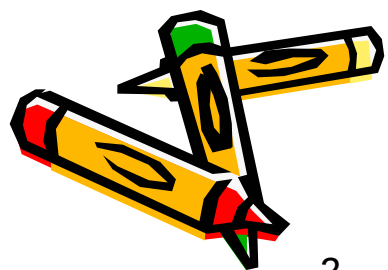
# Assignment

- Strategy for protein-protein interaction
  - Induced proteolysis of yeast DNA replication proteins
    - *Nature*, 2003, 423, 720-724
    - 鄧文豪
  - msSUS for membrane proteins
    - *PNAS*, 2004, 101, 12242-12247
    - 張淳淳
  - Leuciferase complementation in cells and living animals
    - *PNAS*, 2004, 101, 12288-12293
    - 簡禎瑩
  - Luminescence-based mammalian interactome mapping (LUMIER)
    - Science, 2005, 307, 1621-1625
    - 許禮汎
  - TAP for *E. coli* interactome
    - Nature, 2005, 433, 531-537
    - 葉明鑫
- Presentation
  - Date: 3/31, 20 min. each person

# New high-throughput strategies

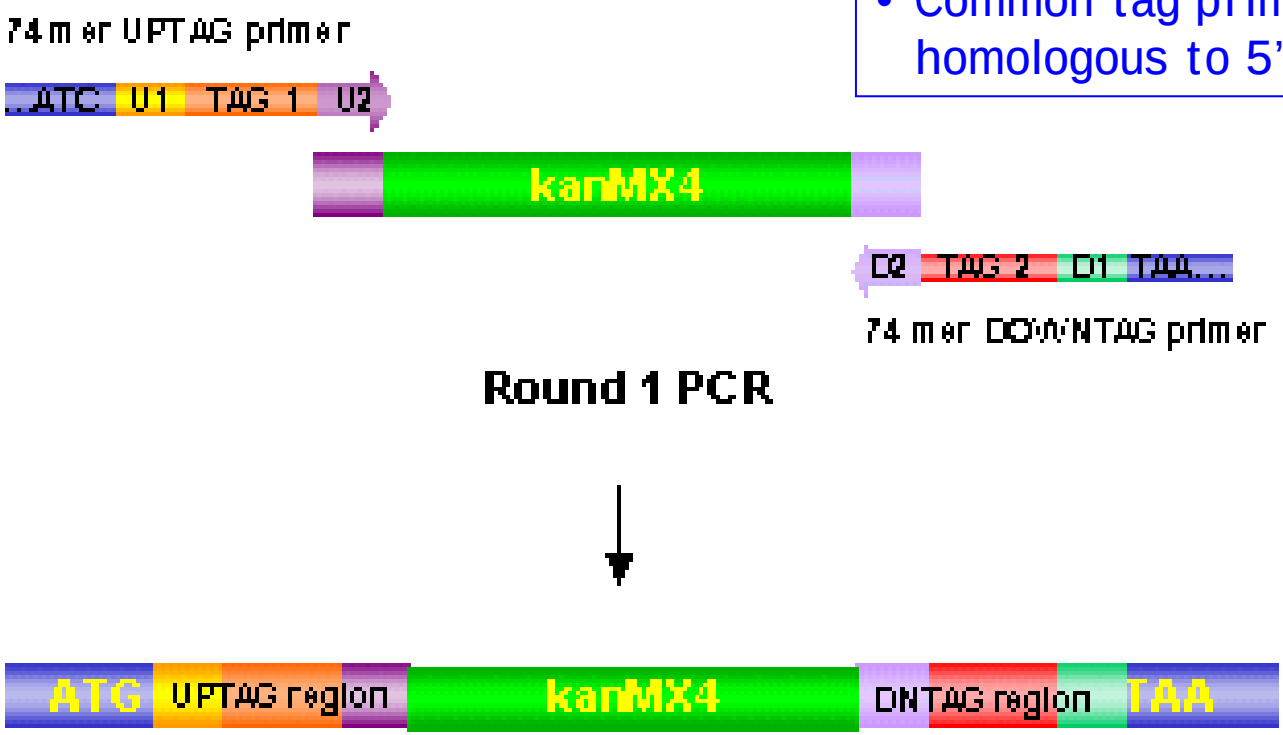
- What it is ?
  - Molecular function
  - *Genomic Knockout*
    - Random transposon tagging (yeast)
      - Michael Snyder at Yale
    - Directed PCR based mutagenesis (yeast)
      - Barcode
      - Ron Davis at Standford
    - RNAi (*C. elegans*)
    - ???



# Primer Design

- Nucleotide Barcode

- Homology to ORF upstream
- Common tag priming site (U1)
- UPTAG (20 bases)
- Common tag priming site (U2) homologous to 5' to the Kan gene

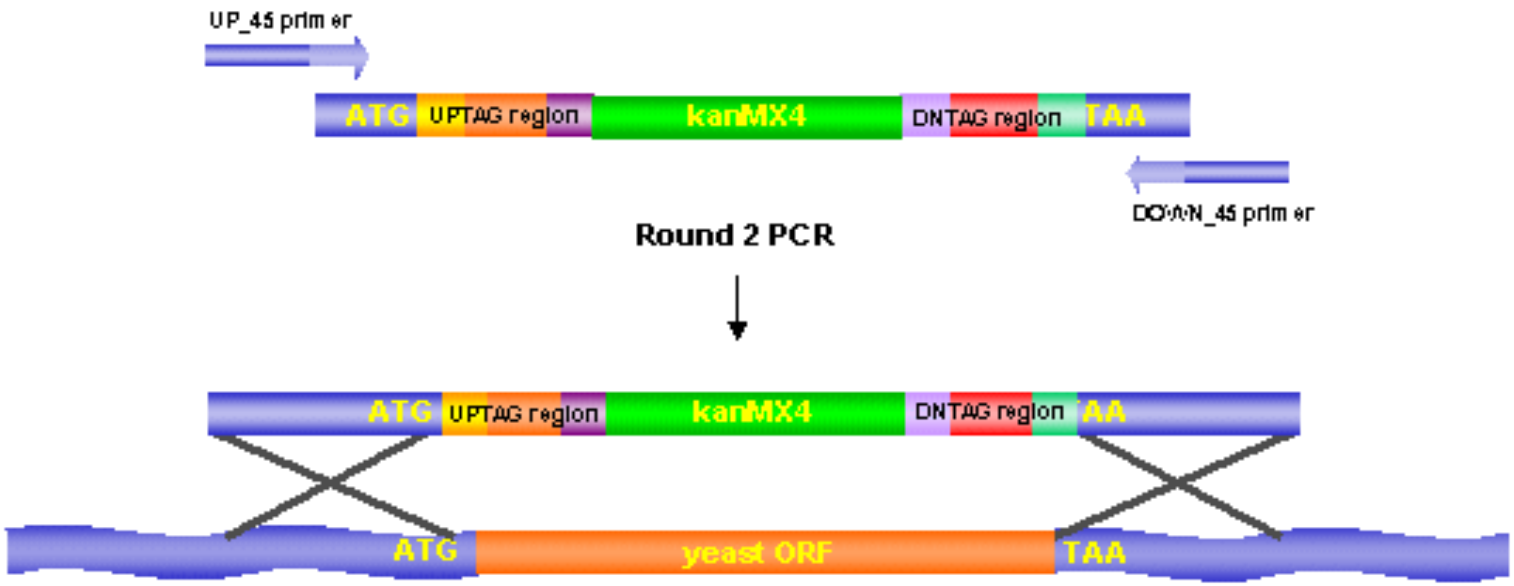





http://www-sequence.stanford.edu/group/yeast_deletion_project/PCR_strategy.html

# PCR-based Gene Deletion

- Deletion strategy
  - PCR primer
    - Barcode tag
    - 50 bp upstream/downstream of a targeted gene
  - Homologous recombination

