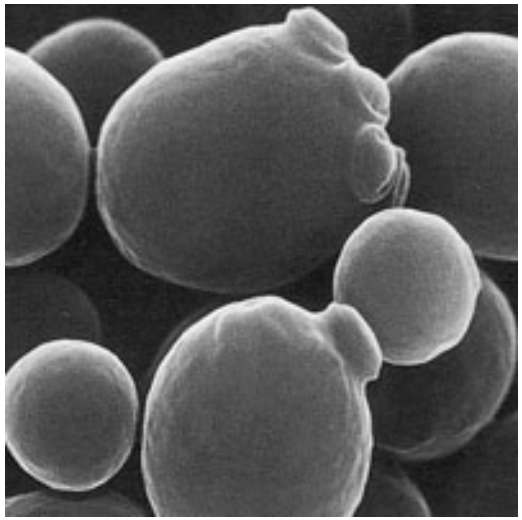
Single Cell Visualization of the DNA repair mechanism *in vivo*



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Objectives

• DNA double-strand break constitutes the most dangerous type of DNA damage

• Inefficient DNA repair may lead to genomic defects, hypersensitivity to cellular stress and resistance to apoptosis, which is characteristic of cancer cells

• Current research on DNA repair has enabled numerous breakthroughs in our understanding of the DNA repair mechanisms at the population level

• Population level measurements have two fundamental shortcomings:

  • The repair process is not visualized
  • They only measure the mean of a distribution and usually hide the cell-to-cell

• To proceed further, we must acknowledge that cells even in genetically identical population, exhibit epigenetic individuality, which may have important implications on the fitness of a population.

Objectives and long term goals:

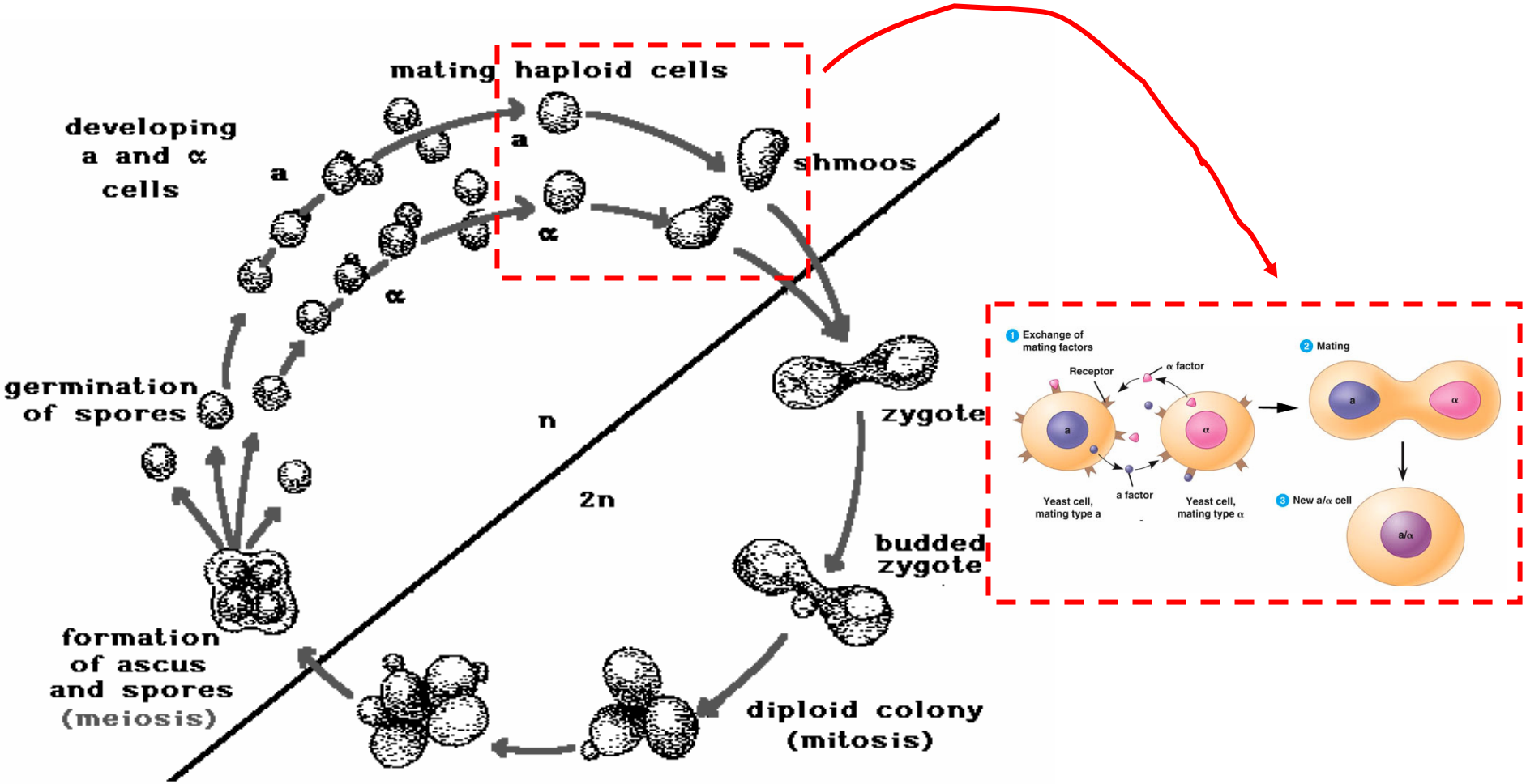
1. To understand the molecular mechanisms of the DNA repair

2. To improve our understanding of cell-to-cell variability in DNA repair and its impact on the fitness of a population

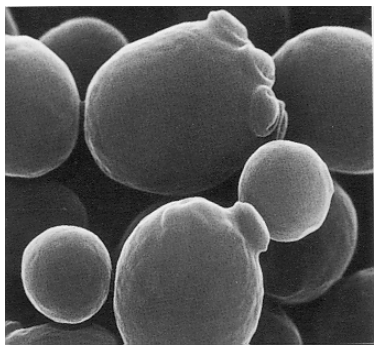
Focus on ‘well characterized' biochemical networks:

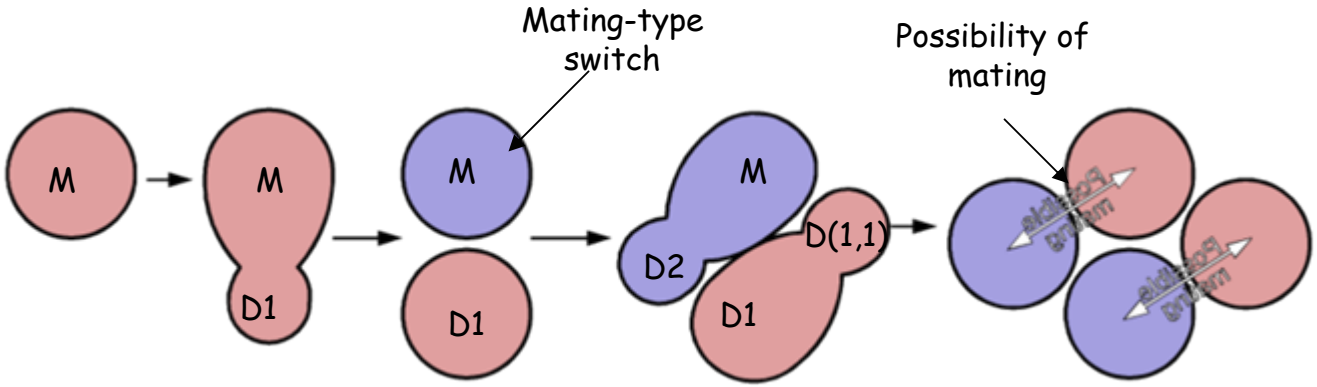
The model system: Budding Yeast

A model system: *Saccharomyces cerevisae* (Budding Yeast)



