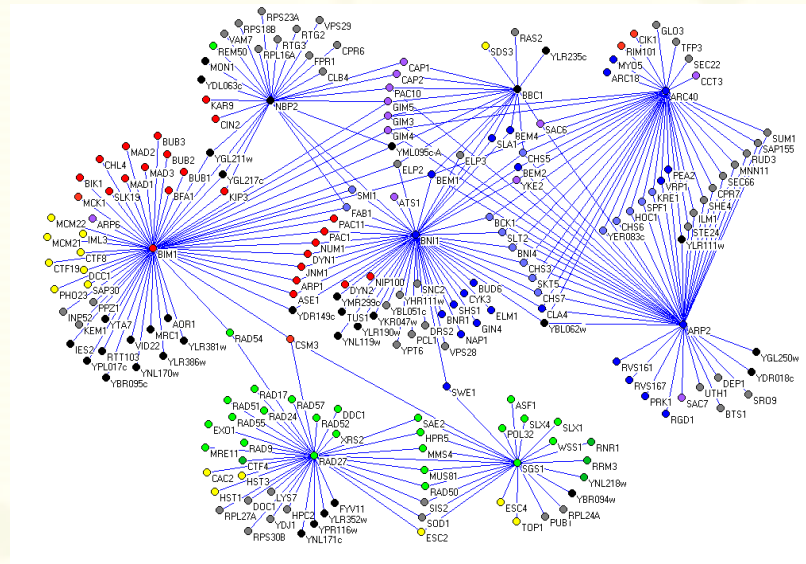


Yeast Genomics: Part III

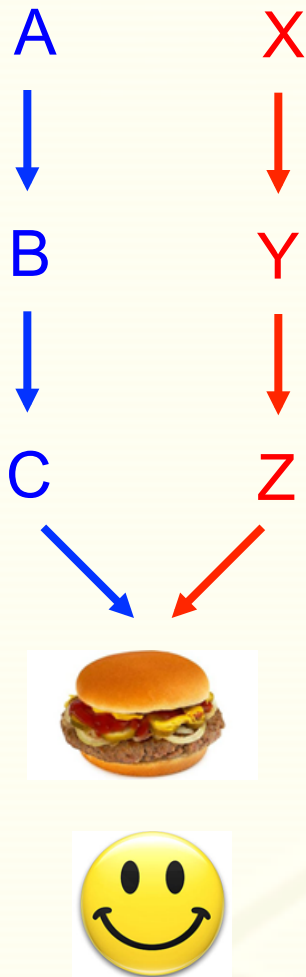
- Now enter the world of genetic interactions



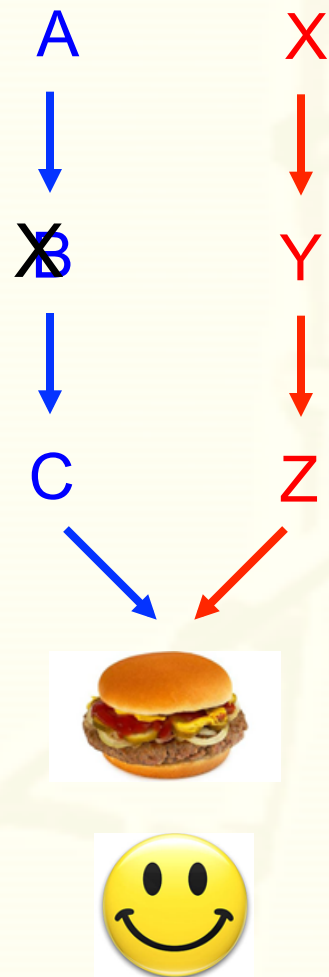
Genetic interactions

- **Epistasis** (genetic interactions): Two mutations have a **genetic interaction** when their combination yields a **surprising phenotype** that cannot be explained simply by the independent effects observed for each mutation alone.
- **Epistasis** effects are also extensively studied in protein folding and RNA folding, in which the effect of the second mutation depends on the first mutation.

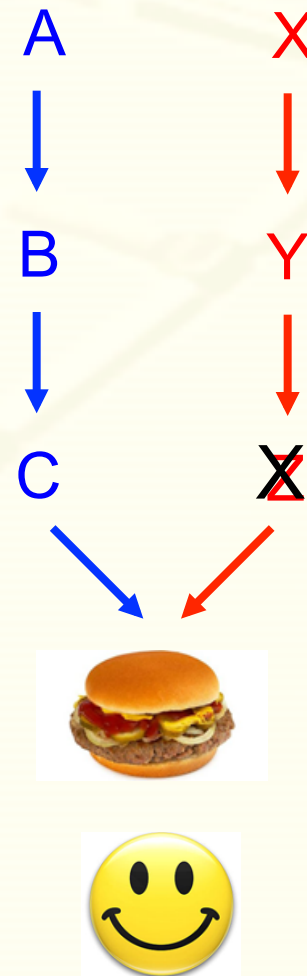
Epistasis in genetic pathways and networks



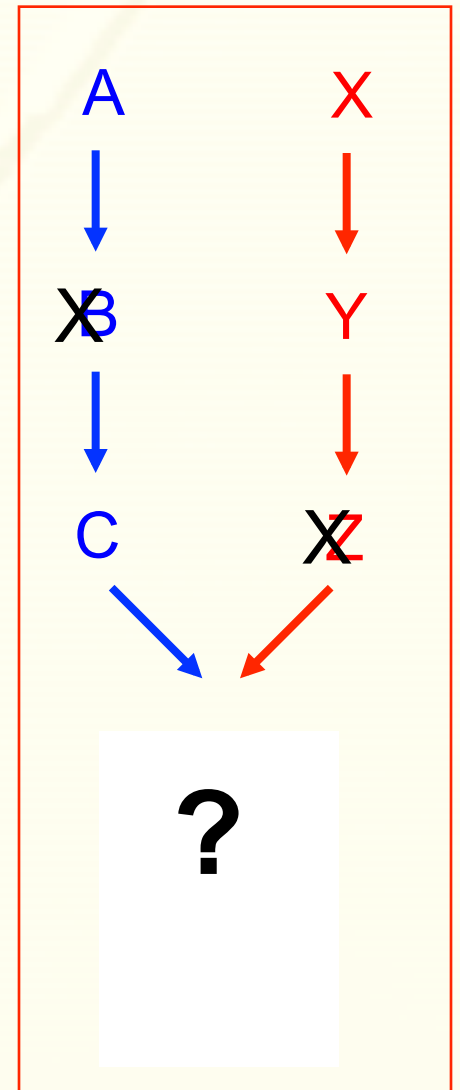
Epistasis in genetic pathways and networks



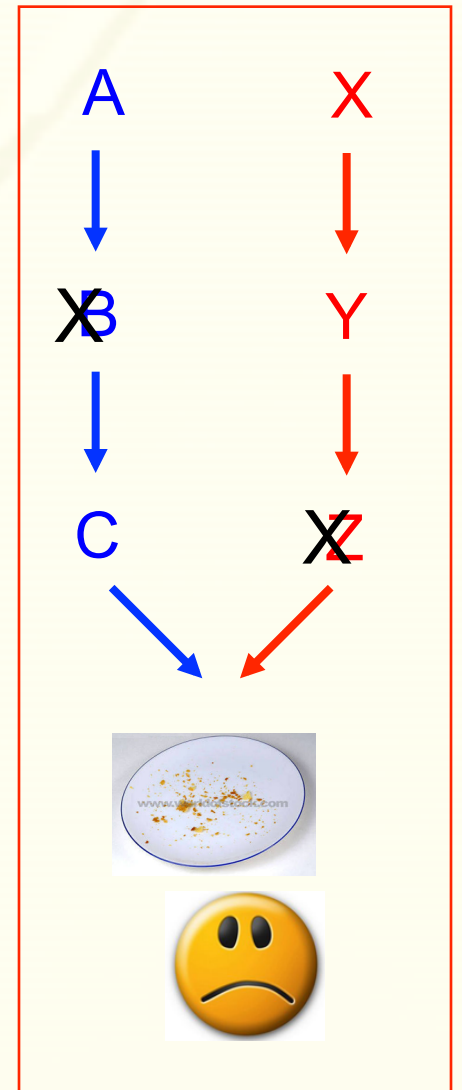
Epistasis in genetic pathways and networks



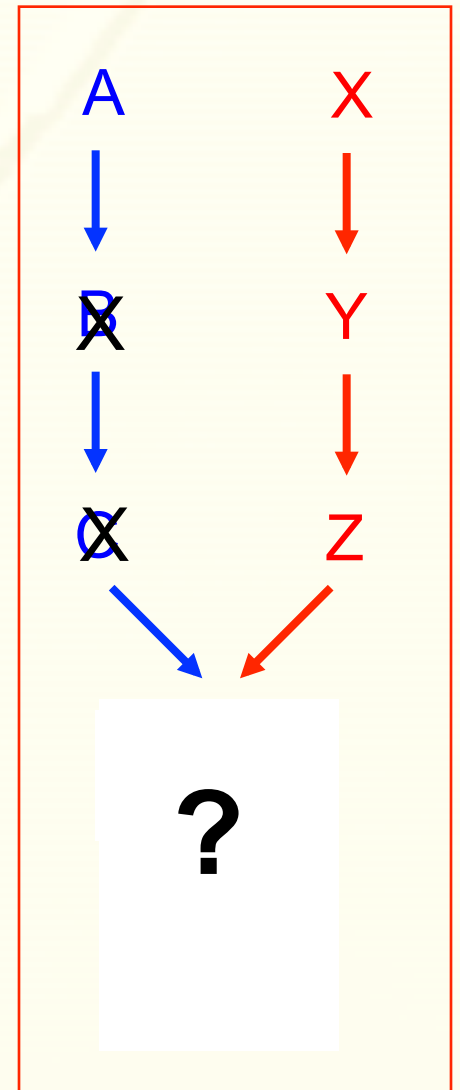
Epistasis in genetic pathways and networks



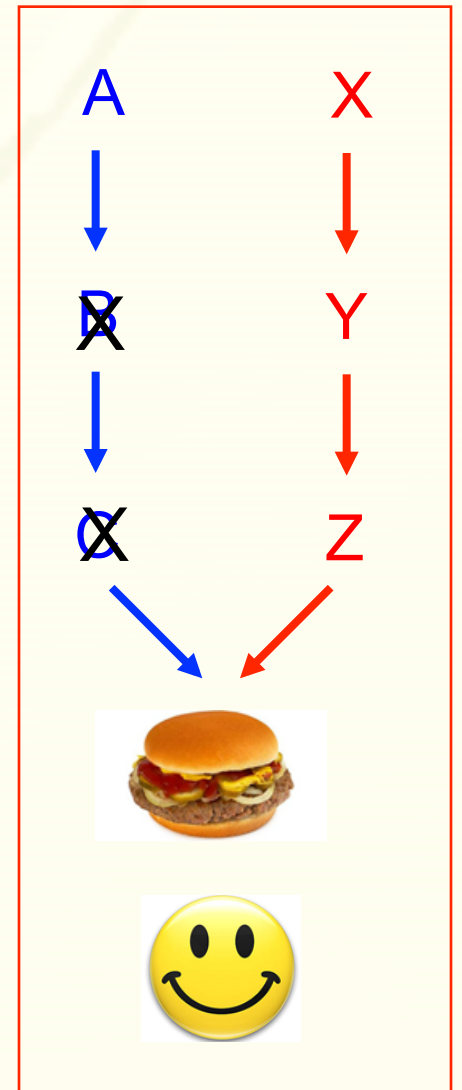
Epistasis in genetic pathways and networks



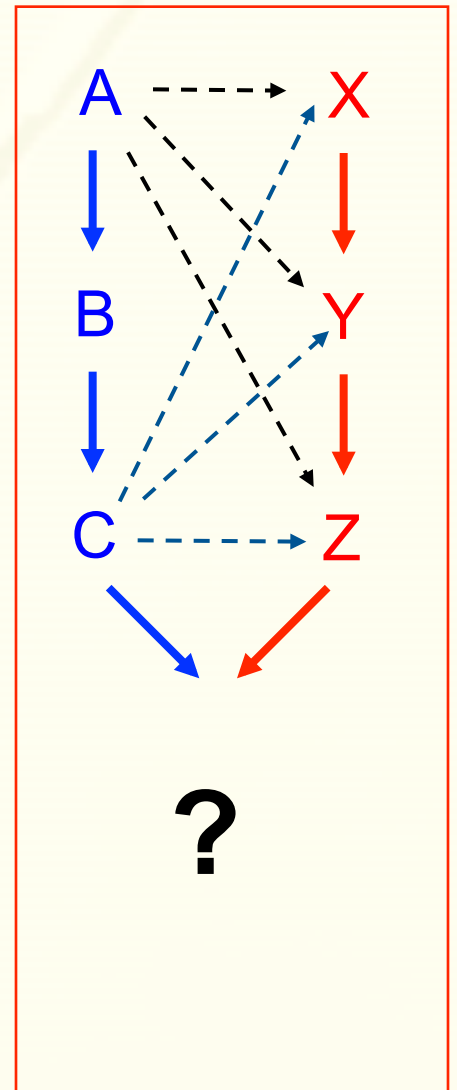
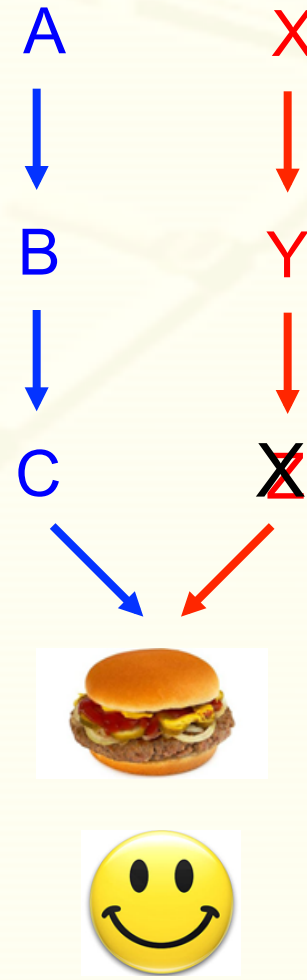
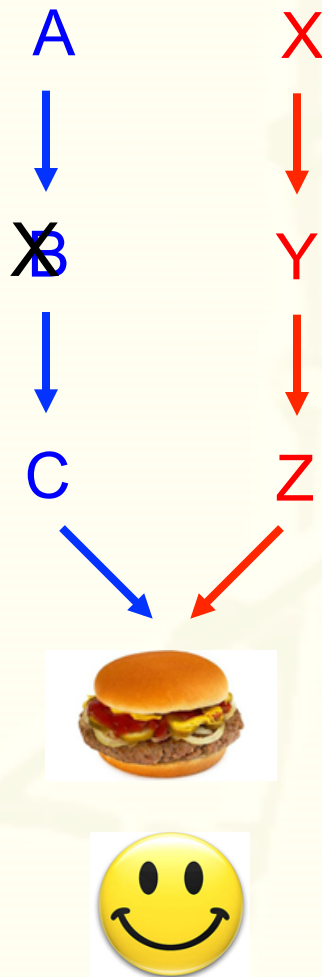
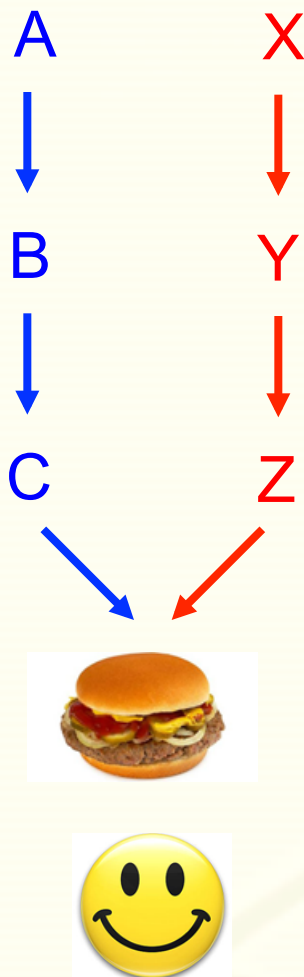
Epistasis in genetic pathways and networks



Epistasis in genetic pathways and networks



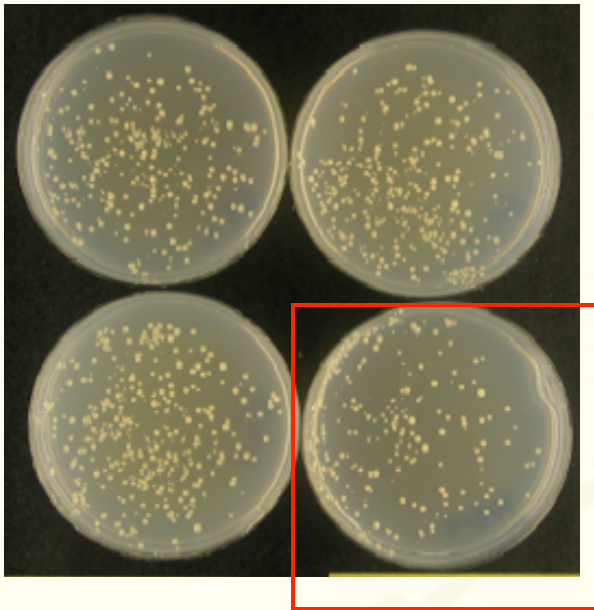
Epistasis in genetic pathways and networks



Low-throughput genetic assay

Control

ΔA



ΔX

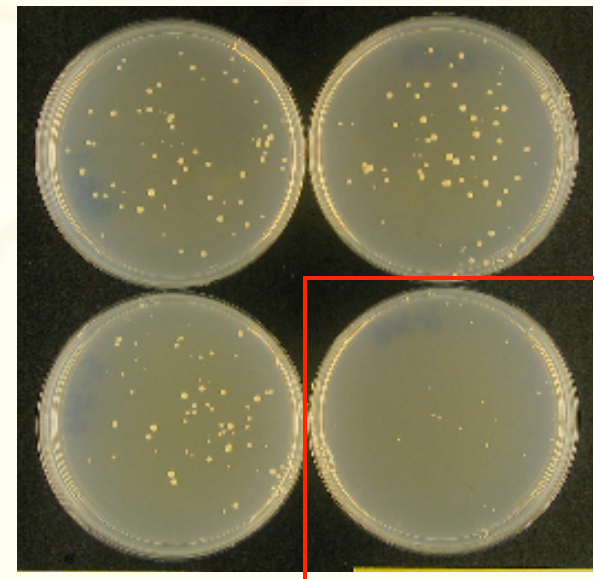
$\Delta A \Delta X$

No Interaction



Control

ΔA



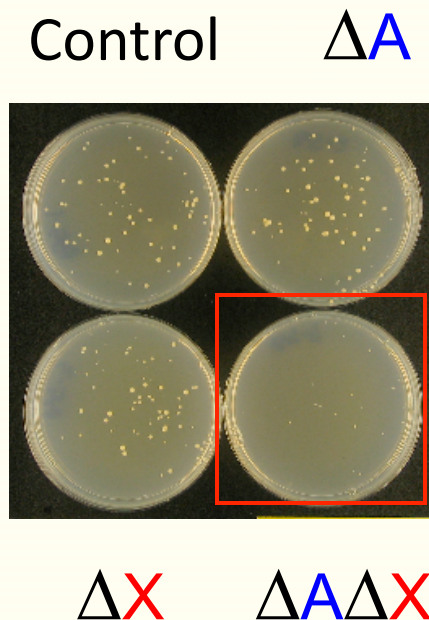
ΔX

$\Delta A \Delta X$

Synthetic Lethal



How to quantify genetic interactions



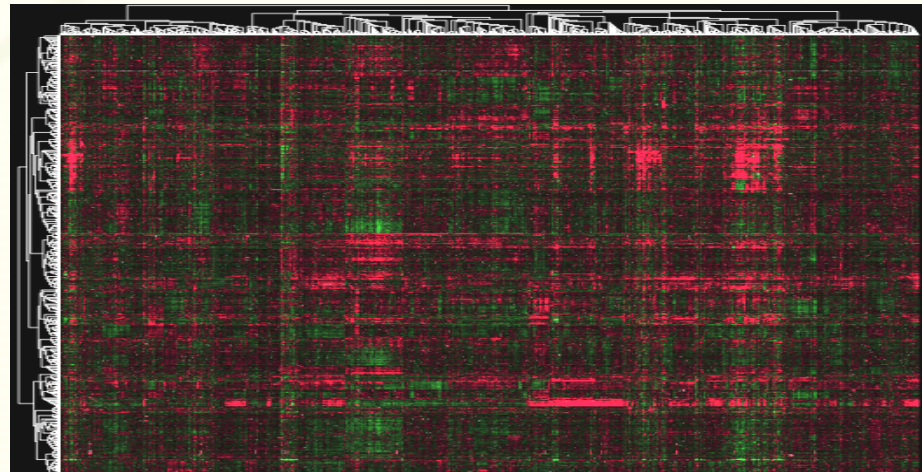
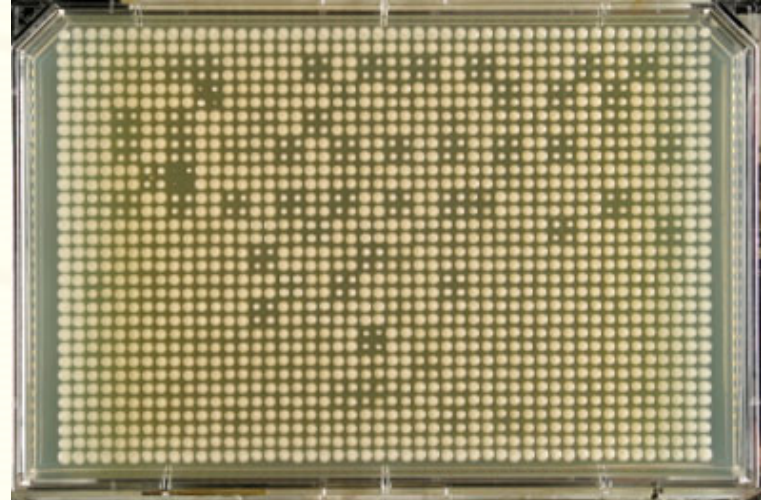
Multiplicative model