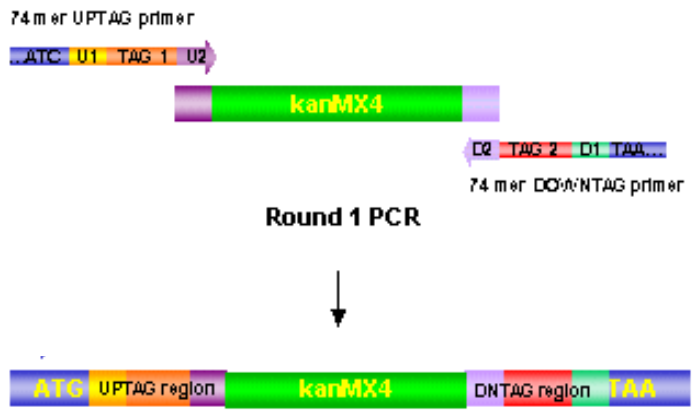



Chromosomal integration by homologous recombination



http://www-sequence.stanford.edu/group/yeast_deletion_project/PCR_strategy.html

# Primer synthesis




- Primers needed
  - 8~10 different primers/ORF
  - Yeast genome: ~ 6000 ORF
- High-throughput primer synthesis
  - Primer-picking scripts
    - Input:
      - ORF data + UPTAG/DOWNTAG list
    - Output:
      - Primer sequences
  - Automated Multiplex Oligonucleotide Synthesizer

# Results

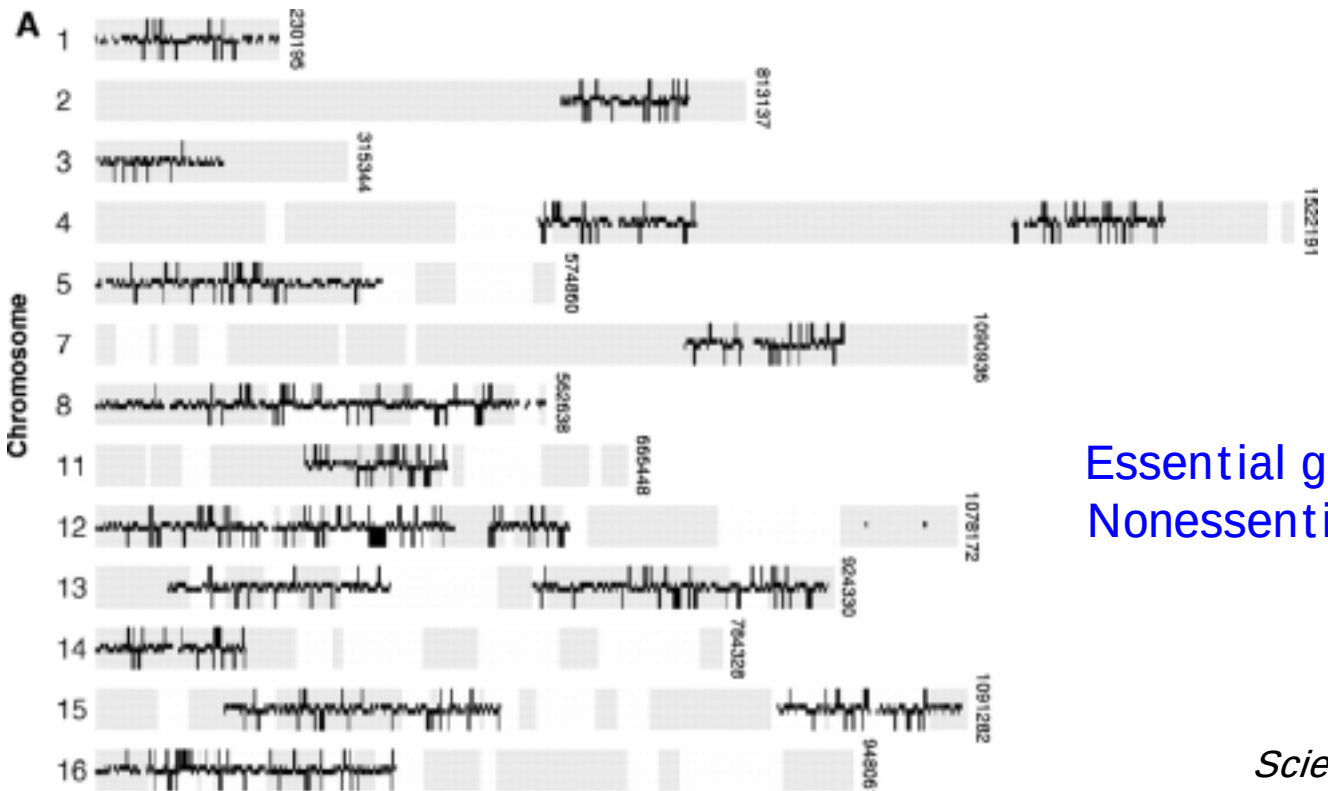
- 6925 deletion strains of yeast constructed…
  - Essential for viability
  - Lack of human homologs
  - Targets for antifungal drugs
- Screen for genes essential for viability
  - Spores from 2026 ORF (1/3 of the genome) heterozygous strains on YPD media at 30°C
    - 356 haploid deletants could not be recovered
    - 1620 ORFs not essential for viability in yeast
      - Construct one additional homozygous and two haploid deletants
- Statistics
  - 8.5% of the identified non-essential ORFs in the yeast genome have a closely related homolog elsewhere (redundancy)
  - 1% of the essential gene have homologs



*Science* 1999 285, 901-906.