Budding Yeast: An experimental model system for DNA repair

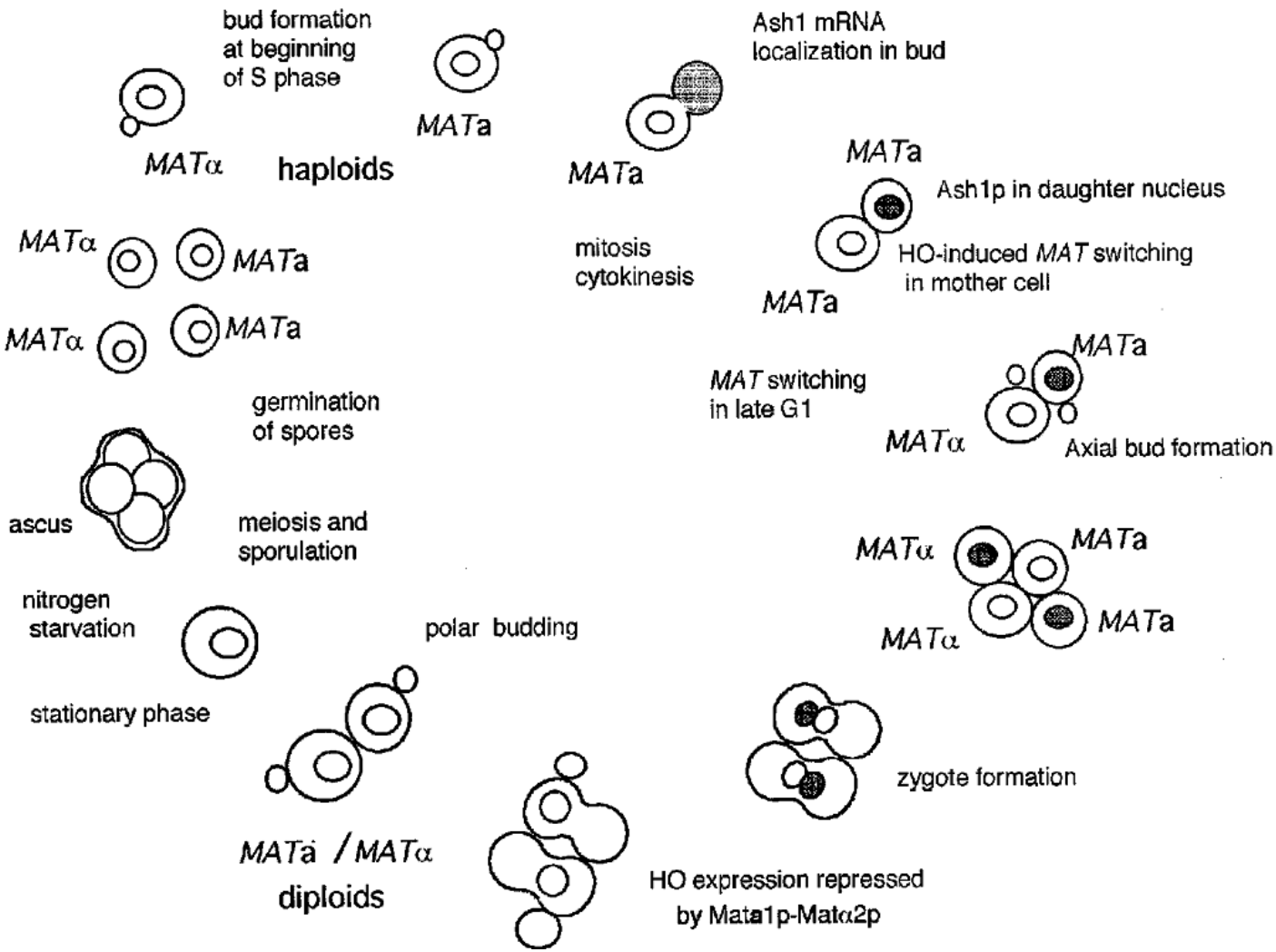




How does mating-type switching relate to the DNA repair?

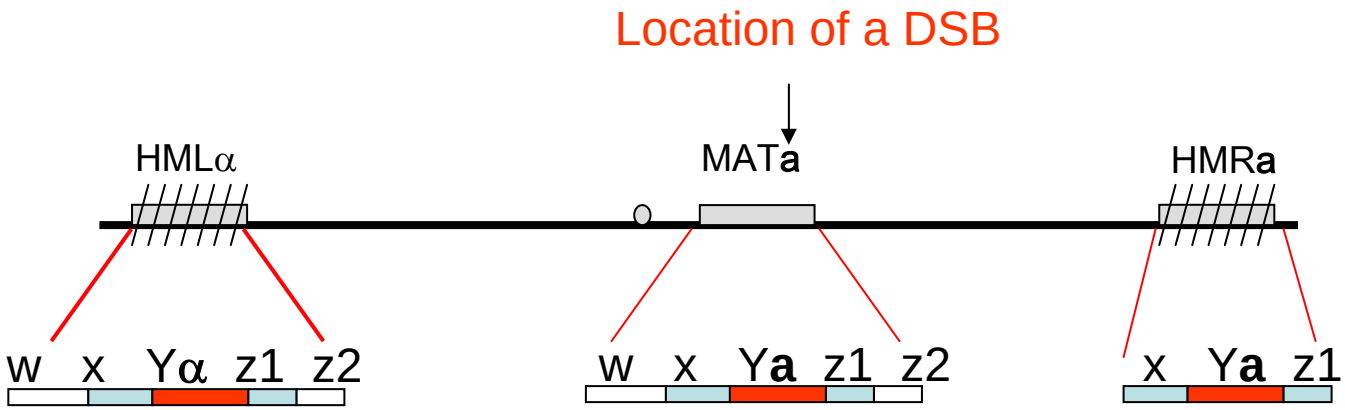
Yeast cells induce a double-strand break in their own genome and then repair it, as a result they change their mating type

