The Abdus Salam
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2038-30

Conference: From DNA-Inspired Physics to Physics-Inspired Biology

1 - 5 June 2009

Finding a match - How do homologous sequences find each other for recombination?

Adi BARZEL

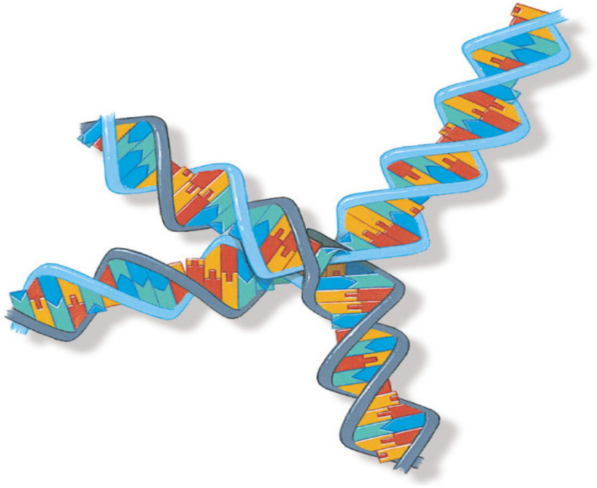
Tel Aviv University
Dept. of Molecular Microbiology & Biotechnology Ramat Aviv
Tel Aviv 69978
ISRAEL

Finding a match-

How do homologous sequences find each other for recombination?

Adi Barzel
The Martin Kupiec lab
Tel Aviv University
Israel

Topics:



- The biological context of recombination
- The way we study recombination
- The open question of homology search

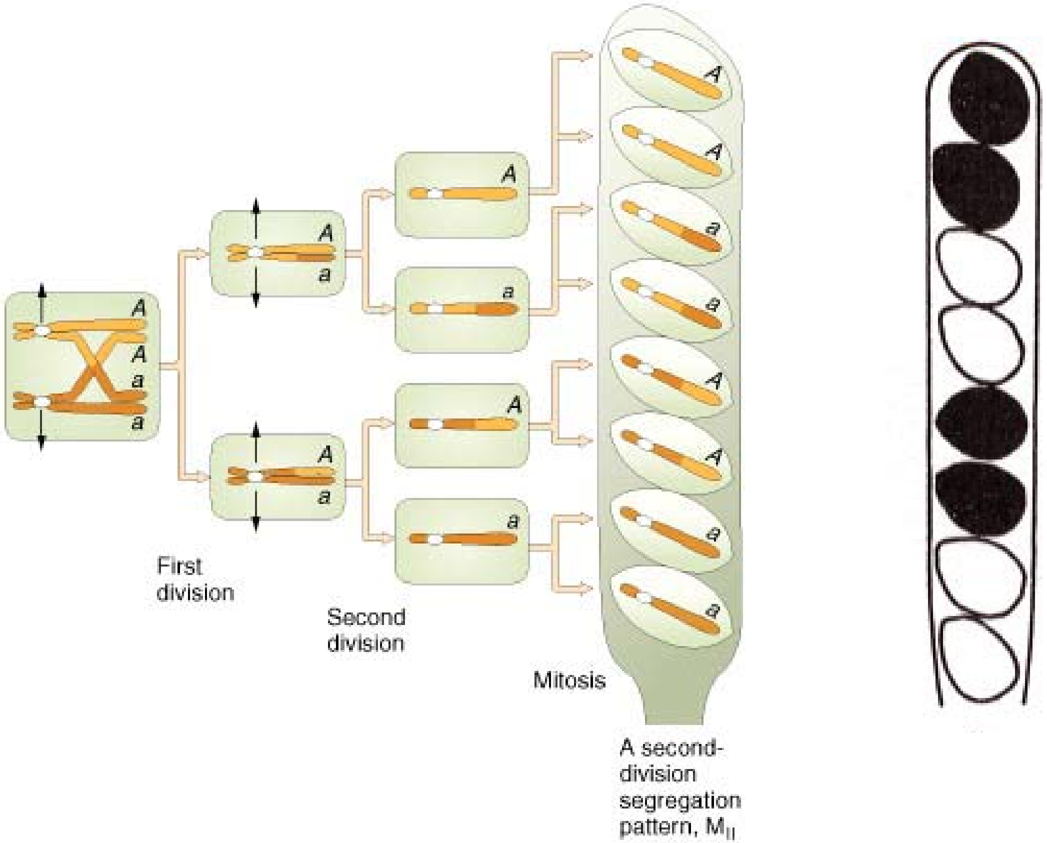
HOMOLOGOUS RECOMBINATION

Two similar DNA molecules exchange information

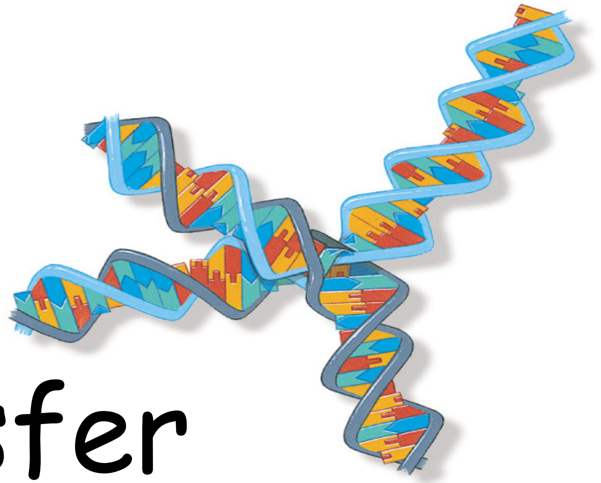
When taking place in Meiosis it produces genetic variability

HOMOLOGOUS RECOMBINATION

producing genetic variability

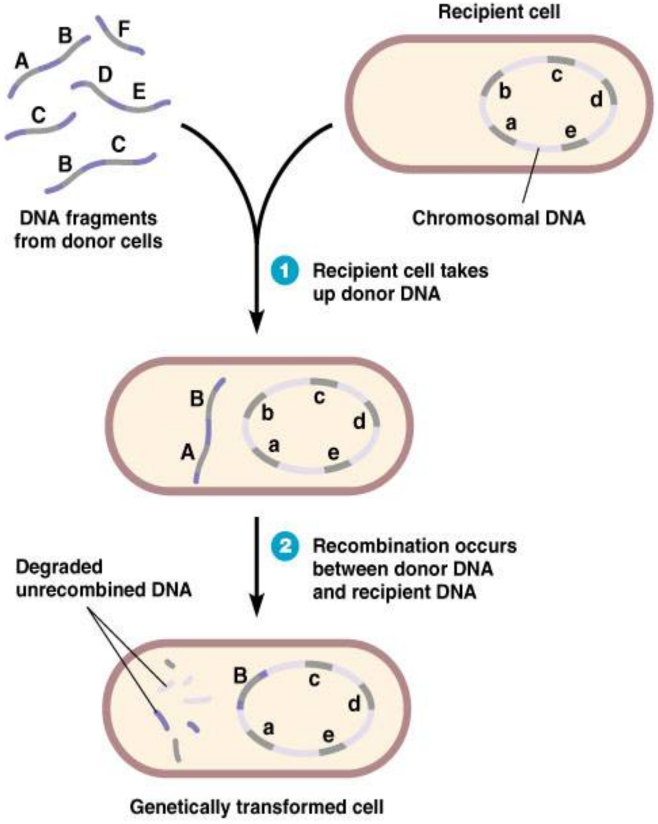


HOMOLOGOUS RECOMBINATION



Horizontal gene transfer

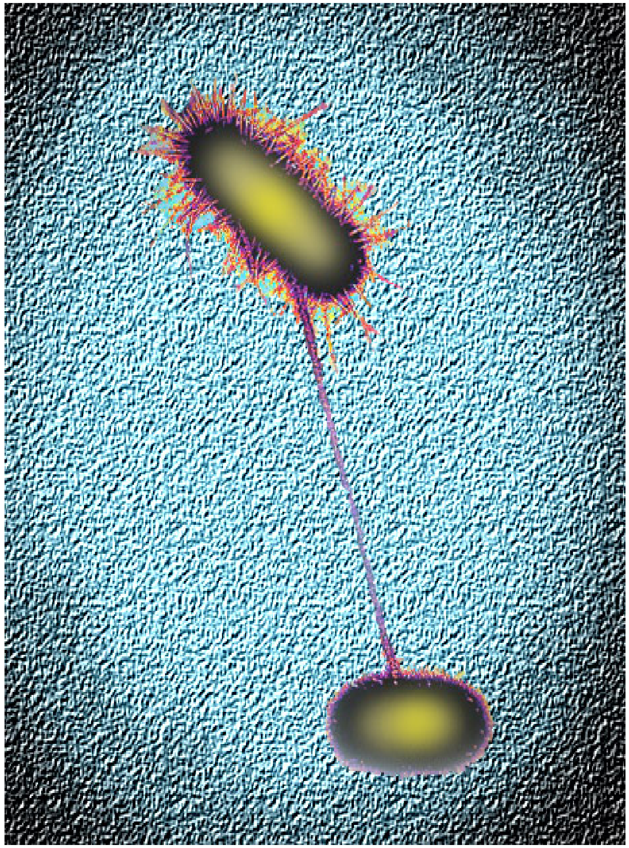
Integrative transformation



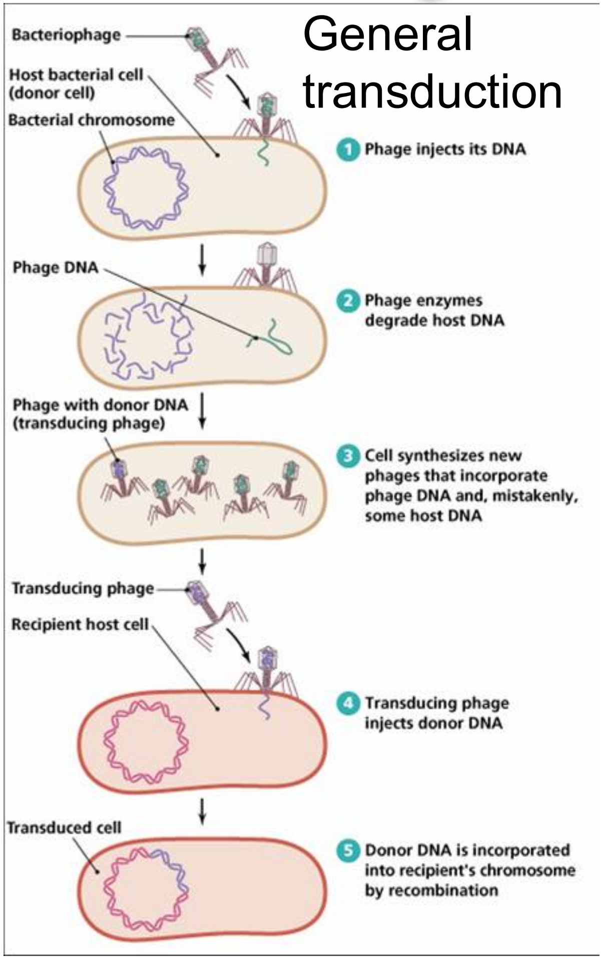
① Recipient cell takes up donor DNA

② Recombination occurs between donor DNA and recipient DNA

Hfr Conjugation



General transduction



① Phage injects its DNA

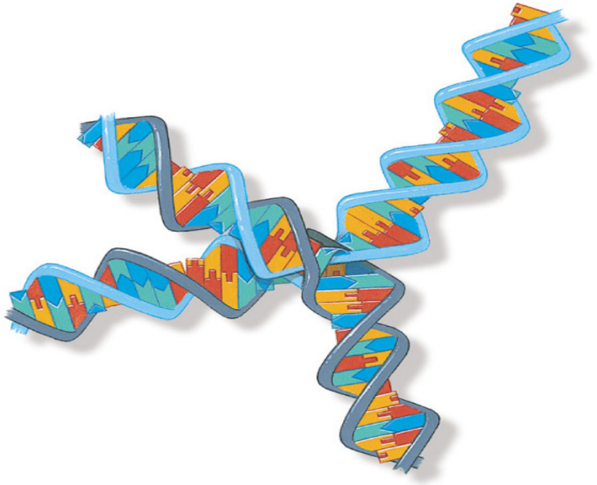
② Phage enzymes degrade host DNA

③ Cell synthesizes new phages that incorporate phage DNA and, mistakenly, some host DNA