# Genomic locations

- Essential genes
  - Distributed evenly across the chromosome
    - Biased toward close to each other (within 5 kb)
    - Not found within 50 kb of the telomeres
  - Heavily transcribed



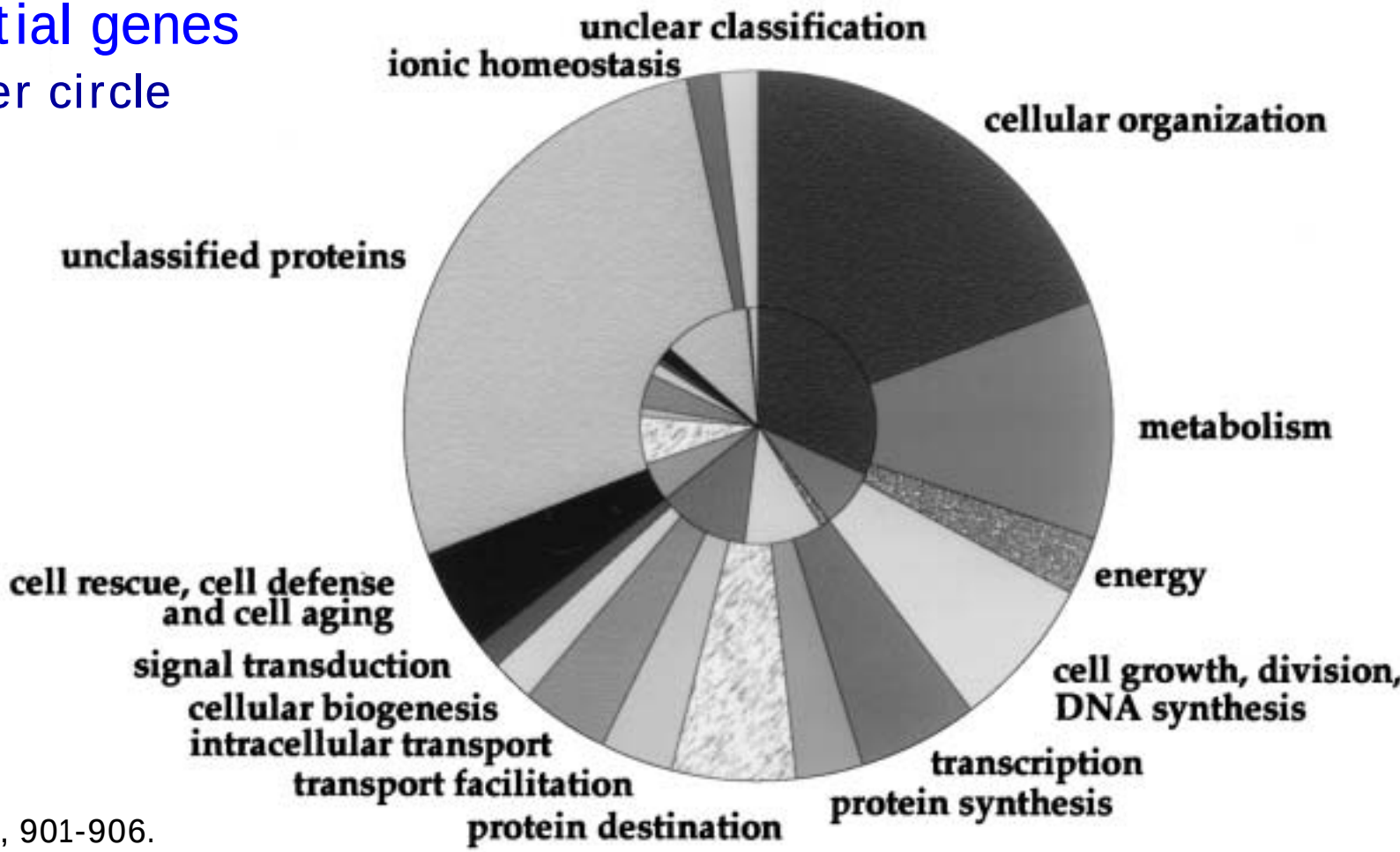

Essential gene: tall bar
Nonessential: short bar



*Science* 1999 285, 901-906.

# Functional class distribution

- Nonessential genes
  - Outer circle
- Essential genes
  - Inner circle




*Science* 1999 285, 901-906.

# Competitive growth assays

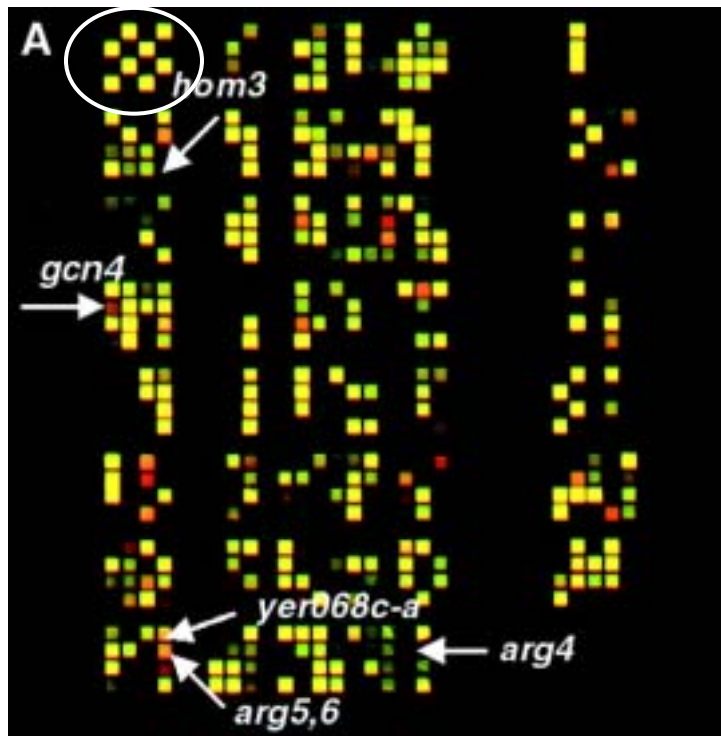
- How to characterize the genes nonessential for viability?
- Pooled functional assay
  - 558 homozygous deletion strains were pooled
  - Grow in rich and minimal media for ~60 generations
  - Remove aliquots from the two pools at various time points
  - Tags were amplified and hybridized to DNA array

Red: grown for 0 hr
Green: grown for 6 hr

Normal growth (expression)

Grow slow (reduced expression)

Grow fast (enhanced expression)




*Science* 1999 285, 901-906.