$$\varepsilon = ab - a \times b$$

↓ ↓ ↓
double array query
mutant mutant mutant

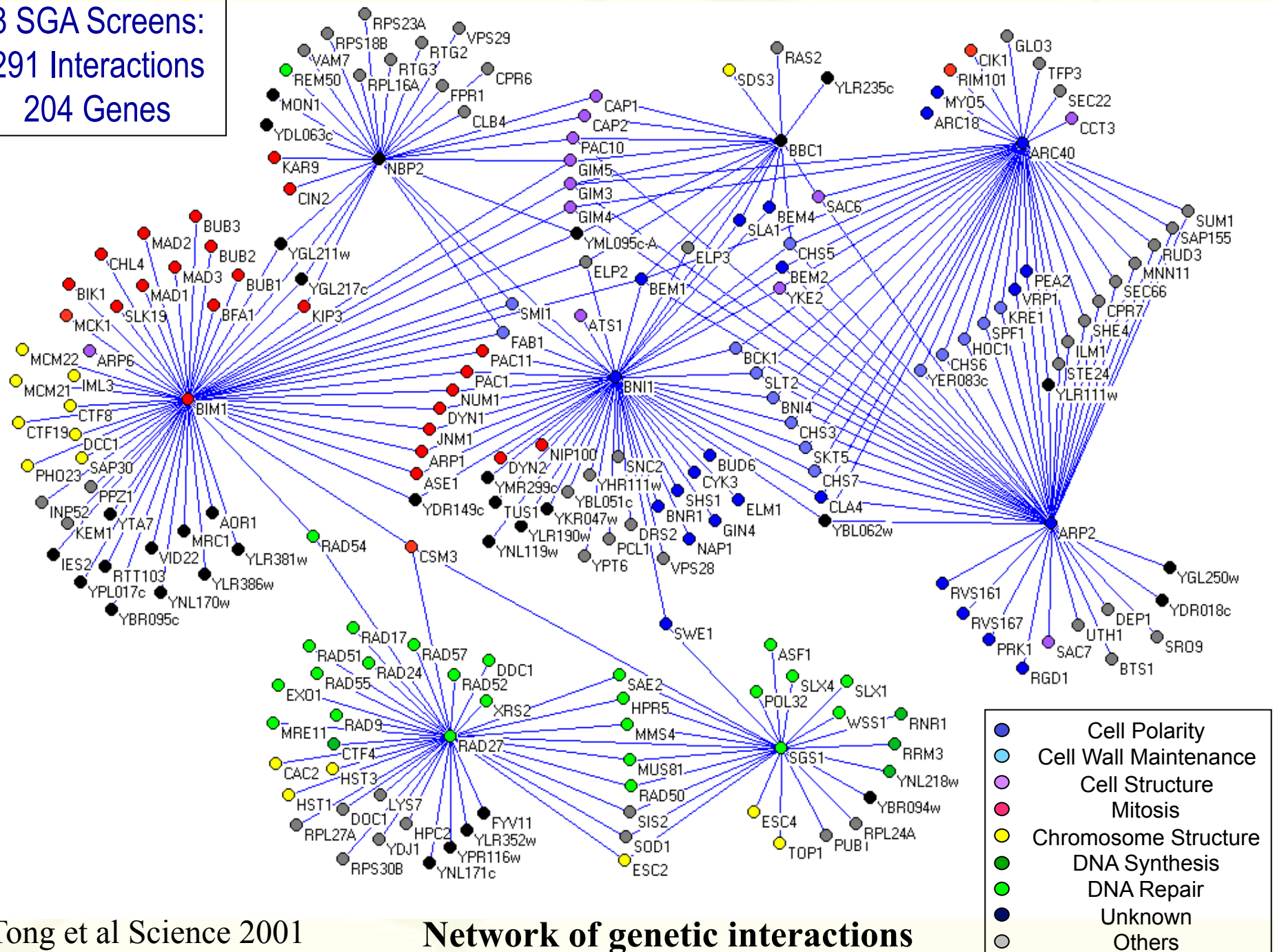
$ab = \text{expected}, \varepsilon = 0$	neutral interaction
$ab > \text{expected}, \varepsilon > 0$	positive interaction (within same complex)
$ab < \text{expected}, \varepsilon < 0$	negative interaction (synthetic lethal or sick)

Automated robotic system



Brenda Andrews / Charlie Boone

8 SGA Screens:
291 Interactions
204 Genes



Tong et al Science 2001

Network of genetic interactions

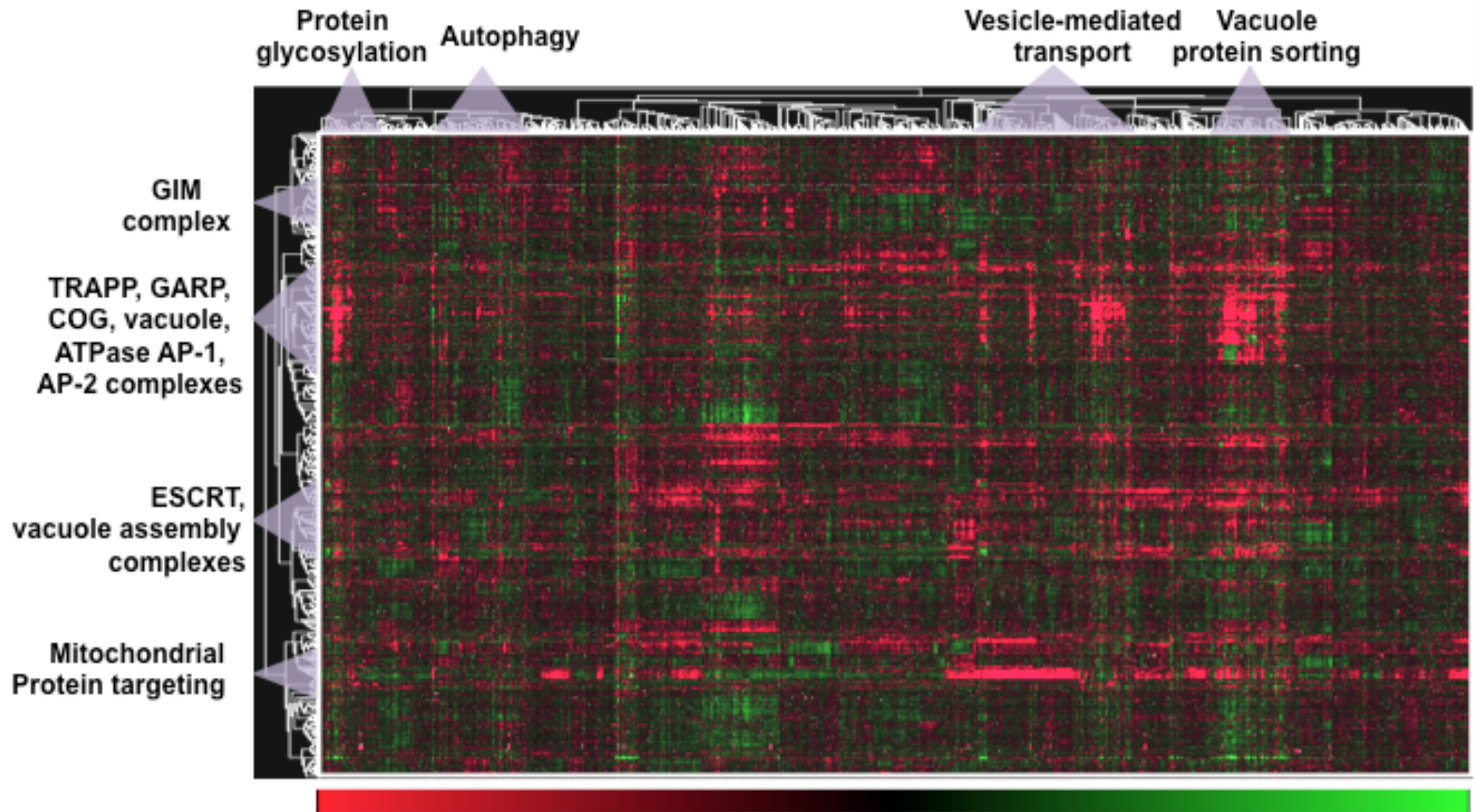
The Genetic Landscape of a Cell

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†}

- Assayed 1,712 genes against 3,900 genes
- A total of 5.5 million gene pairs
- Identified 170,000 genetic interactions, 3% of the surveyed gene pairs have an interaction

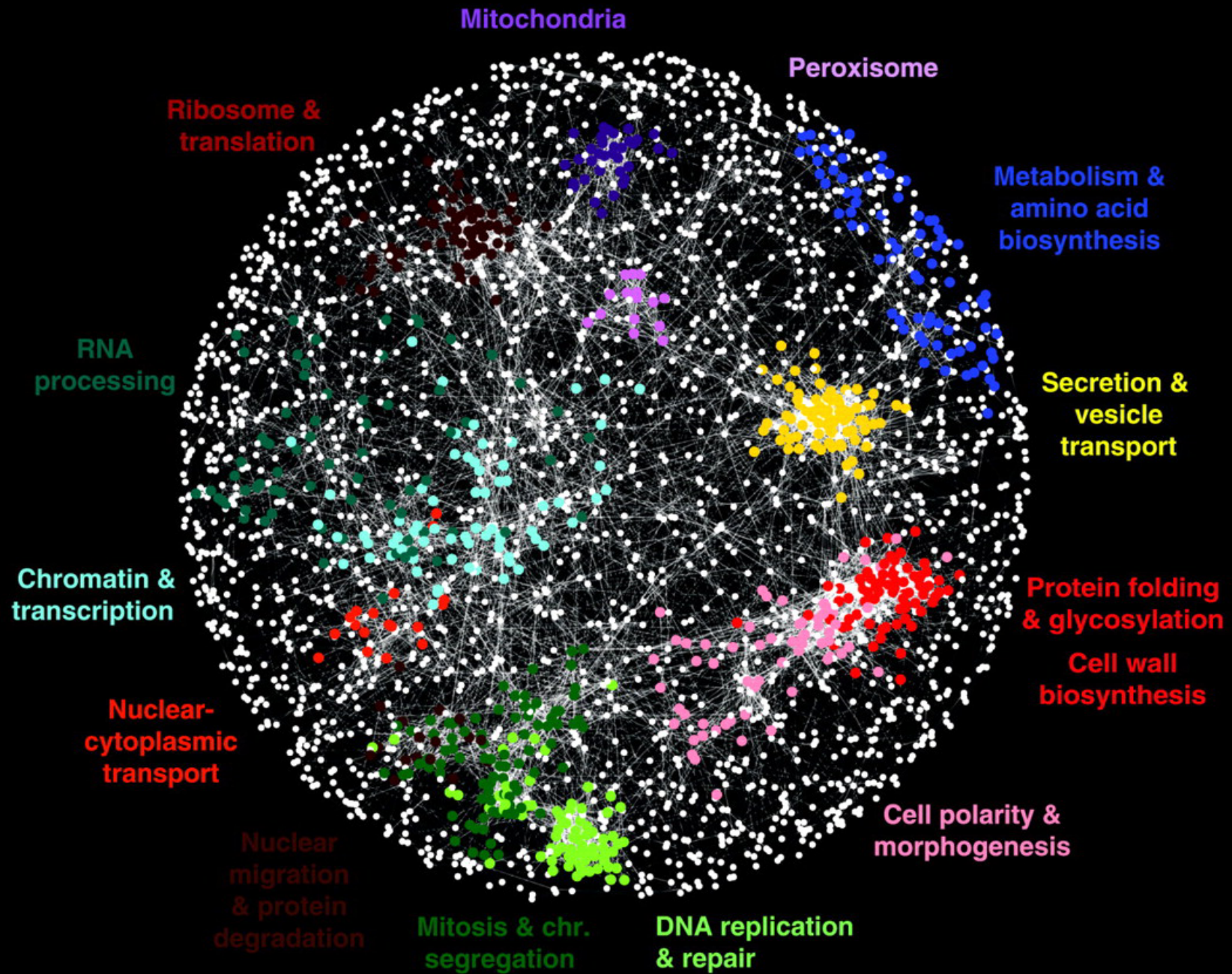
Michael Costanzo, *et al. Science* 327, 425 (2010);

SGA Genetic Interaction Matrix

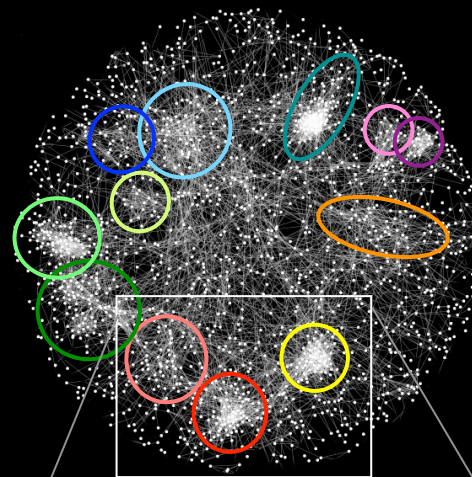


**Negative interactions
(synthetic lethal or sick)**

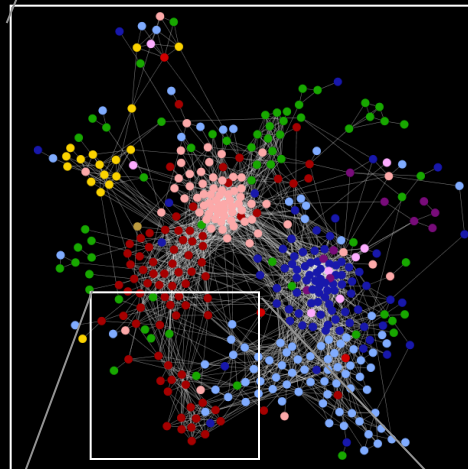
Positive interactions



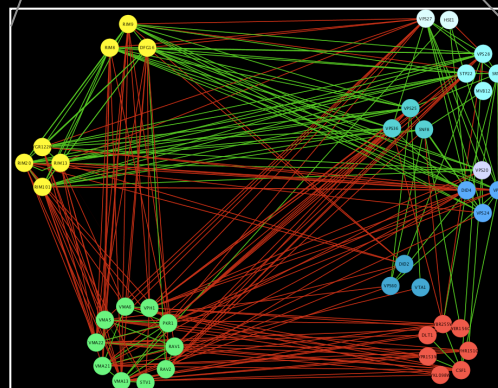
The Cell Map



Global level

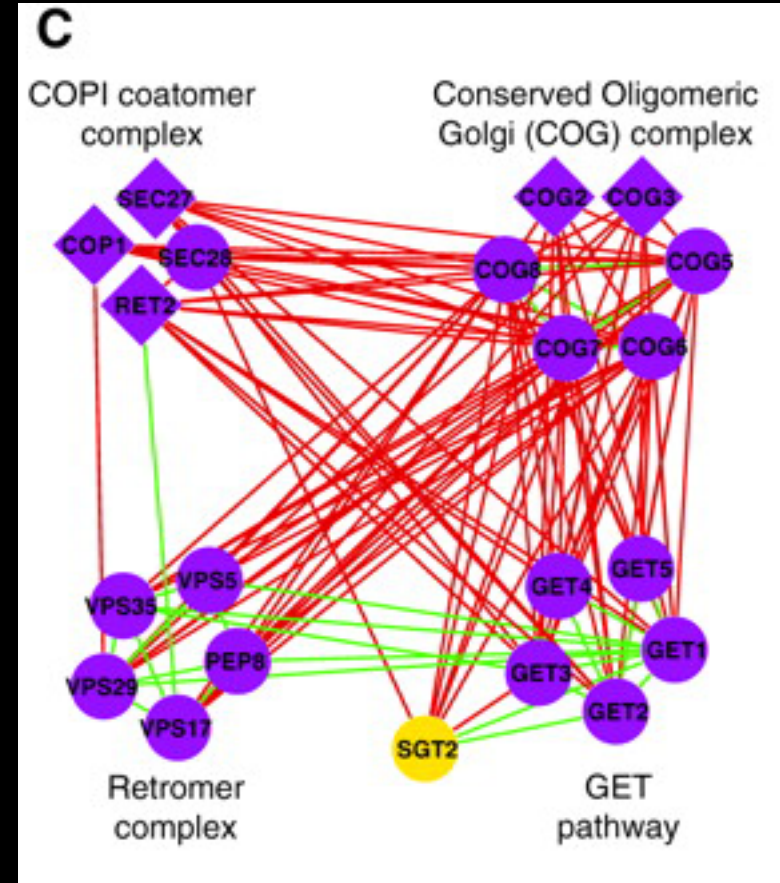
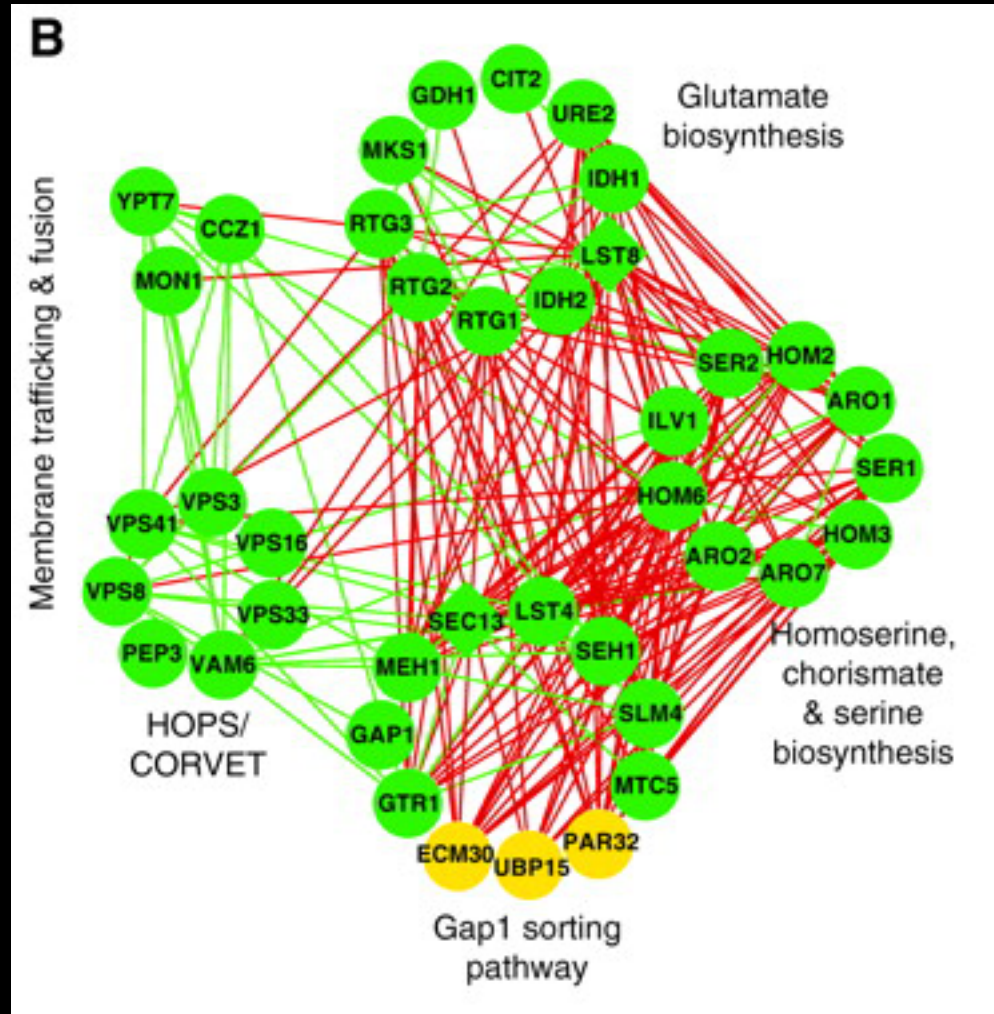


Process level



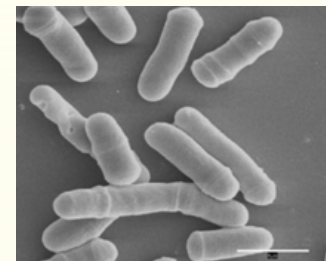
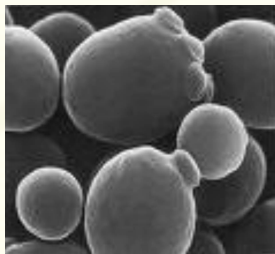
Pathway/complex level

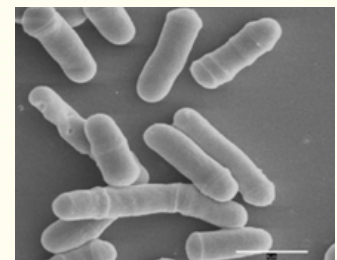
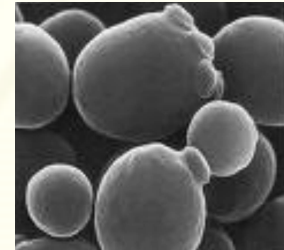
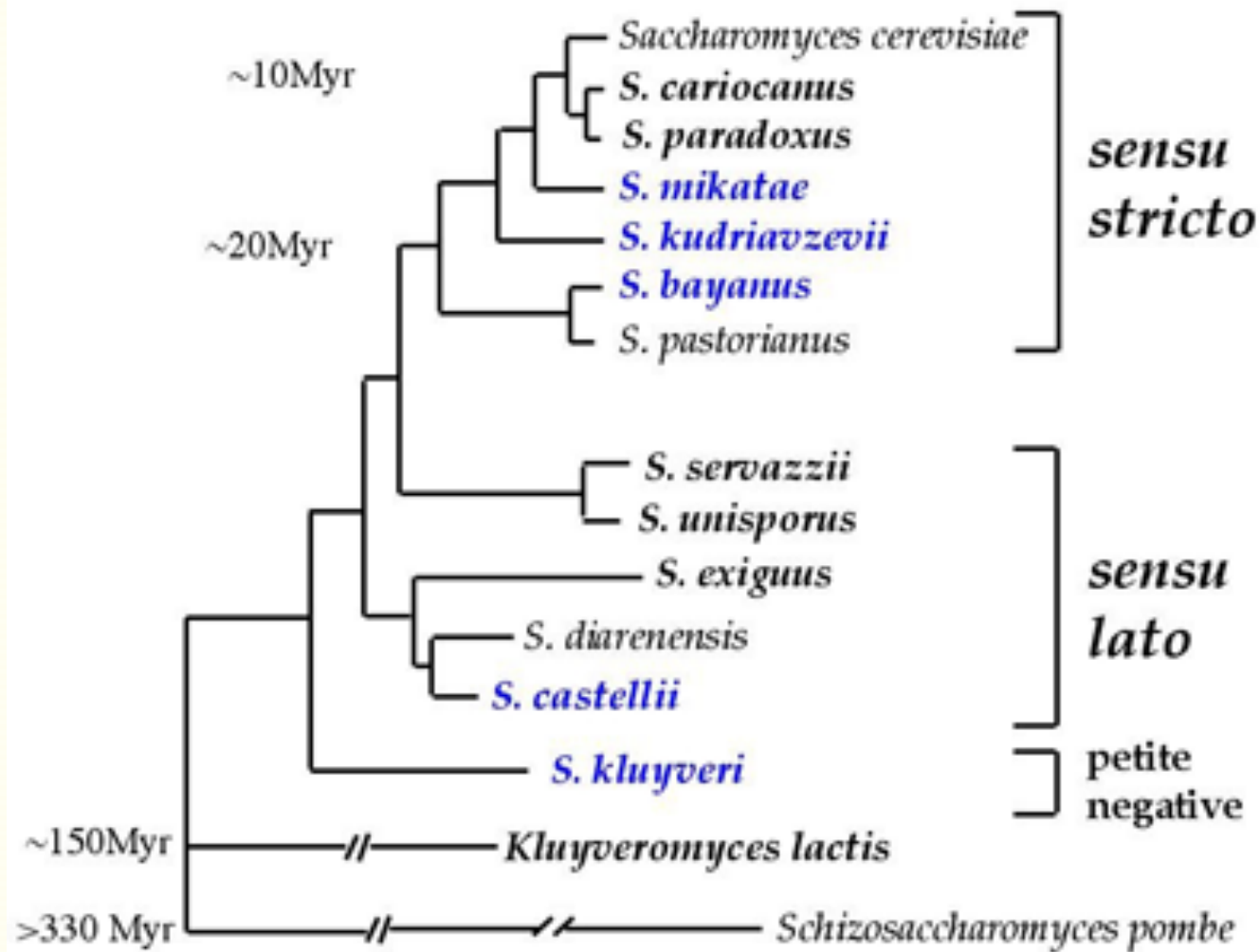
Genetic interactions between protein complexes



Evolutionary conservation of genetic interactions

- How are the genetic interactions in budding yeast conserved in fission yeast ?
- Fission yeast: *Schizosaccharomyces pombe*. *Schizo*: greek for “split:”, *Pombe*: Swahili for “beer”.
- These species diverged at 300 - 400 million years ago, and have very different biology.
- However, 75% of the genes in *S. pombe* have orthologs in *S. cerevisiae*.