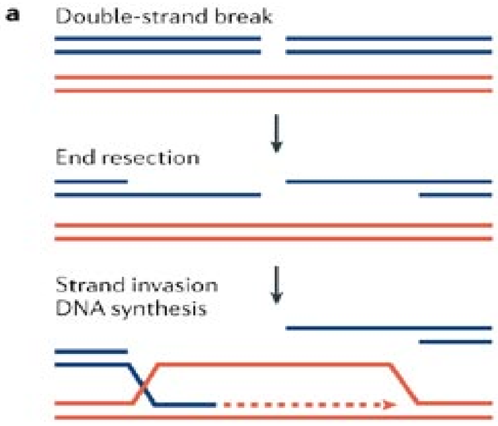
④ Transducing phage injects donor DNA

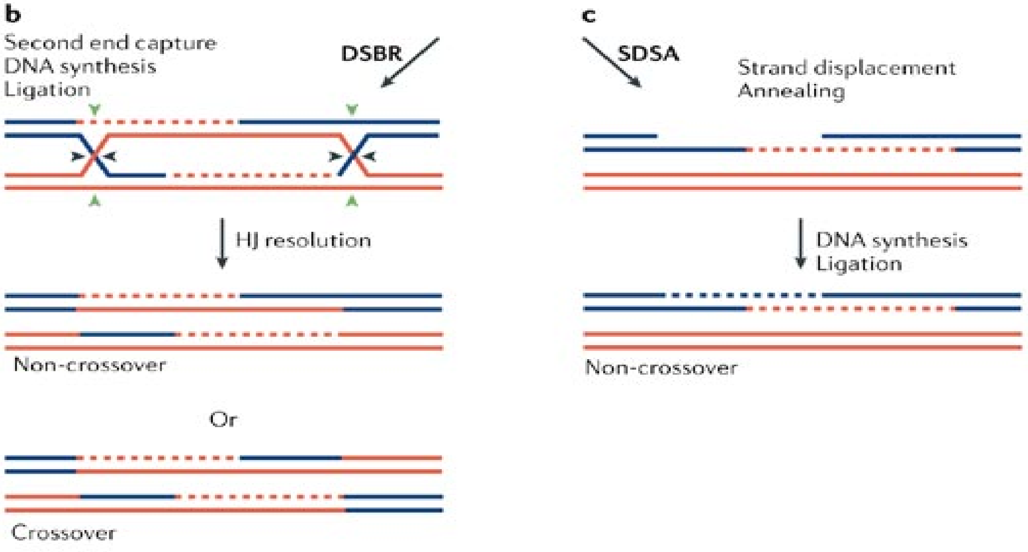
⑤ Donor DNA is incorporated into recipient's chromosome by recombination

HOMOLOGOUS RECOMBINATION



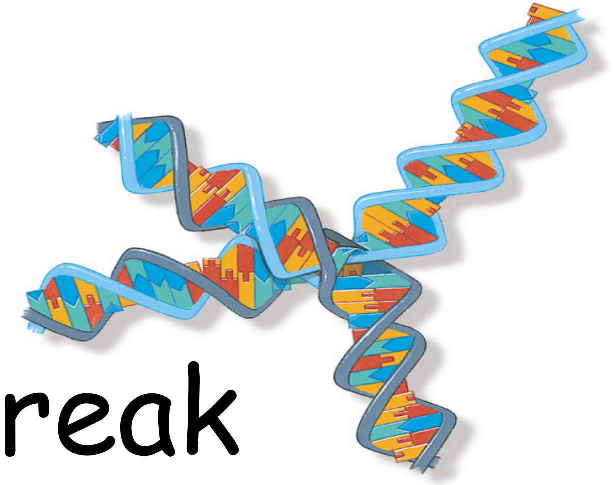
A DNA repair mechanism



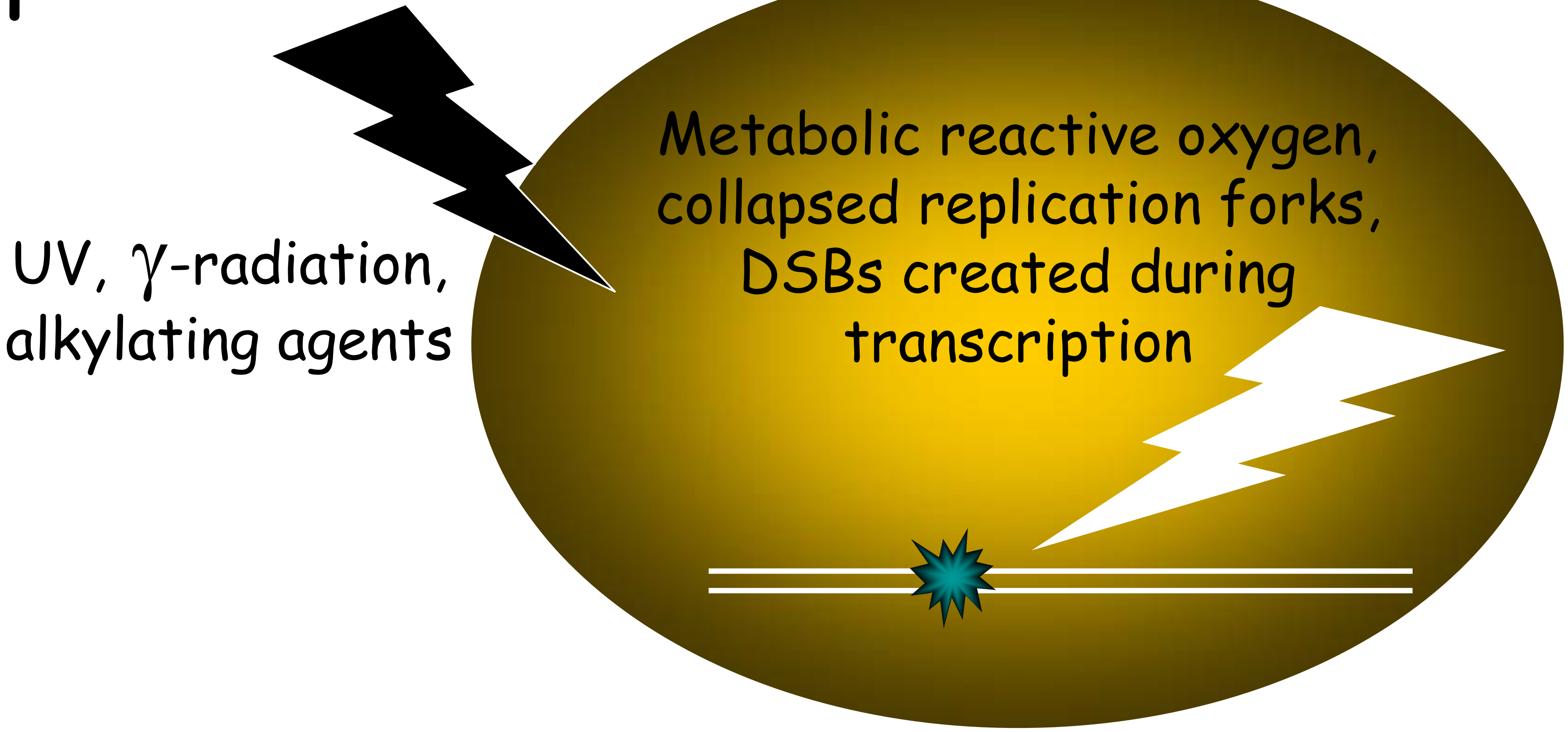


Nature Reviews | Molecular Cell Biology

HOMOLOGOUS RECOMBINATION



A DNA double strand break repair mechanism

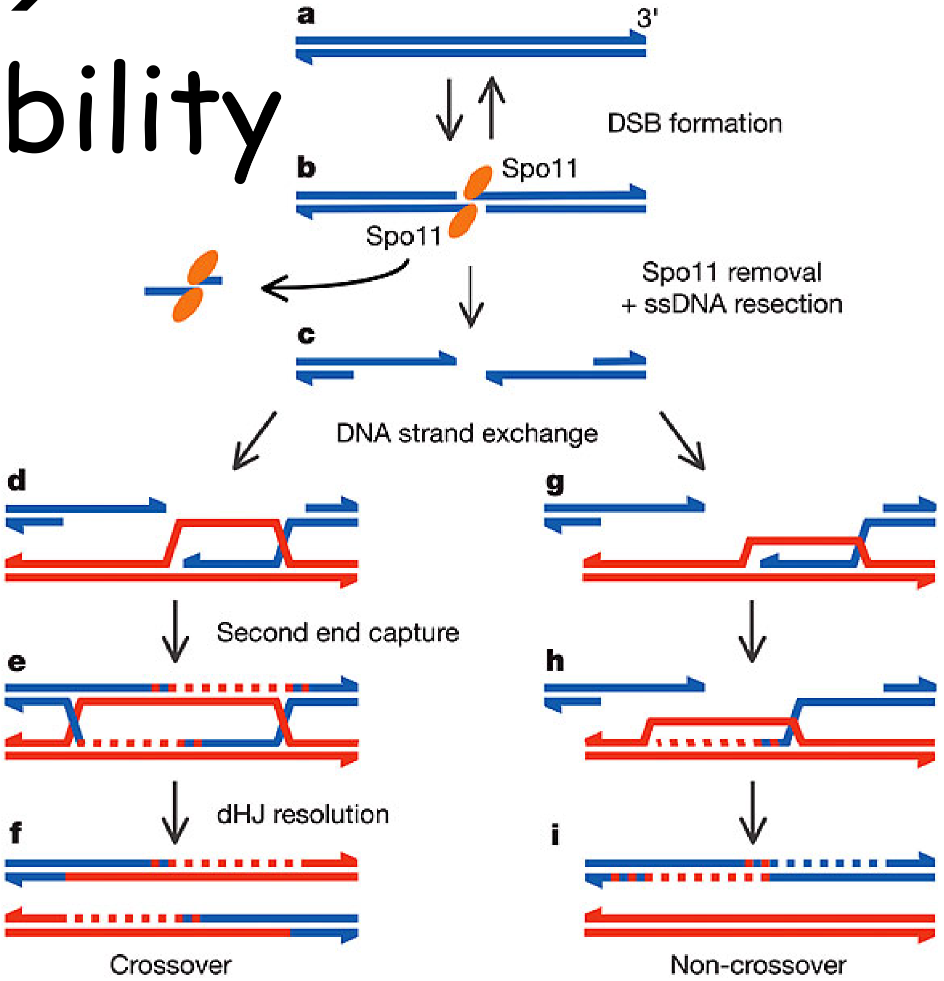


UV, γ-radiation, alkylating agents

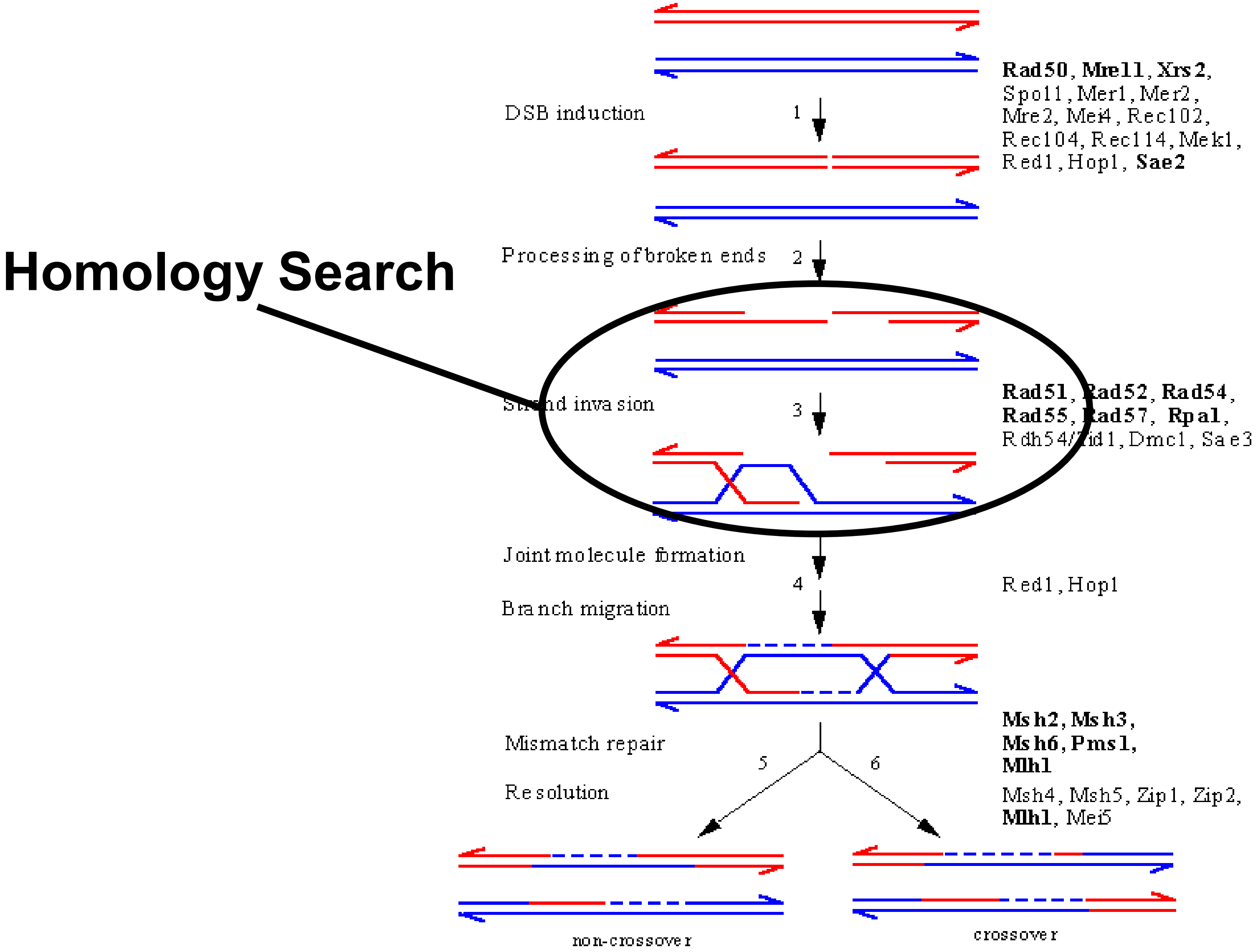
Metabolic reactive oxygen, collapsed replication forks, DSBs created during transcription

HOMOLOGOUS RECOMBINATION

DNA repair → genetic variability



How do you repair a broken chromosome?



