From Networks through Genes to Mechanisms: Yeast cell cycle and Human Hypertension

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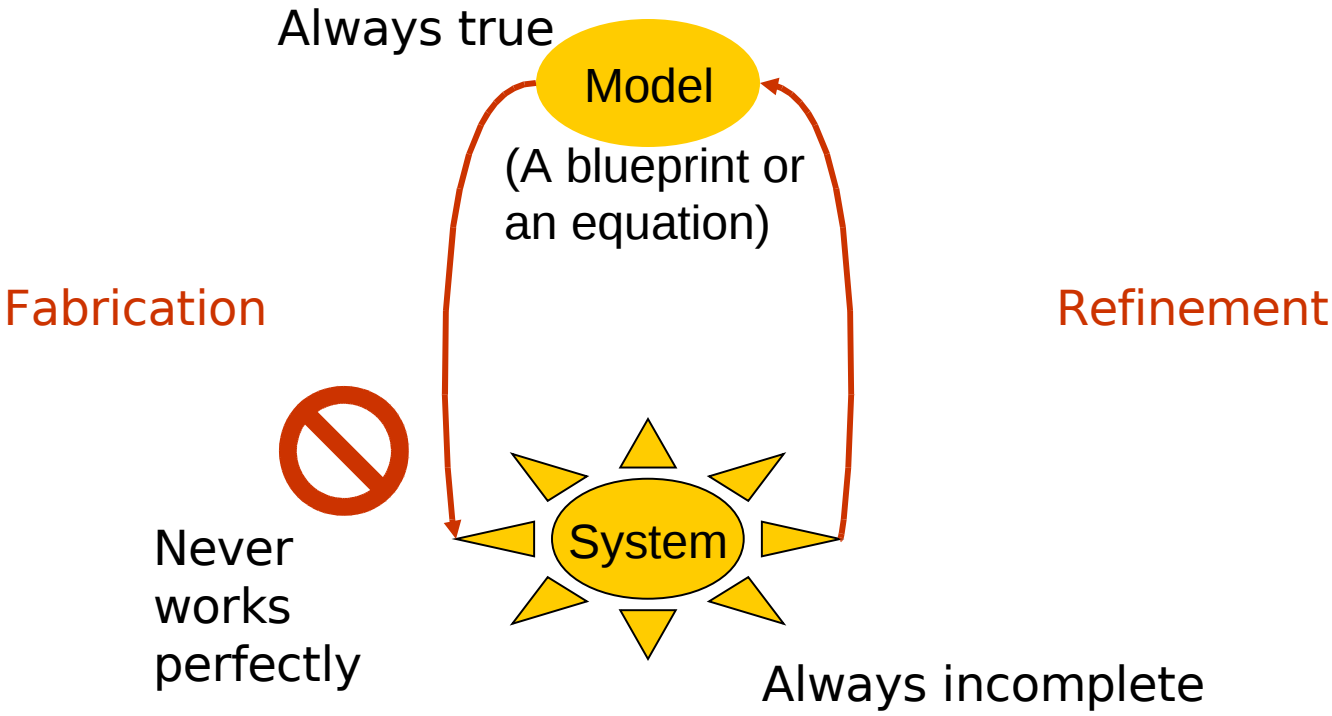
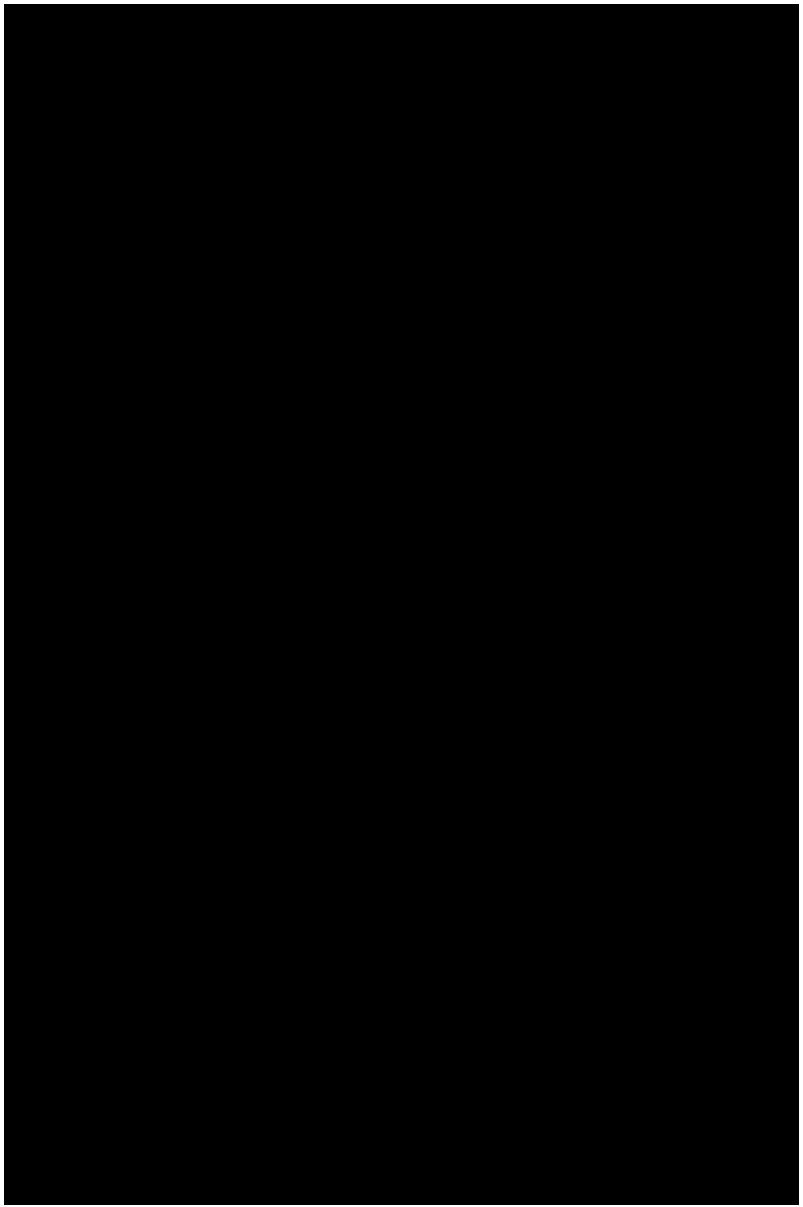
The epistemology of engineering



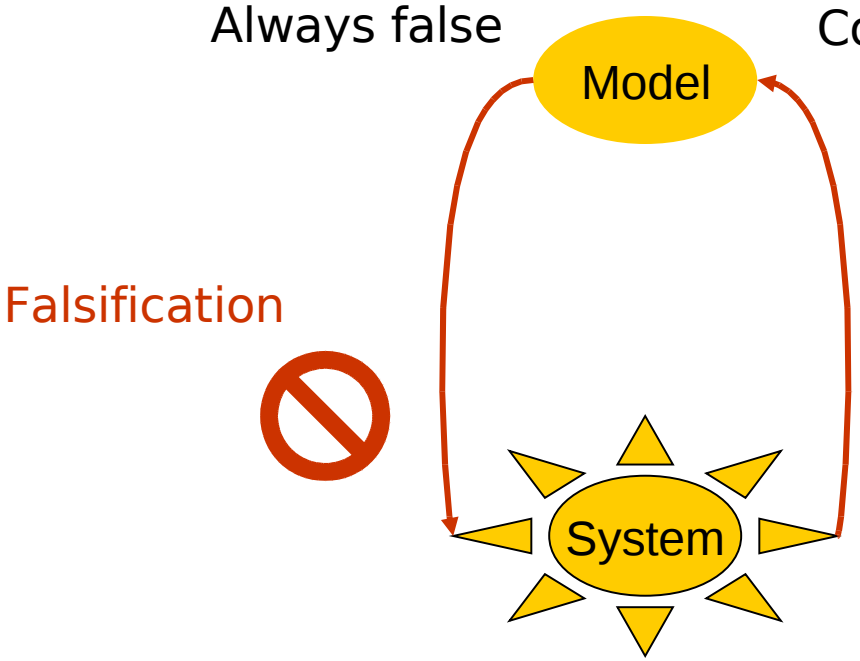
Refrigerating machine with organic solvent

Albert Einstein & Leo Szilard



Andy Delano, Georgia Institute of Technology, with the Einstein-Szilard refrigerator: Works but hardly practical!!

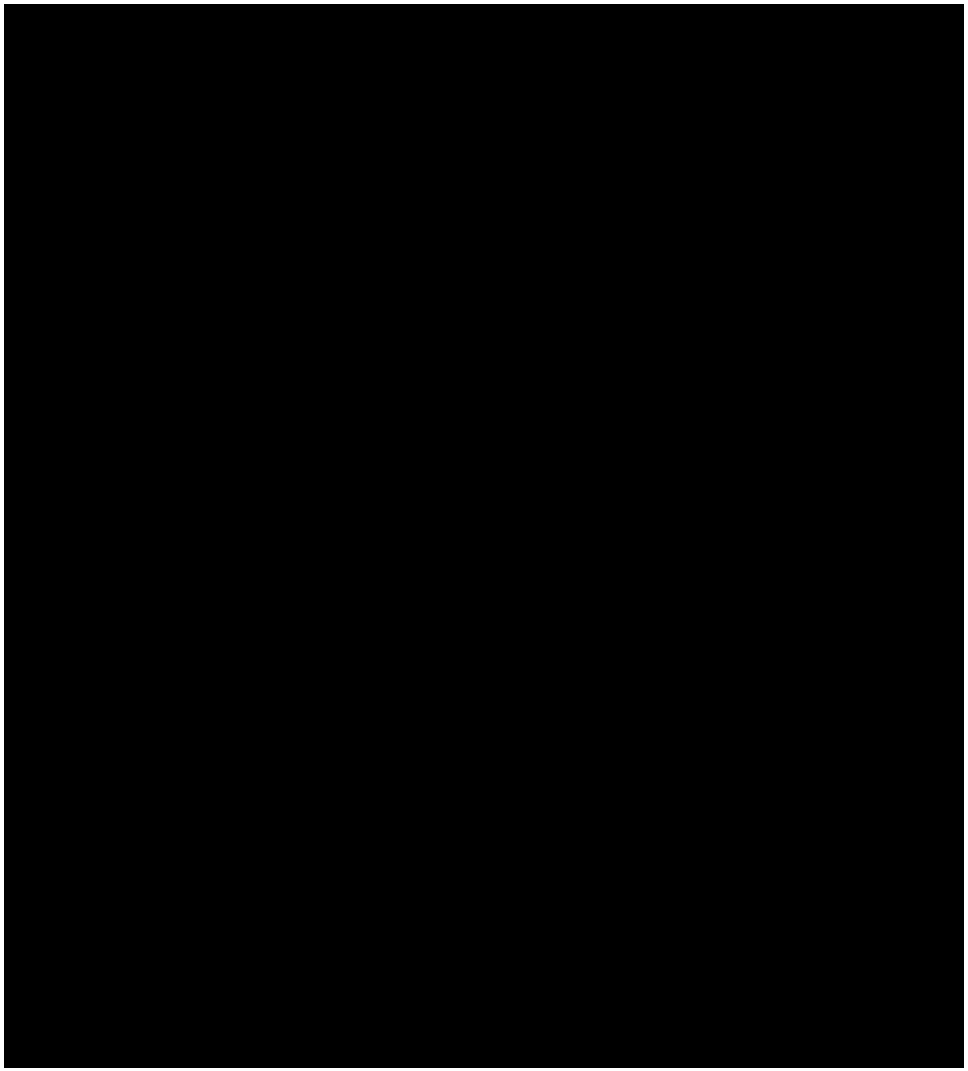
The epistemology of science (Popper)



The double helix
$E = mc^2$
[redacted]

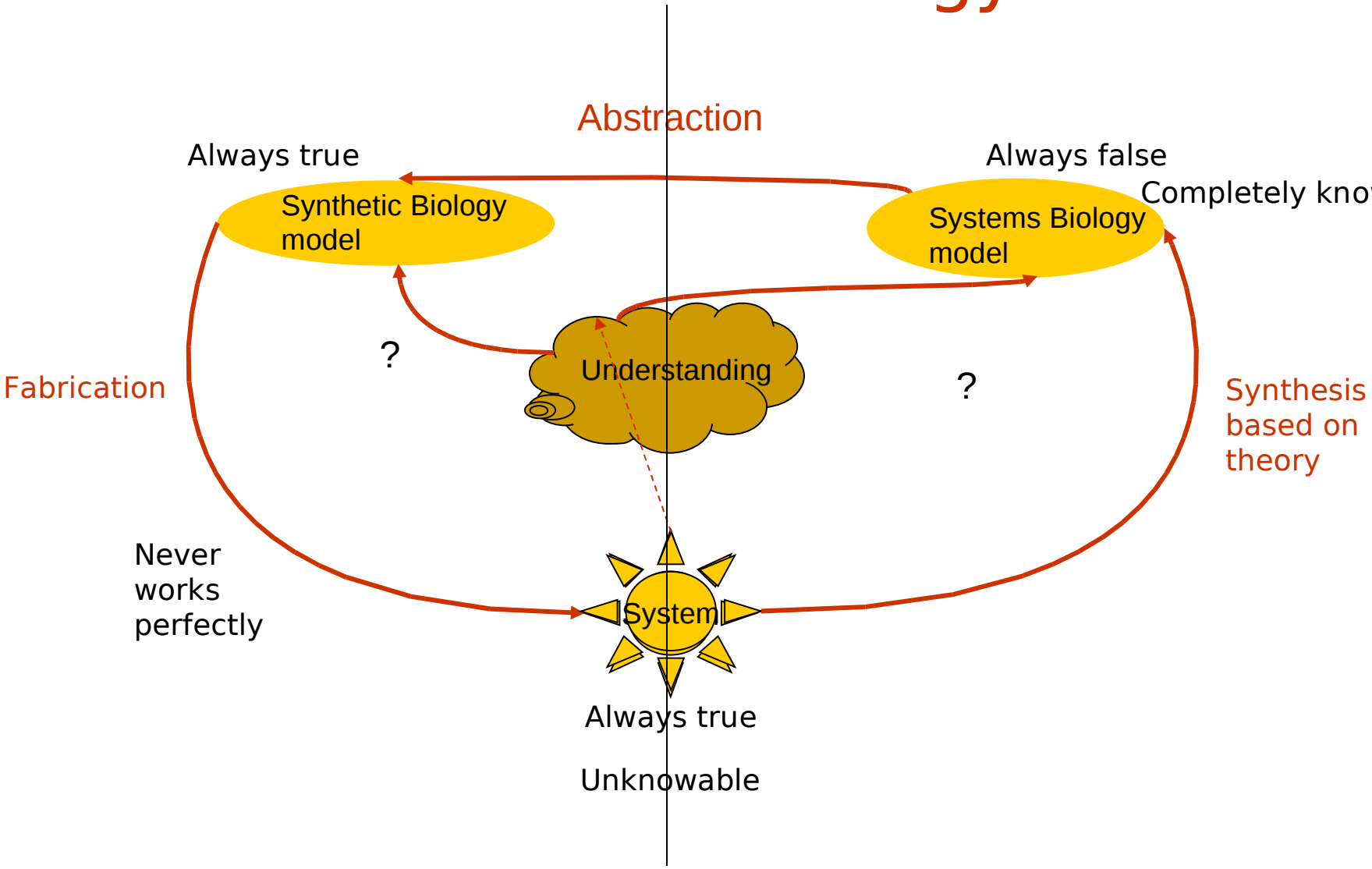
What is a gene?
Physical reality?

What is a gene?



A unit of a phenotype (Mendel)
A unit of mutation (Morgan)
A piece of a DNA chain (Hershey)
A DNA chain encoding a protein (Watson & Crick)
A cistron (Benzer)
A DNA chain encoding a polypeptide (Yanofsky)
A set of exons encoding mRNA (Sharp)
A set of exons with a common set of *cis* regulatory elements
A piece of DNA encoding an miRNA
A piece of DNA encoding an ncRNA
A piece of DNA encoding a piRNA
…etc etc…

The ‘New’ Biology



Theories in Biology

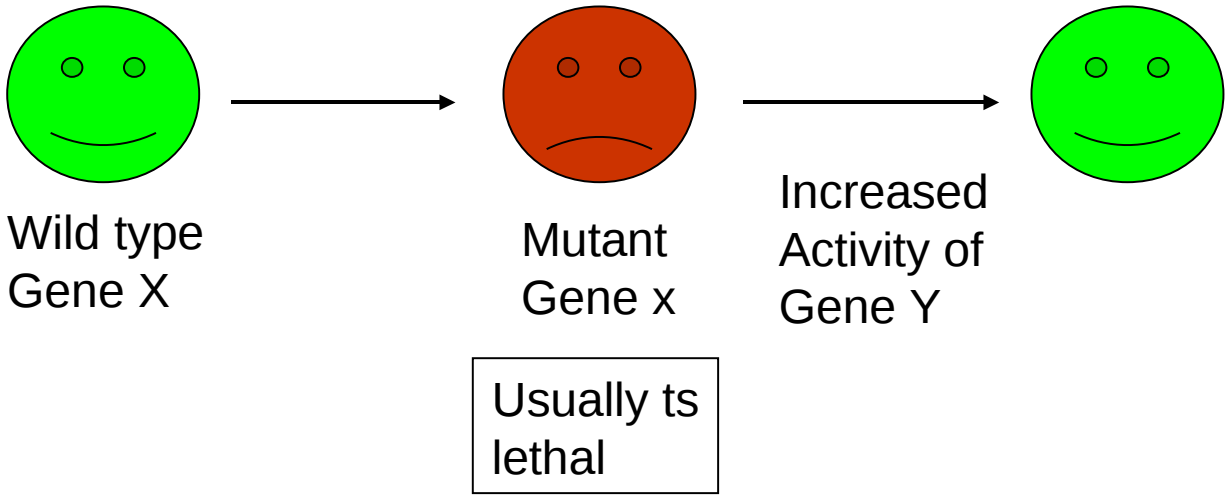
- Theory of Evolution
- Information as the central idea in biology (Schrödinger, Szilard, von Neumann), genetics (Crick, Gamow) and biochemistry (Szent-Gyorgyi, Lipmann)
- Biological networks (Monod and Jacob, Kauffman):
  - Biomolecules interact with one another
  - Their interactions are akin to communication channels
  - Phenotype is encoded by the interaction network in as much as the organizational behavior of a community is encoded by the communication network

Aims today

Present two vignettes that demonstrate the relevance of the network metaphor in understanding biology:

- From genetics through networks to mechanisms
- Networks and the genetics of hypertension

Principle of dosage suppression



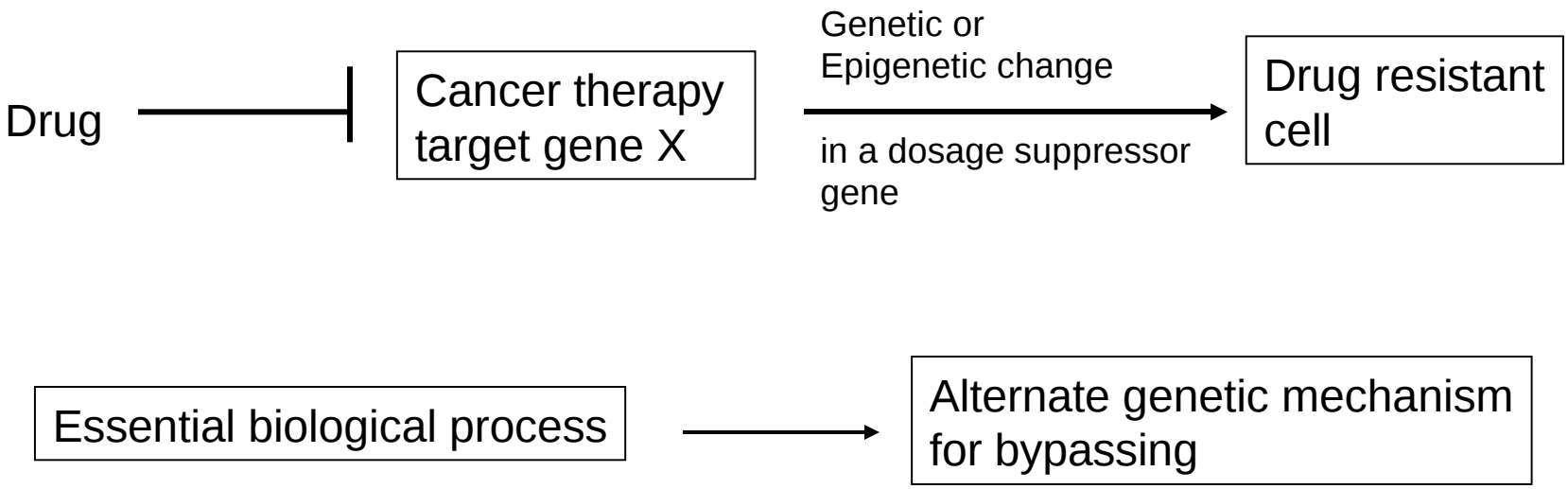
Implications of dosage suppression

- A severe selective pressure on cell survival, coupled with high dosage expression of another gene may reveal archival circuits of survival

- Alternate pathways through the network of interacting genes

- Existence of alternate pathways imply the possibility that some such pathways might be operative in future evolution

Utilities of dosage suppressor genes



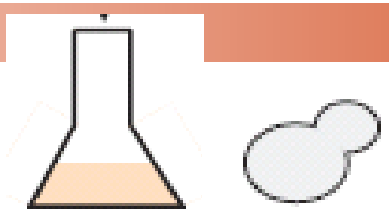
A genome wide dosage suppressor screen of essential genes in yeast

- A collection of 80 ts lethals in a uniform genetic background from Charlie Boone (U Toronto)
- A collection of ~30 preexisting ts lethals including ~18 deletion lethals (from YKO collection)
- Screened against a whole genome Moveable Open Reading Frame (MORF) library: galactose inducible genes in high copy plasmids (Gelperin et al. Genes & Dev. 19:2816-26 (2005)
  - Eric Phizicky and Elizabeth Grayhack (U Rochester)

Suppressor identification

Conditional mutants

Grow at permissive conditions



Transform with MORF Plasmids

(Whole genome ORF collection under Gal control)

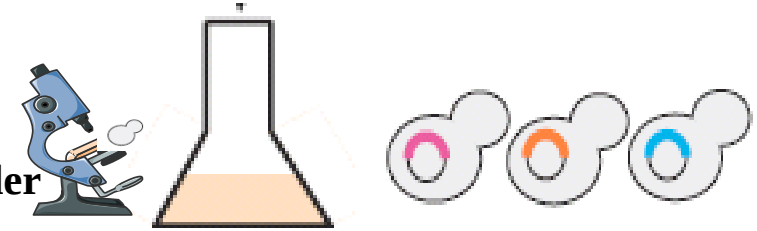
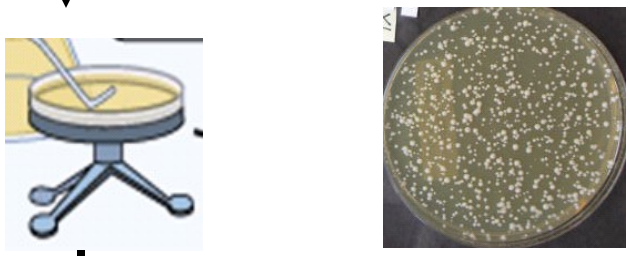


Plate cells and allow them to recover in selective media/permissive temp.



