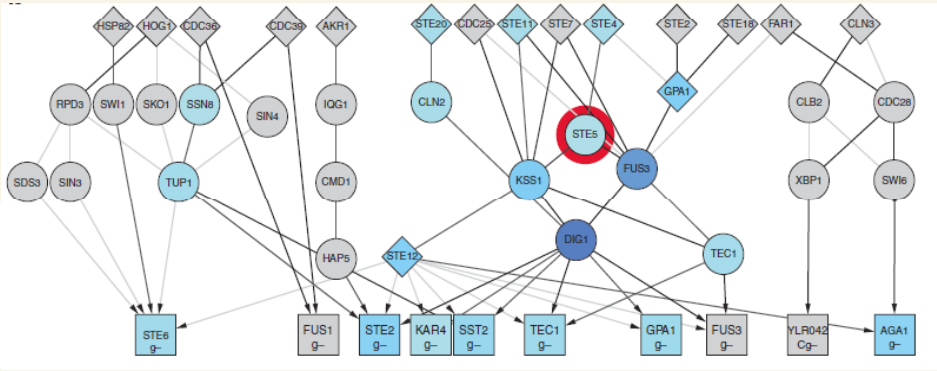
Drosophila: Boutros et al, Genome-Wide RNAi Analysis of Growth and Viability in Drosophila Cells *Science* 2004



- Approach feasible in mammalian systems?
  - experimental variation ↓↓
  - reproducibility ↑↑
  - sensitivity ↑↑

- Automated cell culture
- HTS and HCS cellular assays





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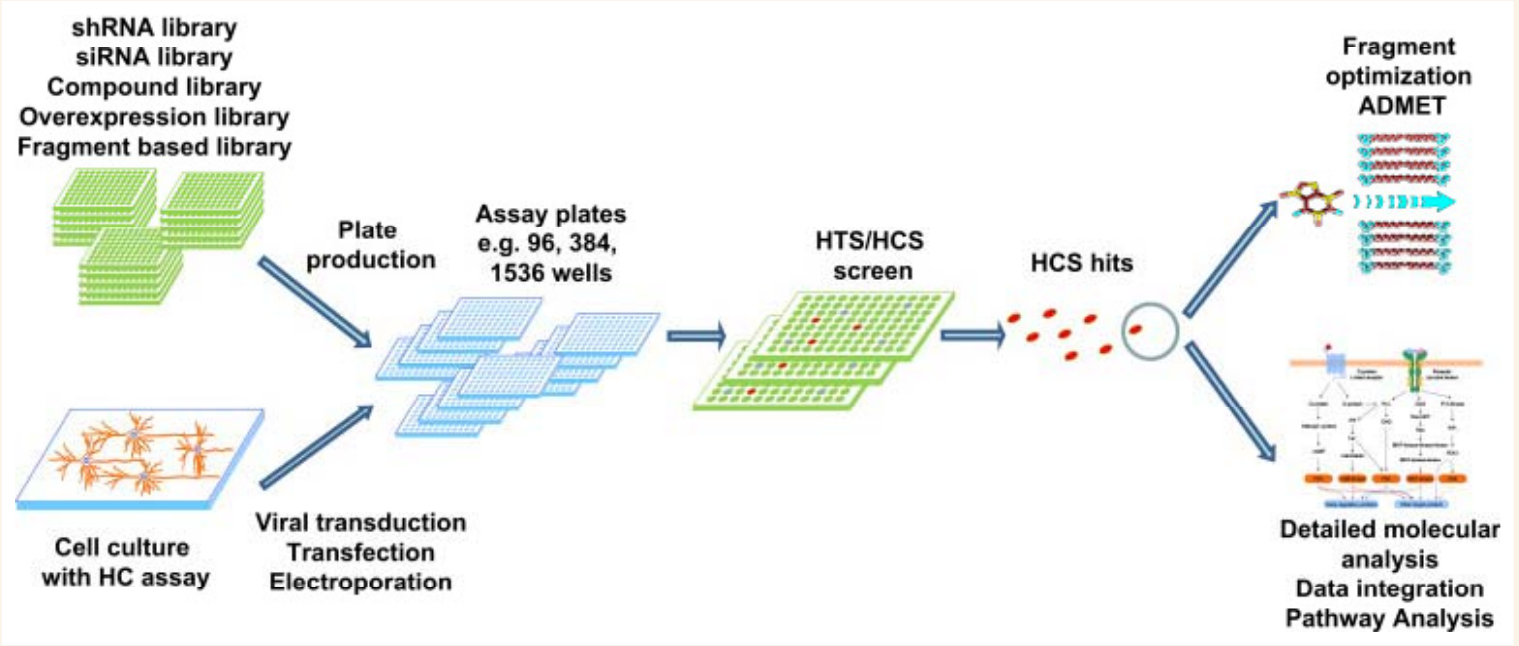
High Throughput-High Content Screens in neuronal cells