Homology Search

DSB induction

1

Rad50, **Mre11**, **Xrs2**, Spo11, Mer1, Mer2, Mre2, Mei4, Rec102, Rec104, Rec114, Mek1, Red1, Hop1, **Sae2**

Processing of broken ends

2

Strand invasion

3

Rad51, **Rad52**, **Rad54**, **Rad55**, **Rad57**, **Rpa1**, Rdh54/Tid1, Dmc1, Sae3

Joint molecule formation

4

Red1, Hop1

Branch migration

Mismatch repair

Msh2, **Msh3**, **Msh6**, **Pms1**, **Mlh1**

5

6

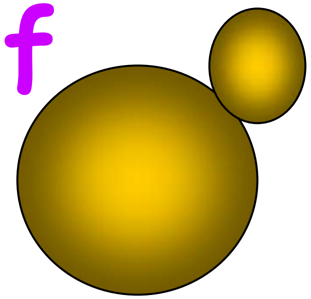
Resolution

Msh4, Msh5, Zip1, Zip2, **Mlh1**, Mei5

non-crossover

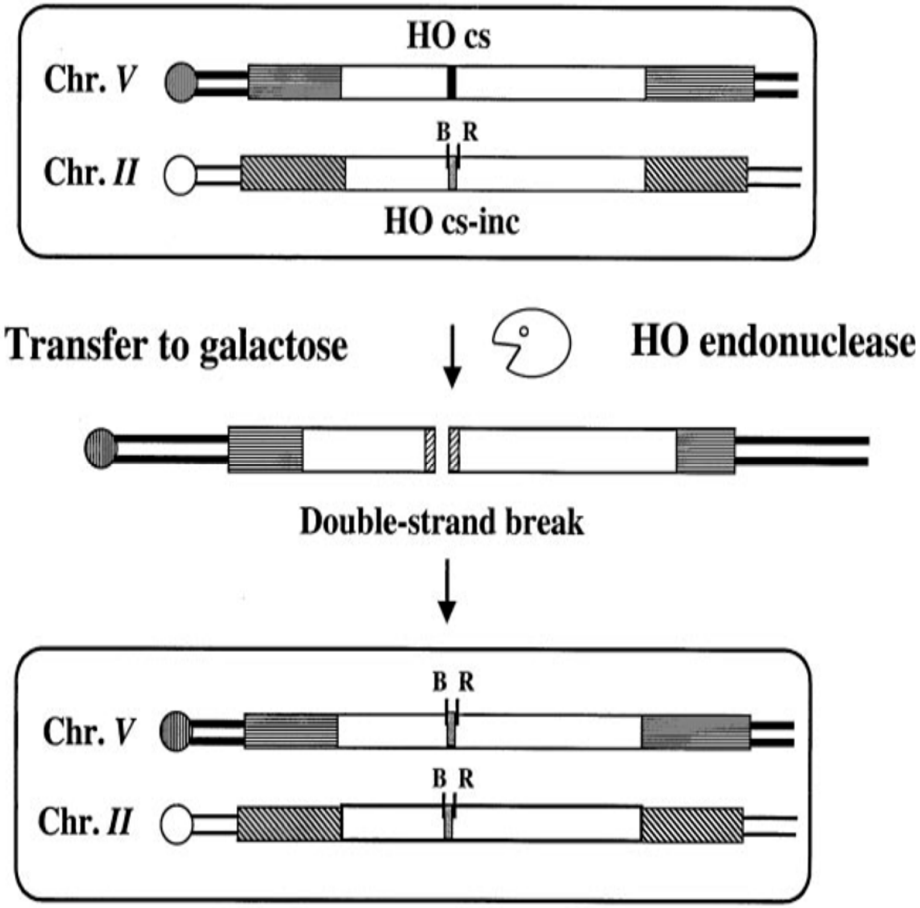
crossover

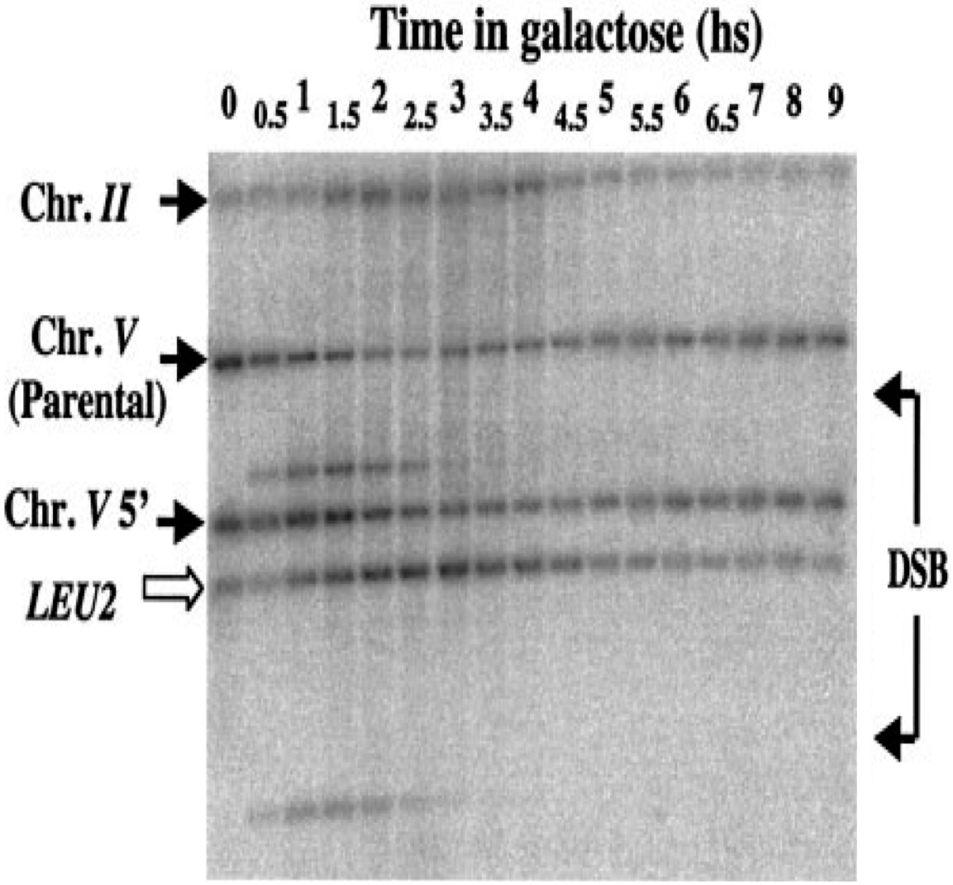
The budding yeast as a model organism for the study of recombination



Pros	Cons
Ease of genetic manipulations	Small nucleus- challenging cytology
High rates of mitotic recombination	Sometimes difficult to extrapolate results to higher eukaryotes
Highly studied	

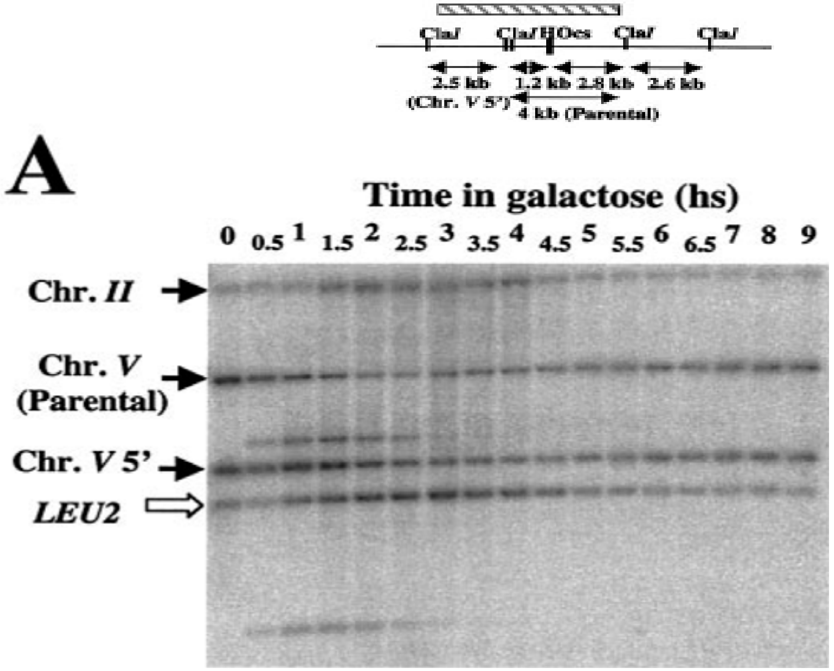
The HO experimental system

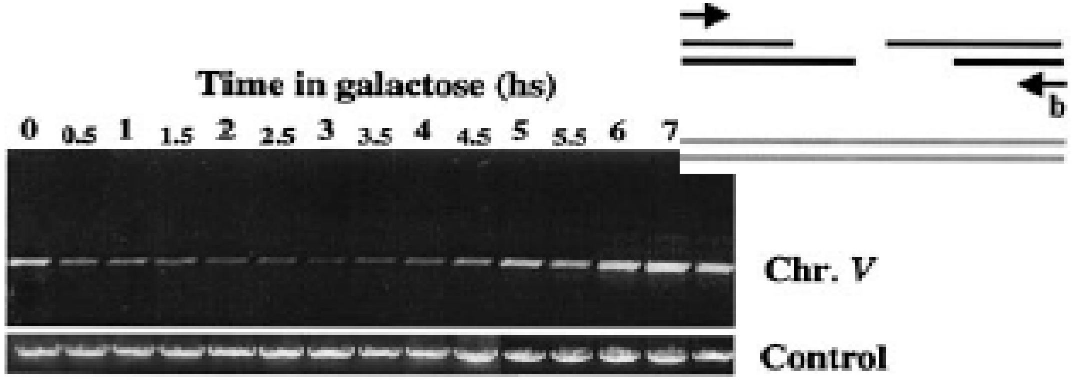


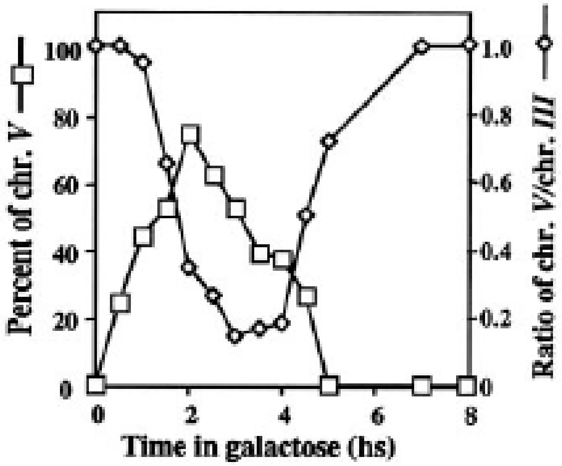


Assays in use

- The double stranded DNA break and its repair are assayed by southern blot and quantitative PCR



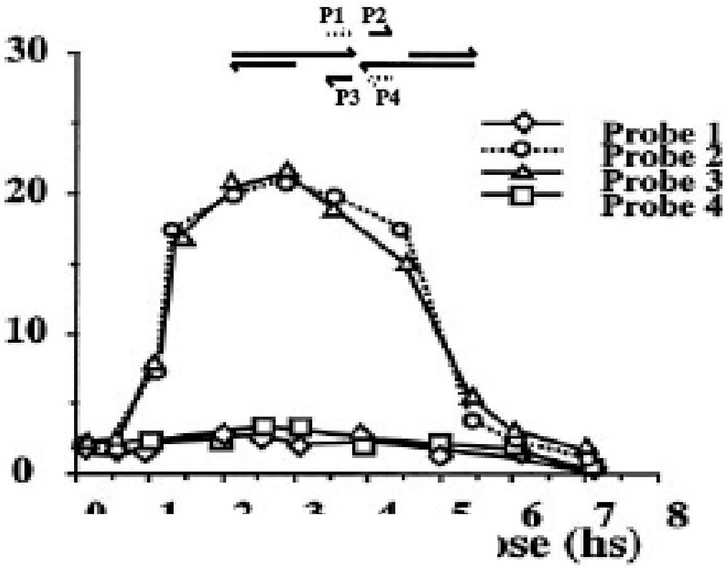


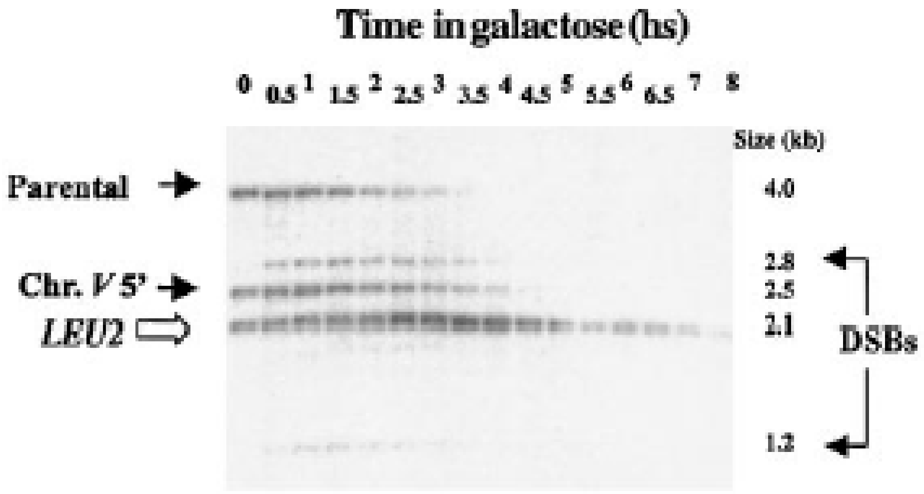


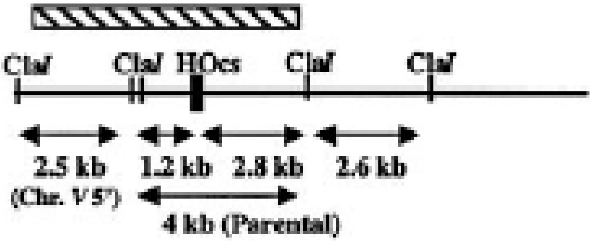
Aylon Y et al Mol Cell Biol Feb 2003 .