Scrape and pool all transformants

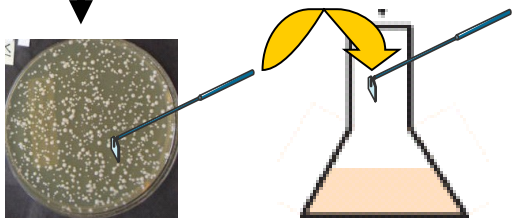
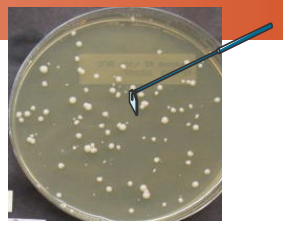


Plate cells in inducing media and shift to restrictive conditions

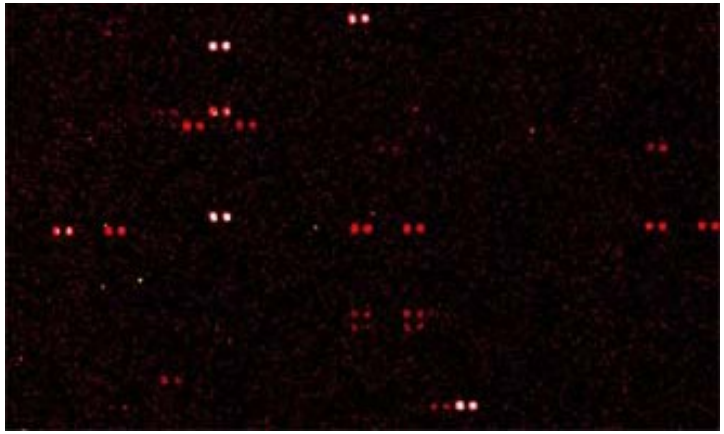
Yeast cells grow if original mutation is suppressed

Scrape and pool all colonies that grow

at restrictive temp.



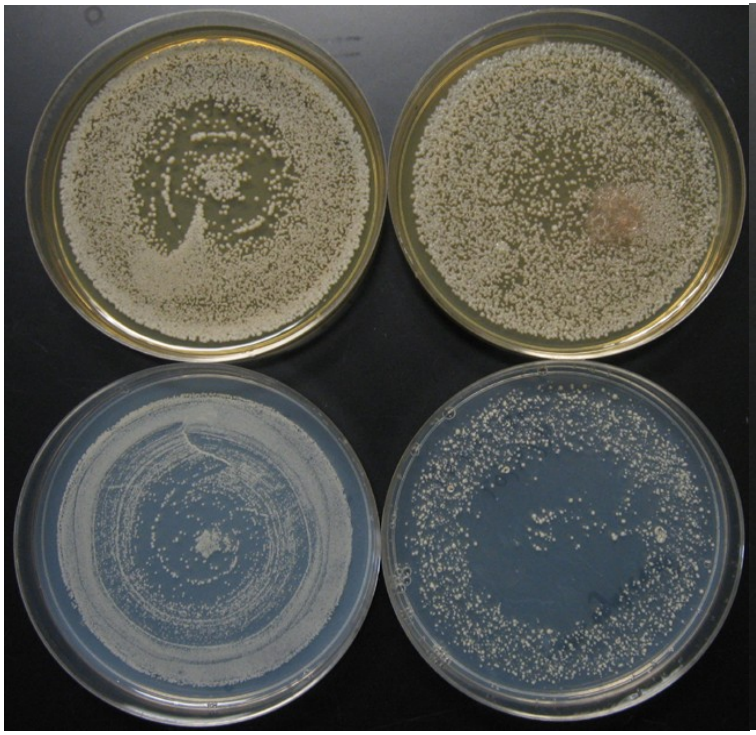
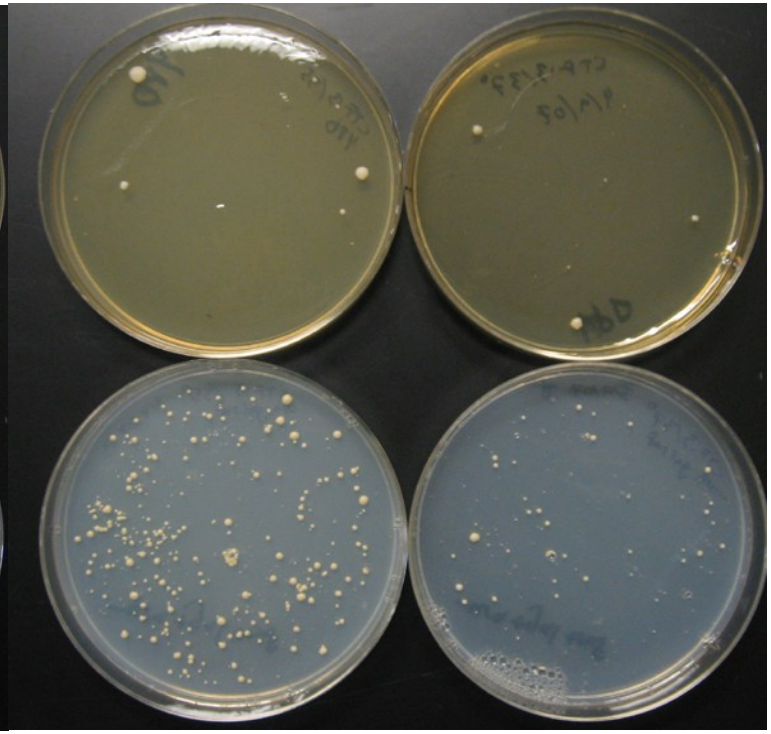
Isolate pooled DNA, PCR labeling and Hybridize to microarray



Identify putative suppressors

Re-isolate from MORF Library
Re-transform for final confirmation

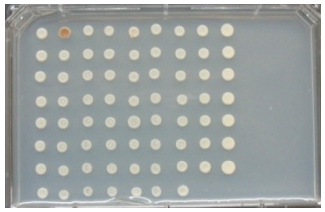
Screening and selection at restrictive temperature

	25	33	35	37
YPD				
-ura + Raf + Gal				

rlg1-4: confirmation test by spot test

(based on microarray result)

-URA R&G	SD-URA
YP R&G	YPD



-URA RAff 25°C

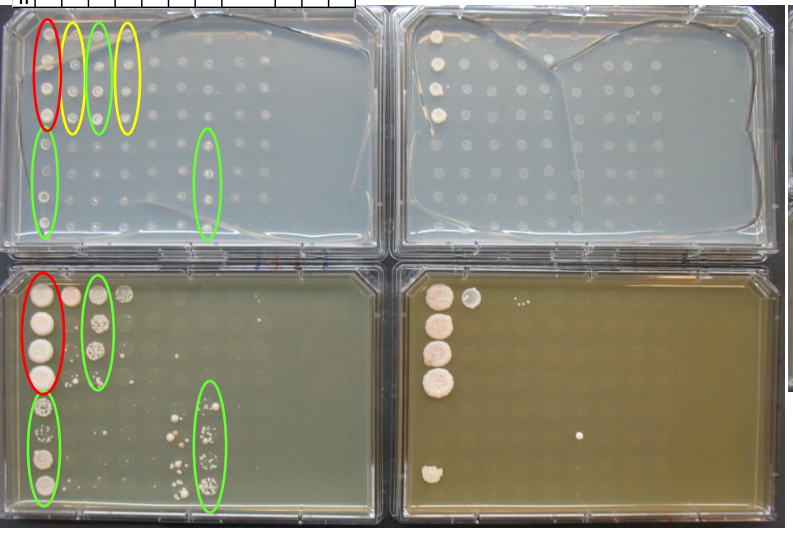
	1	2	3	4	5	6	7	8	9	10	11	12
A	RLG1	GAL80	ALR2	PGD1	YNL260C	YDR249C	YBR012C	CDC50	BG1766(-)			
B												
C												
D												
E	GIS3	CTR86	NOB1	CSI1	CDC55	RPC25	SGN1	YHM2(-)	ERV25(-)			
F												
G												
H								EMPTY				

35°C

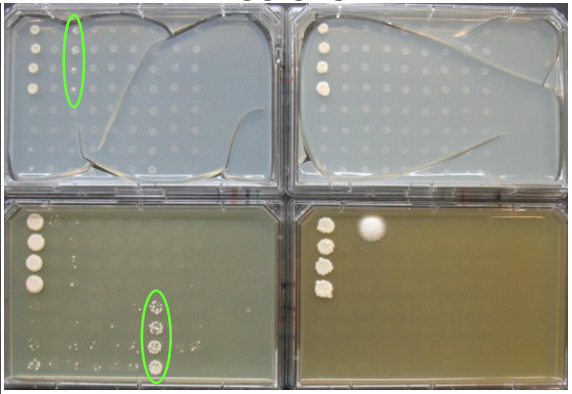
37°C

	1	2	3	4	5	6	7	8	9	10	11	12
A	RLG1	GAL80	ALR2	PGD1	YNL260C	YDR249C	YBR012C	CDC50	BG1766(-)			
B												
C												
D												
E	GIS3	CTR86	NOB1	CSI1	CDC55	RPC25	SGN1	YHM2(-)	ERV25(-)			
F												
G												
H								EMPTY				

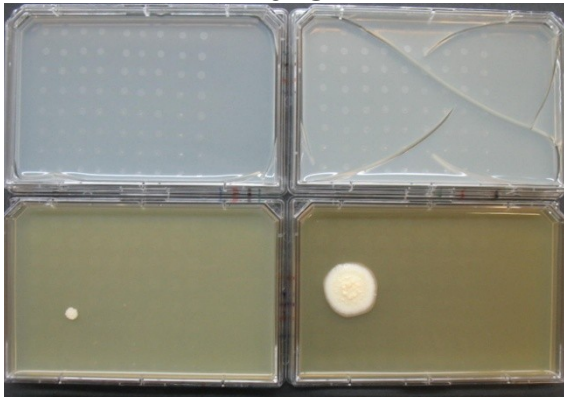
38°C



38.5°C



40°C



80 *ts* strains(CB)

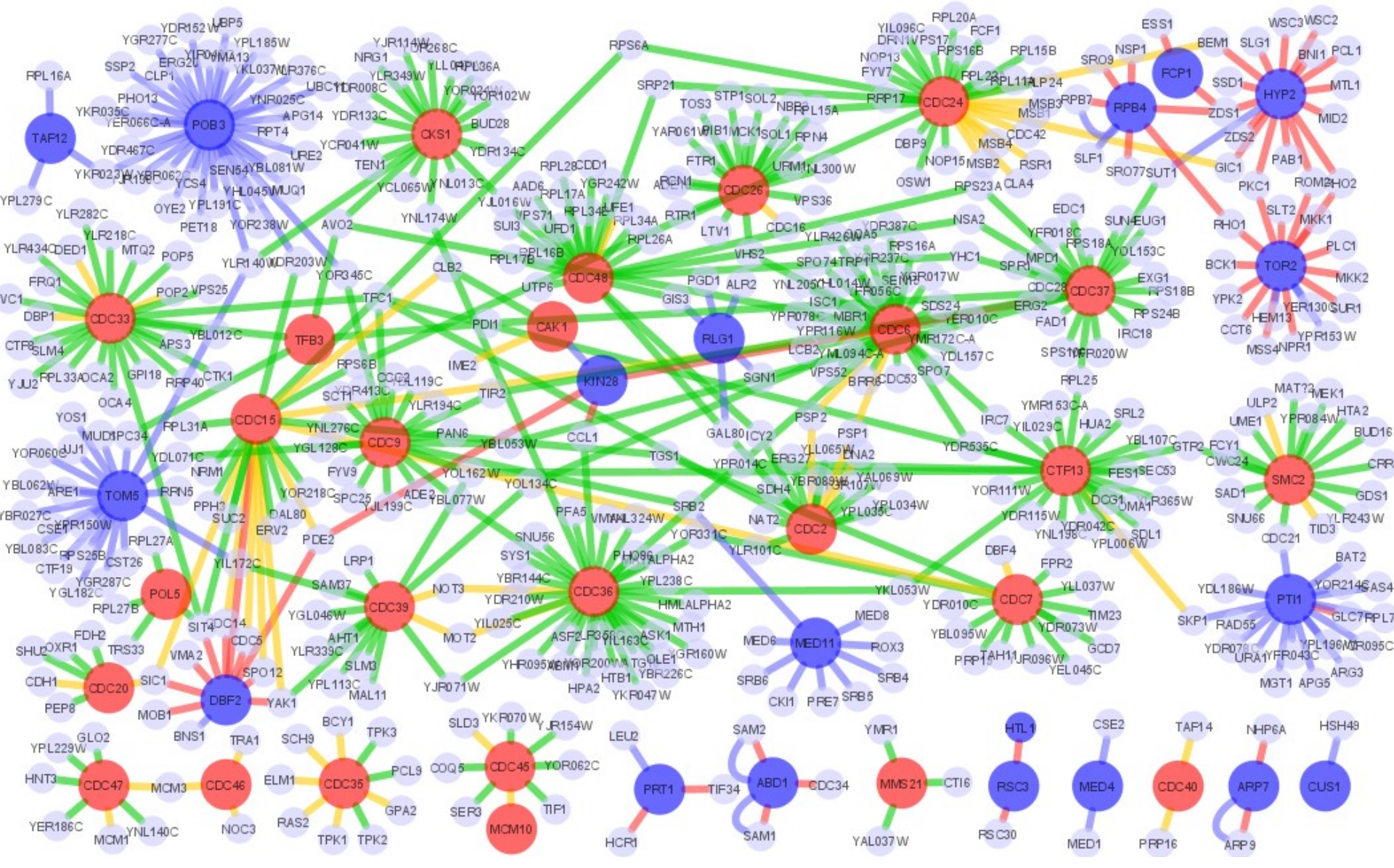
Cell cycle related genes

KGI	
cak1-23	cdc48-9 (Y-F)
cdc123-4	cdc5-1
cdc13-1	cdc6-1
cdc15-2	cdc7-1
cdc16-1	cdc9-1
cdc20-1	cks1-35
cdc24-H	ctf13-30
cdc25-1	kin28-ts
cdc2-7	med11-ts
cdc28-td	med4-6
cdc33-E72G	mms21-1
cdc35-1	pob3-7
cdc36-16	pol5-2
cdc37	pti1-ts7
cdc39-1	rad3-ts14
cdc40-ts	rsp5-1
cdc4-1	smc2-8
cdc45-27	spt6-14
cdc46-1	taf12-9
cdc47	tfb3-ts

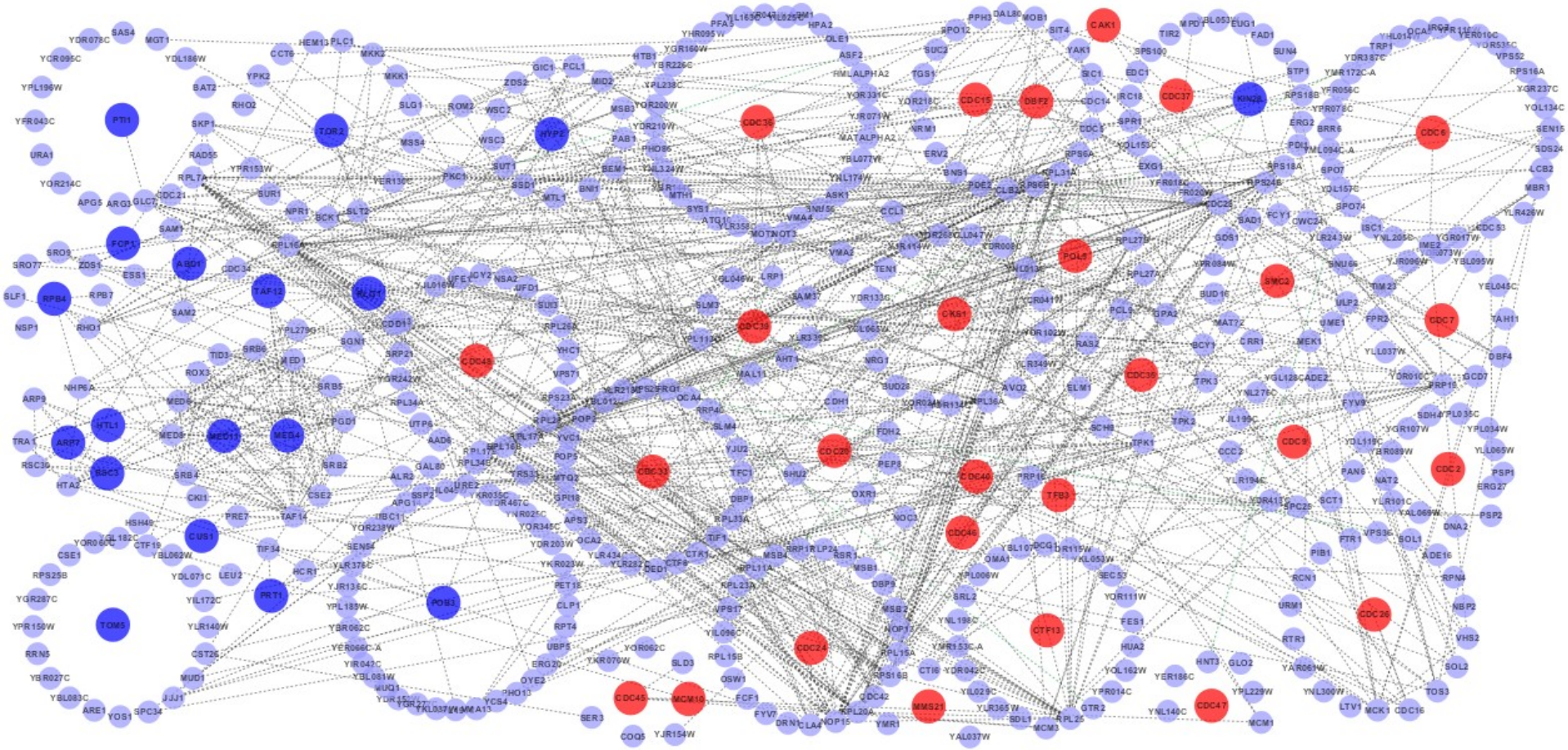
Rochester	
abd1-5	mcm10-1
abf1-102	mcm2-1
afg2-18	mot1-1033
arp4-G161D	nab3-11
arp7-E411K	nog2-1
cft2-1	nop2-3
cus1-3	orc2-1
dbf2-1	orc3-70
dbp5-1	pcf11-ts10
dcp2-7	prt1-1
ded1-199	rap1-1
dim1-2	rat1-1
esa1-L254P	rnt1-ts
ess1-H164R	rsc3-1
fcp1-1	spt15-P65S
gcd10-506	sup35-td
gcd1-502	swd2-1
hsf1-848	tfc1-E447K
hts1-1	tor2-21
hyp2-1	yef3-F650S

