The Yeast Deletion Collection: 6000 Strains

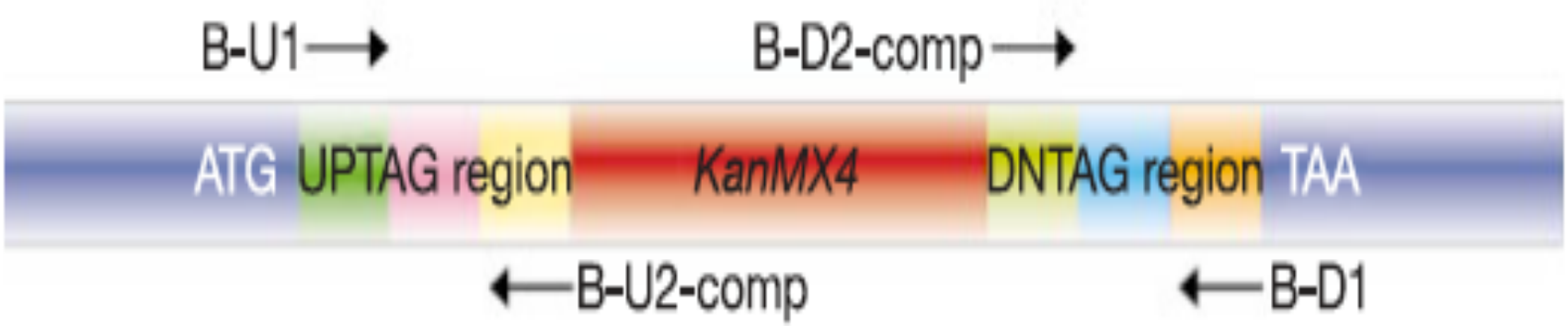
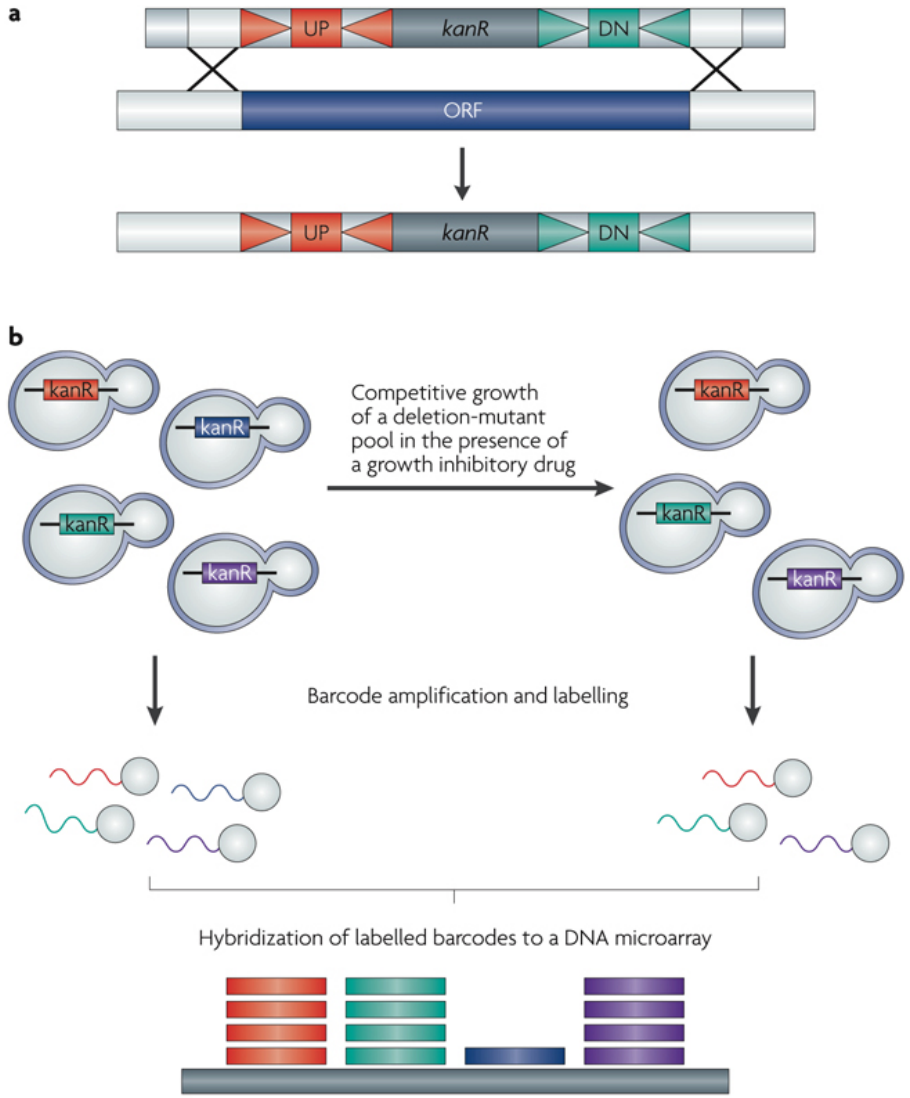
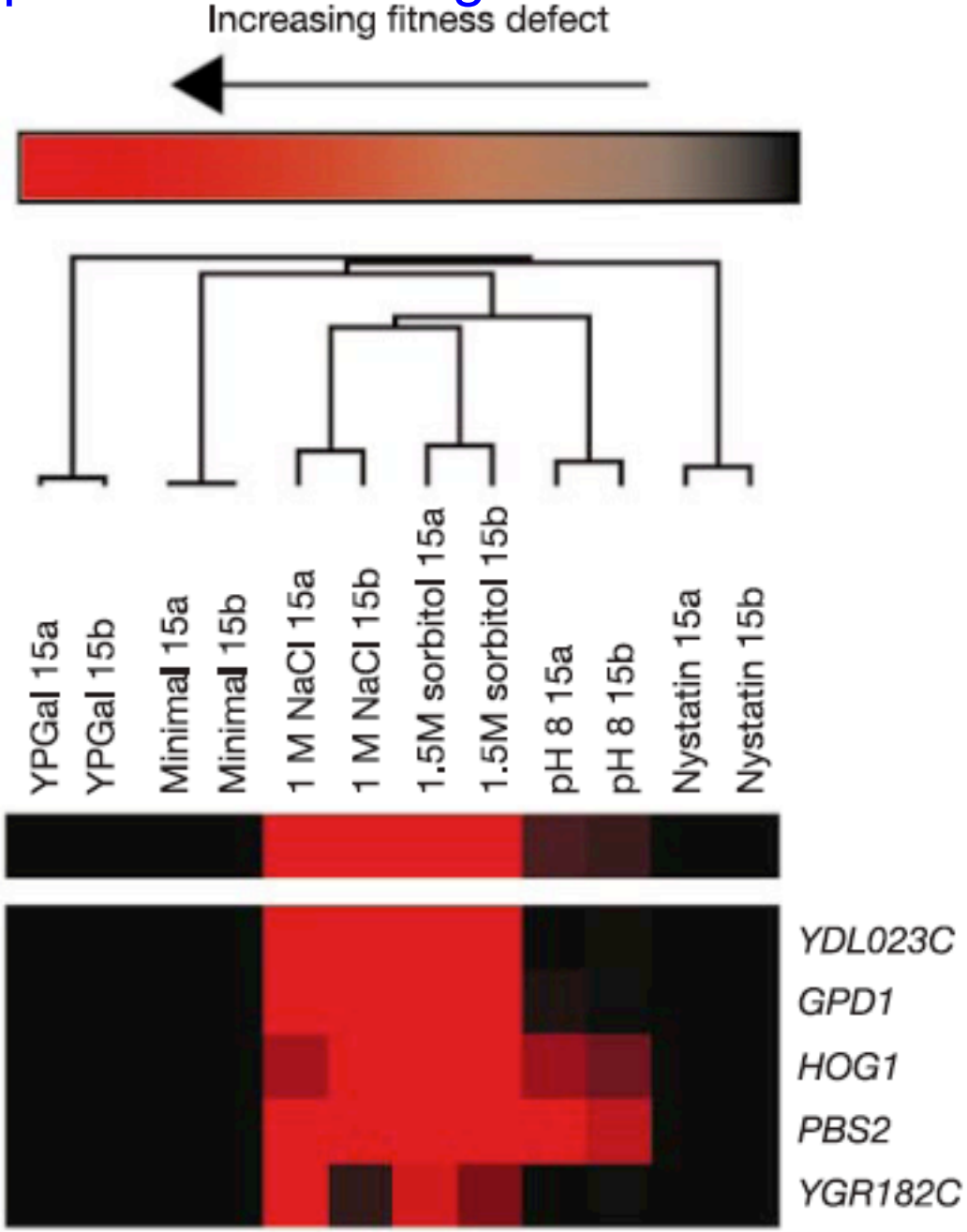


Figure 1 The *KanMX* deletion cassette module. The biotin-labelled, deletion-specific primers (B-U1, B-U2-comp, B-D1 and B-D2-comp; see Methods for structure) are used to amplify the unique UPTAG and DNTAG sequences from genomic preparations generated in the fitness-profiling studies.

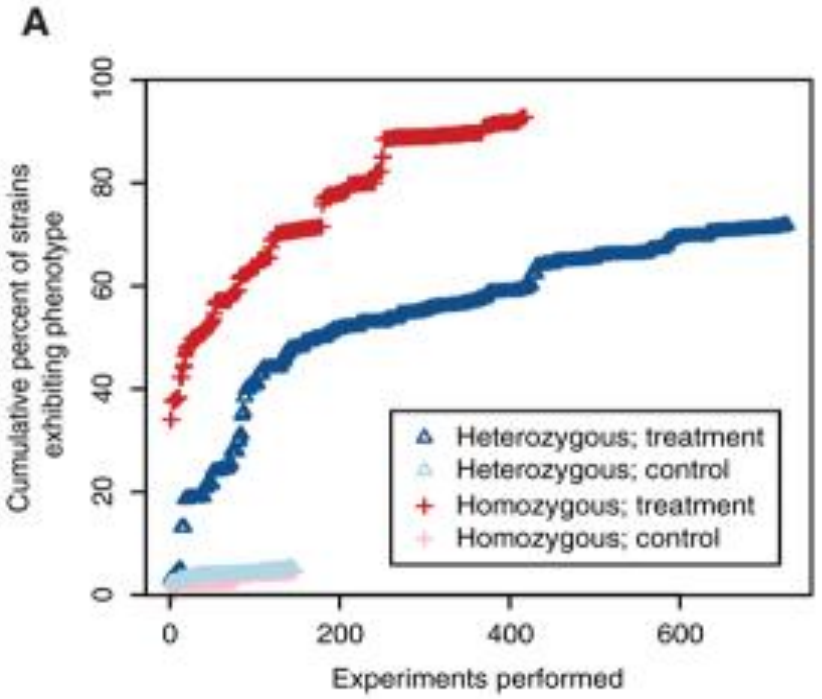
Competitive Fitness Tests

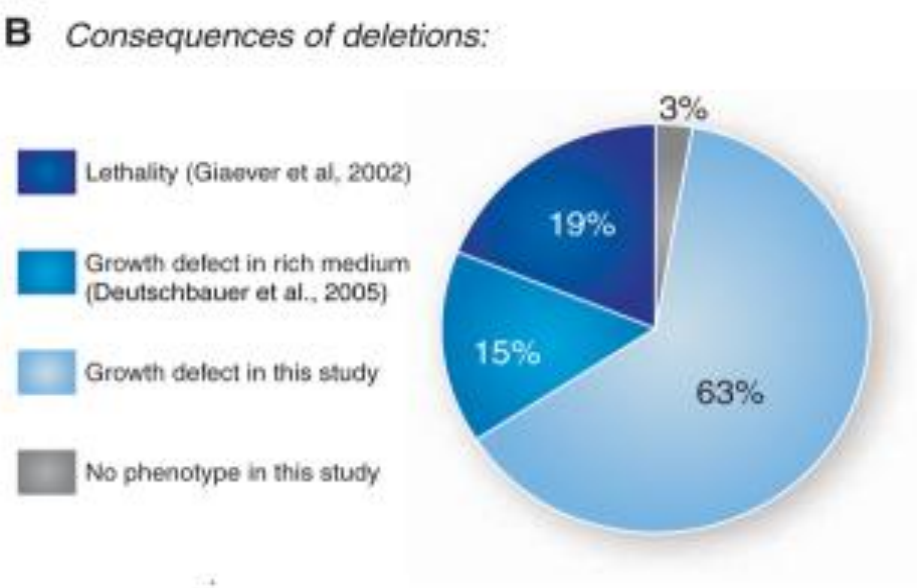


A small sample of tests: new genes sensitive to salt/sorbitol



Chemical genomic profiling with ~700 chemicals reveals a phenotype for virtually every gene deletion

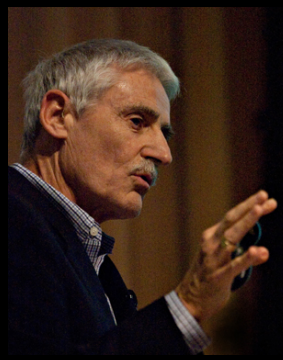


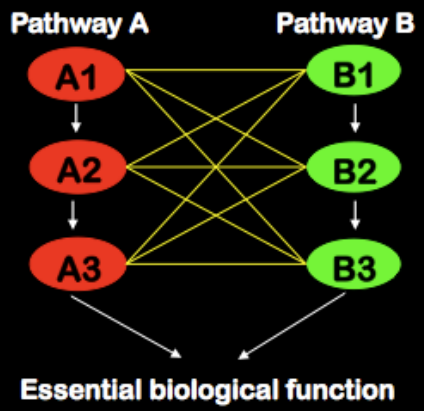


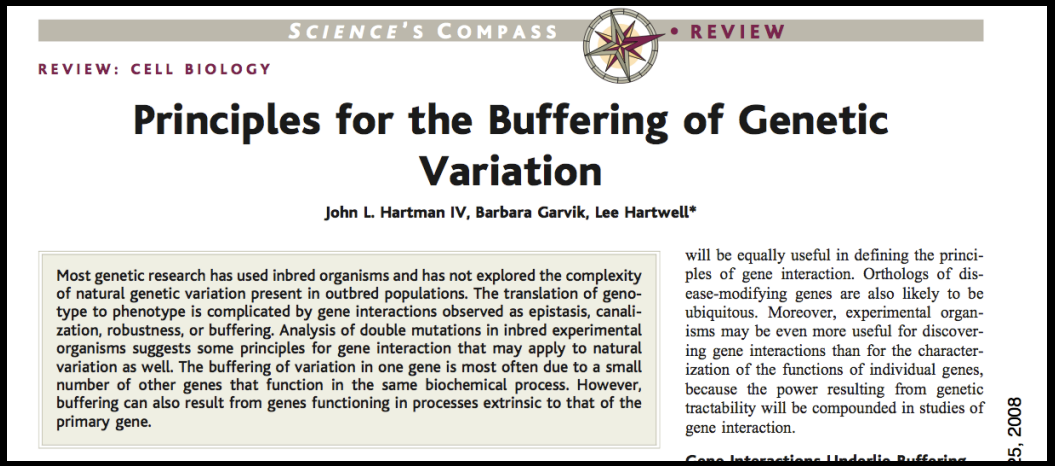
Published in 2008, so Mark Johnston's prediction wasn't far wrong … depending on your definition of "know"

The Hartwell Idea

Synthetic Lethal Networks May Guide Our Understanding of Genotype to Phenotype







SCIENCE'S COMPASS • REVIEW

REVIEW: CELL BIOLOGY

Principles for the Buffering of Genetic Variation

John L. Hartman IV, Barbara Garvik, Lee Hartwell*

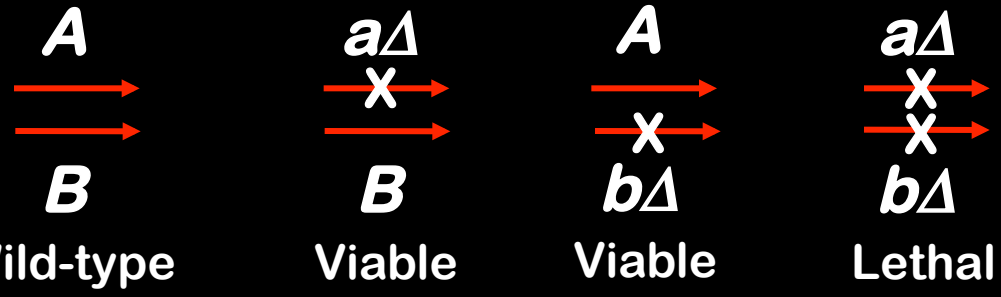
Most genetic research has used inbred organisms and has not explored the complexity of natural genetic variation present in outbred populations. The translation of genotype to phenotype is complicated by gene interactions observed as epistasis, canalization, robustness, or buffering. Analysis of double mutations in inbred experimental organisms suggests some principles for gene interaction that may apply to natural variation as well. The buffering of variation in one gene is most often due to a small number of other genes that function in the same biochemical process. However, buffering can also result from genes functioning in processes extrinsic to that of the primary gene.

will be equally useful in defining the principles of gene interaction. Orthologs of disease-modifying genes are also likely to be ubiquitous. Moreover, experimental organisms may be even more useful for discovering gene interactions than for the characterization of the functions of individual genes, because the power resulting from genetic tractability will be compounded in studies of gene interaction.

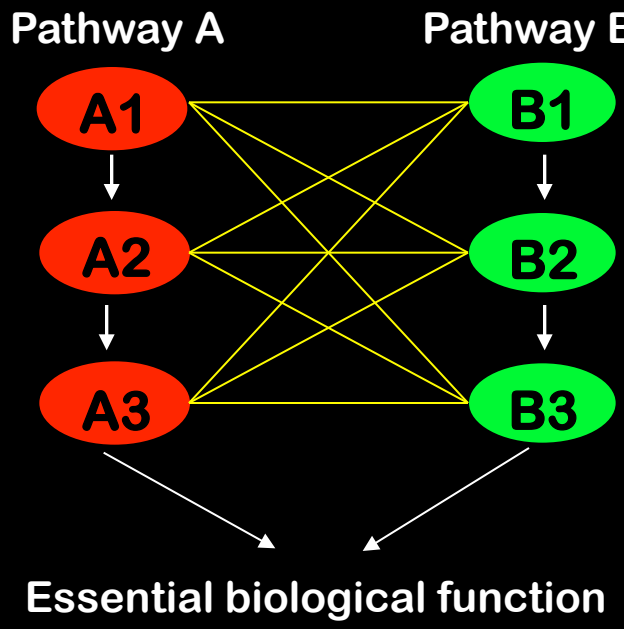
Hartman, Garvik, and Hartwell,
Science, 9 February (2001), Pg. 1001

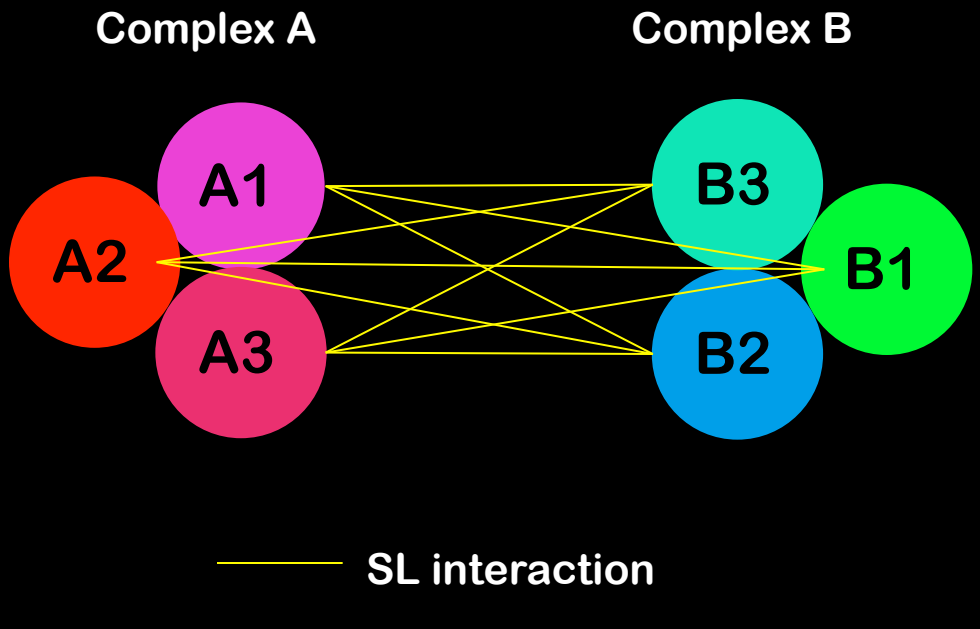
"analysis of double mutations in inbred experimental organisms suggest principles that may apply to natural variation in outbred populations"