Jain & Heutink; Neuron 2010

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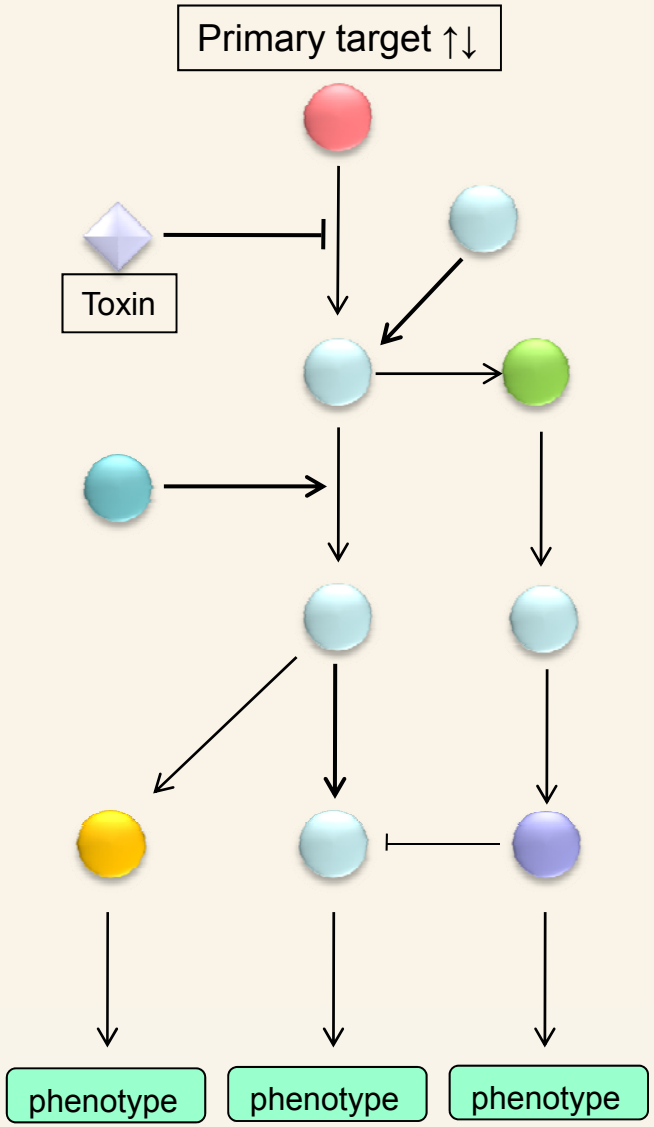
Systematic gene network analysis



- Create robust High Content assays
- (Genome wide) enhancer-suppressor screens
- Secondary screen with additional constructs
- Confirmation in primary cells
- Rescue/complementation experiments
- Test selected multiple-gene comparisons
- Describe functional pathway
- (Define best drug targets)
- (Compound screens for therapy development)

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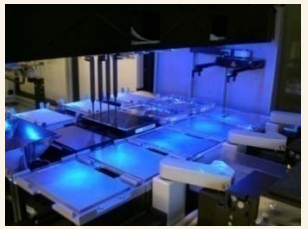
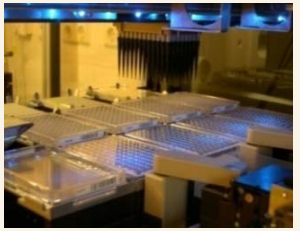
Automated workflow to reduce experimental variation

Incubator

HEPA-Filter Hood

Hamilton ML STAR





Cellavista

Control PC

Robot arm

Culture medium

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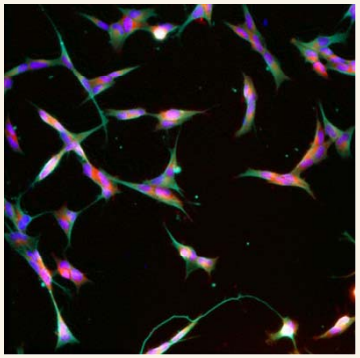
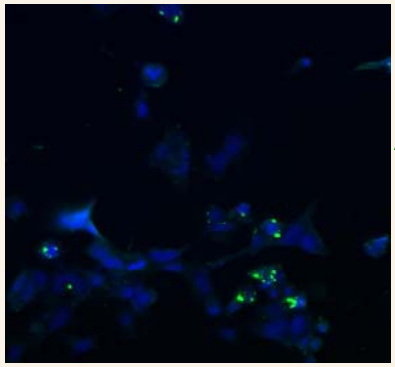
Biological question and assay design



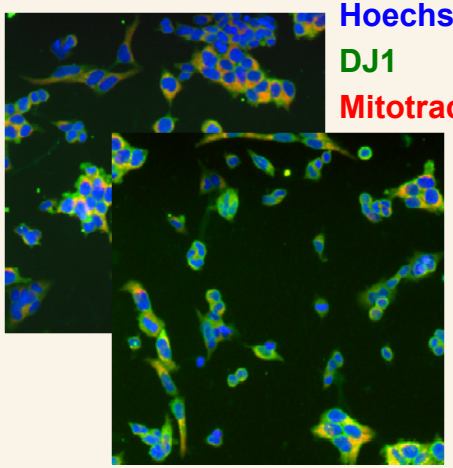
Hoechst
anti-βIII-Tubulin
MitoTracker
Phaloidin