RNA related genes

Suppressors network

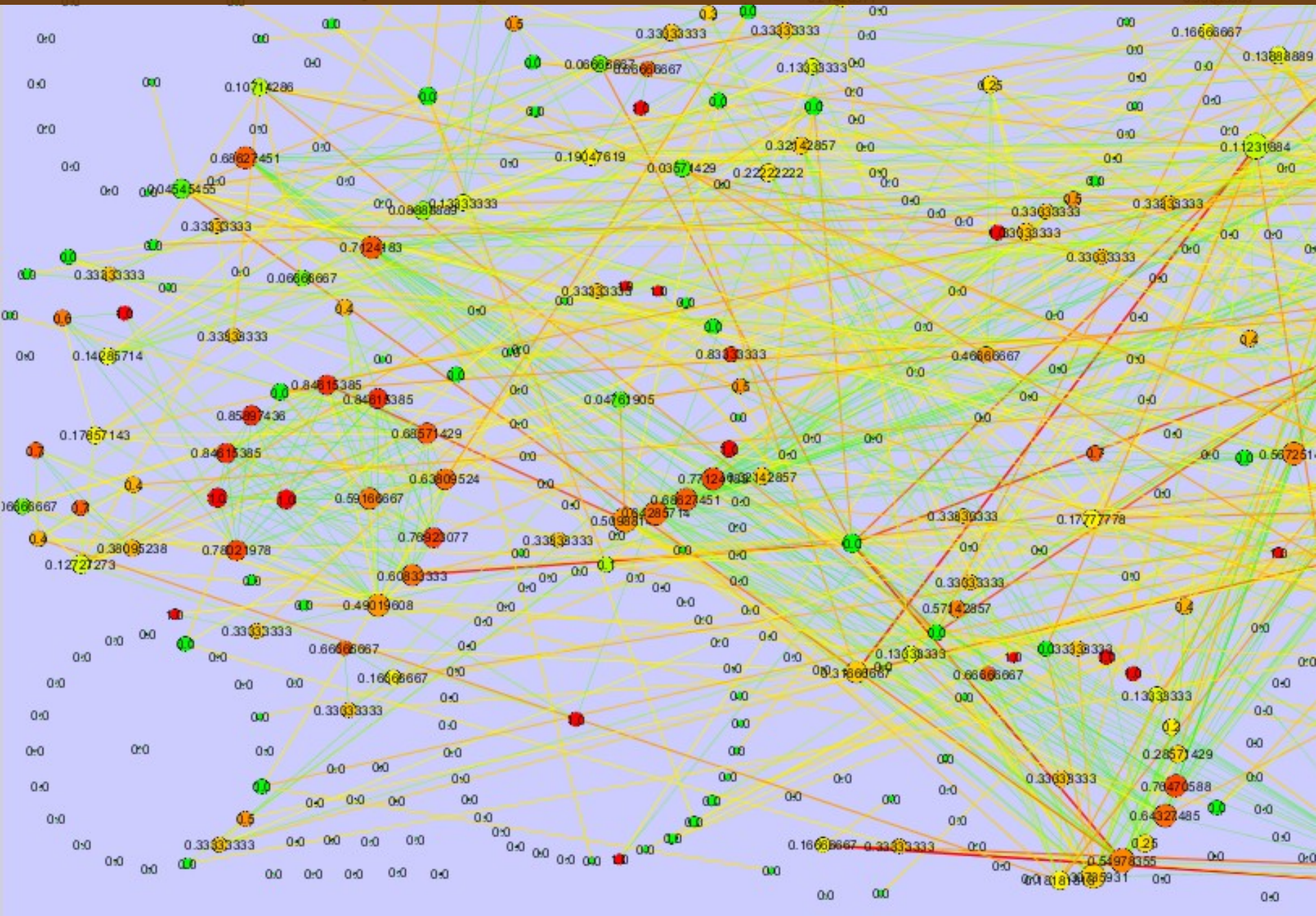


Network of only Physical Protein Interactions among Point mutants and Suppressors

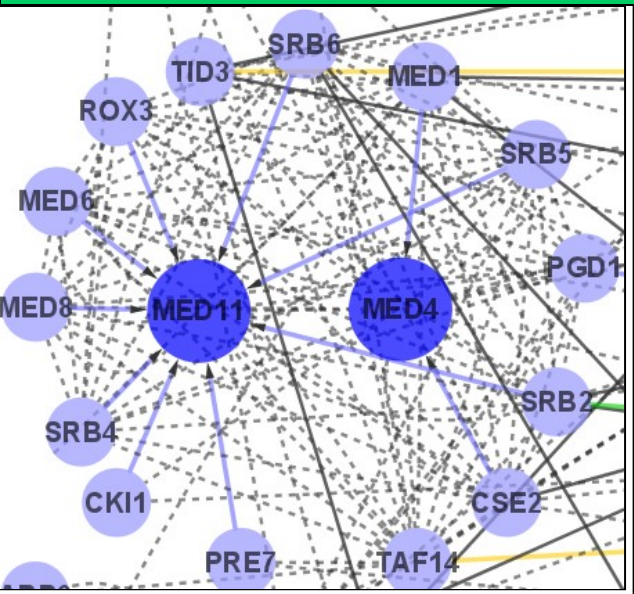


Point Mutants : [Dark Blue]=non-CDC and [Red]=CDC
Light Blue – Suppressors

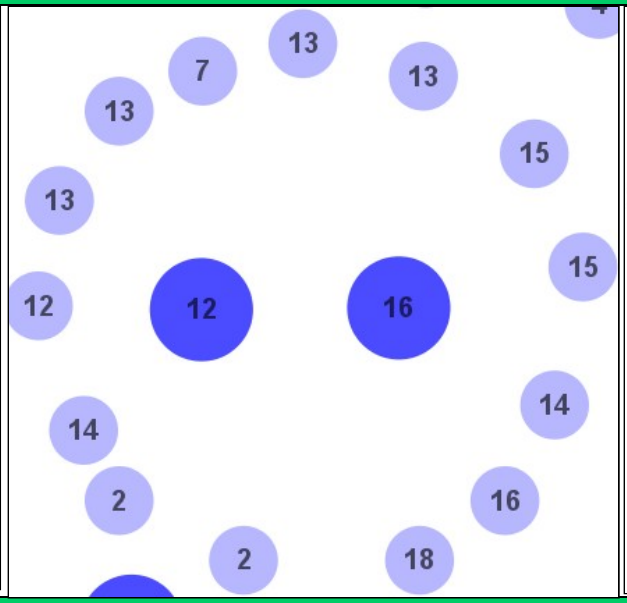
Nodes with Highest Clustering Coefficients (Left Half)



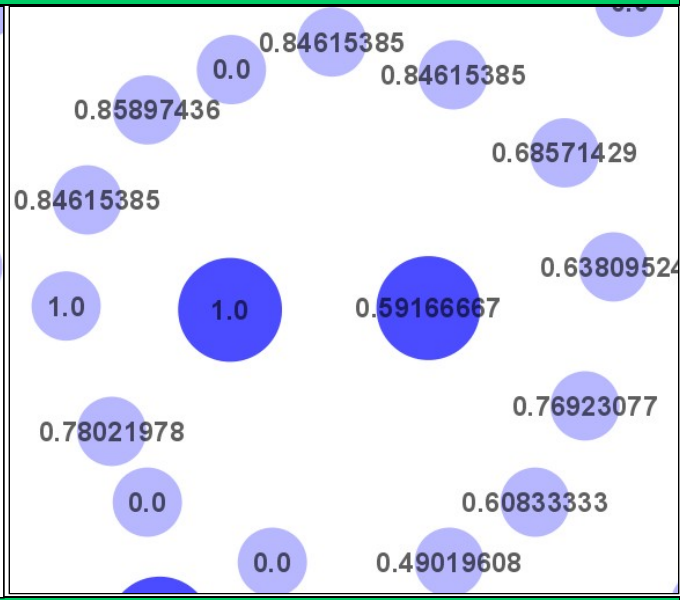
Suppressor Network along With PPI and SL overlay



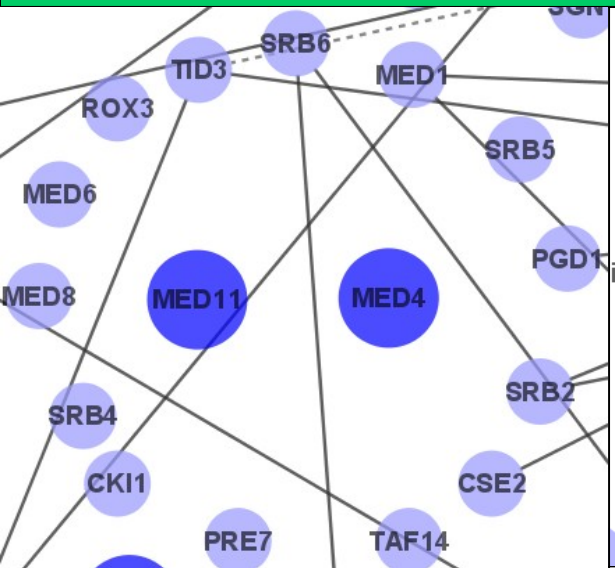
Number of PPI interactions for each suppressor node



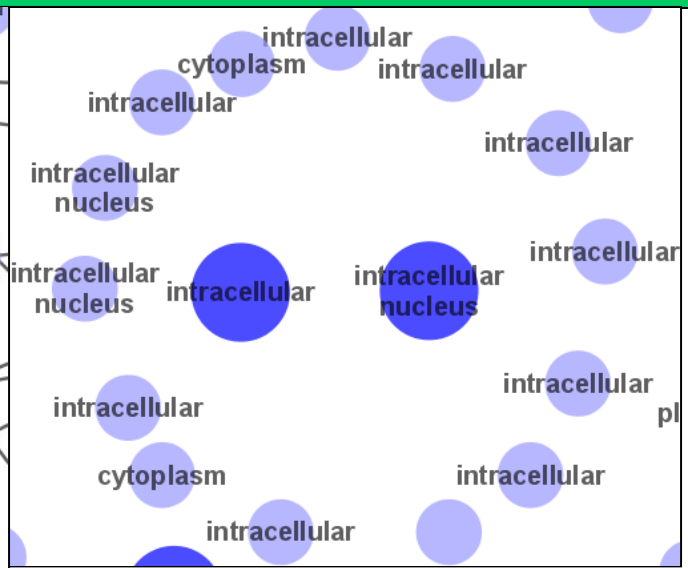
Clustering Coefficient of the Protein-Interaction Network



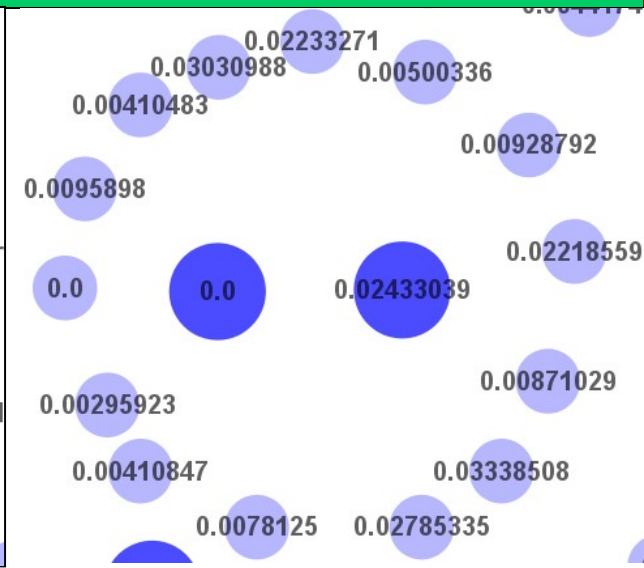
Synthetic Lethal Interaction network



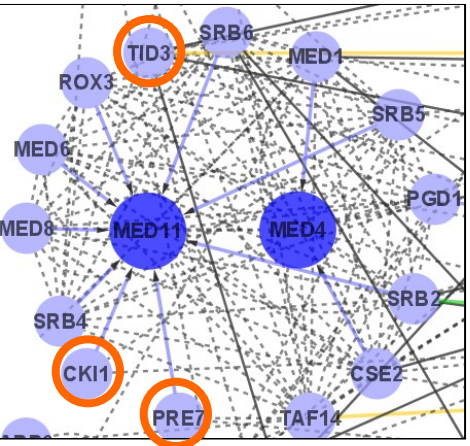
Cellular localization of all suppressor nodes

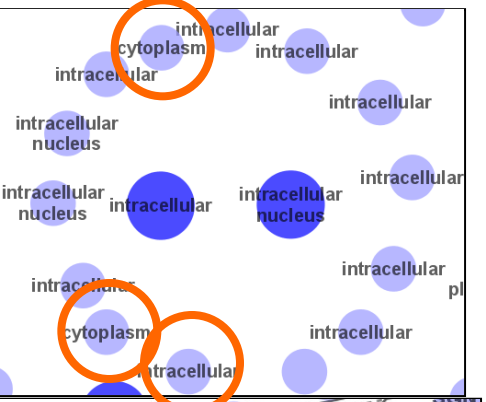


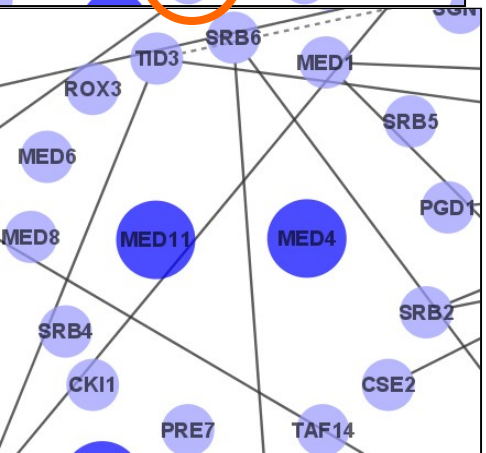
Betweeness Centrality of Suppressor nodes (PIN)



MED4 & MED11 : Results





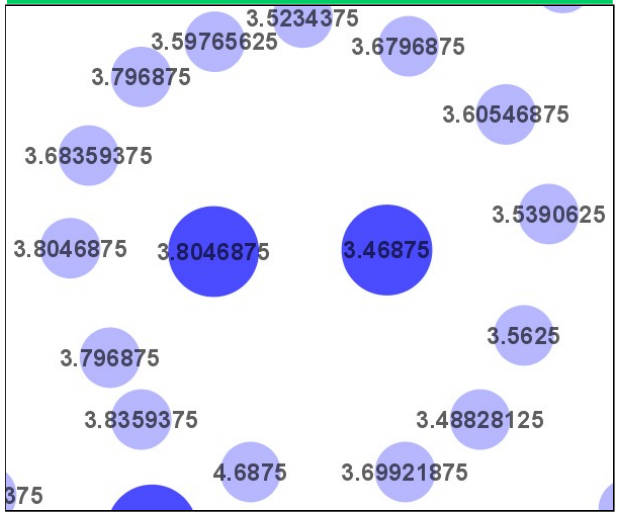


Suppressors for MED4 and MED11 take part in numerous physical interactions. As a result, they have very high clustering coefficients:

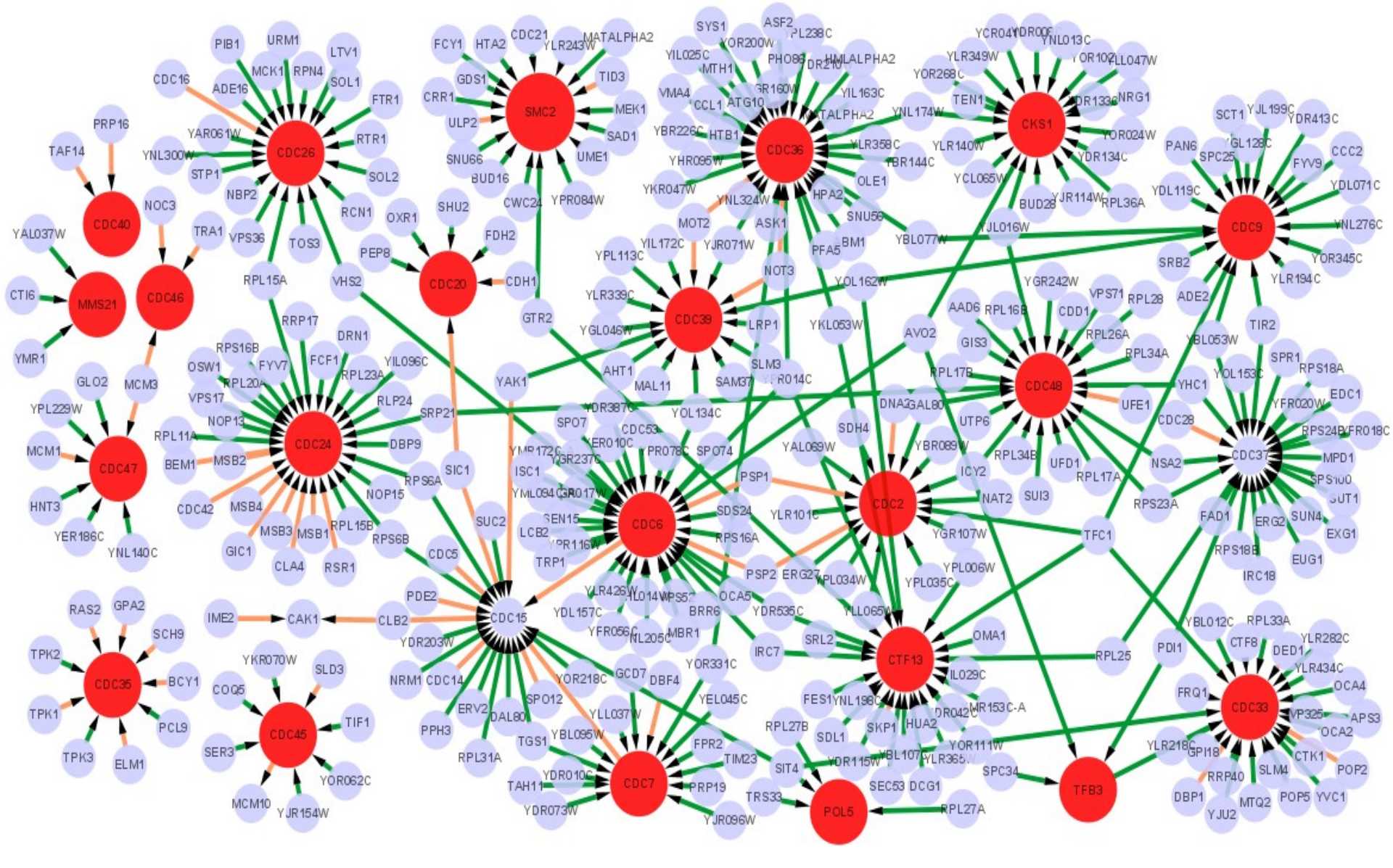
MED8	**1.000**
MED6	**0.846**
ROX3	**0.859**
TID3	**0.000**
SRB6	**0.846**
MED1	**0.846**
SRB5	**0.686**
PGD1	**0.638**
SRB2	**0.769**
CSE2	**0.608**
TAF14	**0.490**
PRE7	**0.000**
CKI1	**0.000**

- TID3 does not appear to belong to this complex (It physically interacts only with MED4)
- TID3 and CKI1 localize to the cytoplasm while all other suppressors of MED4 & MED11 localize to the intracellular region or nucleus.
- Most of the suppressors here rescue MED11. Would they also rescue MED4 ?