Mating-type switching in yeast



Haber, Annu. Rev. Gen. 98

Yeast repairs its genome mostly by finding homologous regions in DNA

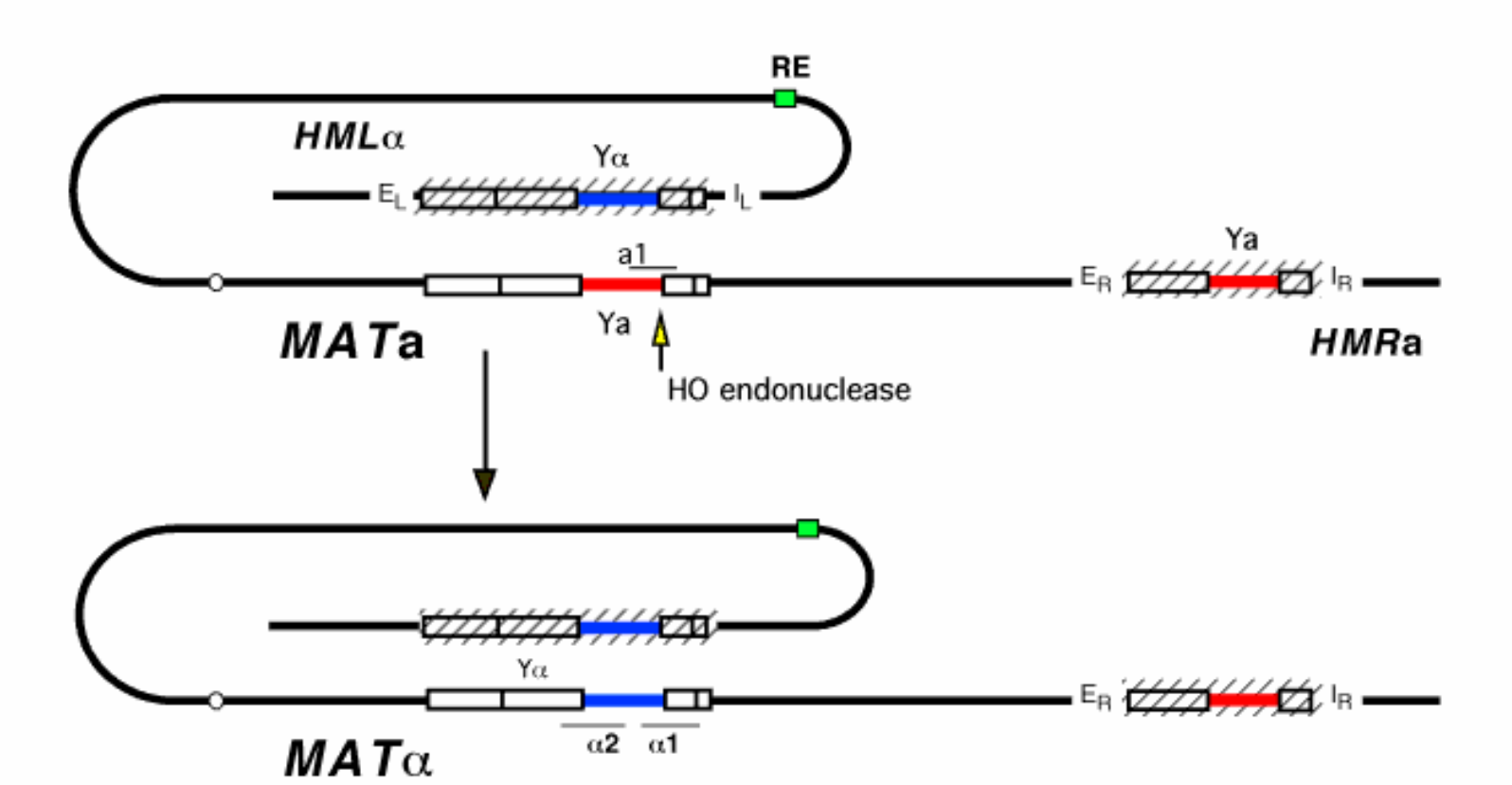


MAT: Mating type determination locus carries **a** or α type genes

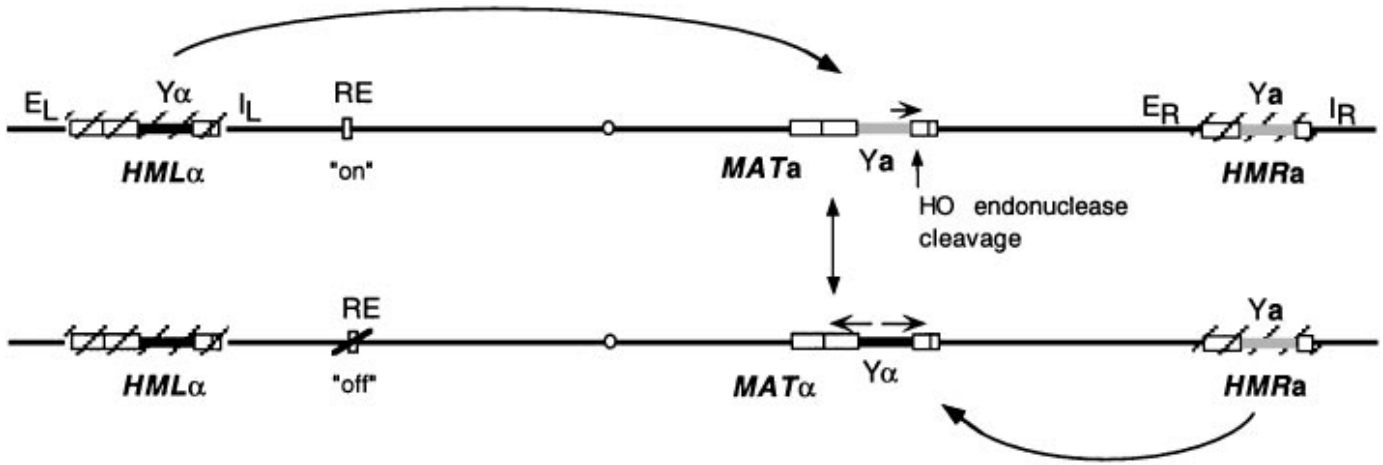
HML : Silent donor on the left arm of the chromosome carries homologous region to MAT plus α type gene

HMR : Silent donor on the right arm of the chromosome carries homologous region to MAT plus **a** type gene

During repair, the information from donor sequence is copied into MAT



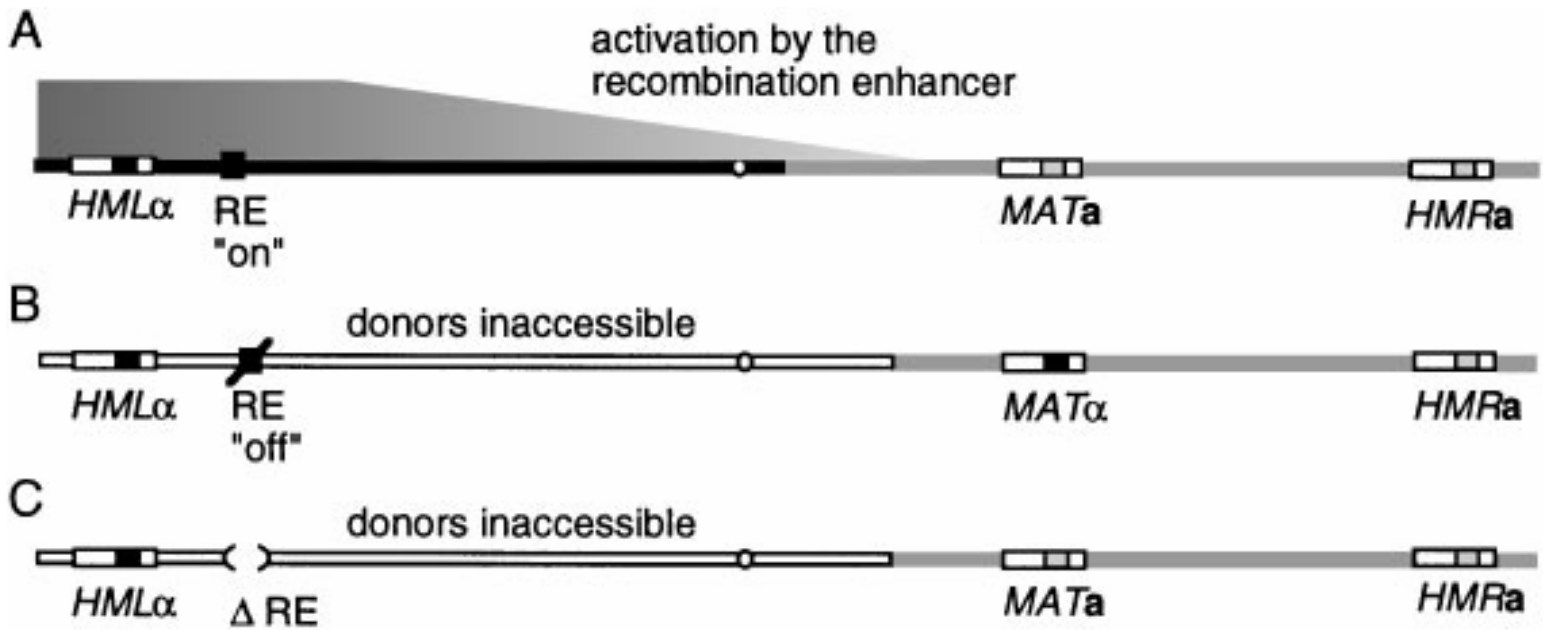
Donor Preference



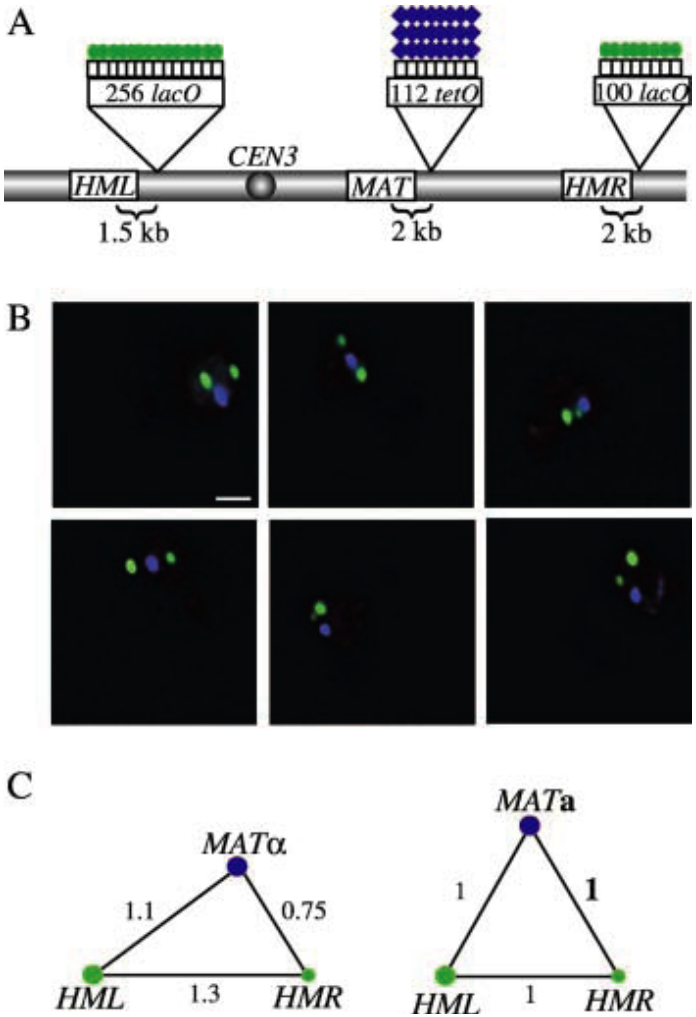
Mata cells mostly use the left arm of the chromosome
Matα cells mostly use the right arm of the chromosome

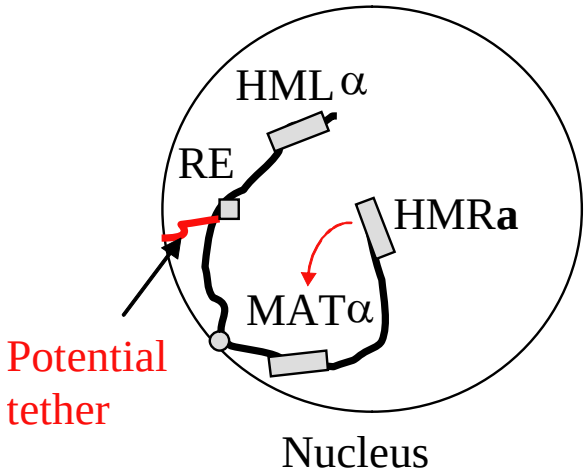
Haber, Annu. Rev. Gen. 98

Recombination enhancer (RE) activates the left arm of the chromosome in MAT**a** cells