Apoptosis



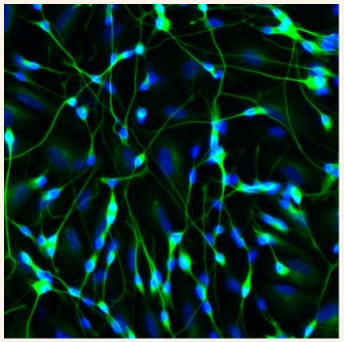
Hoechst
A53T α-synuclein

Protein aggregation



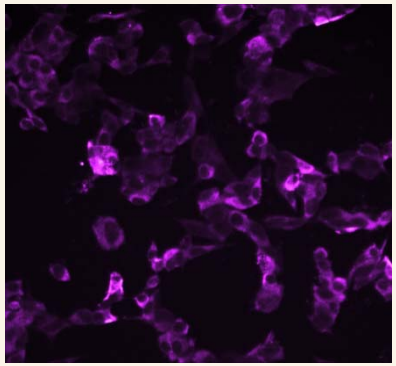
Hoechst
DJ1
Mitotracker

Protein translocation



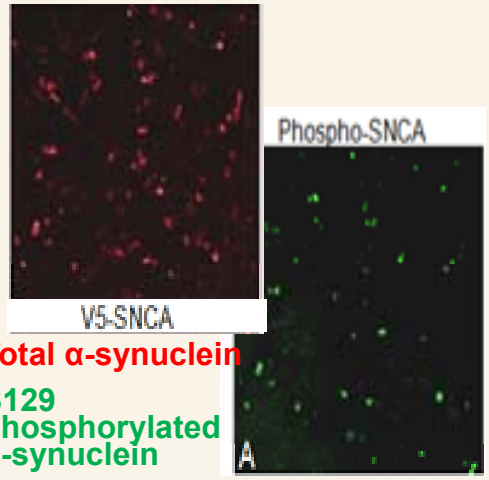
Hoechst
anti-βIII-Tubulin

Neuronal Outgrowth



Hoechst
PDI

ER stress



Total α-synuclein
S129 phosphorylated α-synuclein

Protein phosphorylation

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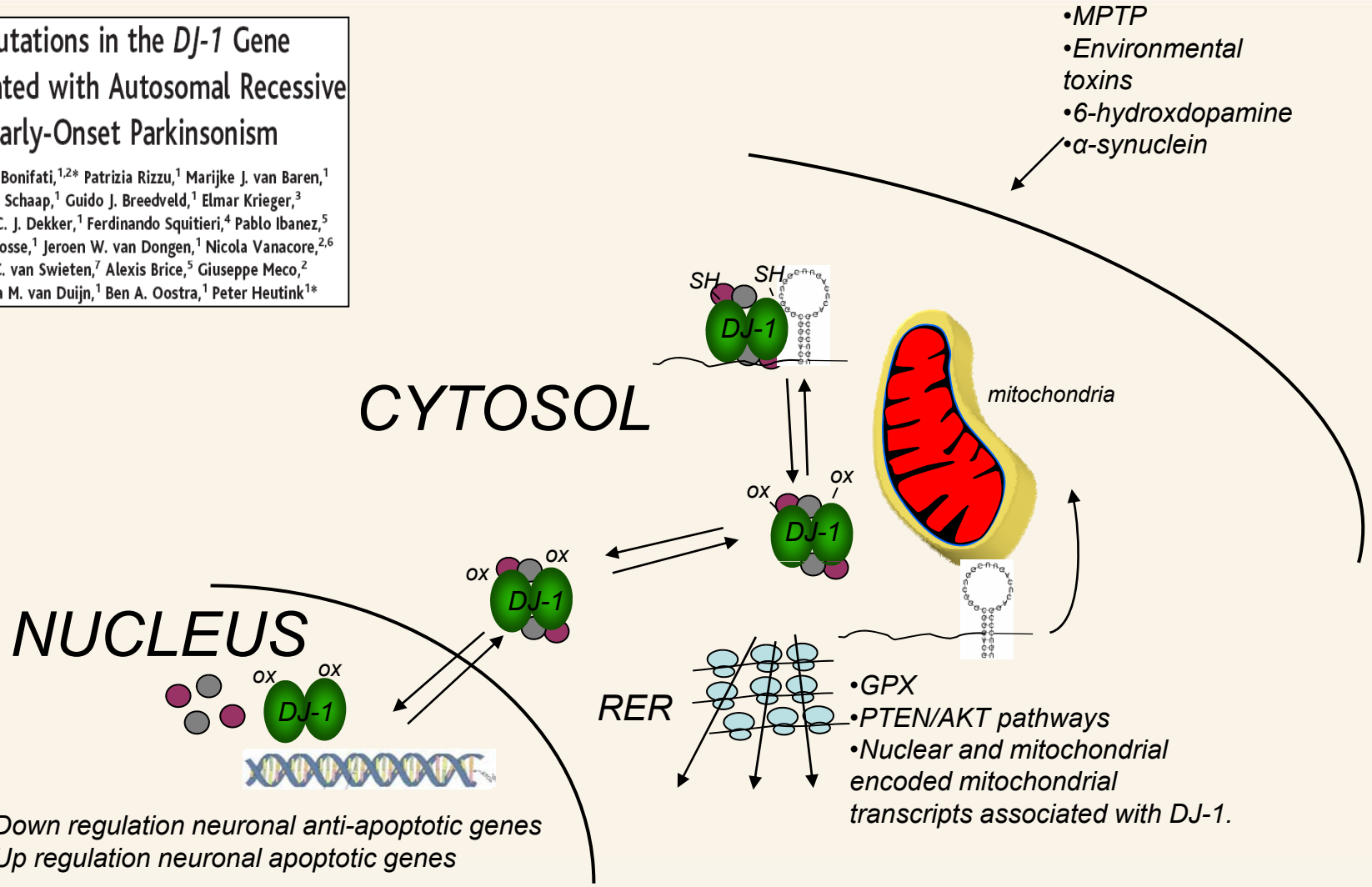
Reported physical and functional interactions DJ-1