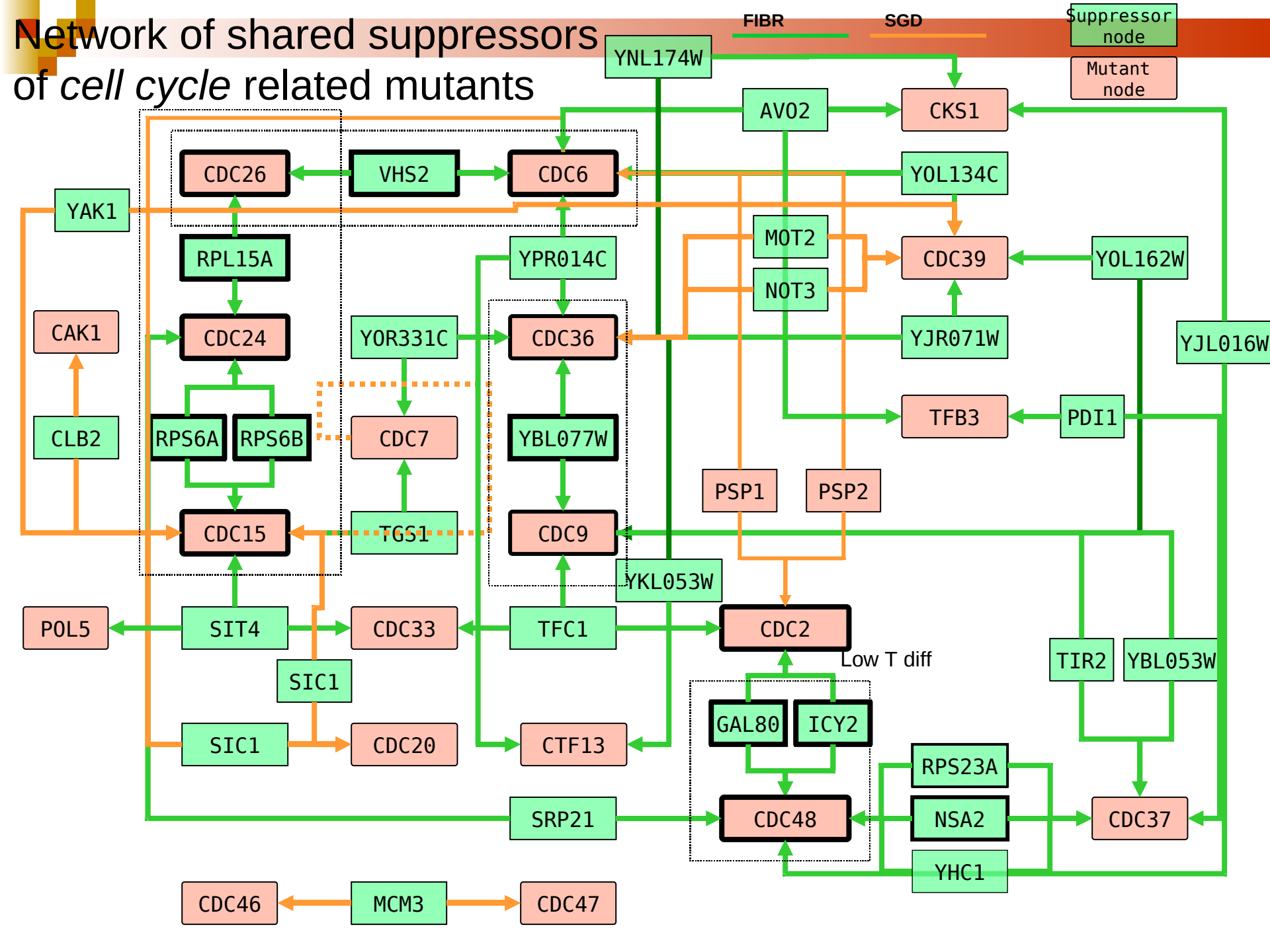
Shortest Node Length

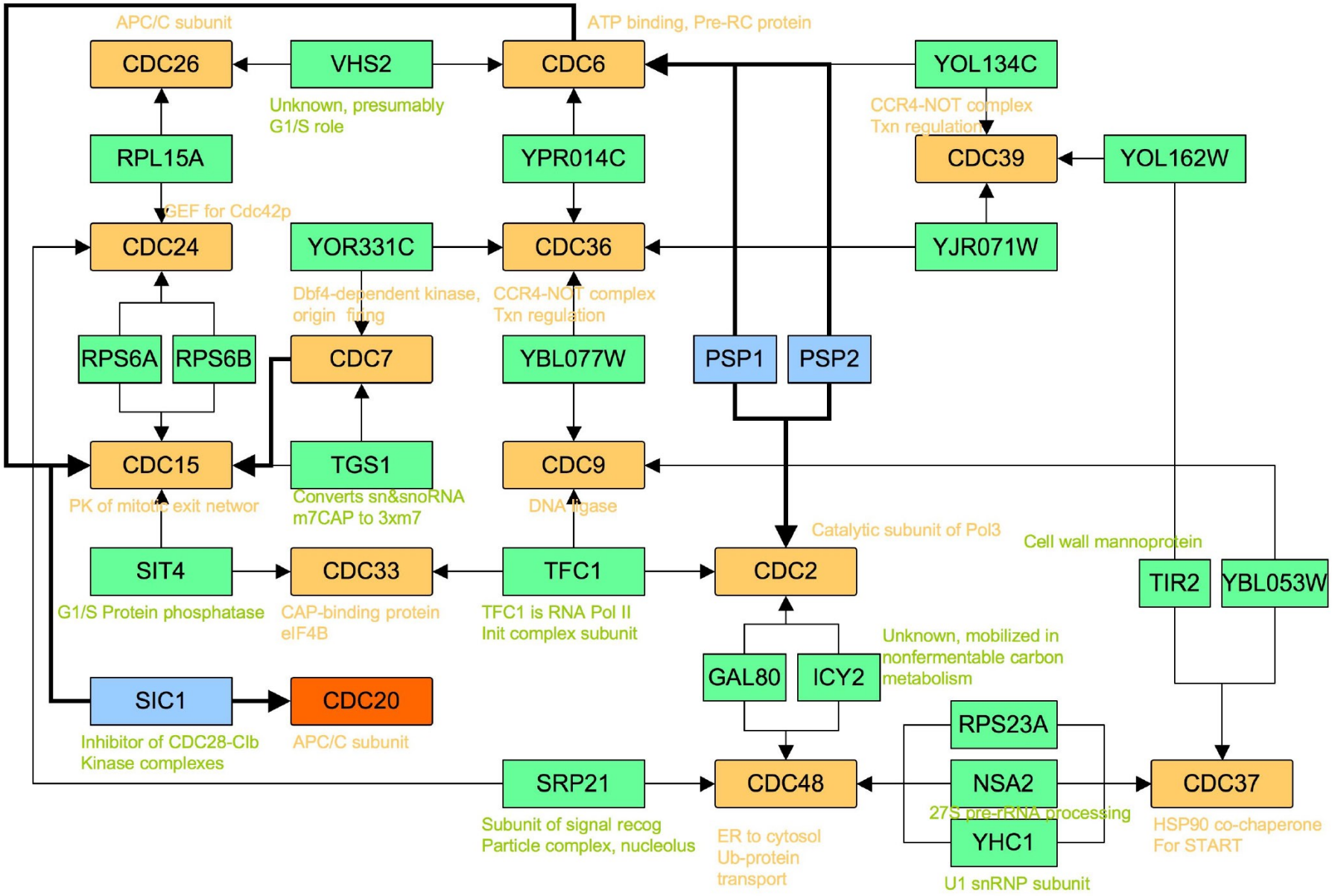


Dosage suppressor network involving *cdc* & related mutant nodes



Network of shared suppressors of *cell cycle* related mutants




APC/C subunit
ATP binding, Pre-RC protein
CDC26
VHS2
CDC6
YOL134C
Unknown, presumably
G1/S role
CCR4-NOT complex
Txn regulation
RPL15A
YPR014C
CDC39
YOL162W
GEF for Cdc42p
CDC24
YOR331C
CDC36
YJR071W
Dbf4-dependent kinase,
origin firing
CCR4-NOT complex
Txn regulation
RPS6A
RPS6B
CDC7
YBL077W
PSP1
PSP2
CDC15
TGS1
CDC9
PK of mitotic exit networ
Converts sn&snoRNA
m7CAP to 3xm7
DNA ligase
Catalytic subunit of Pol3
Cell wall mannoprotein
SIT4
CDC33
TFC1
CDC2
TIR2
YBL053W
G1/S Protein phosphatase
CAP-binding protein
eIF4B
TFC1 is RNA Pol II
Init complex subunit
Unknown, mobilized in
nonfermentable carbon
metabolism
GAL80
ICY2
SIC1
CDC20
RPS23A
Inhibitor of CDC28-Clb
Kinase complexes
APC/C subunit
SRP21
CDC48
NSA2
CDC37
27S pre-rRNA processing
Subunit of signal recog
Particle complex, nucleolus
ER to cytosol
Ub-protein
transport
YHC1
HSP90 co-chaperone
For START
U1 snRNP subunit

Analysis of *smc2-ts* suppression

- *SMC2*: Subunit of the condensin complex; essential SMC chromosomal ATPase family member that forms a complex with Smc4p to form the active ATPase; Smc2p/Smc4p complex binds DNA; required for clustering of tRNA genes at the nucleolus
- Suppressors are:
  - pGAL-UME1 Negative regulator of meiosis, required for repression of a subset of meiotic genes during vegetative growth, binding of histone deacetylase Rpd3p required for activity, contains a NEE box and a WD repeat motif; homologous with Wtm1p
  - pGAL-MEK1 Meiosis-specific serine/threonine protein kinase, functions in meiotic checkpoint, promotes recombination between homologous chromosomes by suppressing double strand break repair between sister chromatids
  - pGAL-HTA2 Histone H2A, core histone protein required for chromatin assembly and chromosome function; one of two nearly identical (see also HTA1) subtypes; DNA damage-dependent phosphorylation by Mec1p facilitates DNA repair; acetylated by Nat4p
  - pGAL-SNU66 Component of the U4/U6.U5 snRNP complex involved in pre-mRNA splicing via spliceosome; has homology to human SART-1 and to an S. pombe protein; snu66 null mutation confers cold-sensitivity but is not lethal at normal growth temperatures

Strains: smc2/pGAL-SMC2, smc2/pGAL-UME1, smc2/pGAL-MEK1, smc2/pGAL-HTA2, smc2/pGAL-SNU66, smc2/pGAL-

negative control

- Six strains were grown in YP-Raf-Gal at 25^{o}C, then shifted in fresh medium to the restrictive temp for smc2-ts
- RNA sampled at t = 0 & 3h were hybridized to Affy arrays
- Analysis results: Sporulation/meiosis genes are over-expressed in all suppressors in 3 h relative to pGAL-SMC2, normalized wrt the negative control plasmid
- Question: Why sporulation/meiosis genes are over-expressed in haploid suppressed strains in YP-Raff-Gal?

MA-Plots of 12 hyb experiments

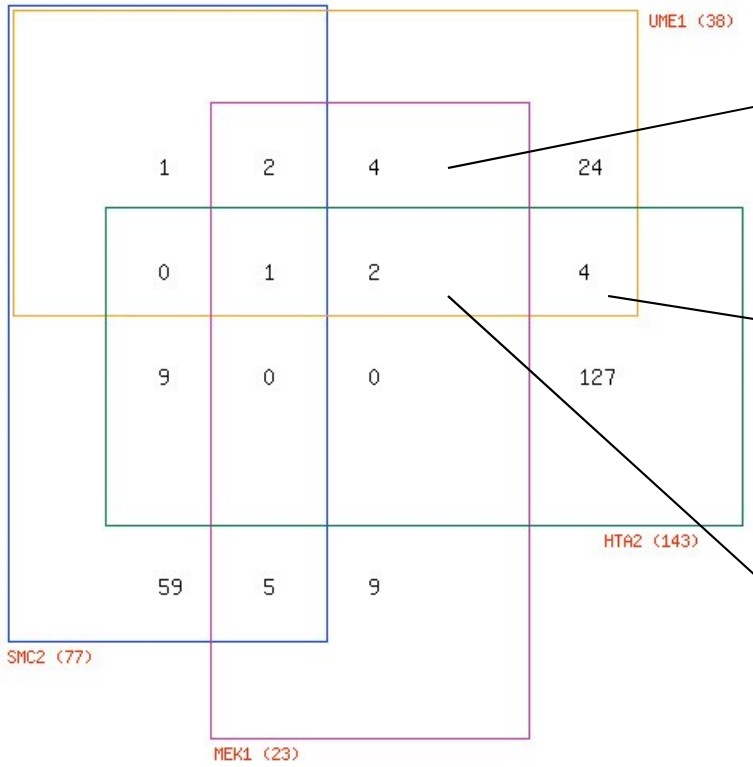
Smooth medians (red lines): low noise

Differentially expressed genes at 3h

- Normalized expression ratios (to the negative control) were filtered for outliers beyond ± 2 SD

- Venn diagrams of outlier genes (normalized to the negative controls)

Venn Diagram of genes over-expressed at 3h in *smc2*/pGAL-SMC2, *smc2*/pGAL-UME1, *smc2*/pGAL-MEK1 and *smc2*/pGAL-HTA2 above 2SD relative to negative control



ENV7
HMS1
AAC3
ATG9

PK

DNA binding, tsx regulation

YCL042W
PAU17
SMA2
YMR084W

EDS1

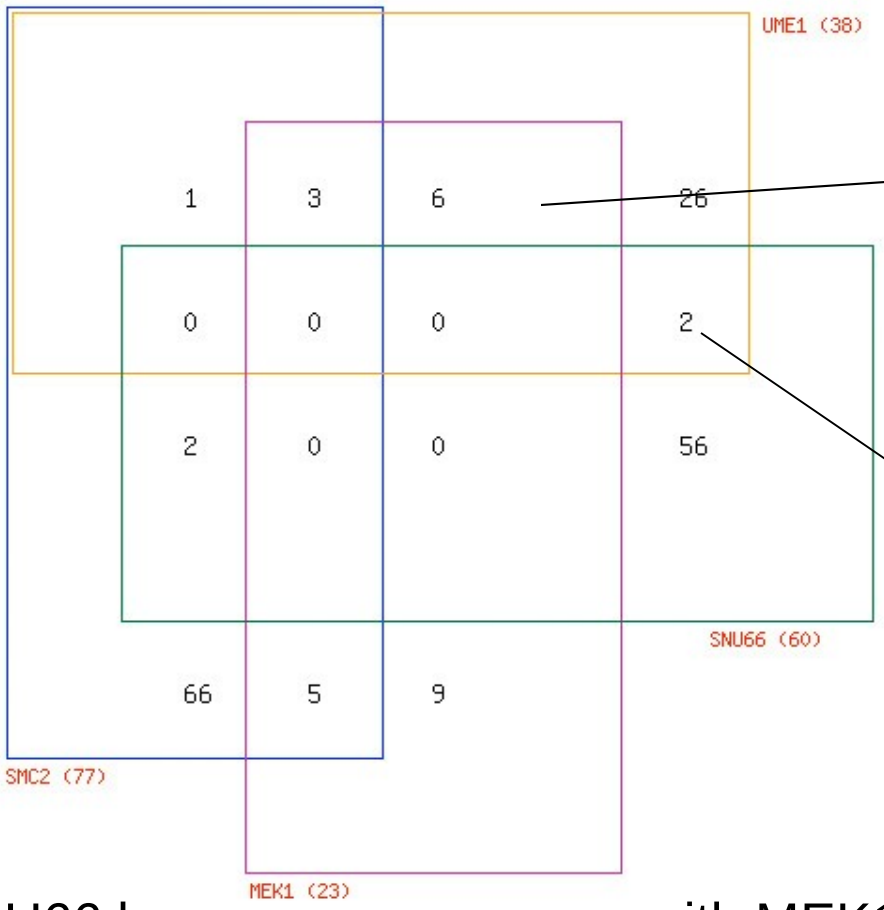
Putative Zn cluster protein

MEK1

Meiosis-specific protein kinase

UME1 and HTA2 both over-express MEK1, which is itself a suppressor of *smc2* --> Does suppression by each of these three genes occur through the common MEK1 pathway?

Venn Diagram of genes over-expressed at 3h in *smc2*/pGAL-SMC2, *smc2*/pGAL-UME1, *smc2*/pGAL-MEK1 and *smc2*/pGAL-SNU66 above 2SD relative to negative control



ENV7
HMS1
AAC3
ATG9
EDS1
MEK1

PRR2
MND1

ST-PK, inhibits MAPK at Ste12p TF level

Forms a complex with Hop2p,
Meiotic recombination
Chromosome pairing
DSB repair

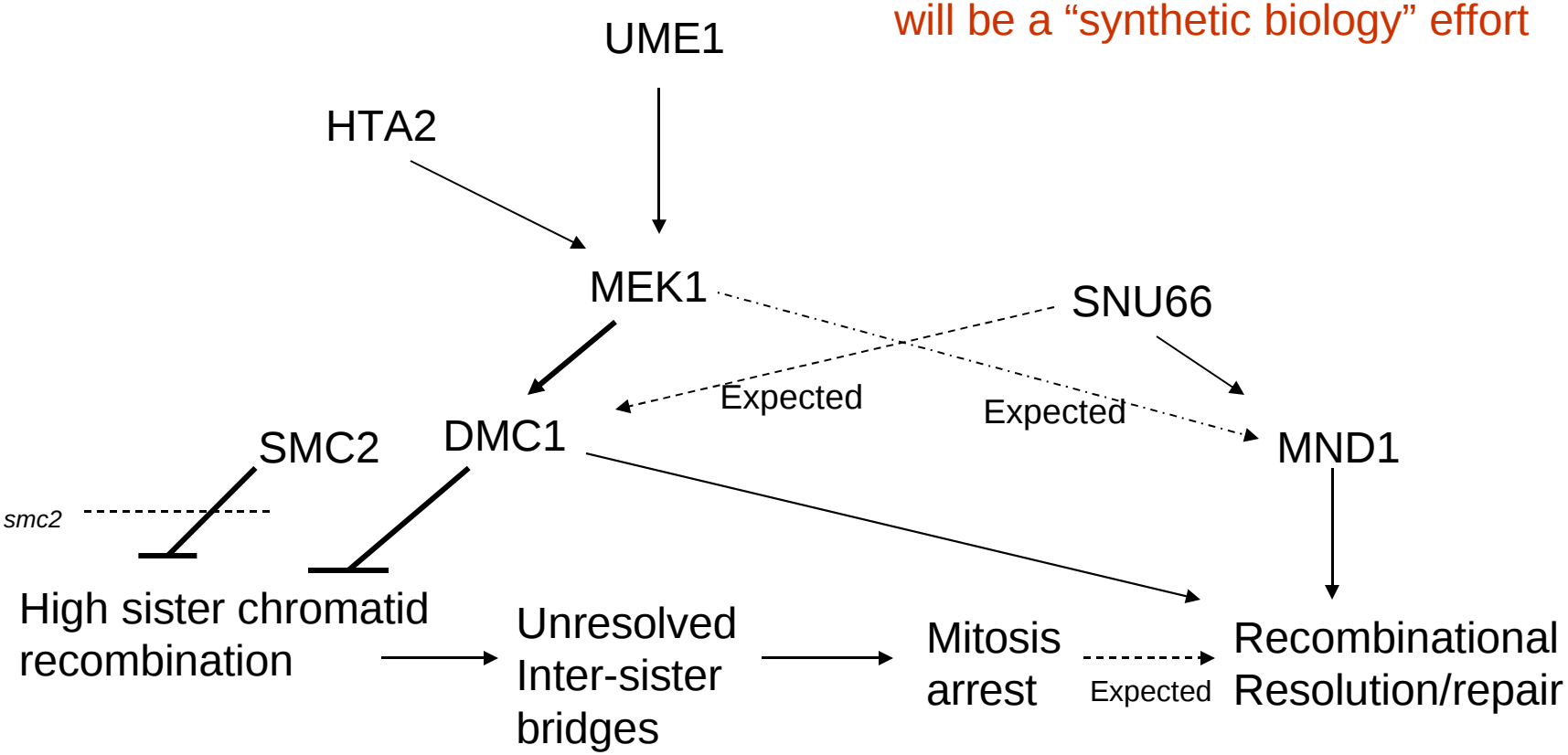
pGAL-SNU66 has no common gene with MEK1, so may have a different pathway of suppression. MND1 may be involved in repair of chromosome breaks in *smc2* mutant--> *smc2 mnd1* double cannot be suppressed by SNU66

Models

- Smc2p is part of the condensin complex that promotes mitotic and meiotic chromosome pairing. Absence of resolution of sister chromatid bridges (presumably, intermediates in sister chromatid recombination) during haploid mitosis in smc2 mutant causes aneuploidy and also mitotic arrest phenotype
- From the observed role of Smc2p in meiosis, where Smc2p promotes homolog pairing by discouraging sister chromatid pairing (Yu and Koshland, 2003 JCB, 163: 937), it was speculated that Smc2p may also inhibit sister chromatid pairing/recombination in mitosis
- Mek1p, a meiosis-specific kinase, is activated by DSB and induces DSB repair by interhomolog recombination but inhibits repair by sister chromosome recombination (Nieu et al, MCB 2007, doi:10.1128/MCB.00416-07)
- Thus, absence of Smc2p causes haploid mitotic arrest due to sister chromatid bridges, which in principle may be avoided by Mek1p over-production. [But other proteins, such as Red1 and Hop1 are known to be involved in Mek1p activation. Are these proteins present in suppressed cells or is there another mechanism?]
- Since MEK1 is over-expressed in both UME1 and HTA2, MEK1-mediated suppression may work through MEK1. Prediction: smc2 mek1 cannot be suppressed by UME1 or HTA2.
- Since SNU66 suppressor does not over express MEK1, SNU66 works through a different pathway. Prediction: smc2 mek1 can be suppressed by SNU66
- Since MND1 is over-expressed in SNU66, it might allow repair recombination to occur in SNU66 over-expressed strain. Test: smc2 mnd1 cannot be suppressed by SNU66
- To do: Interaction network around differentially expressed genes to obtain further hypothesis