Synthetic Lethality

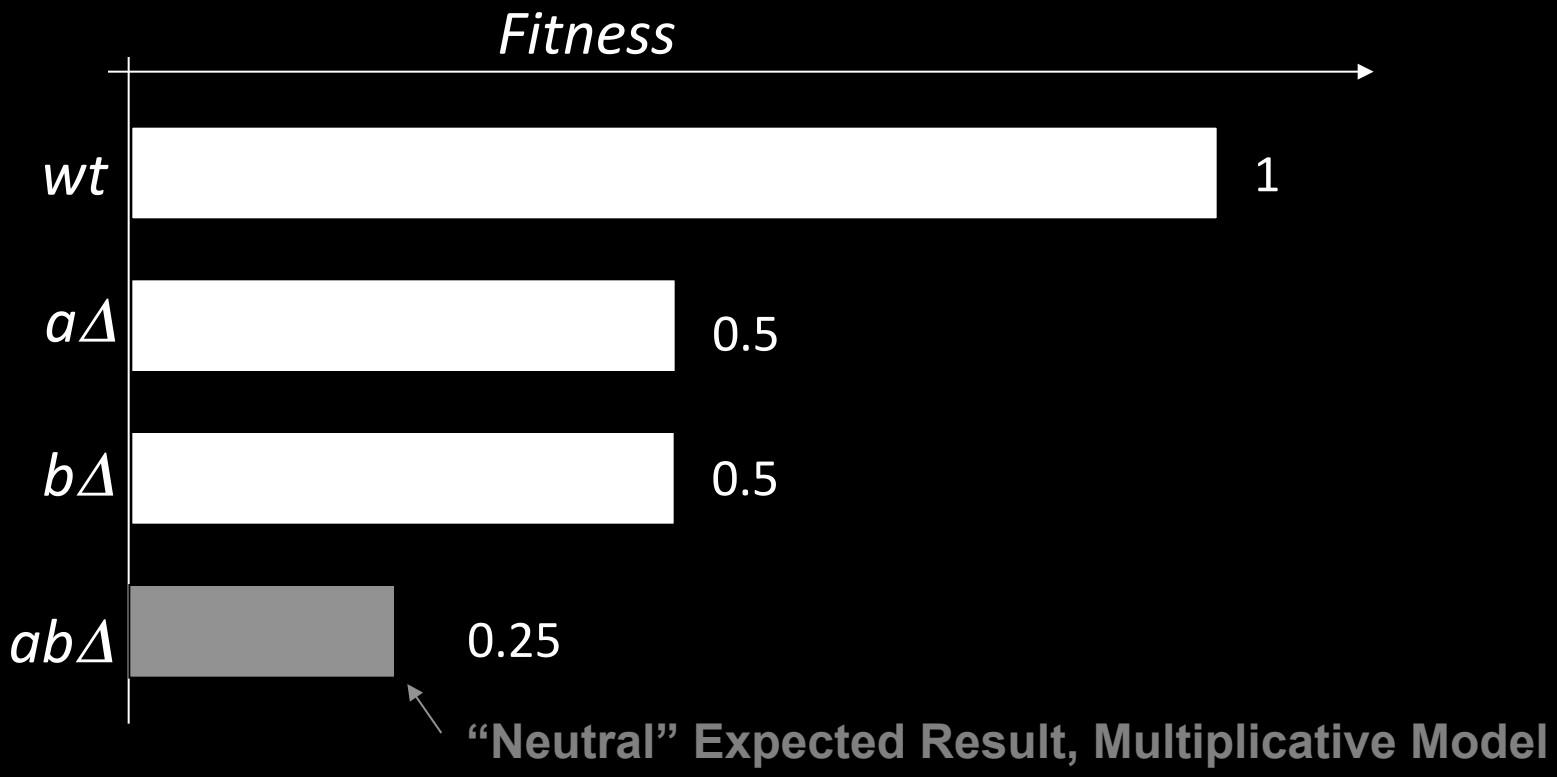


Functional Relationships





Multiplicative Model
Expected Double Mutant Fitness

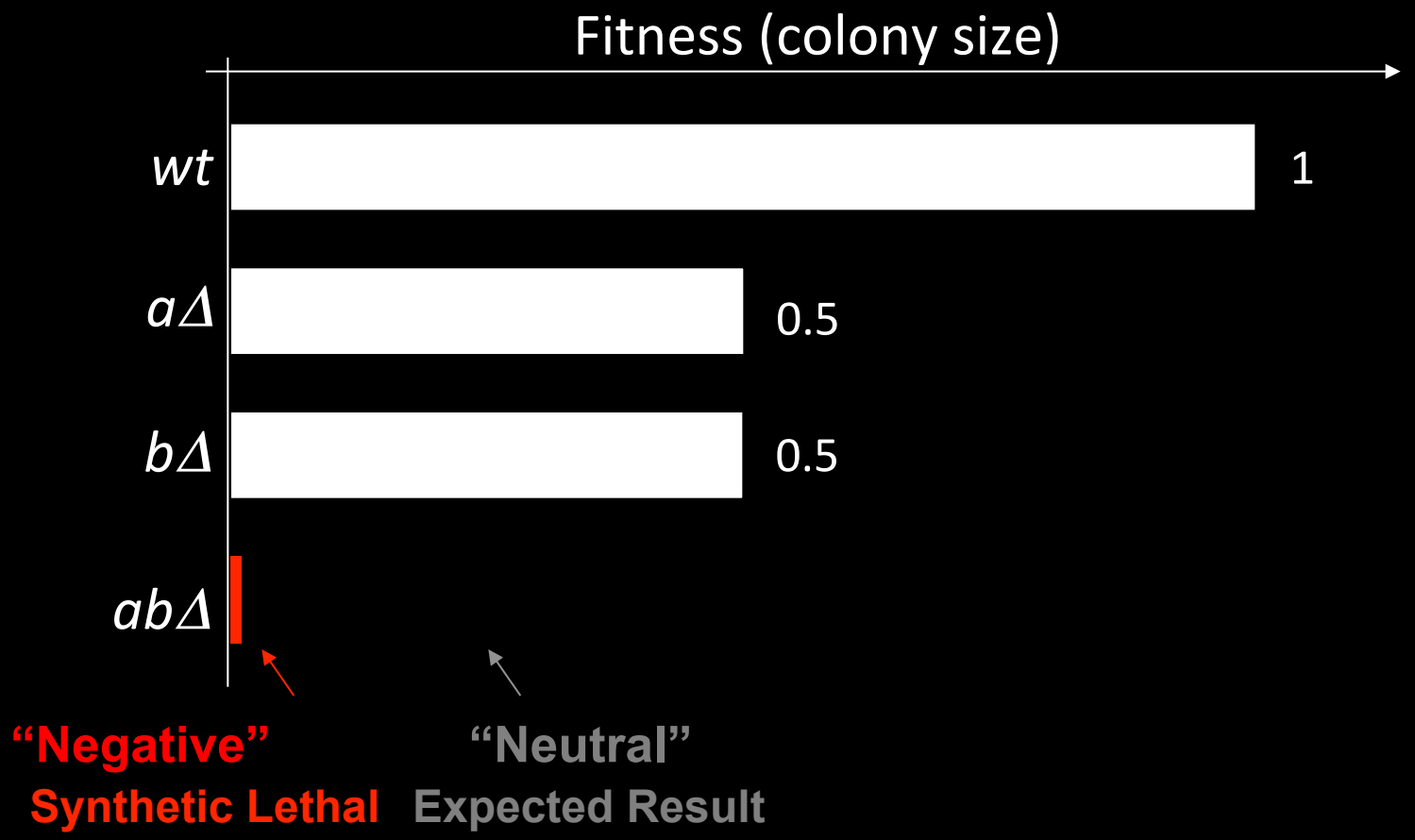


D. Segre, R. Kishony et al., *Nature Genetics* **37**, 77 - 83 (2004)

S. R. Collins, N. Krogan, J. Weissman, *Genome Biol* 2006;7:R63. "S-Score""

R. Mani F. Roth et. al., *PNAS* 2008 Mar 4;105(9):3461-6. Epub 2008 Feb 27

Two Basic Types of Genetic Interactions



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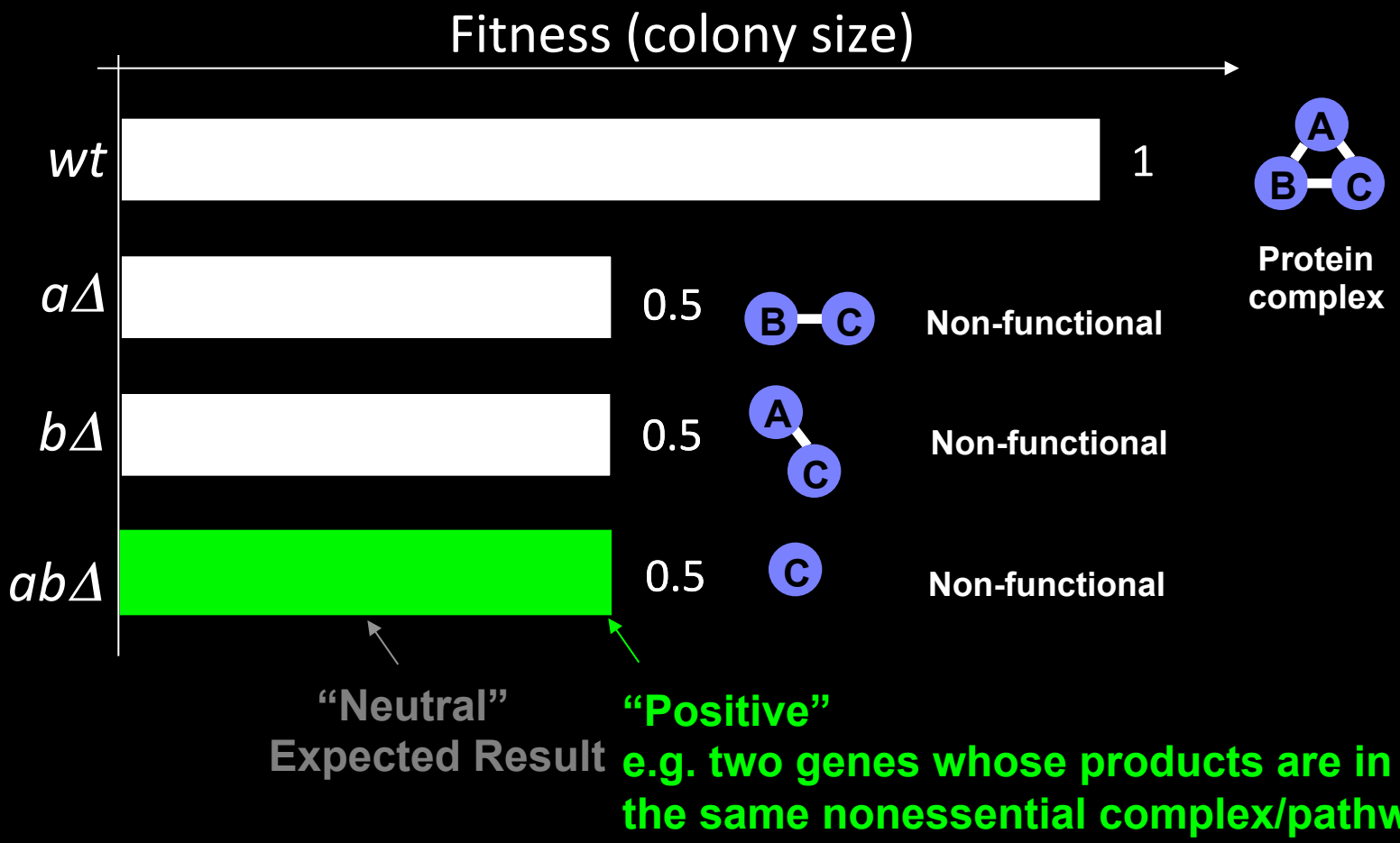
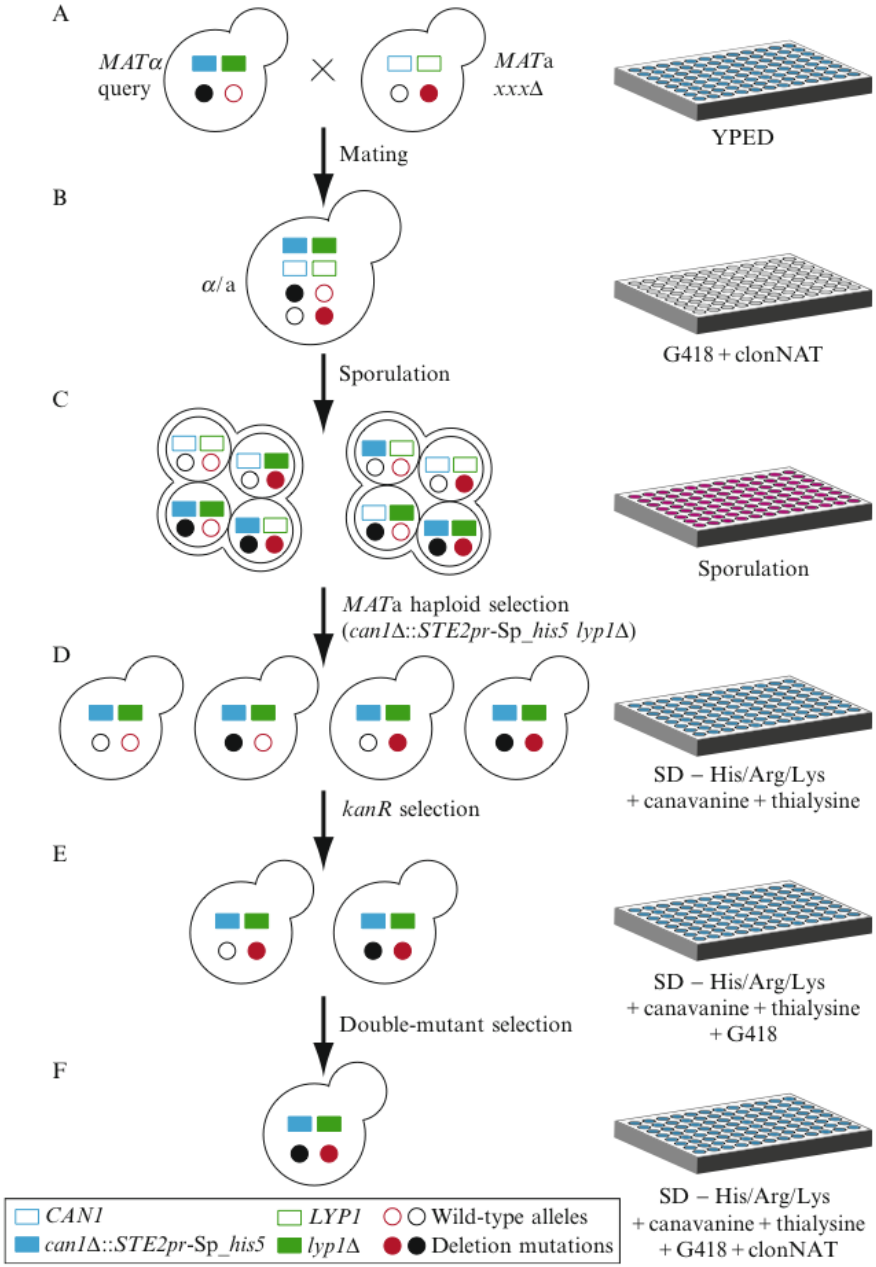
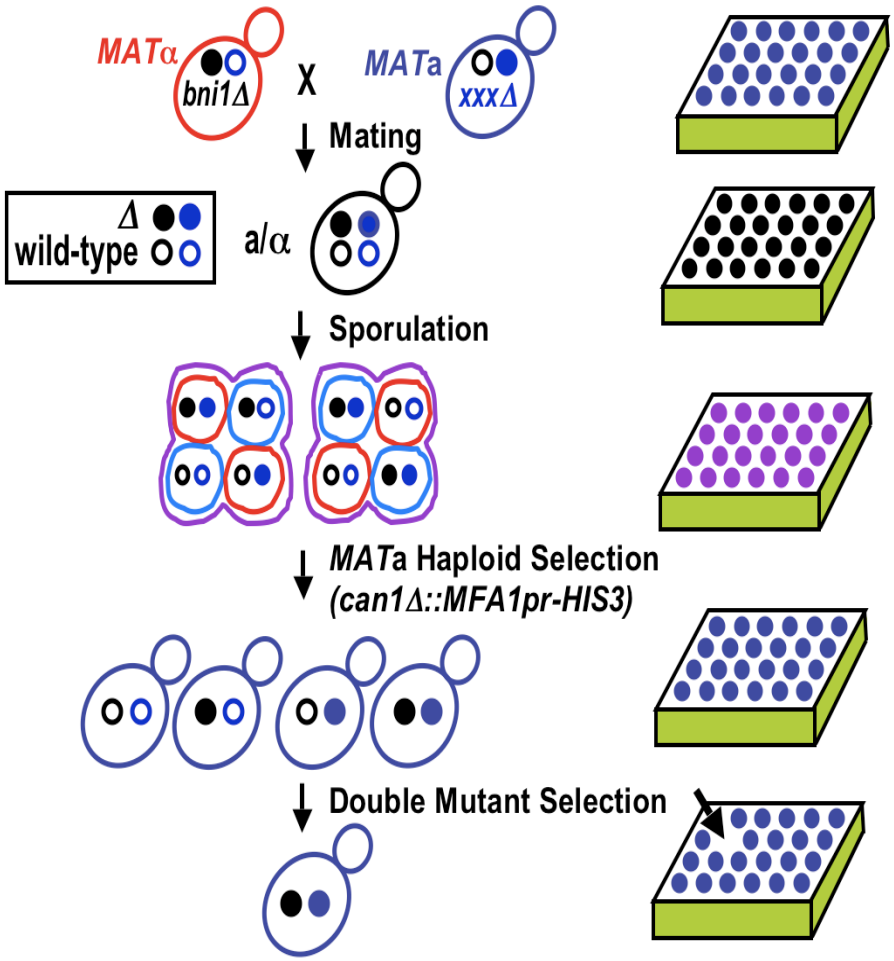


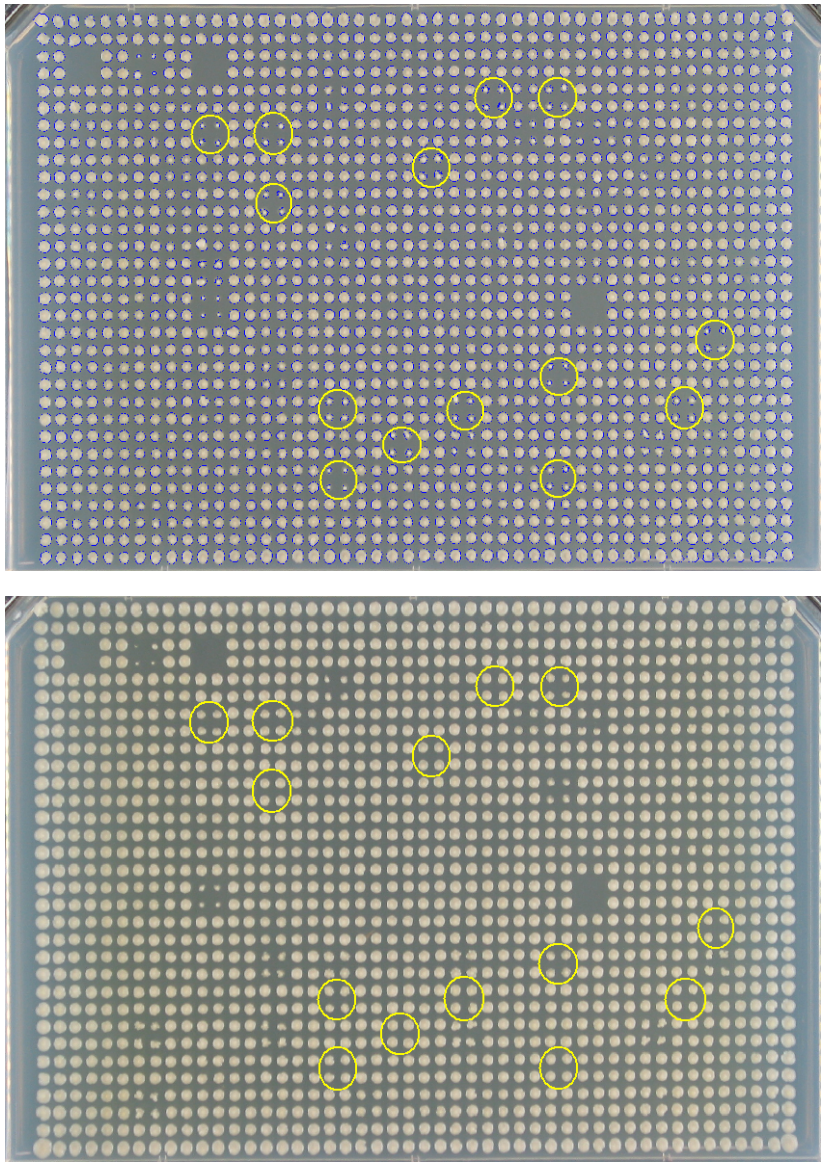
Synthetic Genetic Array (SGA)