Mutations in the *DJ-1* Gene Associated with Autosomal Recessive Early-Onset Parkinsonism

Vincenzo Bonifati,[1,2*] Patrizia Rizzu,[1] Marijke J. van Baren,[1] Onno Schaap,[1] Guido J. Breedveld,[1] Elmar Krieger,[3] Marieke C. J. Dekker,[1] Ferdinando Squitieri,[4] Pablo Ibanez,[5] Marijke Joosse,[1] Jeroen W. van Dongen,[1] Nicola Vanacore,[2,6] John C. van Swieten,[7] Alexis Brice,[5] Giuseppe Meco,[2] Cornelia M. van Duijn,[1] Ben A. Oostra,[1] Peter Heutink[1*]



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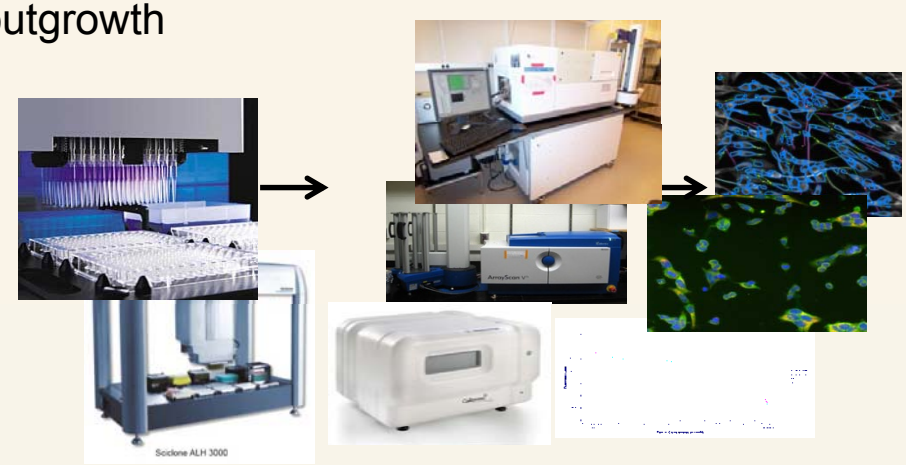
Experimental design to verify 42 interactors

- WT and DJ-1 KD cell lines plated into 96 well plates: 5000 cells per well
  - Experiments performed in triplicate
  - shRNA knockdown of 42 genes/5 shRNA's per gene (TRC1 shRNA library)
  - Virus pre-plated into assay plates – known interactors of DJ1
  - Negative controls per plate – scrambled shRNA
  - Positive controls per plate – cells incubated with 0.1% saponin for 10mins
  - Wells with no cells for background (used as control for data acquisition)

- SH-SY5Y cells were infected and subsequently differentiated for 7 days
  - On day 5 toxin added for 24 hours
  - Cells assayed for viability
  - Automated antibody staining for DJ-1 translocation
  - Automated antibody staining for neurite outgrowth