Summary model

To be able to reconstruct these molecular steps in a model cell will be a “synthetic biology” effort



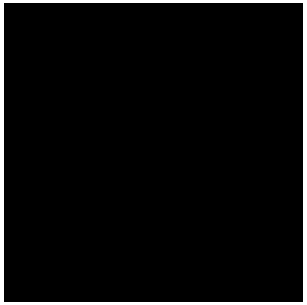
Summary

- Suppression network reveals unexpected robustness of the genome
- In the presence of strong selection pressure, such as growth arrest, novel bypass circuits emerge
- What is possible in future evolution is likely predicated by the previous evolutionary history of the organisms

Your shadow at morning striding behind you
Or your shadow at evening rising to meet you…
T.S. Elliot, in *The Waste Land*, 1922

Acknowledgement

- Biranchi Patra, Anthony Sevold
- Yoshiko Kan
- Ravi Rao
- Eric Phizicky, Elizabeth Grayhack
- Charles Boone
- David Galas



Disease network: How rare diseases can inform the understanding of common diseases

With Amarnath Gupta's and Dan O'Connor's labs

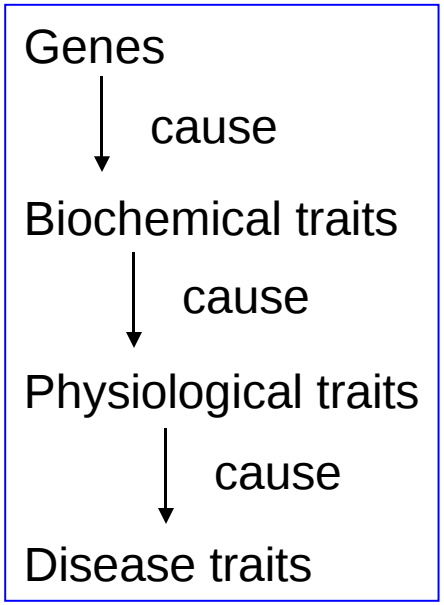
UCSD

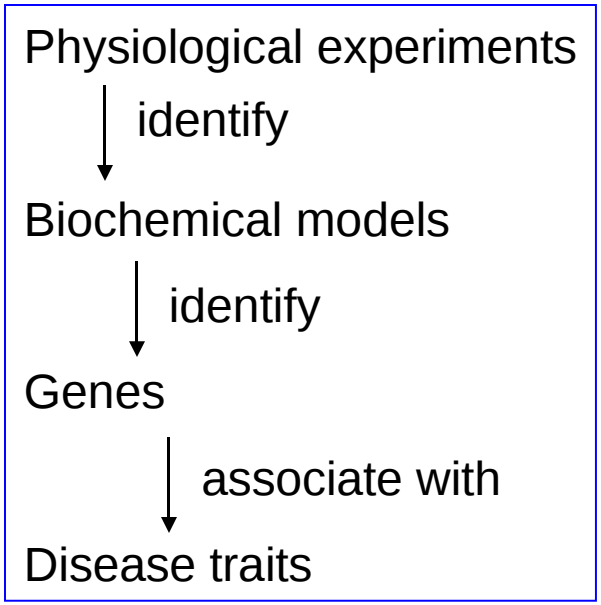
Differential transcriptome of hypertensive vs. hypotensive mouse model

The order of presentation

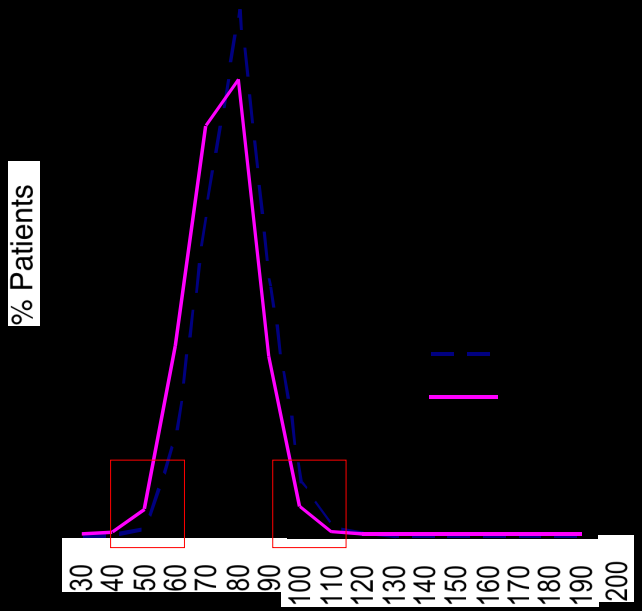
- Provide a background of why we take this approach
- Clarify the hypothesis and assumptions behind this approach
- Present the method: *Rare disease--> common disease*
- Present our salient results
- A model of complex disease development
- Conclude

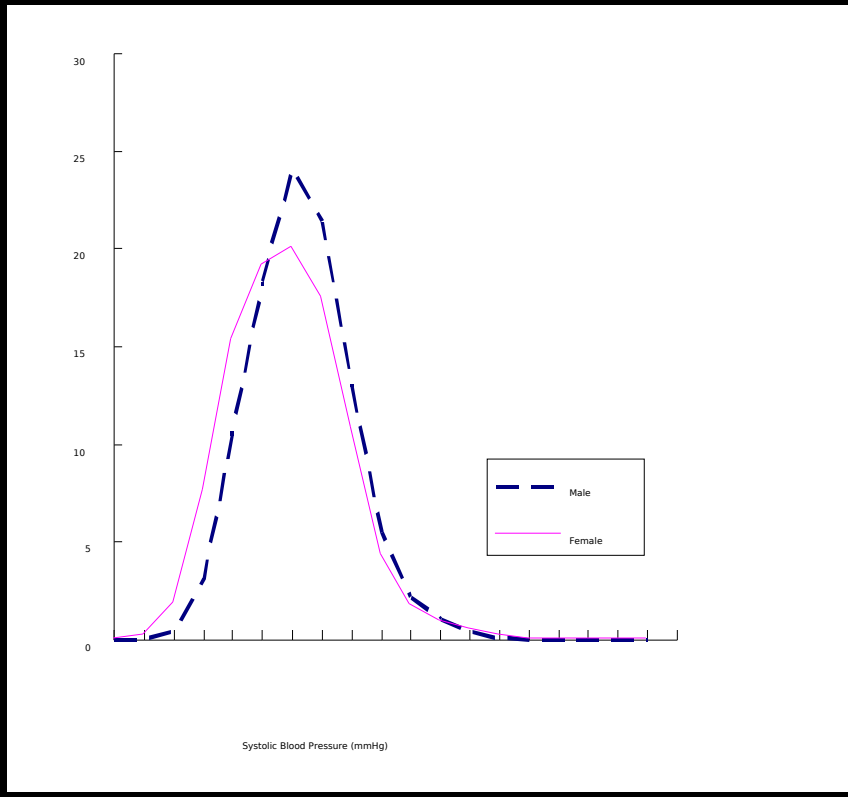
Concepts vs. Epistemology





Rao et al. (2007)


% Patients
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200


30
25
20
15
10
5
0
Male
Female
Systolic Blood Pressure (mmHg)

Renin-Angiotensin system

ungs

From: R. E. Klabunde http://www.cvphysiology.com/Blood%20Pressure/BP015.htm

Classical Approach provides a hypothesis-driven rationale for discovering genetic factors of hypertension

- Rana et al. Hypertension 49: 96 (2007)
- Adrenergic and renal pathway genes (renin-angiotensin system) that are known to be important in hypertension were tested for polymorphisms linked to hypertension in a very large population
- Monitored 35 loci
- One α_{2A}-adrenergic receptor haplotype (in females), two angiotensinogen haplotypes (in males) influenced blood pressure
- Evidence of epistatic effects between gene (allele) pairs (2 pairs discovered)
- Extremely successful method for determining genetic variants associated with hypertension phenotypes

Problem with classical approach

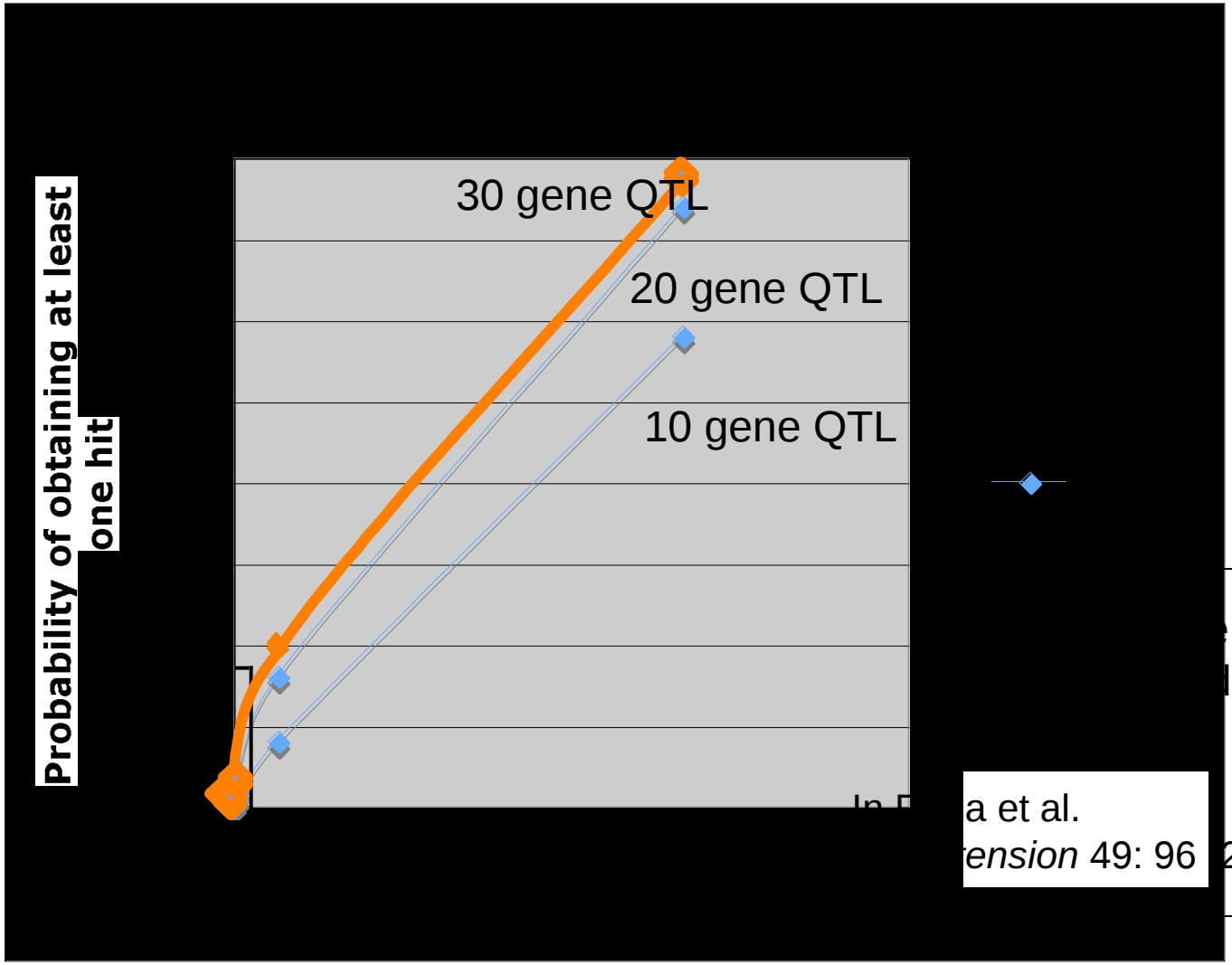
- We have a *very* incomplete understanding of complex diseases (*e.g.*, hypertension)
- Mechanistic hypotheses behind the choice of candidate markers for association studies are often incompletely posed
- How good are current rational approaches to finding genes to serve as markers of hypertension propensity?
- If genes are associated at random with phenotype, the hypergeometric probability distribution finds an association

Hypergeometric probability distribution: worst case

- Out of 23,000 genes, assume that there are 10, 20, or 30 genes that determine hypertension propensity: QTLs
- If genes are chosen randomly, then the probability that a hypertension-related gene is chosen by chance among *n* chosen genes is given by hypergeometric distribution

$$\frac{\binom{m}{k}\binom{N-m}{n-k}}{\binom{N}{n}}$$

N = total number of genes
m = number of causal genes
k = number of candidate genes among those tested
n = number of candidates tested



loci tested
associated

a et al.
ension 49: 96 2007)

This does *NOT* mean that the gene set chosen represented a random sampling

- It means that if the experiment were to be carried out a very large number of times, with randomly chosen sets of ~35 genes in every experiment, and if there were ~30-50 hypertension-related QTLs, then we expect the same frequency as obtained

- It also may mean that either the number of QTLs is small (< 20), or that Rana et al's choice of gene set for looking at association was an expert choice, much better than random--> can we do even better?

Translation of the problem

- Is it possible to expand the collection of loci to be tested for association in a rational, hypothesis-driven manner?

- Does systems level thinking provide *additional* (*NOT alternative*) approaches?

Our Rationale

- Observation: A set of differentially expressed genes in hypertensive animals (Hypertension-induced genes) (Friese et al., 2005)
- Hypothesis: *If some genes are coexpressed, they may share at least one regulator* (There is no function-based operator bias on our selection)
- Inference: *If two genes share a regulator, their expression should be correlated in many other conditions, not just hypertension*
- Test:
  - Mine GEO database for correlated expression of hypertension-specific gene pairs
  - Find phylogenetically conserved transcription factor binding sites in cis-regions of the highest correlated gene pairs
  - Find regulators of transcription factors, which also show correlated expression with hypertension-specific genes
  - Find hypertension-related disease phenotype that map to these regulators (TxF) and regulators of regulators (Kinases etc)
  - If strong support of hypertension-related phenotype-genotype association, then advance model to search for polymorphism-association with hypertension

Hypotheses

- Pairs of genes with highly correlated expression across many different conditions share common transcription factors
- Common transcription factors are phylogenetically conserved
- Their binding sites show strong alignment with respect to one another

Phylogenetic footprinting by PhyloCon: Gary Stormo (WashU, St. Louis)

Method

1. A list of over-expressed genes from Friese et al. 2005 (25 genes)
2. Downloaded all GEO data, extracted mouse expression data (630 experiments, 20,000-40,000 genes each)
3. Take all pairs of genes from (1) and determine Pearson correlation coefficient in normalized expression values in all of (2)

   a) take a pair of genes: geneA and geneB from our list

   b) number of experiments where GeneA is present is N_a, and N_b for GeneB

   c) find number of experiments where both GeneA and GeneB are present: N_{ab}

   d) for every GeneA and GeneB, repeating take the mean

   e) calculate Pearson for GeneA and GeneB

Method (contd)

5. Within each dataset, calculate Pearson correlations between every pair of genes.

- Filter Pearson *R > 0.7* [11 genes]

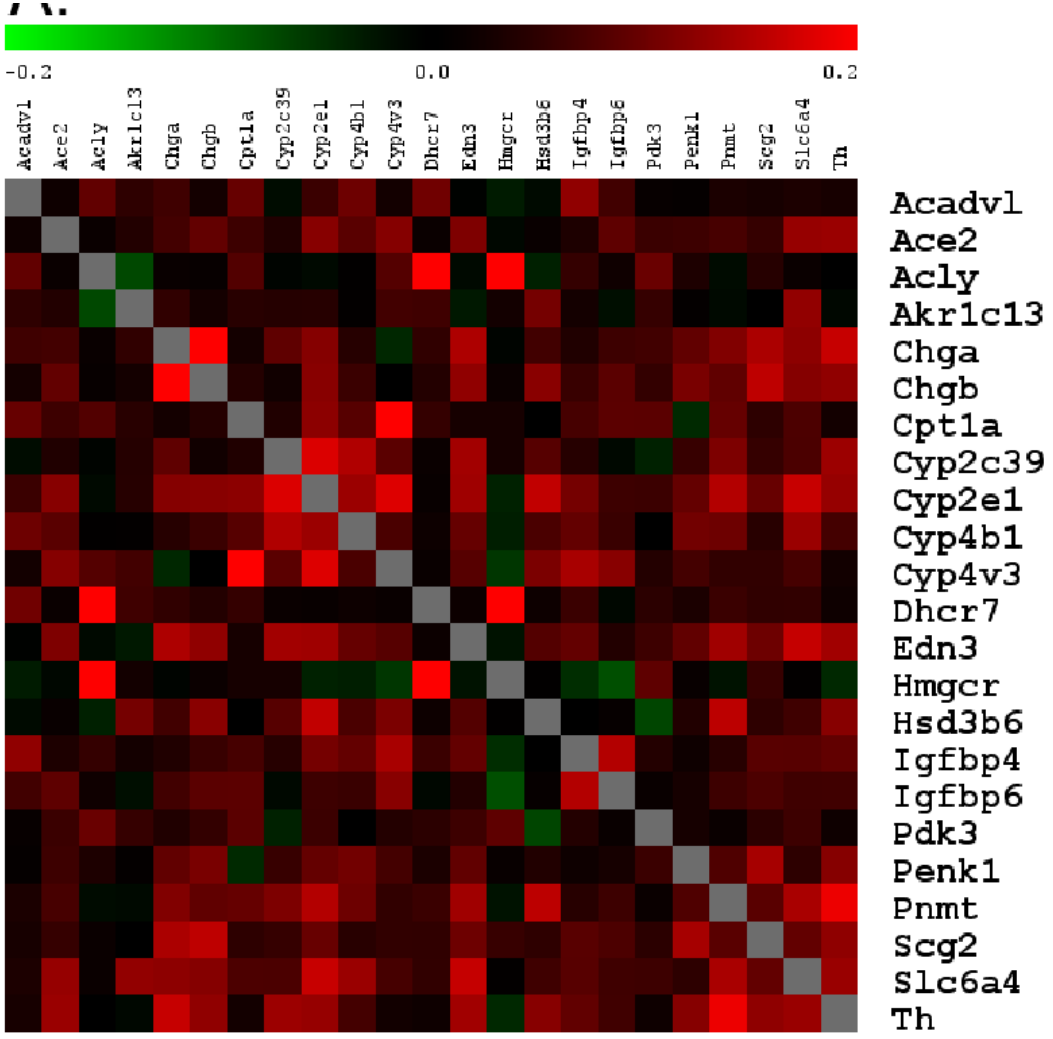
7. Using PAP workbench API (Phylogenetic Foot Printing), extracted potential Transcription Factors and respective *R*-scores for pairs of coexpressed genes.