Synthetic Lethal Scoring Program

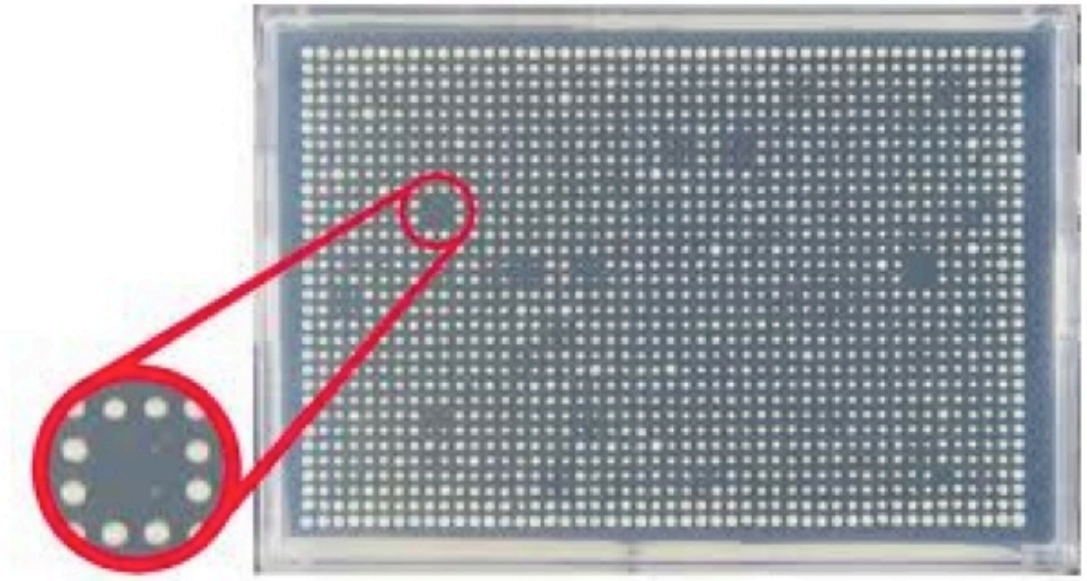


Query: *VMA6*Δ

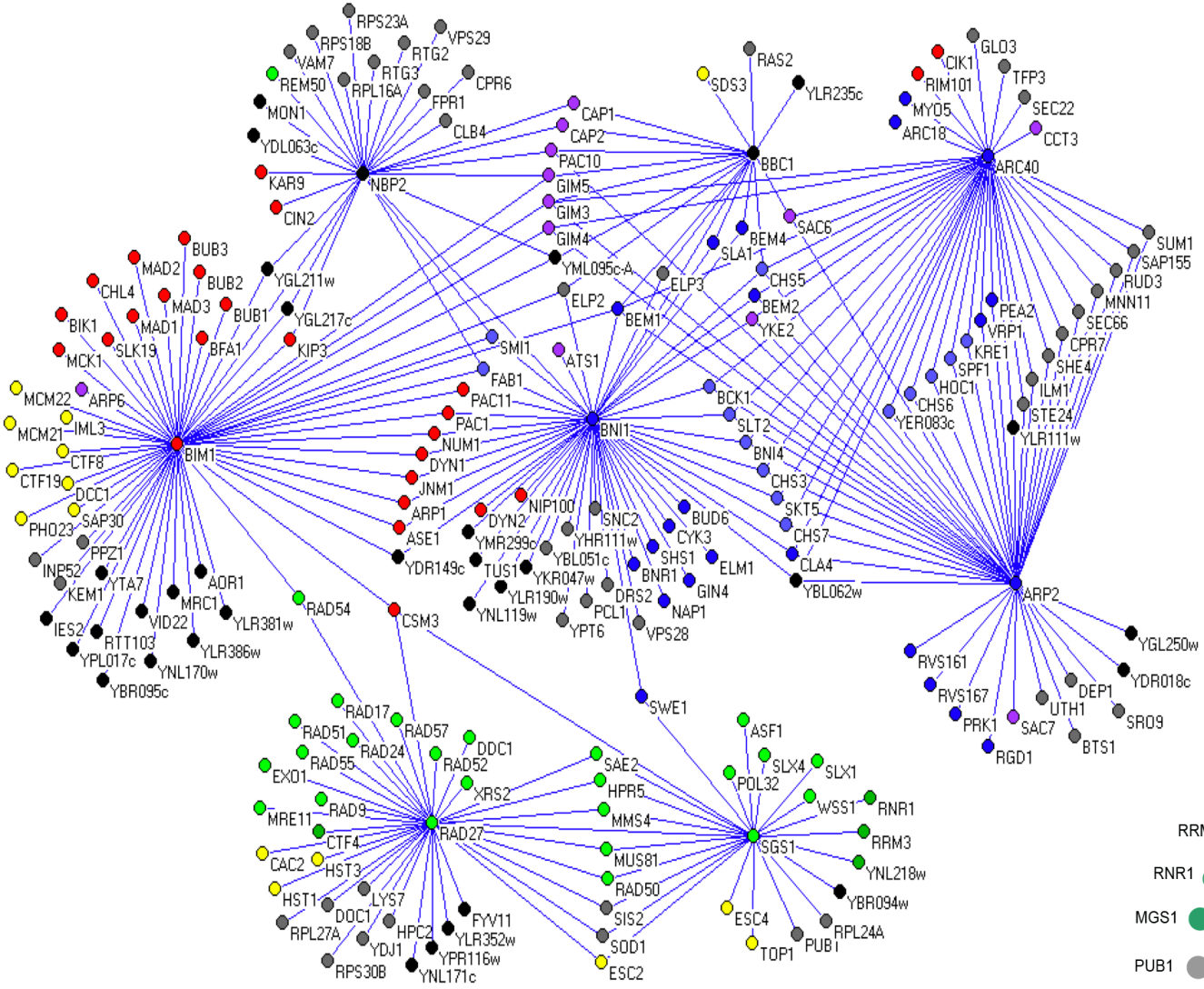


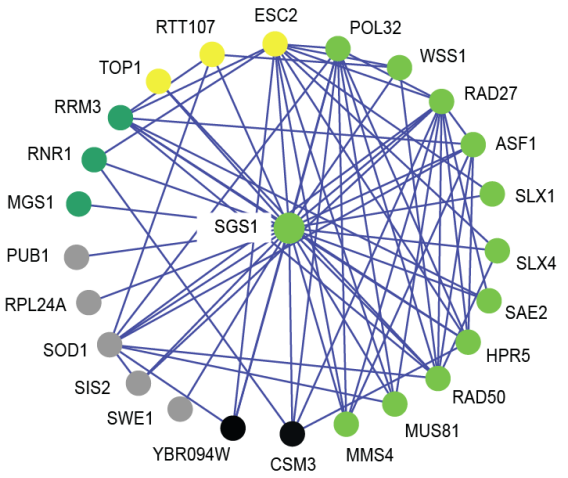
Wildtype control

A blow-up, showing the 4-fold replica for each double mutant



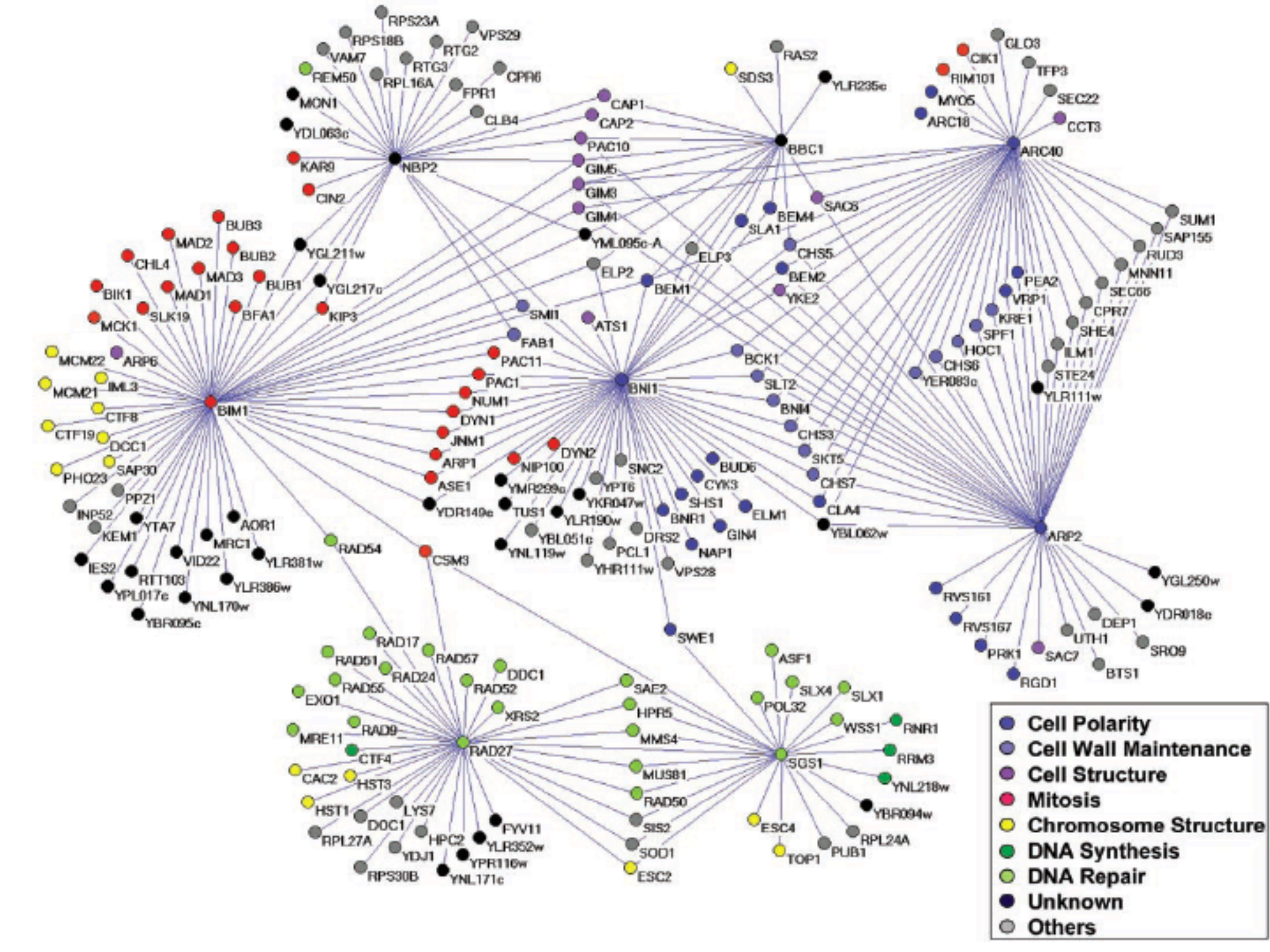
First genetic interaction (synthetic lethal) network



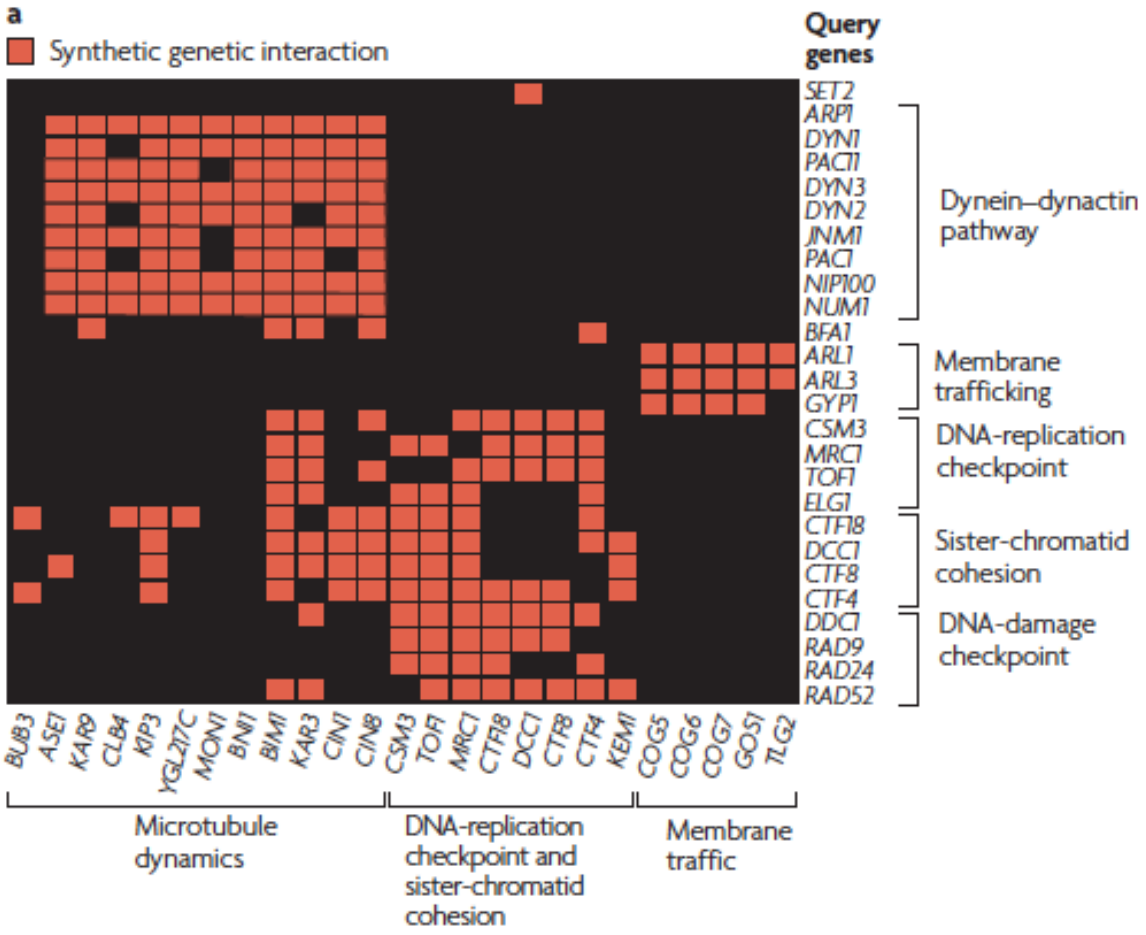


8 screens
291 interactions
204 genes

Tong *et al.*, Science (2001)


RPS23A
RPS18B
VPS29
VAM7
RTG2
RTG3
REM50
RPL16A
CPR6
FPR1
MON1
CLB4
YDL063c
KAR9
NBP2
CIN2
YGL211w
BUB3
MAD2
BUB2
CHL4
MAD3
BUB1
YGL217c
BIK1
MAD1
SLK19
BFA1
MCK1
KIP3
MCM22
ARP6
MCM21
IML3
CTF8
CTF19
DCC1
PHO23
SAP30
PPZ1
INP52
KEM1
YTA7
AOR1
MRC1
IES2
VID22
YLR381w
RTT103
YLR386w
YPL017c
YNL179w
YBR095c
BIM1
RAD54
CSM3
CAP1
CAP2
PAC10
GIM5
GIM3
GIM4
YML095c-A
ELP2
ELP3
BEM1
SMI1
ATS1
FAB1
PAC11
PAC1
NUM1
DYN1
JNM1
ARP1
ASE1
YDR149c
BNI1
DYN2
NIP100
YMR299c
YKR047w
TUS1
YLR190w
YBL051c
YNL119w
PCL1
YHR111w
SNC2
YPT6
DRS2
VPS28
BUD6
CYK3
SHS1
BNR1
NAP1
ELM1
GIN4
SWE1
RAS2
SDS3
YLR235c
BBC1
SAC6
BEM4
SLA1
BEM2
YKE2
BCK1
SLT2
BNI4
CHS3
SKT5
CHS7
CLA4
YBL062w
GLO3
CIK1
TFP3
RIM101
SEC22
MYO5
CCT3
ARC18
ARC40
SUM1
SAP155
RUD3
MNN11
SEC66
PEA2
VRP1
CPR7
KRE1
SHE4
SPF1
HOC1
ILM1
CHS6
STE24
YER083c
YLR111w
ARP2
YGL250w
RVS161
YDR018c
RVS167
DEP1
UTH1
SRO9
PRK1
SAC7
BTS1
RGD1
RAD17
RAD51
RAD57
RAD24
DDC1
RAD55
RAD52
EXO1
XRS2
MRE11
RAD9
RAD27
CTF4
CAC2
HST3
HST1
LYS7
DOC1
RPL27A
HPC2
FYV11
YDJ1
YLR352w
YPR116w
RPS30B
YNL171c
SAE2
HPR5
MMS4
MUS81
RAD50
SIS2
SOD1
ESC2
ASF1
SLX4
SLX1
POL32
WSS1
RNR1
SGS1
RRM3
YNL218w
YBR094w
ESC4
RPL24A
TOP1
PUB1
Cell Polarity
Cell Wall Maintenance
Cell Structure
Mitosis
Chromosome Structure
DNA Synthesis
DNA Repair
Unknown
Others

Another view of the data: hierarchical clustering



~2000 Quantitative SGA Screens

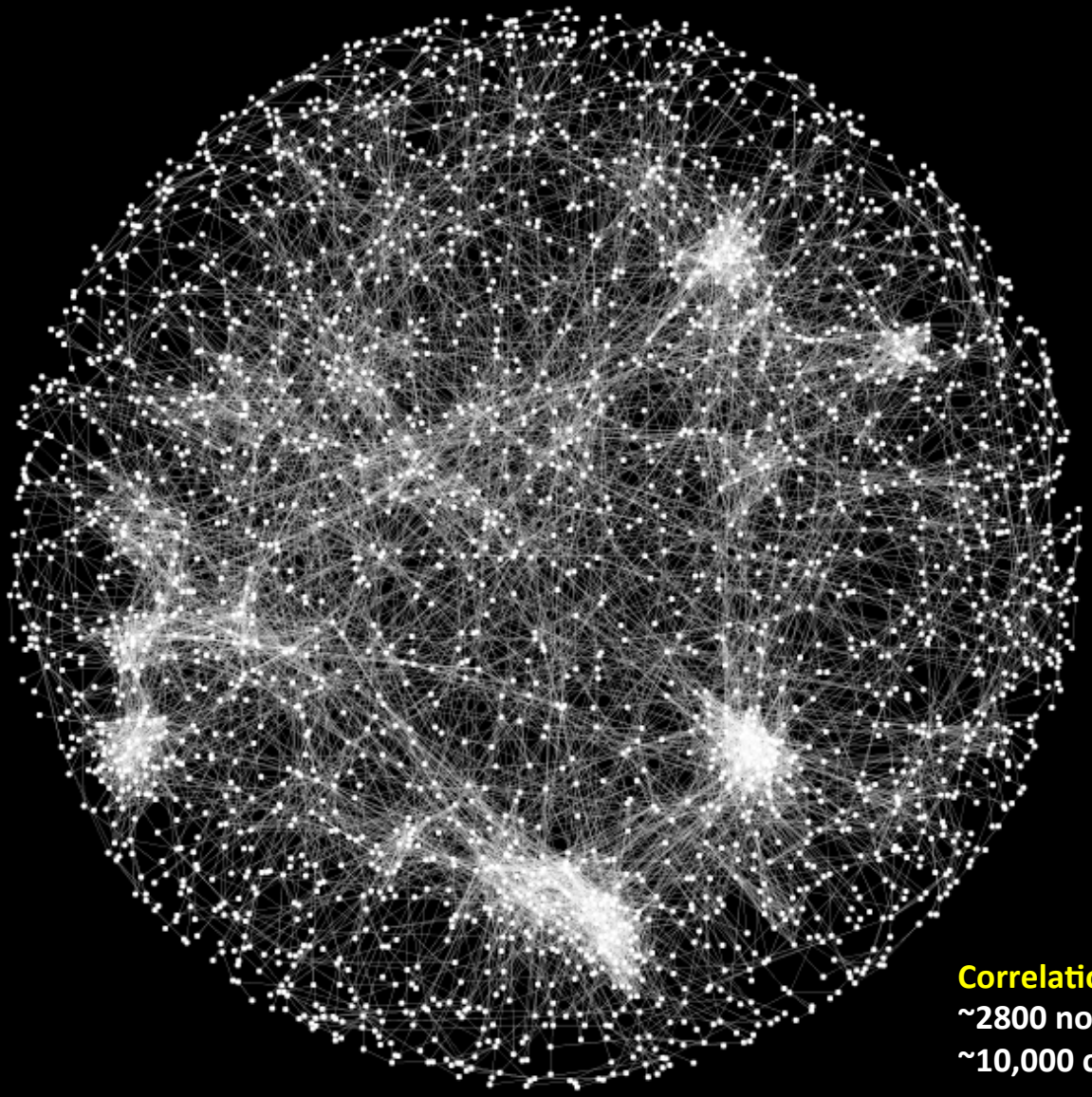
5.4 million gene-gene pairs, ~30% of Total Network
170,000 Interactions, ~2/3 Negative 1/3 Positive