The left arm of the chromosome maybe tethered in MATα cells

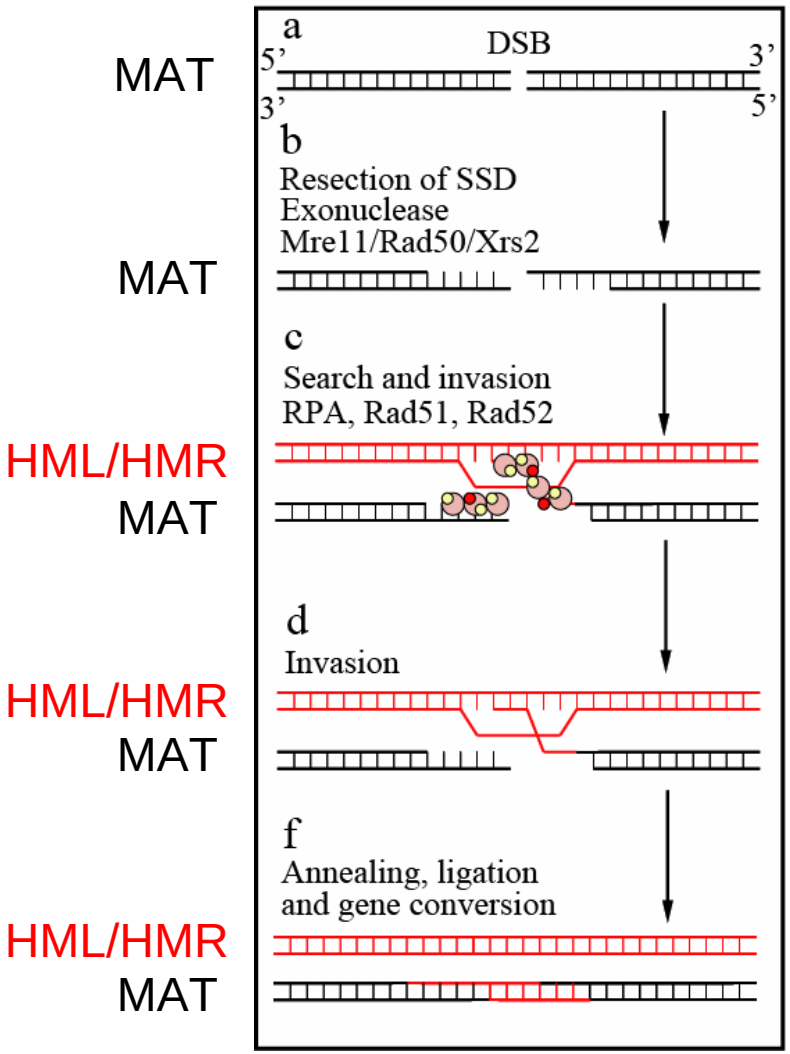


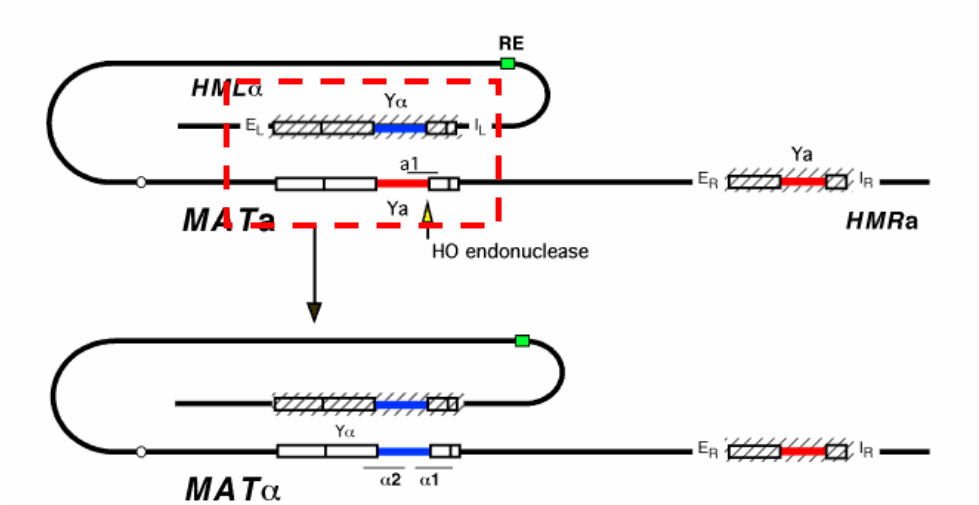


RE may be removing the tether in MAT**a** cells

Bressan et al. JCB 04

Classic view of homologous recombination





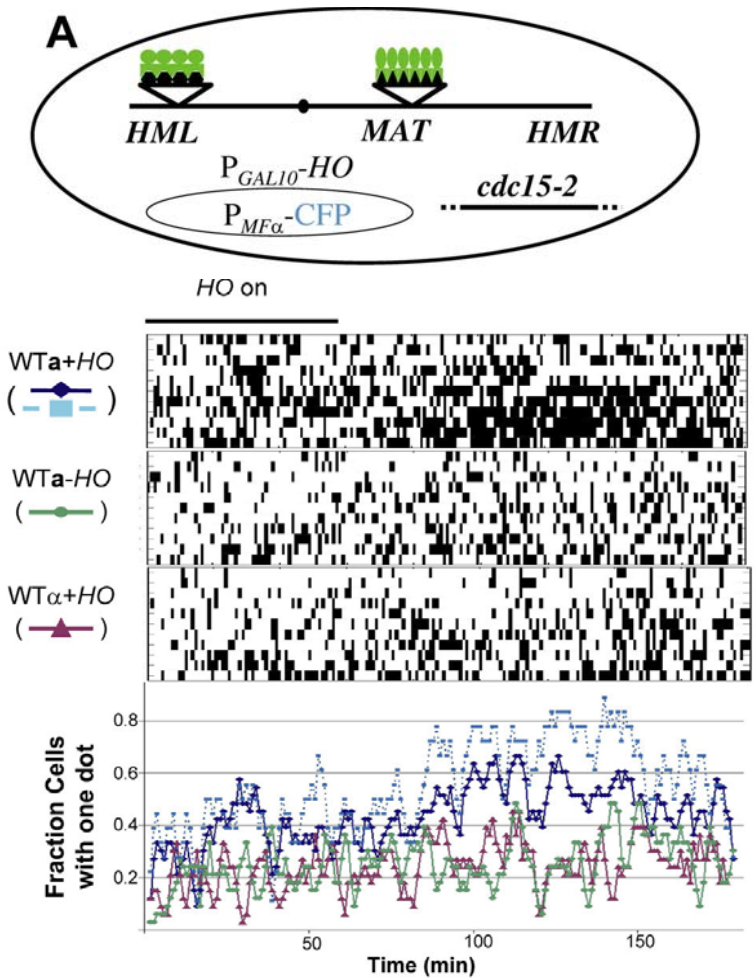
- The donor and recipient DNA have to be close to each other
- They have to form a successful synapse

• Recent single cell measurements suggests that the rate of collisions between the recipient and donor sequences increases during the repair

• A significant association time between the donor and recipient DNA has not been observed

