Mechanisms of DNA Repair by Recombination

Jim Haber
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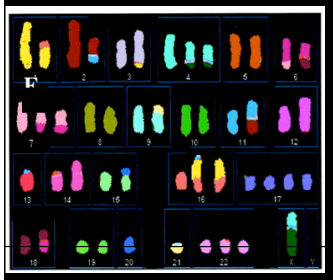
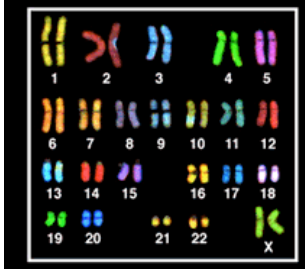




Genome instability in tumor cells

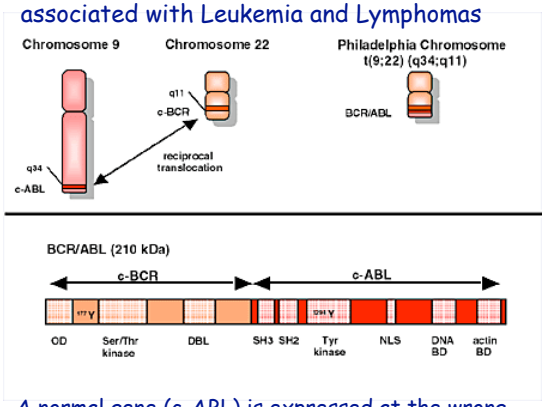
Translocations
Truncations
Inversions
Deletions
Copy Number Variation

Mutations

Missegregation

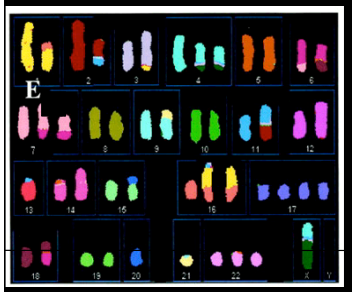
Abdel-Rahman et al. PNAS 98: 2538 (2001)

Some chromosome rearrangements are directly implicated in the etiology of cancer: The "Philadelphia chromosome" translocation is associated with Leukemia and Lymphomas



A normal gene (c-ABL) is expressed at the wrong time/place by being translocated adjacent to DNA sequences at BCR that control the timing and amount of gene expression

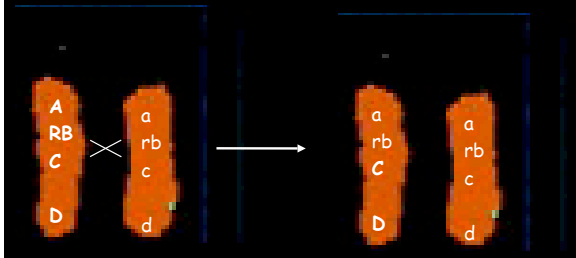
http://www.medscape.com/pi/editorial/cmecircle/2001/259/griffin/slide04.gif



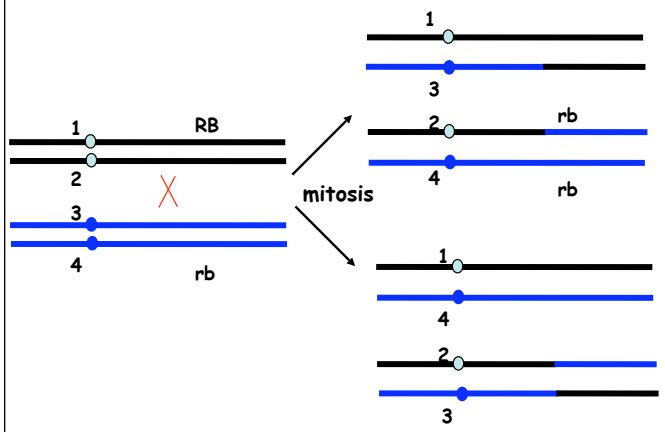
Genome instability in tumor cells

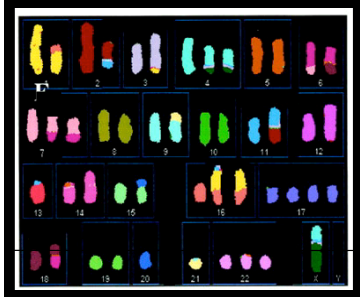
Loss of heterozygozity (LOH)