- Data is collected using several instruments



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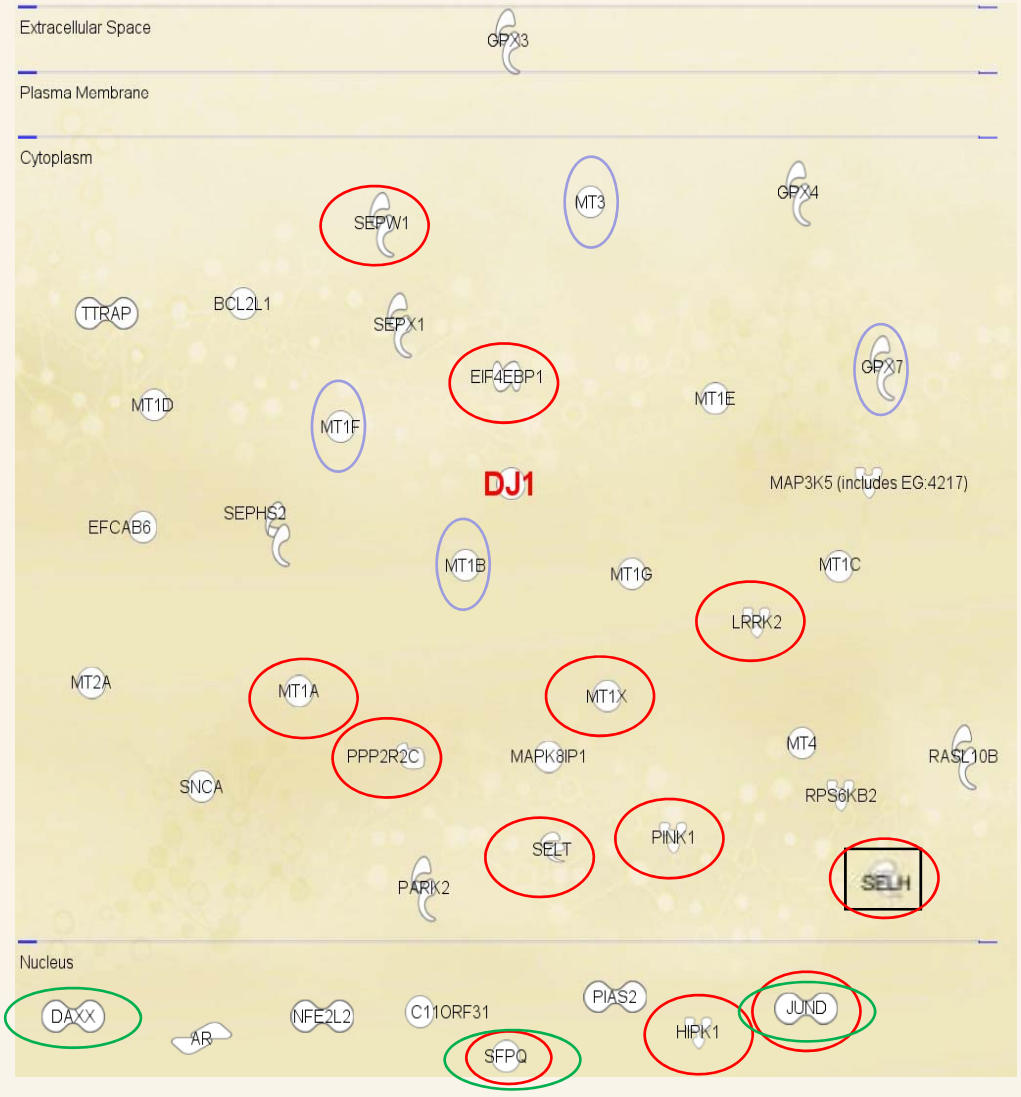
Modifiers of DJ1 function



Cell viability (red); Neurite outgrowth (green); DJ-1 translocation (purple)

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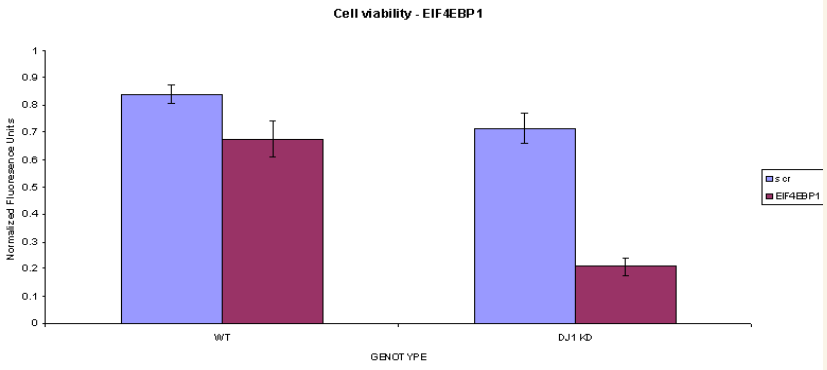
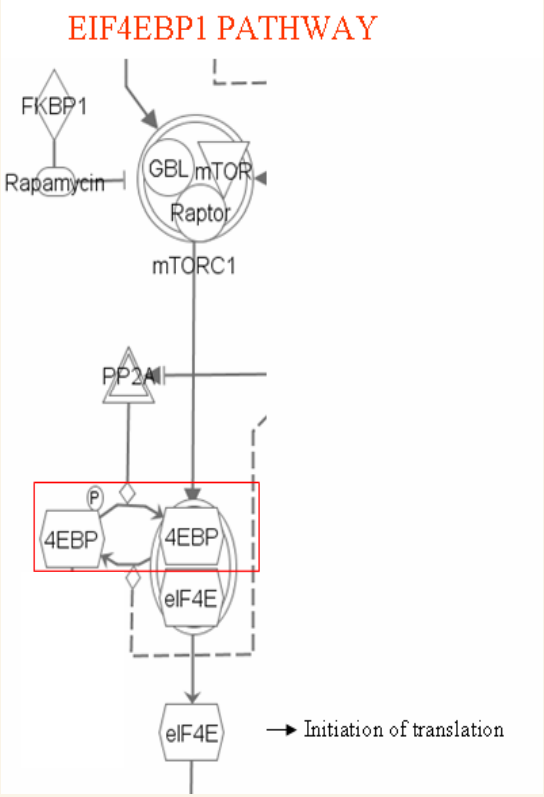
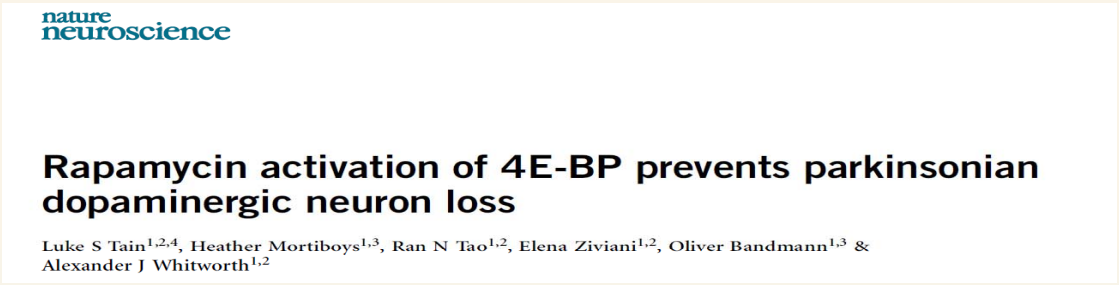
Modifiers of DJ1 function – Cell viability