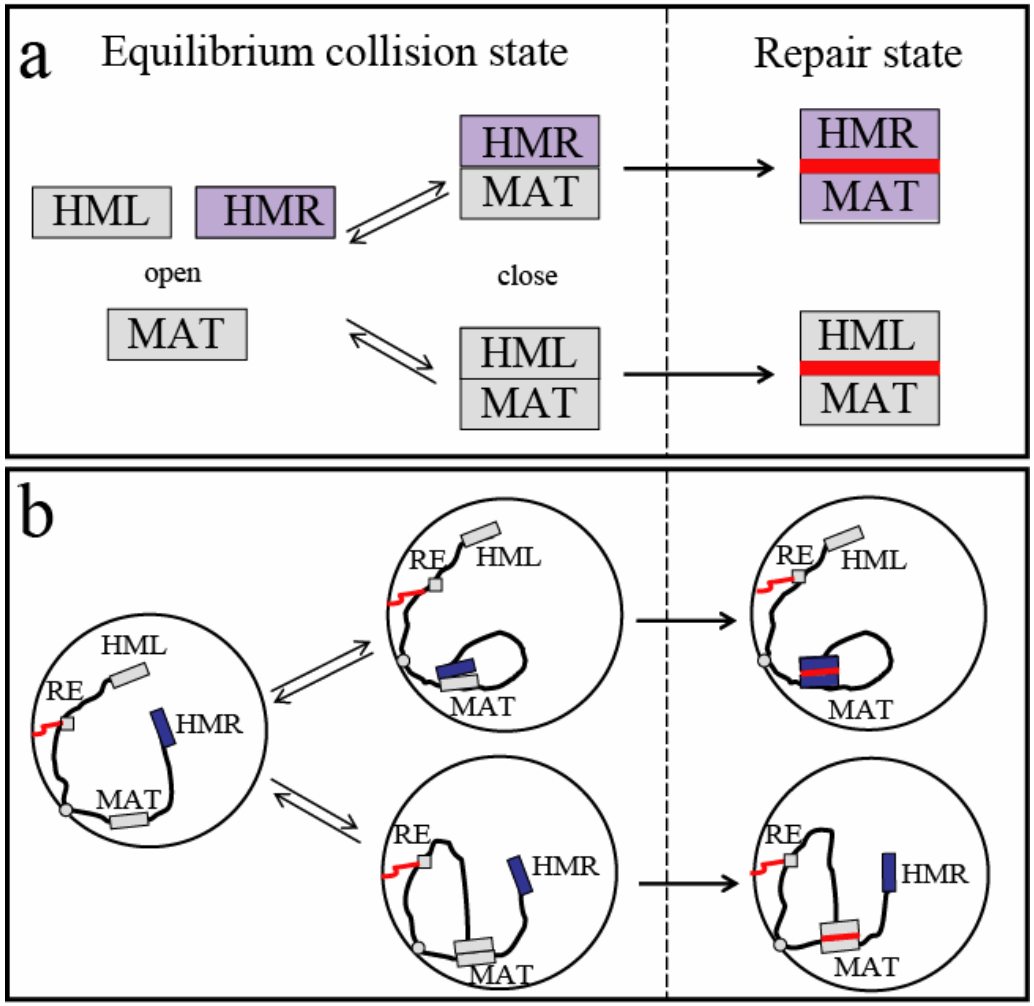
• The data is based on 6 single cells

• The distribution of distances during the repair is unknown



Houston et. al, PLoS Genet. 2006

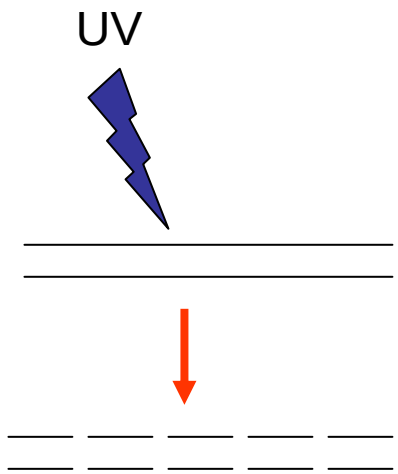
A thermodynamic model of recombination



J. Kondev

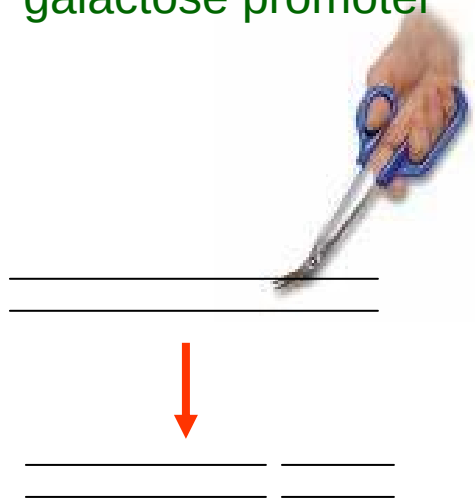
Controlled induction of a double stranded break in the genome of a genetically-engineered yeast

UV radiation



Number and locations of DSB are not controllable

Controlling the induction of HO by placing it under galactose promoter

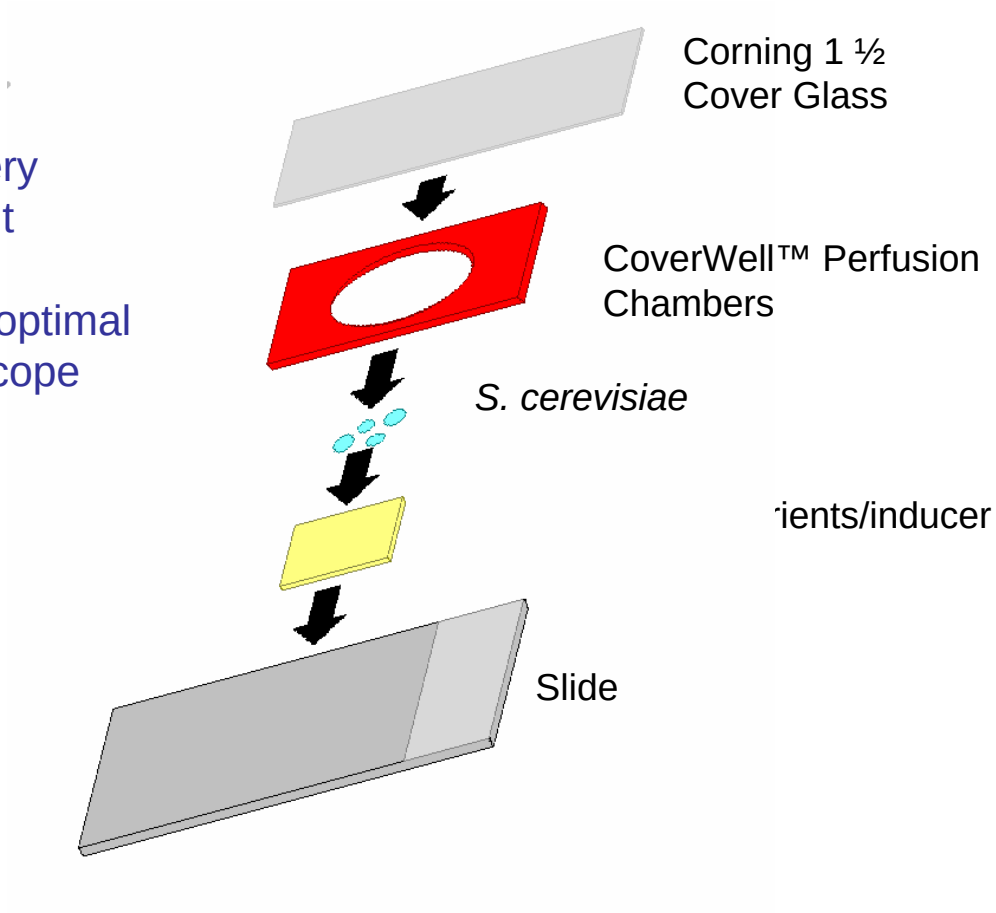


Number and locations of DSB are precisely controllable

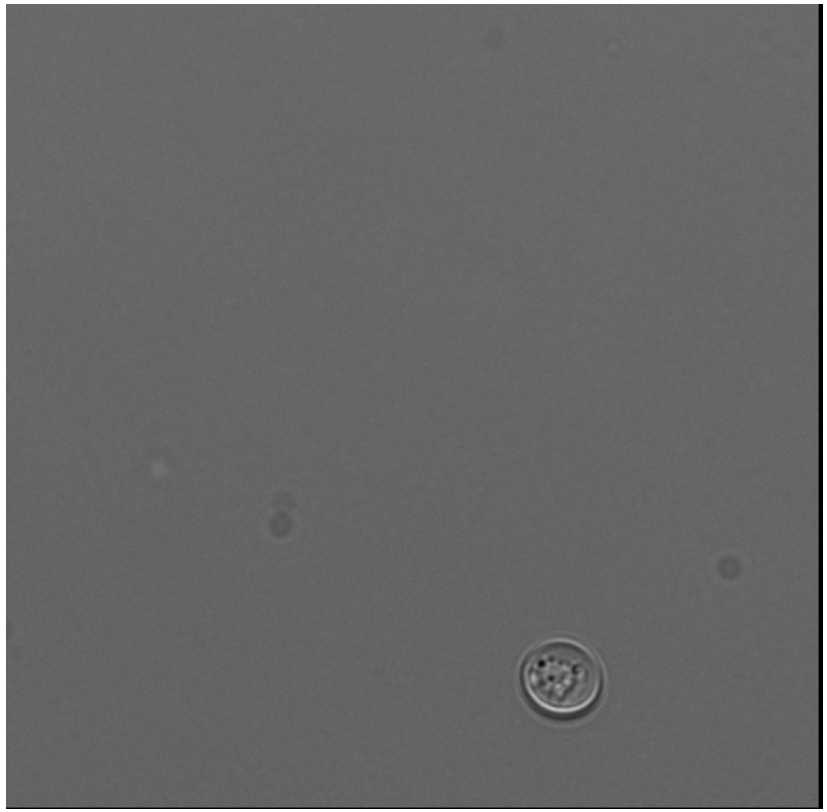
Genetic engineering allows us to induce a well-defined DSB in the genome of yeast, by simply putting Galactose into the media