e.g. Retinoblastoma (RB) tumor suppressor genes



LOH by mitotic crossing-over





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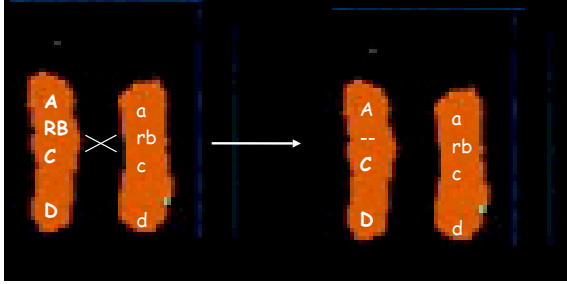
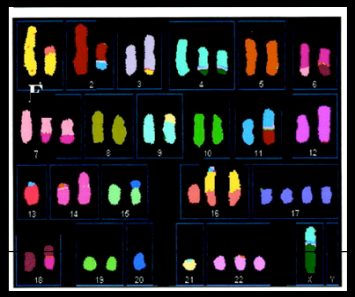
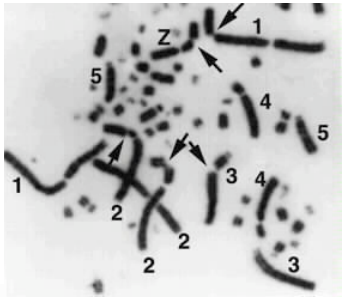




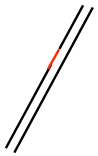
Associated with	Examples
Defects in repair of chromosome breaks	BRCA2 BLM
or	
Defects in the DNA damage checkpoint	ATM p53

In vertebrates, Double-Strand Break repair is essential for life

DSBs arise during normal DNA replication



DSBs arising in vertebrate cells after Rad51 is depleted



Repaired by Rad51 recombinase

Sonoda et al. EMBO J. 1998

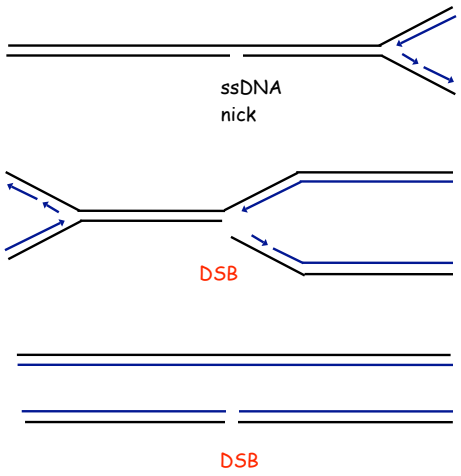
An Oncogene-Induced DNA Damage Model for Cancer Development

Thanos D. Halazonetis,[1]* Vassilis G. Gorgoulis,[2] Jiri Bartek[3]

"Of all types of DNA damage, DNA double-strand breaks (DSBs) pose the greatest challenge to cells… Recent experimental findings suggest that, in both precancerous lesions and cancers, activated oncogenes induce stalling and collapse of DNA replication forks, in turn leads to the formation of DNA DSBs. This continuous formation of DNA DSBs may contribute to the genomic instability that characterizes the vast majority of human cancers."

Science 2008 319:1352

Broken chromosomes can arise by replication over a ssDNA nick



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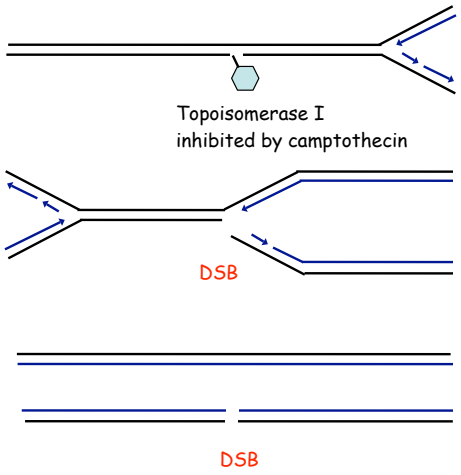
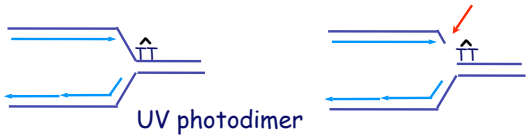
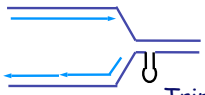