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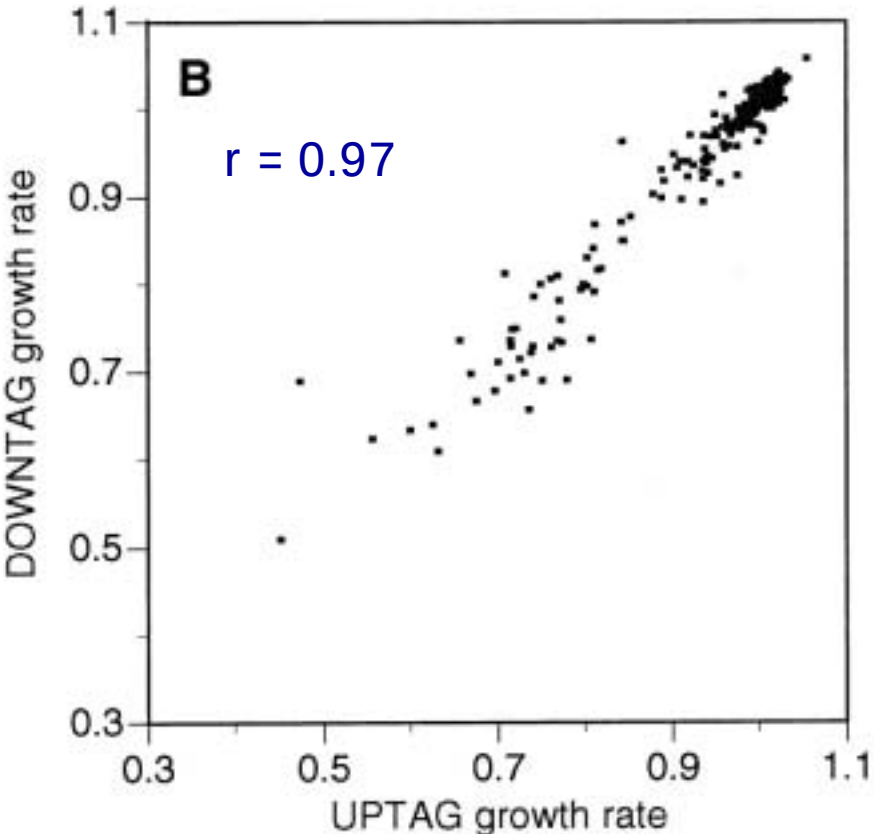
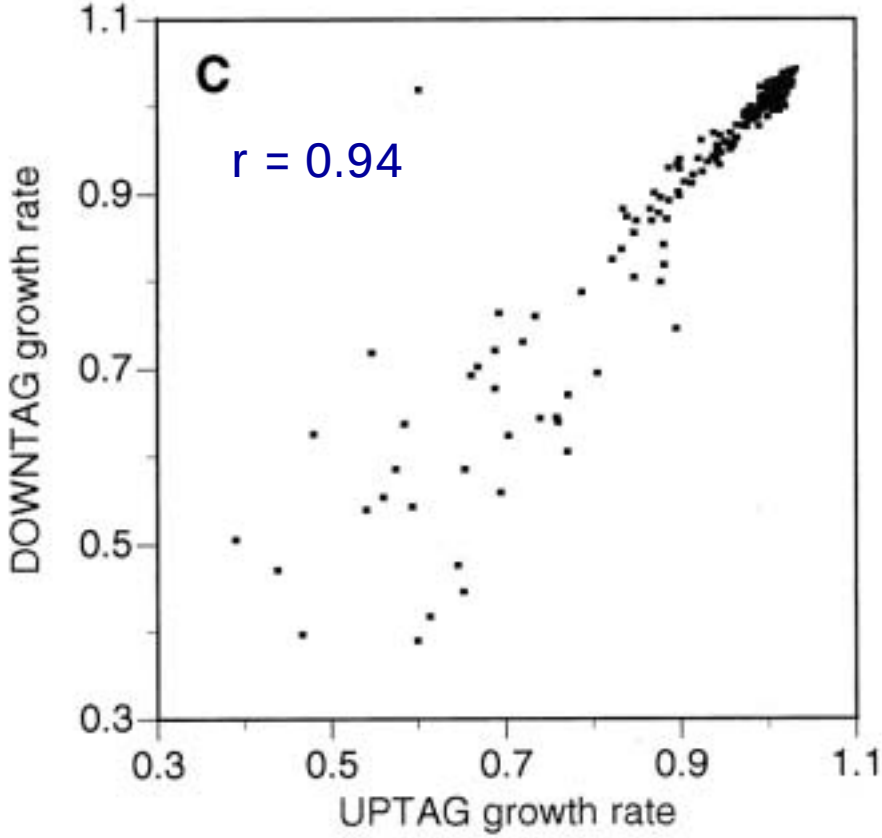
# Correlation of growth rate

- Assumption
  - Growth rate for each strain obtained independently with the UPTAG and DOWNTAG signals would be the same
- Where is the wild type ?
- Why is the correlation coefficient (r) is lower for growth in mim. medium?

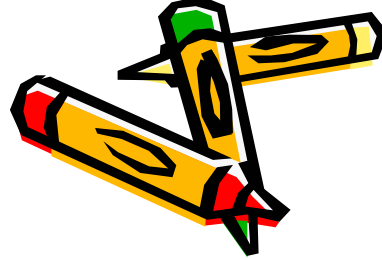
In rich medium




In minimal medium



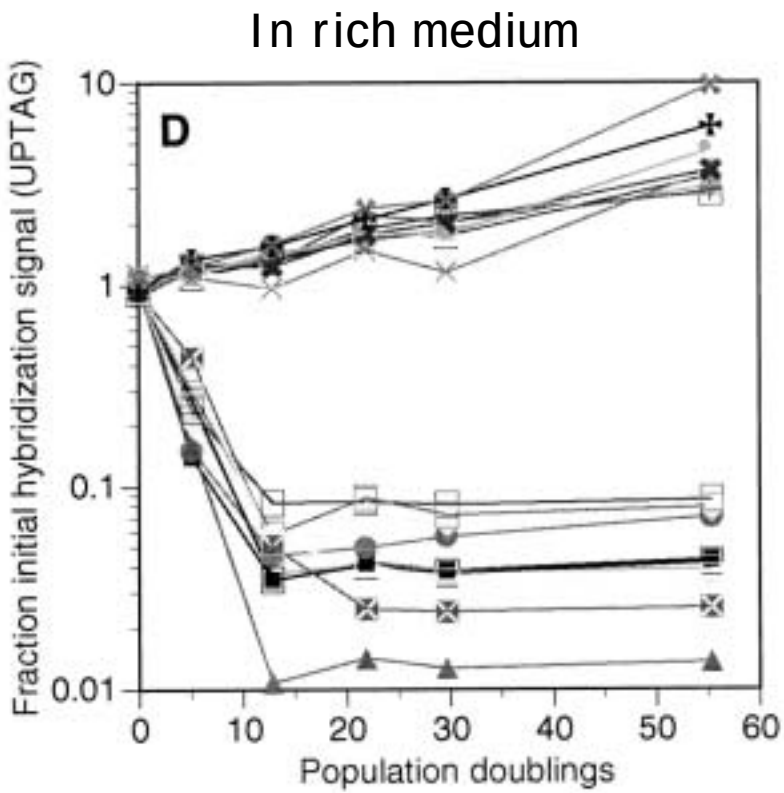
# Normalized growth rate



- Hybridization intensity = growth rate
  - Normal growth = 1
  - Grown fast (abundant) > 1
  - Grown slow (fewer) < 1

Q:

Predict what might happen if only the slowest growing strains were incubated together.