The Genetic Landscape of a Cell

Michael Costanzo,[1,2]* Anastasia Baryshnikova,[1,2]* Jeremy Bellay,[3] Yungil Kim,[3] Eric D. Spear,[4] Carolyn S. Sevier,[4] Huiming Ding,[1,2] Judice L.Y. Koh,[1,2] Kiana Toufighi,[1,2] Sara Mostafavi,[1,5] Jeany Prinz,[1,2] Robert P. St. Onge,[6] Benjamin VanderSluis,[3] Taras Makhnevych,[7] Franco J. Vizeacoumar,[1,2] Solmaz Alizadeh,[1,2] Sondra Bahr,[1,2] Renee L. Brost,[1,2] Yiqun Chen,[1,2] Murat Cokol,[8] Raamesh Deshpande,[3] Zhijian Li,[1,2] Zhen-Yuan Lin,[9] Wendy Liang,[1,2] Michaela Marback,[1,2] Jadine Paw,[1,2] Bryan-Joseph San Luis,[1,2] Ermira Shuteriqi,[1,2] Amy Hin Yan Tong,[1,2] Nydia van Dyk,[1,2] Iain M. Wallace,[1,2,10] Joseph A. Whitney,[1,5] Matthew T. Weirauch,[11] Guoqing Zhong,[1,2] Hongwei Zhu,[1,2] Walid A. Houry,[7] Michael Brudno,[1,5] Sasan Ragibizadeh,[12] Balázs Papp,[13] Csaba Pál,[13] Frederick P. Roth,[8] Guri Giaever,[2,10] Corey Nislow,[1,2] Olga G. Troyanskaya,[14] Howard Bussey,[15] Gary D. Bader,[1,2] Anne-Claude Gingras,[9] Quaid D. Morris,[1,2,5] Philip M. Kim,[1,2] Chris A. Kaiser,[4] Chad L. Myers,[3]† Brenda J. Andrews,[1,2]† Charles Boone[1,2]†

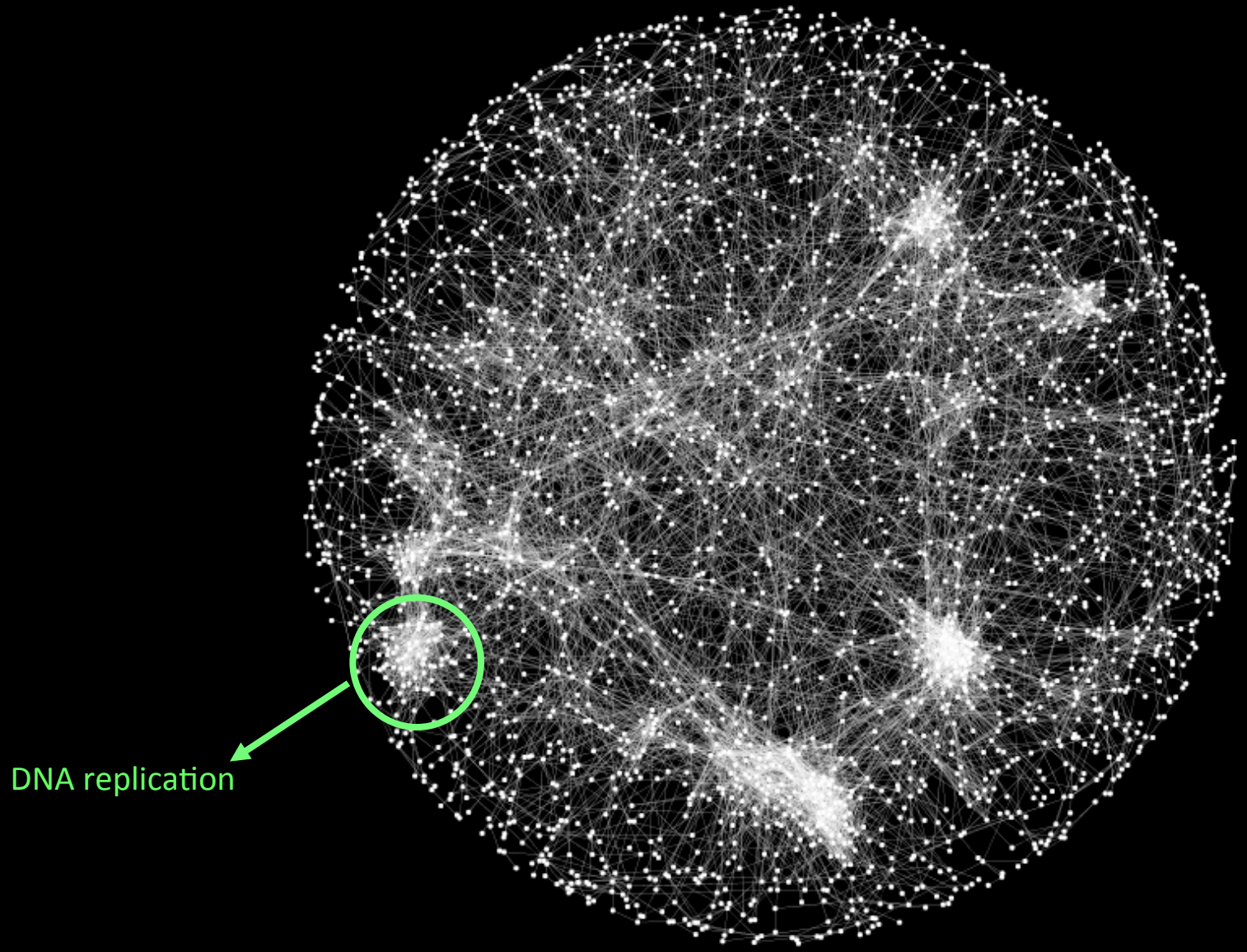
A genome-scale genetic interaction map was constructed by examining 5.4 million gene-gene pairs for synthetic genetic interactions, generating quantitative genetic interaction profiles for ~75% of all genes in the budding yeast, *Saccharomyces cerevisiae*. A network based on genetic interaction profiles reveals a functional map of the cell in which genes of similar biological processes cluster together in coherent subsets, and highly correlated profiles delineate specific pathways to define gene function. The global network identifies functional cross-connections between all bioprocesses, mapping a cellular wiring diagram of pleiotropy. Genetic interaction degree correlated with a number of different gene attributes, which may be informative about genetic network hubs in other organisms. We also demonstrate that extensive and unbiased mapping of the genetic landscape provides a key for interpretation of chemical-genetic interactions and drug target identification.

Yeast Genetic Interaction Network
Global Level



Correlation-based network
~2800 nodes
~10,000 correlation edges

Yeast Genetic Interaction Network
Global Level



Yeast Genetic Interaction Network
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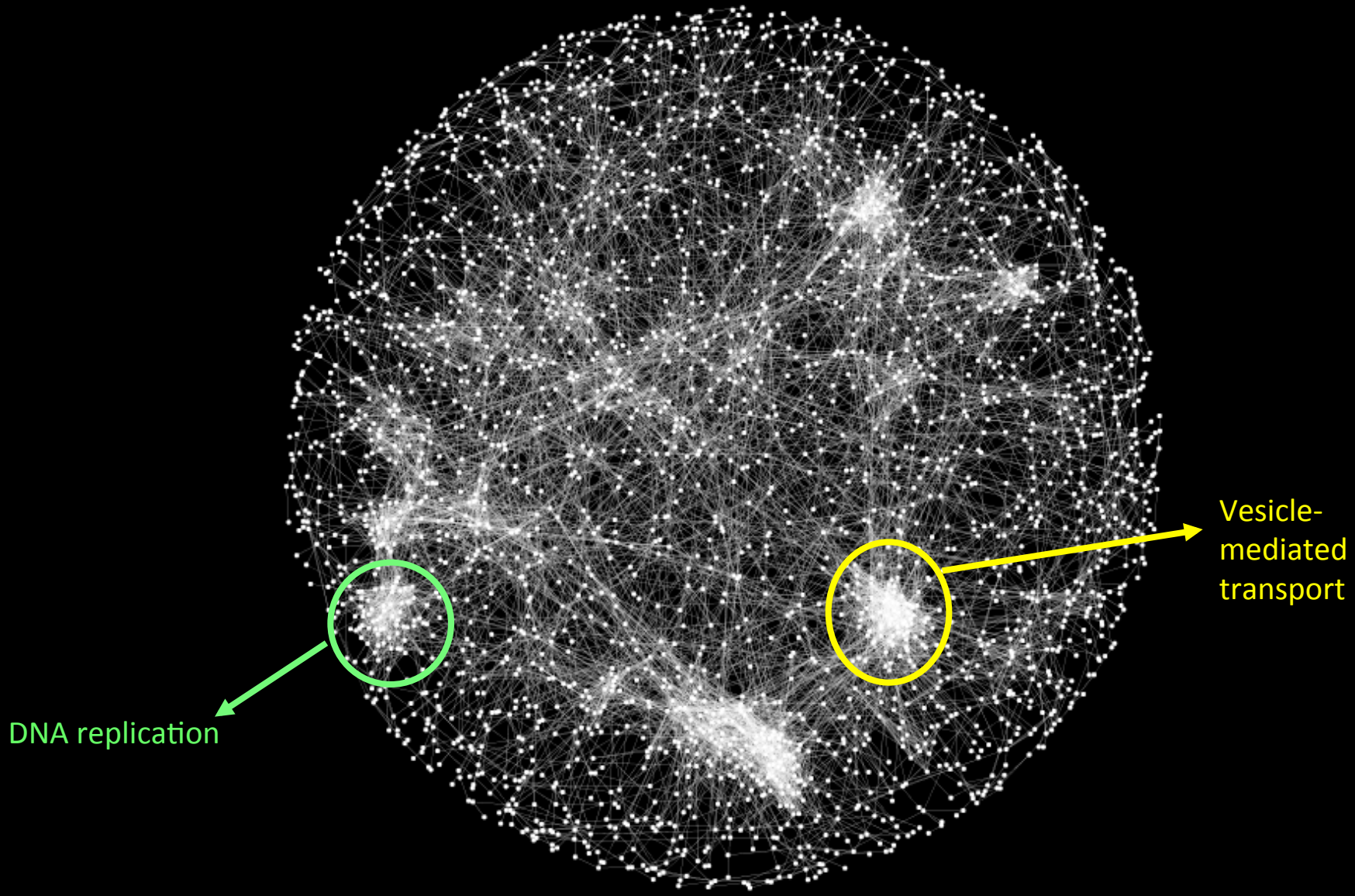

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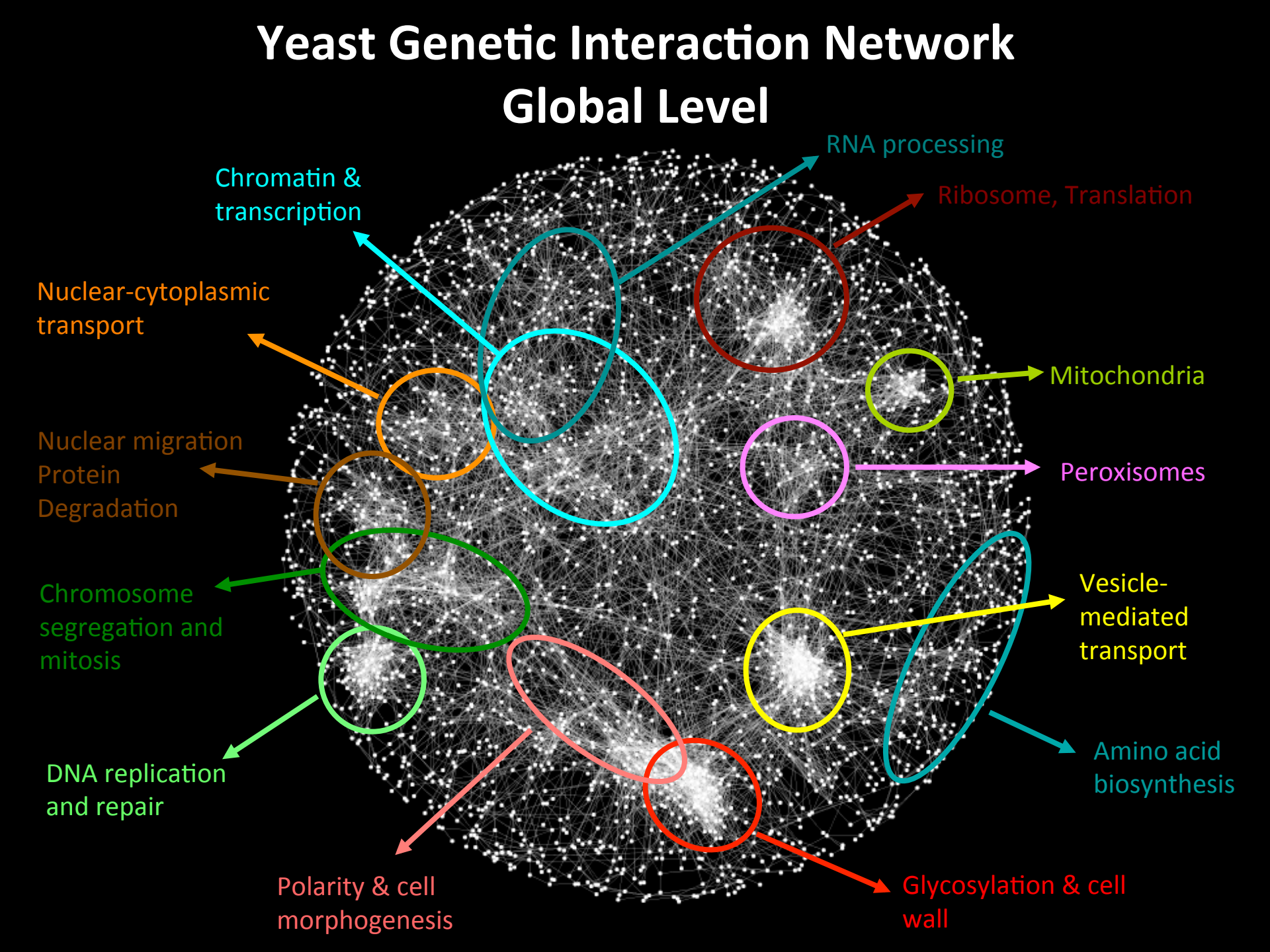

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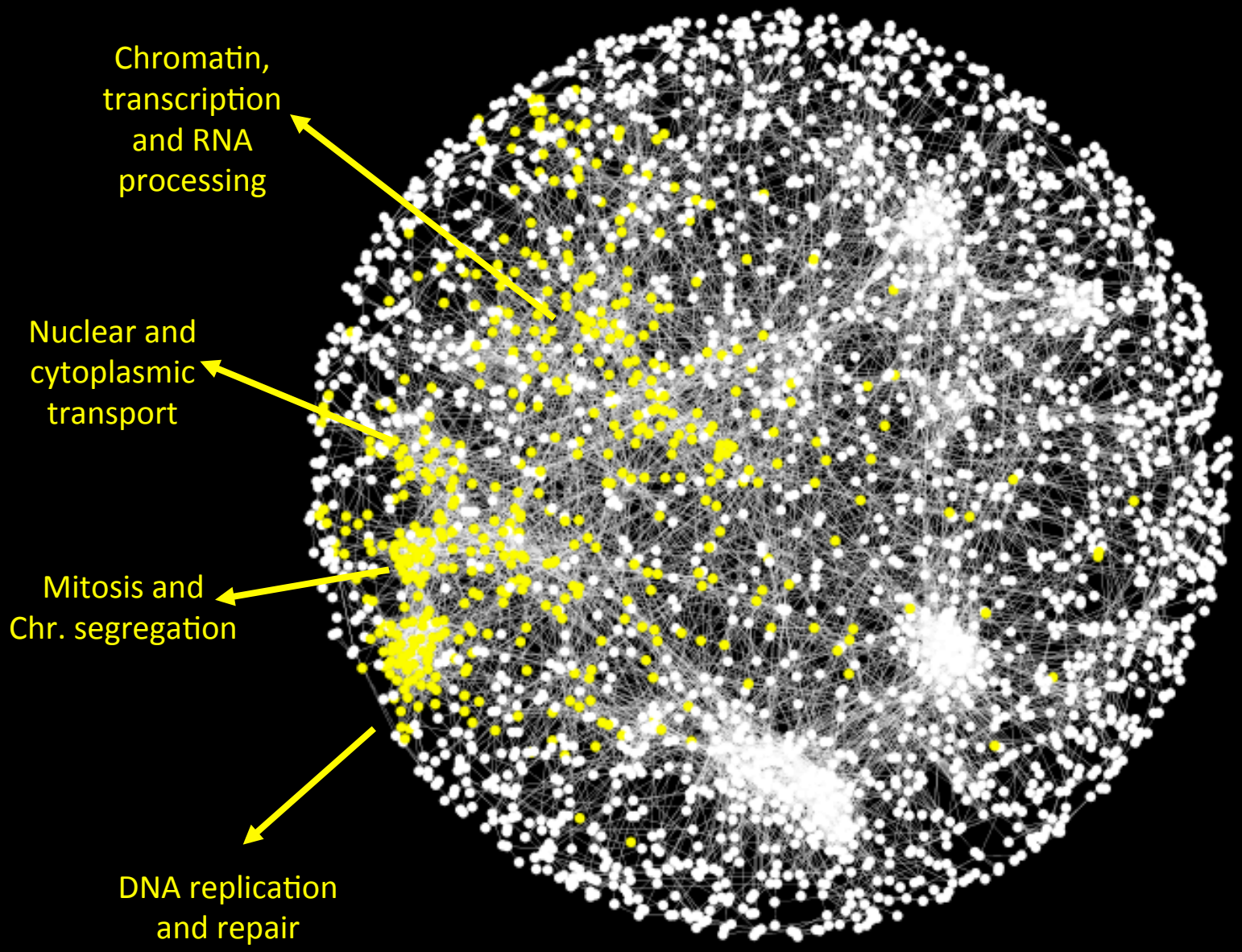
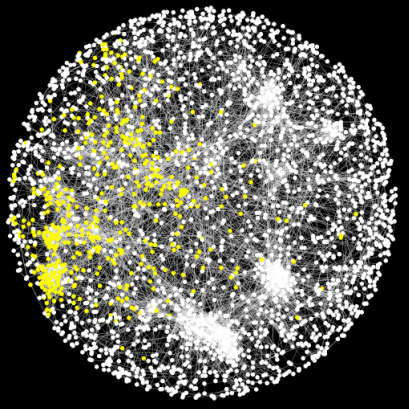
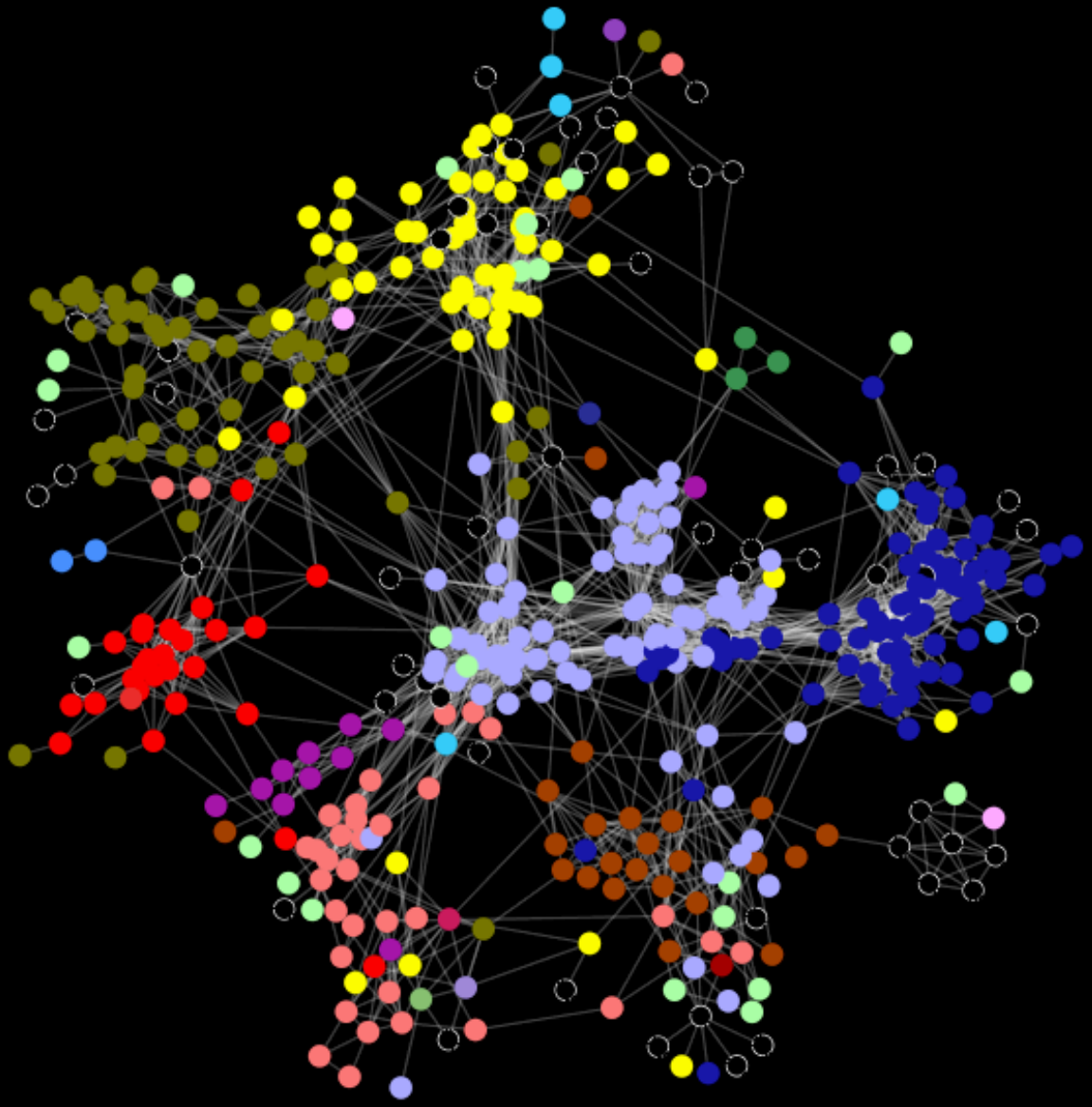




Yeast Genetic Interaction Network
Process Level



DRYGIN

Data Repository of Yeast Genetic INteractions

Quick gene search:
enter gene names or orfs

submit

Problem with searching ?