Single cell visualization of repair

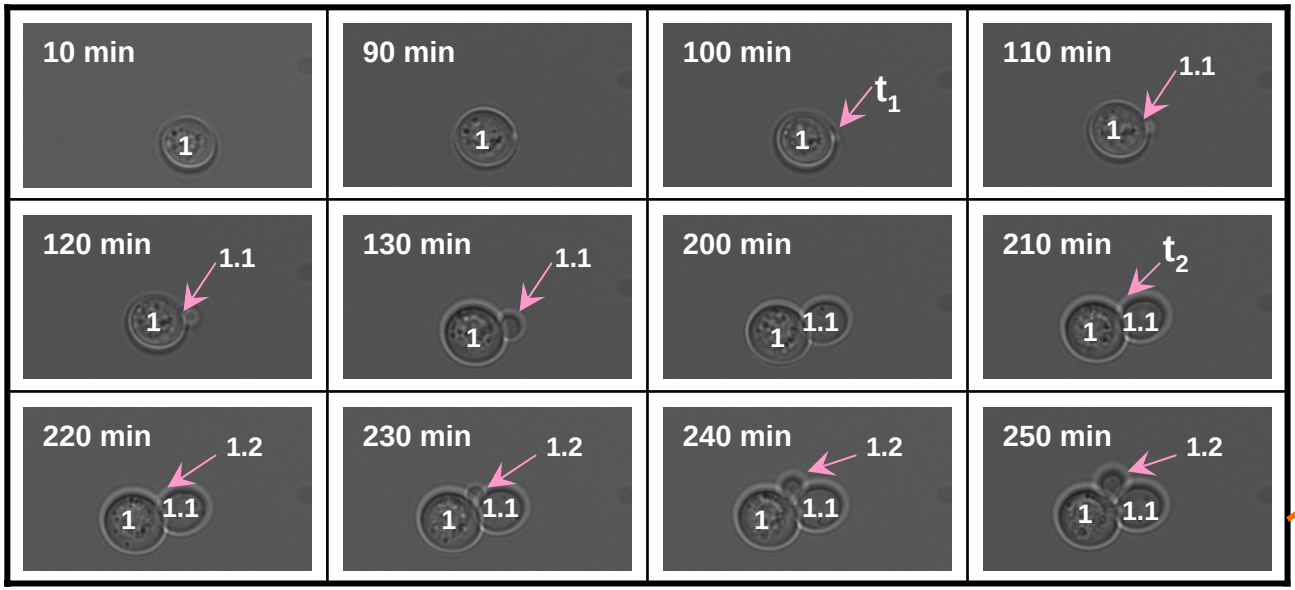
- Cells with broken DNA are very sensitive to the fluorescent light
- We need to keep the cells in optimal growth condition under microscope

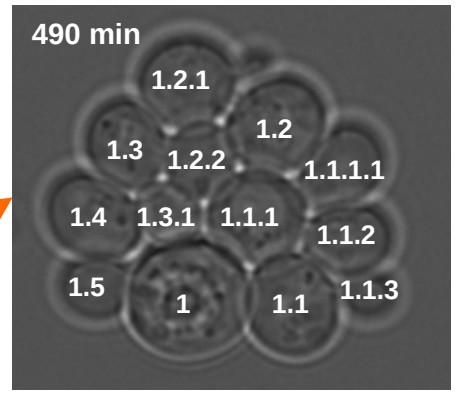


Long-term single cell observation



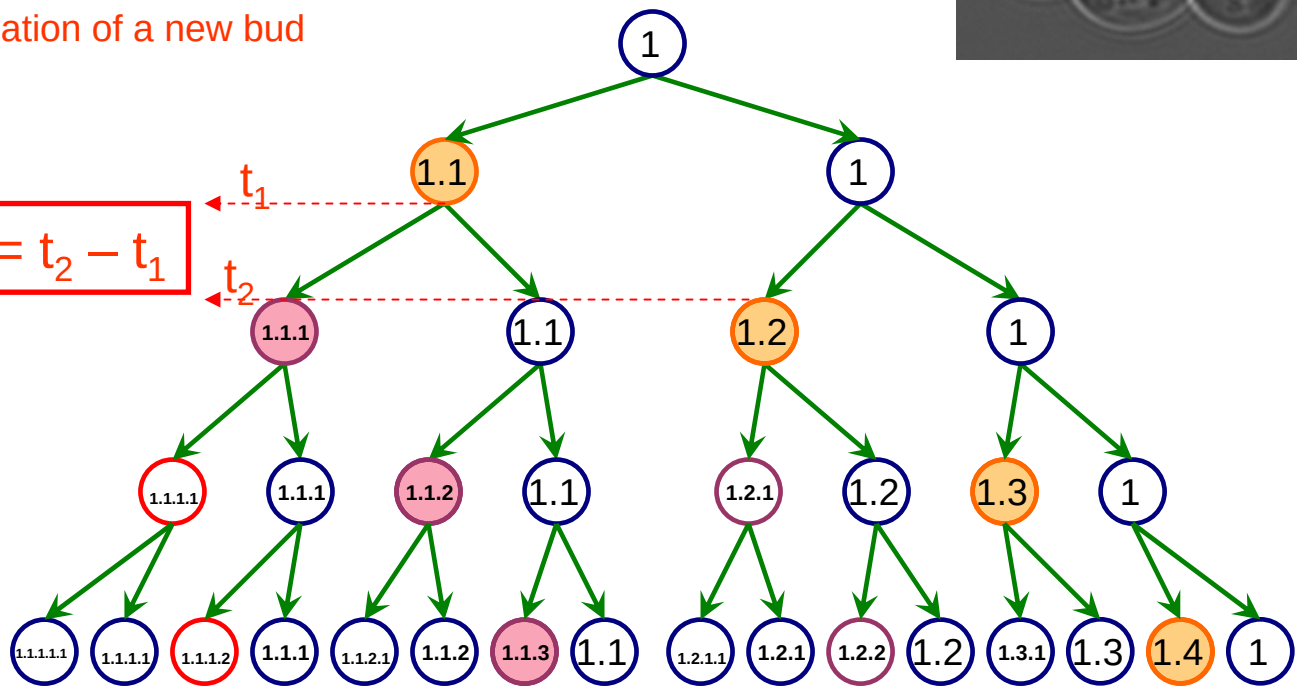
Total time = 14 hours



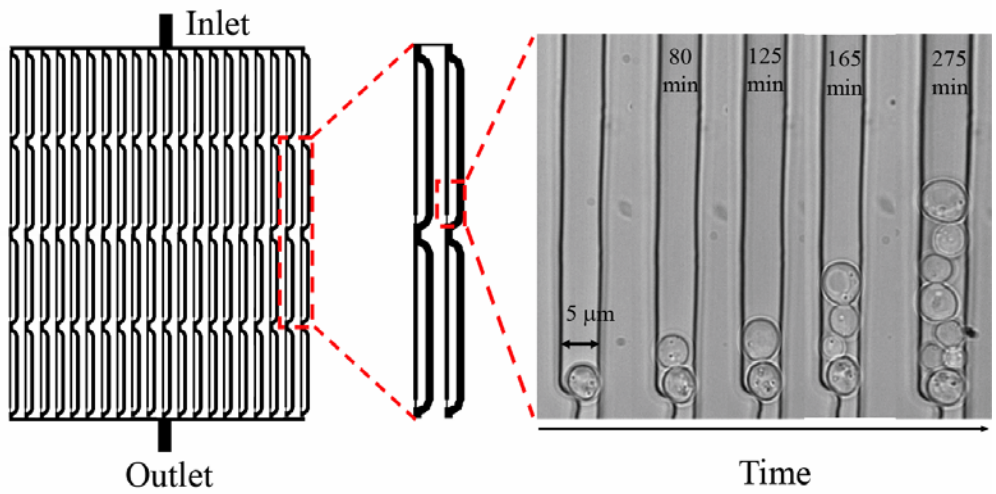


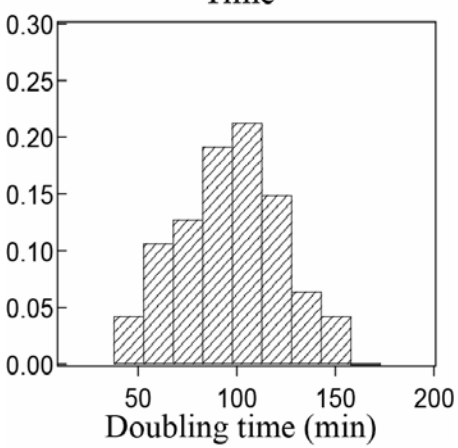
Arrows indicate the formation of a new bud

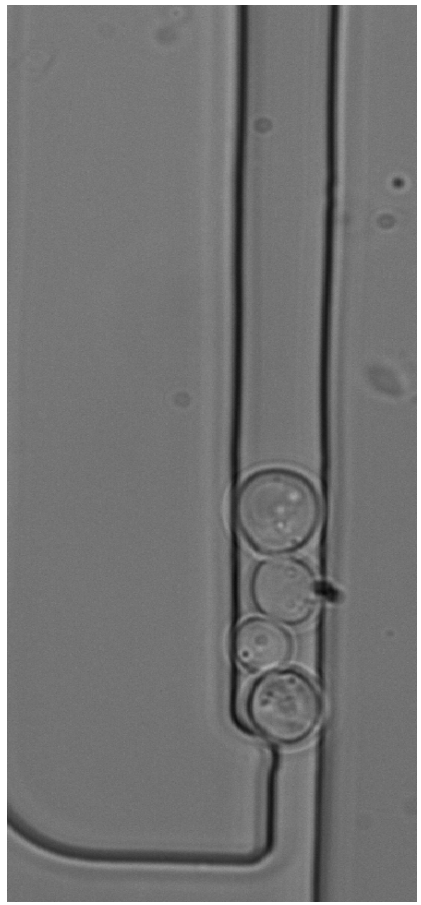
Doubling Time = $t_2 - t_1$



The progeny chamber



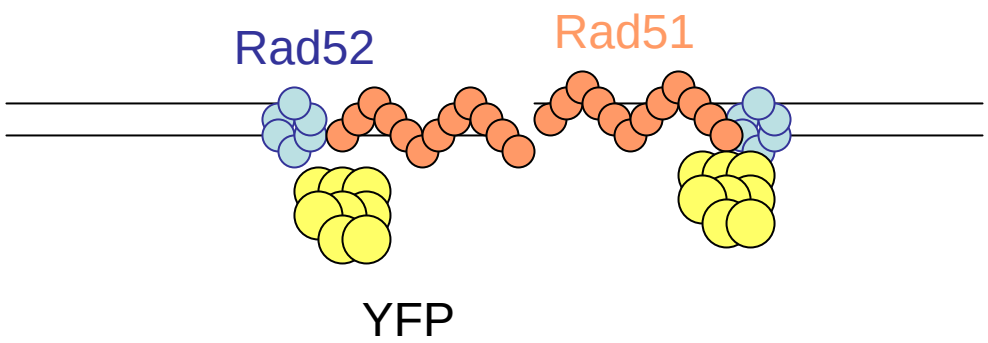




- The depth of the channels ~ size of the cells

- Cells stay in focus for many generations

How do we measure the repair time?