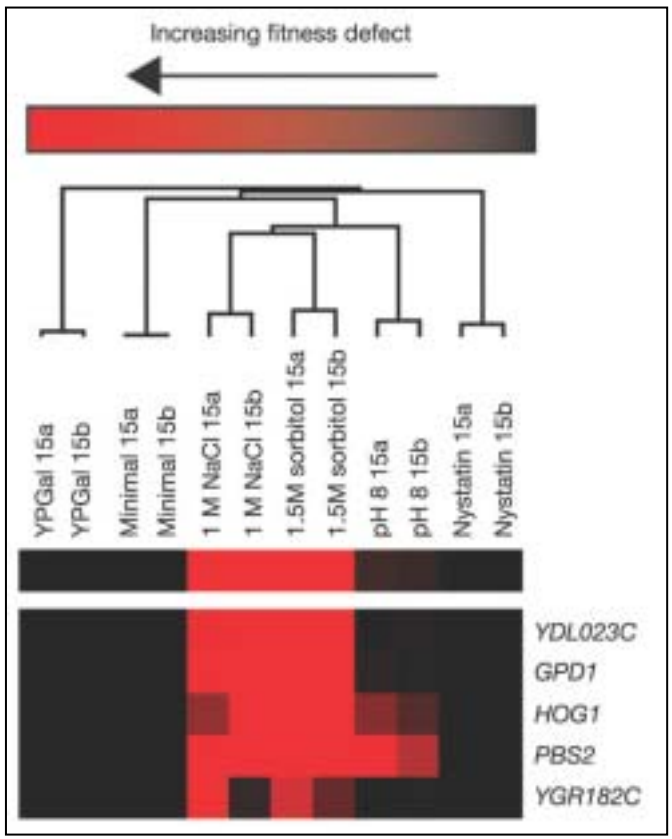

*Science* 1999 285, 901-906.

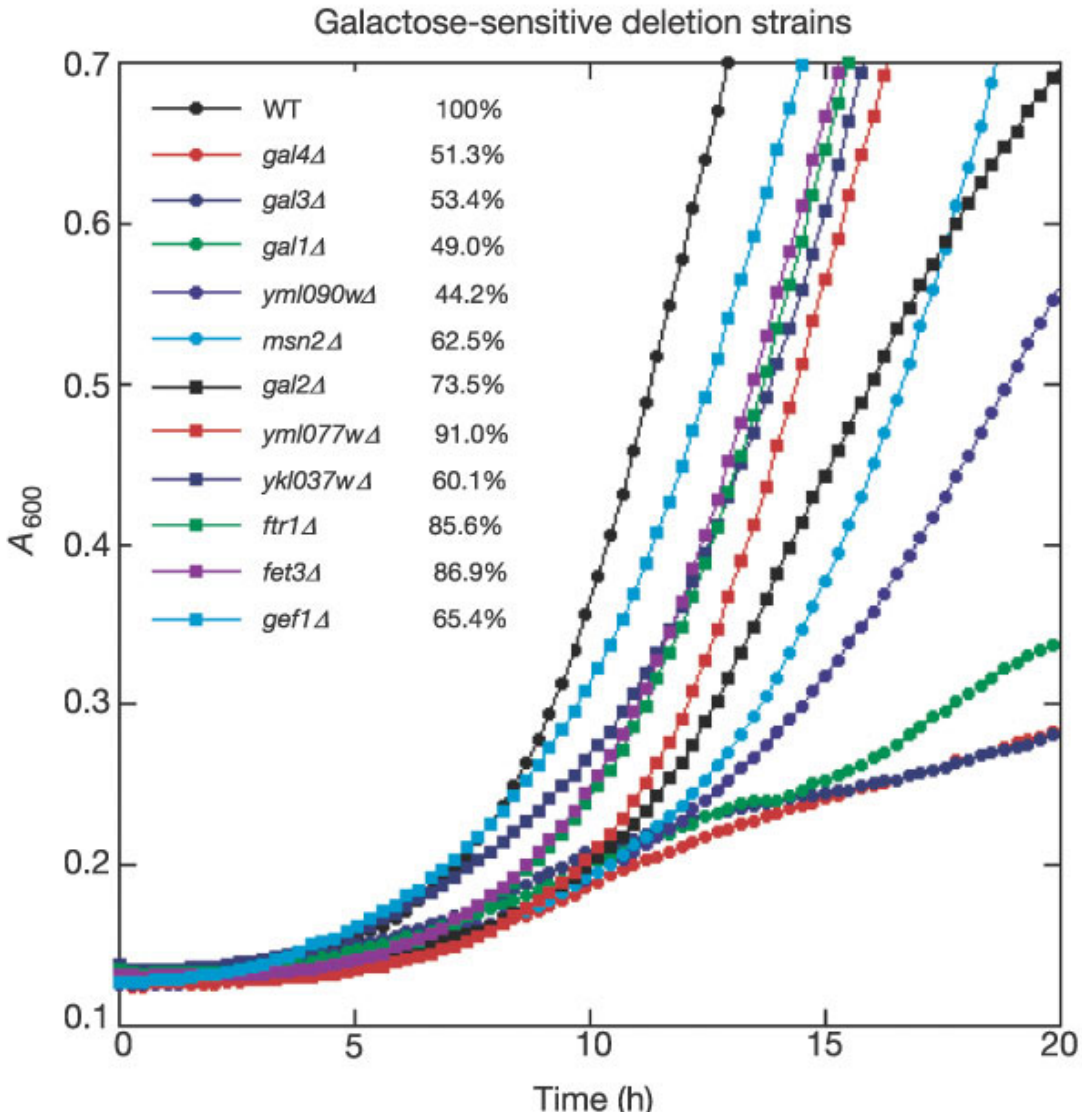
# Further studies …

- “Barcode” mutants
  - High salt
  - Sorbitol
  - Galactose
  - pH 8
  - Minimal medium
  - Nystatin treatment

# Whole-genome parallel analysis

- Fitness profiling
  - C source
- Clustering
  - Osmoregulation




Nature 2002 418, 387-391.