DRYGIN is a database of quantitative genetic interactions of *S. Cerevisiae* derived from the SGA double-mutant arrays conducted in Boone lab at Terrence Donnelly Centre for Cellular and Biochemical Research, University of Toronto.

Current version of DRYGIN is 1.0 (DRYGIN documentation).

Latest Database statistics

- Total SGA genetic interactions in DRYGIN: 5,482,948
- Total genes screened: 1,711 query (1,672 ORFs) X 3,885 array (3,885 ORFs)
- **Updates:**
  2010-02-10: The database was updated with the latest values from Costanzo et al., 2010.
  2010-11-18: The *clc1Δ* screen was removed because an allele discrepancy was noticed.

For description of the database content, please refer to Judice L.Y. Koh, Huiming Ding, Michael Costanzo, Anastasia Baryshnikova, Kiana Toufighi, Gary D. Bader, Chad L. Myers, Brenda J. Andrews, and Charles Boone. DRYGIN: a database of quantitative genetic interaction networks in yeast. *Nucleic Acids Research*, 2010, Vol. 38, Database issue D502-D507.

Search genes - Query SGA genetic interactions using gene names or orfs. Download interactions or visualize network or clustergram, with genes color-coded according to functional annotations.

Sub-networks - Search and visualize sub-networks of genetic interactions between a group of genes.

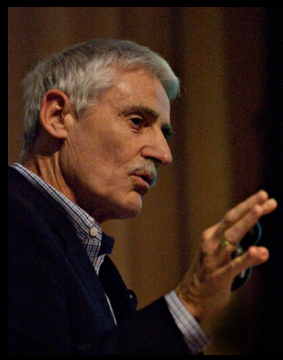
Search complexes/pathways - Search and visualize SGA genetic interactions in known protein complexes and pathways.

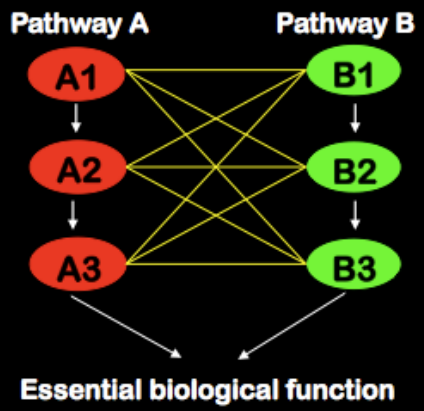
Overlap with BIOGRID - Search for SGA genetic interactions that overlap with BIOGRID genetic and physical interactions.

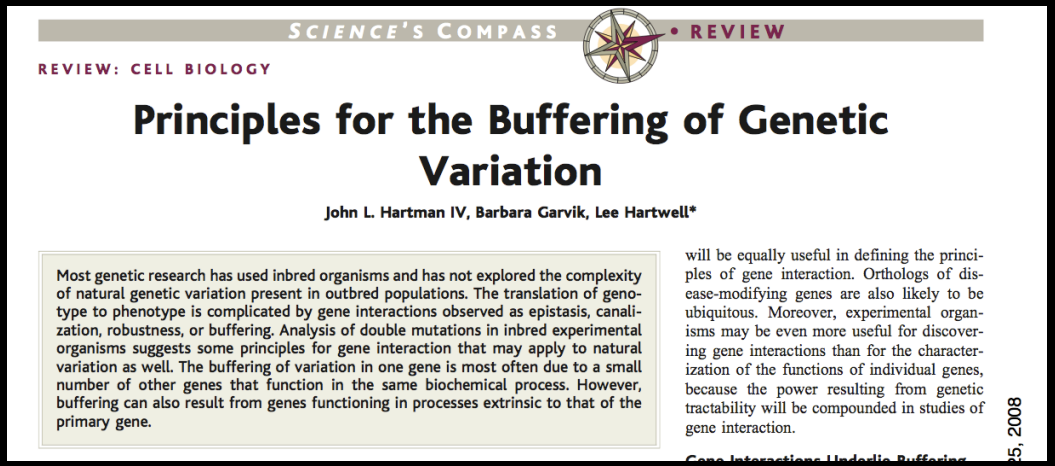
Download - Download latest SGA genetic interactions in Cytoscape SIF, tab-delimited, or Java Treeview CDT formats.

The Hartwell Idea

Synthetic Lethal Networks May Guide Our Understanding of Genotype to Phenotype







SCIENCE'S COMPASS • REVIEW

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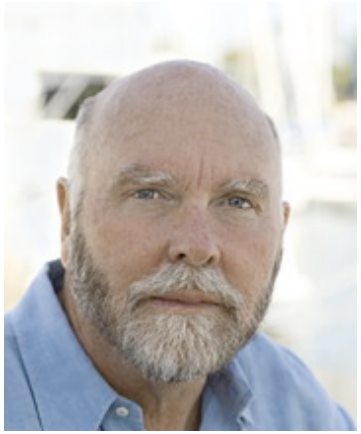
Hartman, Garvik, and Hartwell,
Science, 9 February (2001), Pg. 1001

"analysis of double mutations in inbred experimental organisms suggest principles that may apply to natural variation in outbred populations"

The Fink Idea

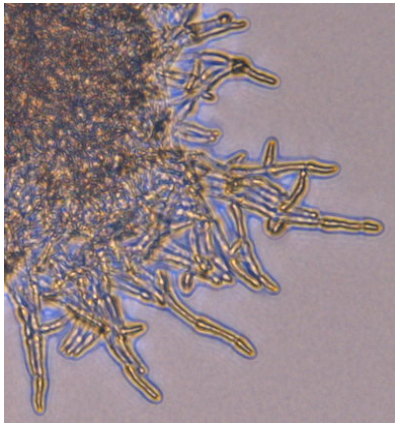
Σ1278b

S288c

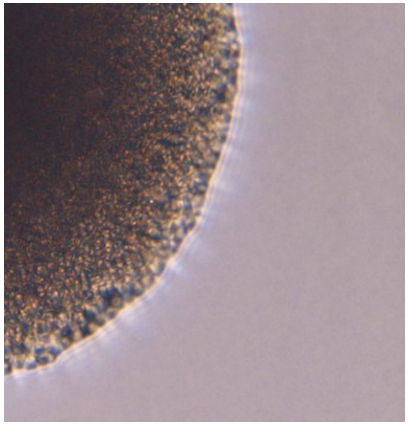


Genotype/Phenotype of *S. cerevisae* Strains

Σ1278b



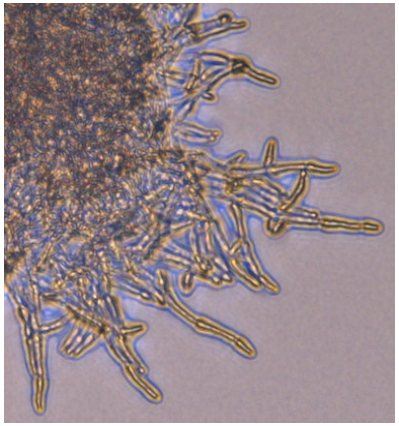
S288c



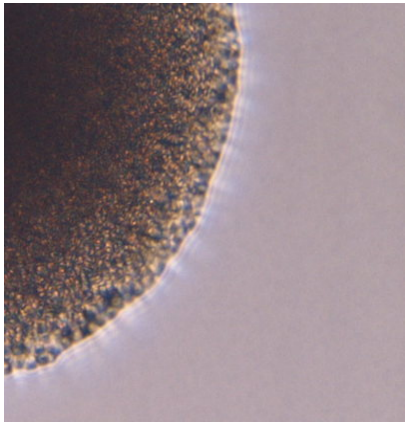
- **Strains Mate, Meiotic Progeny are Viable**

Genotype/Phenotype of *S. cerevisae* Strains

Σ1278b



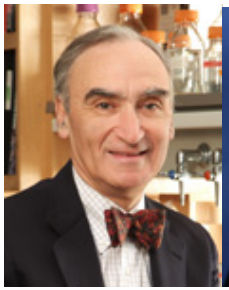
S288c



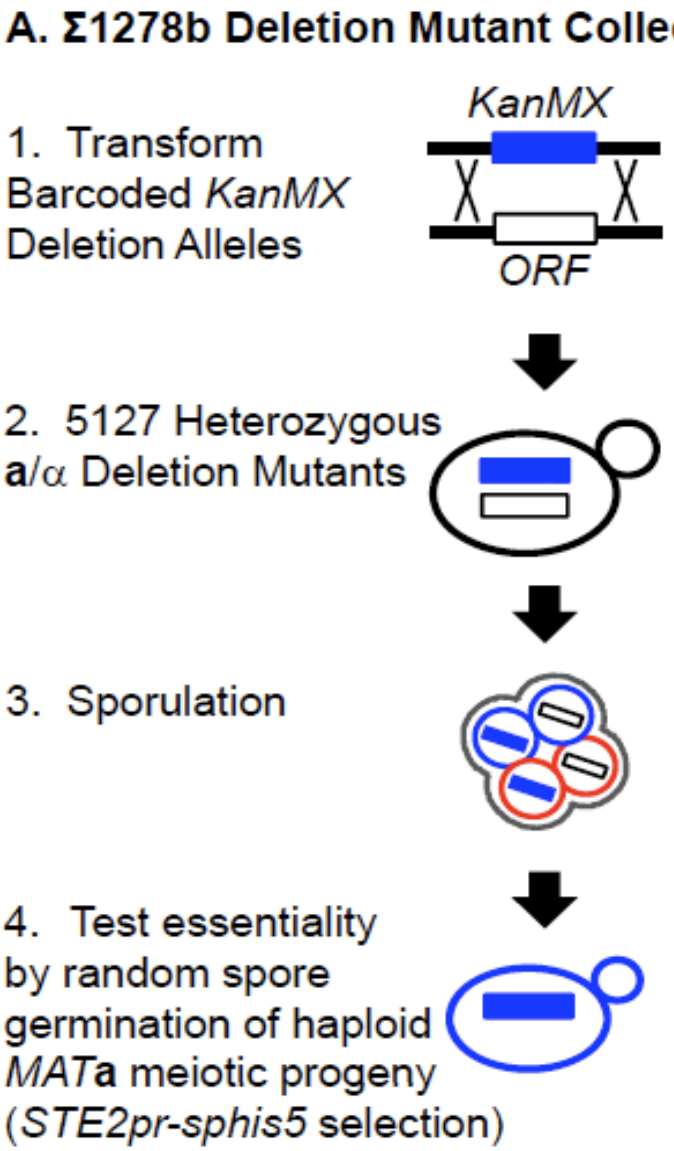
- **Strains Mate, Meiotic Progeny are Viable**

- **G. Fink, R Dowell, and D. Gifford (MIT) sequenced Σ1278b.**

~0.2% natural variation between strains



Σ1278b Deletion Mutant Collection
Exploring Conditional or Background-specific Phenotypes



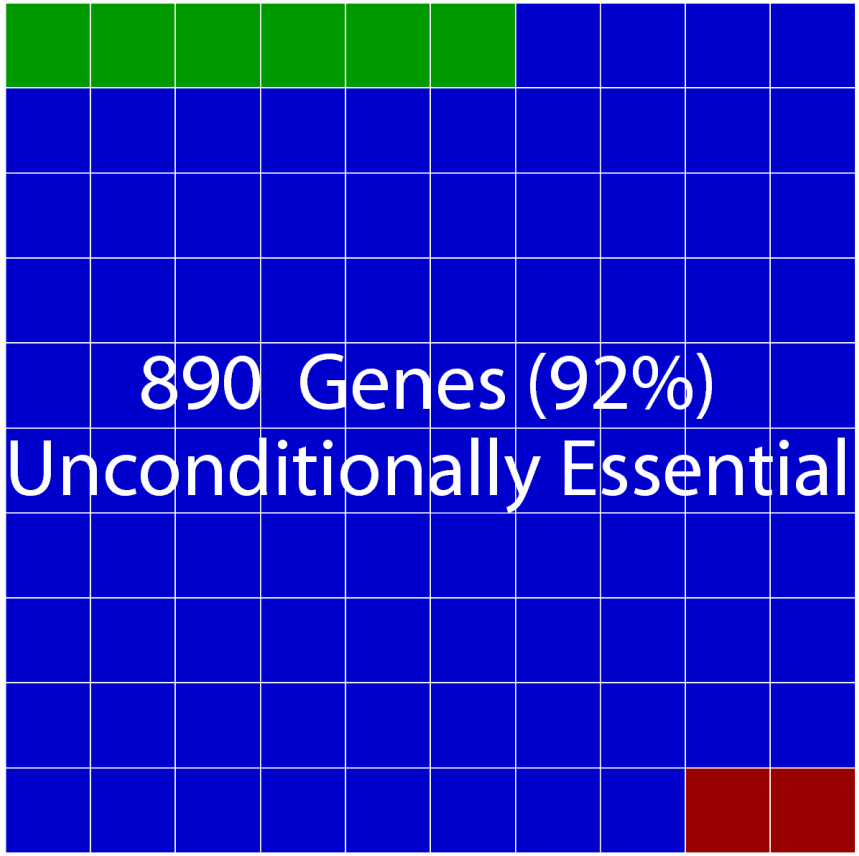
Owen Ryan

Deletion Mutant Analysis in Σ1278b should reveal two kinds of essential genes:

1. Unconditional Essentials, those shared by S288c
2. Conditional Essentials, those specific to either Σ1278b or S288c

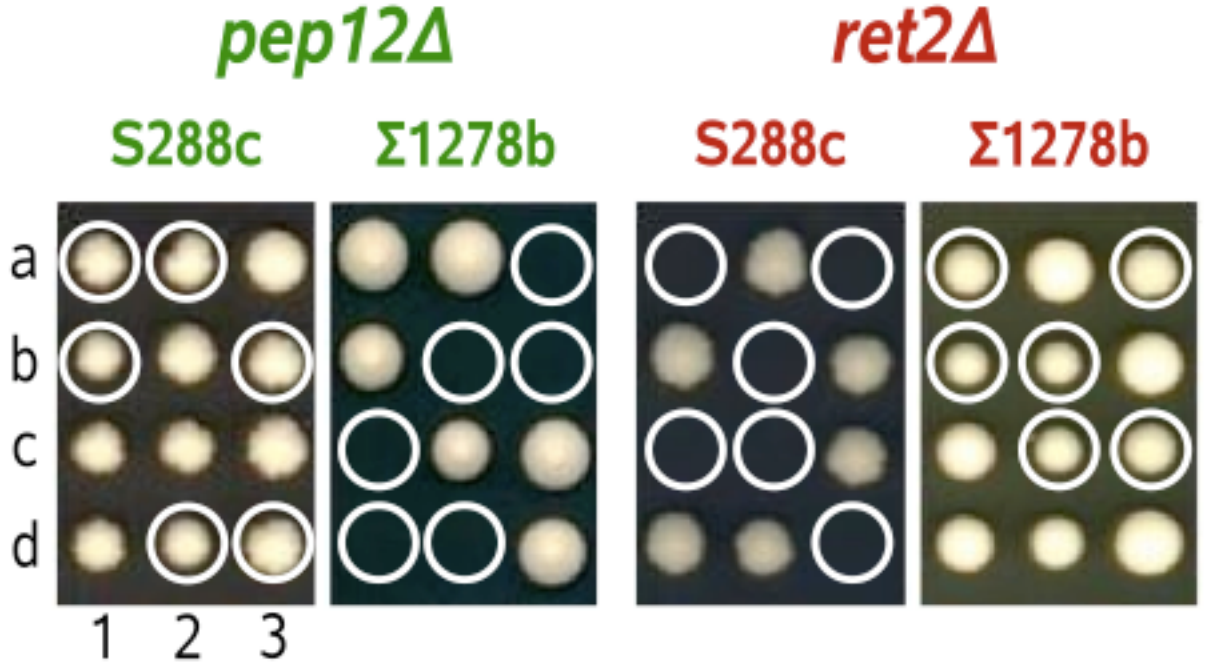
Conditional Loci may identify background-specific polymorphisms that cause synthetic lethality.

55 Genes (6%)
Σ1278b Conditionally Essential



20 Genes (2%)
S288c Conditionally Essential

Conditional Essential Genes:
Genes Required for Life in One Individual but not the Other

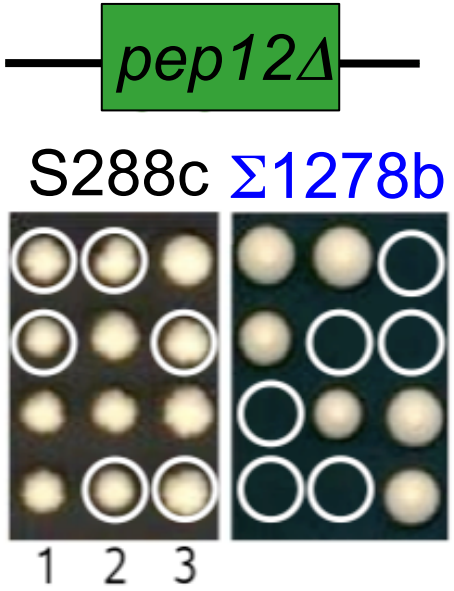


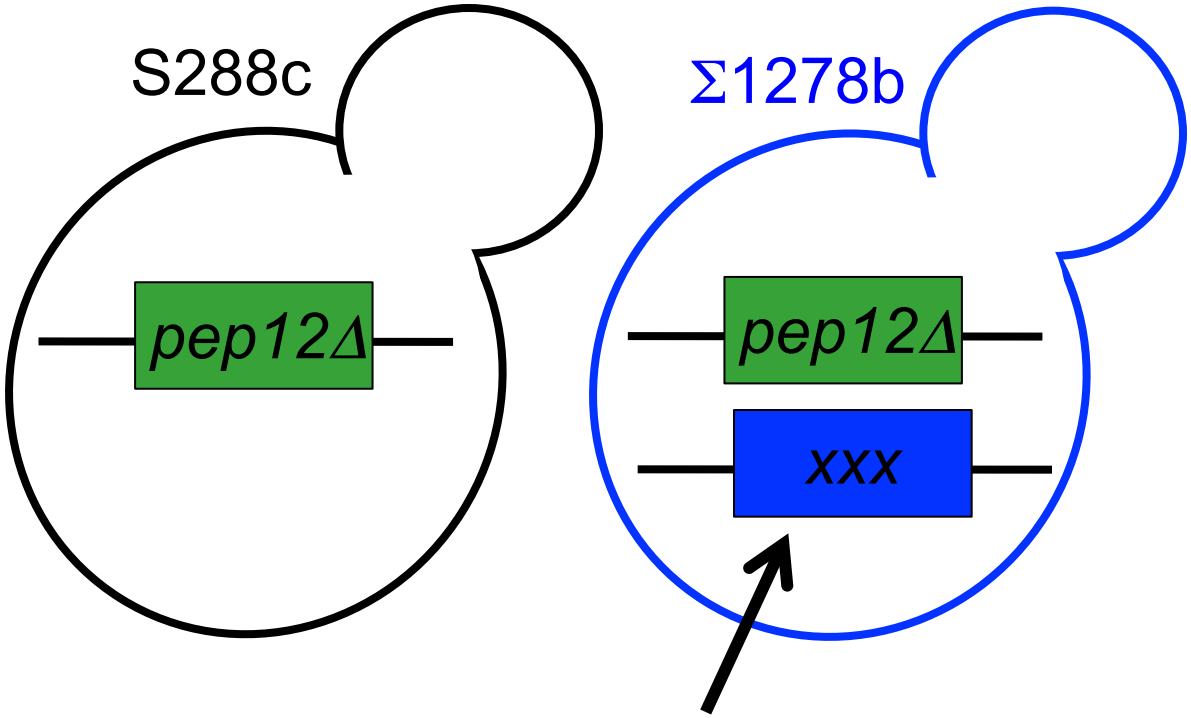
Tetrad dissection: 4 spores (a,b, c, and d) from a single meiosis are separated and allowed to grow into colonies. In this experiment, 2 different diploid genotypes were put through meiosis, *PEP12*/*pep12* in one case and *RET2*/*ret2* in the other.