Stalled replication forks may break

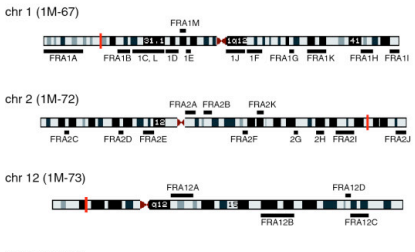




Triplet repeat and
other difficult-to-replicate
sequences

DSBs at "fragile sites"

Partial inhibition of the DNA replication machinery with aphidicolin (APH) exacerbates fragile site breakage



http://genomebiology.com/content/figures/gb-2007-8-6-r120-7-1.jpg

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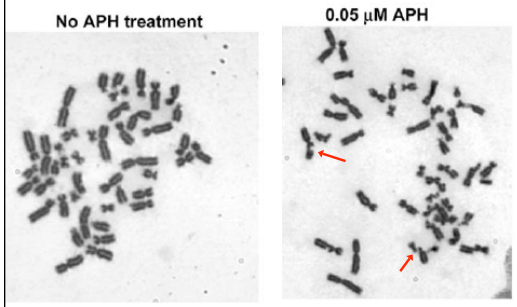
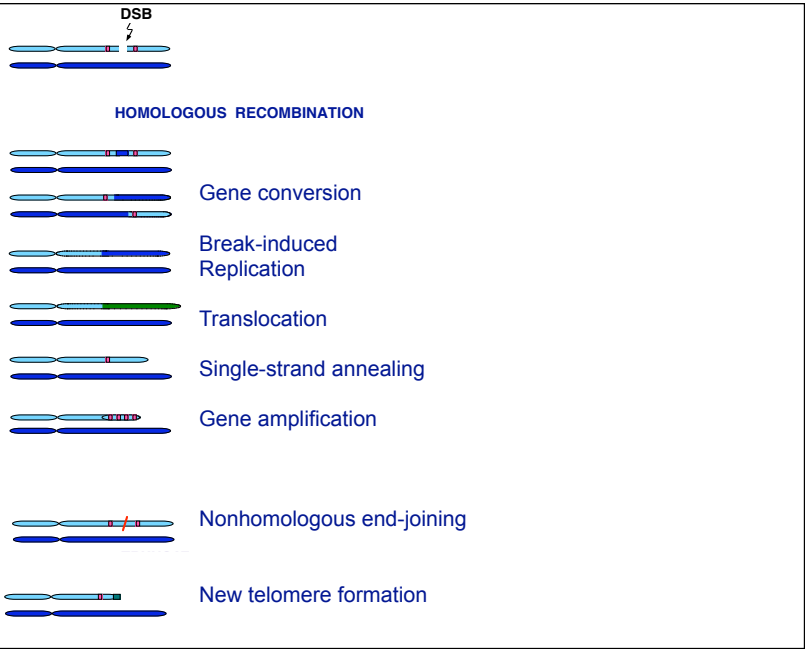
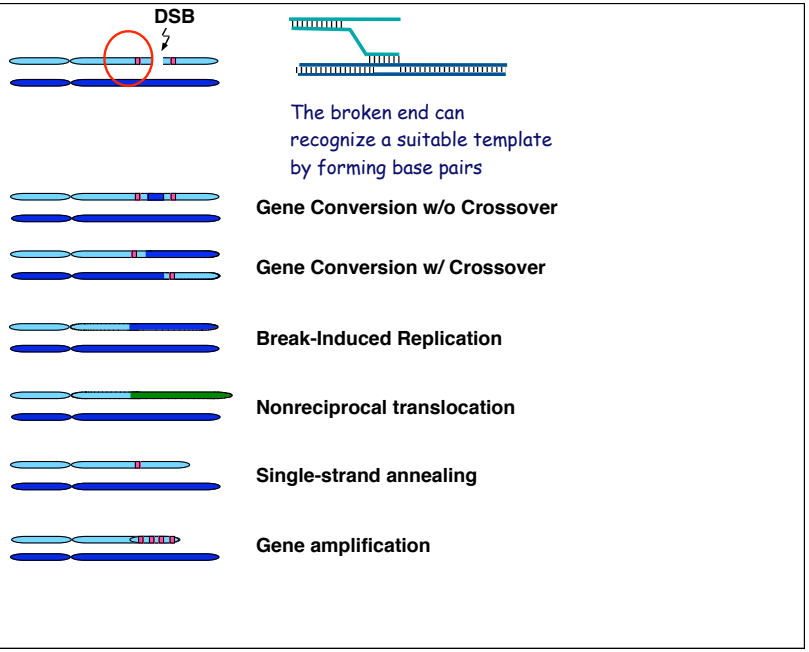
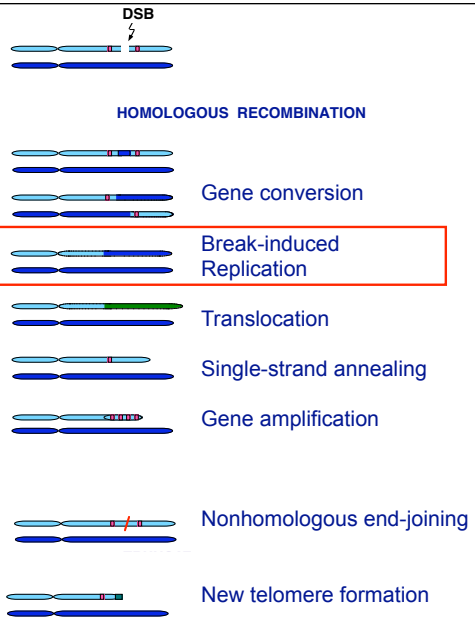