# Fitness vs. Expression profiling

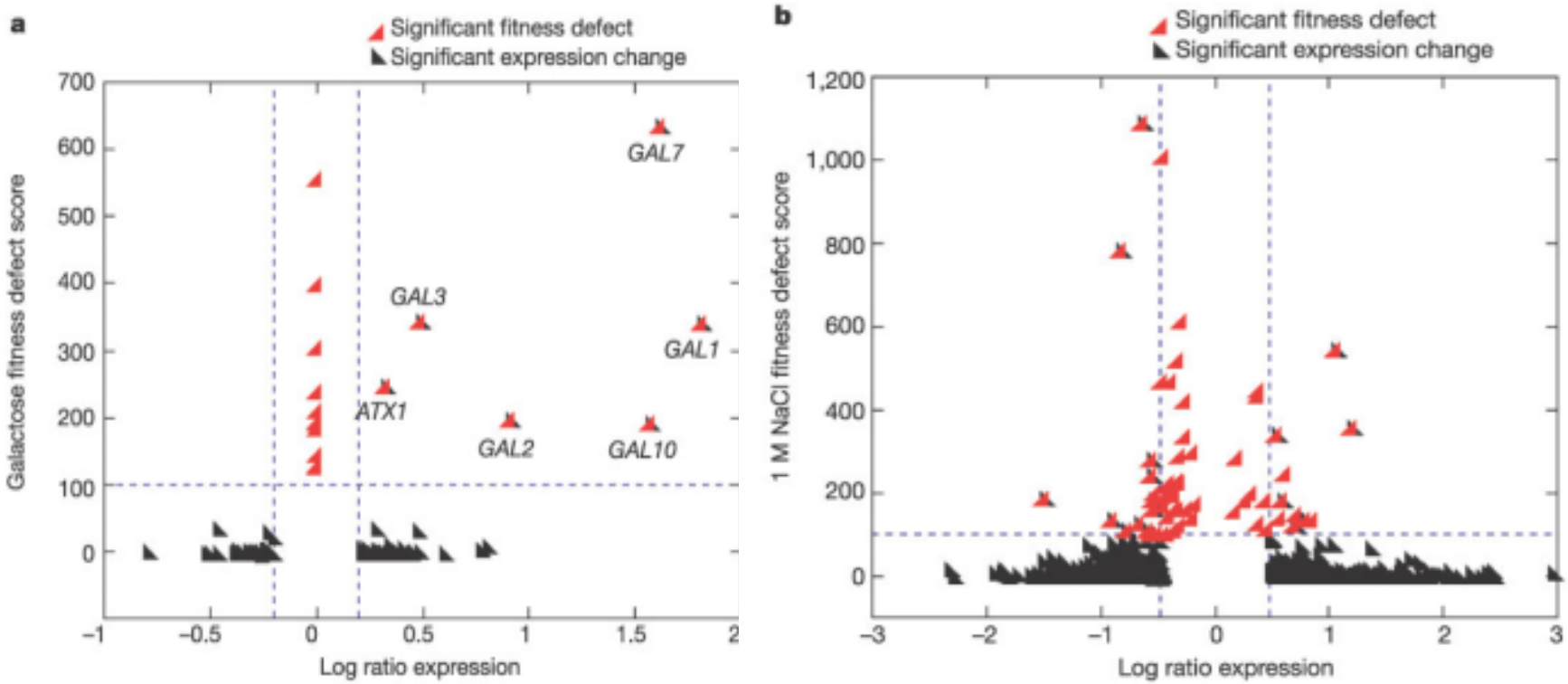
- $H_o$: in a given condition, if a gene expression ↑, then growth ↑.
  - Good fitness → good expression
  - Good expression → good fitness

| Condition | Measured genes | Up*-regulated | Down*-regulated | % Up*-reg. & Fitness defect* | % Down*-reg. & Fitness defect* |
|---|---|---|---|---|---|
| Galactose | 4682 | 99 | 84 | 6.06 | 0.00 |
| Alkali | 4711 | 434 | 464 | 3.00 | 3.23 |
| 1M NaCl | 4711 | 679 | 1047 | 0.88 | 1.15 |
| 1.5 M Sorbitol | 4711 | 588 | 1024 | 0.34 | 0.0 |



Nature 2002 418, 387-391.

# Fitness vs. Expression




Q: (1) Fitness ↑, no Δ expression, *why?*

(2) Expression ↑, no Δ fitness, *why?*



Nature 2002 418, 387-391.

# Comparison

- mTn method
- Pros:
- Cons:

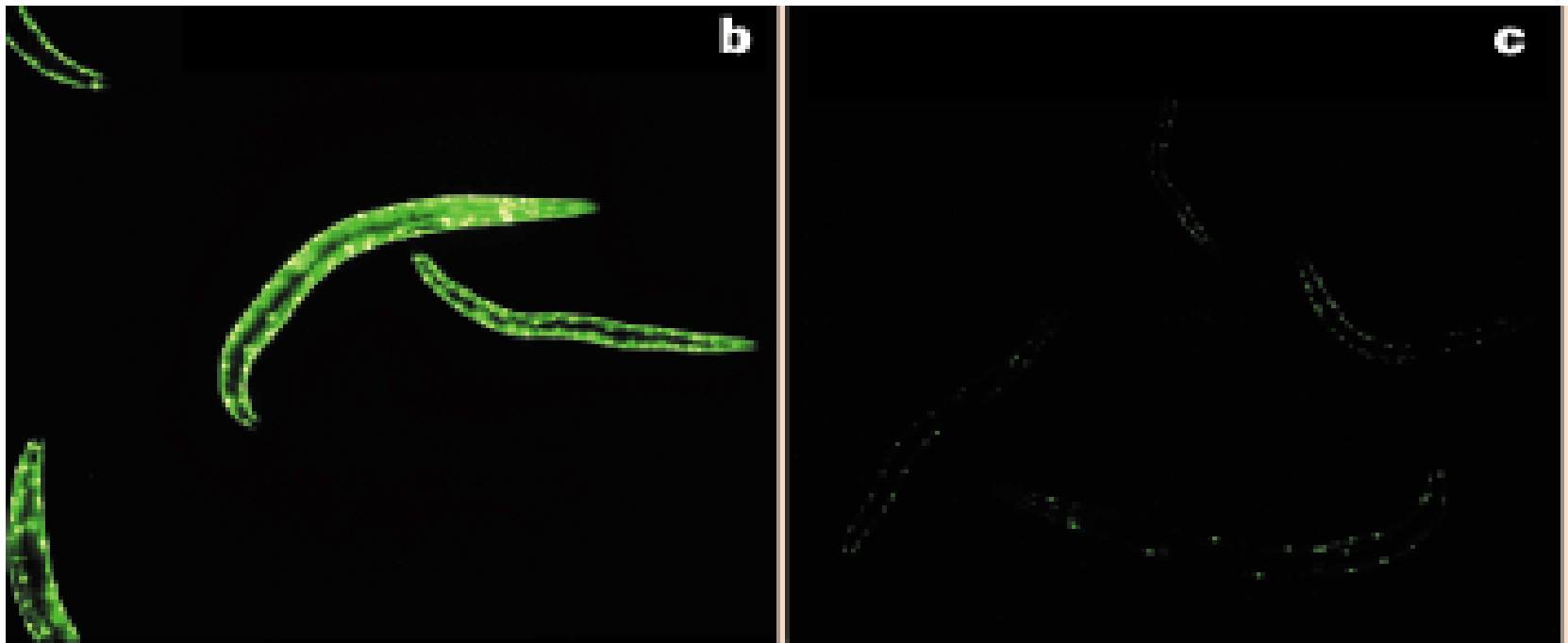
- PCR based method
  - bar code
- Pros:
- Cons:

# Completed genome

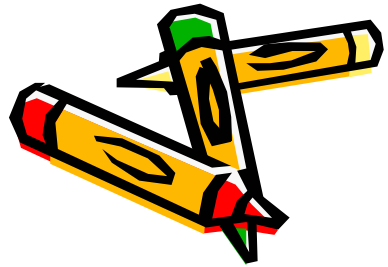
- Unicellular eukaryotes
  - Budding yeast, *Saccharomyces cerevisiae*
- Multicellular eukaryotes
  - Nematode, *Caenorhabditis elegans*
  - Fruit fly, *Drosophila melanogaster*