Circles indicate the segregants carrying the deletion allele

Mechanism of Conditional Lethality

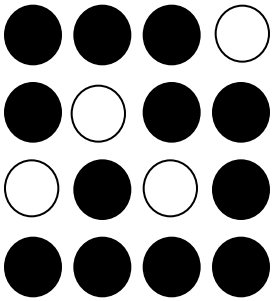
Maybe Σ1278b Carries a Modifier Locus that Leads to Classic Digenic Synthetic Lethality with a *pep12Δ* Mutation



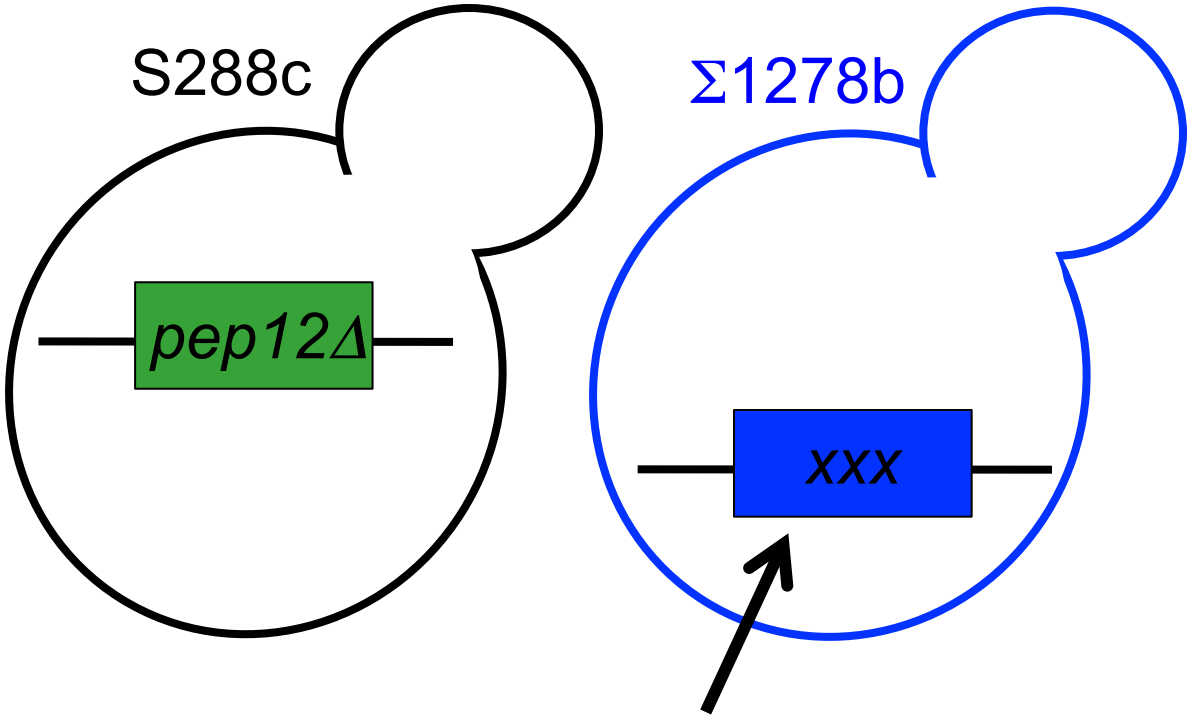


Maybe a Modifier Causes Synthetic Lethality

Testing Number of Modifiers Underlying of Conditional Lethality

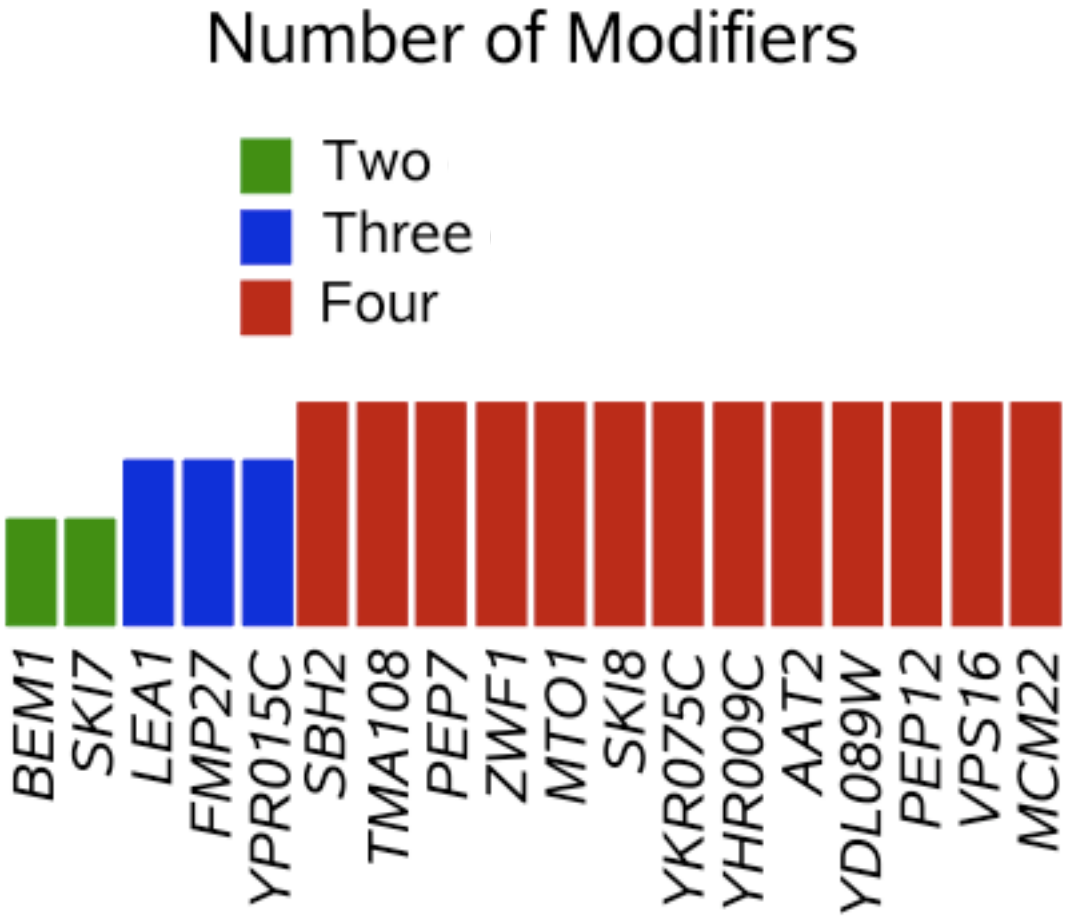


¼ Spores will die with 1 synthetic lethal modifier

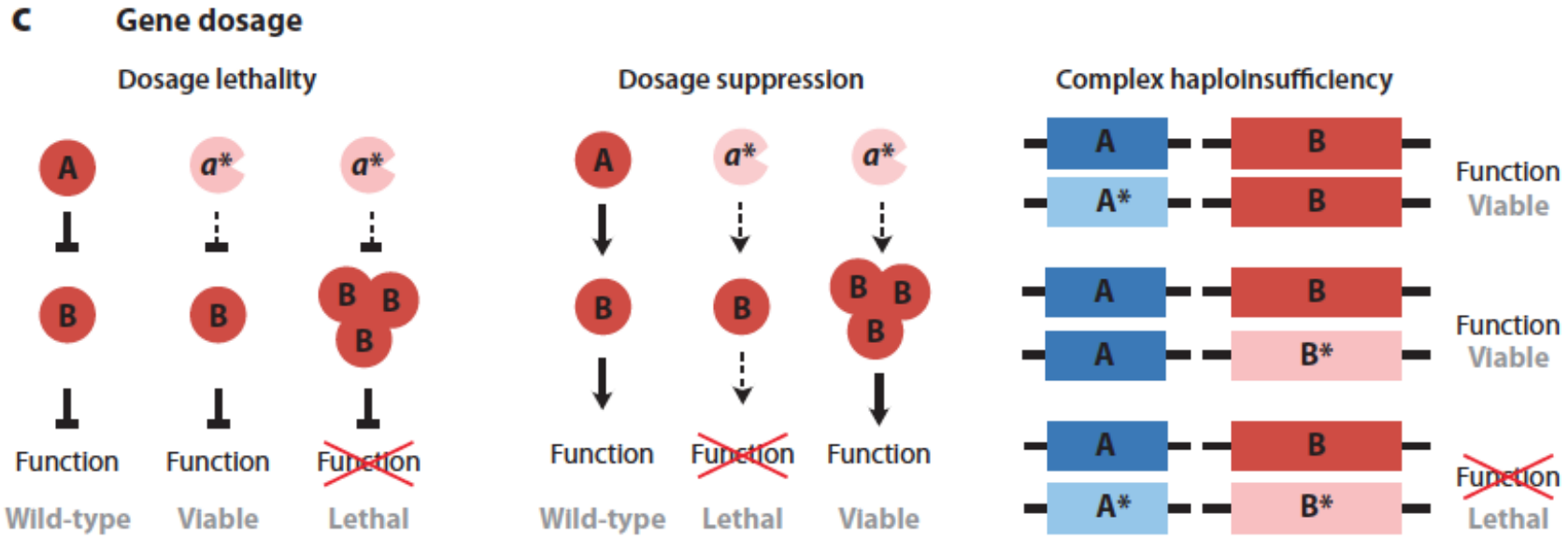


Maybe a Modifier Causes Synthetic Lethality

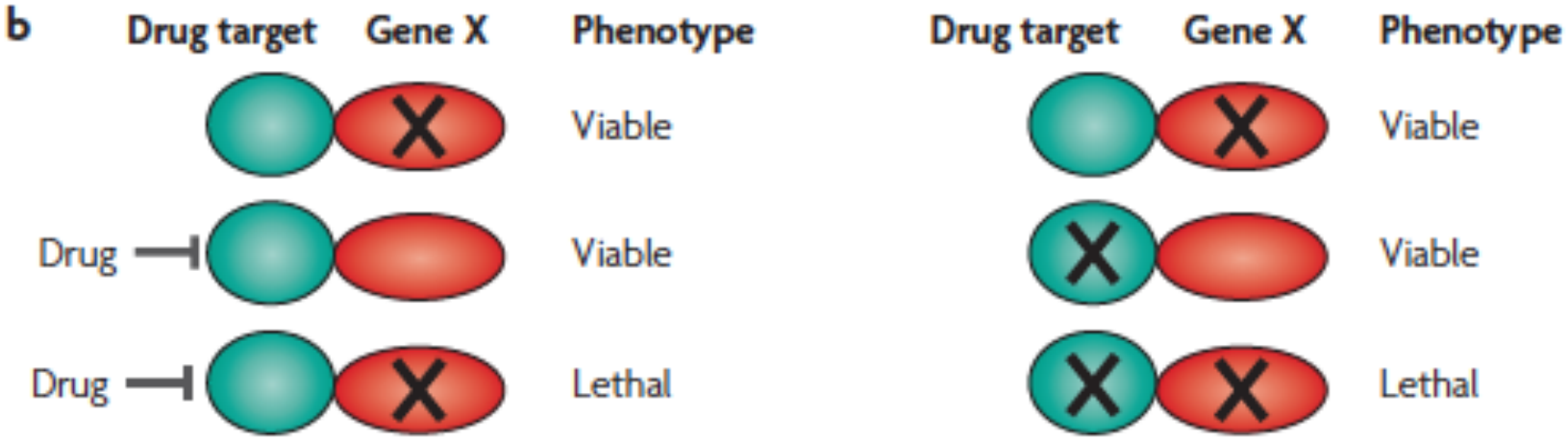
Complex Synthetic Lethality Drives Conditional Essentiality



Variations on the synthetic lethal approach

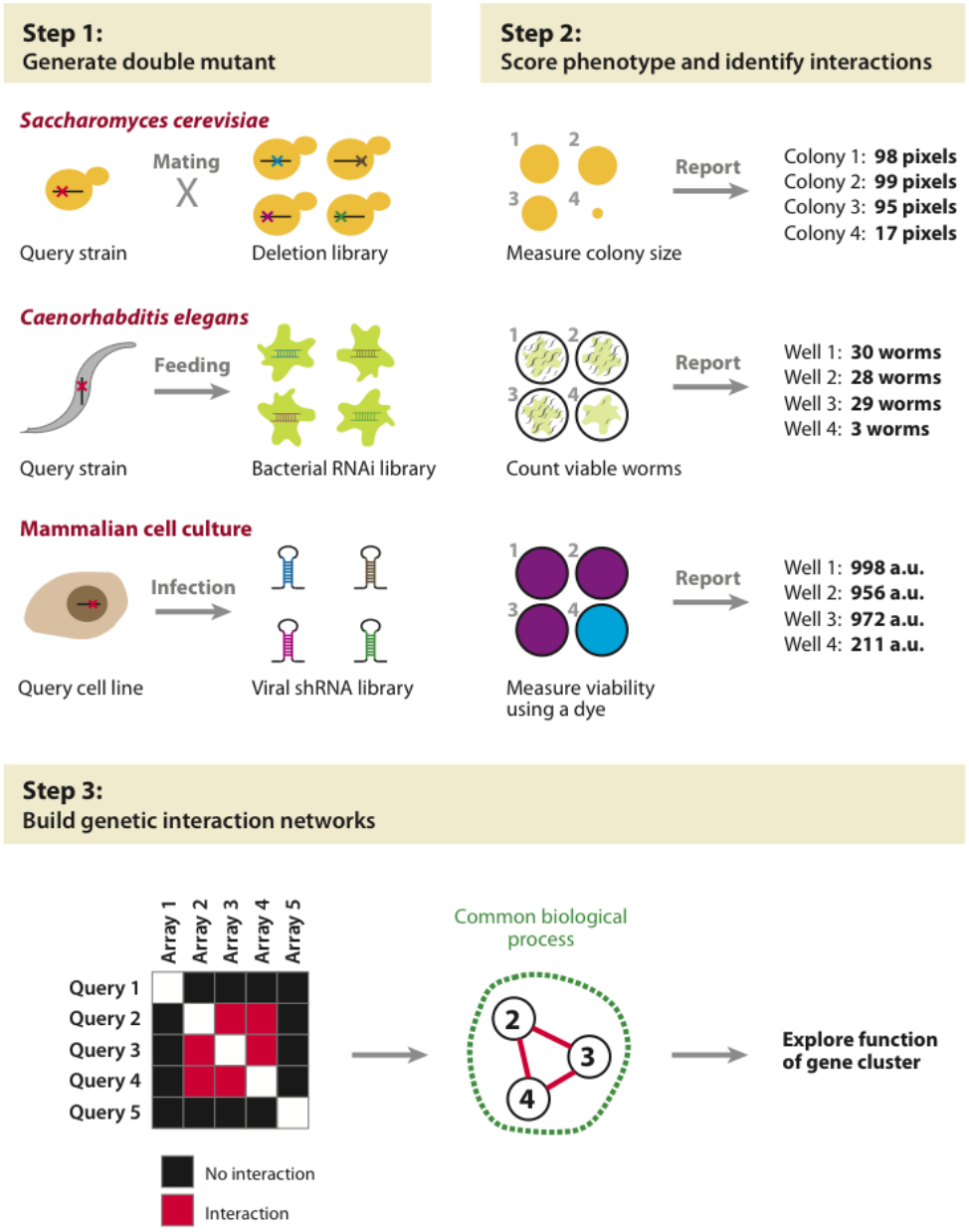


Variations, continued

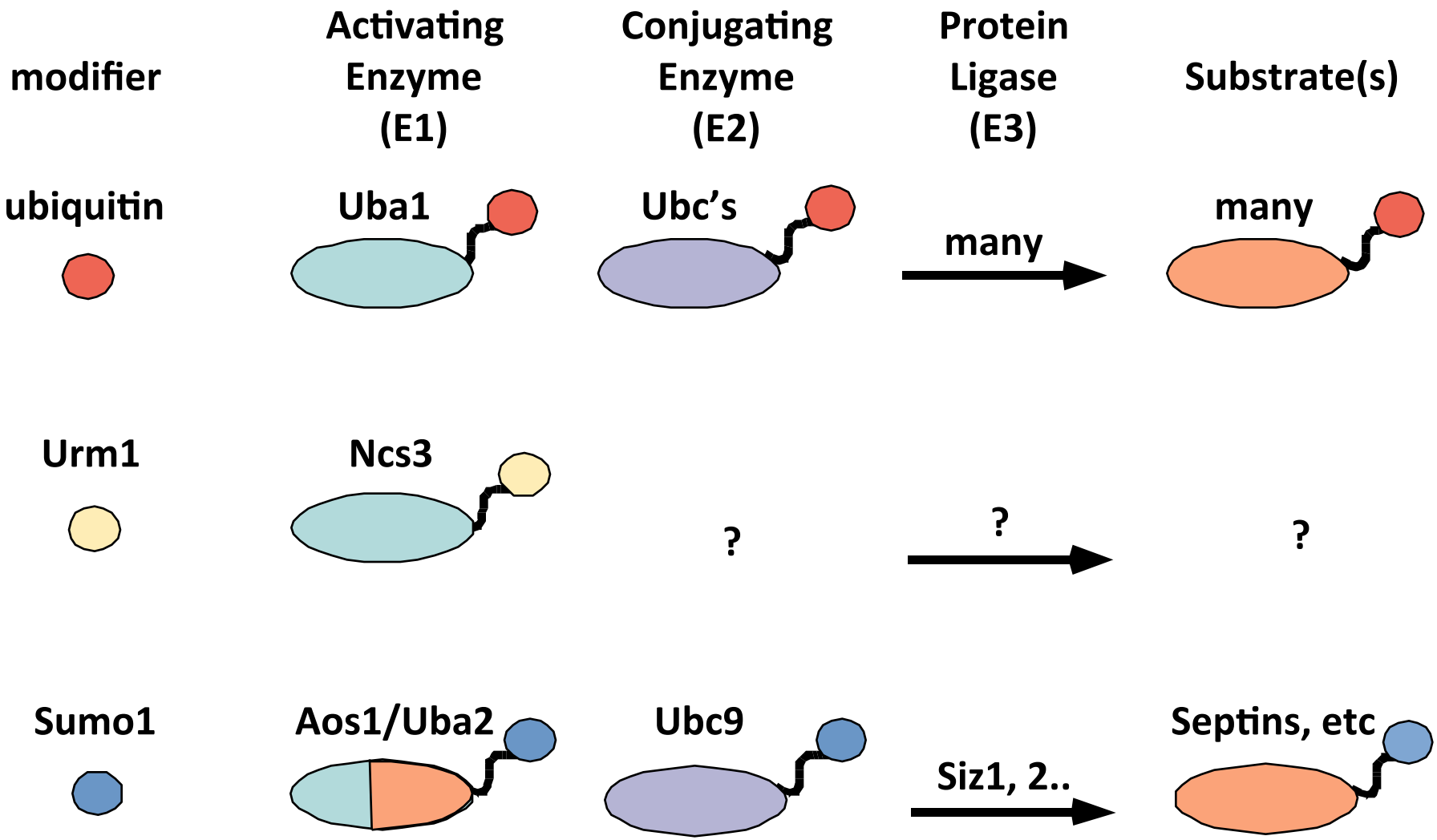


Screen for new drugs or find targets of existing drugs

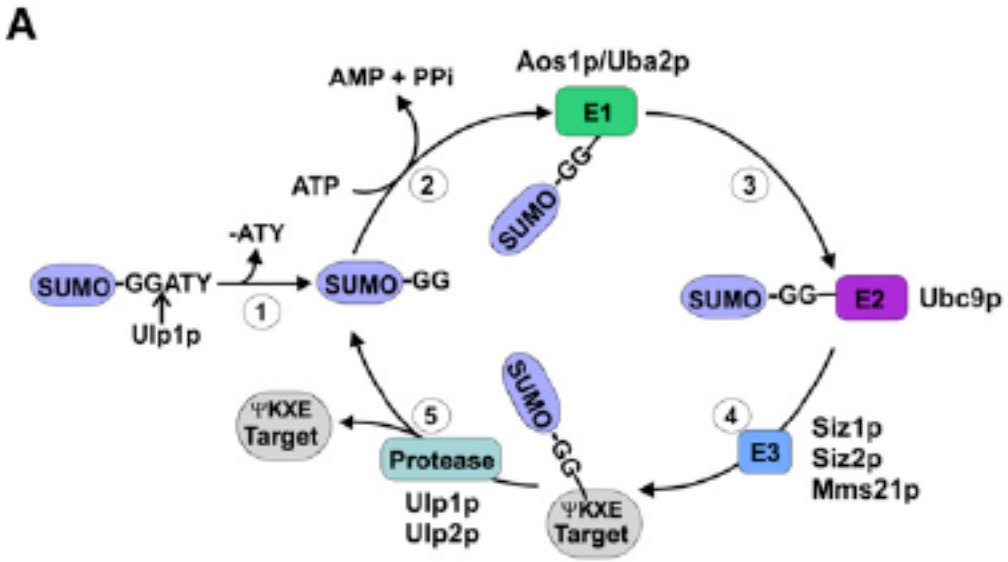
Systematic genetic screens in yeast, worms and mammalian cells