Cell, Vol. 111, 779–789, I


DSB
HOMOLOGOUS RECOMBINATION
Gene conversion
Break-induced
Replication
Translocation
Single-strand annealing
Gene amplification
Nonhomologous end-joining
New telomere formation


DSB
The broken end can
recognize a suitable template
by forming base pairs
Gene Conversion w/o Crossover
Gene Conversion w/ Crossover
Break-Induced Replication
Nonreciprocal translocation
Single-strand annealing
Gene amplification

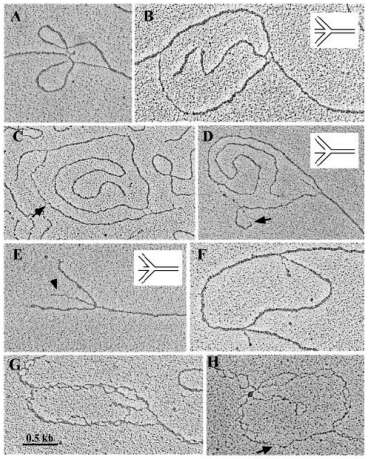


Replication fork regression

Formation of a Holliday junction by annealing of newly-copied strands

This allows access for DNA repair enzymes

Holliday junction formation

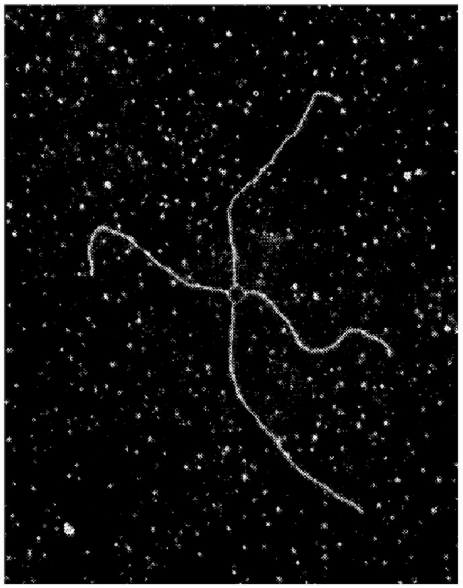


Fork Reversal and ssDNA Accumulation at Stalled Replication Forks Owing to Checkpoint Defects

