1. Haploid
2. Easy to grow
3. Homologous recombination
4. Transformation
   a. Plasmids
   b. Integration
1. Plasmids

   a. High copy number
      1. endogenous plasmid  2μ
      2. 50-100 copies/cell

   b. ARS/ori plasmid

   bacterial plasmid → φ → yeast DNA

   sequence allow maintenance
ARS - autonomous replicating sequences
- origin of replication

1. AT rich
2. 50-100 copies/cell
3. Selectable marker
   a) Kanamycin resistance
   b) URA3

uracil ← uridine monophosphate
orotidine-5-phosphate decarboxylase → URA3

5-fluoro-orotic acid (5FOA) NOT TOXIC

\[ \downarrow \text{URA3} \]
\[ \downarrow \text{URAS} \]
\[ 5\text{-fluoro-uracil} \quad \text{This kills yeast cells: TOXIC} \]

\[ 5\text{-FOA} \]
\[ \downarrow \text{if cells have a ura3 mutant} \]
\[ \downarrow \text{It blocks production of} \]
\[ \downarrow 5\text{FOA} \]
This scheme allows selection for

\[ \text{URA3}^+ \quad \text{ura3}^- \]

OR

\[ \text{URA3}^- \quad \text{ura3}^+ \]

dies with uracil mutant in the medium.

Can select for prototrophy and wild-type URA3 gene

\[ \text{ura3}^- \quad \text{URA3}^- \]

medium with 5-FOA, wild-type cells die

URA3- mutant survive
CEN plasmid (Centromere)

1. John Carbon and Louise Clark

a) LEU2 - from tetrad analysis they knew it was linked
   - walked and looked for rescue of an E. coli mutant (levB)

b) CDC10 - linked to LEU2
   - rescued the temperature-sensitive growth phenotype

c) This plasmid was “more stable”
d. Isolate additional DNA sequences that provided "stability"

e. found they shared ~100 bps of sequence and showed reductive division at meiosis I

f. copy number went from 50–60 to 1–2

2. YAC vectors
What does increased length do for segregation?
Yeast deletion collection

- 5916 genes deleted
  - 4811 are non-essential
  - 1105 are essential in the lab on rich medium
  - 67% have homolog
  - 8.57% have paralog
  - 82% have homolog in other organisms
  - 19% have paralog
Bar codes allow population based experiments

sequence
bar codes

condition X

sequence
bar codes

some strains are increased
others are decreased
a. fitness (survival / reproduction)

b. drug resistance

c. phenotypes
   1. Bud site selection
      found 127 strains
   2. Killer toxin (dsRNA)
      226 strains showed resistance
Haploinsufficiency using diploids that are heterozygous for a deletion.

Drug targets have been hard to identify in haploid

\[
\Delta (\text{anti-malarial}) + \Delta 6100 + \text{look for hyper sensitivity seq. bar codes}
\]

cladosporin
anti-bacterial
arginin
anti-fungal
triazole pyrimidine
sulfonamide
lysyl tRNA synthetase
mitochondrial elongation factor G
acetolactate synthase

reduced fitness
Epistasis in biosynthetic pathways

1. ADE3 $\rightarrow$ 2 $\rightarrow$ 3 $\rightarrow$ 4 $\rightarrow$ 5 $\rightarrow$ 6 $\rightarrow$ 7

1. all mutants require adenine to be added to medium
2. adel1, adel2 mutant accumulate a pigment and turn red
Δ ade 3 white
Δ ade 2 red
Δ adel red
ade 3-101 while