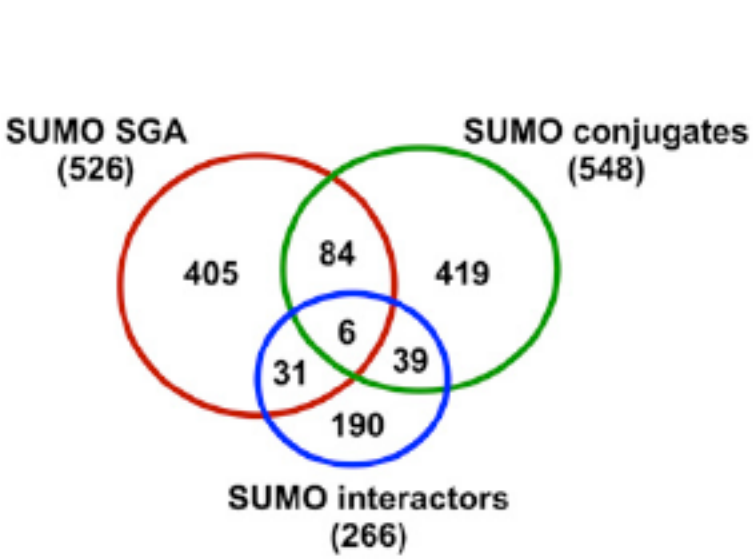


Ubiquitin and Ubiquitin like conjugation pathways

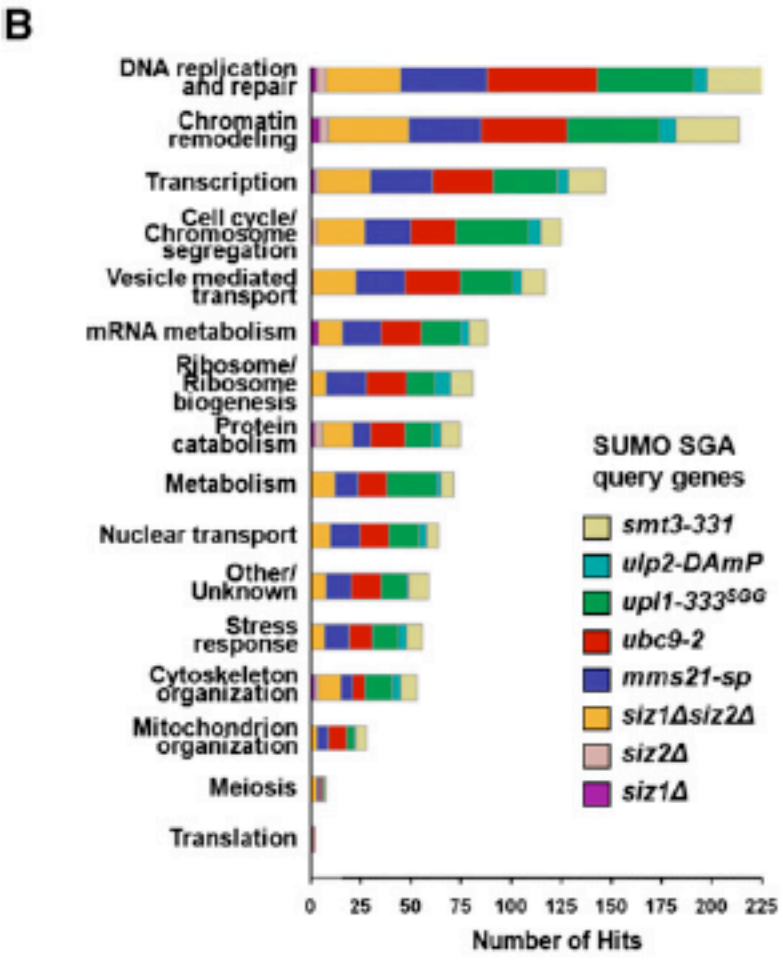


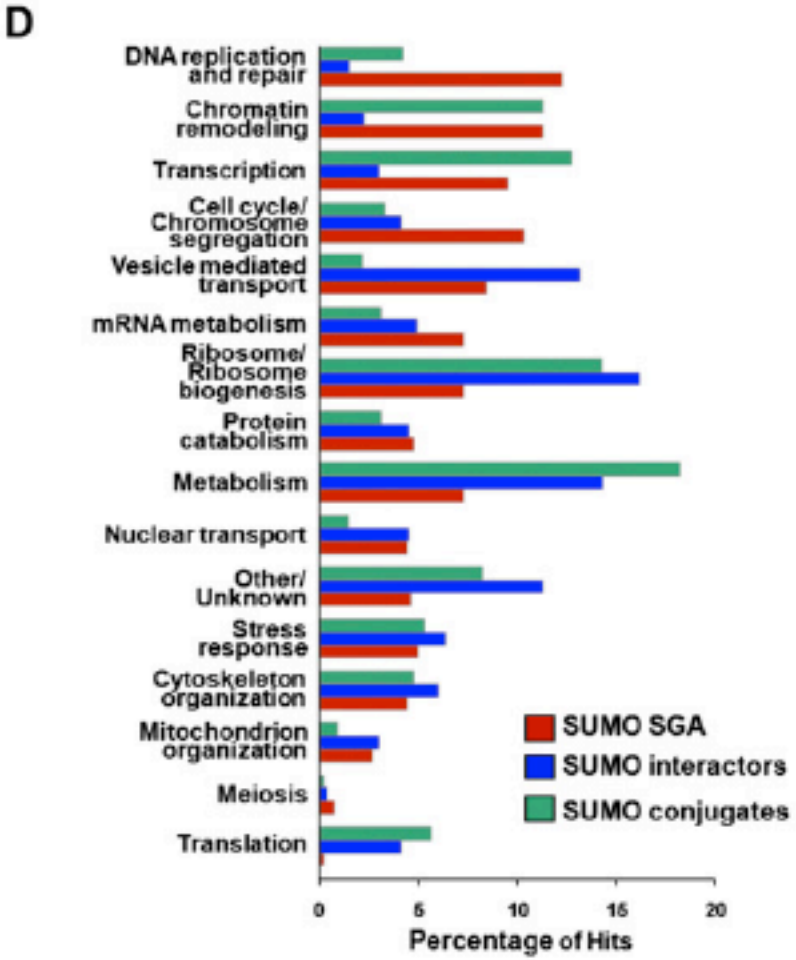
SUMO pathway and “interactors”





SGA analysis using different SUMO pathway mutants yields similar results

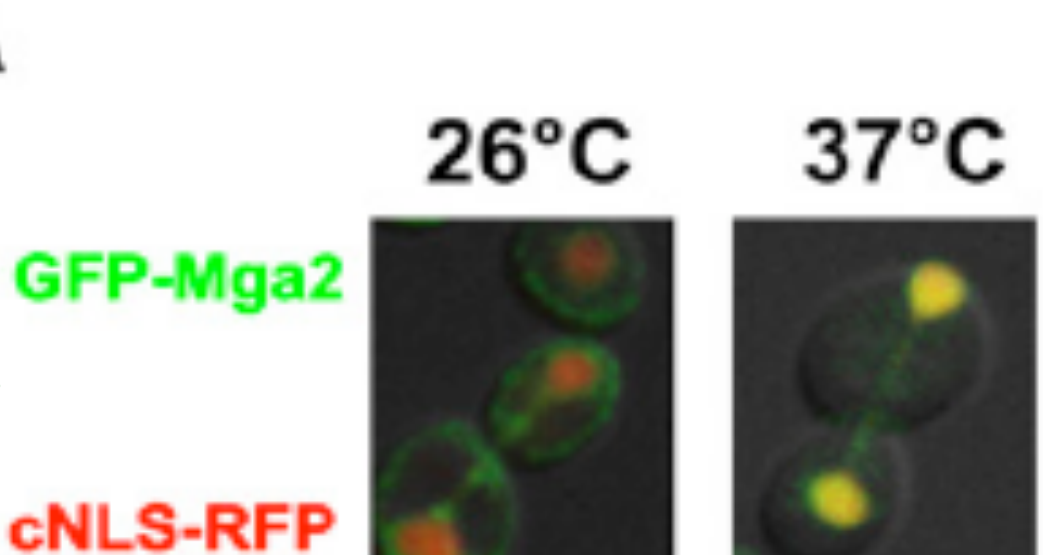




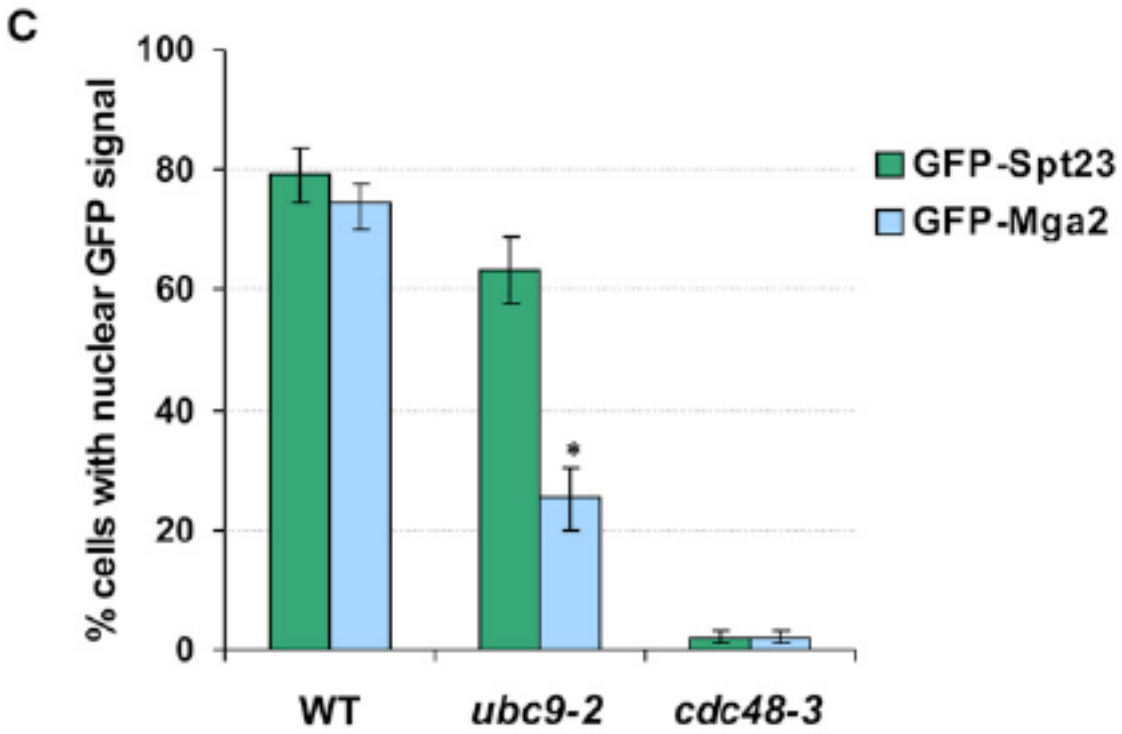
But biological processes are represented differently in the 3 analysis methods

SUMO regulates nuclear localization of a transcription factor

Wildtype cells



Quantitation in wildtype and mutants



Haploinsufficiency and complex haploinsufficiency

Haploinsufficiency

In a diploid, “wildtype” allele on one chromosome and loss-of-function allele on the other results in a phenotype

Hence, haploinsufficiency is a kind of dominance. How would you distinguish this sort of dominant allele from a gain-of-function dominant allele?

Complex Haploinsufficiency

In a diploid, loss-of-function alleles at two (or more) different loci, eg GENEX+/genex- GENEY+/geney-, confers a phenotype whereas neither single loss-of-function allele does. The thinking is that complex haploinsufficiency may be akin to the genetics of disease susceptibility in humans

Actin has many roles in the cell

Membrane trafficking

Polarized cell growth

Cytokinesis

Organelle positioning and segregation

Nuclear structure

More

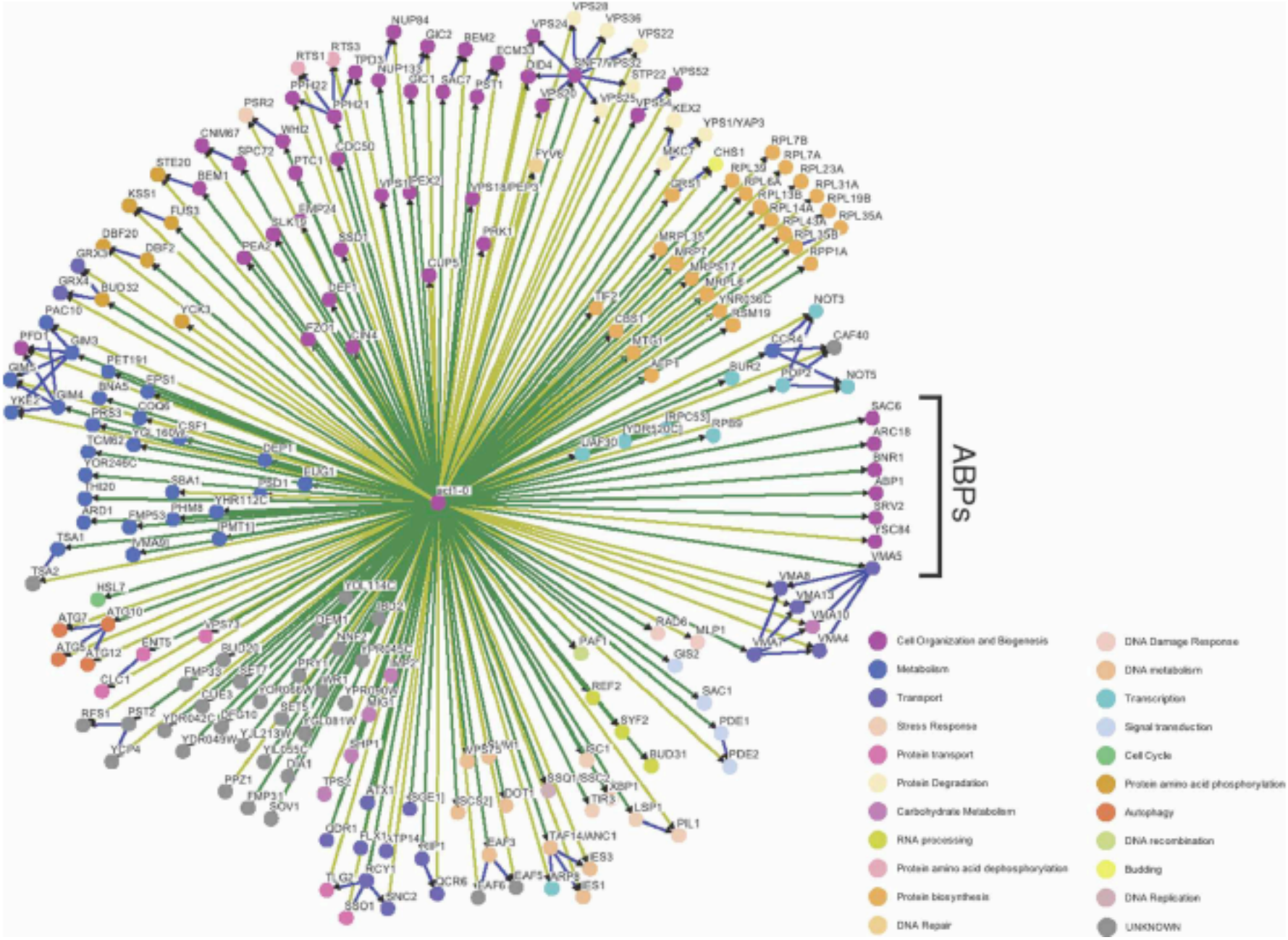
Complex Haploinsufficiency Tests with Actin

act1Δ strain
Carrying plasmid-borne wildtype *ACT1* gene

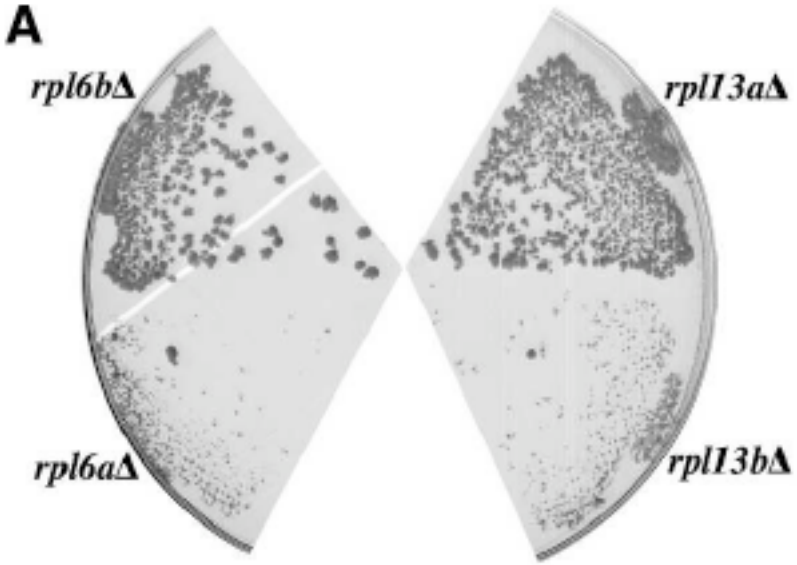
Mate to deletion collection of nonessential genes and select diploids

Determine whether each resulting diploid strain can lose the plasmid. If not, a CHI between actin ands the corresponding gene in the deletion array is suggested

Actin shows complex haploinsufficiency with many nonessential genes



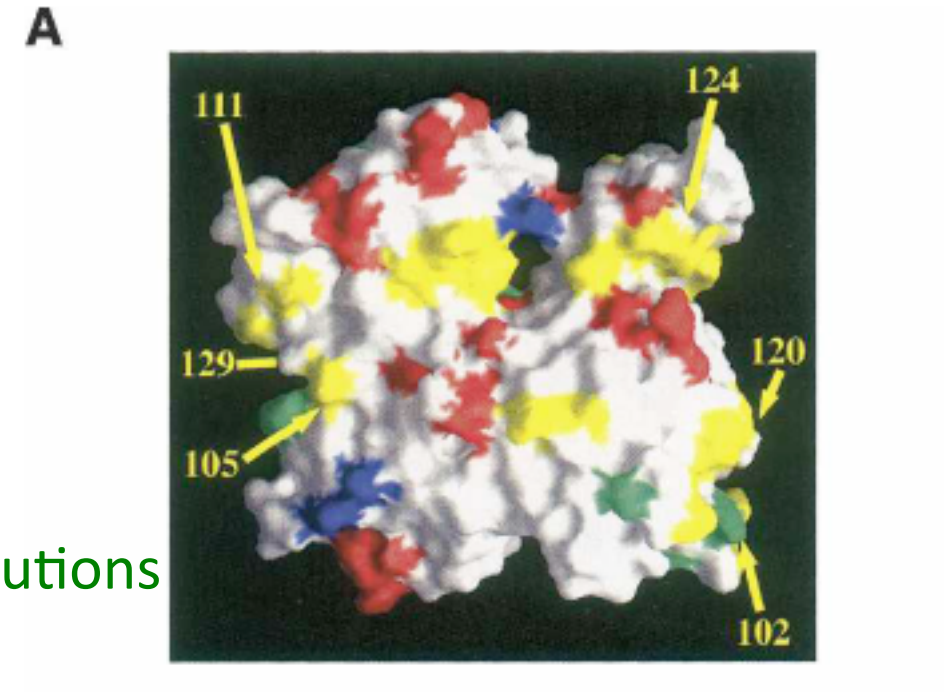
Ribosome large subunit paralogs show different interactions with actin



Genotypes: heterozygous for deletion of *ACT1* and for each of the indicated genes

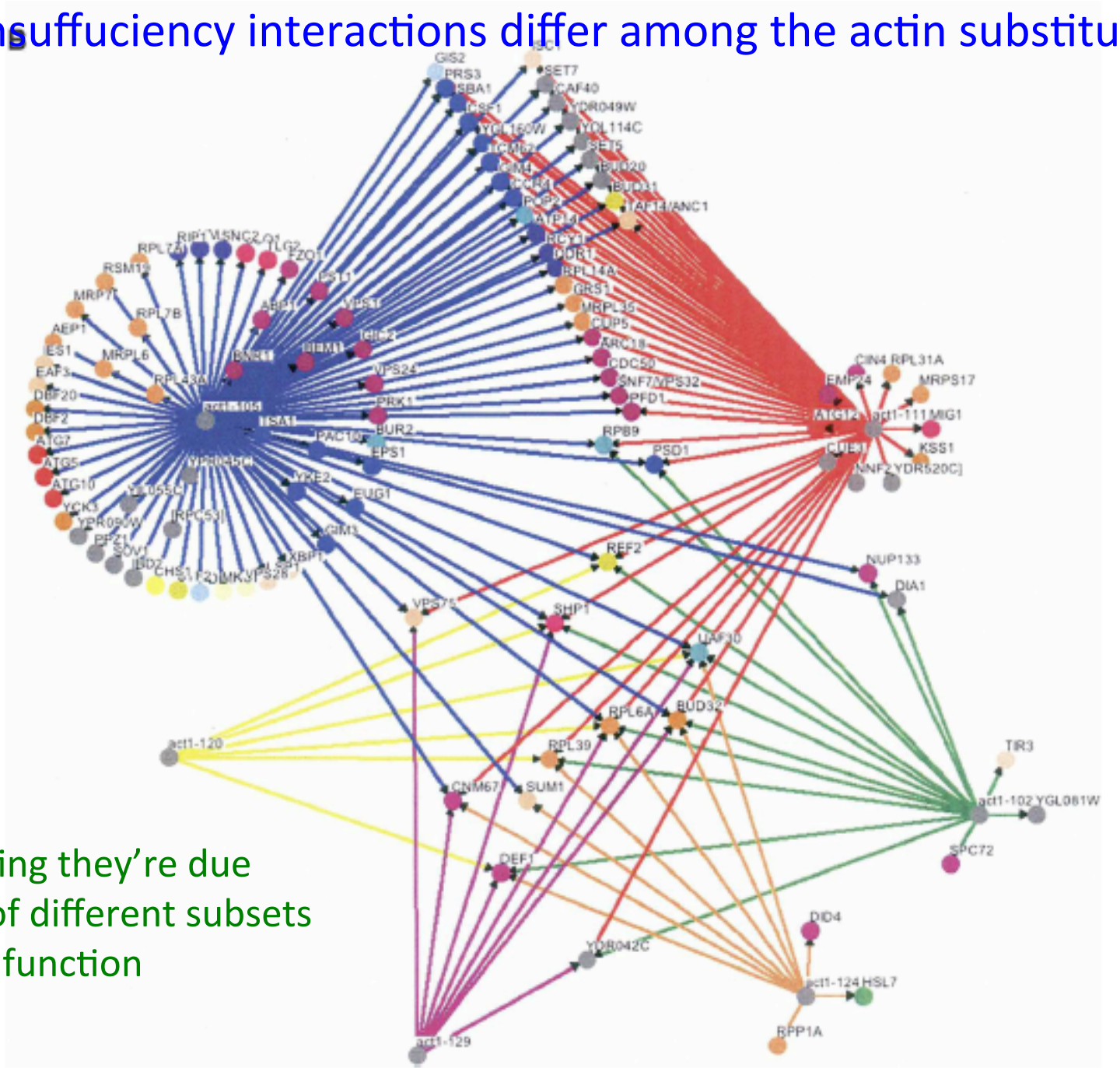
Positions of actin substitutions used in haploinsufficiency analysis