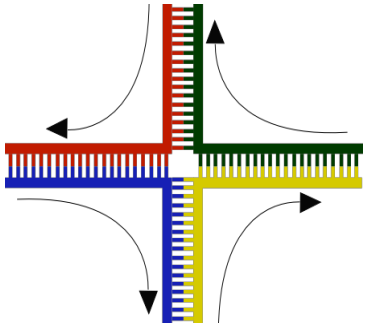
José M. Sogo,[1*] Massimo Lopes,[2*] Marco Foiani[2†]

Stalled replication forks can regress to form 4-armed "chicken foot" structures

These are known as Holliday junctions



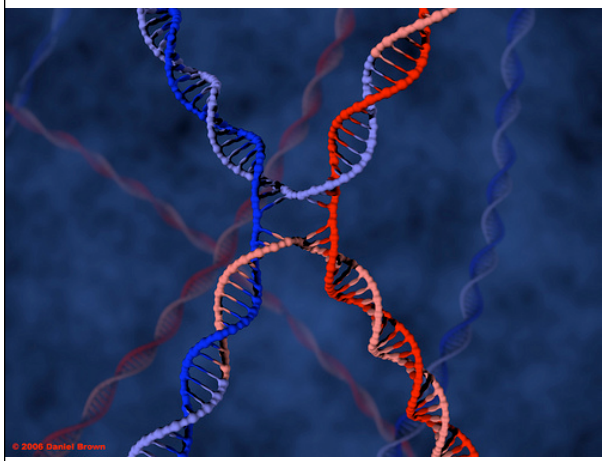
http://upload.wikimedia.org/wikipedia/commons/archive/9/9b/20090331165557!Holliday_Junction_cropped.png



All base pairs can be formed

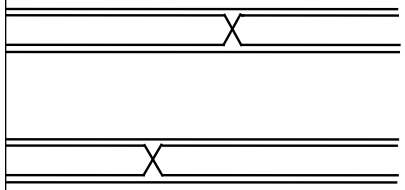
http://en.wikipedia.org/wiki/File:Holliday_Junction.png

The HJ is a symmetric structure in which all base pairs can be formed



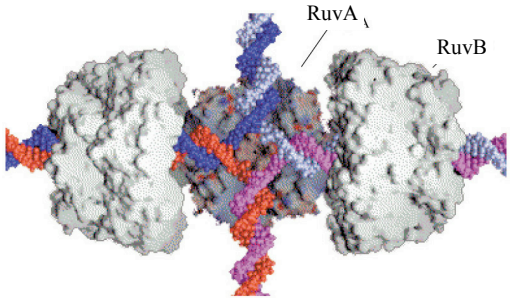
http://www.flickr.com/photos/irradiatus/2235730200/

Holliday junctions can branch migrate



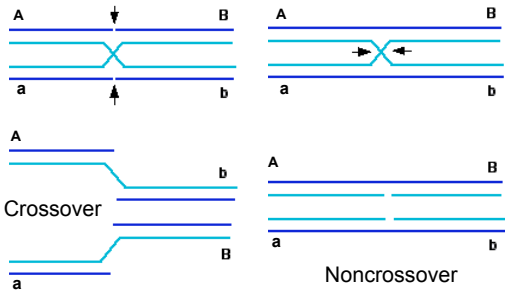
Branch migration requires no net expenditure of energy, as each step involves the breaking and making of 2 base pairs

In E. coli, RuvAB drives branch migration



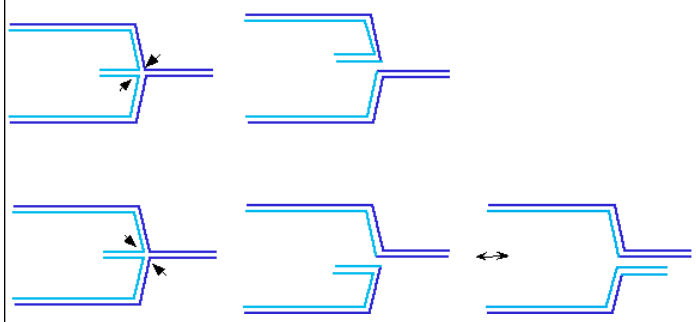
Animation at http://www.shef.ac.uk/mbb/ruva