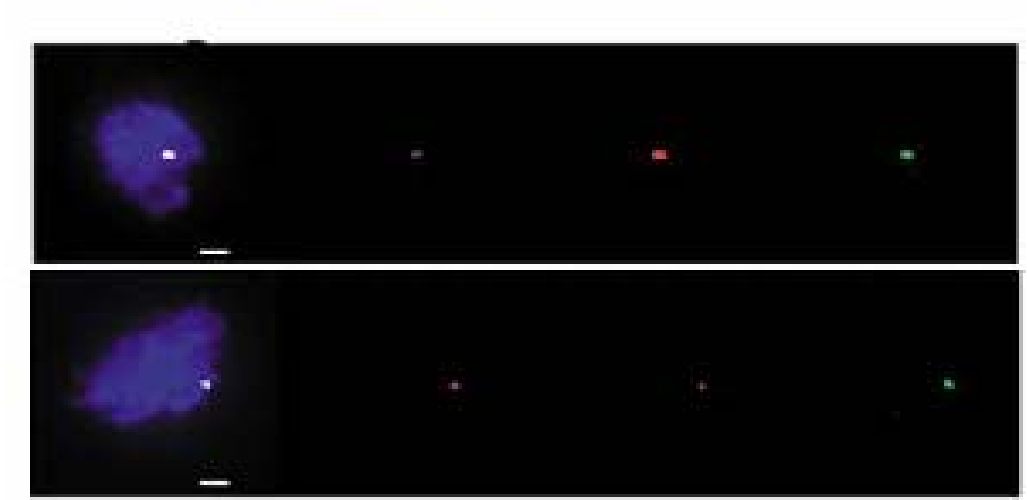
- Rad52 is one the first proteins recruited to the site of a DSB

- The formation of Rad52 foci was observed to begin at induction and end with the DSB repair event

- Therefore the timing of the disappearance of the Rad52 foci can be used as a measure of the completion of the repair event

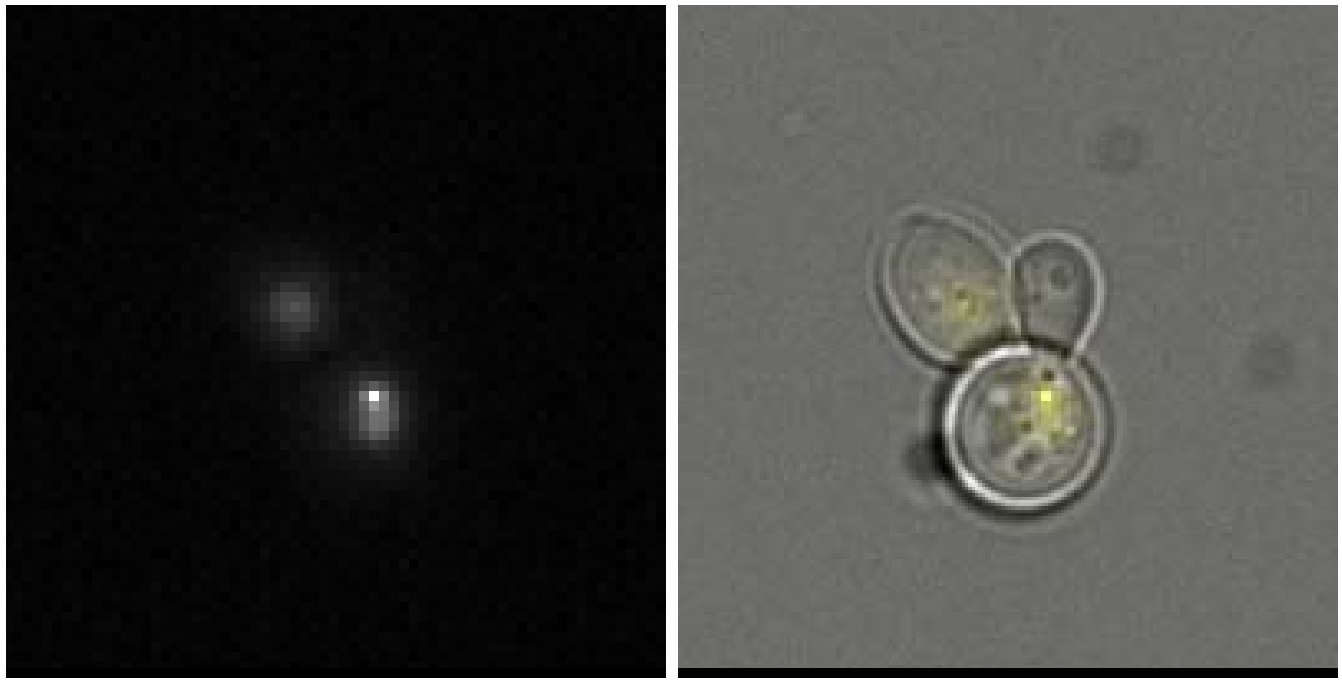
Rad52 co-localizes with the site of double-strand break