Assays in use

- DNA resection is assayed by slot blot and southern blot



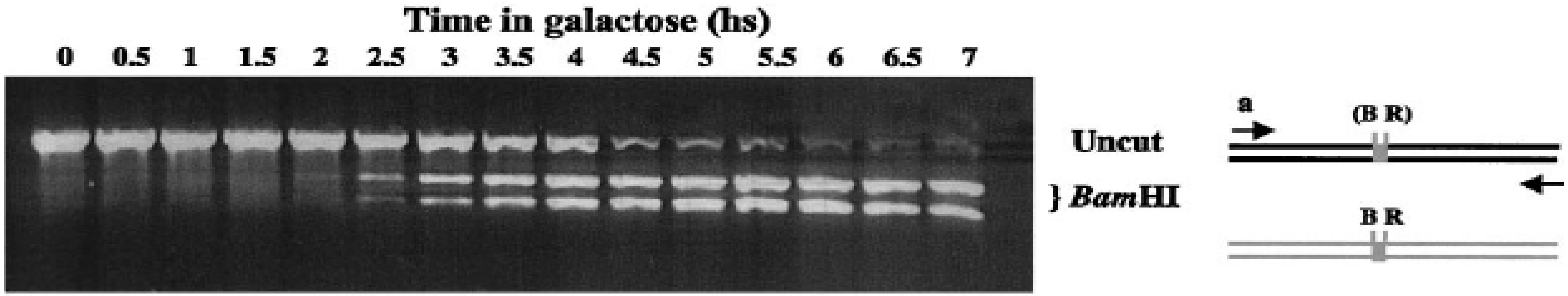




Aylon Y et al Mol Cell Biol Feb 2003 .

Assays in use

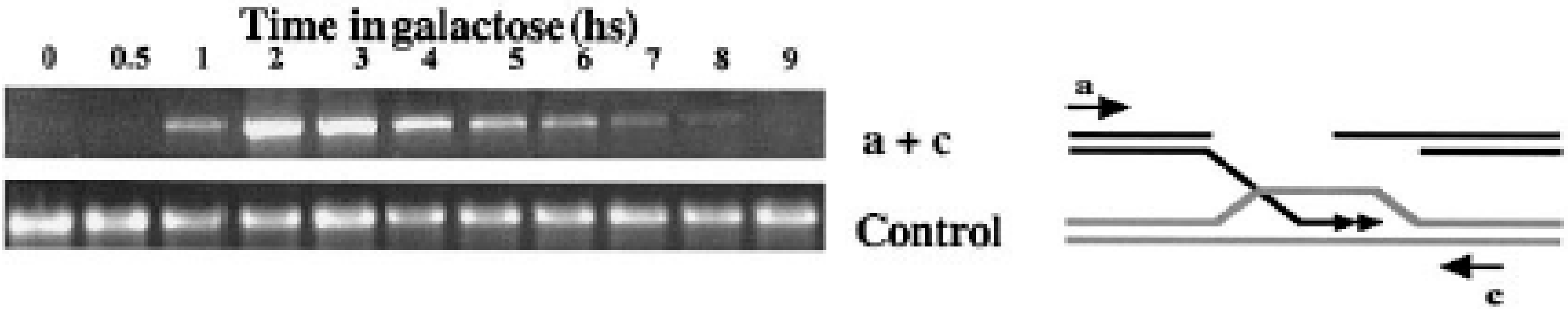
- Gene conversion rates are assessed using restriction sites polymorphisms.



Aylon Y et al Mol Cell Biol Feb 2003 .

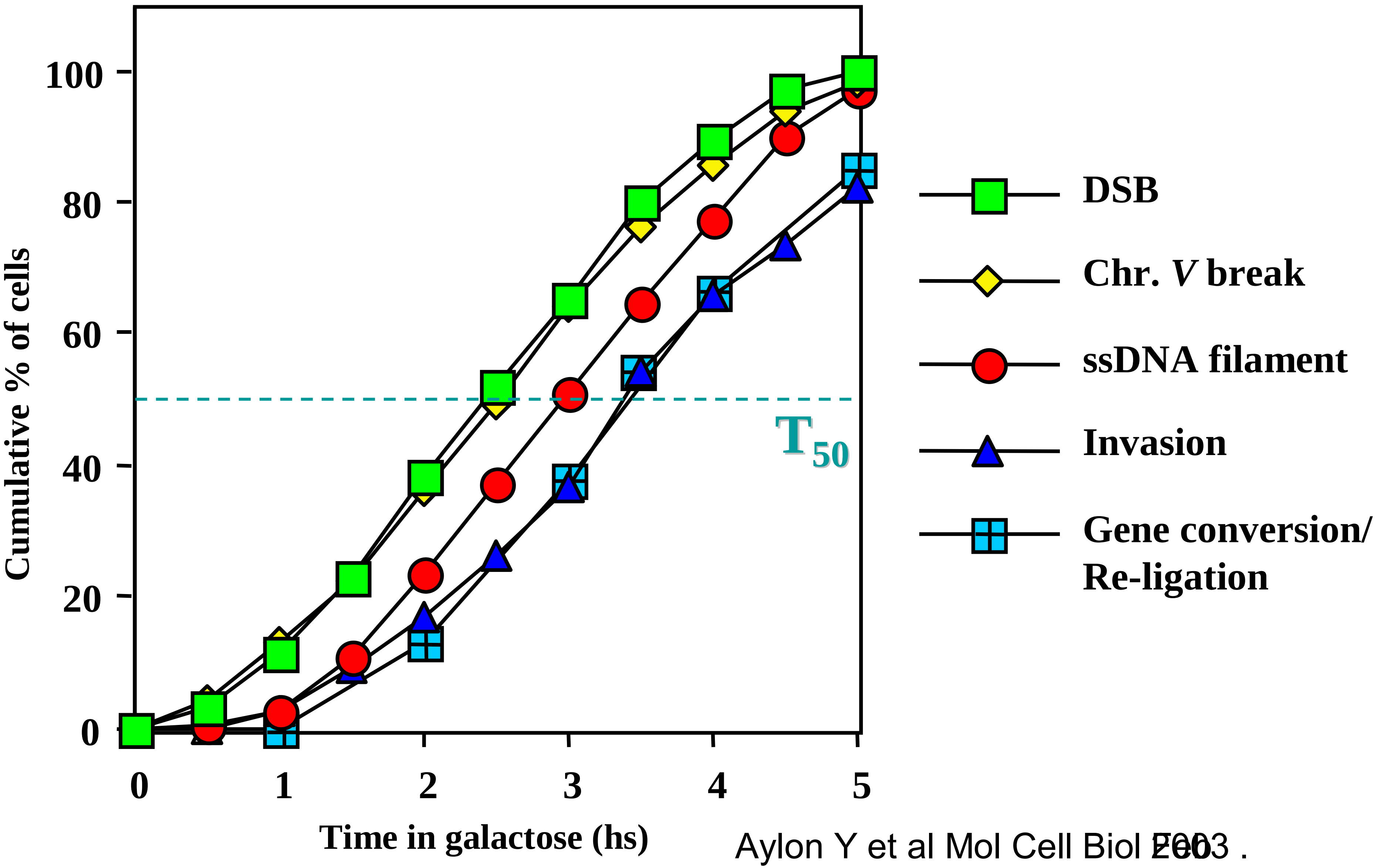
Assays in use

- Post-invasion polymerization is assayed by PCR.



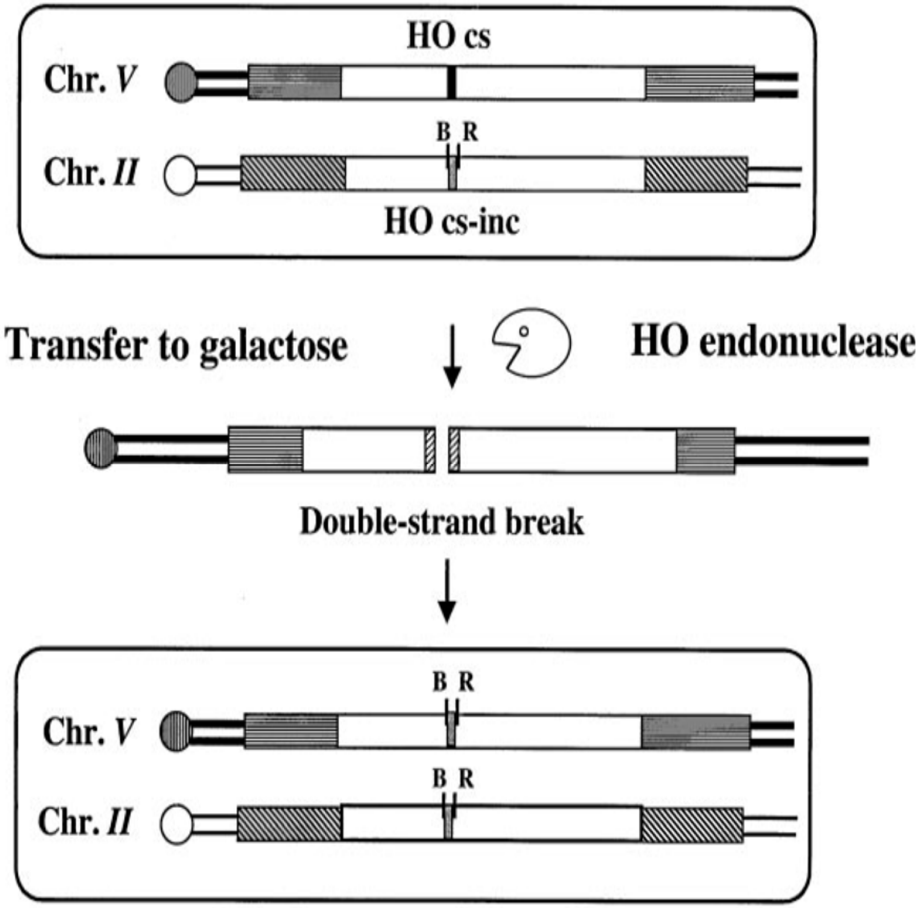
Aylon Y et al Mol Cell Biol Feb 2003 .

