Yeast: *Saccharomyces cerevisiae*

I. Why study yeast?

A. Provides connection of genes/proteins with function
   1. 5800 protein-coding genes
   2. Biological role annotated for about 85%

B. Many techniques are easier in yeast
   1. 12 Mb genome
   2. Few introns
   3. Small (< 1 kb) promoters
   4. Transformation
      a. Homologous
      b. Episomal

C. Many techniques developed or refined in yeast
   1. Yeast two hybrid
   2. Microarrays
c. Genomic-wide deletion collection

d. ChIP-chip

e. ChIP-seq

1. Roles of translation
   2. mRNA stability

f. Synthetic lethality \( \rightarrow \) SGA

g. Evolution via tetraploidy

h. Subcellular distribution of proteins

i. Non-Mendelian inheritance

j. Model for human disease

D. Genetic techniques

1. haploid

2. 2 mating types

3. meiosis

4. tetrad

5. Forward genetic screens with large numbers
Life cycle

90 minutes for one cell cycle

Two mating types

haploid 17 chromosomes
mating

zygote

cell division

mitosis

mitosis, cytokinesis

mother

daughter

bud

spore
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(A) Homologous chromosomes pair.

(B) Homologous chromosomes separate.

(C) Daughter nuclei

(D) Centromeres split and chromatids separate.

(E) Four daughter nuclei, the products of meiosis
Figure 5.5: Chromosome behavior during meiosis in an organism with two pairs of homologous chromosomes (red/rice and green/blue). At each stage, the small diagram represents the entire cell and the larger diagram is an expanded view of the chromosomes at that stage.
Pairing of homologs

1. Search for homology
2. Stabilize interactions
3. "Stirring" the contents
   → SUN mediated link to the nuclear envelope that connects telomeres to cytoplasmic motors
   → yeast (mouse, maize as well)
   telomeres cluster near the nuclear envelope to make a "bouquet"
4. Mutations in SUN proteins slow/block movement and pairing of short chromosomes but long chromosomes pair.
homologs

1. Sister chromatids
2. MI prophase
3. Initiation Double strand breaks (DSB)
4. NHEJ
5. DSB repair
6. Gene conversion
7. Crossingover
8. Sister chromatid exchange

Ecotropic (nonallelic) Recombination

- Spn → Spell
- Rad → Rad 51

1 - 100 bps

How were the proteins involved identified?
Meiosis-specific processes

1. Pairing after replication of homologous chromosomes

2. Reciprocal recombination of maternal and paternal chromosomes that is initiated by double-strand breaks created by Spo11


4. Prevention of bi-orientation of sister chromatids via kinetochores to allow sisters to move to one pole

5. Tension on all homolog pairs allow chiasmata to be resolved. Rec8 is cleaved by separase

6. Protection of cohesion near centromeres at meiosis I when chiasmata are resolved

7. Complex in S. cerevisiae to promote mono-orientation of sister kinetochores in Meiosis I.
4. Vocabulary

a. Prophase
b. Metaphase
c. Homolog
d. Kinetochore
e. Centromere
f. Synaptonemal complex
g. Recombination
h. Sporulation
i. Euploidy
j. Aneuploidy
k. Trisomy
l. Chromatid
m. Sister chromatid
n. Chiasma (chiasmata)
o. Reductional division
p. Equational division
Tetrads

\[
\begin{align*}
\text{MAT}^a \times \text{MAT}^a \\
\downarrow \\
\text{Diploid} \quad a/\alpha \\
\downarrow \\
- N \\
+ \text{acetate} \\
\circ \circ \quad \text{Ascus} \\
glass \text{needle} \leftarrow \\
\text{fiber} \\
0 \quad a \\
4 \text{ haploid progeny} \\
0 \quad a \\
0 \quad \alpha \\
0 \quad a
\end{align*}
\]