Evolutionary conservation of genetic interactions

Proc Natl Acad Sci U S A. 2008 Oct 28;105(43):16653-8. Epub 2008 Oct 17.

Significant conservation of synthetic lethal genetic interaction networks between distantly related eukaryotes.

Dixon SJ, Fedyshyn Y, Koh JL, Prasad TS, Chahwan C, Chua G, Toufighi K, Baryshnikova A, Hayles J, Hoe KL, Kim DU, Park HO, Myers CL, Pandey A, Durocher D, Andrews BJ, **Boone C**.

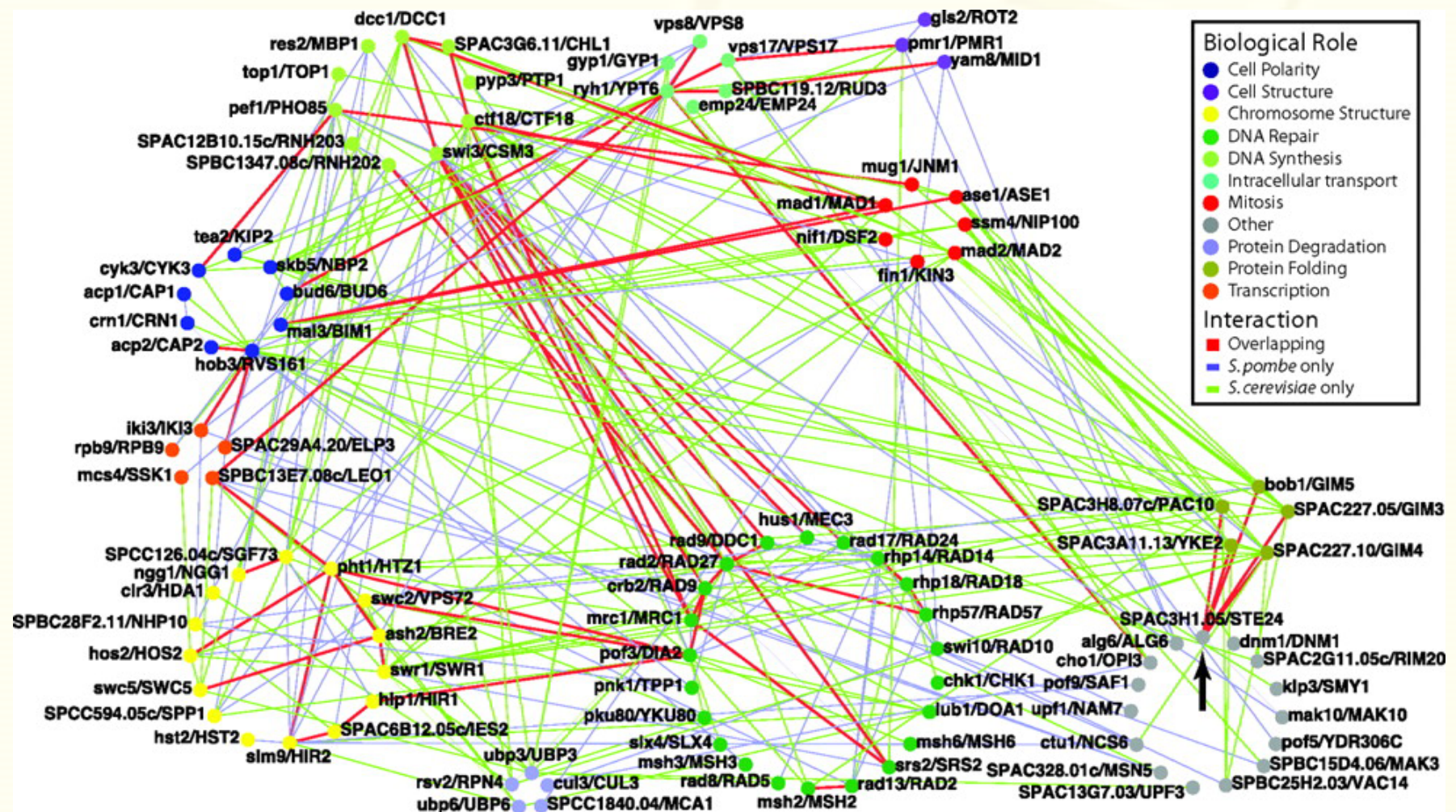
Science. 2008 Oct 17;322(5900):405-10. Epub 2008 Sep 25.

Conservation and rewiring of functional modules revealed by an epistasis map in fission yeast.

Roguev A, Bandyopadhyay S, Zofall M, Zhang K, Fischer T, Collins SR, Qu H, Shales M, Park HO, Hayles J, Hoe KL, Kim DU, Ideker T, Grewal SI, Weissman JS, Krogan NJ.

Department of Cellular and Molecular Pharmacology, University of California, San Francisco, CA 94158, USA.

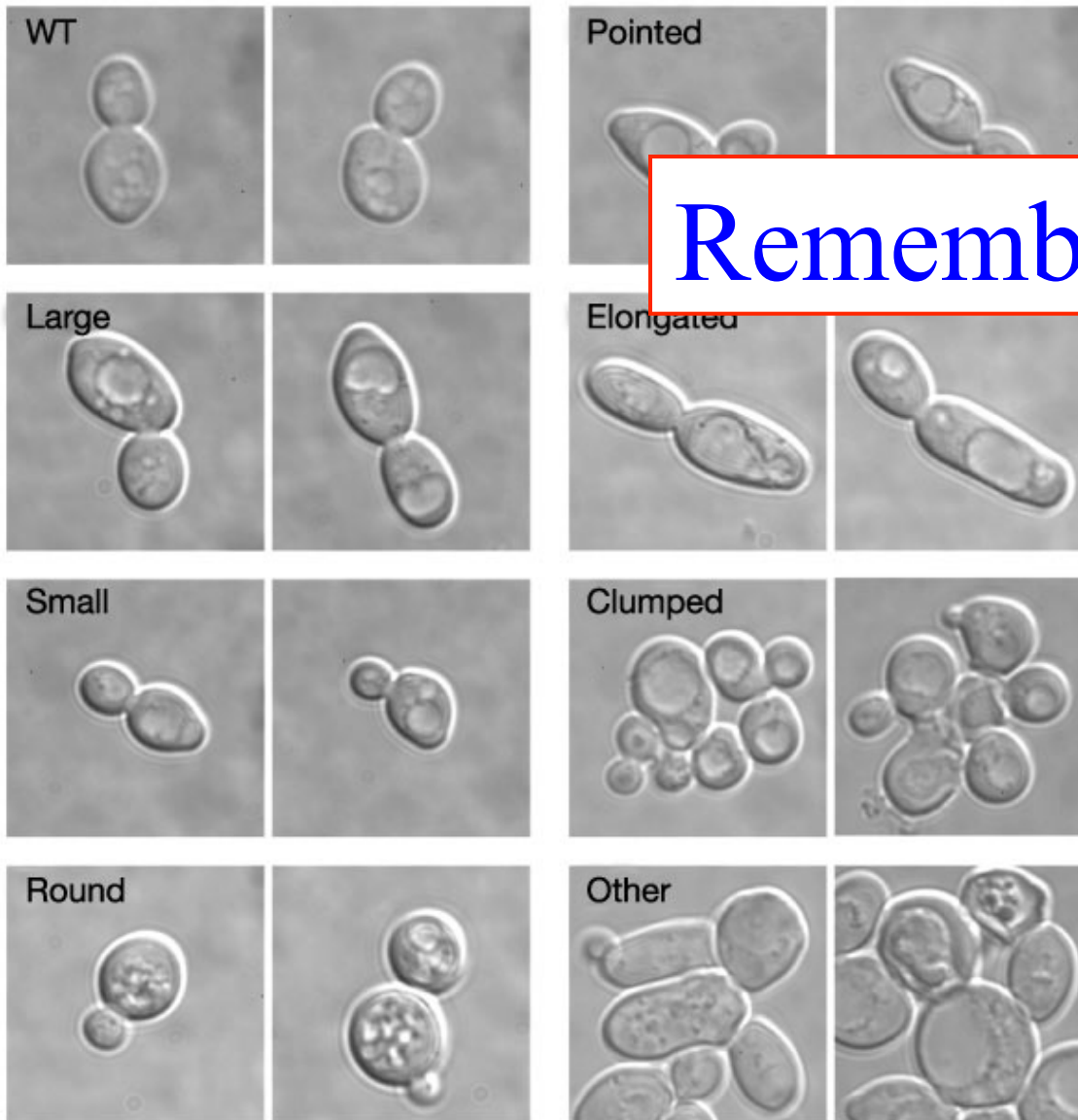
About 23-30% of the genetic interactions are shared between these distantly related yeast species



Dixon et al PNAS 2008

Phenomics:
**High-content cell imaging: detecting
phenotypic changes at high resolution**

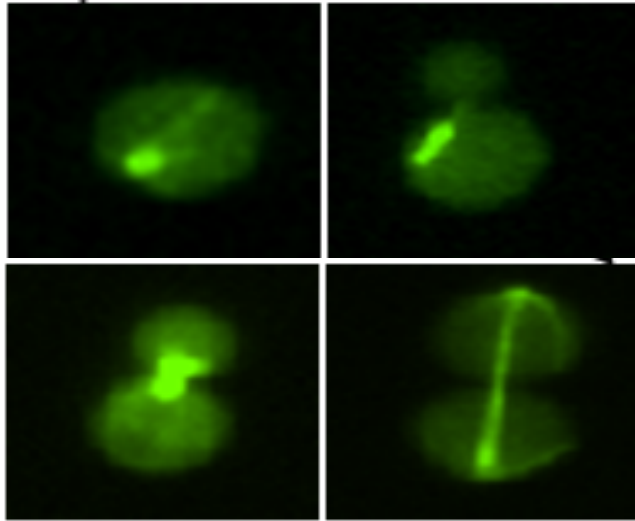
Deletion of nonessential genes have **morphological** phenotypes



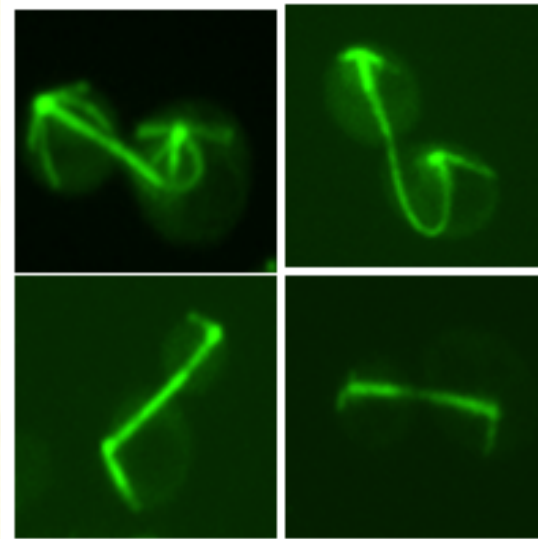
Remember this slide ?

Giaever et al *Functional profiling of the *Saccharomyces cerevisiae* genome* Nature 2002

Combining genetics + cell Imaging: what happen to the cells after removing a gene ?



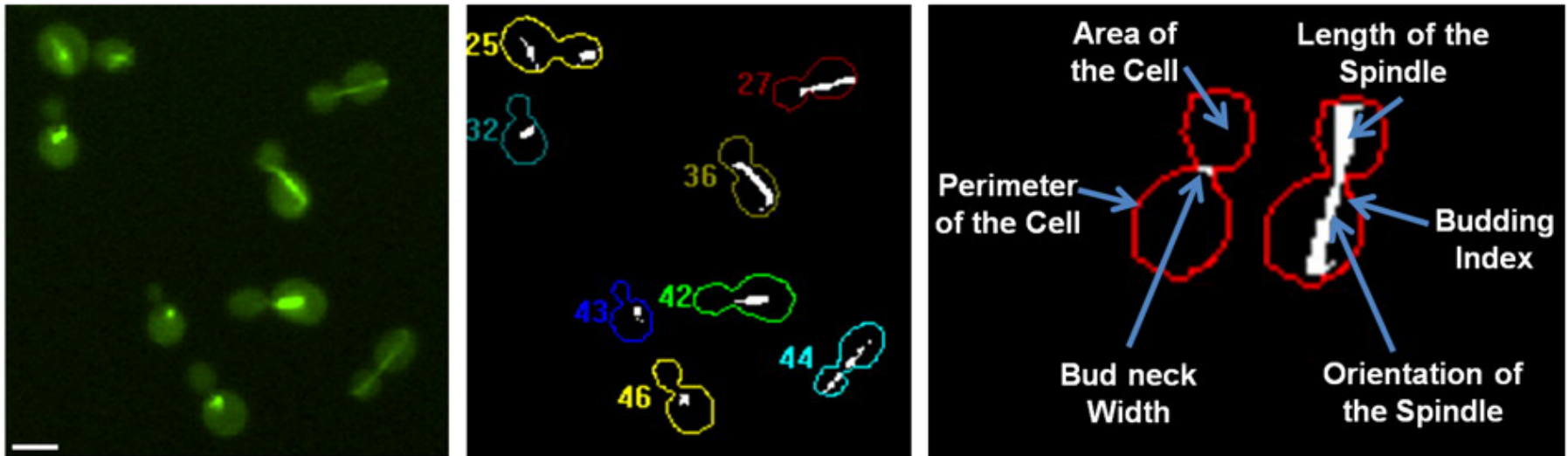
Wild type
Normal spindle



ΔA
Single gene mutant
“fishhook” spindle

From Franco Vizeacoumar

Automated image analysis

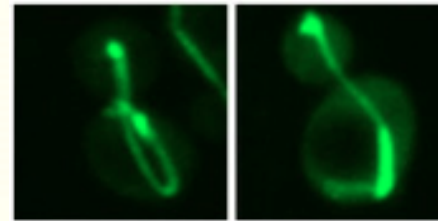
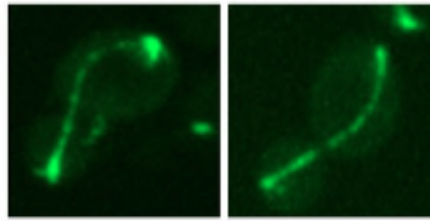


What happens after removing 2 genes ?

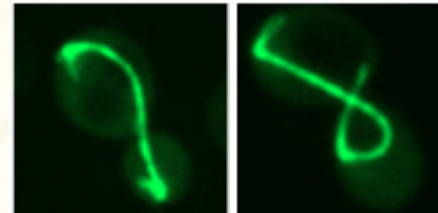
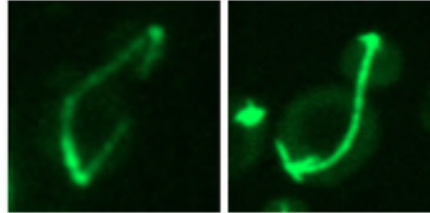
Single mutant

Double mutants with EMI2

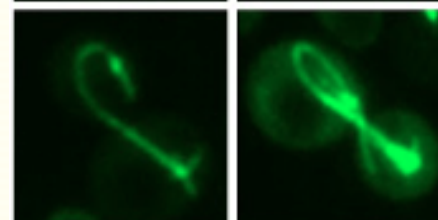
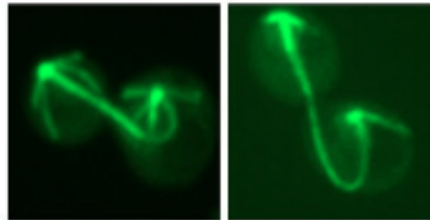
bns1Δ



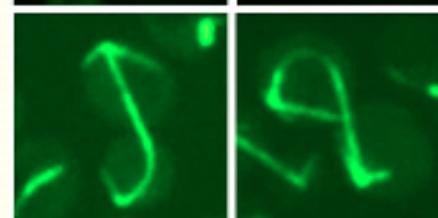
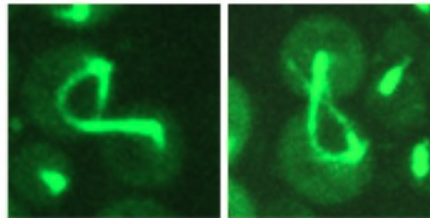
spo12Δ



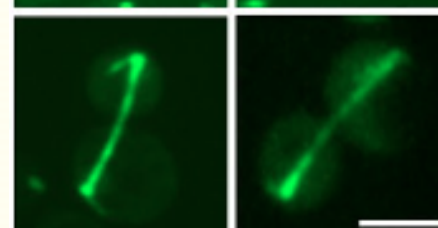
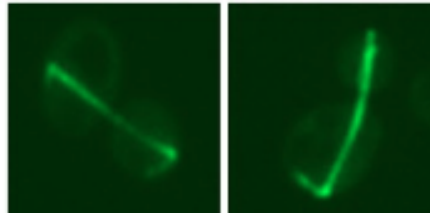
slk19Δ