EIF4EBP1 - eukaryotic initiation factor 4E binding protein

- Negative regulator of eIF
- Loss of DJ1 leads to increased translation of specific mRNA targets
- Loss of eIF4EBP1 may add to the deregulation of translation


nature neuroscience

Rapamycin activation of 4E-BP prevents parkinsonian dopaminergic neuron loss

Luke S Tain[1,2,4], Heather Mortiboys[1,3], Ran N Tao[1,2], Elena Ziviani[1,2], Oliver Bandmann[1,3] & Alexander J Whitworth[1,2]

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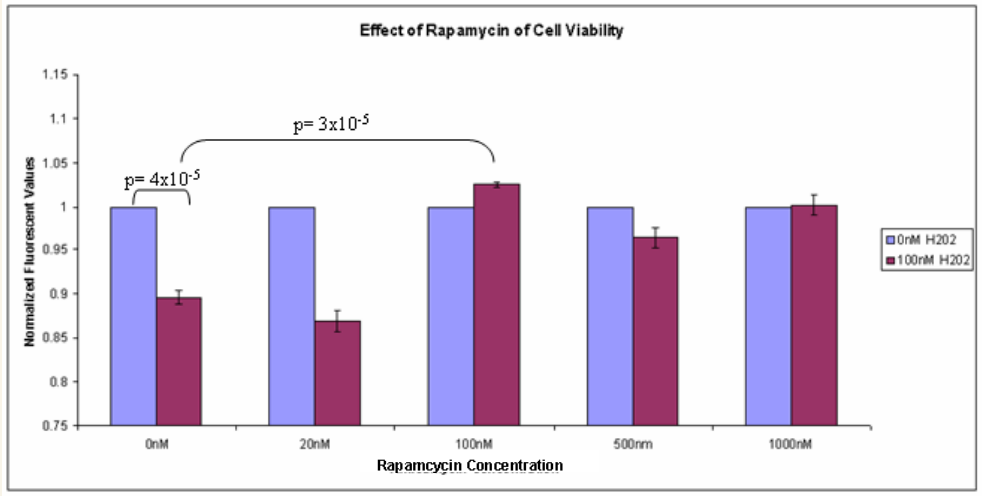
Modifiers of DJ1 function – EIF4EBP1

- Effect validated with additional shRNA clones
- Inhibition of EIF4EBP-1 activation by rapamycin leads to reduced levels of protein translation and hypothetically prevent cell death
- Addition of rapamycin to DJ1 deficient cells prevent cell loss.
- Rapamycin used by NIH consortium for ADMET testing



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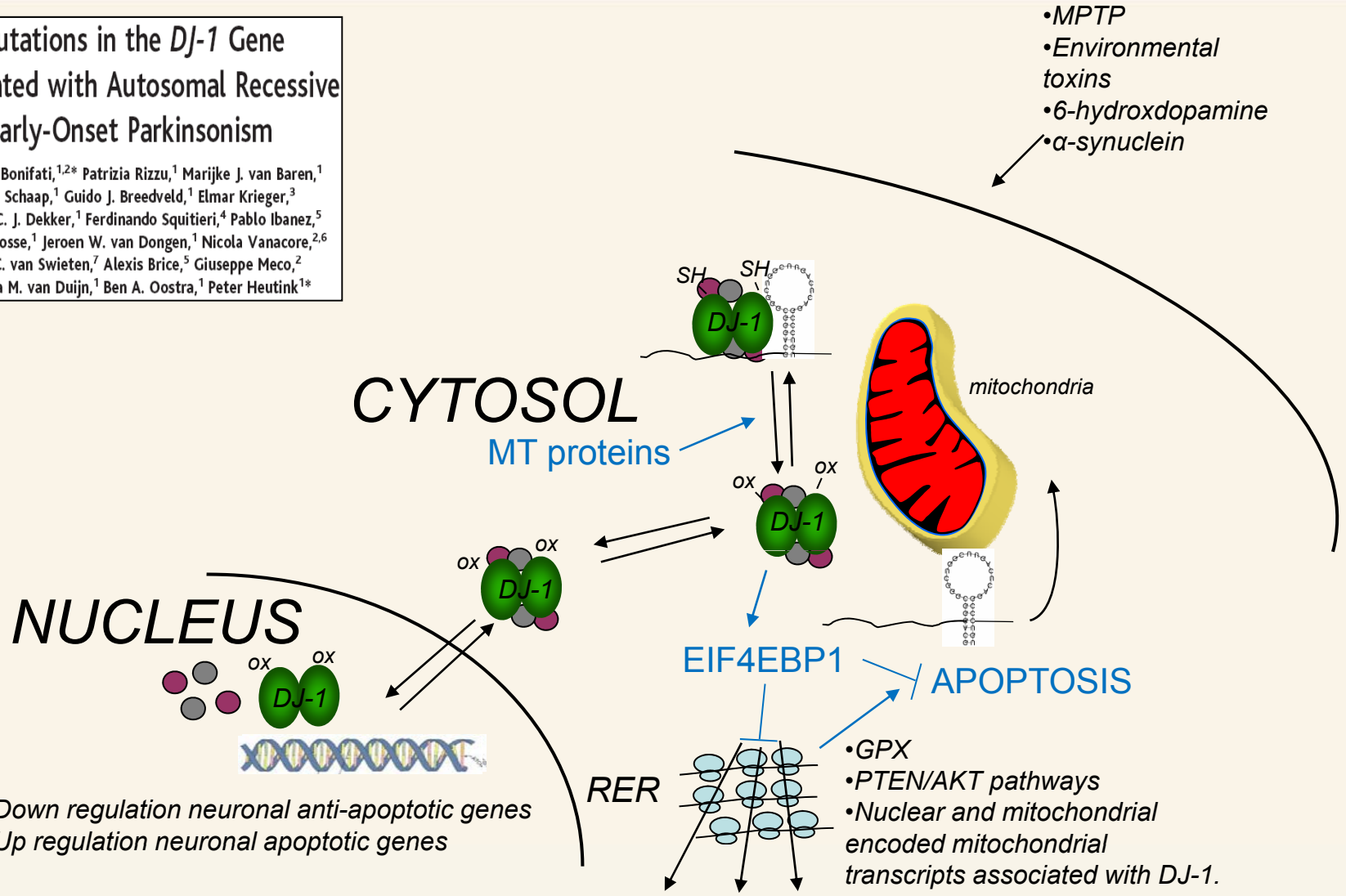