In addition to branch migration, Holliday junctions can be "resolved" by a Holliday junction resolvase

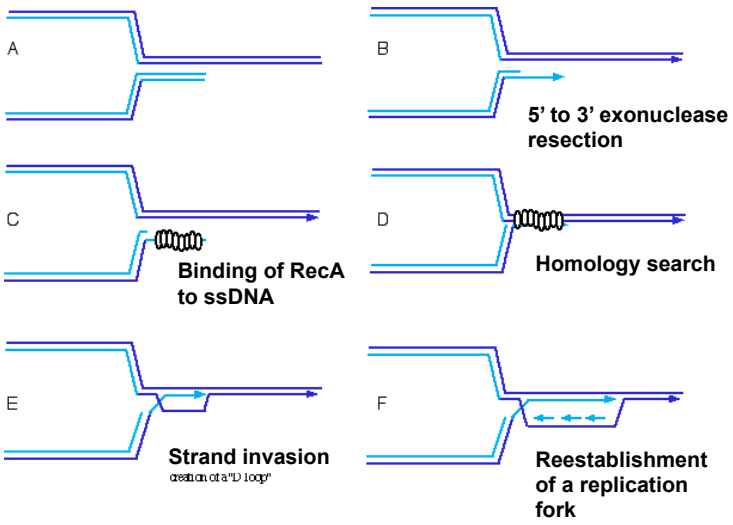


Nicks can be ligated

Cleavage of a Holliday junction at a stalled replication fork produces an intact template and a broken-ended molecule

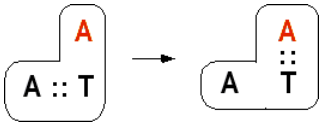


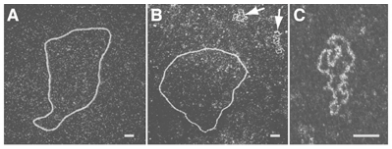
Replication re-start by homologous recombination
Break-Induced Replication (BIR)



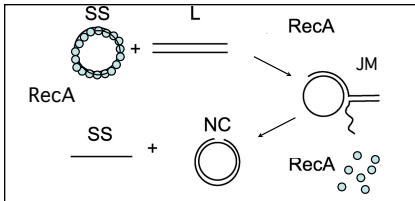
Basic strand exchange

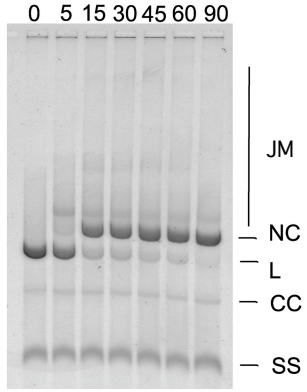
RecA/Rad51 binds both single-stranded DNA and double-stranded DNA and catalyzes base pair exchange



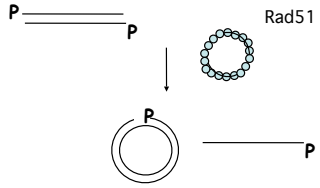


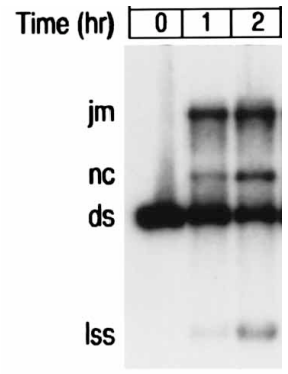
- **RecA or Rad51 filaments stretch and unwind both ssDNA and dsDNA about 1.5 x 18.6 bp/turn**