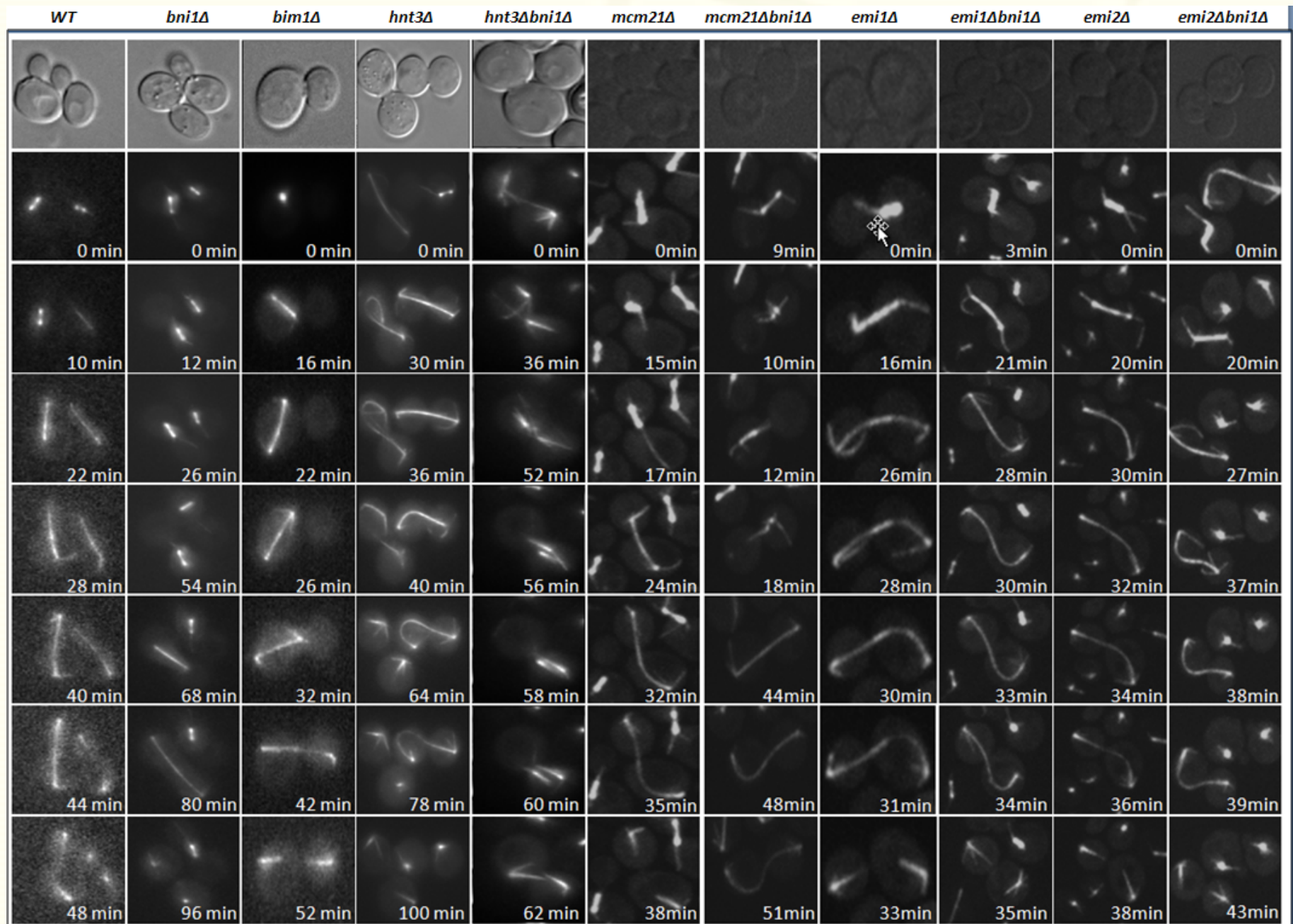


lte1Δ



bub2Δ



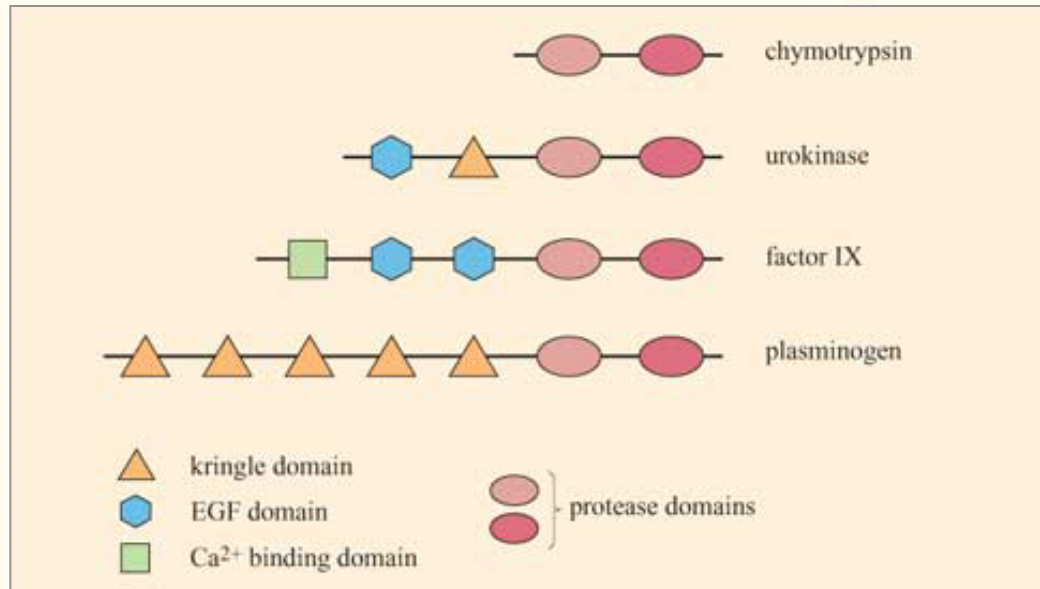


Franco Vizeacoumar, Charlie Boone, Brenda Andrews

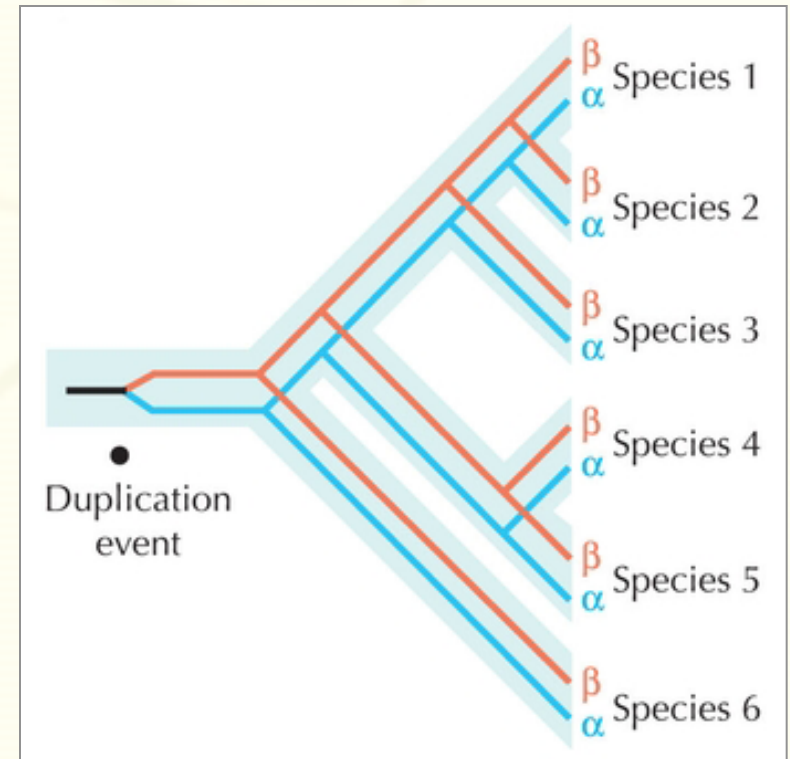
Now something different ...

- We have discussed the concepts of gene expression analysis, gene regulation by transcription factors (TF), protein-protein interactions, genetic interactions etc.
- Now we put these into action to a real evolutionary problem: **study of duplicated genes in *S. cerevisiae***

We have already encountered duplication events earlier in the course



Duplication of protein domains



Duplication of single genes

Gene duplication is an important mechanism to generate new genes

Molecular mechanisms for creating new genes

- Exon shuffling
- • Gene duplication
- • Retrotransposition
- Mobile (repetitive) elements
- Lateral gene transfer
- Gene fusion / fission
- De novo origination

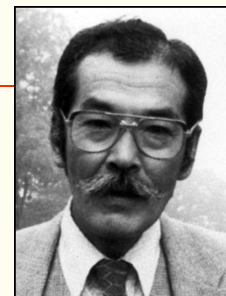
Nat Rev Genet. 2003 Nov;4(11):865-75.

The origin of new genes: glimpses from the young and old.

Long M, Betrán E, Thornton K, Wang W.

Evolution by Gene Duplication

- Susumu Ohno (大野 乾): **Evolution by Gene Duplication**, 1970
- “(without gene duplication), The creation of metazoans, vertebrates, and finally mammals from unicellular organisms would have been quite impossible, for **such big leaps in evolution** required the creation of new gene loci with previously nonexistent function”
- “Natural selection merely modified while **redundancy** created”

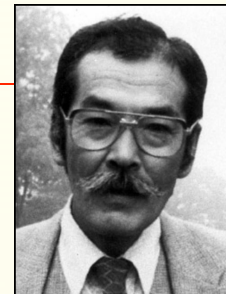


Evolution by Gene Duplication

- Susumu Ohno (大野 乾): **Evolution by Gene Duplication**, 1970

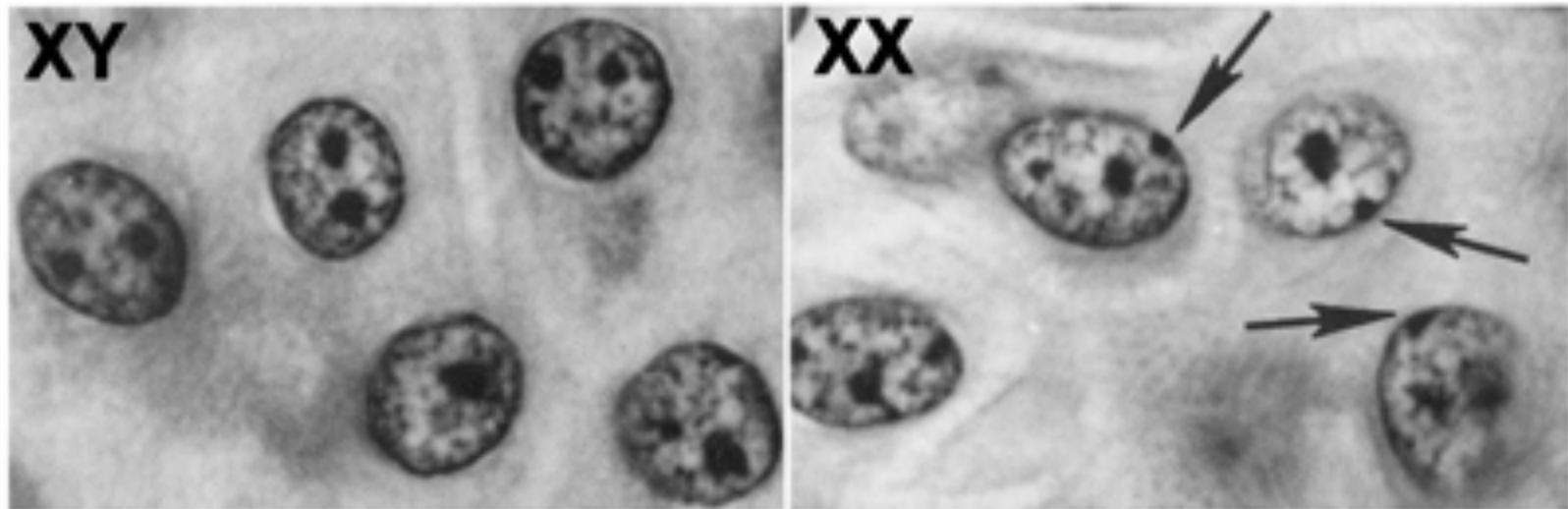
- What does he mean?

- (i) It is easier to duplicate an existing gene and evolve new functions than creating a new gene from scratch.
 - (ii) Without duplication, it is difficult to modify the existing gene and evolve new function without generating deleterious effects.

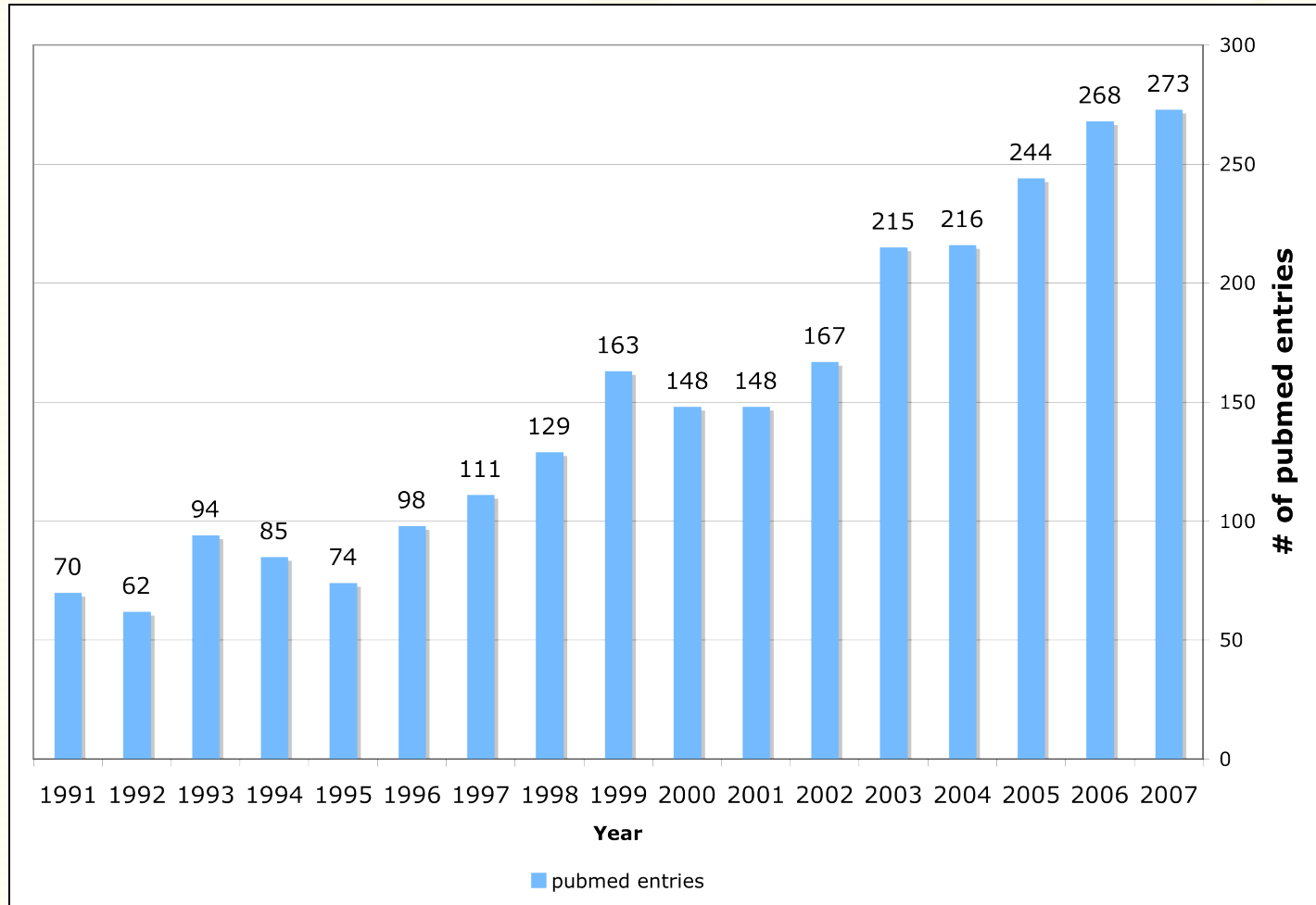


Other contributions by Susumo Ohno

- Ohno discovered in 1956 that the “**Barr body**” of mammalian female nuclei was in fact a condensed and inactivated X chromosome (due to dosage compensation mechanism).
- Proposed that vertebrate genome may be the result of one or **two rounds of whole genome duplications**.

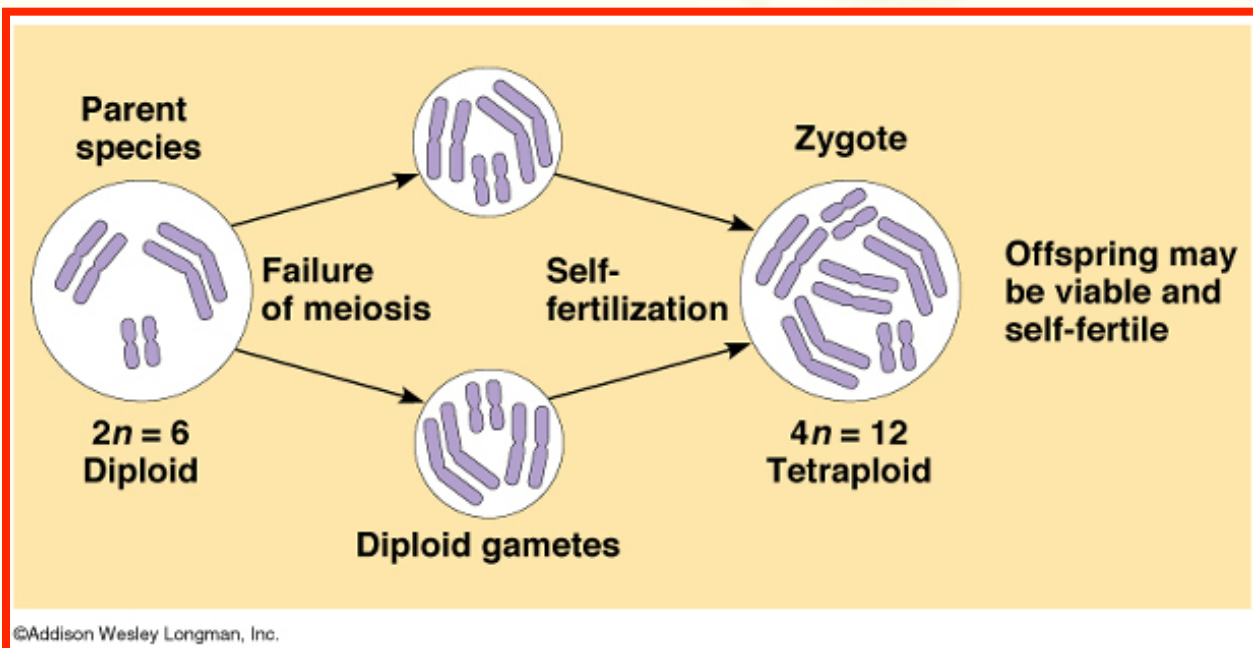
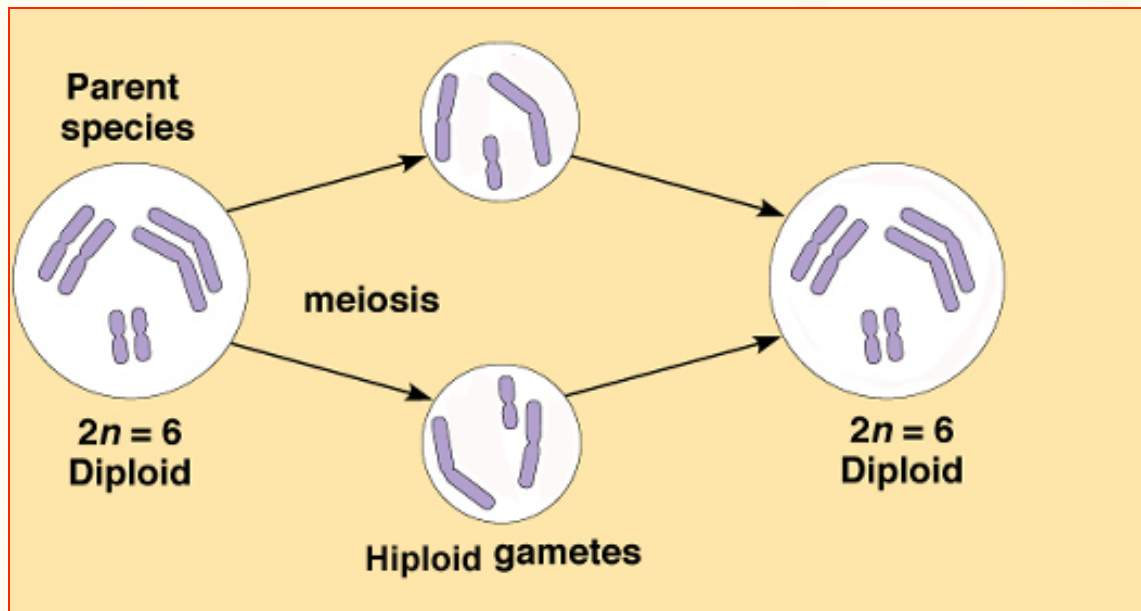


“Gene Duplication” in Pubmed

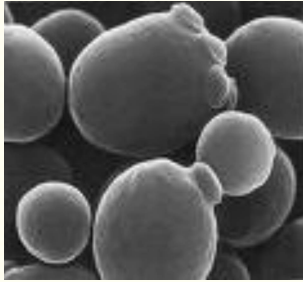


Pubmed: “gene duplication”[title/abstract]: 3,080

Whole Genome Duplication (WGD)



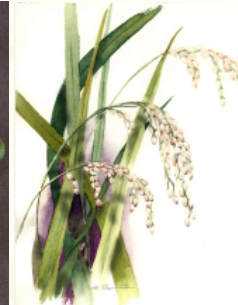
Whole Genome Duplication



S. Cerevisiae 100
million years ago



Paramecium, 3
rounds of dup.



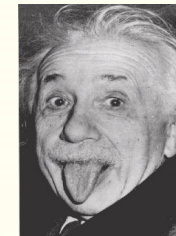
Arabidopsis,
25-40 mya;
50-70 mya



X.laevis vs *X. tropicalis*
40 millions years ago



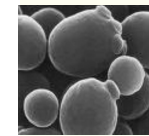
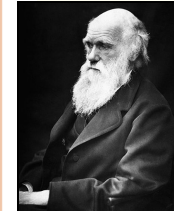
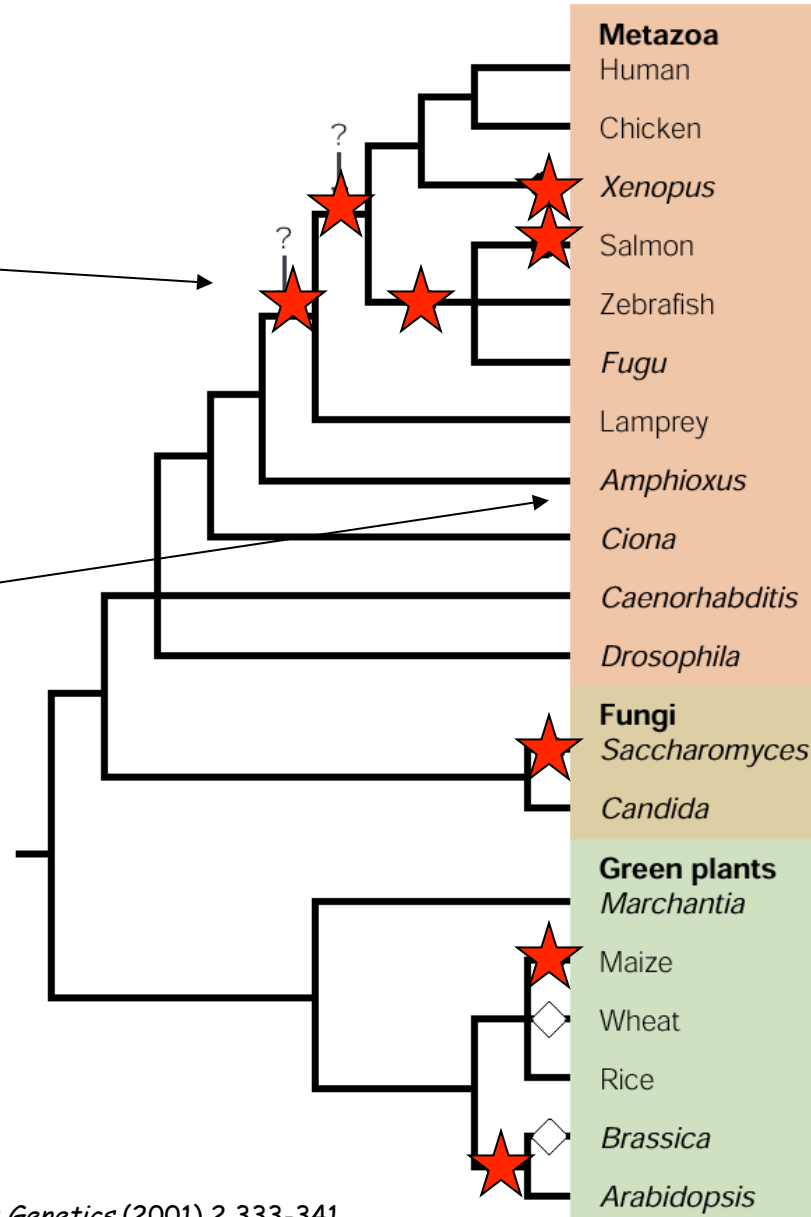
Pufferfish, 350 mya



2 rounds ?