- Used functions that explore Phylogenetic foot printing on three species (Human, Rat, and Mouse), thus these functions look for conservative binding sites for a pair coexpressed genes over Hs, Rn, Mm.
- Filtered results for: Pearson R > 0.7, TF R-score = 3 (p ~0.05).
- **Choose only the top 25 highest scoring Transcription Factors (Up-Up)**
- **Find for each transcription factor in (10) all protein-protein interactions (TF-TF, TF-Kinase, P-P)**
- **Determine which of these pairs have high expression correlation in GEO**
- Combine interactions among all pairs of highly co-expressed genes (6), TFs that bind their upstream sites (9) AND have correlated expression (12), and make an integrated interaction network
- Determine all co-expressed interaction edges
- **Select genes that show strongest correlated expression AND physical interaction with the set of 11 co-expressed genes**
- **Search for phenotypic relation of genes (in 15) to diseases in the OMIM database**

Top 25 over-expressed genes

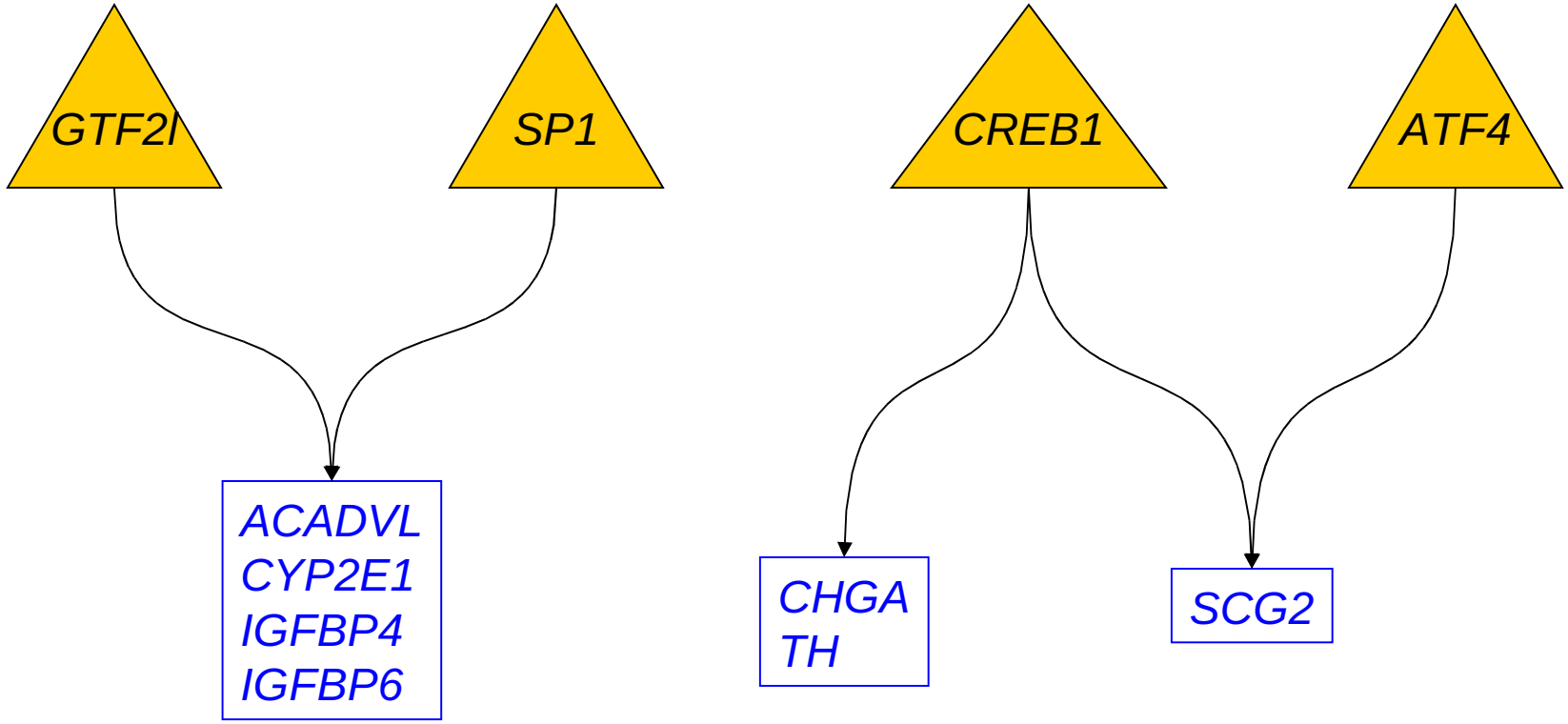
#	Name	Descripion
1	Acadvl	acyl-Coenzyme A dehydrogenase, long chain
[redacted]	[redacted]	[redacted]
3	Acly	ATP citrate lyase
4	Akr1c13	aldo-keto reductase family 1, member C13
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
7	Cpt1a	carnitine palmitoyltransferase 1a, liver
8	Cyp2c39	cytochrome P450, family 2, subfamily c
9	Cyp2e1	cytochrome P450, family 2, subfamily e
10	Cyp4b1	cytochrome P450, family 4, subfamily b
11	Cyp4v3	cytochrome P450, family 4, subfamily v
12	Dhcr7	7-dehydrocholesterol reductase
13	Edn3	endothelin 3
14	Facl4	acyl-CoA synthetase long-chain family member 4
15	Gch	GTP cyclohydrolase 1
16	Hmgcr	3-hydroxy-3-methylglutaryl-Coenzyme A reductase
17	Hsd3b6	hydroxysteroid dehydrogenase-6
18	Igfbp4	insulin-like growth factor binding protein 4
19	Igfbp6	insulin-like growth factor binding protein 6
20	Pdk3	pyruvate dehydrogenase kinase, isoenzyme 3
21	Penk1	preproenkephalin 1
22	Pnmt	phenylethanolamine-N-methyltransferase
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
25	Th	tyrosine hydroxylase

11 Up-regulated genes in Friese et al., which also show highly pair-wise correlated expression over 600 unrelated microarray experiments in GEO



FDR $P < 10^{-14}$

Phylogenetically conserved transcription factor binding sites in upstream of correlated gene pairs



Human, mouse, rat
18/25 conserved promoters from at least 2 species were identified

Conserved TFBS --> TFs --> Interacting proteins

- 18 conserved promoters
- 103 conserved transcription factors at the binding sites, with highly correlated expression in GEO (with 25 hypertension related genes)
- ~300 interactors (P-P, P-PK) with highly correlated expression in GEO (with 25 Hypertension related genes)

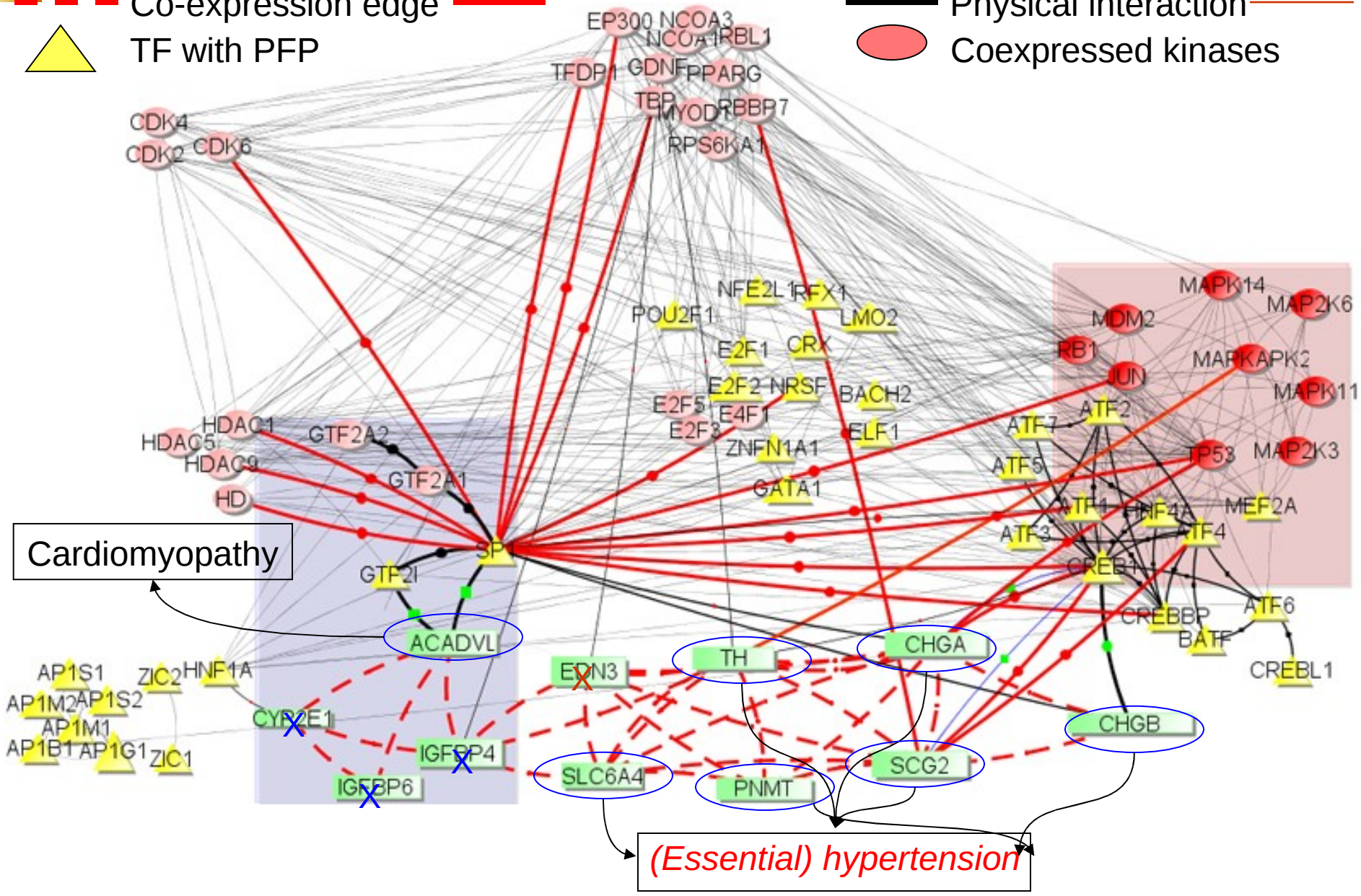
Highly co-expressed (GEO) gene pairs from Friese et al.

Co-expression edge

Physical interaction

TF with PFP

Coexpressed kinases



CYP11B1 & B2, CYP17 (corticosteroid metabolism enzymes)

If the network is functionally significant, then co-expressed AND interacting genes should show functional relation to hypertension

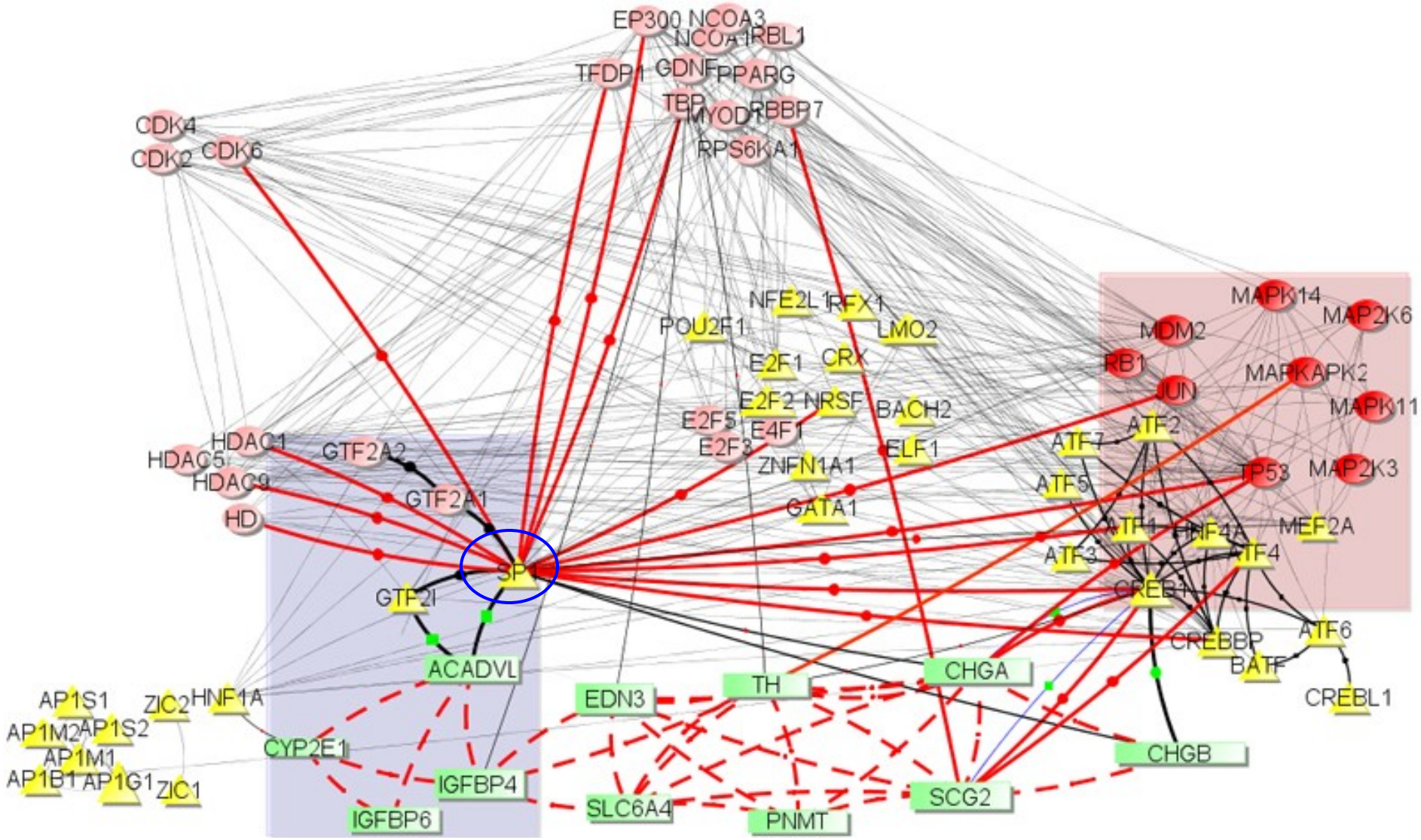
- Test if such genes have disease phenotypes overlapping cardiovascular or renal functions

(Assumption: Drastic mutations causing major disease syndromes may reveal subtle allele effects on chronic diseases)

- Query OMIM database for association of query gene mutation with a set of hypertension-related expert-chosen key words

OMIM search term enrichment

- 11,304 OMIM genes
- 504 are hypertension search term associated (4.9%)
- 32/103 hypertension coregulation network genes are hypertension search term associated (31%)

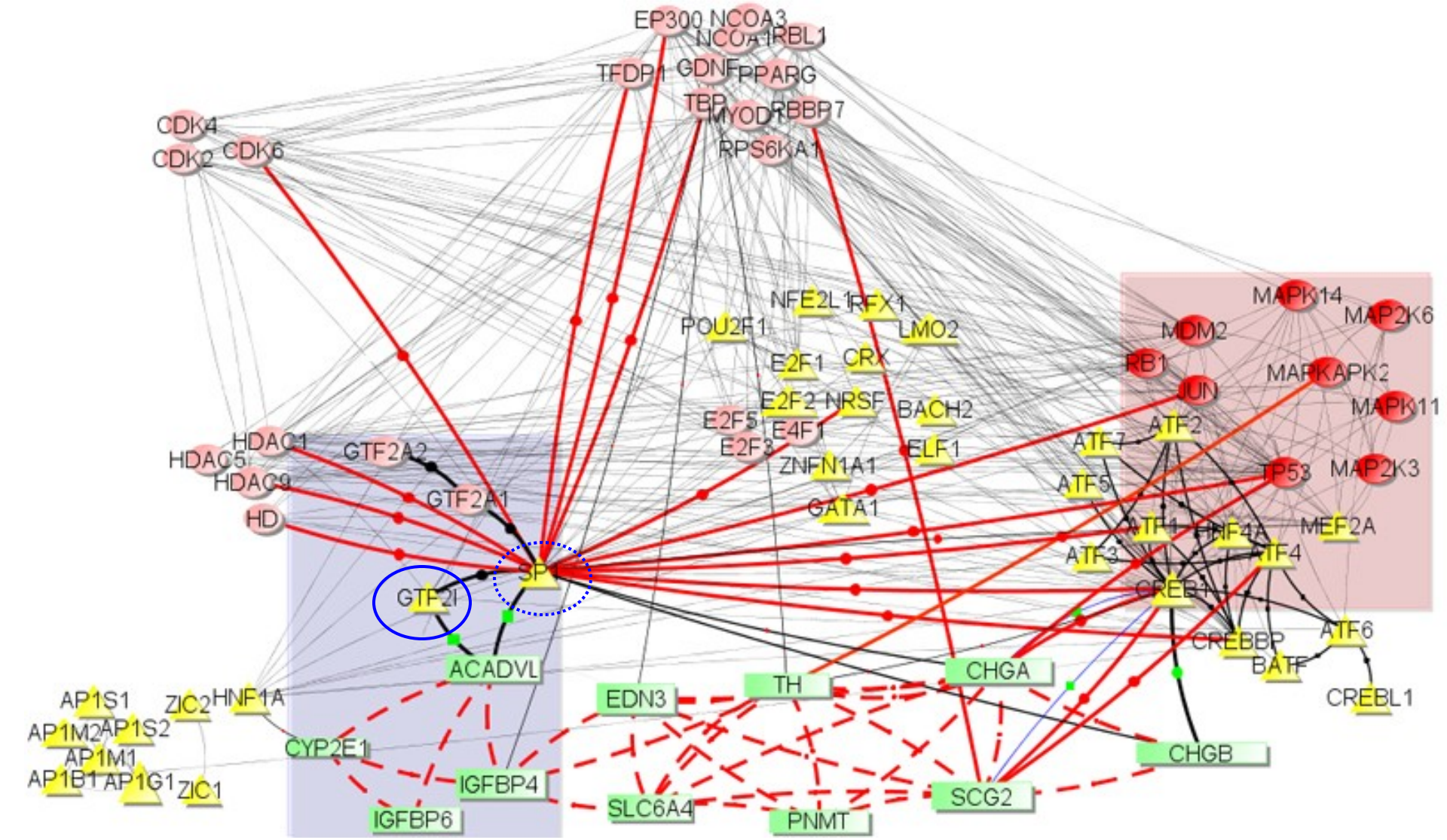


CYP11B1 & B2, CYP17 (corticosteroid metabolism enzymes)

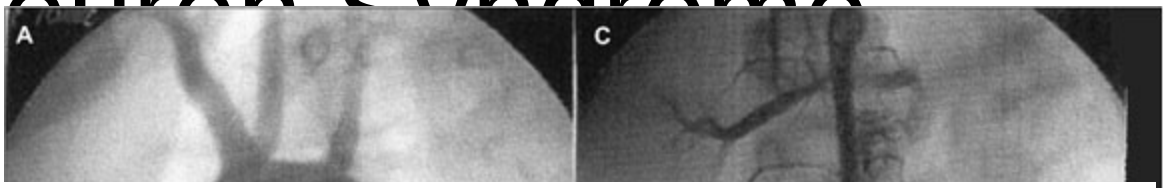
SP1

- Natriuretic peptides form important components of Essential Hypertension
- Transcription of Type B Natriuretic Peptide Receptor gene is controlled by SP1 transcription factor binding to its promoter
  - Rahmutula D, Cui J, Chen S, Gardner DG. Transcriptional regulation of type B human natriuretic Peptide receptor gene promoter: dependence on Sp1.
    - *Hypertension*. 2004 Sep;44(3):283-8. Epub 2004 Jul 19.

- *Polymorphisms of SP1 binding sites on the receptor gene promoter might influence hypertension development, but so might subtle regulatory or structural variations of SP1 itself*

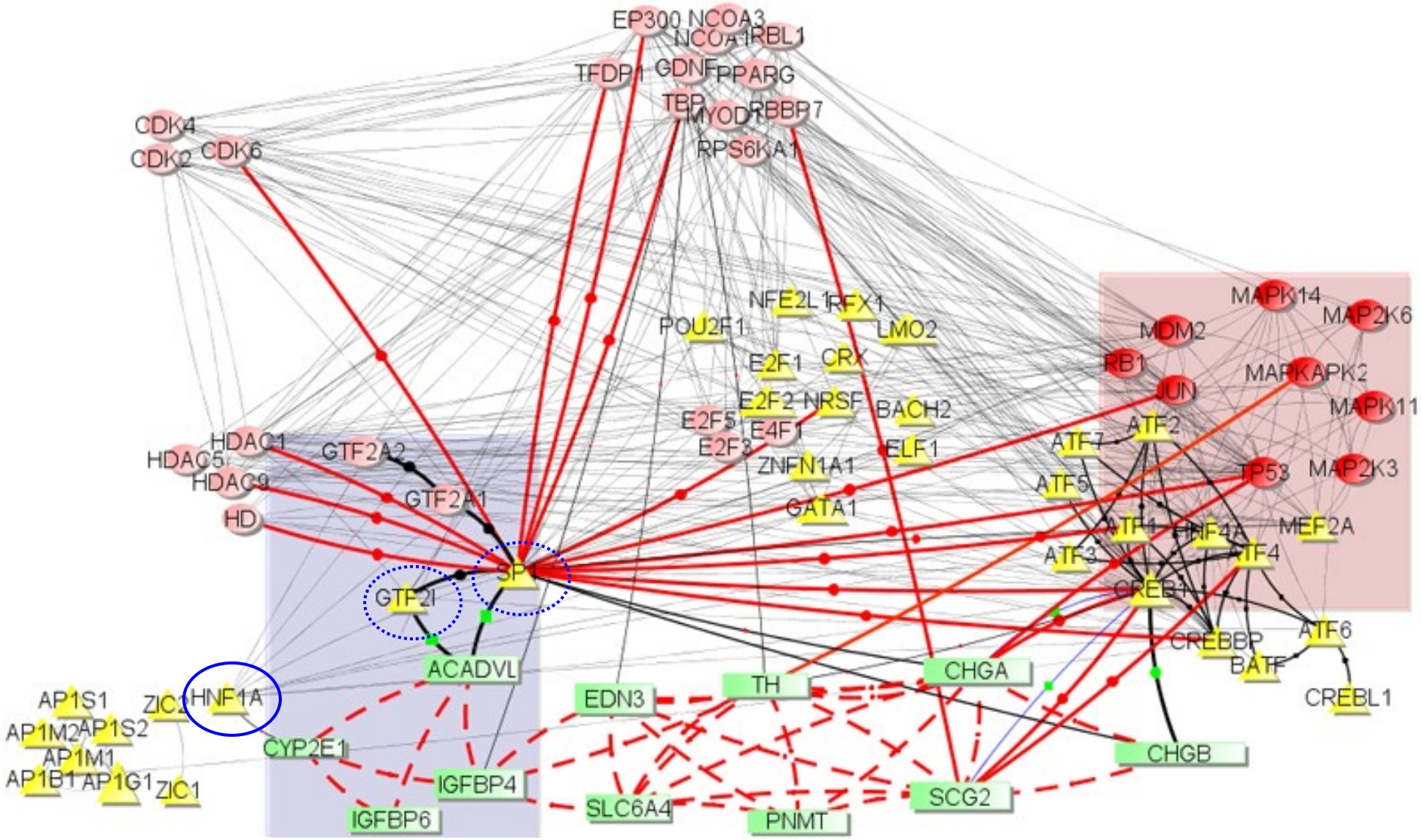

EP300
NCOA3
NCOA1
RBL1
TFDP1
GDNF
PPARG
TBP
MYOD1
RBBP7
RPS6KA1
CDK4
CDK2
CDK6
NFE2L1
RFX1
POU2F1
LMO2
E2F1
CRX
E2F2
NRSF
BACH2
E2F5
E4F1
E2F3
ELF1
ZNFN1A1
GATA1
HDAC1
HDAC5
HDAC9
HD
GTF2A2
GTF2A1
SP
GTF2I
ACADVL
HNF1A
ZIC2
AP1S1
AP1M2
AP1S2
AP1M1
AP1B1
AP1G1
ZIC1
CYP2E1
IGFBP4
IGFBP6
EDN3
SLC6A4
TH
PNMT
CHGA
SCG2
CHGB
MAPK14
MAP2K6
MDM2
RB1
MAPKAPK2
JUN
MAPK11
ATF2
ATF7
ATF5
TP53
MAP2K3
ATF1
HNF4A
MEF2A
ATF3
ATF4
CREB1
CREBBP
ATF6
BATF
CREBL1

Williams-Beuren Syndrome



- Autosomal dominant
- Hemizygous deletion overlapping CLIP2, ELN, GTF2l, GTF2lRD1 & LIMK1 genes
- Supravulvular aortic stenosis, transient hypercalcemia, general hypertension, ‘elfin’ face, arterial stenosis, stroke, mental retardation (autistic)
- ELN is thought to be responsible for stenosis (no god reason), GTF2 haplo-insufficiency thought to be the cause of other pleiotropic defects
- *We suggest a possible direct effect through GTF2l on ACADVL*--> Polymorphic GTF2l?