# RNAi

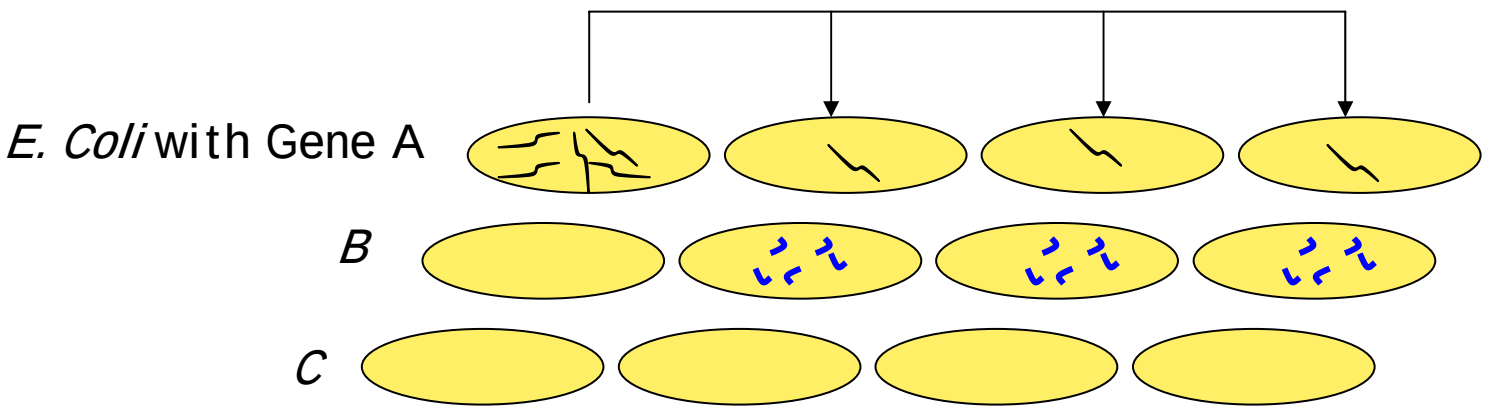
- RNA interference by Andy Fire, 1998
- RNAi transiently inhibits the activity of a target gene with a dsRNA in eukaryotes



# RNAi and *C. elegans*

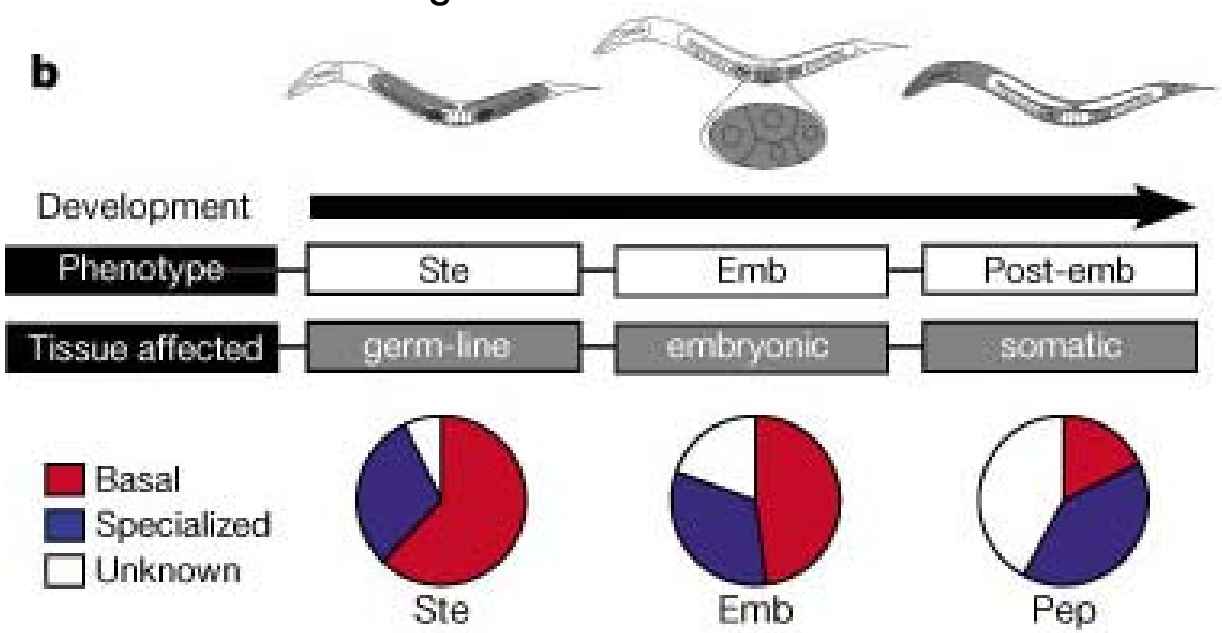


- *C. elegans* eats *E. coli* expressing specific dsRNA
- Observe phenotypes of adult and embryo development



# Functional Distribution

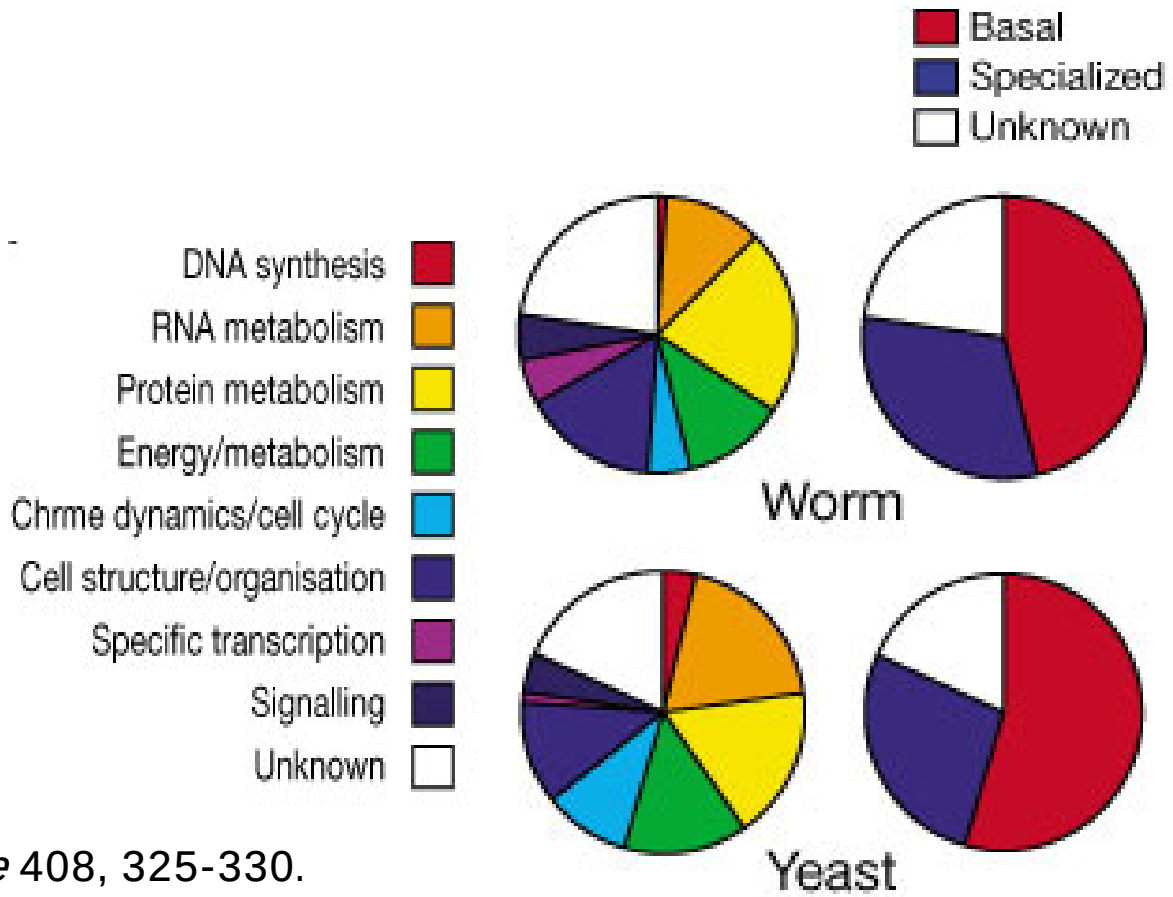
- Genes on chromosome I of *C. elegans*
  - Ste: sterile
  - Emb: embryonic phenotype
  - Pep: post-embryonic phenotype
- Basal metabolic process vs. Specialized functions
  - Germline function/embryonic viability
  - Later developmental process
  - Fractions of unknown genes




