Genetics, Molecular and Cell Biology of Yeast

1. Introduction
2. Genetic elements
3. Classical genetics
4. Molecular biology of yeast
5. Cell biology
6. Genomics
Objectives

• Understand the advantage of genetic-approaches to biochemical- and cell biological questions.

• Understand the use of molecular biology techniques to address specific problems.

• Understand the use and results obtained from “genome-wide” approaches.
## Model Organisms

Why do you want to work with a “model organism”?

<table>
<thead>
<tr>
<th>Advantage</th>
<th>Disadvantage</th>
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<tbody>
<tr>
<td>Bacteriophage, Viruses</td>
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<td>Bacteria, <em>E. coli</em>, Cyanobacteria</td>
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<tr>
<td>Unicellular eukaryote, yeast <em>Dictiostelium</em></td>
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<tr>
<td>Multicellular, <em>C. elegans</em></td>
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<td>Insect, <em>Drosophila</em></td>
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<td>Vertebrae, zebra fish</td>
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<td>Mammals, mice, rat, dogs, monkey</td>
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<td>Plant, <em>Arabidopsis</em></td>
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<td>Xenopus egg</td>
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<tr>
<td>Cell culture</td>
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Why work with yeast?

1. Eukaryote, unicellular => model for cellular processes that also take place in our body, basic research
   - Easy and cheap to cultivate, as bacteria
   - Fast generation time, 90min
   - Lines/strains can be stored/frozen
   - Very strong genetic system, high frequency of homologous recombination
   - First sequenced eukaryotic genome
   - Small genes, few introns
   - Model to develop new technologies with future applications to animal cells

2. Fungal pathogens are industrially relevant, *ie* agriculture, and a growing health problem (*Candida* etc)
http://www.yeastgenome.org

http://mips.gsf.de/genre/proj/yeast/
Saccharomyces is a budding yeast
Yeast adapt to vast changes in Osmolarity, Nitrogen, Carbon, Temperature.
What are Yeast?

- phylum *Ascomycetes* (*Schlauchpilz* [Morchel, Trüffel] ≠ *basidomycetes*),
  order *Saccharomycetales* (*Zuckerhefen*)
- wide dispersion of natural habitats, plant leaves, wine grapes
- ferment sugars to ethanol
  - Production of wine, beer etc.
  - Baking, raise dough through production of CO₂
  - Sparkling wine
  - Used by man aprox. 6000 years B.C.
- difficult to define a wild-type
- vitamin supplement in food production
- divide by budding: *Saccharomyces (cerevisiae)*
- divide by division: *Schizosaccharomyces (pombe)*
- human pathogen: *Candida albicans*
Yeast life cycle
Cell polarity of diploid vs haploid cells

Diploid, egg-shaped, radial (bipolar) budding

Haploid, round, axial budding
a Axial budding

- Bud scar

- Bipolar budding

M D

b Daughter cell (bud)

- Myo2-driven vesicle delivery

- Actin cap

- Exocyst/SNAREs

- Myo2-driven microtubule capture

- Actin cable anchoring

- Astral microtubule

- Bud scar

- Kar9 Bim1

- Myo2p Kar9p Bim1p

- Myo2p

- Formins (Bni1p/Bnr1p)

- Arp2/3-activating complex

- Polarisome complex

- p21-activated kinases

- Type I myosins

- Actin cables

- Anchor/nucleate actin cables

- Assemble actin cap (scaffolds)

- Bud site selection

- ‘Landmark’ proteins

- Bud1p/ Rsr1p GTPase

- Cdc42 GTPase

- Rho GTPases

- Target vesicles

- Type V myosin (Myo2p)

- Sec4 GTPase

- Exocyst/SNAREs

- Polarized membrane growth

Regulation → Process → Machinery
Pseudohyphal growth
Meiosis

DNA SYNTHESIS  PAIRING EXCHANGE  MI DIVISION REDUCTIONAL  MII DIVISION EQUATIONAL  MEIOTIC PRODUCTS

Diploids

2n -> 4n -> 4x 1n

haploid spores
**Meiosis**

\[ \text{MAT}^{a} \times \text{MAT}^{a} \rightarrow \text{MAT}^{a}/\text{MAT}^{a} \ (2n) \rightarrow \text{synthesis} \ (4n) \rightarrow \text{meiotic div. I} \rightarrow \text{meiotic div. II} \rightarrow \text{spores: MAT}^{a}; \text{MAT}^{a}; \text{MAT}^{a}; \text{MAT}^{a} \]