Whole Genome Duplication

2R: two rounds of duplication



evolutionary questions on gene duplication

- how to detect whole genome duplications ?
- how do duplicate genes evolve ?
- why are so many duplicates maintained in the genome ?

Detecting Genome Duplication

1. by genomic DNA alignment
2. by number of paralogs per gene
3. by molecular dating of paralogs pairs

By number of paralogs



By number of paralogs



Most of the genes in the genome will have just **1** paralog

By number of paralogs

A 

Ancestral genome

B 

Whole genome duplication (1R)

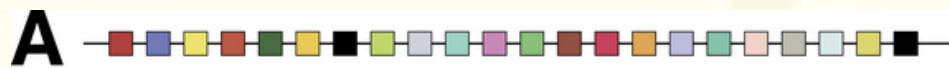
C 

Divergence after duplication

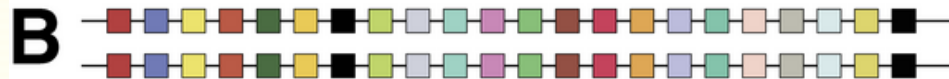
D 

Whole genome duplication (2R)

By number of paralogs



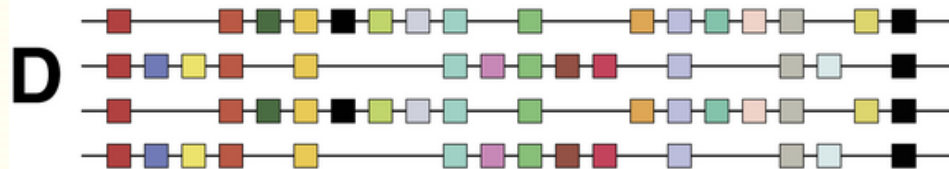
Ancestral genome



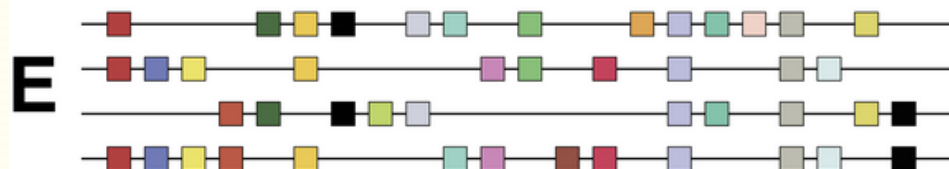
Whole genome duplication (1R)



Divergence after duplication



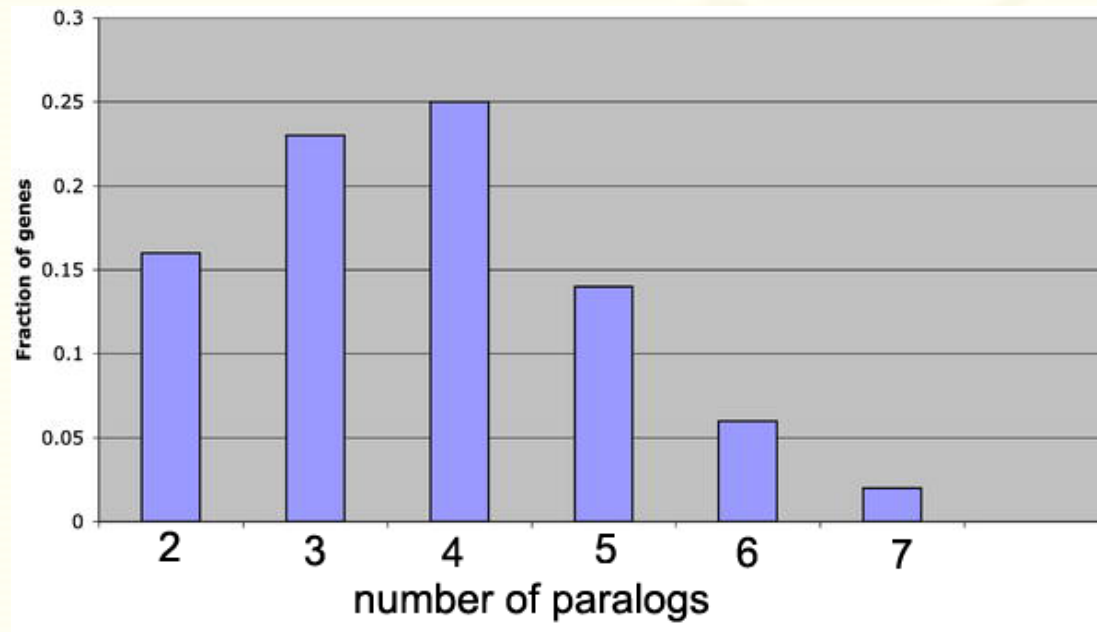
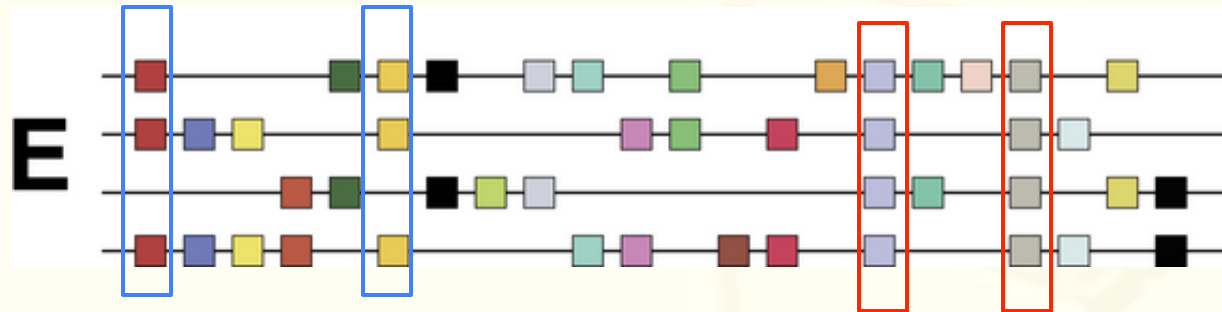
Whole genome duplication (2R)



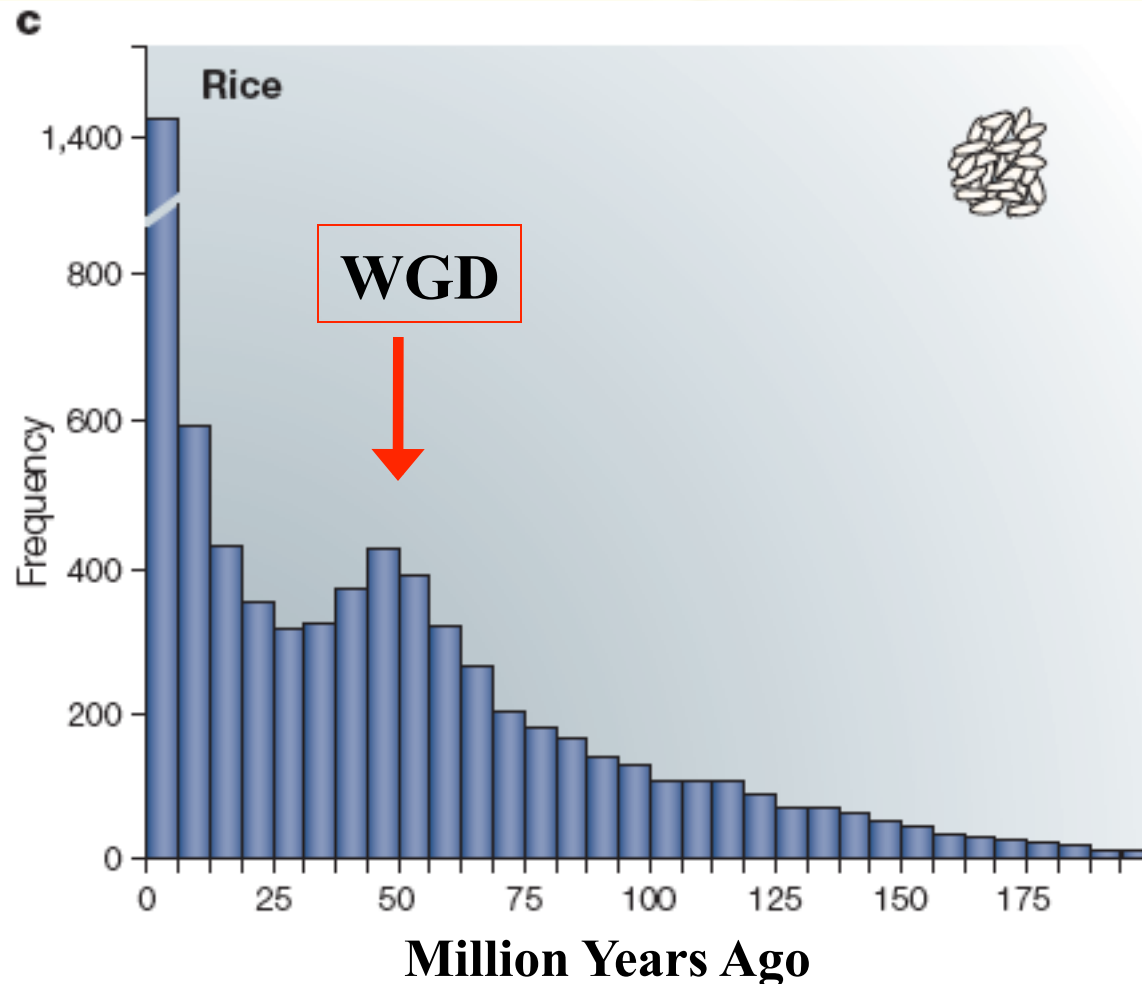
Divergence after duplication

Most of the genes in the genome will have 4 paralogs

Have modern vertebrates had 2 rounds of genome duplications ?



detect genome duplication by age distribution of paralog pairs

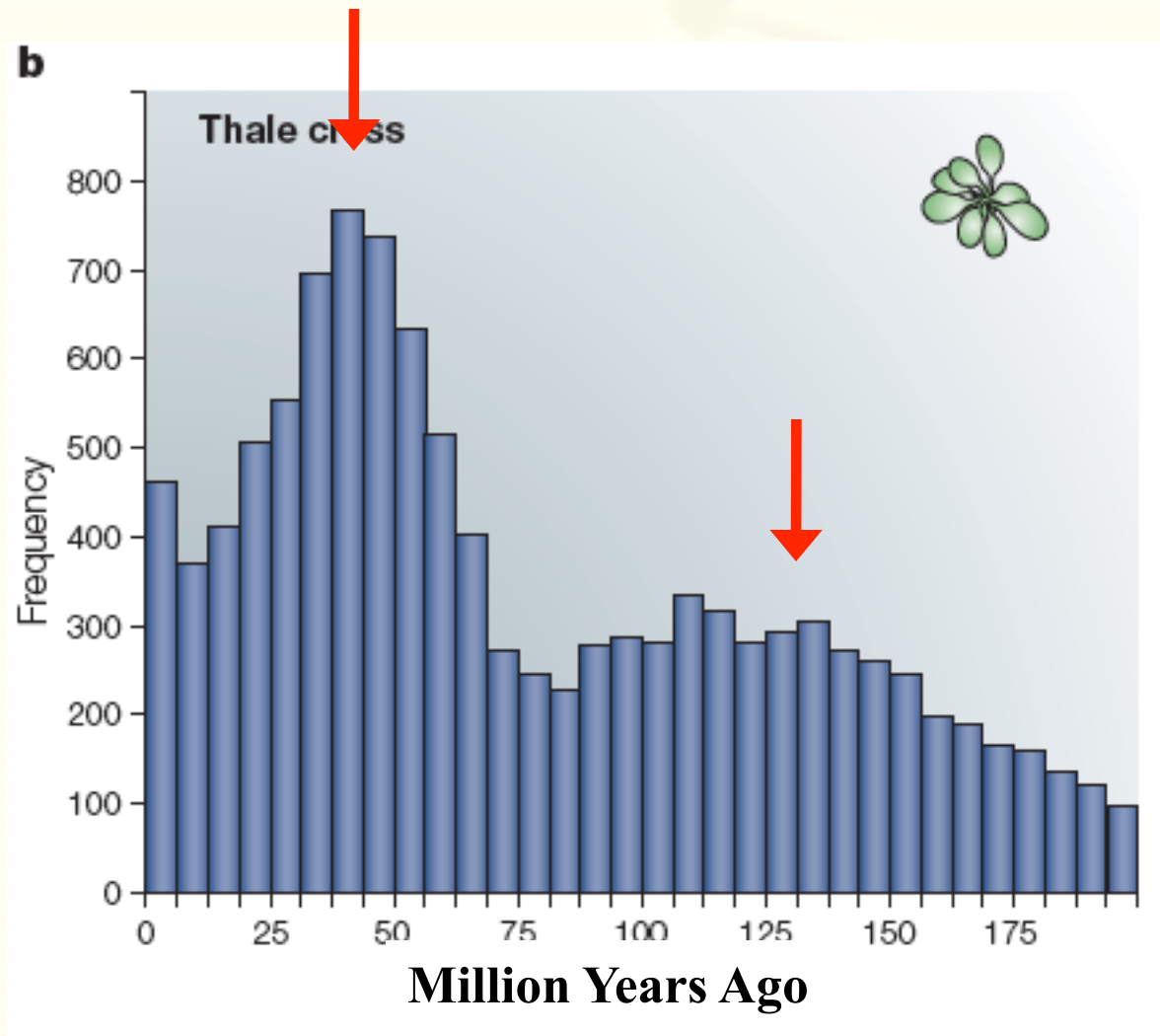


Rice

Van de Peer
Nature Rev Gen 2004

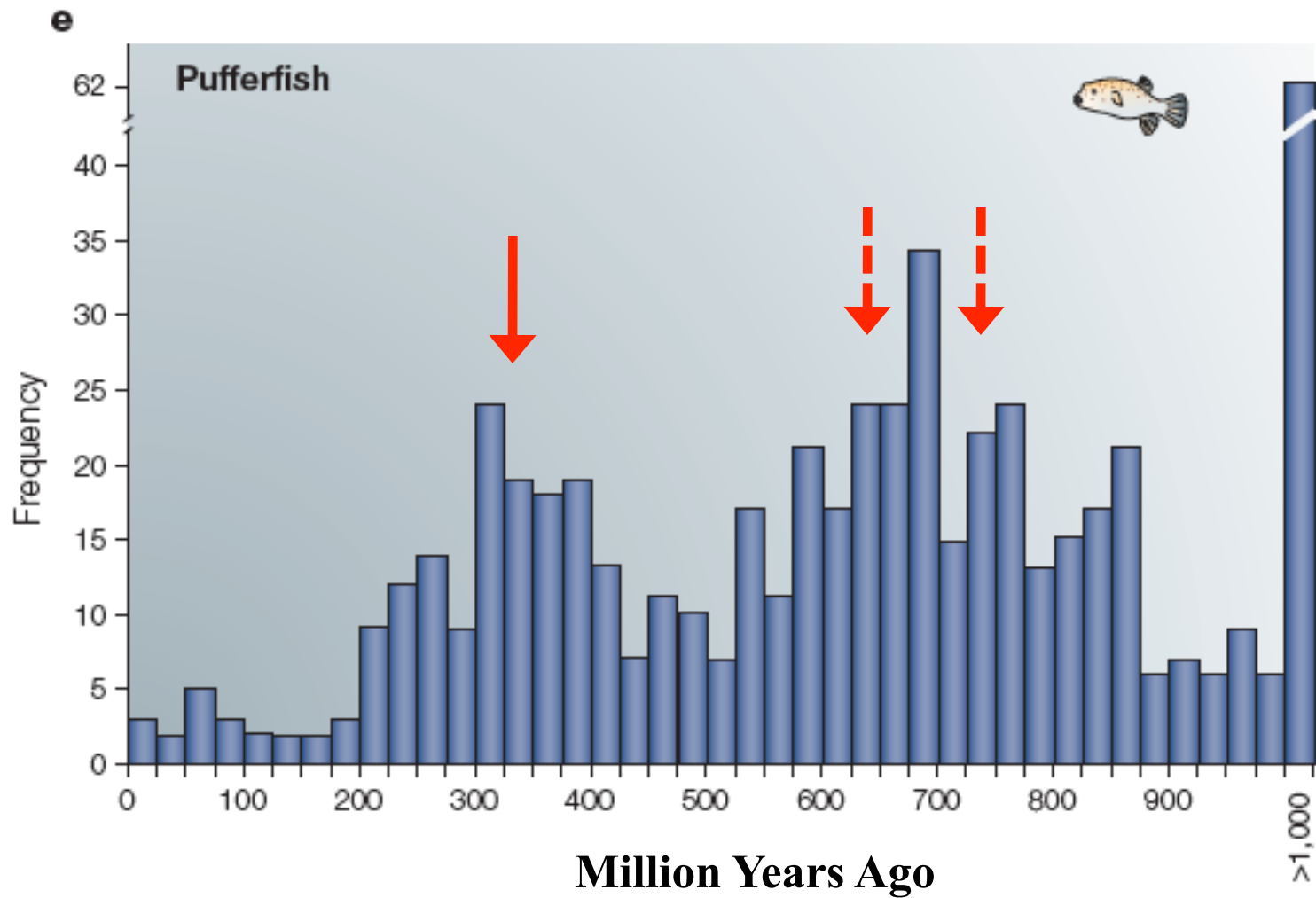
**Divergence time between paralogs:
Calculated by number of pair-wise synonymous substitutions**

detect genome duplication by age distribution of paralog pairs

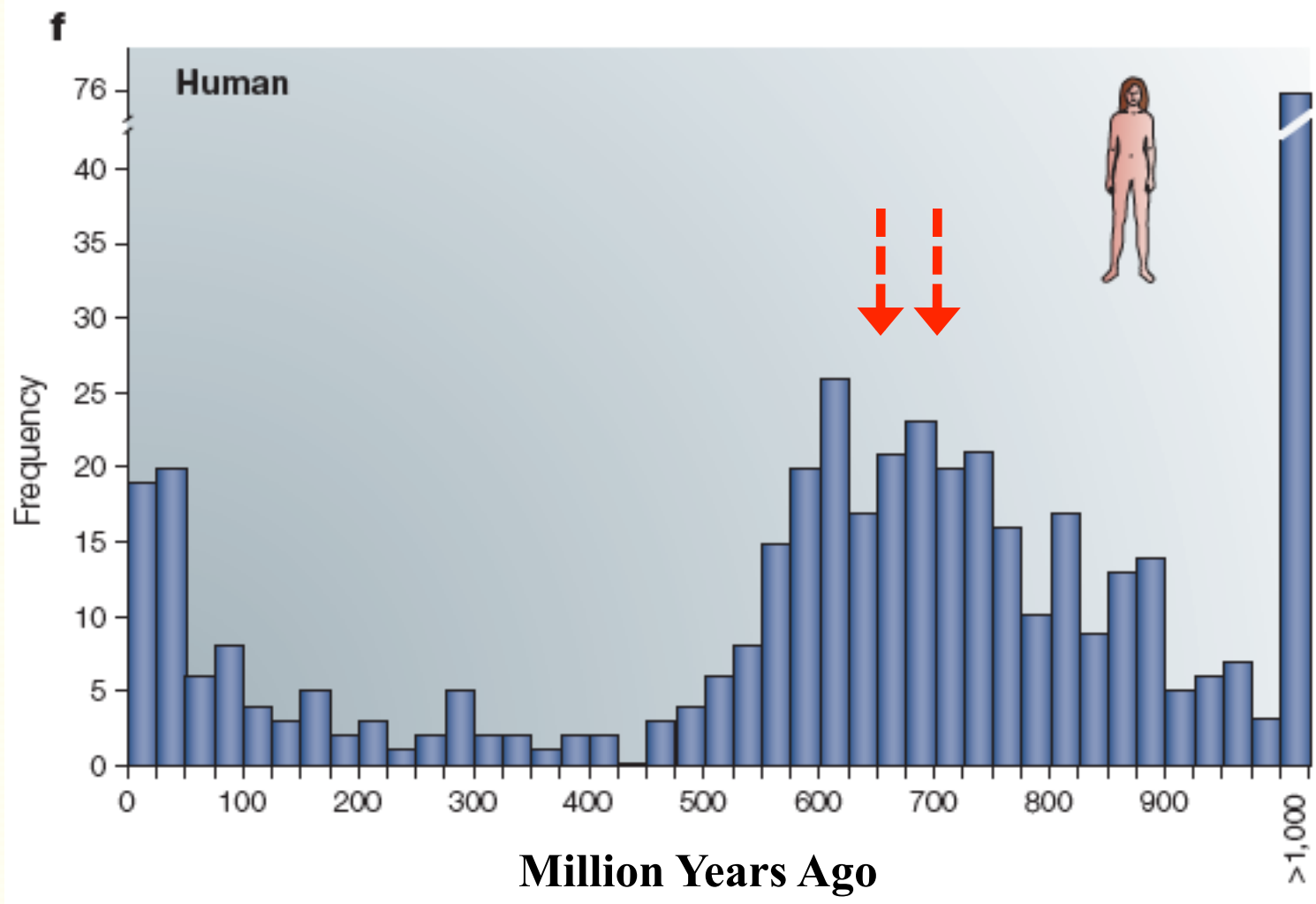


Arabidopsis

detect genome duplication by age distribution of paralog pairs



detect genome duplication by age distribution of paralog pairs



Whole-Genome-Duplication in *S. cerevisiae*

- **Ken Wolfe** reported in 1997 the *S. cerevisiae* genome had undergone a whole-genome duplication, shortly after the publication of the yeast genome (1996)
- *“We propose ... this species is a degenerate tetraploid resulting from a whole-genome duplication ...”*
- *“Only a small fraction of the genes were subsequently retained in duplicate (most were deleted), and gene order was rearranged by many reciprocal translocations between chromosomes.”*

Nature. 1997 Jun 12;387(6634):708-13.