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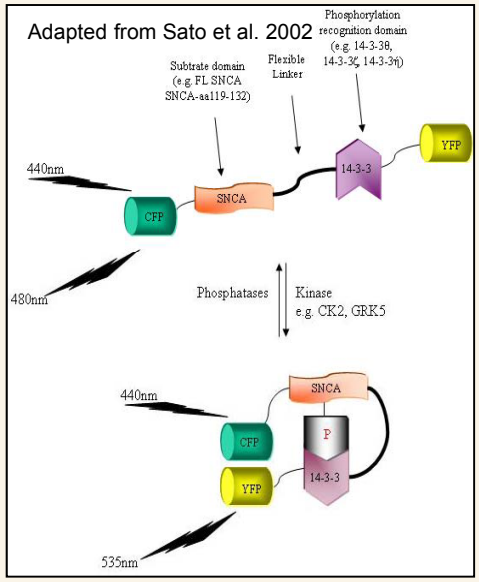
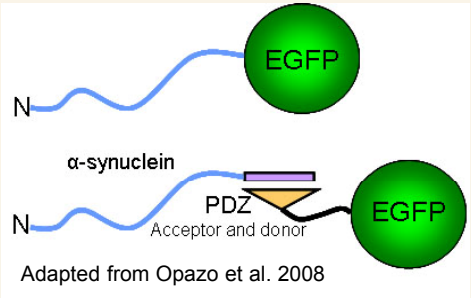
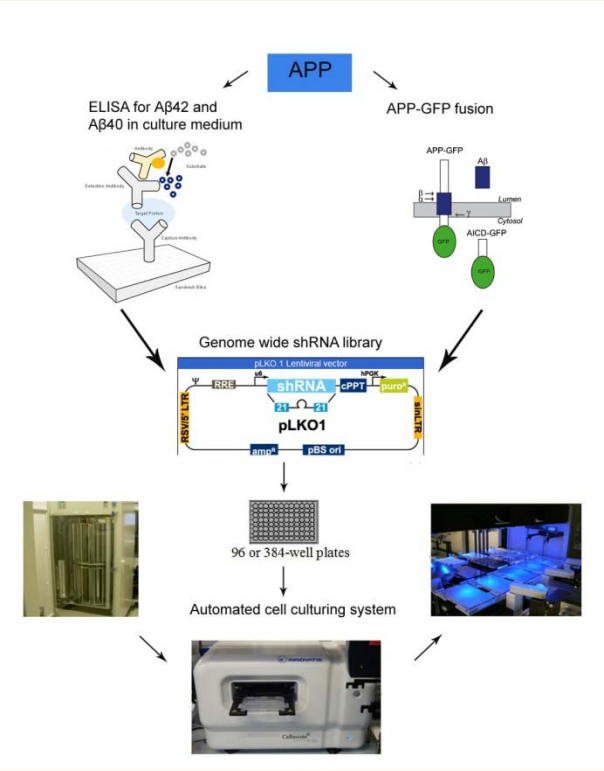
Assay building and validation