Auxotrophic mutations

\[
\begin{align*}
\text{trp1 (requires tryptophan to grow)} \\
\text{leu1 (requires leucine to grow)} \\
\text{trp1} \cdot \text{LEU1} \times \text{leu1} \cdot \text{TRP1}
\end{align*}
\]
Diploid \[ \frac{TRP1}{trp1} \ \frac{LEU1}{LEU1} \]

Tetrad Analysis
- look at the 4 products of meiosis together

\[
\begin{array}{cccc}
TRP1 & LEU1 & TRP1 & LEU1 & TRP1 & LEU1 & TRP1 & LEU1 \\
TRP1 & LEU1 & TRP1 & LEU1 & trp1 & LEU1 & trp1 & LEU1 \\
trp1 & LEU1 & trp1 & LEU1 & trp1 & LEU1 & trp1 & LEU1 \\
trp1 & LEU1 & trp1 & LEU1 & trp1 & LEU1 & trp1 & LEU1 \\
\end{array}
\]

2 genotypes 2 genotypes 4 genotypes
that resemble Neither are the 2 parental
two parents same as parents 2 non parental

Parental Di-typenon Parental Di-type Tetrotype

PD NPD T

TT

Map distance: \[ \frac{\% \text{ recombinant progeny}}{\text{Total}} \times 100 \]
\[ \text{trp} \ j \ \text{LEU} \ \text{I} \ x \ \text{TRPL} \ j \ \text{LEU} \ I \]

<table>
<thead>
<tr>
<th>90 PD</th>
<th>10 TT</th>
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<tbody>
<tr>
<td>trp LEU</td>
<td>trp LEU</td>
</tr>
<tr>
<td>trp LEU</td>
<td>TRP LEU</td>
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<tr>
<td>TRP LEU</td>
<td>trp LEU</td>
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<td>TRP LEU</td>
<td>TRP LEU</td>
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How far apart (map units) are TRP1 + LEU1

1 mu = 1% recombination

≈ 3 Kb in yeast

On average ≈ 100 Kb in flies

≈ 1 Mb in humans
<table>
<thead>
<tr>
<th>TRP1; cdc4 x trp1; CDC4</th>
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<tbody>
<tr>
<td>50 PD</td>
</tr>
<tr>
<td>TRP; cdc4</td>
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<tr>
<td>TRP; cdc4</td>
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<tr>
<td>trp; CDC4</td>
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<tr>
<td>trp; CDC4</td>
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<tr>
<td>50 NPD</td>
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<tr>
<td>trp1; cdc4</td>
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<td>TRP; CDC4</td>
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<td>TRP; CDC4</td>
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</tbody>
</table>
\[
\text{metL \ lys7} \times \text{METl \ lys7}
\]

<table>
<thead>
<tr>
<th>PD</th>
<th>NPD</th>
<th>TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>2</td>
<td>38</td>
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</table>

| metL \ lys7 | metL \ YS | met \ lys |
| metL \ lys7 | metL \ YS | MET \ lys |
| MET \ YS | MET \ lys | met \ lys |
| MET \ YS | MET \ lys | MET \ lys |
gal4; ura3 × GAL4; URA3

PD : NPD : TT

45 45 10

<table>
<thead>
<tr>
<th>gal4 ura3</th>
<th>gal4 URA3</th>
<th>gal URA</th>
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<td>GAL4 URA3</td>
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<td>GAL4 URA3</td>
<td>GAL ura</td>
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Problems

\[ \text{HIS}^3 \text{ MAT}^a \times \text{HIS}^3 \text{MAT}^a \]

\[ \begin{array}{ccc}
\text{PD} & \text{NPD} & \text{TT} \\
82 & 0 & 18
\end{array} \]

*What is the linkage relationship between HIS and MAT?*

\[ \text{TRP}^1 \text{ HIS}^3 \times \text{trp}^1 \text{ his}^3 \]

\[ \begin{array}{ccc}
\text{PD} & \text{NPD} & \text{TT} \\
35 : 35 : 30
\end{array} \]