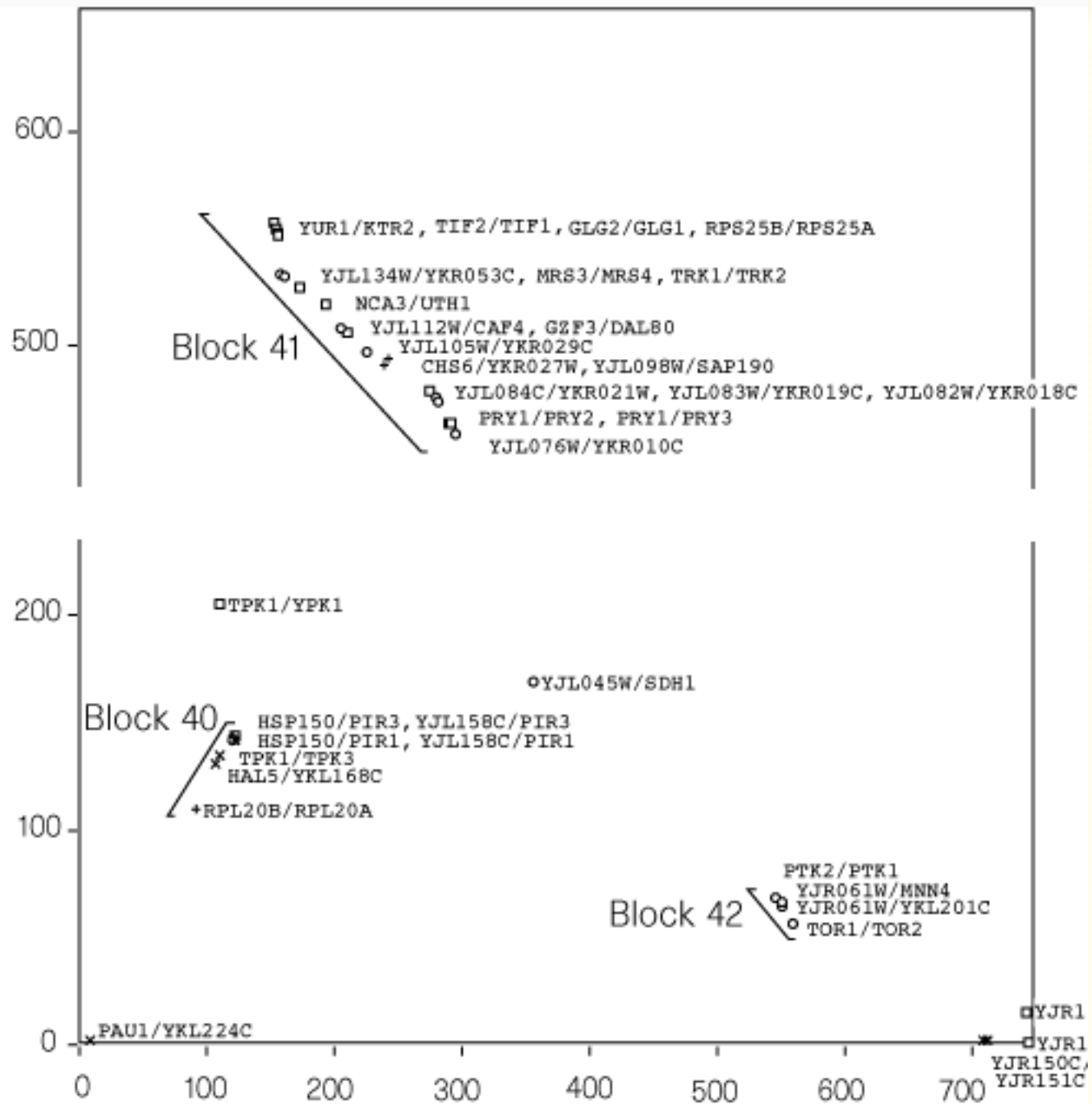
Molecular evidence for an ancient duplication of the entire yeast genome.

Wolfe KH, Shields DC.

Whole-Genome-Duplication in *S. cerevisiae*

- We searched systematically for duplicated regions in the complete yeast genome by using **BLASTP** amino-acid sequence similarity searches of **all yeast proteins against one another**, and plotted the results on dot matrices. Duplicate regions are visible as a diagonal series of 'hits' with conserved gene orientation.
- In the whole genome, 55 duplicate regions were identified containing **376 pairs** of homologous genes.
- The criteria used to define a duplicate region were: (1) **BLASTP** high scores of 200 for each gene pair ($P = 10^{-18}$ or less); (2) at least three pairs of homologues with intergenic distances of 50 kilobases (kb) on each chromosome; and (3) conservation of gene order and orientations

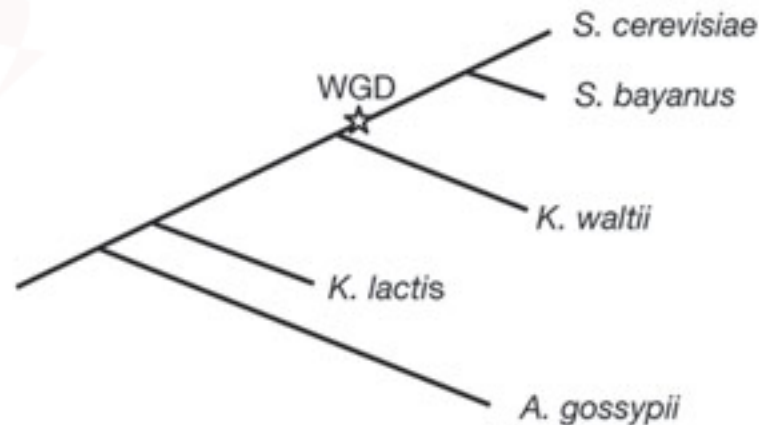
Distance along **chromosome XI** (kb)



Distance along **chromosome X** (kb)

Seven years and \$\$\$ later

- Kellis et al sequenced the genomes of *Kluyveromyces waltii*, which presumably diverged before the putative WGD event.
- *K waltii* genome is of similar length as *S. cerevisiae* (10.7 Mb vs 23 Mb) and has comparable number of genes (5200 vs 5800)
- The authors identified 457 gene pairs resulted from **whole-genome duplication**, and 130 gene pairs from **small-scale duplications**.

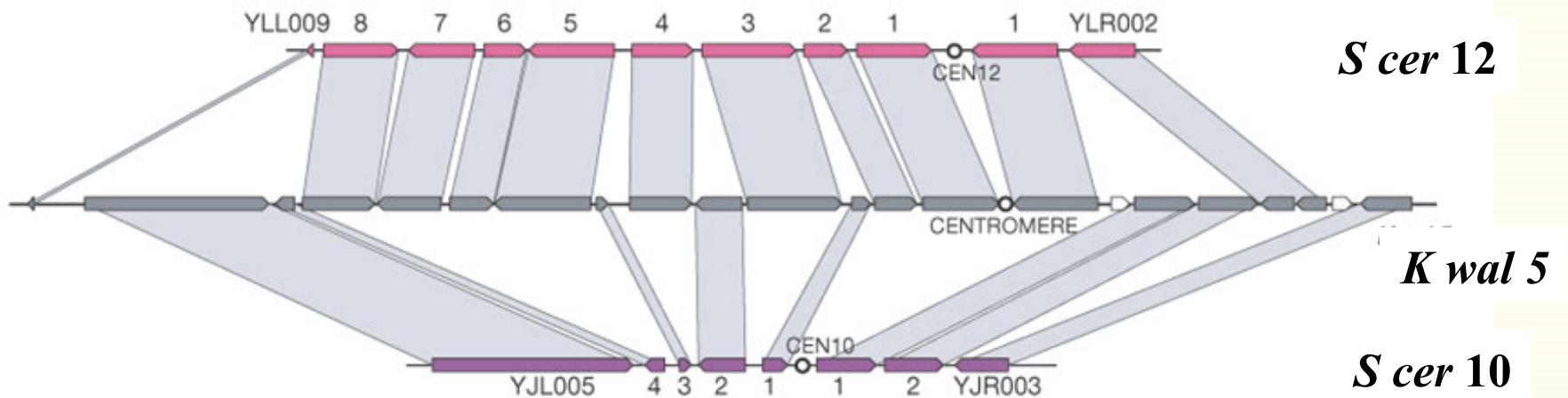


Nature. 2004 Apr 8;428(6983):617-24. Epub 2004 Mar 7.

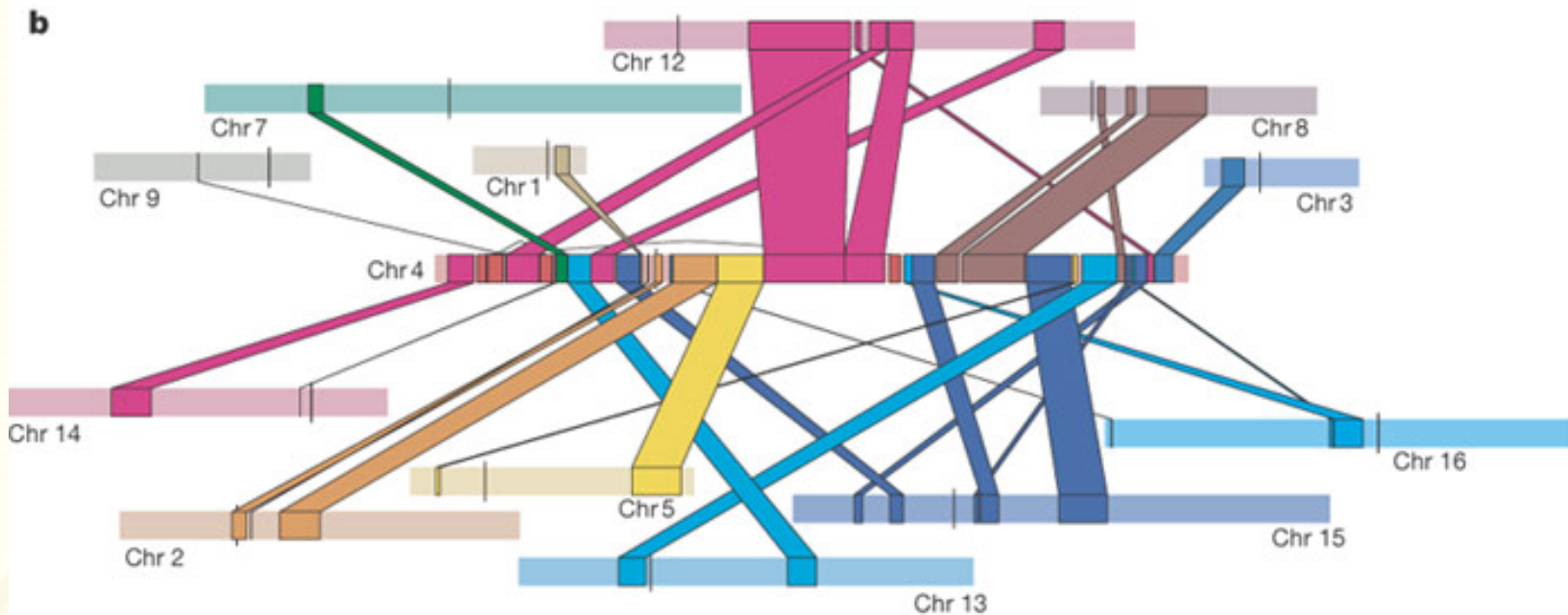
Proof and evolutionary analysis of ancient genome duplication in the yeast *Saccharomyces cerevisiae*.

Kellis M, Birren BW, Lander ES.

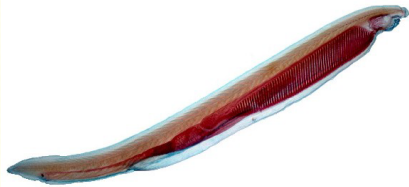
Alignment between *K. waltii* and *S. cerevisiae*



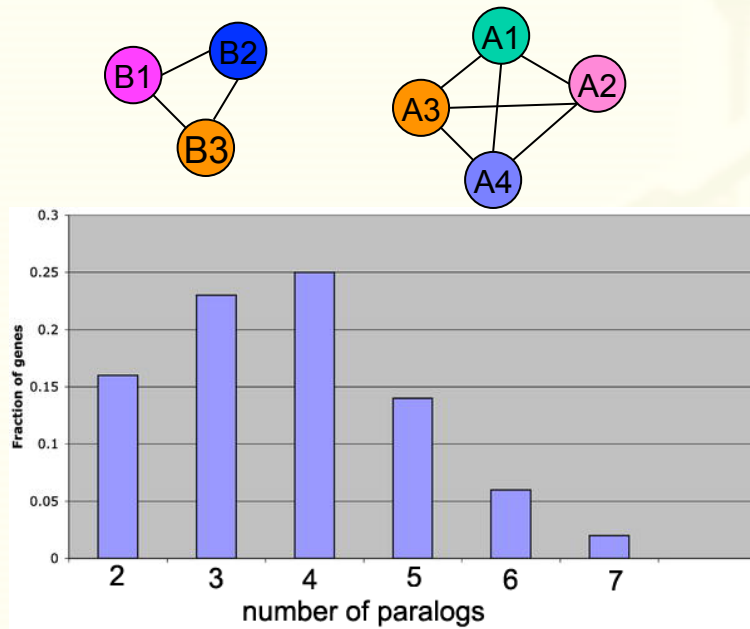
Alignment among chromosomes in *S. cerevisiae*



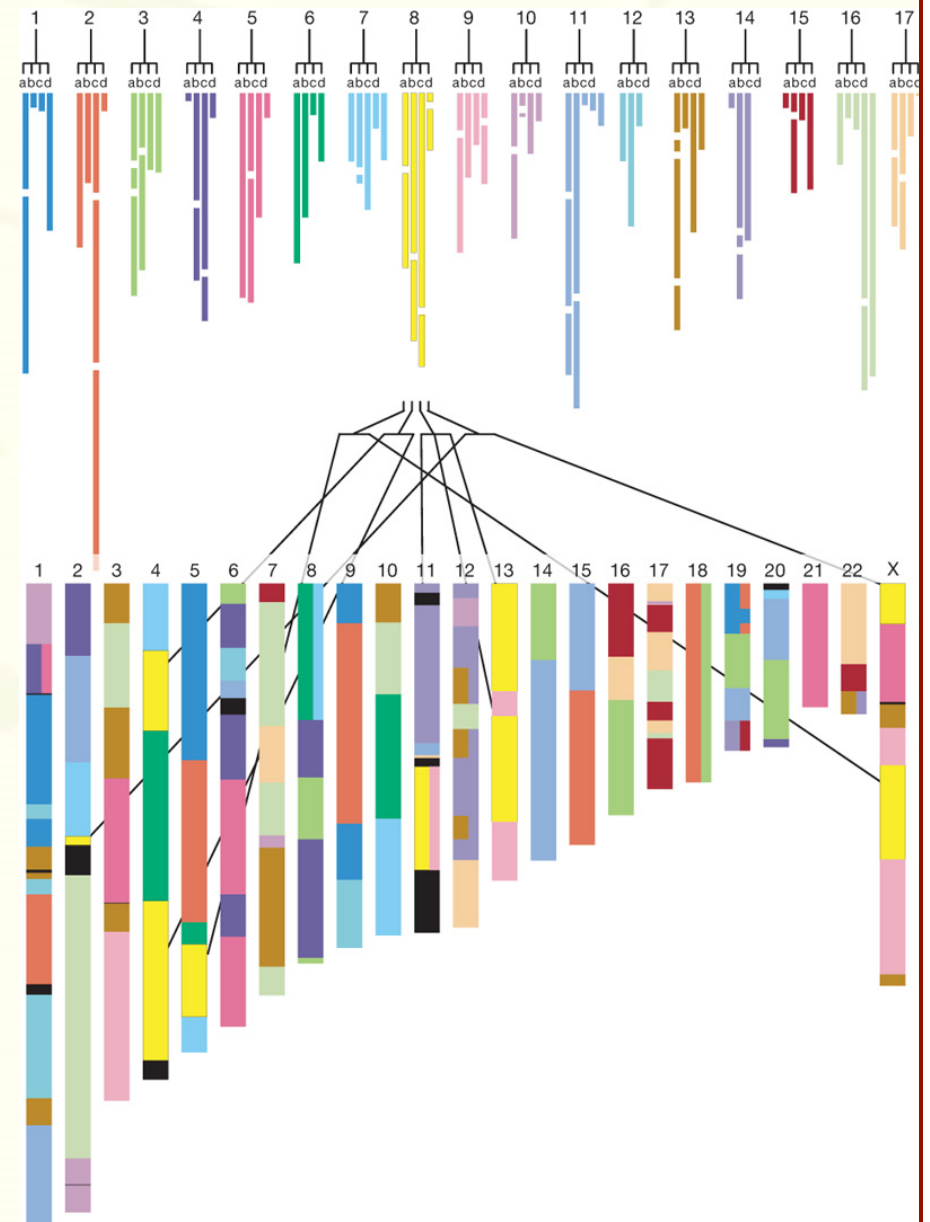
2 rounds of Genome Duplications in Vertebrates



The amphioxus genome and the evolution of the chordate karyotype
Nature 2008



Evidence 1: cluster of 4 paralogs



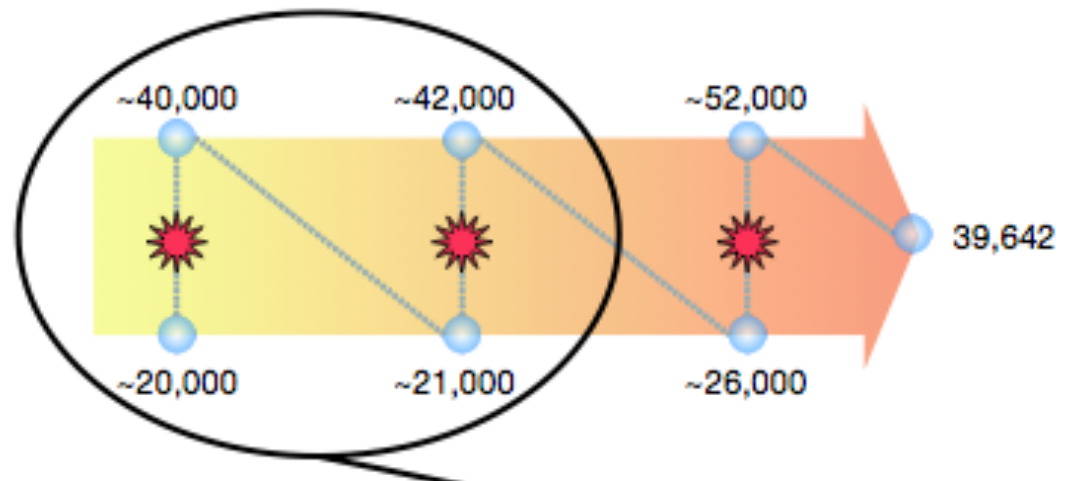
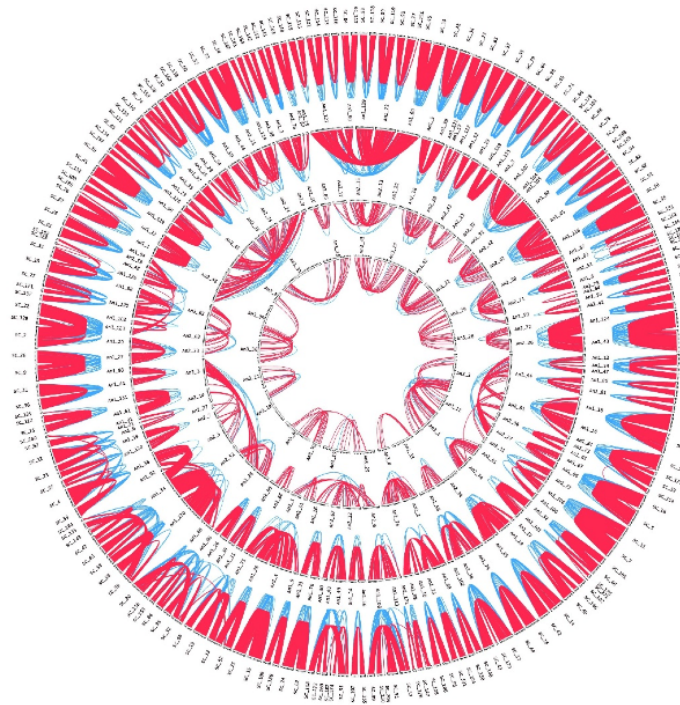
Evidence 2: genome alignment

Paramecium: 3 rounds of duplication

Nature **444**, 171-178 (9 November 2006) | doi:10.1038/nature05230; Received 4 May 2006;
Accepted 31 August 2006; Published online 1 November 2006

Global trends of whole-genome duplications revealed
by the ciliate *Paramecium tetraurelia*