Numbers indicate position of substitutions tested



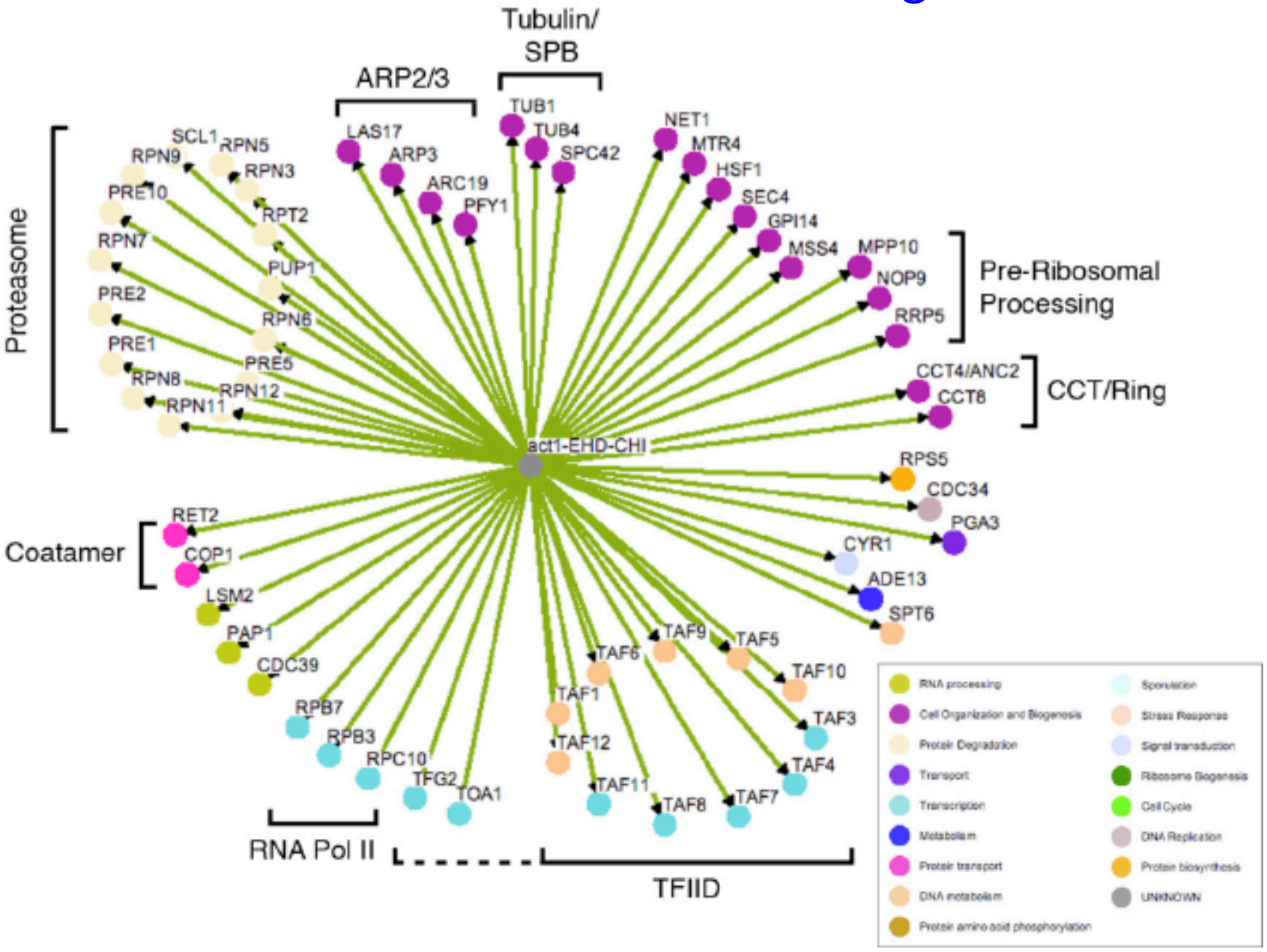
Colors indicate growth phenotype of substitutions in that region.
Green – wildtype; yellow – conditional;
Red - lethal

Haploinsuffuciency interactions differ among the actin substitutions

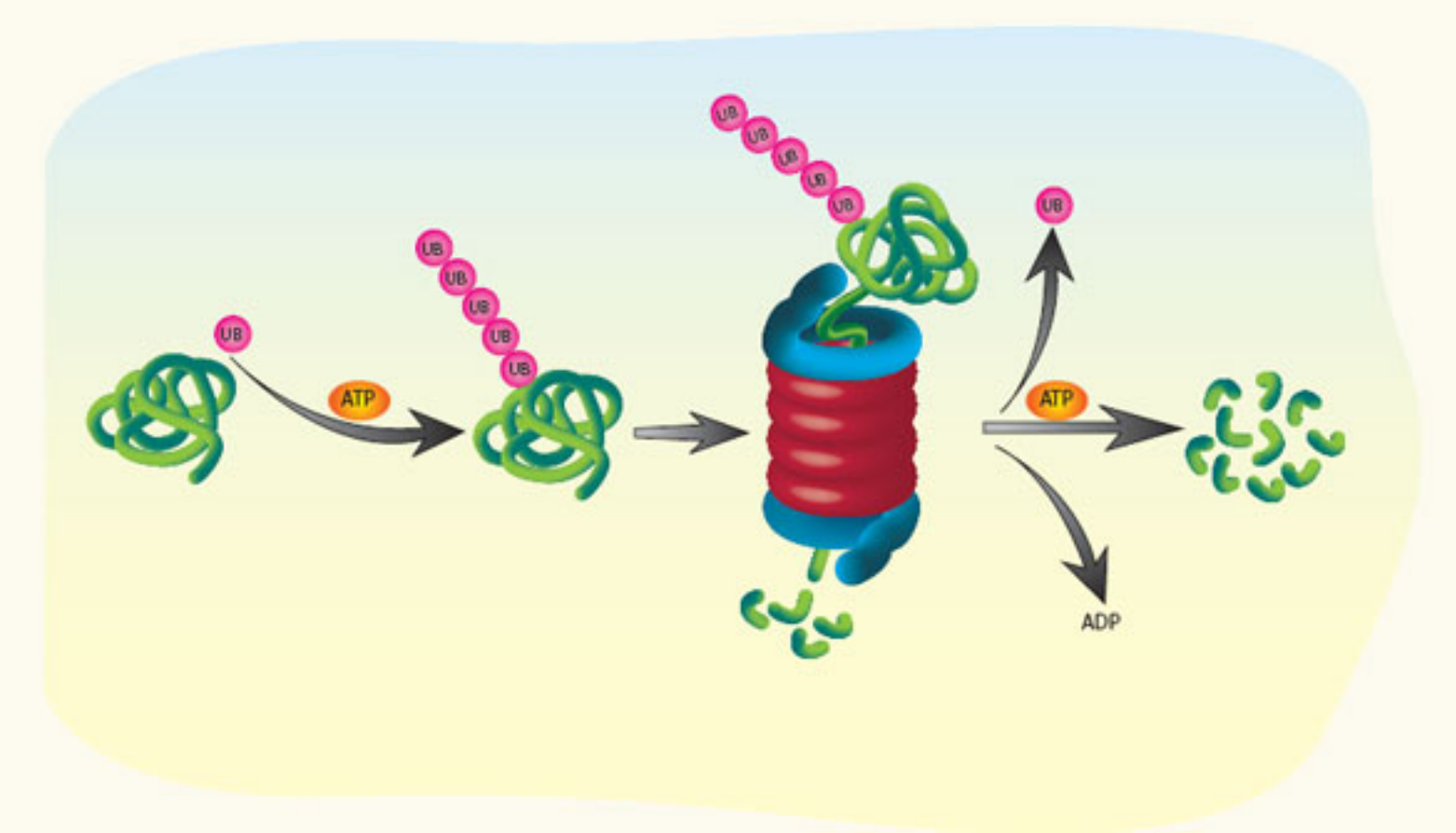


Suggesting they're due
to loss of different subsets
of actin function

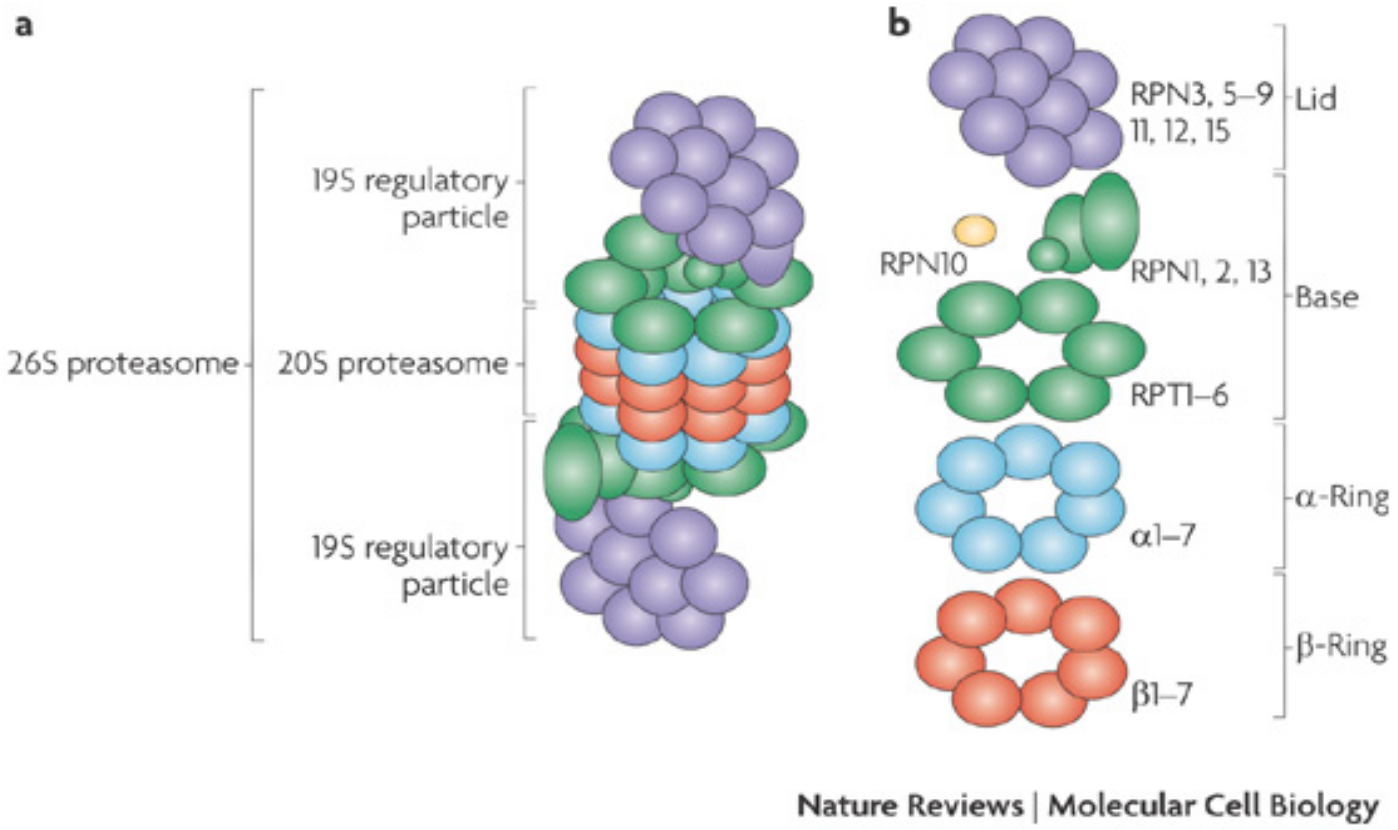
CHI with actin and essential genes



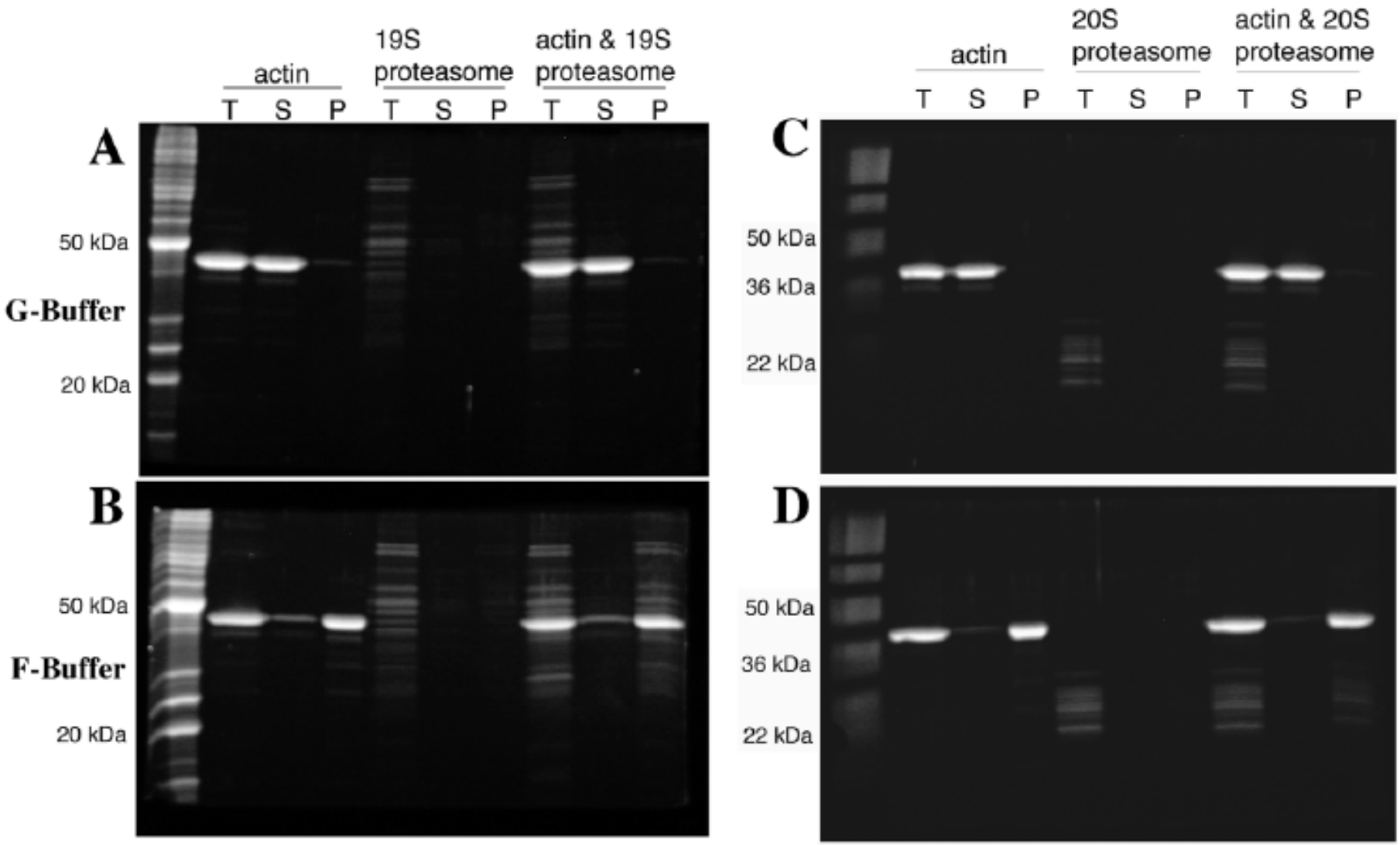
The proteasome and ubiquitin-dependent protein degradation

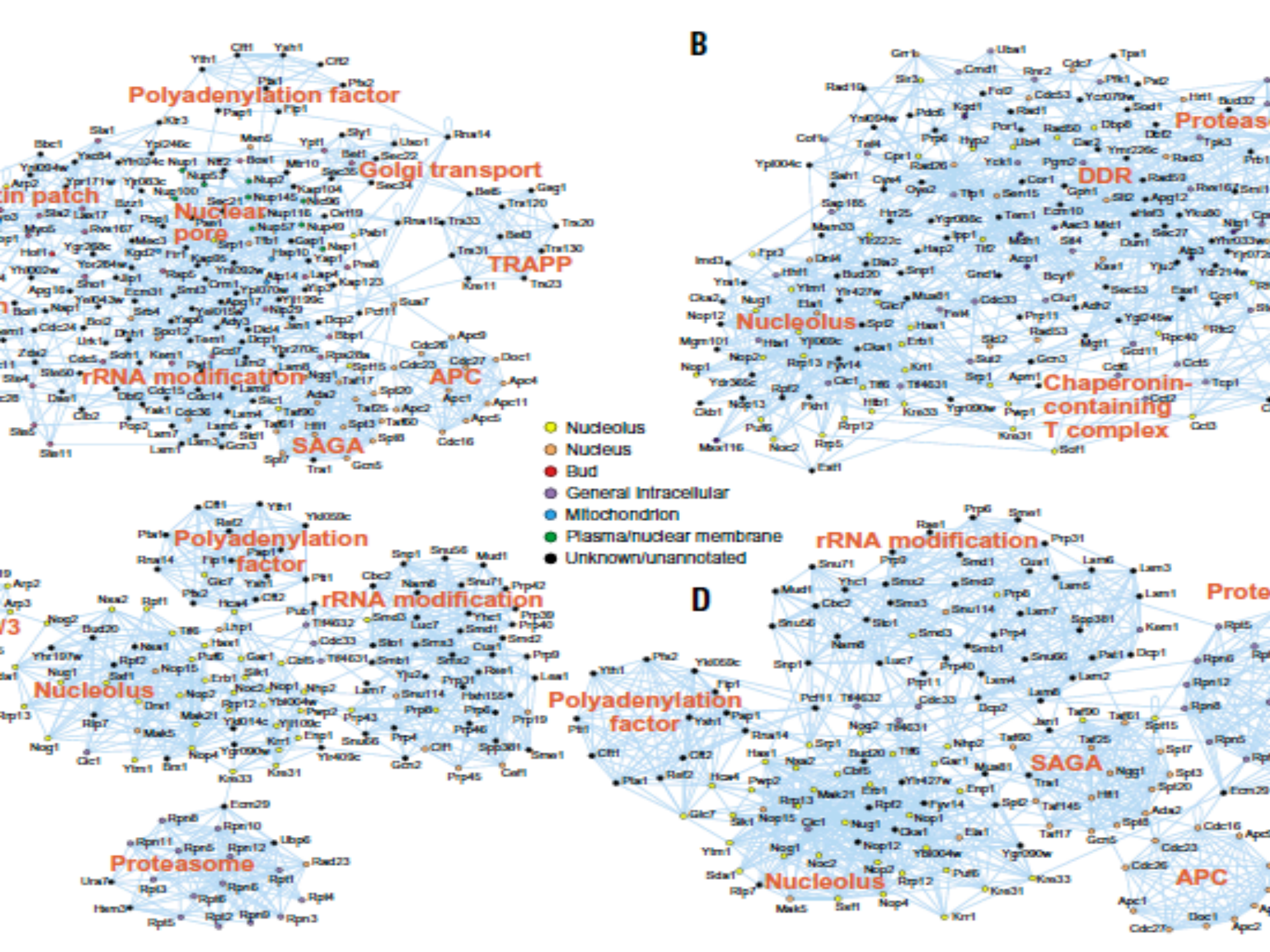


The proteasome consists of two subparticles, 19S and 20S



Actin associates with the proteasome




B
D
Polyadenylation factor
Golgi transport
Nuclear pore
TRAPP
rRNA modification
APC
SAGA
Nucleolus
Nucleus
Bud
General Intracellular
Mitochondrion
Plasma/nuclear membrane
Unknown/unannotated
DDR
Chaperonin-containing T complex
Proteasome
Polyadenylation factor
rRNA modification
SAGA
Nucleolus
APC