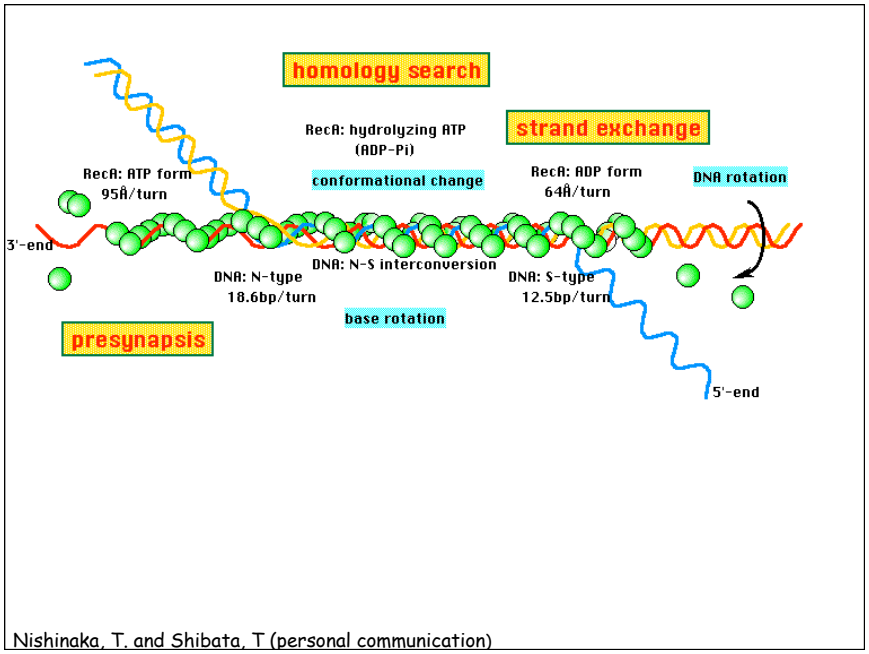


PNAS 101: 14174-14179 Fig.3

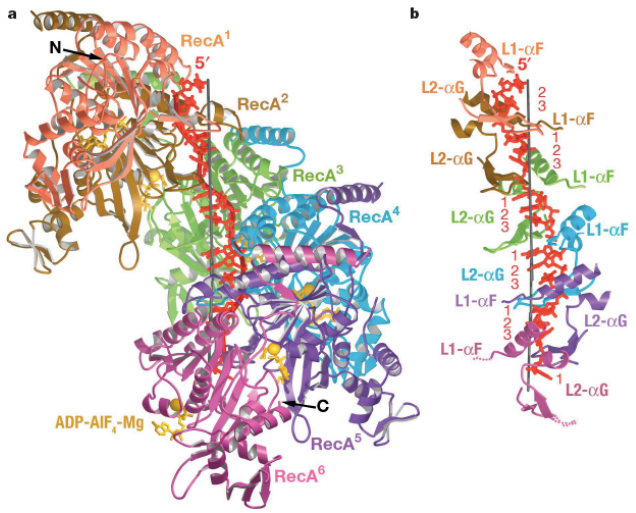




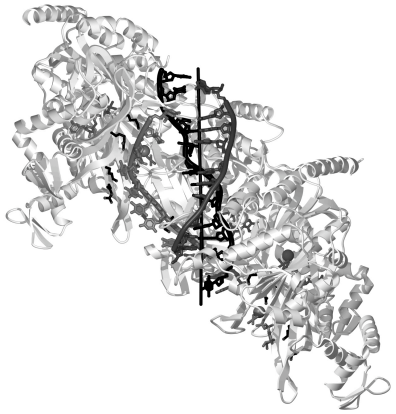
Mol Cell Biol 1997 17: 5359 (Fig. 2)



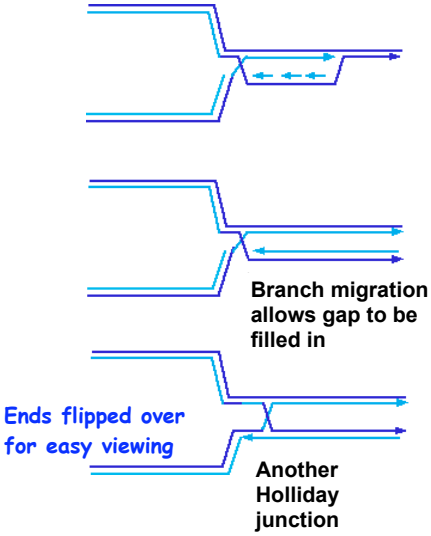
Nishinaka, T. and Shibata, T (personal communication)