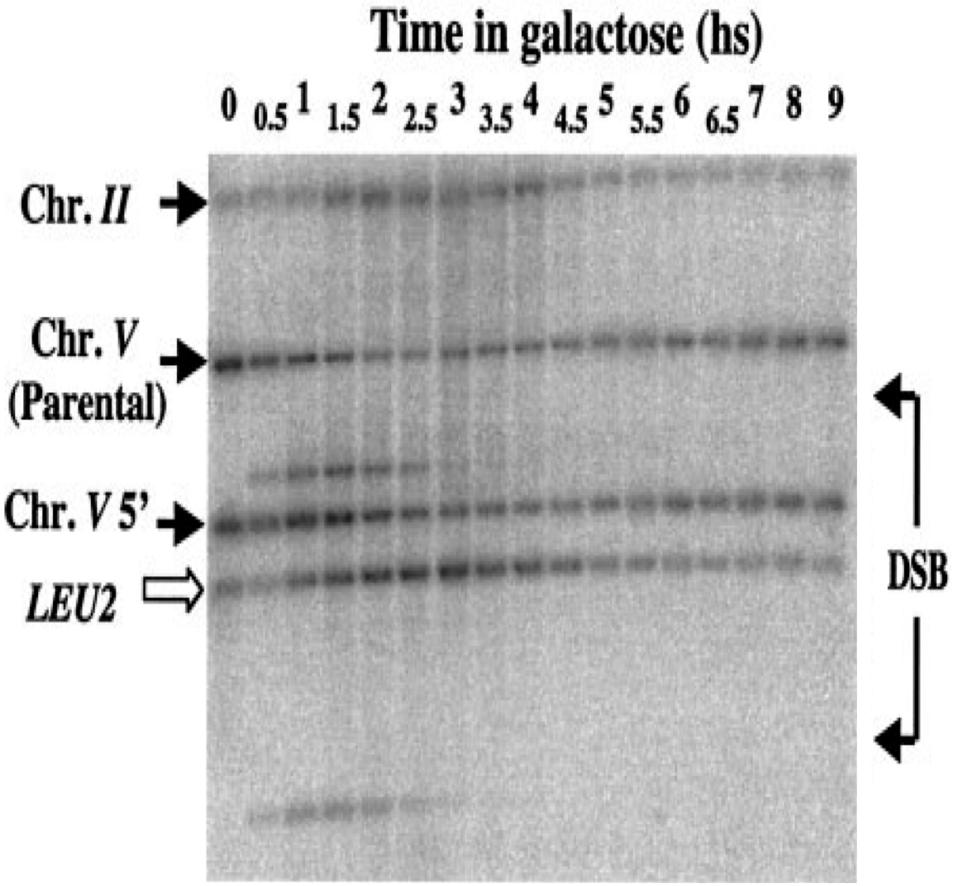
Dissection of yeast mitotic recombination



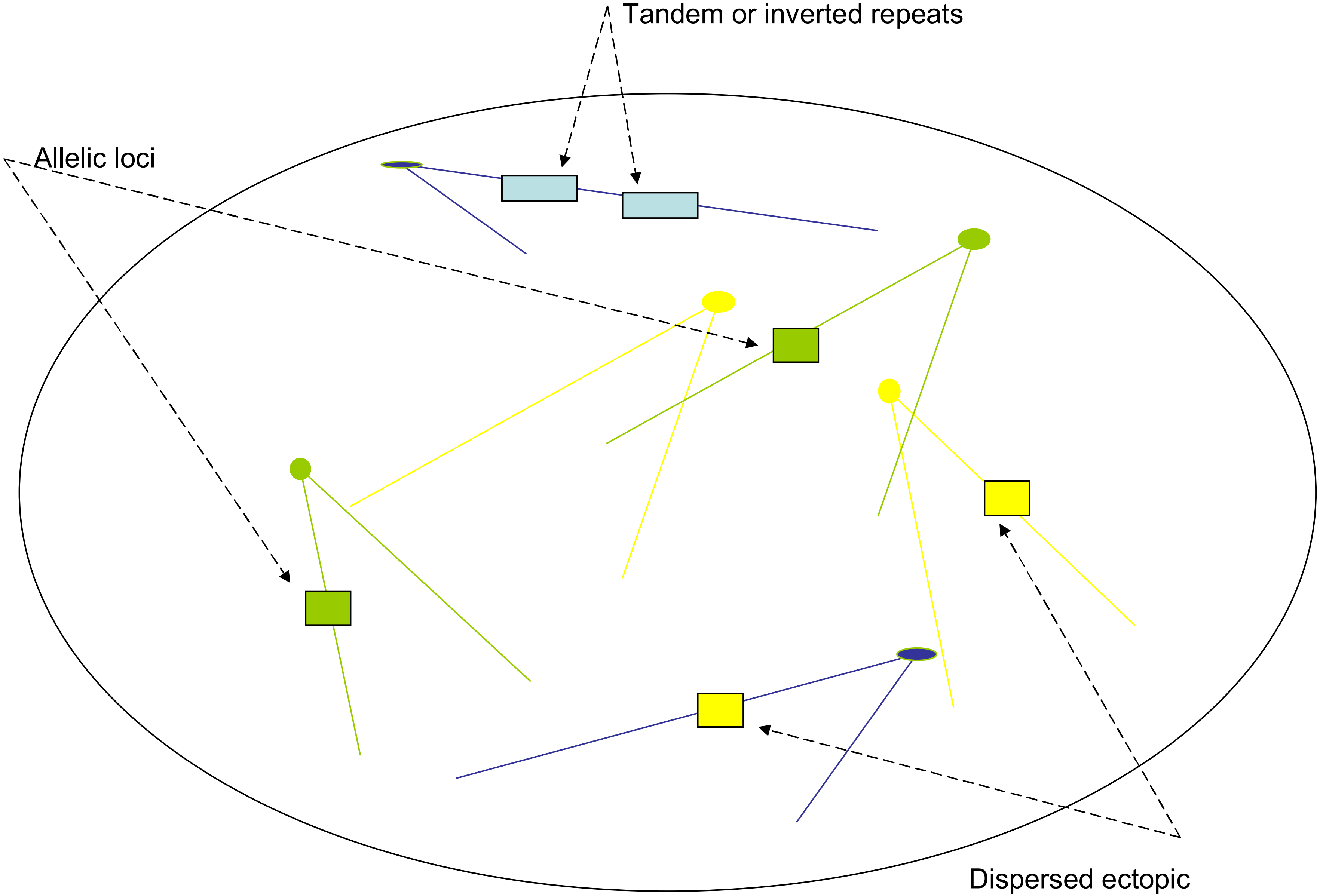
Aylon Y et al Mol Cell Biol Feb 2003 .

The HO experimental system





Alternative homologous counterparts



Tandem or inverted repeats

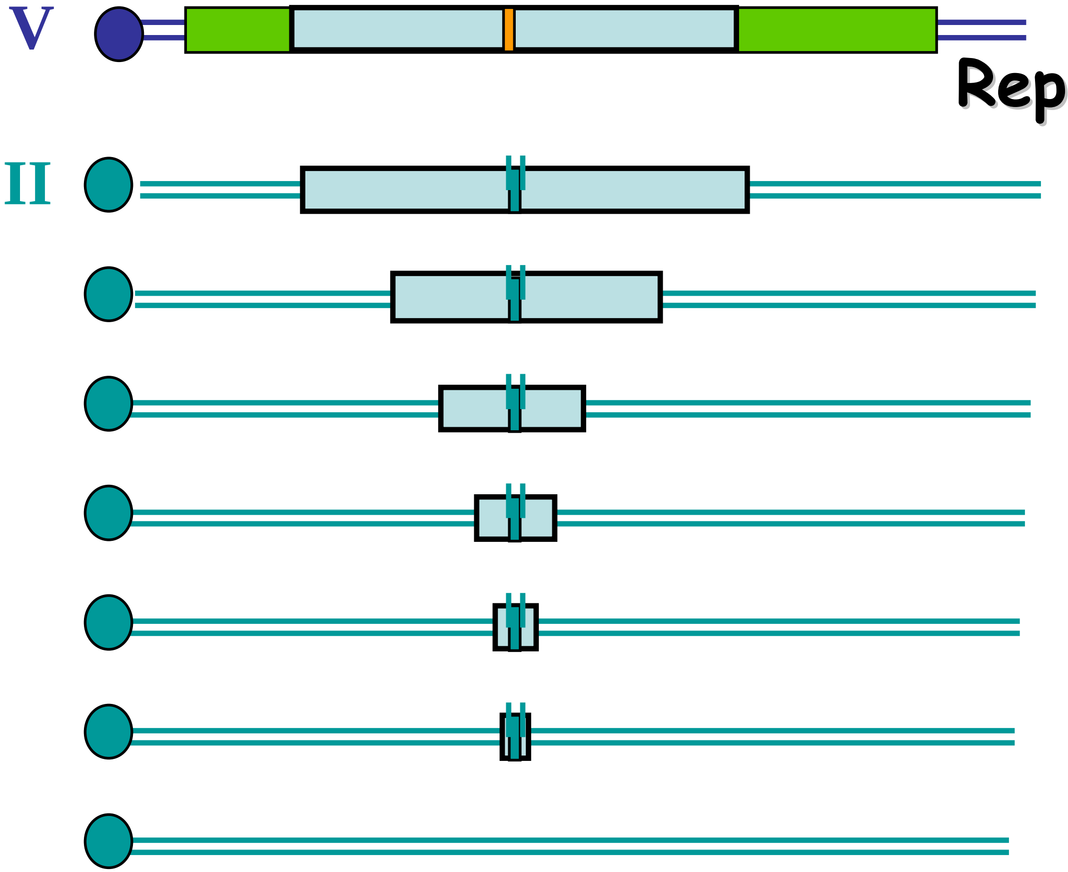
Allelic loci

Dispersed ectopic homologues

How is the needle in the haystack found?

Size does matter

HR efficiency positively correlates with homology length



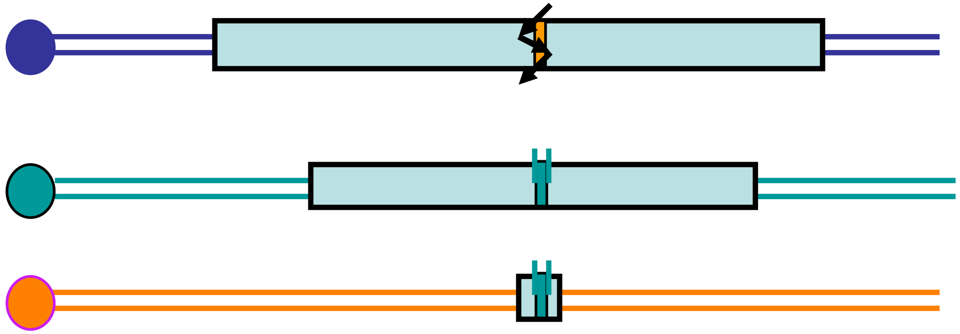
Donor size	Repair efficiency
10 kb	100%
5 kb	100%
2 kb	100%
1.2 kb	90%
0.5 kb	25%
0.2 kb	0.1%
0	0.001%

Lifshitz B et al unpublished

Free competition

We have constructed yeast strains that contain two alternative donor sequences, a long one and a short one. Will repair be as efficient as that in the strain containing:

- Only the short donor?
- Only the long donor?
- The sum of both?



Recipient
The one that breaks V

The long donor II

The short donor XV

The winner doesn't take it all

Strains with competing donor sequences are characterized by intermediate repair efficiencies

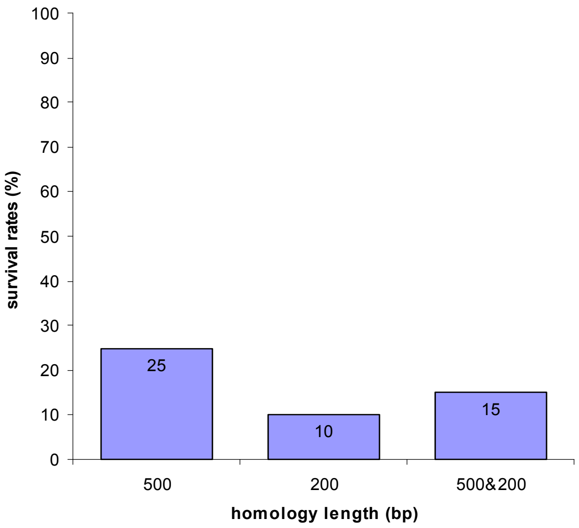
Conclusions:

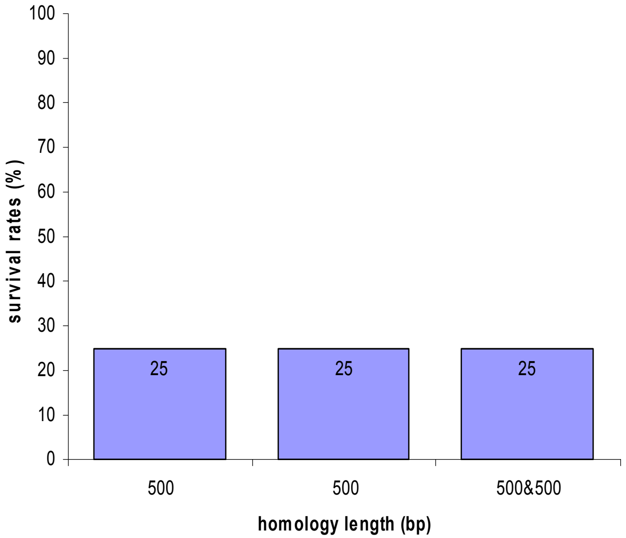
1. Shorter and longer homologies are found with similar frequencies.

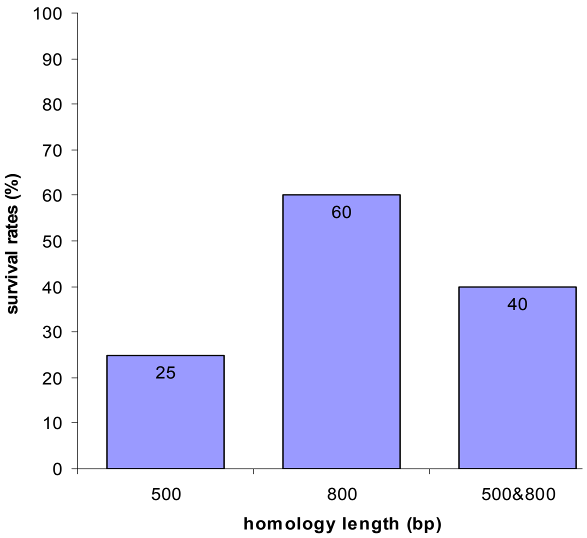
2. The attempt to recombine with a short homolog is sometimes devastating.