Fraser et al., 2000 *Nature* 408, 325-330.

# Function and RNAi phenotype

- Worm vs. Yeast
  - Genes important for viability
    - Similar distribution within the different functional classes



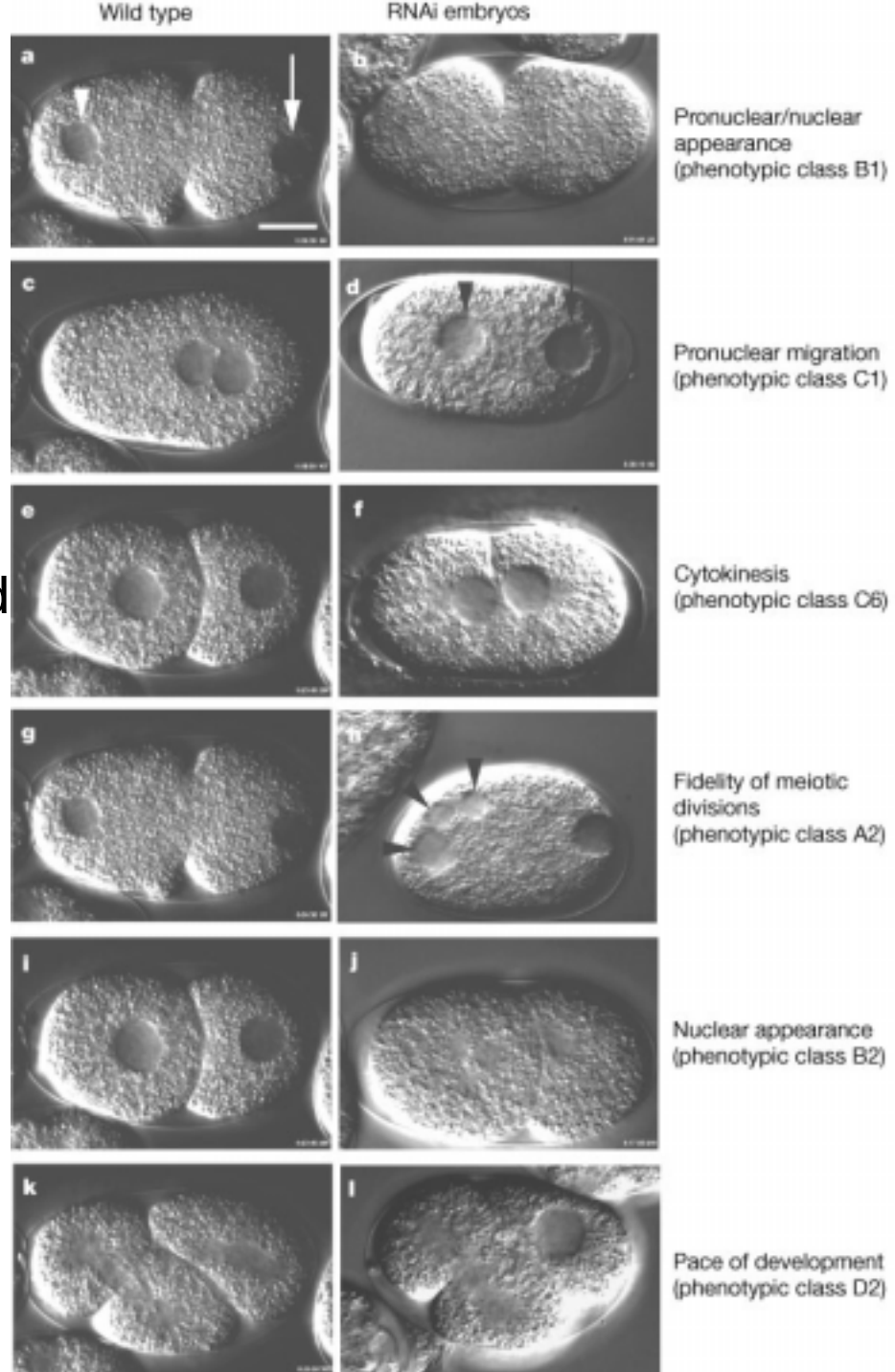

Fraser et al., 2000 *Nature* 408, 325-330.

# Comparisons

- The British group
  - Chromosome I (cDNA library: 2445 clones = 2416 genes)
    - Bacterial expressed dsRNA
    - By feeding
    - Viability, and observable phenotypes
  - Phenotype related gene: from 70 to 378
- The German group
  - Chromosome III (2232 ORF, ~96%, cell division process)
    - PCR amplified, in vitro transcription ssRNA, annealed to generate dsRNA
    - By microinjection
    - Cell-division process (time-lapse differential interference contrast microscopy)
  - df

# DIC images

- Phenotype classes
  - Pronuclei visible vs. non-visible
  - Pronuclei together vs. separated
  - Daughter cells separated vs. unseparated
  - No cytokinesis
  - Nuclei not visible
  - Nuclear envelope breakdown vs. intact



# RNAi strategy

- Pros
  - Specificity
  - Potency
  - Simple protocol (feeding)
- Cons
  - Inhibition efficiency is not 100% for all genes
  - Multiple genes targeted
  - Subtle or conditional phenotypes undetected