α-synuclein phosporylation and aggregation

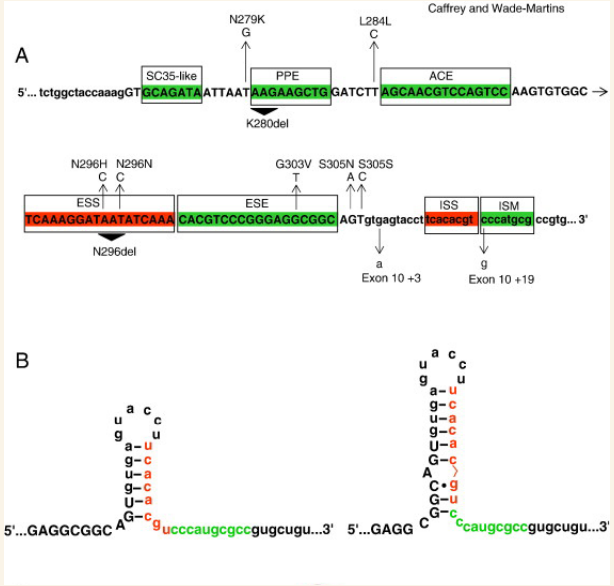




APP processing



MAPT splicing



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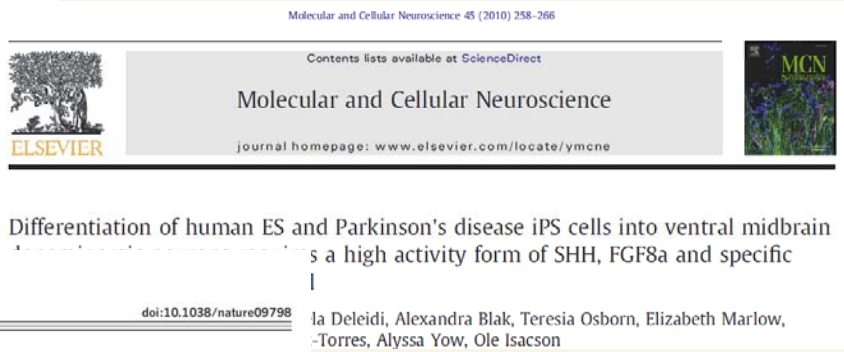
But how to model multifactorial disease?

- To model the combined effects of tens to hundreds of (weak) genetic risk factors, differentiated patient specific iPS cells holds great promise but:
  - differentiation protocols need to be improved.
  - iPS need to be extensively characterized

Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Cell

Kazutoshi Takahashi,[1] Koji Tanabe,[1] Mari Ohnuki,[1] Megumi Narita,[1,2] Tomoko Ichisaka,[1,2] Kiichiro Tomoda,[3] and Shinya Yamanaka[1,2,3,4,*]



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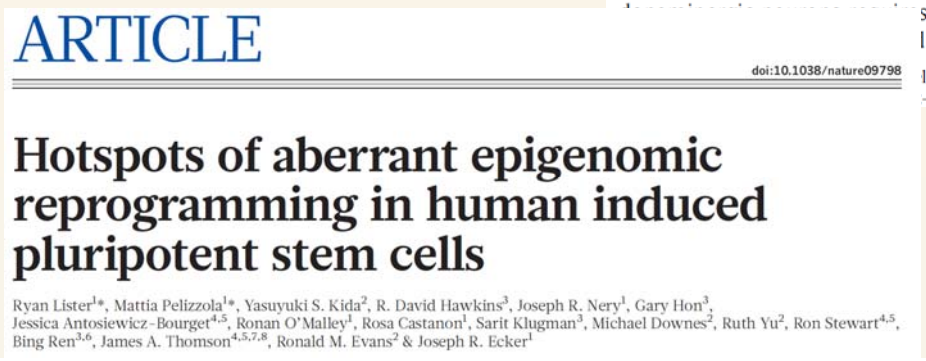
Differentiation of human ES and Parkinson's disease iPS cells into ventral midbrain
s a high activity form of SHH, FGF8a and specific

la Deleidi, Alexandra Blak, Teresia Osborn, Elizabeth Marlow,
-Torres, Alyssa Yow, Ole Isacson



ARTICLE

doi:10.1038/nature09798

Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells

Ryan Lister[1]*, Mattia Pelizzola[1]*, Yasuyuki S. Kida[2], R. David Hawkins[3], Joseph R. Nery[1], Gary Hon[3], Jessica Antosiewicz-Bourget[4,5], Ronan O'Malley[1], Rosa Castanon[1], Sarit Klugman[3], Michael Downes[2], Ruth Yu[2], Ron Stewart[4,5], Bing Ren[3,6], James A. Thomson[4,5,7,8], Ronald M. Evans[2] & Joseph R. Ecker[1]

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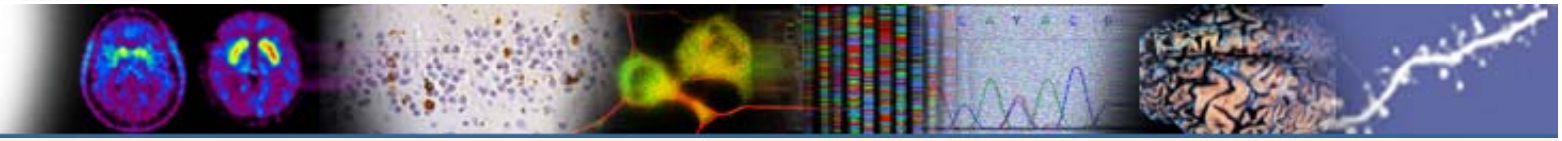
MEDICAL GENOMICS



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