Fig. 3 - Catheterization. A, B) Progressive aortic narrowing, mainly of the descending aorta; C) stenosis of the right renal artery; D) obstruction of the left renal artery with collaterals. MSC, 6 years, 2000.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E2F1
CRX
E2F2
NRSF
BACH2
E2F5
E4F1
E2F3
ELF1
ZNFN1A1
GATA1
HDAC1
HDAC5
HDAC9
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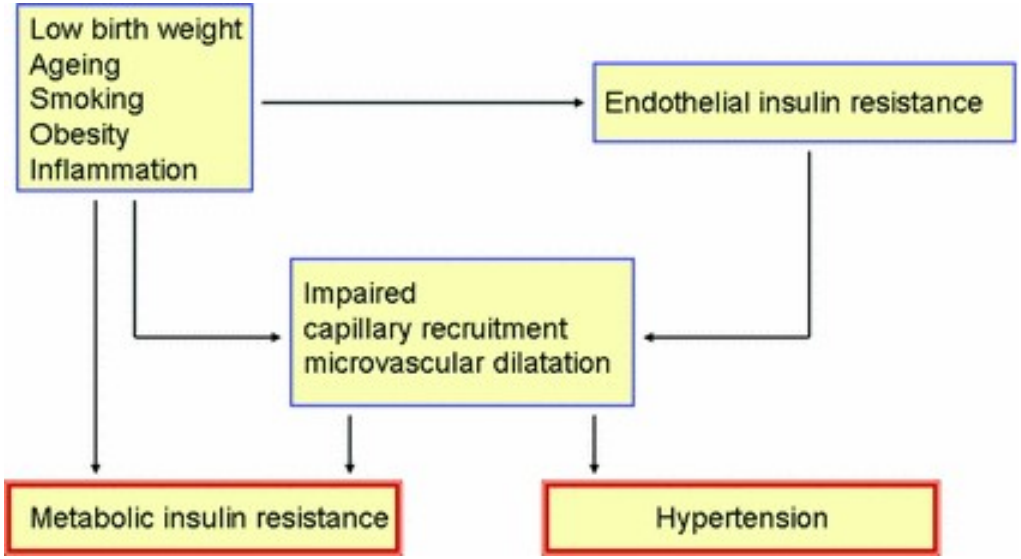
GTF2A2
GTF2A1
SP1
GTF2I
ACADVL
HNF1A
AP1S1
ZIC2
AP1M2
AP1S2
AP1M1
AP1B1
AP1G1
ZIC1
CYP2E1
IGFBP4
IGFBP6
EDN3
TH
SLC6A4
PNMT
CHGA
CHGB
SCG2
MAPK14
MAP2K6
MDM2
RB1
MAPKAPK2
JUN
MAPK11
ATF2
ATF7
ATF5
TP53
MAP2K3
ATF1
HNF4A
MEF2A
ATF3
ATF4
CREB1
CREBBP
ATF6
BATF
CREBL1

MODY3

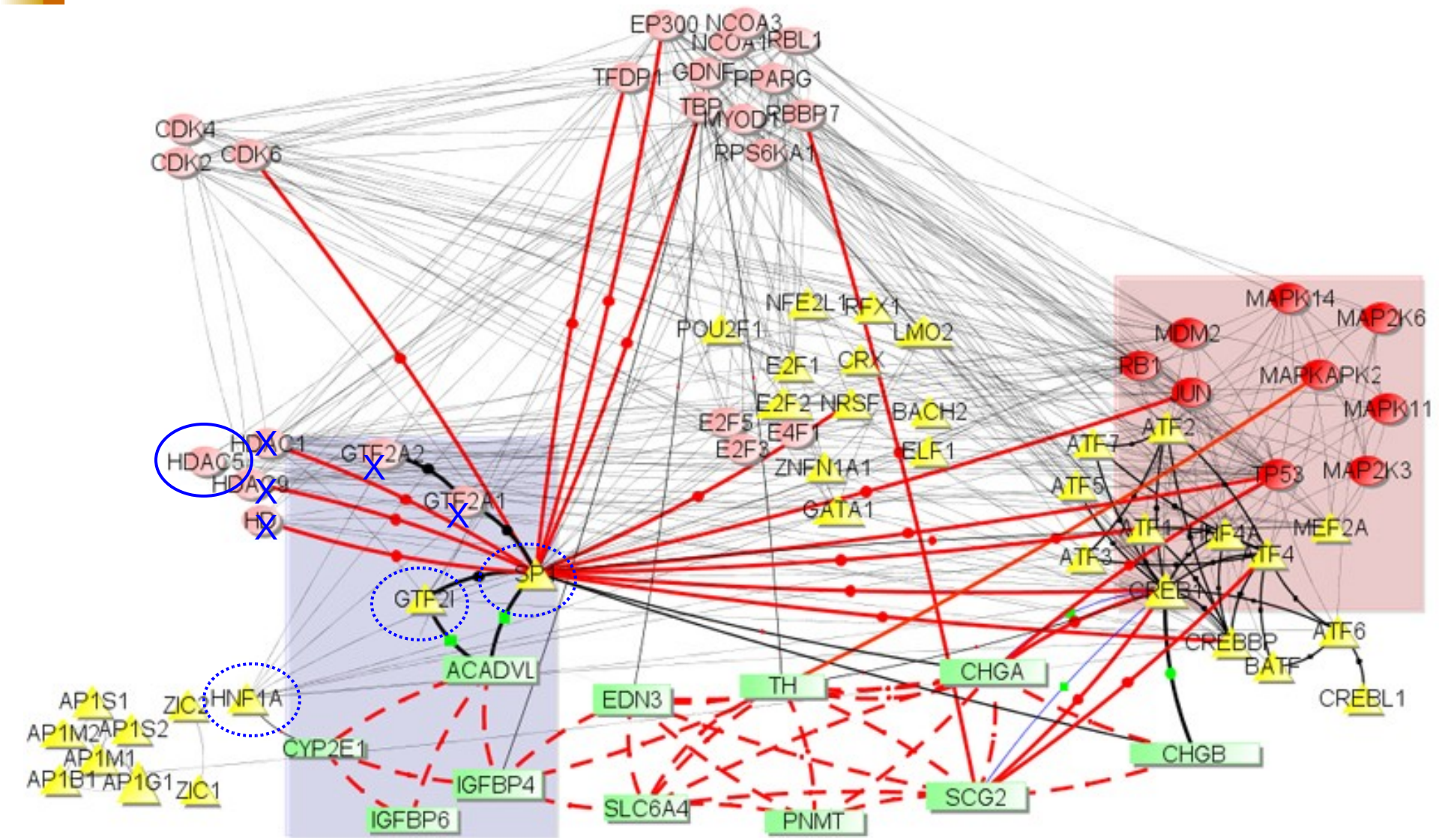
- HNF1A is a liver-specific homeo-domain transcription factor
- HNF1A defects (autosomal dominant) cause Maturity Onset Diabetes of the Young type 3
- Severe insulin secretory defect, hyperglycemia with microvascular complications

Microvascular Complication in Diabetes and Chronic Heart Diseases



From: Casper G. SCHALKWIJK*† and Coen D. A. STEHOUWER
Clinical Science (2005) 109: 143–159
Vascular complications in diabetes mellitus: the role of endothelial dysfunction

- *Could polymorphic HNF1A explain subtle dysregulation of CYP2E1 and ACADVL in hypertensive patients?*
- *Could epigenetic processes be involved?*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CDK6
NFE2L1
RFX1
POU2F1
LMO2
E2F1
CRX
E2F2
NRSF
BACH2
E2F5
E4F1
E2F3
ELF1
ZNFN1A1
GATA1
HDAC1
HDAC5
HDAC9
HD
GTF2A2
GTF2A1
SP1
GTF2I
ACADVL
HNF1A
AP1S1
ZIC2
AP1M2
AP1S2
AP1M1
AP1B1
AP1G1
ZIC1
CYP2E1
IGFBP4
IGFBP6
EDN3
TH
SLC6A4
PNMT
CHGA
CHGB
SCG2
MAPK14
MAP2K6
MDM2
RB1
MAPKAPK2
JUN
MAPK11
ATF2
ATF7
ATF5
TP53
MAP2K3
ATF1
HNF4A
MEF2A
ATF3
ATF4
CREB1
CREBBP
ATF6
BATF
CREBL1

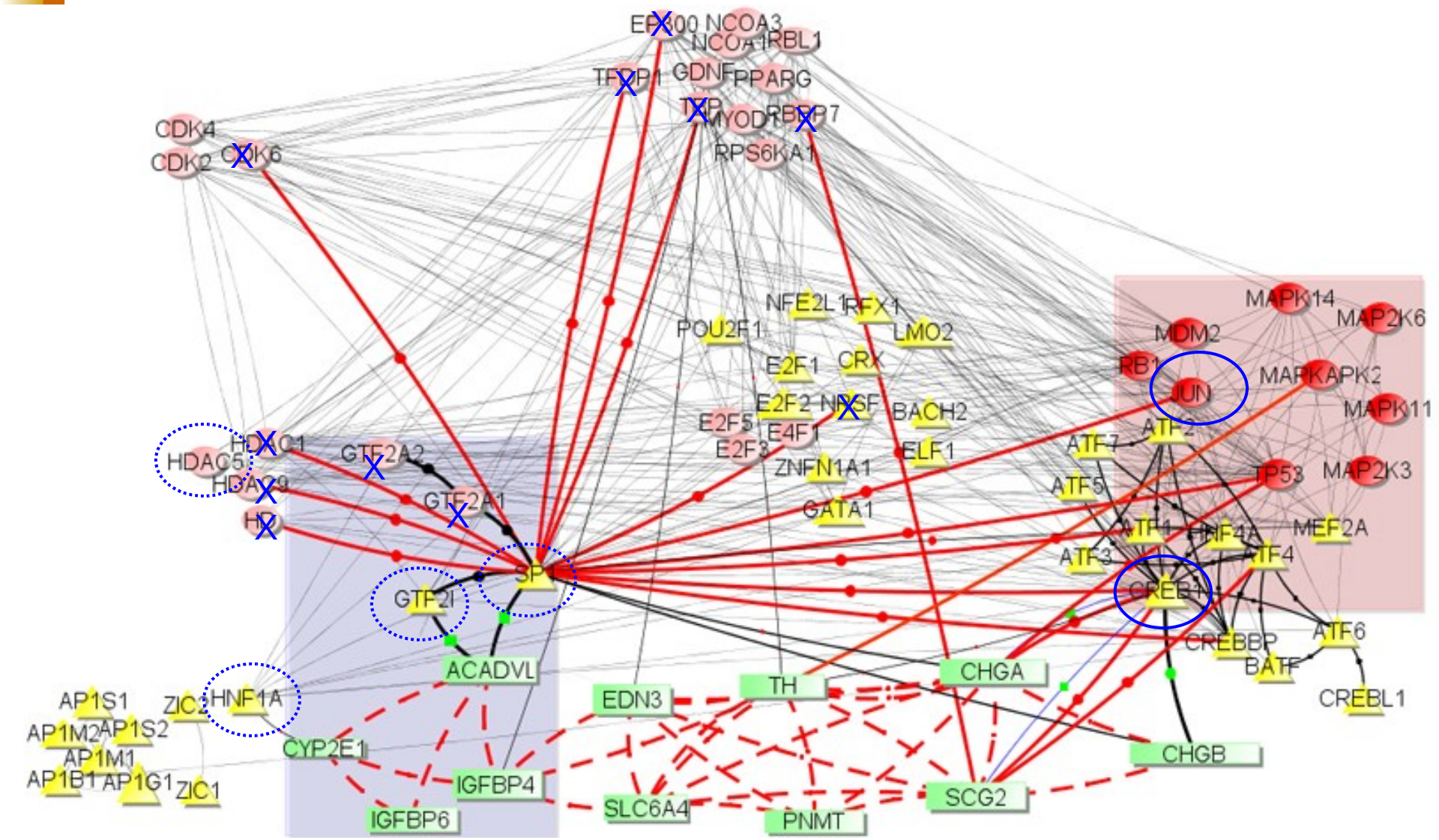
HDAC5

- Mutations in HDAC5 are associated with cardiac hypertrophy, heart failure
- HDAC5 nucleo-cytoplasmic shuttling is dependent on CaM Kinase-Ca^{++} waves in ventricular myocytes
  - Molkentin JD. *J Clin Invest.* 2006 Mar;116(3):623-6.
  - Comment on: J Clin Invest. 2006 Mar;116(3):675-82.

"Dichotomy of Ca2+ in the heart: contraction versus intracellular signaling"

- Direct binding of CaM Kinase-Ca^{++} to HDAC5 inhibits repressor core binding of HDAC5 to Myocyte Enhancer Factor 2 gene

Could HDAC5 polymorphisms be linked to hypertension? Could any of the Epigenetically regulated genes be regulated through HDAC5?


NCOA3
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E2F1
CRX
E2F2
NFSF
BACH2
E2F5
E4F1
E2F3
ELF1
ZNFN1A1
GATA1
HDAC5
HDAC1
GTF2A2
GTF2A1
SP1
GTF2I
ACADVL
HNF1A
ZIC2
AP1S1
AP1M2
AP1S2
AP1M1
AP1B1
AP1G1
ZIC1
CYP2E1
IGFBP4
IGFBP6
EDN3
TH
SLC6A4
PNMT
CHGA
CHGB
SCG2
MAPK14
MAP2K6
MDM2
RB1
JUN
MAPKAPK2
MAPK11
ATF2
ATF7
TP53
MAP2K3
ATF5
ATF1
HNF4A
MEF2A
ATF3
ATF4
CREB1
CREBBP
ATF6
BATF
CREBL1

JUN & CREB1

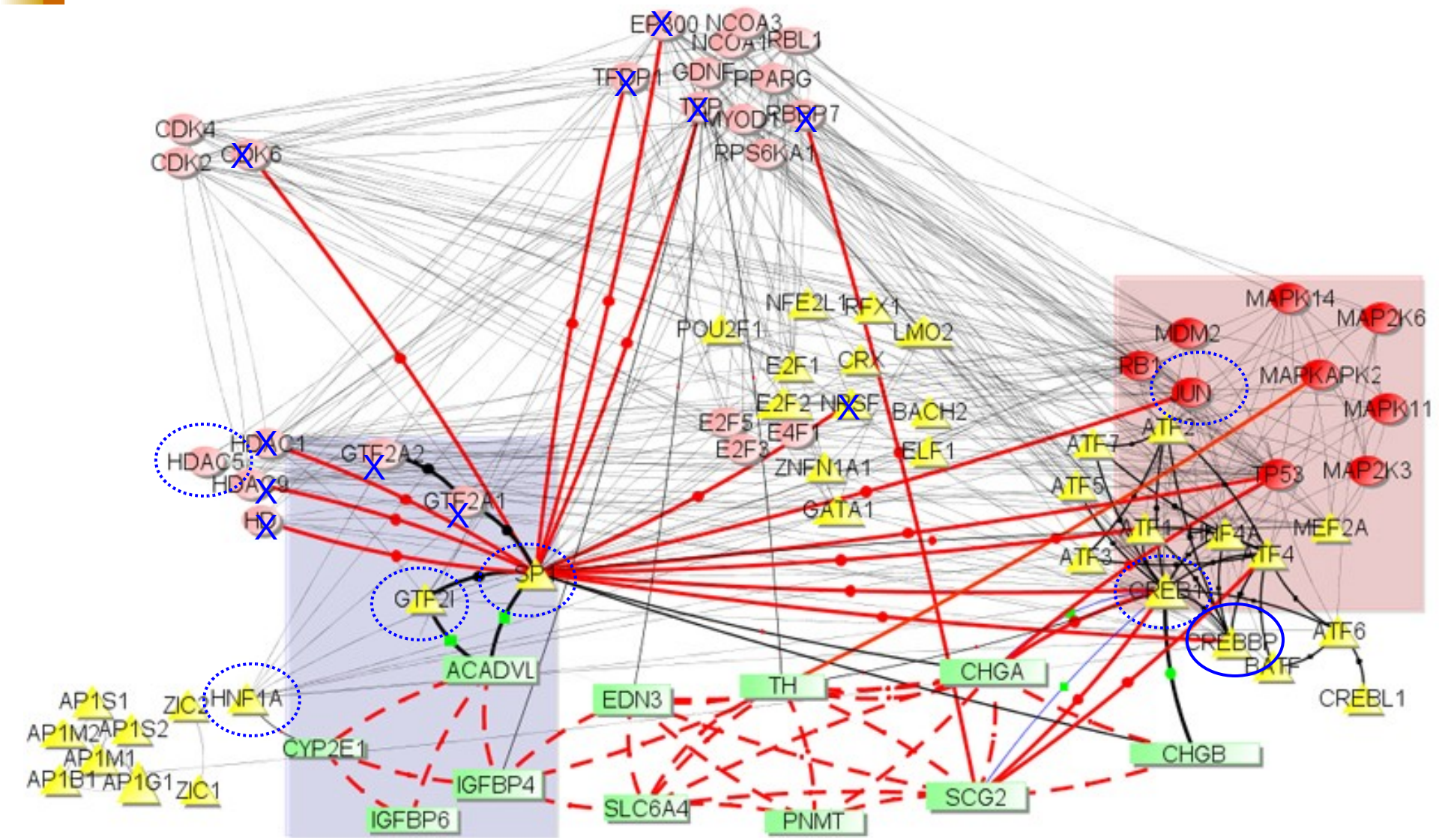
1. Rose P, Bond J, Tighe S, Toth MJ, Wellman TL, de Montiano EM, Lewinter MM, Lounsbury KM.

"Genes overexpressed in cerebral arteries following salt-induced hypertensive disease are regulated by angiotensin II, JunB, and CREB"

Am J Physiol Heart Circ Physiol. 2008 Feb;294(2):H1075-85. Epub 2007 Dec 21.