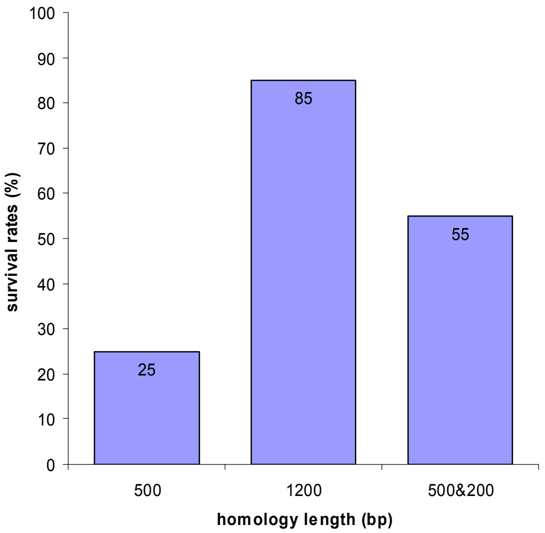
Bottom line: homology search is highly efficient even for very short ectopic homologs`

Lifshitz B et al unpublished









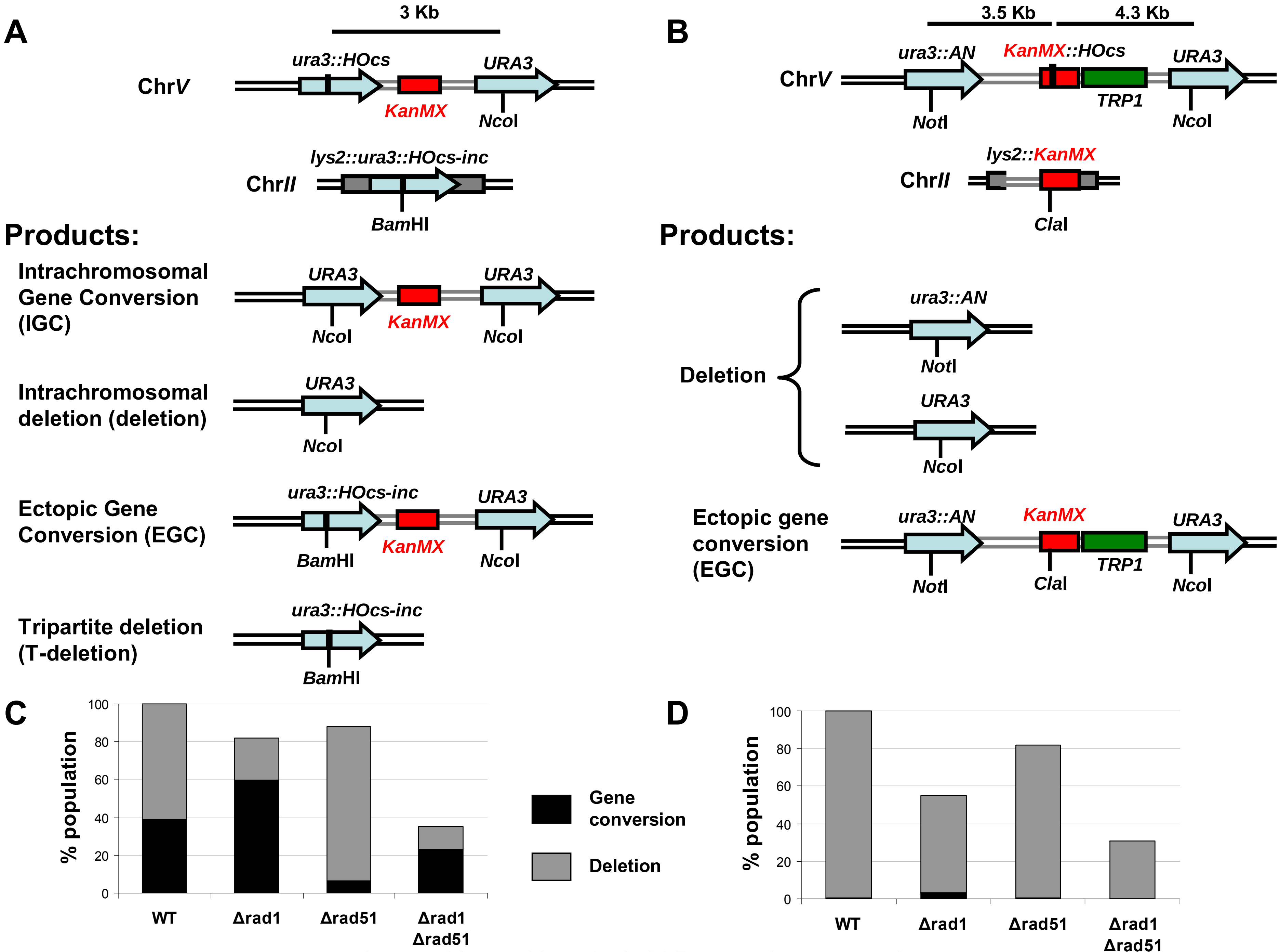


Figure 2

Agmon N et al Nucleic Acid Research- accepted

The Null hypothesis: homologous-pairing can be accomplished by random diffusion alone

$3*10^7$ bp in a G2 haploid *S. cerevisiae* genome

Each bp might mark the beginning of the desired homologous segment

Therefore: It would take $3*10^7$ sequential independent trials on average for a sequence to find its ectopic homolog

The Null hypothesis- some math even a biologist can do…

The homology search in each cell takes between one and two hours.
The time from the beginning of one trial to the beginning of the next should thus be approximately $2.5*10^{-4}$ of a second to allow for the null model
(Because: $2.5*10^{-4}*3*10^{7}$=7500sec=125min)

That’s 40 times less than the times it takes DNA polymerase to add a single nucleotide to a growing DNA chain

The Null hypothesis- limitations

The homologous sequence might contain mismatches, insertions or gaps- The number of candidates is therefore higher by orders of magnitude.

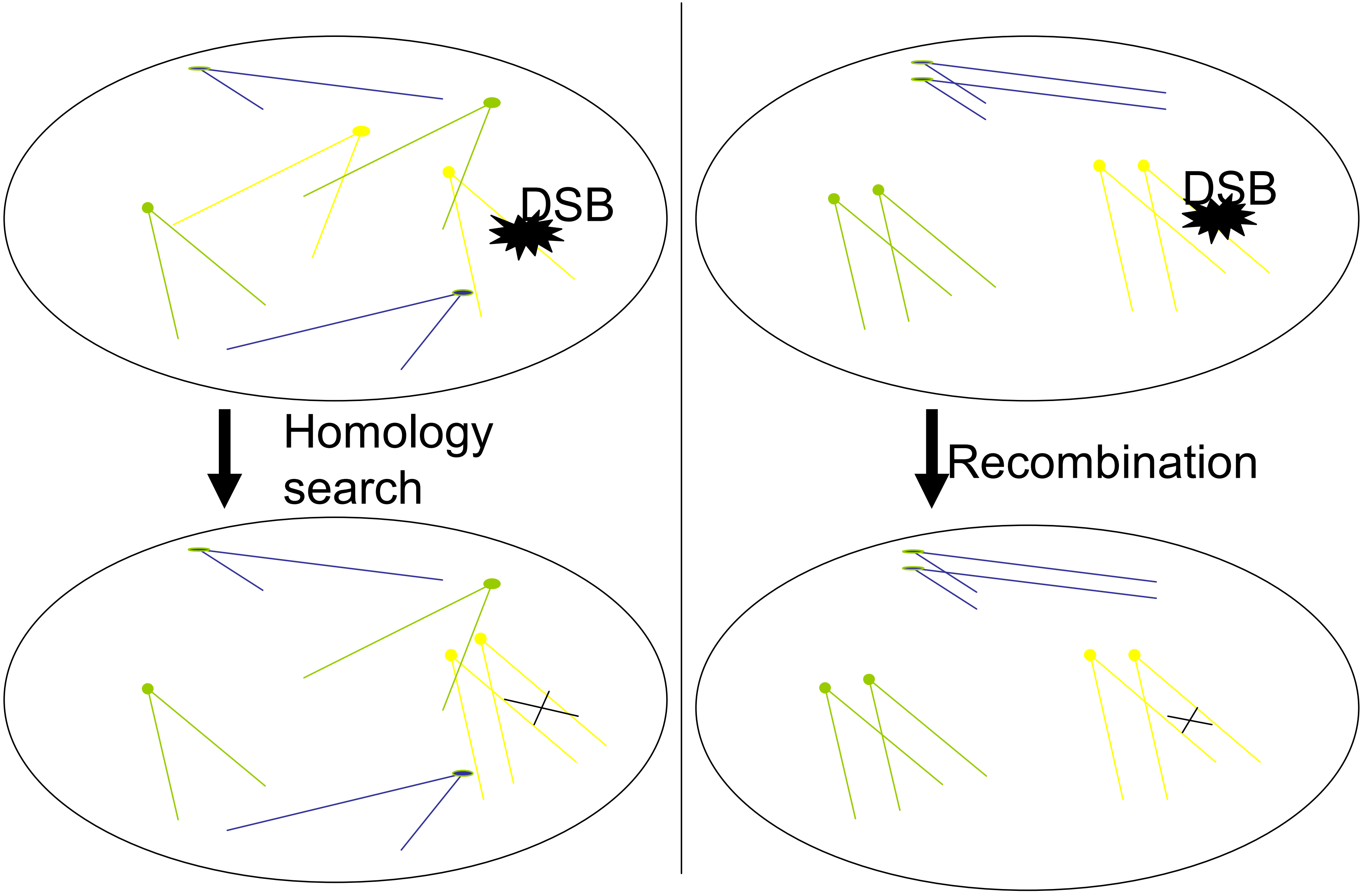
Chromosomes are large molecules that diffuse very slowly- any large scale movement requires a lot of energy

The Null hypothesis- just imagine the intertwining….

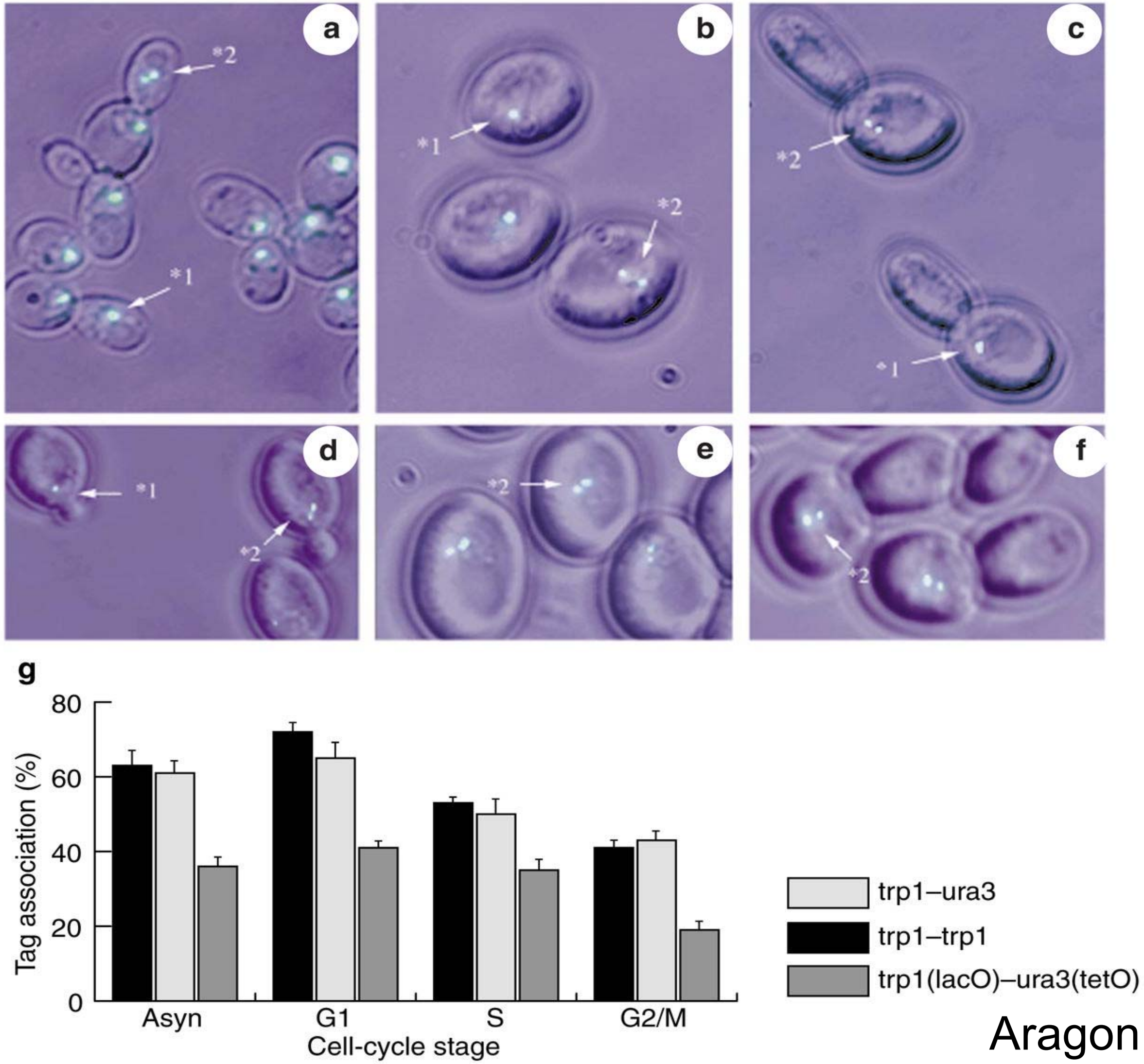
Alternative hypothesis:

The pairing of homologous sequences is an enduring and general feature of the genomic architecture that facilitates homologous recombination whenever and wherever damage occurs

Figure 4: Damage induced homology search or constitutive homologue-pairing?