Four possible outcomes for double mutants

\[
\begin{align*}
\text{a} & \rightarrow \text{b} & \rightarrow \text{“a”} \\
\text{b} & \rightarrow \text{a} & \rightarrow \text{“b”} \\
\text{a.b} & \rightarrow & = \\
\begin{array}{c}
\text{a} \\
\text{b}
\end{array} & \rightarrow & \text{unique}
\end{align*}
\]

ade 2; ade 6

ade 2; ade 3

ade 2; ade 3-101 pink
Phil Heiter

Synthetic lethality to develop cancer drugs

-CIN genes
chrome loss - 692 genes $\Rightarrow$ cin phenotype
70% have human homologs
323 essential genes

\[
\begin{align*}
\text{yfg1} & \quad \text{viable} \\
\text{yfg2} & \quad \text{viable} \\
\text{mre11} & \quad \text{47% of colorectal cancers} \\
\text{cdc4} & \quad \text{11%}
\end{align*}
\]

but

\[
\begin{align*}
\text{yfg1 yfg2} & \quad \text{die}
\end{align*}
\]
<table>
<thead>
<tr>
<th>Gene</th>
<th>2° gene</th>
<th>doubleT</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAD54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAD51</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGS I</td>
<td>CTF18</td>
<td>any combination is lethal</td>
</tr>
<tr>
<td>BUB I</td>
<td>DCC I</td>
<td></td>
</tr>
<tr>
<td>MAD1</td>
<td>CTF4</td>
<td></td>
</tr>
<tr>
<td>MRE11</td>
<td>RAD27</td>
<td></td>
</tr>
<tr>
<td>CDC4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMC1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- CTF18 - part of RFC-like complex
- CTF4 - replication complex
- DCC1 - sister chromatid cohesion
- RAD27 -
16/20 double mutants showed decreased viability in mammalian cells

van Pel et al. 2013
How to find a role if your mutant has no phenotype?

\[ \text{mutx} \rightarrow \text{weak or no phenotype} \]

Does it have a "synthetic phenotype"?

\[ \text{ade} 2\Delta; \text{ade} 3\Delta; \text{mutx} + \text{plasmid (2u)} \]
\[ + \text{plasmid RED} \]
\[ - \text{plasmid WHITE} \]

Mutagenize to \( \sim 10\% \) viability to find \( yfg(\text{SL}) \)
Question

is there a genotype (mutx; muty) that shows lethality?

mutx  no phenotype
muty  no phenotype
mutx; muty  dead
petri plate w/2000 colonies

The plasmid is required for viability

most colonies red with a white sector

The white sector arises when the plasmid is lost

No sector suggest that loss of plasmid

petri plate w/2000 colonies

The plasmid is required for viability

most colonies red with a white sector

The white sector arises when the plasmid is lost

No sector suggest that loss of plasmid
Some general rules of synthetic lethal screens

1. The majority of synthetic lethal relationships occur among genes acting in a single pathway.

2. Some in biochemically distinct circuits - repair and recombination.

3. Each gene has only a small number of synthetic interactions.
Revertants / Suppressors

Change in

Same gene

UGA (stop codon)

氨酸 acid (trp) UGG

Change in
different gene
Revertants → tightly linked to original mutation by meiotic results.

WT
CAG (Glutamine)
CTG Leuine

Variant
TAG Glutamine
CGG Proline

CAG
TTG
TGG
CAG
AAG
Leu
Lysine

23 3 1 2 2

CTG
TCG
CAG
TTG
Leucine
Serine
Glutamine
Leucine

Lin et al. 2015
Suppressors

1. Informational

2. Interaction

3. Bypass

Suppressors - Extragenic
restore a more "wild-type"
phenotype
Informational = altering part of the central dogma

- alter the anticodon to read a stop codon
- frame shift that read 4 bases instead of 3

Why are these suppressors viable?
In *C. elegans*

- *unc-54*
- *lin-29*
- *emb-36*
- *unc-76*

**SMG**

- mRNA stability increased
- Nonsense mediated decay

**NMD**

1. *smg* restore function to all of these mutants
2. Surveillance pathway - removes mRNA transcripts that contain premature stop codons.