*Movie!*
Advantages of meiosis (sexual reproduction)

- Chromosome mixing, $2^{16} = 65,536$ possibilities
- Crossing over $\rightarrow$ indefinite number of new combinations (45x2)
- Gene conversion
- Gene repair
- Generation of new alleles
- 2 different isolates of yeast differ every 100bp
The yeast cell cycle

- **START**
- **Cell separation**
- **Cytokinesis**
- **Initiation of DNA synthesis**
- **Late nuclear division**
- **Medial nuclear division**
- **Bud emergence**
- **Nuclear migration**

- **G1**
- **S**
- **G2**
- **M**
Checkpoints monitor cell cycle progression

The Cell Cycle and the Checkpoints
Yeast can be propagated as haploids or diploids. This greatly simplifies genetic analysis of yeast.
The yeast cell cycle
Spindle dynamics

1. Pre-anaphase: one SPB remains on mother bud neck, newly synthesized SPB traverses nuclear envelope
2. Spindle remains constant in length or elongates very slowly
3. Spindle rapidly elongates
4. Movements of the spindle within mother-bud neck governed by cytoplasmic microtubules
5. Slower spindle movement and transition to ‘hour-glass’ shape
6. Nucleus divides shortly before cytokinesis
Factors mediating the process of microtubule attachment with the bud cell cortex are Bim1p and Kar9p. Bim1p can directly bind to microtubules and is required for the high dynamic instability of microtubules that is characteristic of cells before spindle assembly. Kar9p has been implicated in the orientation of functional microtubule attachments in relation to the bud during vegetative growth. It is delivered to the bud by a Myo2-dependent mechanism presumably tracking on actin cables. Interaction of the two factors, Bim1p and Kar9p, appears to provide a functional linkage between the actin and microtubule cytoskeletons. In addition, Bud3p, a protein for axial budding of haploid cells, accumulates at the bud neck and is required for the efficient association of Bud6p to the neck region. Further, a variety of motor proteins are necessary in spindle morphogenesis: dynein and the kinesin-like proteins Kip2p and Kip3p, as well as Kar3p are involved in regulating microtubule dynamics, mediating nuclear migration to the bud neck and facilitating spindle translocation (Figure 10-13).

10.2.4 Sister Chromatid Cohesion and Separation

Sister chromatid cohesion is essential for accurate chromosome segregation during the cell cycle [Nasmyth, 1999; Biggins & Murray, 1999; Robert et al.; Nasmyth, 2002; Carnobel & Cohen-Fix; 2002; Uhlmann, 2004]. A number of structural proteins are required for sister chromatid cohesion and there seems to be a link in some organisms between the processes of cohesion and condensation. Likewise, a number of proteins that induce and regulate the separation of sister chromatids have been identified.

Chromosome splitting is an irreversible event and must therefore be highly regulated. Once sister chromatids separate from one another, damage to the genome cannot easily be repaired by recombination nor can mistakes in chromosome alignment be corrected. Sister chromatids are pulled to opposite 'halves' of the cell by microtubules that emanate from opposite spindle poles. These microtubules interdigitate and keep the two poles apart. Subsequently, a second set of microtubules attaches to chromosomes through specialized 'kinetochores' and pulls them to the poles. In this way, sister chromatides separate and start to move into opposing poles (Figure 10-14).

However, chromosomes do not remain inactive at this process: cohesion between sister chromatids generates the tension by which cells align them on the metaphase plate. Cohesion also prevents chromosomes falling apart because of double-stranded breaks and facilitates their repair by recombination.

Figure 10-13: Fluorescence imaging of microtubules.
The power of genetics

Leland Hartwell, Nobel Prize 2001
the $cdc$-screen