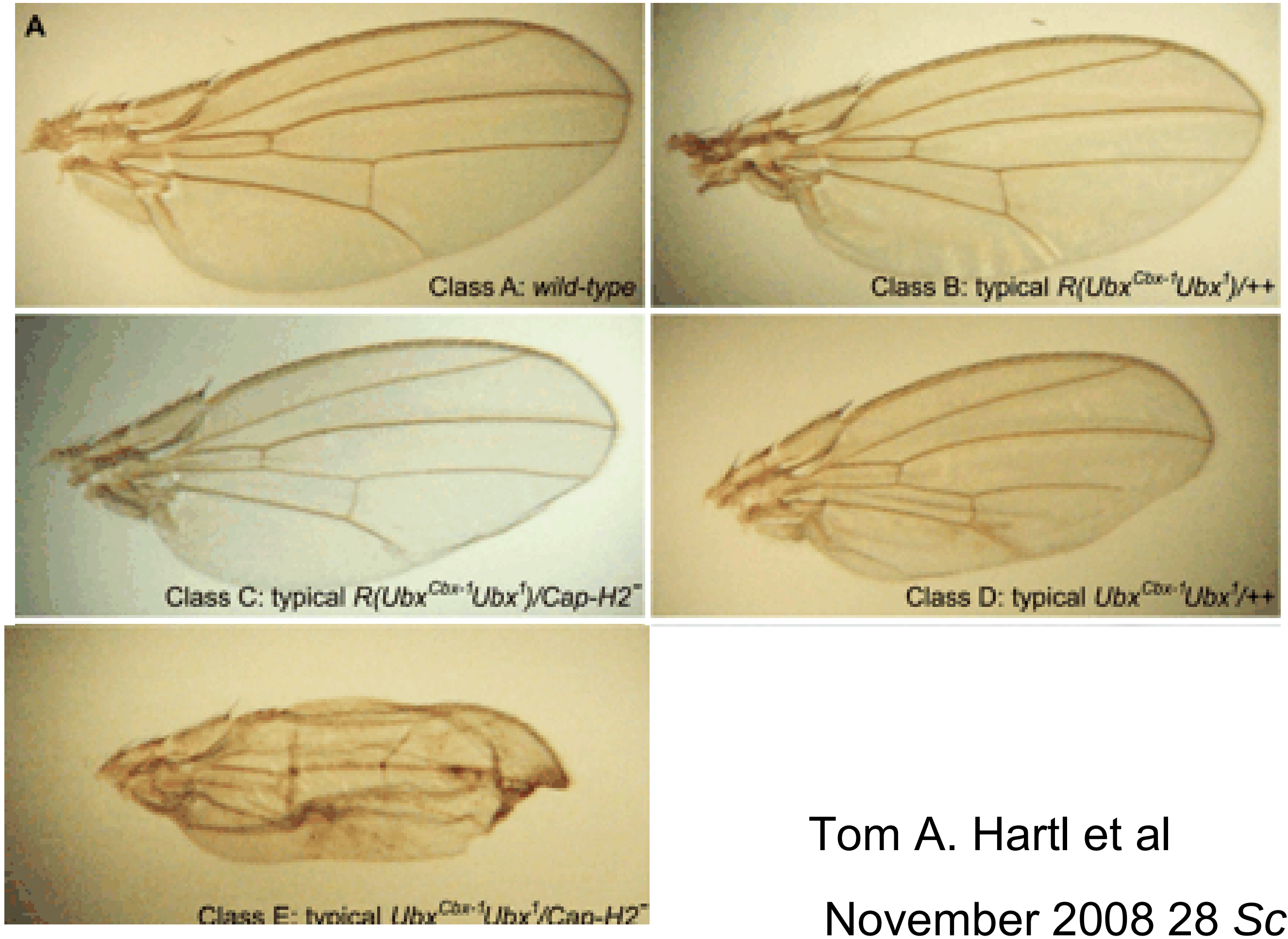


Examples of prior pairing- fluorescence imaging



Aragon Alcaide L et al
Nat Cell Biol. 2000 Nov

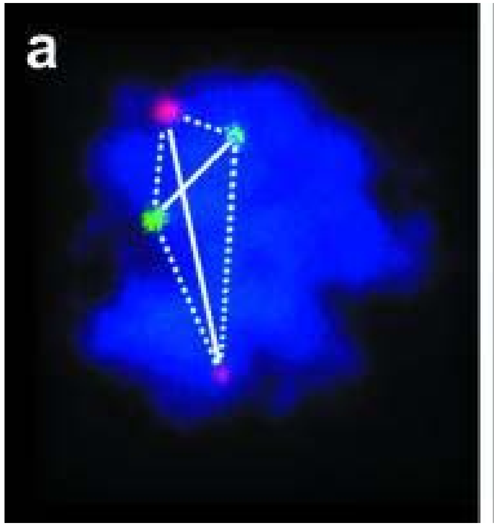
Examples of prior pairing-Transvection

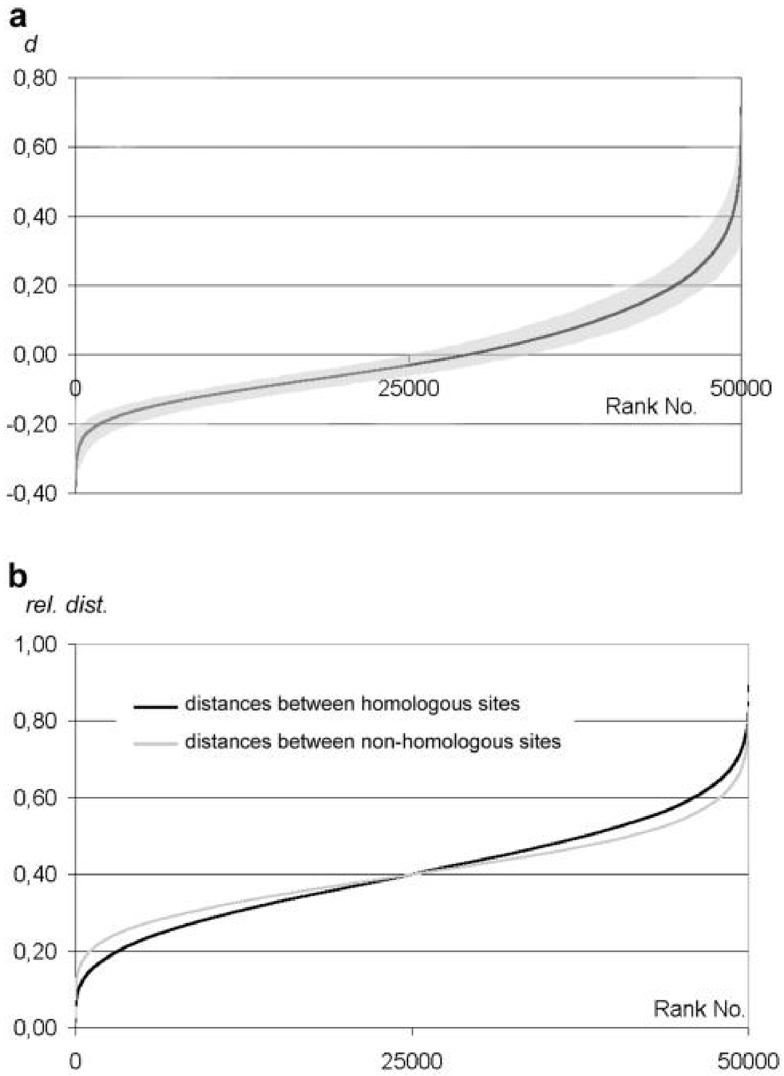


Tom A. Hartl et al

November 2008 28 *Science*

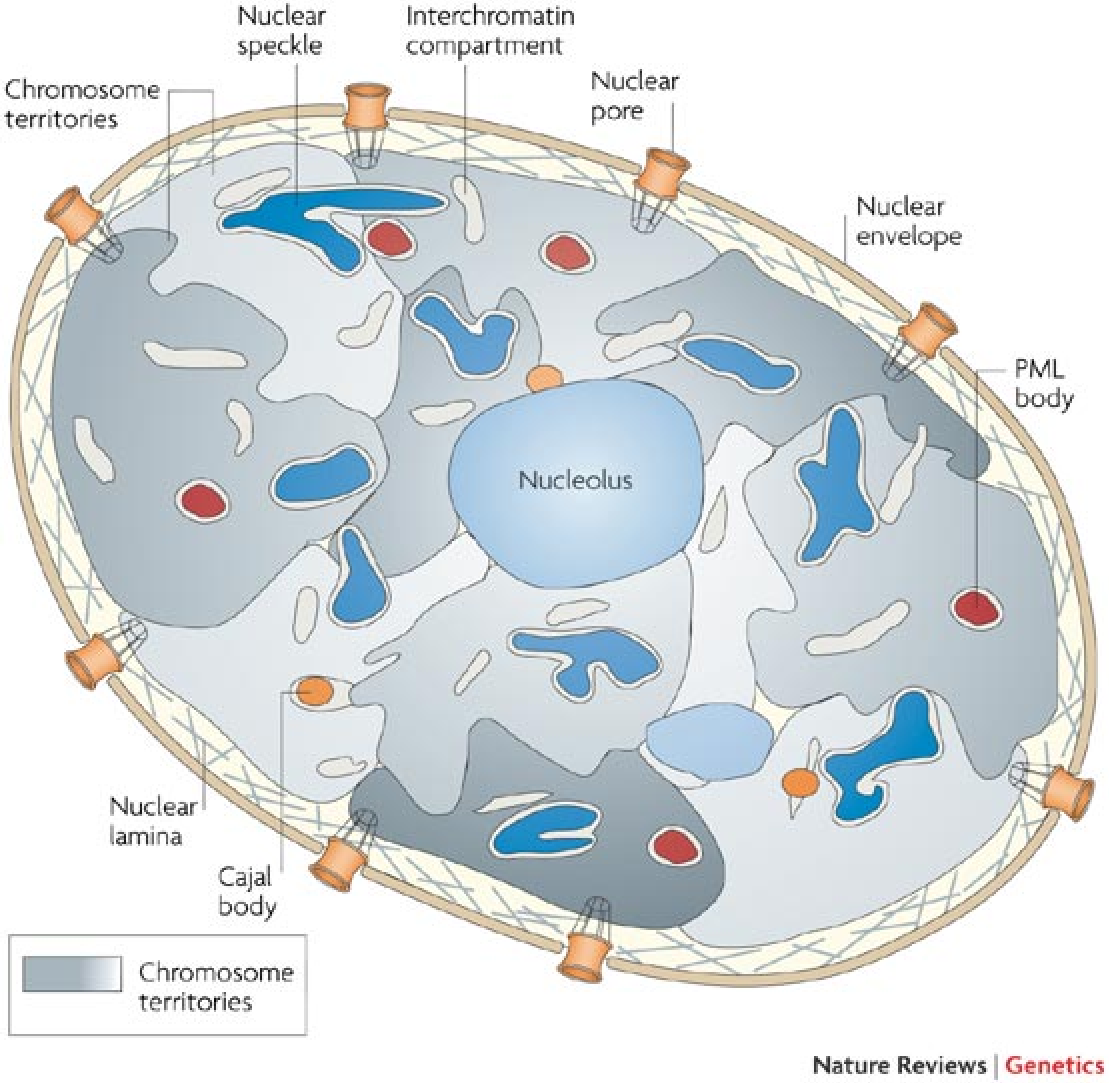
Counter examples- Fluorescence imaging





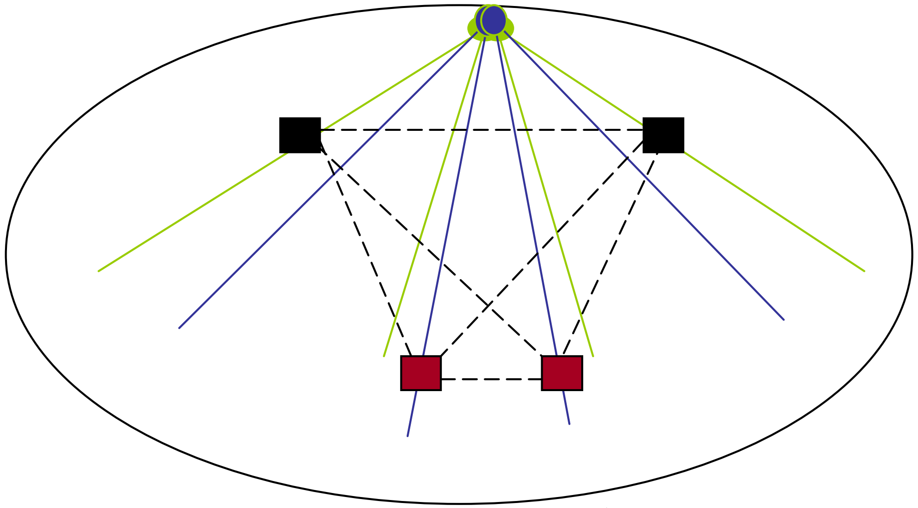
Alexander Lorenz et al
Eukaryot Cell 2003 .October