1. Not all premature stop codons are suppressed
2. Some premature stop codons + *smg* become dominant
Interactive suppressors

Correct the phenotypic defect by mutations in two genes

RARE

- actin
  - SAC1: phosphatidylinositol phosphate phosphatase
  - SAC2: golgi associated retrograde protein (VPS52)
  - SAC3: mRNA export factor
  - SAC6: fimbrin, actin bundling

1/4 interactive suppressors
Spc97^− (gamma tubulin complex protein 2)
Spc98^−

temperature-sensitive
so arrest at high temperature
suppressors identified in α-tubulin
that were cold-sensitive

γtubcs suppressed by mutants in α-tubulin
Bypass suppressors

Correct phenotypic defect by mutants in another pathways or later in the same pathway

\[ \downarrow \text{mut} \]

\[ \downarrow \text{sup} \]

\[ 1 \rightarrow 2 \rightarrow 3 \]

but if mutant in 1

\[ 2 \rightarrow 1 \rightarrow \text{blocks process} \]

bypass suppressor in 2

\[ \times \rightarrow \times \rightarrow \rightarrow \text{allow process to work} \]
Enhancers or second site noncomplementation

1. Recessive mutant

\[
\begin{align*}
&\text{mut}^1 \quad \text{wild-type} \\
&\quad \text{MUT}^2 \\
&\text{mut}^2 \quad \text{wild-type} \\
&\quad \text{MUT}^2 \\
&\text{mut}^1 \quad \text{MUT}^1^+ \\
&\quad \text{MUT}^2^+ \\
&\quad \text{mutant phenotype}
\end{align*}
\]
mut1 = α-tubulin

in yeast

\[
\frac{\text{mut1}}{+} + \frac{+}{\text{mut2}}
\]

mut2 = β-tubulin

mutants with null alleles

dosage

in flies

\[
\frac{\text{mut1}}{+} + \frac{+}{\text{mut2}}
\]

mutants with missense alleles

dosage

interaction
Multi-copy suppression or regulated promoter (GAL4)

mut + genomic library on high copy plasmid

- works better with loss of function alleles rather than null alleles
- ↑ dosage may stabilize the mutant protein
Histones - suppression by dosage

1. 2 copies of each gene
   H2A, H2B, H3, H4

Questions

1. Is copy number important?

<table>
<thead>
<tr>
<th>chromosome loss</th>
<th>yos+ cyh2</th>
<th>adcb6</th>
<th>+</th>
<th>measure in Int -1 disome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ aro</td>
<td>+</td>
<td>+</td>
<td>adce3</td>
</tr>
</tbody>
</table>

↑ H2A, H2B
↑ H3, H4
↑ H2A, H2B, H3, H4

10 fold increase in loss
10 fold increase in loss
no increase
Intragenic revertants in human genes

Can you see "evolution" in action using revertants?
Comparing conservation of loci in patients to 200 vertebrates

Identifying pathogenic variants in patients

5-12% patient missense variant

why?

1

200

a) Lack of conservation in other vertebrates not conserved in all vertebrates

these vertebrates have an intragenic revertant
RPGRIPIL
R937L

The changes are functional in zebrafish assays
P189, R937 mut
F193, R937 WT
T961, R937 WT

4/200 are homozygous for 937L

Do these vertebrates have a second mutation in RPGRIPIL?

P189 F193 R937 R961

L  L  L  L  T
L  L  L  L  T
L  L  L  L  T
L  L  L  L  T
BBS4

N165H is a known human variant
H366T may be an intragenic revertant
one example of suppressor screens

mating-type switching

α

mother O daughter

α

α

a a α α

screen for turning on the silent copies

silenced

HML MAT HMR
It double strand cut by Ho endonuclease
Four genes
SIR1
SIR2
SIR3
SIR3

\{ \text{NAD dependent histone deacetylase}
\text{Silencing protein}
\text{Silencing protein} \}

set of chromatin domains that are silenced by SIR2-SIR3-SIR4 complex that interact with histones
What controls H0 expression?

H0 promoter → URA3 → SFOA resistance

H0 promoter → β-gal → White colonies

Screen for suppressors

H0 promoter → β-gal; swi 1 white so mutagenize and screen for blue (suppressed)

4 sup 30 sup
swi 4 swi 5

5FOA resistance
White colonies

swi 1 1 swi 1 2 swi 1 3 swi 1 4 swi 1 5
swi/swf complex for chromatin remodeling

TF
SIN1  -  polyadenylation
SIN2 (HHT)  Histone H3
SIN3  Histone deacetylase
SIN4  Part of Mediator RNAPII complex

suppressors identify chromatin factors