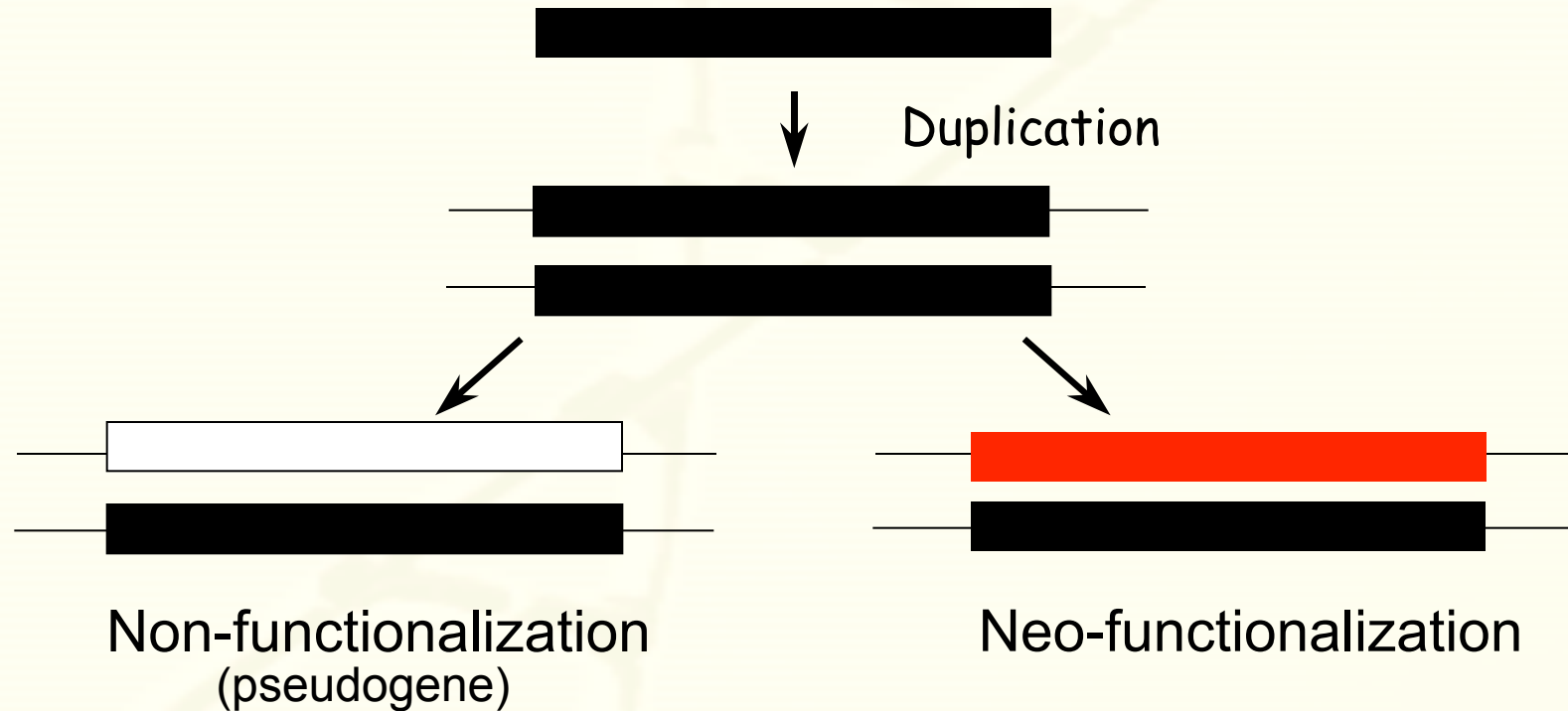
Jean-Marc Aury^{1,9}, Olivier Jaillon^{1,9}, Laurent Duret², Benjamin Noel¹, Claire



Evolution of duplicate genes

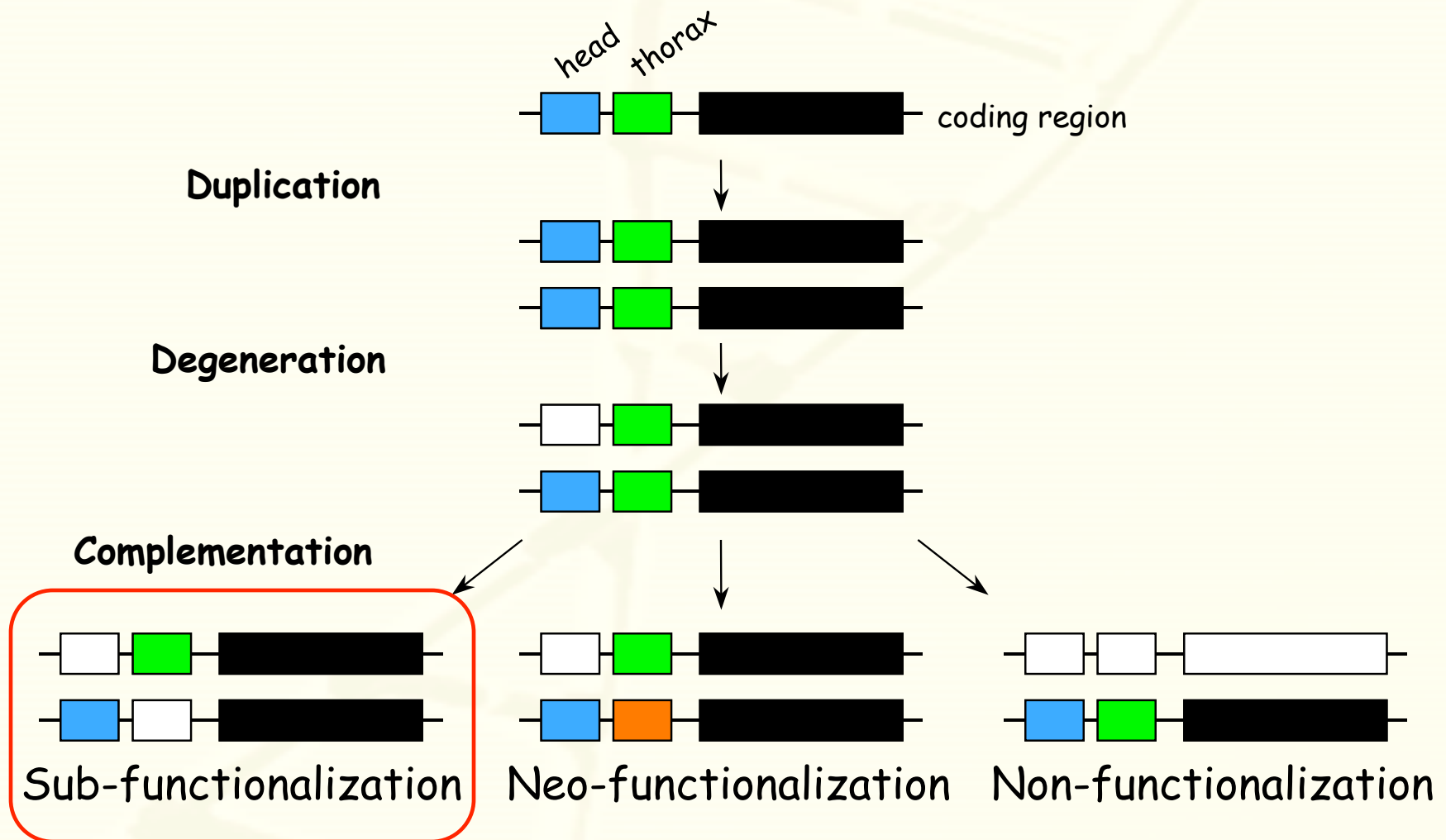
- **Question:** how do duplicate genes (sequence, expression etc) evolve after duplication event ? Do they evolve differently from singleton genes ?
- Yeast has 5800 genes, but has 457 WGD pairs and 130 SSD pairs. i.e. 20% of all the genes.

The Fates of duplicated genes: the classical models



Ohno, 1970s

The duplication-degeneration-complementation (DDC) model



Often the debate on neo- vs sub- functionalization depends on what metric is used to quantify the functional divergence between paralogs.

- Divergence in protein coding sequence
- Divergence in gene expression and transcriptional regulation
- Divergence in protein-protein interaction and protein complexes
- Divergence in fitness effect (measured in deletion mutants)
- Divergence in protein localization
- Divergence in phosphorylation

Trends Genet. 2007 Jun;23(6):266-9. Epub 2007 Apr 10.

Retention of protein complex membership by ancient duplicated gene products in budding yeast.

Musso G, Zhang Z, Emili A.

Proc Natl Acad Sci U S A. 2008 Jan 22;105(3):950-4. Epub 2008 Jan 16.

The evolutionary dynamics of the *Saccharomyces cerevisiae* protein interaction network after duplication.

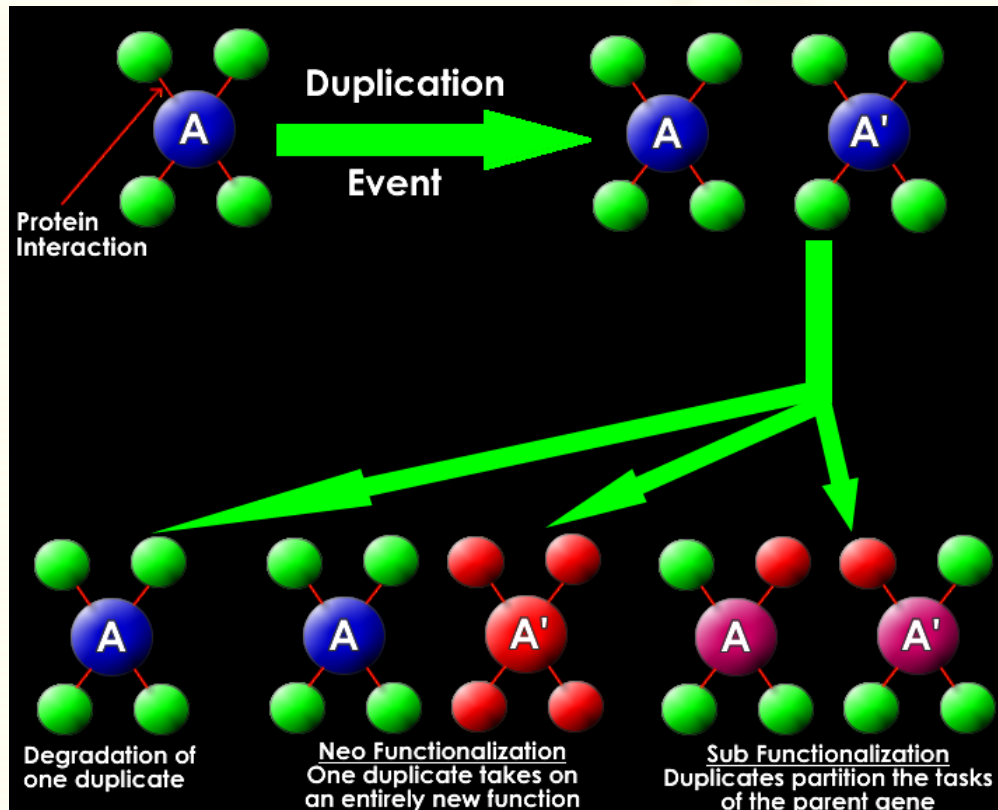
Presser A, Elowitz MB, Kellis M, Kishony R.

Genome Biol. 2008;9(8):R132. Epub 2008 Aug 26.

Preferential regulation of duplicated genes by microRNAs in mammals.

Li J, Musso G, Zhang Z.

Divergence in protein-protein interactions



60% of paralogs are
in the same complex

Genome Analysis

Retention of protein complex membership by ancient duplicated gene products in budding yeast

Musso et al Trends in Genetics 2007

The Cellular Robustness by Genetic Redundancy in Budding Yeast

Jingjing Li^{1,2,3*}, Zineng Yuan^{1,2,3}, Zhaolei Zhang^{1,2,3*}

[Mol Biol Evol.](#) 2010 Jul;27(7):1461-6. Epub 2010 Feb 5.

Revisiting the contribution of cis-elements to expression divergence between duplicated genes: the role of chromatin structure.

[Li J](#), [Yuan Z](#), [Zhang Z](#).

[Nucleic Acids Res.](#) 2011 Feb;39(3):837-47. Epub 2010 Oct 8.

Evidences for increased expression variation of duplicate genes in budding yeast: from cis- to trans-regulation effects.

[Dong D](#), [Yuan Z](#), [Zhang Z](#).

Posttranslational regulation impacts the fate of duplicated genes

Grigoris D. Amoutzias^{a,b,1}, Ying He^{a,b,1}, Jonathan Gordon^{a,b}, Dimitris Mossialos^c, Stephen G. Oliver^{d,2}, and Yves Van de Peer^{a,b,3}

Phosphorylation network rewiring by gene duplication

Luca Freschi^{1,2,3}, Mathieu Courcelles^{4,5}, Pierre Thibault^{4,5}, Stephen W Michnick⁵ and Christian R Landry^{1,2,3,*}

Functional diversification of duplicate genes through subcellular adaptation of encoded proteins

Ana C Marques, Nicolas Vinckenbosch, David Brawand and Henrik Kaessmann

Evolution of *cis*-regulatory elements in duplicated genes of yeast

Balázs Papp^{1,2}, Csaba Pál^{1,2} and Laurence D. Hurst¹

Rapid evolution of expression and regulatory divergences after yeast gene duplication

Xun Gu^{†‡§}, Zhongqi Zhang[‡], and Wei Huang[‡]

Preferential Duplication of Conserved Proteins in Eukaryotic Genomes

Jerel C. Davis^{*} and Dmitri A. Petrov

Functional Diversification of Paralogous Transcription Factors via Divergence in DNA Binding Site Motif and in Expression

Larry N. Singh, Sridhar Hannenhalli^{*}

Pervasive and Persistent Redundancy among Duplicated Genes in Yeast

E. Jedediah Dean^{1*}, Jerel C. Davis², Ronald W. Davis¹, Dmitri A. Petrov²

Functional diversification of duplicate genes through subcellular adaptation of encoded proteins

Ana C Marques, Nicolas Vinckenbosch, David Brawand and
Henrik Kaessmann

Prevalence of intron gain over intron loss in the evolution of paralogous gene families

Vladimir N. Babenko, Igor B. Rogozin, Sergei L. Mekhedov and Eugene V. Koonin*

Dosage sensitivity and the evolution of gene families in yeast

Balázs Papp*†, Csaba Pál*† & Laurence D. Hurst*

The (In)dependence of Alternative Splicing and Gene Duplication

David Talavera^{1,2}*, Christine Vogel^{3,4}*, Modesto Orozco^{1,2,5,6}, Sarah A. Teichmann³, Xavier de la Cruz^{1,2,7}*

Alternative splicing and gene duplication are inversely correlated evolutionary mechanisms

Naama M Kopelman¹, Doron Lancet¹ & Itai Yanai^{1,2}

The Role of *Cis*-Regulatory Motifs and Genetical Control of Expression in the Divergence of Yeast Duplicate Genes

Lindsey J. Leach, Ze Zhang,* Chenqi Lu,† Michael J. Kearsey,* and Zewei Luo*†*

Preferential Duplication in the Sparse Part of Yeast Protein Interaction Network

Li Li, Yingwu Huang, Xuefeng Xia, and Zhirong Sun

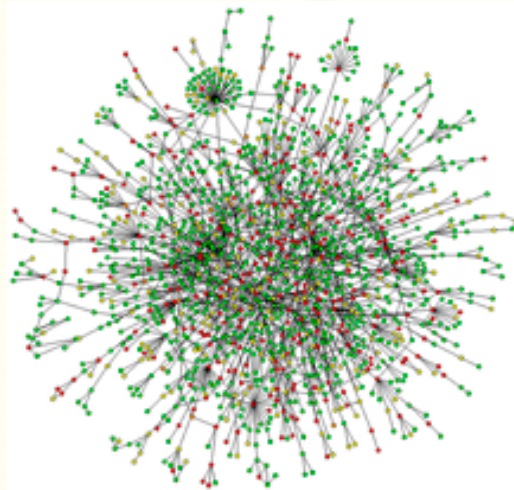
Protein Function, Connectivity, and Duplicability in Yeast

Anuphap Prachumwat and Wen-Hsiung Li†*

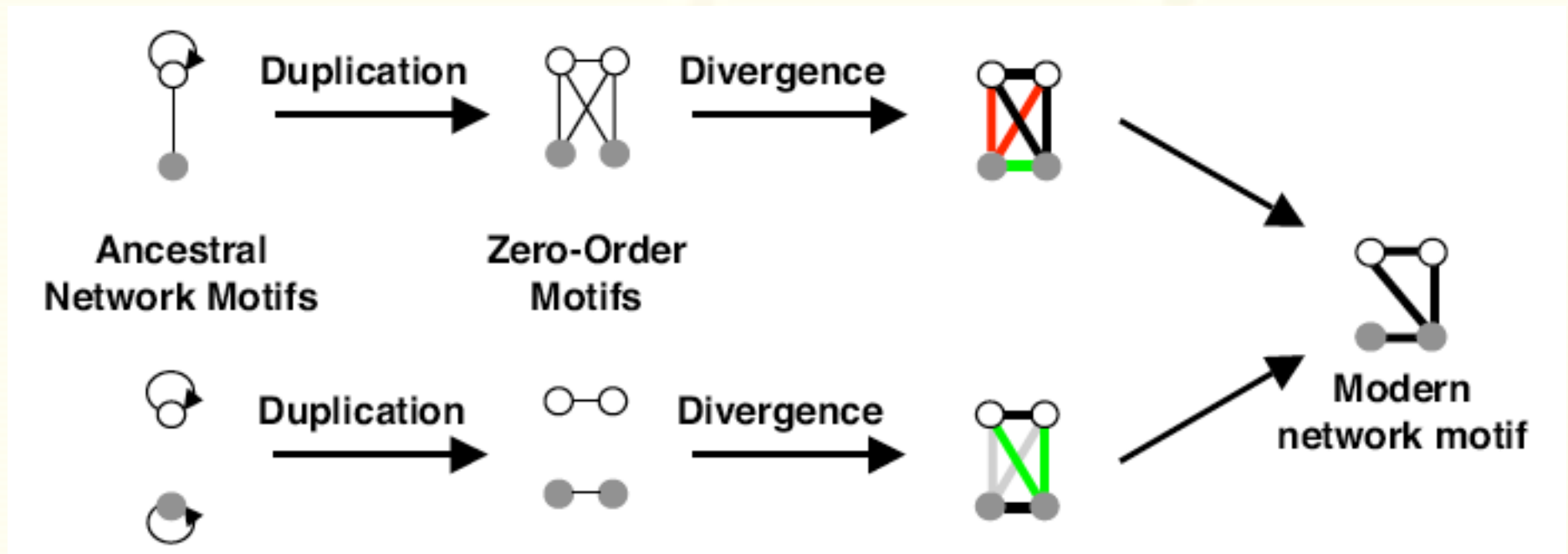
The complex relationship of gene duplication and essentiality

Takashi Makino, Karsten Hokamp and Aoife McLysaght

How do PPI network evolve after WGD ?



Duplication and divergence of network motifs



Gain of interactions



Loss of interactions

Duplicate genes provide robustness against null mutations

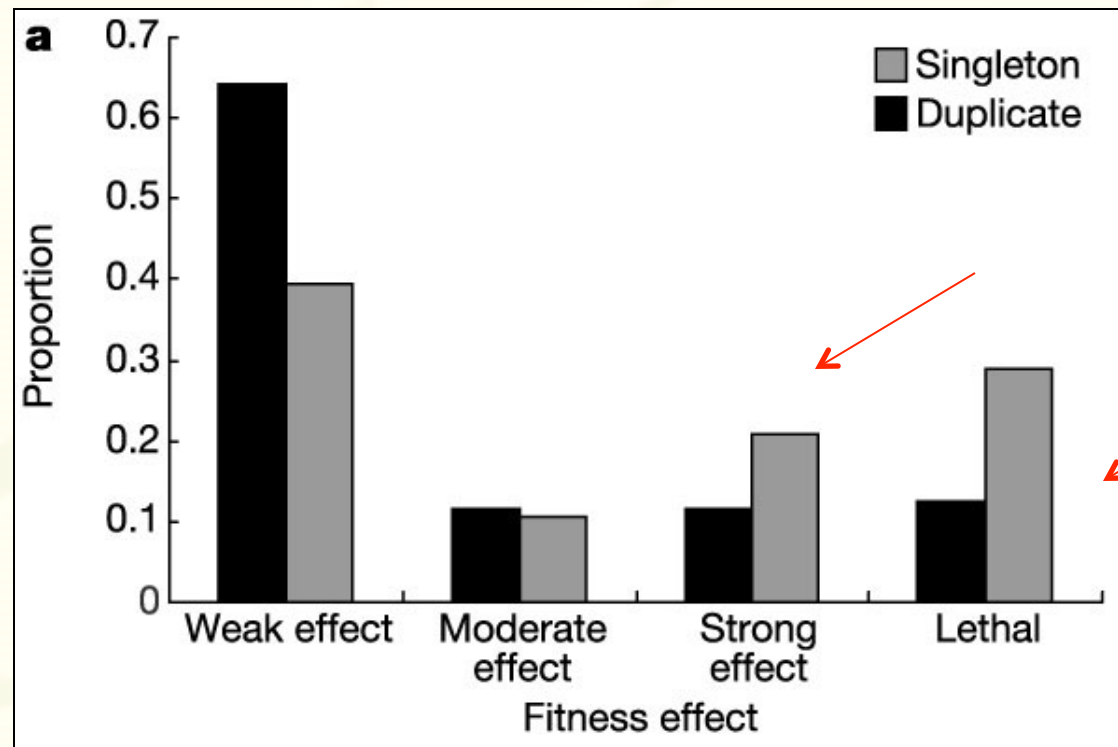
- **Question:** Why are so many duplicate genes kept in the genome ?
- **Hypothesis:** these duplicate genes are maintained because they are needed to provide protection against deleterious mutations on a single gene.
- **Testable experiments:**
 - Deleting a duplicate gene has less fitness effect than deleting a singleton gene
 - Deleting both paralogs will have severe fitness effects

Deleting just one paralog

Nature **421**, 63-66 (2 January 2003) | doi:10.1038/nature01198; Received 29 July 2002; Accepted 16 September 2002

Role of duplicate genes in genetic robustness against null mutations

Zhenglong Gu^{1,2}, Lars M. Steinmetz^{3,2}, Xun Gu⁴, Curt Scharfe³, Ronald W. Davis³ & Wen-Hsiung Li¹



Deleting both paralogs (using SGA)

Genome Res. 2008 Jul;18(7):1092-9. Epub 2008 May 7.