Counter examples- Nuclear compartmentalization

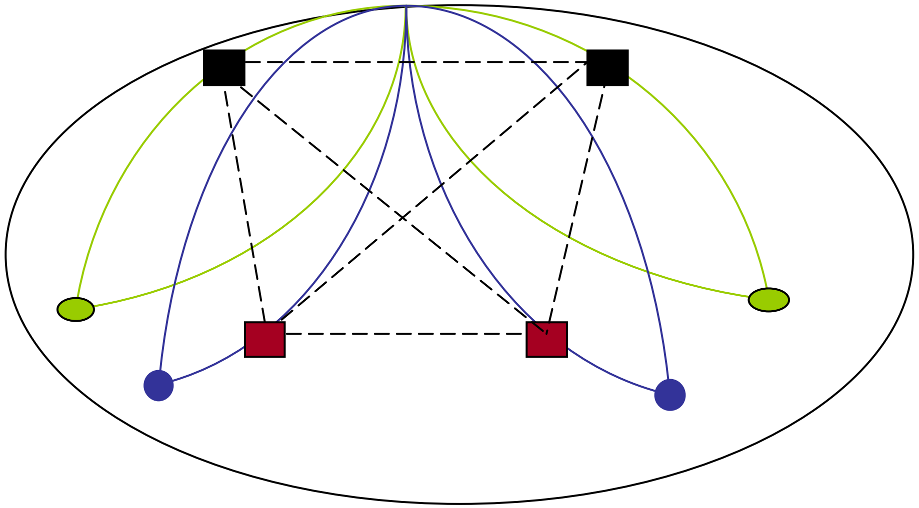


Predispositions- pairing centers, bouquet

The Rabl configuration of interphase chromosomes

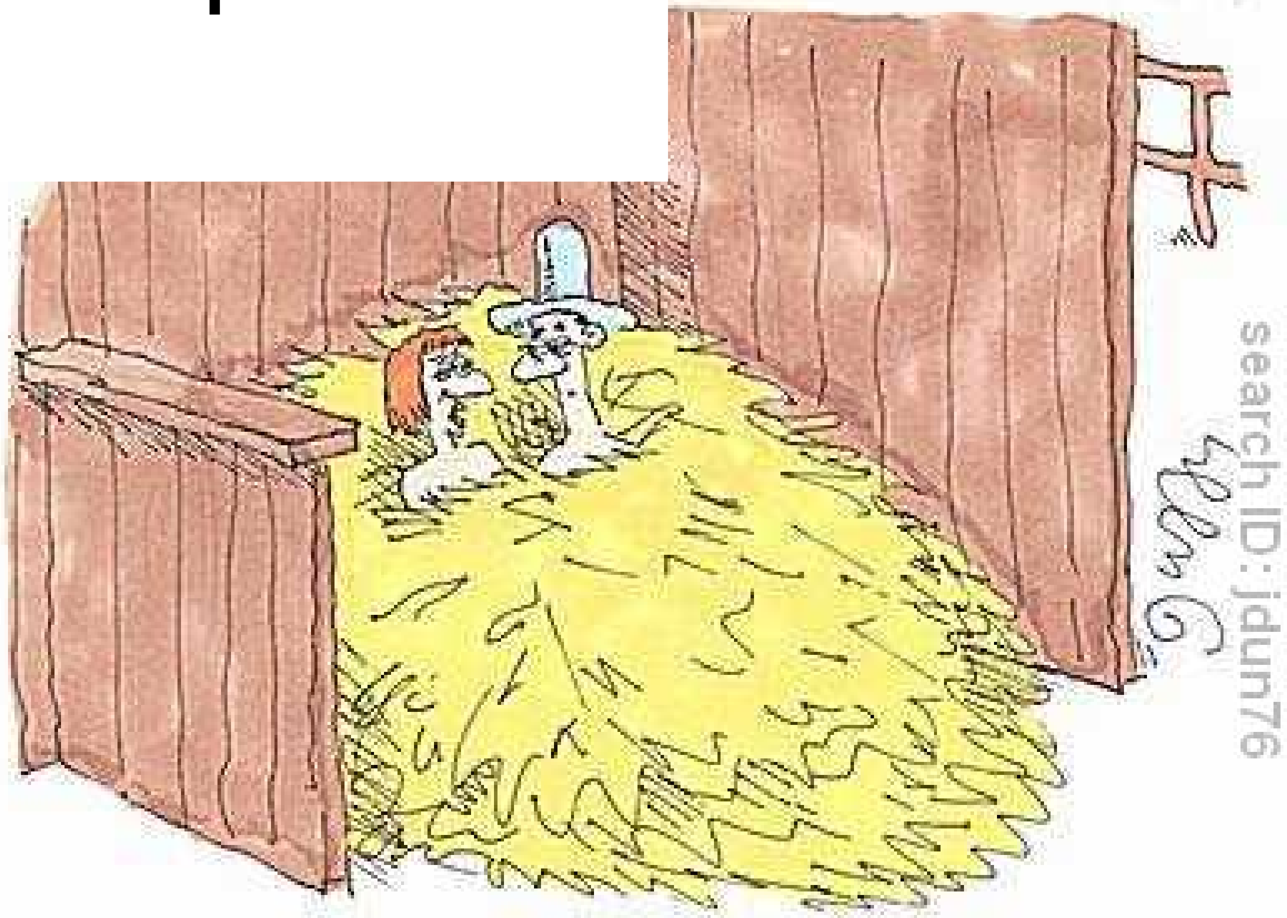


The Bouquet configuration of meiotic chromosomes



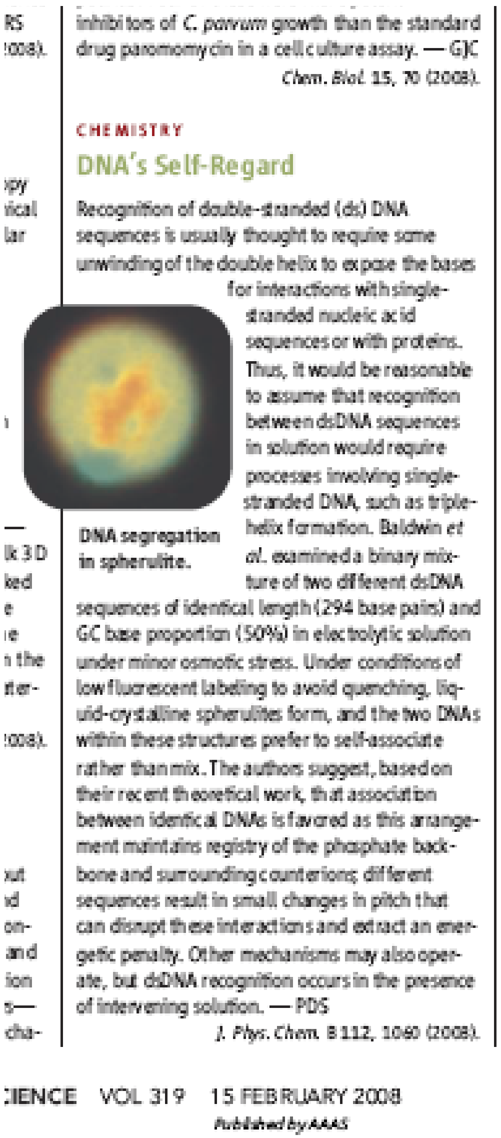
In both cases, allelic loci tend to be closer together than ectopic loci, because the latter usually have different distances from their respective centromere/telomere.

The question is still open



"Ouch! Boy, just try finding a needle in a haystack when you want to!"

Perhaps some Kornyshev long distance interactions?



inhibitors of *C. parvum* growth than the standard drug paromomycin in a cell culture assay. — GJC

Chem. Biol. 15, 70 (2008).

CHEMISTRY

DNA's Self-Regard

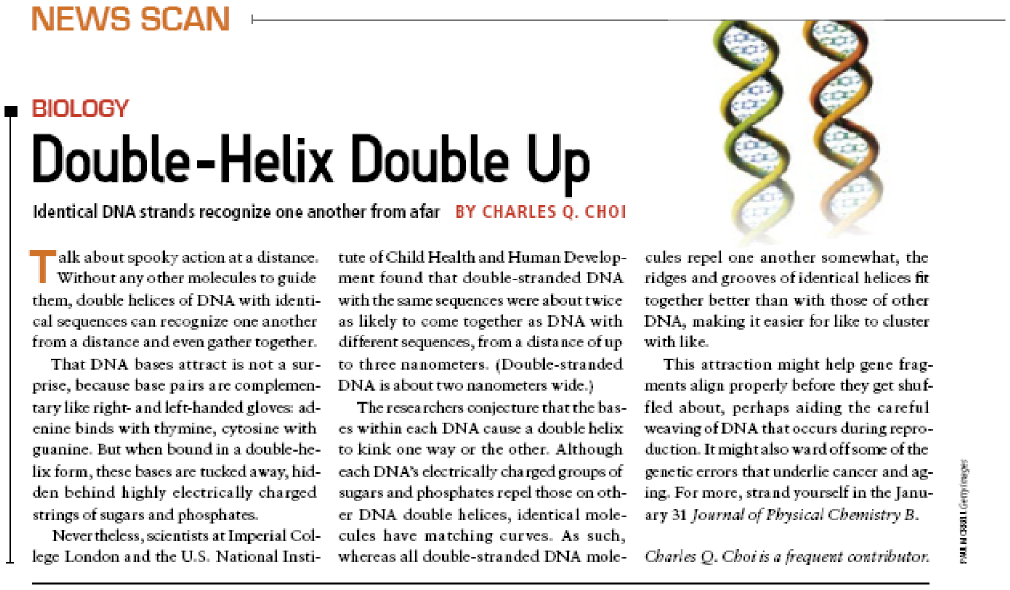
Recognition of double-stranded (ds) DNA sequences is usually thought to require some unwinding of the double helix to expose the bases for interactions with single-stranded nucleic acid sequences or with proteins. Thus, it would be reasonable to assume that recognition between dsDNA sequences in solution would require processes involving single-stranded DNA, such as triple-helix formation. Baldwin *et al.* examined a binary mixture of two different dsDNA sequences of identical length (294 base pairs) and GC base proportion (50%) in electrolytic solution under minor osmotic stress. Under conditions of low fluorescent labeling to avoid quenching, liquid-crystalline spherulites form, and the two DNAs within these structures prefer to self-associate rather than mix. The authors suggest, based on their recent theoretical work, that association between identical DNAs is favored as this arrangement maintains registry of the phosphate backbone and surrounding counterions; different sequences result in small changes in pitch that can disrupt these interactions and extract an energetic penalty. Other mechanisms may also operate, but dsDNA recognition occurs in the presence of intervening solution. — PDS

DNA segregation in spherulite.

J. Phys. Chem. B 112, 1060 (2008).

IENCE VOL 319 15 FEBRUARY 2008

Published by AAAS



NEWS SCAN

BIOLOGY

Double-Helix Double Up

Identical DNA strands recognize one another from afar **BY CHARLES Q. CHOI**

Talk about spooky action at a distance. Without any other molecules to guide them, double helices of DNA with identical sequences can recognize one another from a distance and even gather together.

That DNA bases attract is not a surprise, because base pairs are complementary like right- and left-handed gloves: adenine binds with thymine, cytosine with guanine. But when bound in a double-helix form, these bases are tucked away, hidden behind highly electrically charged strings of sugars and phosphates.

Nevertheless, scientists at Imperial College London and the U.S. National Institute of Child Health and Human Development found that double-stranded DNA with the same sequences were about twice as likely to come together as DNA with different sequences, from a distance of up to three nanometers. (Double-stranded DNA is about two nanometers wide.)

The researchers conjecture that the bases within each DNA cause a double helix to kink one way or the other. Although each DNA's electrically charged groups of sugars and phosphates repel those on other DNA double helices, identical molecules have matching curves. As such, whereas all double-stranded DNA molecules repel one another somewhat, the ridges and grooves of identical helices fit together better than with those of other DNA, making it easier for like to cluster with like.

This attraction might help gene fragments align properly before they get shuffled about, perhaps aiding the careful weaving of DNA that occurs during reproduction. It might also ward off some of the genetic errors that underlie cancer and aging. For more, strand yourself in the January 31 *Journal of Physical Chemistry B.*

Charles Q. Choi is a frequent contributor.

Any ideas anyone?

Thanks

- Yael Aylon
- Batia Lifshitz
- Neta Agmon
- Martin Kupiec