*What is the linkage relationship between HIS and TRP?*

\[ \text{TRP}^1 \text{ MAT}^a \times \text{trp}^1 \text{ MAT}^a \]

\[ \begin{array}{ccc}
\text{PD} & \text{NPD} & \text{TT} \\
42 : 43 : 15
\end{array} \]

*What is the linkage relationship?*

Draw genetic map for these three genes.
mapping function

\[
\frac{TT + 6\ NPD}{2 \times \text{Total}} \times 100 = \text{map distance}
\]
nco (non-crossover)

sco (single crossover)

dco (double crossover)

Mapping function

\[
\frac{\text{sco} + 2 \cdot \text{dco}}{\text{Total}} \times 100 = \text{map distance}
\]

Turn it into PD, NPD, TT

\[
\frac{\text{TT} + 6 \cdot \text{NPD}}{2 \cdot \text{Total}} \times 100 = \text{map distance}
\]
\[ \text{SCO} \]

\[ TT = (3\text{stdco}) + 2 \left( \frac{20t, 30t, 40t}{2} \right) - 2 \times \text{NPD} - 2 \times 4 \times \text{NPD} \]

\[ 2 \times \text{PD} + \text{NDD} + TT \]
Erythromycin $R \times$ Erythromycin $S$

\[ \downarrow \]

Phenotype: Intermediate resistance

Ery$^R$ / Ery$^S$ Diploid

\[ \downarrow \]

0000

4 ery$^R$ : 0 ery$^S$

1) It is not 2$^R$ : 2$^S$

2) Mitochondrial

3) Mitochondrial DNA is not required for viability in yeast

4) gly$^-$ x gly$^+$

\[ \downarrow \]

4 Gly$^+$ : 0 Gly$^-$

petite x grande

\[ \downarrow \]

4 grande : 0 petite

petite strains grow more slowly and lack mitochondrial DNA.
Mapping in the present day

1. High density oligonucleotide arrays
2. 2 yeast strains with ~1% polymorphism
3. Hybridization to 2 arrays → differences
4. Mapped with 2 parental strains and 14 progeny
5. 94.57% showed 2:2 segregation
   44 known markers mapped to correct region
   1 unknown marker → XVI
   based on 3714 markers.

Winzeler et al. 1998
Science 281: 1194-1197
PD = NPD

Yes

No

<100% crossovers

100% PD

close linked

linked

Unlinked

Yes

TT

No

NPDs

Yes

No

TT < 2/3

TT + 6 NPD x 100
Total

1/2 TT
Total

x 100 = map distance

Yes

No

at least one gene unlinked to centromere

1/2 TT
Total

x 100
A. Plasmids

1. High copy number
   a. endogenous plasmid: Zu
   b. 50-100 copies/cell

2. ARS plasmids
   a. AT rich sequence
   b. autonomous replication
   c. 50-100 copies/cell
   d. selectable marker
      1. KAN R
      2. URA3

      orotic acid S-phosphate dehydrogenase

      URA3 ↓
      URA3 - uracil
      Uridine monophosphate
      to grow
      uracil
5-fluoro-orotic acid 5-FOA

ura3 are not killed by 5-FOA

5-FOA suicide substrate 5-fluorouracil is toxic

Select both positive (URA3 or ura1 prototroph)
or negative (growth on 5-FOA)

3. CEN plasmid
   a. contain ~100 kb of centromeric sequence
   b. reduce copy number to 1-2 copies
   c. maintain stability of plasmid but not as well as full length linear chromosomes

4. YAC vectors
   a. ARS
   b. CEN
   c. TEL
   d. "Length"
1. Loss of function
2. Gene is identified

ATG

KAN Hx

... 20bp tag

barcodes

5,916 genes deleted (96.5% of genes)
1,109 essential on rich (+glucose) medium
1,106 48.11

1. 62% have homolog
   in another organism
2. 17% have paralog
2. 8.5% have paralog

1. haploid
2. heterozygous and homozygous diploid