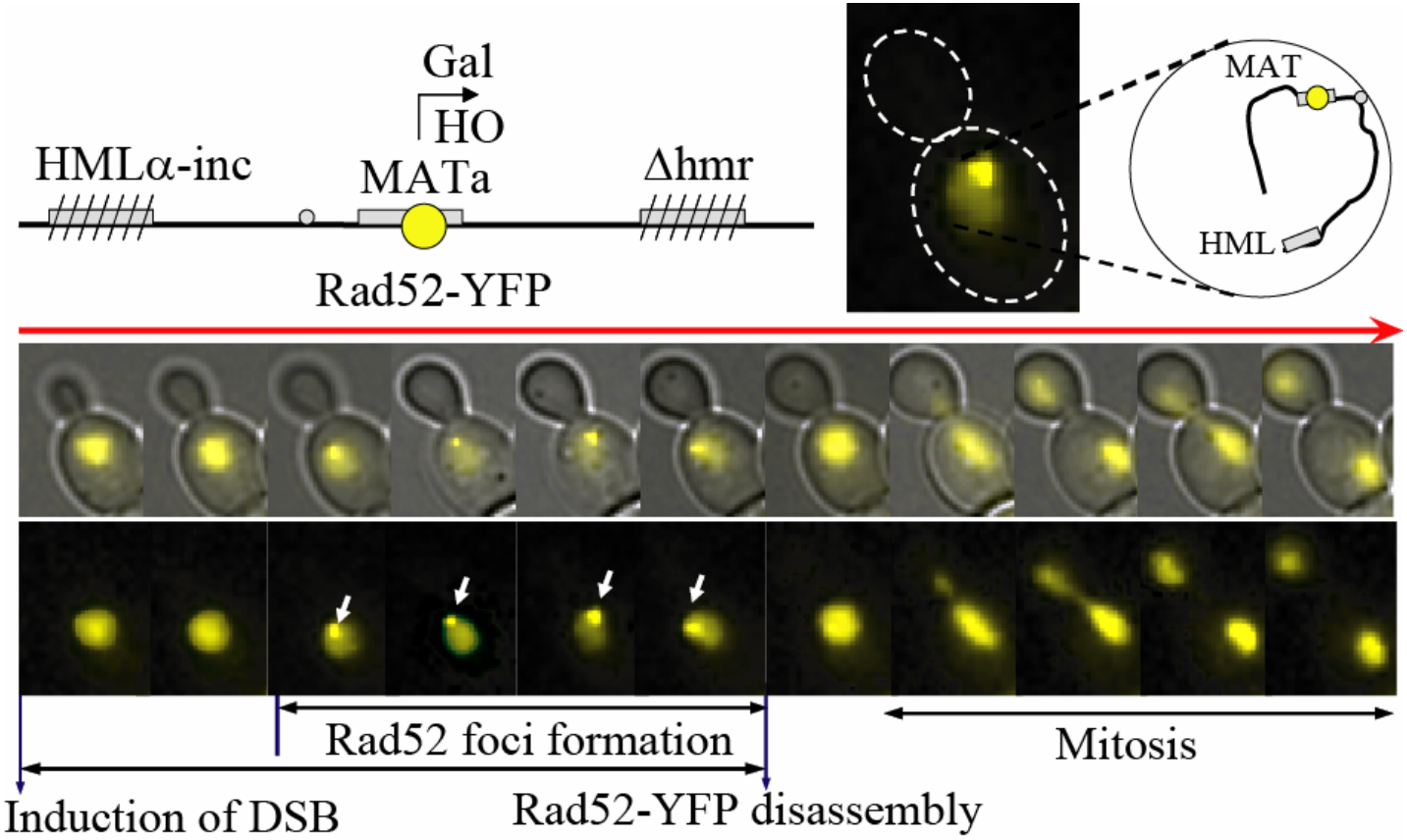
Rad51 Rad52 HO cut-site

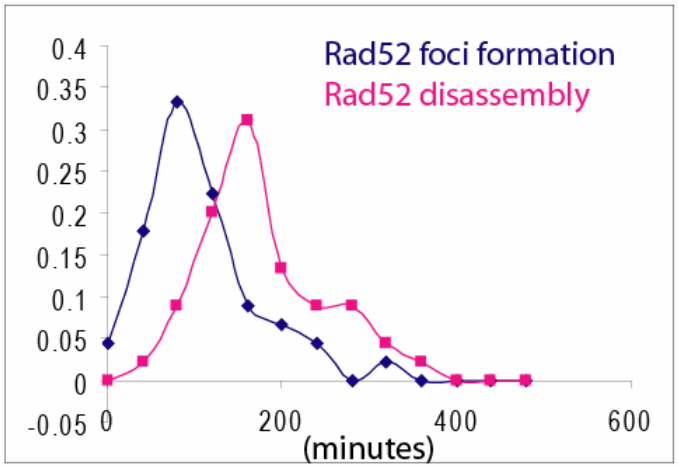


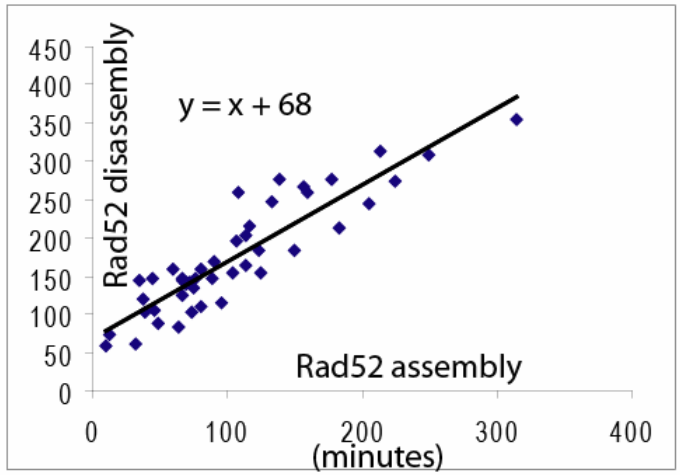
Miyazaki, EMBO 2004

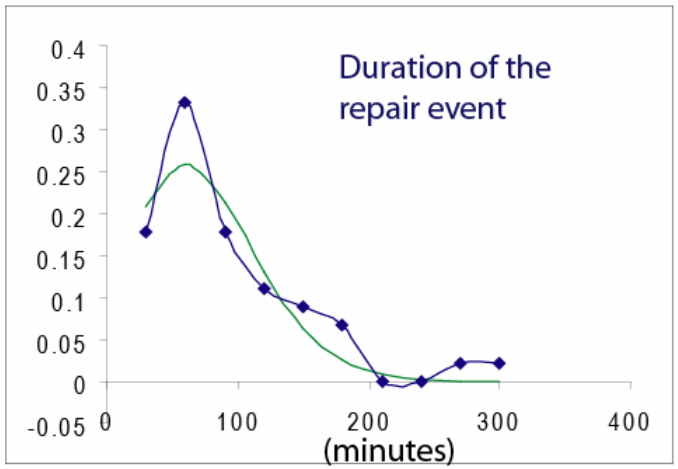
Visualization of the DNA repair process

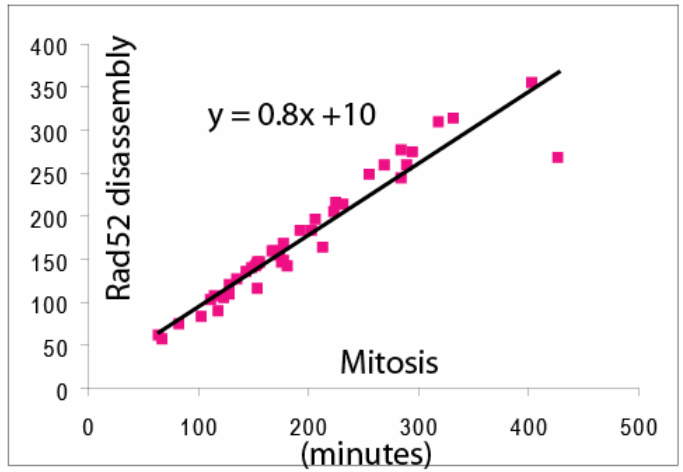



Gal
HO
HMLα-inc
MATa
Δhmr
Rad52-YFP
MAT
HML
Rad52 foci formation
Mitosis
Induction of DSB
Rad52-YFP disassembly

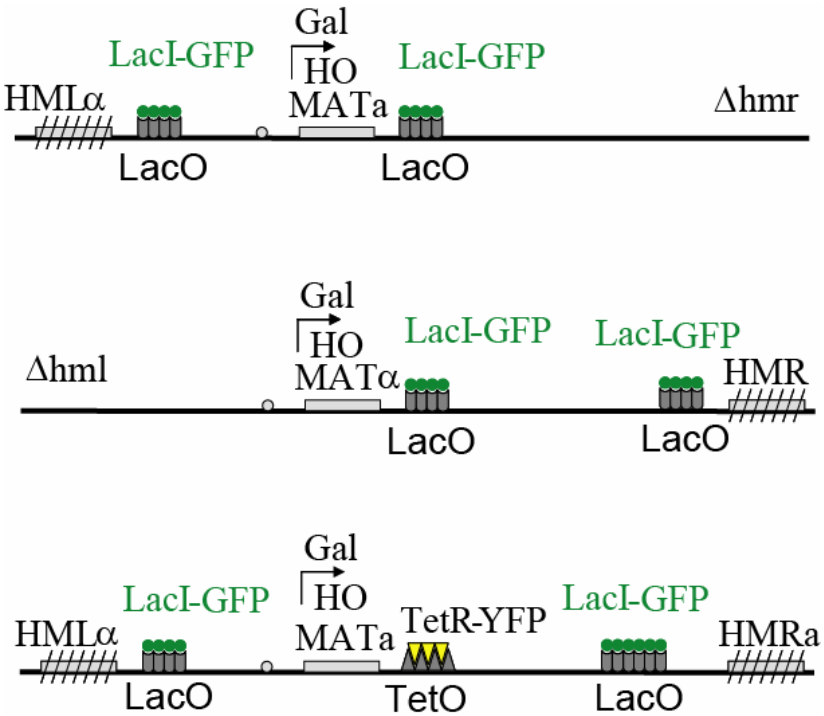

Rad52 foci formation
Rad52 disassembly
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
0
200
400
600
(minutes)

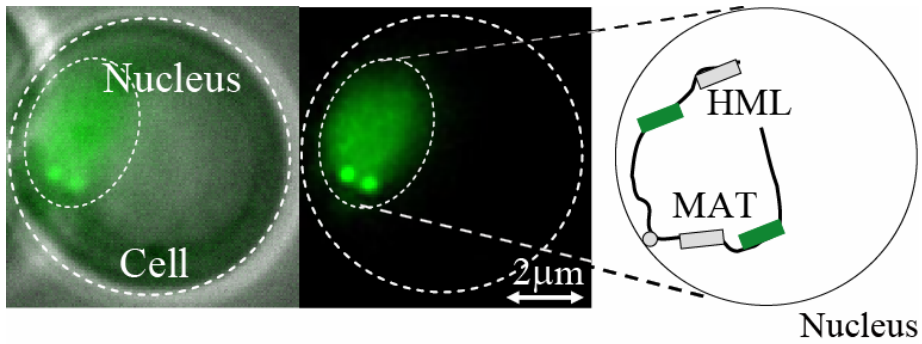

y = x + 68
Rad52 disassembly
Rad52 assembly
450
400
350
300
250
200
150
100
50
0
0
100
200
300
400
(minutes)


Duration of the
repair event
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
0
100
200
300
400
(minutes)


y = 0.8x +10
Rad52 disassembly
Mitosis
400
350
300
250
200
150
100
50
0
0
100
200
300
400
500
(minutes)

Single cell visualization of the DNA repair *in vivo*





- Single cell measurements will teach us about the mechanism of the repair

- Our preliminary data demonstrates that even in a population of genetically identical cells, the duration of repair event varies greatly from cell-to-cell.

- So far only biological processes that regulate cell functions, such as noise at the level of gene transcription and translation have been recognized as major contributions to population heterogeneity.

- However besides a biological reason for production of heterogeneity other physical factors may be in play, for example the variation in timing of the repair event is partially due to the randomness of the search event itself, which is purely a physical mechanism.