The extensive and condition-dependent nature of epistasis among whole-genome duplicates in yeast.

Musso G, Costanzo M, Huangfu M, Smith AM, Paw J, San Luis BJ, Boone C, Glaever G, Nislow C, Emili A, Zhang Z.

OPEN  ACCESS Freely available online

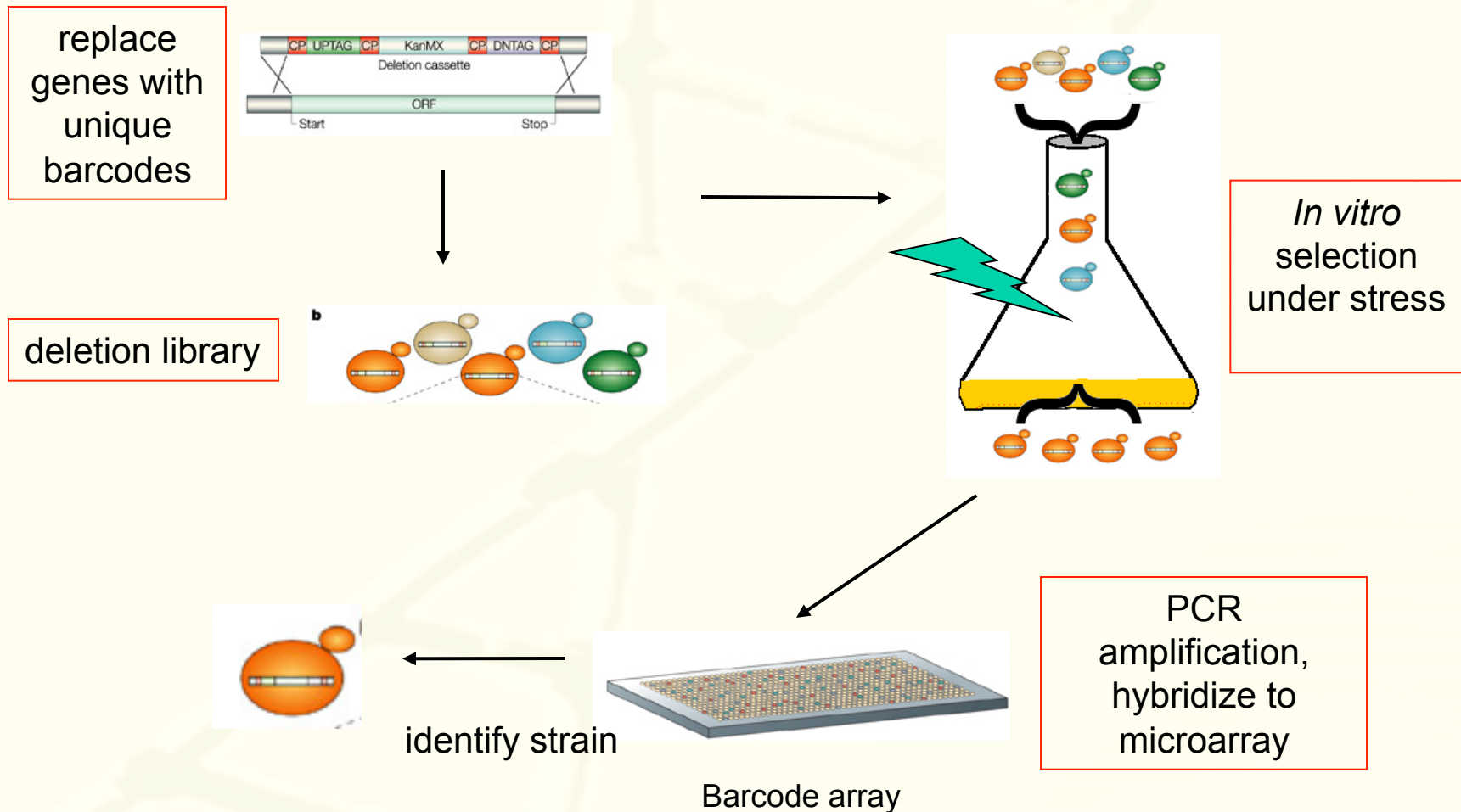
PLoS GENETICS

Pervasive and Persistent Redundancy among Duplicated Genes in Yeast

E. Jedediah Dean^{1*}, Jerel C. Davis², Ronald W. Davis¹, Dmitri A. Petrov²

For more than 1/3 of duplicate pairs,
double mutants are inviable

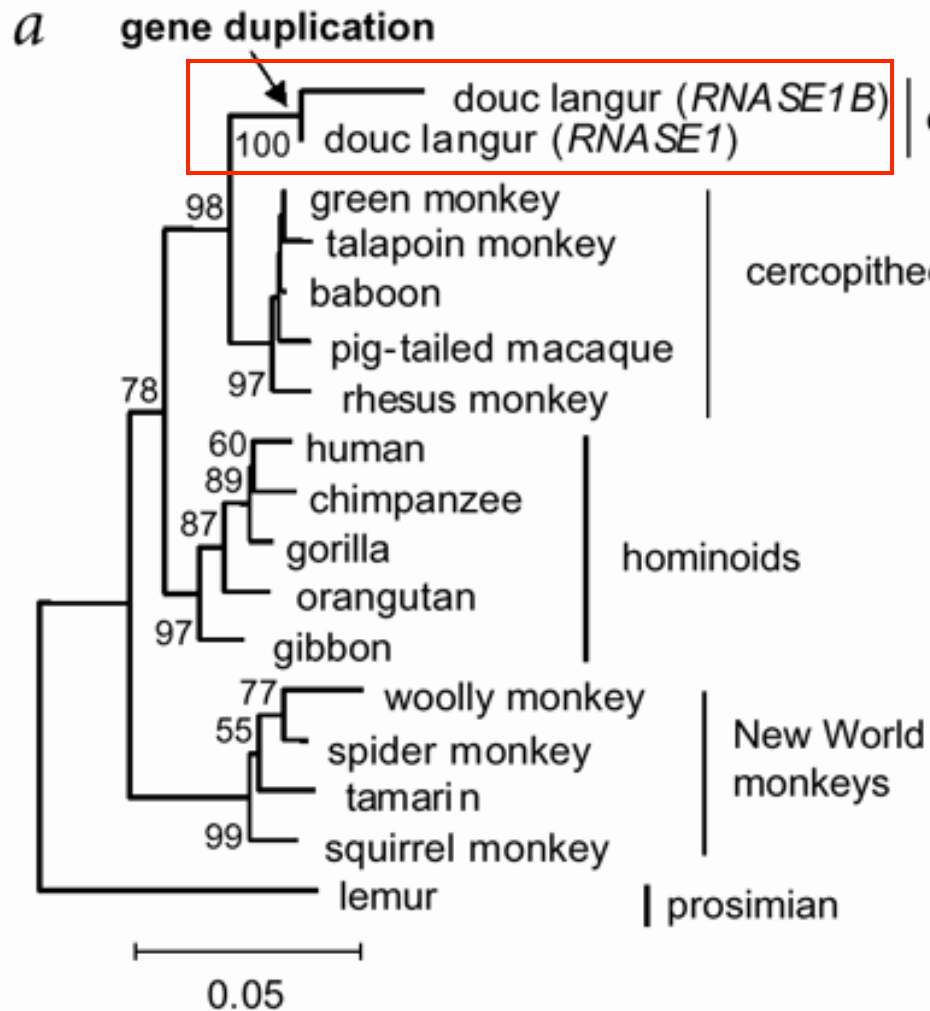
Many more gene pairs are required for survival when cells are under stress or environmental perturbation



Gene duplication can facilitate speciation

- **Two major mechanisms:**
- **Hybrid incompatibility:**
 - Functional divergence of paralogs create reproduction barrier
- **Gain of function:**
 - adaptive evolution of one paralog gene accelerates the adaptation to specific habitat or diet

Gene duplication can facilitate speciation event



Leaf-eating monkeys

The duplicated RNase allows the monkey to digest leaves, the only species of primates.



Curr Biol. 2009 Oct 13;19(19):1642-7. Epub 2009 Sep 3.

Duplication of *fgfr1* permits Fgf signaling to serve as a target for selection during domestication.

Rohner N, Bercsényi M, Orbán L, Kolanczyk ME, Linke D, Brand M, Nüsslein-Volhard C, Harris MP.

- Fgfr is an essential gene, important in vertebrate embryonic development.
- Duplication of this gene releases selective pressure on this gene and allows mutation accumulation, which result in reduction of fish scale.



common carp

wikipedia

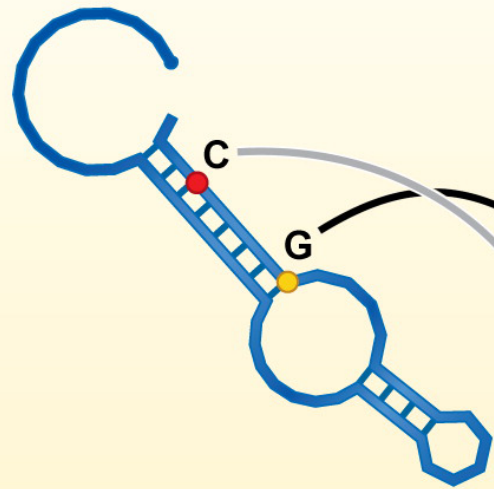


mirror carp

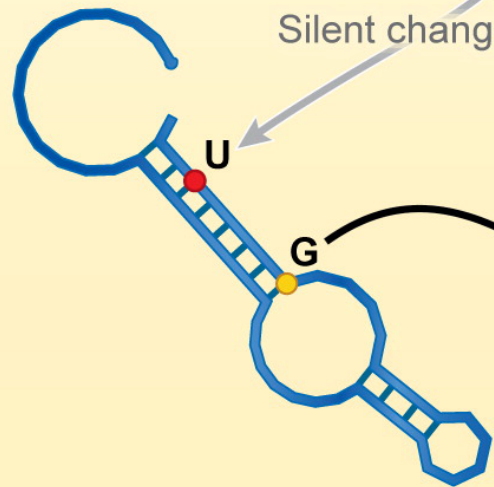
Summary

- Gene duplication and genome duplication are very prevalent.
- Three different evolutionary fates of duplicate genes
- Gene duplication can provide protection against mutations and deletions
- Gene duplication can also facilitate speciation.

Wild-type genotypes

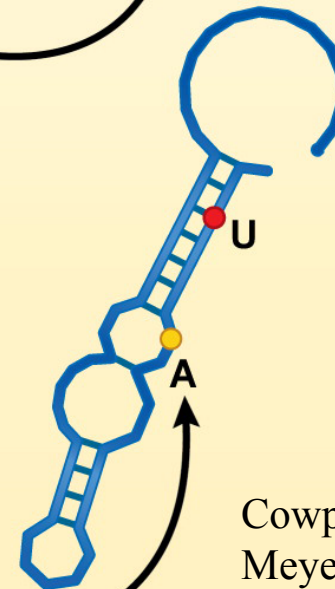
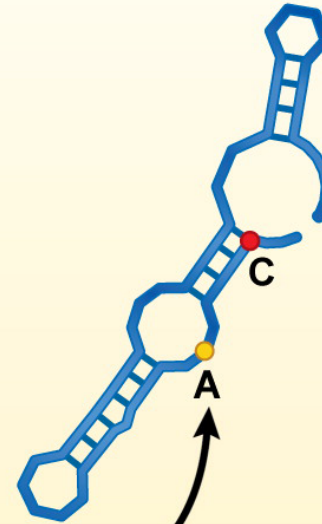


Silent change



Subsequent mutation

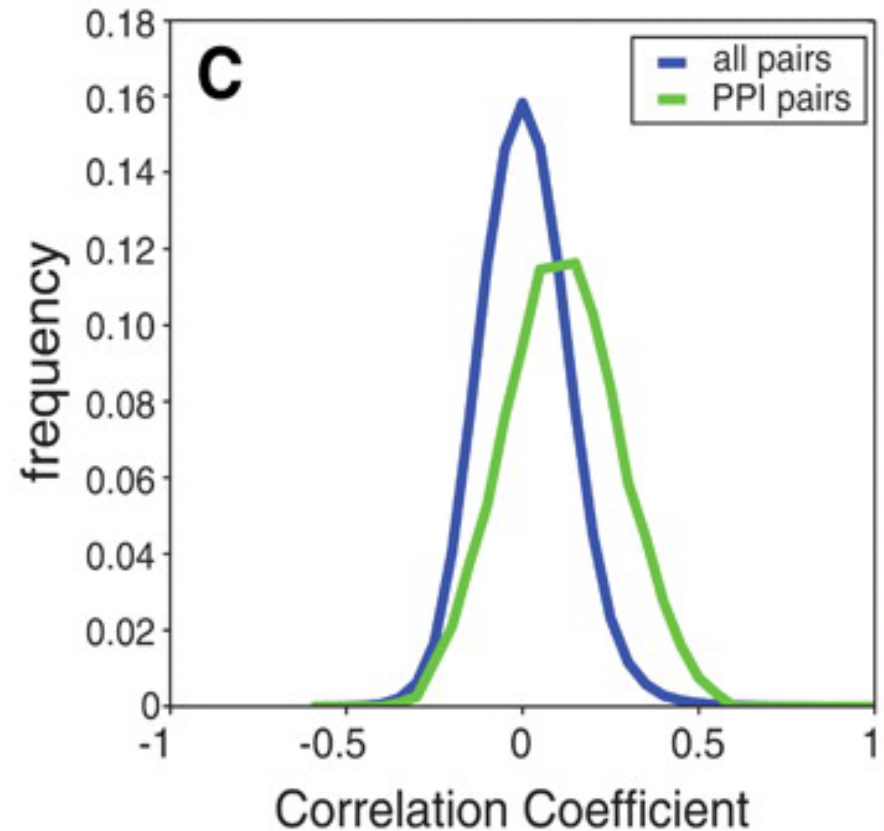
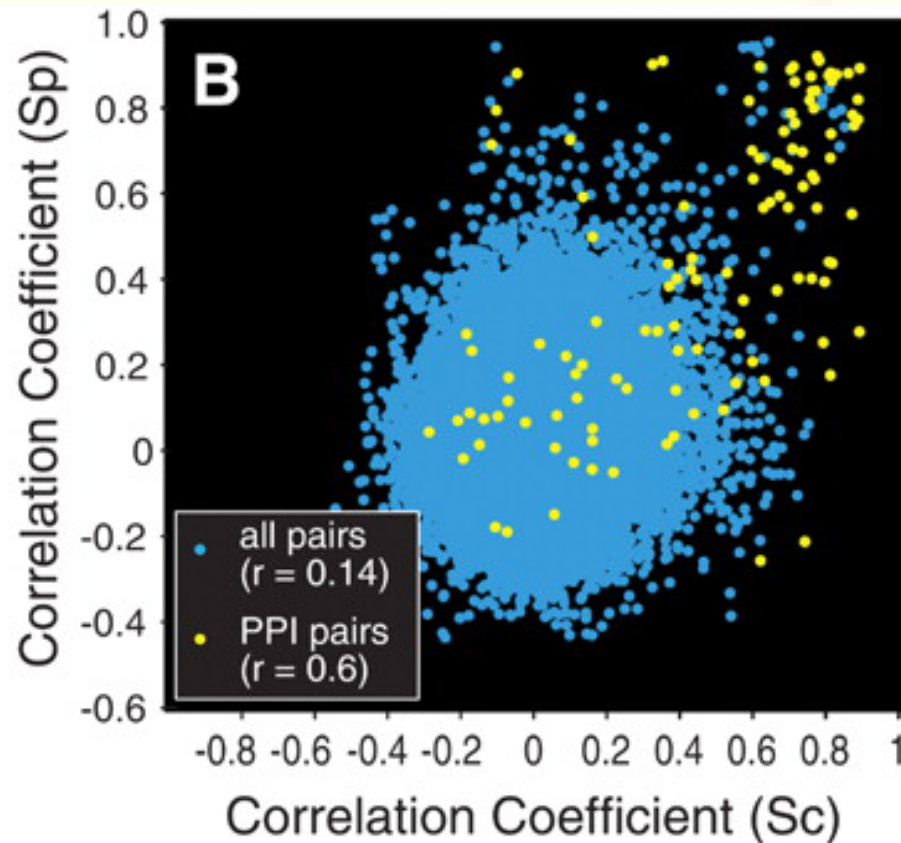
Mutant genotypes



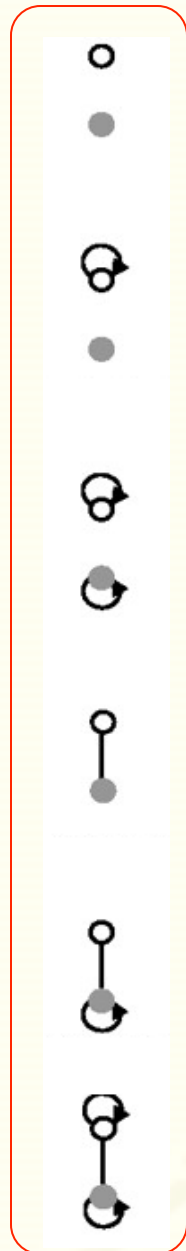
Epistasis in RNA evolution

Cowperthwaite and Lauren Ancel Meyers: How Mutational Networks Shape Evolution: Lessons from RNA Models, Annual Review of Ecology, Evolution, 2007

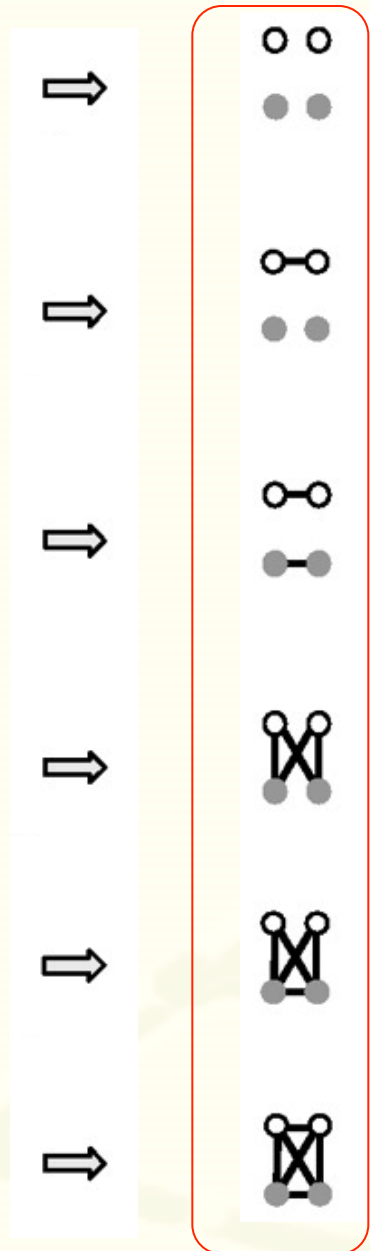
Genetic interactions between genes that physically interact are more conserved



ancestral

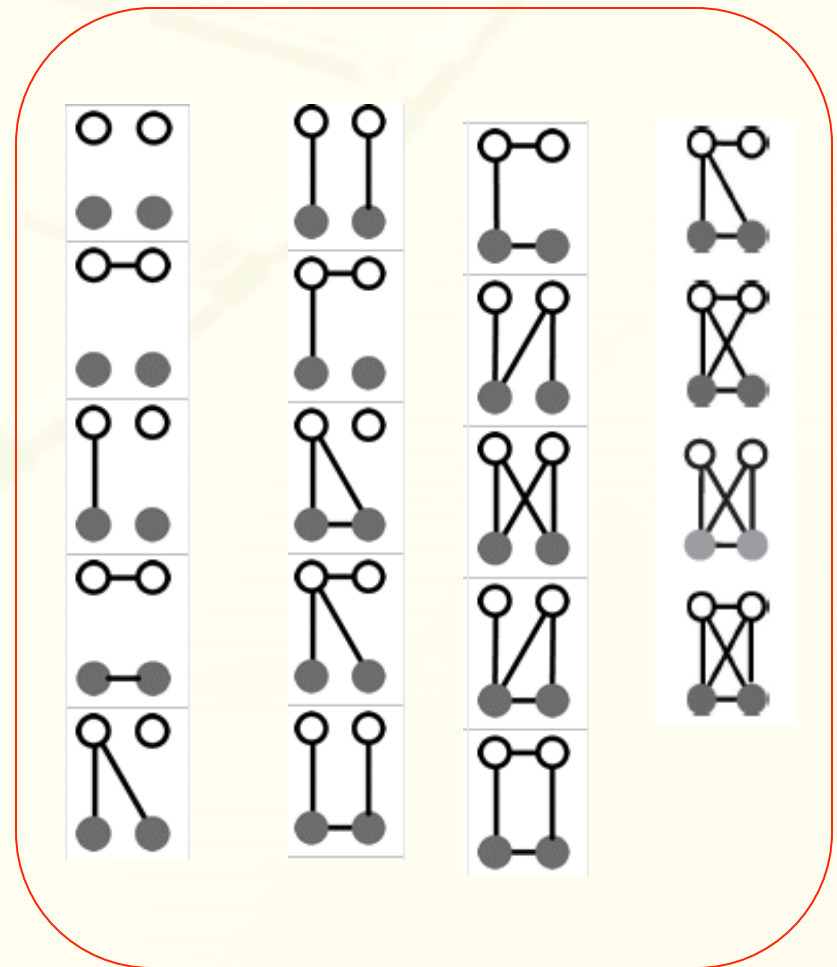


After WGD



How do PPI network evolve after WGD ?

Present



Presser, et al, PNAS 2008. *The Evolutionary dynamics of the yeast protein interaction network after duplication*