2. "Coffin-Lowry" Syndrome, which involves heart and kidney dysfunction, is correlated with reduced RSK2-mediated CREB1 phosphorylation

Monitor Jun and CREB1 polymorphisms or their binding site polymorphisms (or methylation) for hypertension linkage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PPARG
MYOD1
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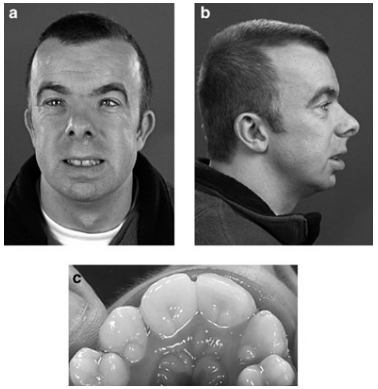
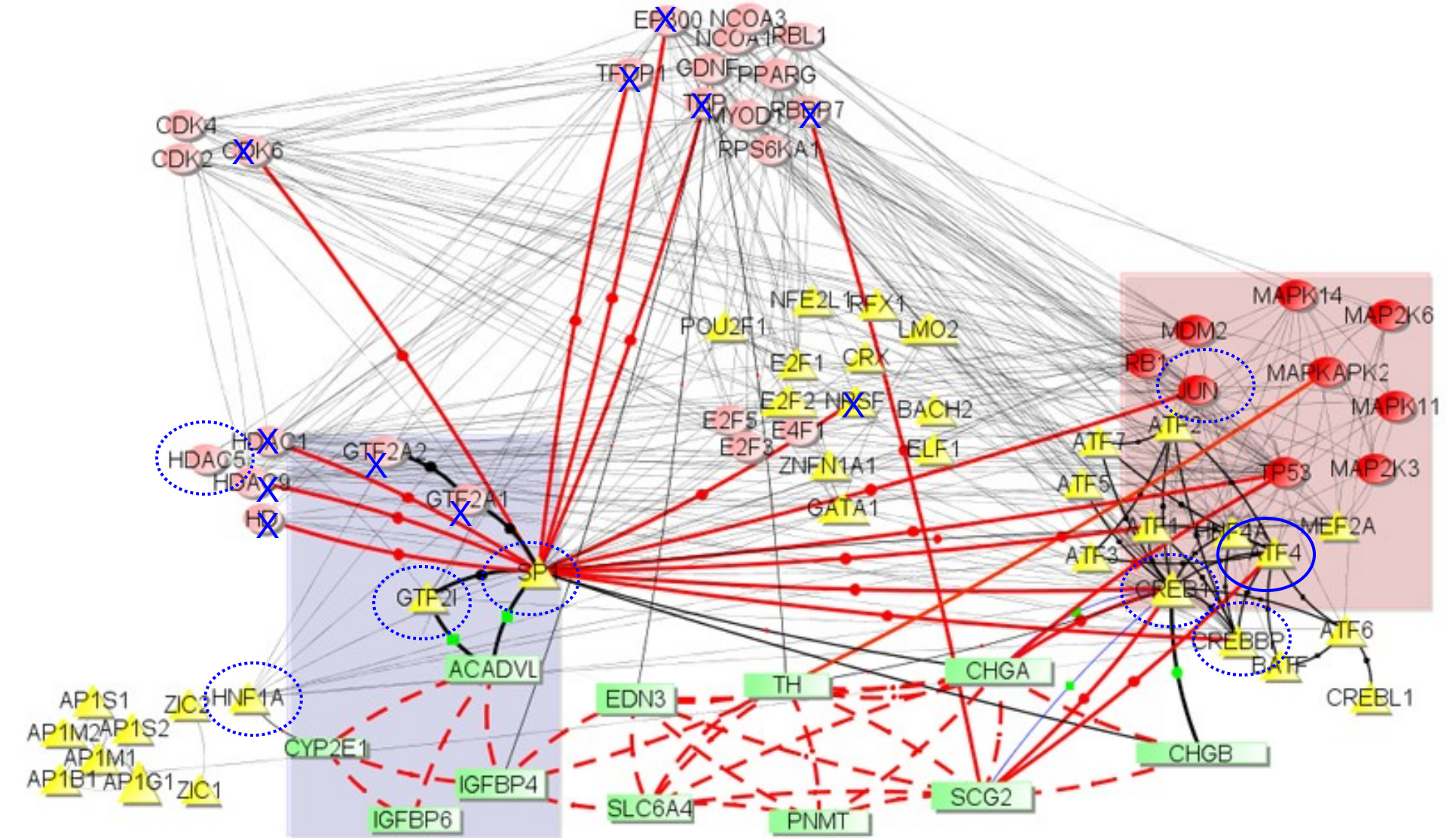
CDK4
CDK2
CDK6
NFE2L1
RFX1
POU2F1
LMO2
E2F1
CRX
E2F2
NFSF
BACH2
E2F5
E4F1
E2F3
ELF1
ZNFN1A1
GATA1
HDAC5
HDAC1
HDAC9
GTF2A2
GTF2A1
SP1
GTF2I
ACADVL
HNF1A
AP1S1
ZIC2
AP1M2
AP1S2
AP1M1
AP1B1
AP1G1
ZIC1
CYP2E1
IGFBP4
IGFBP6
EDN3
TH
SLC6A4
PNMT
CHGA
SCG2
CHGB
MAPK14
MAP2K6
MDM2
RB1
JUN
MAPKAPK2
MAPK11
ATF2
ATF7
TP53
MAP2K3
ATF5
ATF1
HNF4A
MEF2A
ATF3
ATF4
CREB1
CREBBP
ATF6
BATF
CREBL1

Rubinstein-Taybi Syndrome

- Autosomal dominant mutation produces mental retardation, limb abnormality (broad thumb and toes), short stature
- CREBBP hemizygous deletion
- Kanjilal D, Basir MA, Verma RS, Rajegowda BK, Lala R, Nagaraj A. *New dysmorphic features in Rubinstein-Taybi syndrome*. J Med Genet. 1992 Sep;29(9):669-70. (Pulmonary hypertension)

CREBBP polymorphisms linked to hypertension?

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BACH2
E2F5
E4F1
E2F3
ELF1
ZNFN1A1
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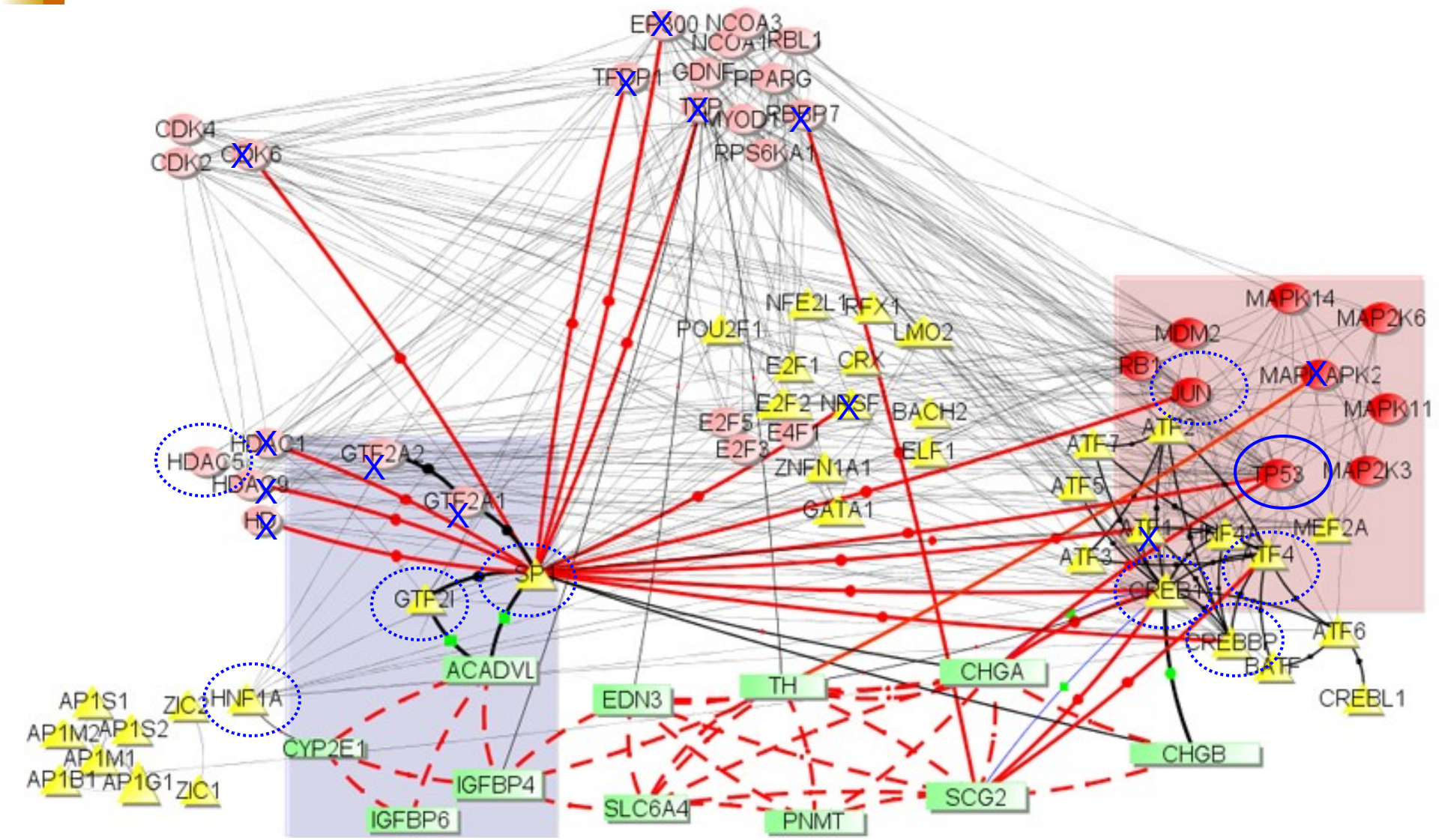
ATF3
TP53
MAP2K3
MEF2A
ATF4
CREB1
CREBBP
BATF
ATF6
CREBL1

ATF4

- Increased expression of ATF4 is associated with ischemic response
- Unfolded protein response (ER stress) is transmitted through ATF4 in ischemia
  - Toth A, Nickson P, Mandl A, Bannister ML, Toth K, Erhardt P. "Endoplasmic reticulum stress as a novel therapeutic target in heart diseases"
    Cardiovasc Hematol Disord Drug Targets. 2007 Sep;7(3):205-18.

ATF4 function in hypertension?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GATA1
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HDAC1
GTF2A2
GTF2A1
SP1
GTF2I
ACADVL
HNF1A
AP1S1
ZIC2
AP1M2
AP1S2
AP1M1
AP1B1
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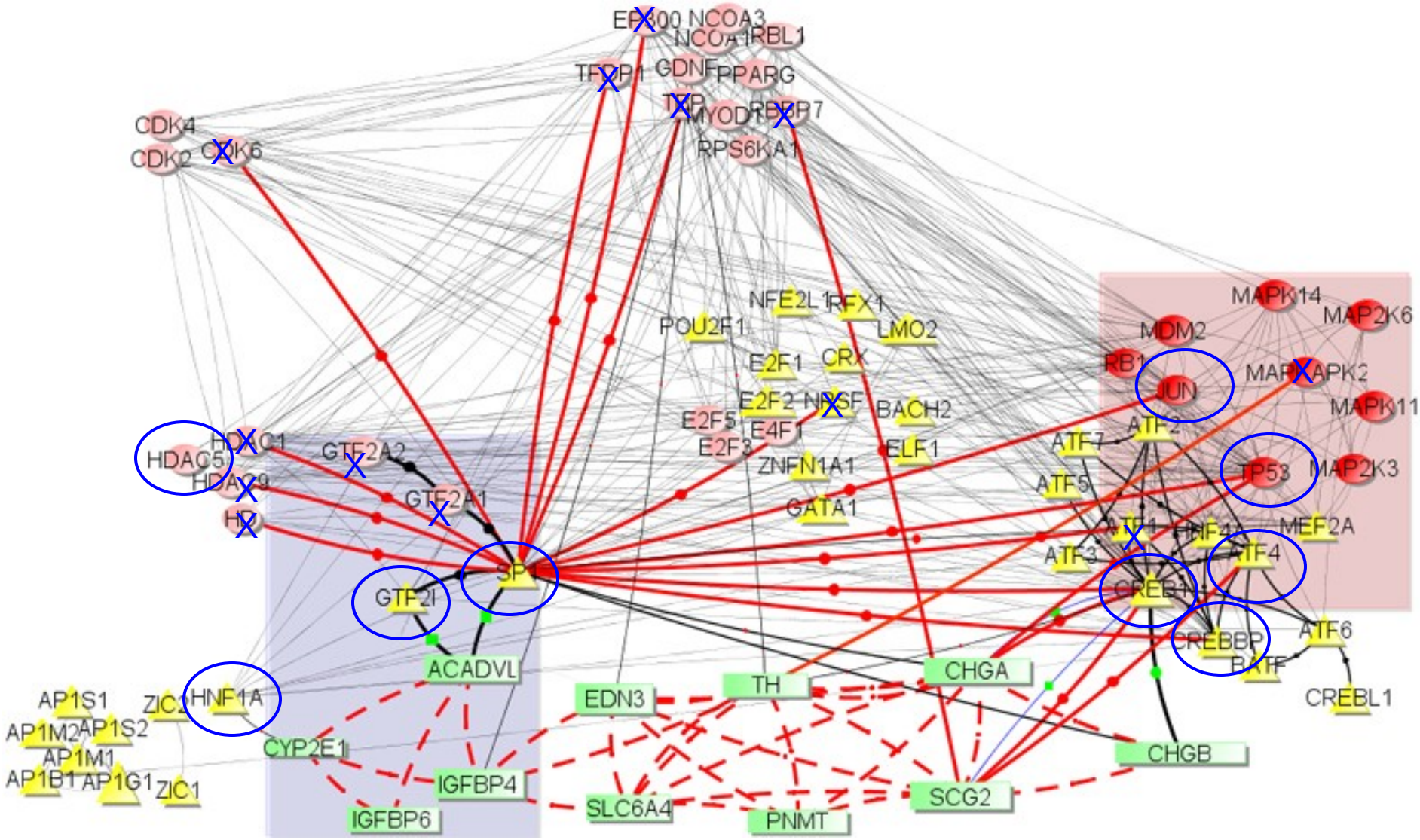
JUN
MAPKAPK2
MAPK11
ATF2
ATF7
TP53
MAP2K3
ATF5
MEF2A
ATF3
ATF4
CREB1
CREBBP
ATF6
BATF
CREBL1

TP53

- A significant association between TP53 Arg72Pro polymorphism and Leber's Hereditary Optic Neuropathy (LHON)
  - *Japanese J. Opthalmology* 49: 121-126 (2005)
  - LHON+ is sometimes associated with cardiac arrythmia
  - The condition is associated with dysfunctional oxidative metabolism

What is the role of TP53 in hypertension or other cardiovascular diseases?



The remaining genes are not eliminated-->provide new opportunities for hypothesis testing: siRNA-mediated interference testing, for example

Are variations in these genes associated with hypertension?

- Find polymorphic SNP markers close to the genes

- Determine frequency of haplotypes within high systolic and high diastolic blood pressure populations and the corresponding frequencies in the normal blood pressure groups

- Determine the probability of the observed frequency due to chance

Dan O'Connor's group (UCSD)

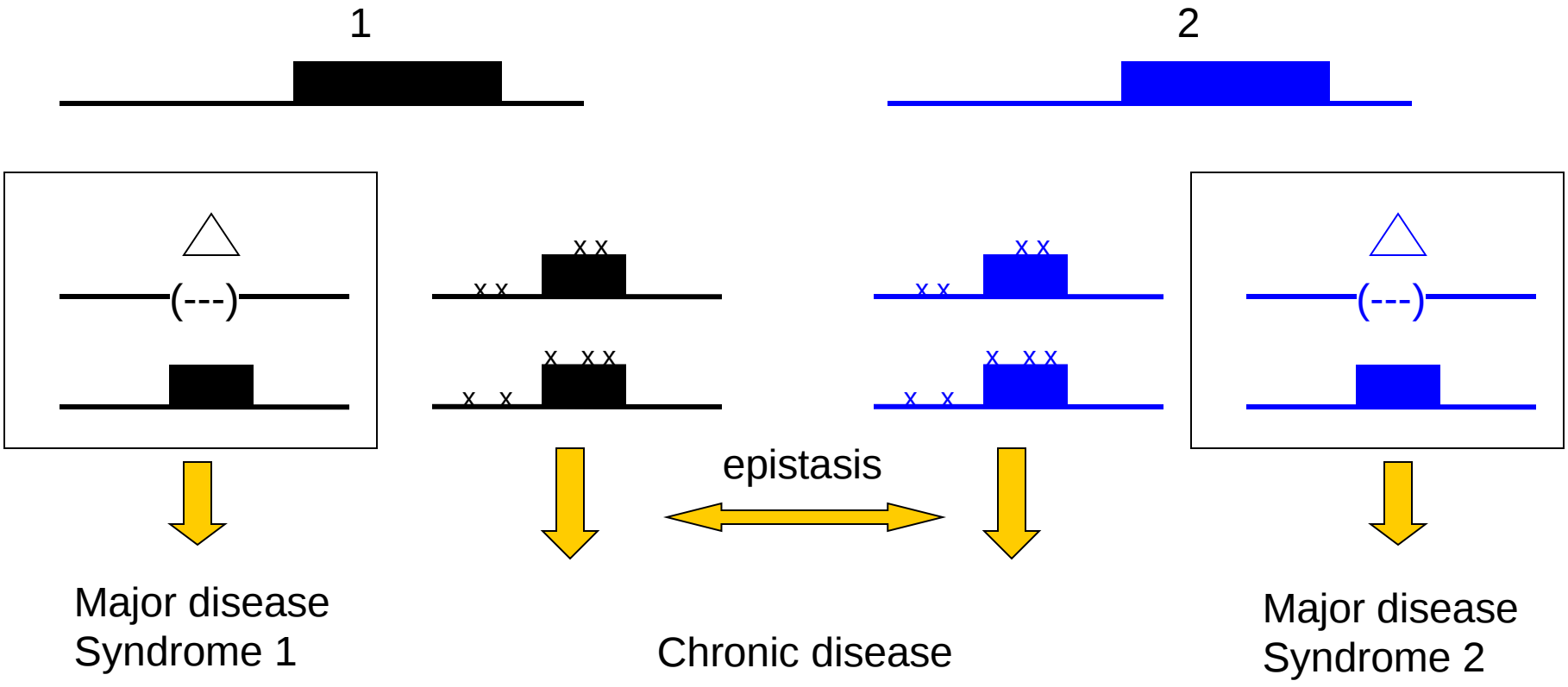
Category	Gene	Gene symbol	Gene name	"Tagging" SNP(s)	Individual association (p) SBP	DBP
Transcription factors	1	**SP1**	Stimulatory protein I	rs10876449	**0.00071**	**0.00024**
	2	**GTF2I**	General transcription factor II, i	rs13238568	**0.04293**	0.09158
				rs2527366	**0.00782**	**0.00796**
				rs17515241	**0.038**	**0.001**
	3	**HNF1A = TCF1=HNF**	Hepatocyte nuclear factor 1 alpha	rs7979473	**0.00367**	**0.01292**
				rs12427353	**0.00195**	**0.03335**
				rs1169307	**0.01255**	**0.00377**
	4	**CREB1**	cAMP response element binding protein	rs10932201	**0.00013**	**0.00034**
				rs11904814	**0.00032**	**1.389E-05**
	5	**CREBBP = CBP**	CREB binding protein	rs130021	**0.01782**	**0.01547**
				rs886528	**3.212E-06**	**2.296E-08**
				rs11076785	**1.658E-05**	**2.299E-09**
	6	**ATF4**	Activating transcription factor 4	rs6519194	**0.00930**	**0.00102**
	7	**TP53**	Tumor protein 53 kDa	rs1042522	**0.00018**	**0.00017**
	8	**HDAC5**	Histone deacetylase 5	rs615070	0.76000	0.28000
Chromogranin /Secretogranin	9	**CHGA**	Chromogranin A	rs7610	**0.01600**	**0.00300**
	10	**CHGB**	Chromogranin B	rs236141	**<0.001**	**0.00100**
	11	**SCG2**	Secretogranin 2	rs3754632	0.49	0.71
				rs11679135	**0.00418**	**0.00196**
Transmitter synthesis /transport	12	**GCH1**	GTP cyclohydrolase	rs841	**0.00700**	**0.00200**
	13	**PNMT**	Phenylethanolamine N-methyltransferase	rs876493	0.40700	0.27900
	14	**SLC6A4 =SERT**	Serotonin transporter	rs8076005	**0.01**	**0.017**
Metabolism	15	**ACADVL**	AcylCoA dehydrogenase	rs2074222	**<0.001**	**<0.001**
	16	**IGFBP4**	Insulin-like growth factor binding protein 4	rs4890114	**<0.001**	**<0.001**
				rs1009728	**0.043**	**0.004**
	17	**IGFBP6**	Insulin-like growth factor binding protein 6	rs2280699	**0.018**	**0.001**
	18	**CYP2E1**	Cytochrome P450 2E1	rs915908	0.09000	0.41000
Endothelium	19	**EDN3**	Endothelin 3	rs6064764	**0.00057**	**0.01042**
				rs2268689	**0.00290**	**0.01265**

Average

Chi-square: Observed (15/19) versus expected (0/19)
Chi-square = 21.6, p<0.0001

A Model

Reconstruction of this complex disease model in a simpler organism will be a synthetic biology effort



Polymorphic loci may also exhibit differential epigenetic signatures, or may respond to epigenetic regulations differentially

Collaborators

- Amarnath Gupta, Michael Baitaluk (UCSD)
- Sri Paladugu
- Dan O’Connor, Ryan Friese (UCSD Medical School)

The ‘New’ Biology