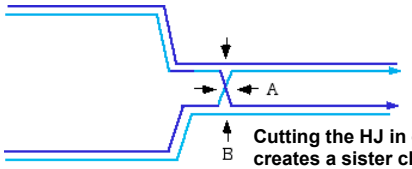
Vol 453|22 May 2008|doi:10.1038/nature06971



Z.Chen, H.Yang & N.P. Pavletich personal communication
Based on Nature 453: 492 Fig. 4

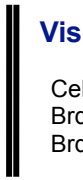


Crossovers accompanying repair of sister chromatids.

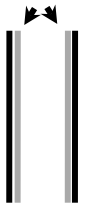


Cutting the HJ in orientation B creates a sister chromatid exchange

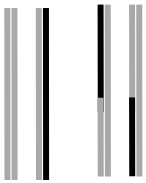
Visualizing sister-chromatid exchange with BrdU



Cells are grown in the presence of BrdU, an analog of dT to produce BrdU-labeled DNA

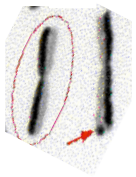


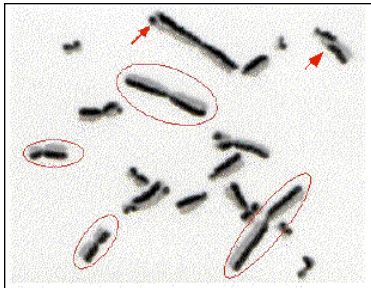
Cells are then grown for two generations without BrdU, so that each pair of sister chromatids will have only one BrdU-containing strand



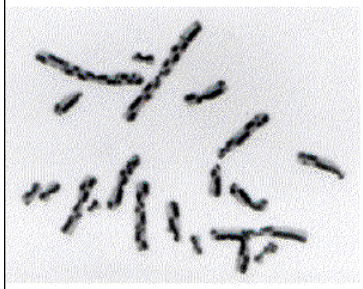
Normally all the label will be along only one of the sisters, unless there has been a SCE

SCE





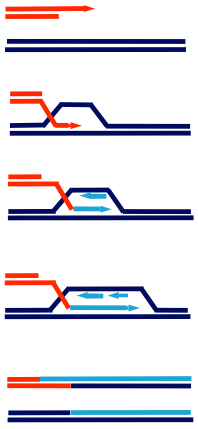
SCEs are not rare. Each time cells replicate one can see approximately 10 such events, illustrating the importance of repair by recombination for cell viability



http://media-2.web.britannica.com/eb-media/73/36873-004-29ADDB36.jpg

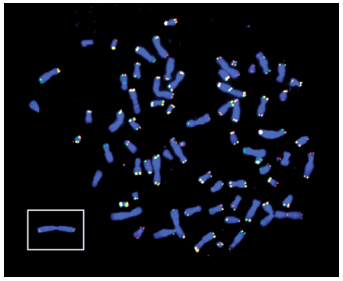
http://www.ultranet.com/~jkimball/BiologyPages/H/Harlequin.html

Break-Induced Replication (BIR)



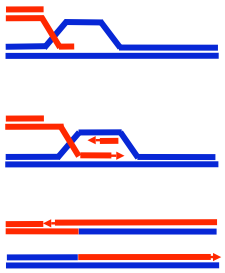
Replication Re-start

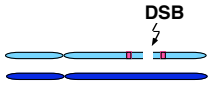
Immortalized transformed cell lines either re-activate telomerase or they use recombination-dependent Alternative Lengthening of Telomeres (ALT)



http://cancerres.aacrjournals.org/content/vol64/issue10/cover.shtml

In budding yeast telomerase-independent telomere maintenance uses two BIR-related mechanisms – one Rad51-dependent and one Rad51-independent – both depending on a nonessential DNA polymerase subunit Pol32.



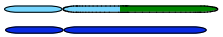


HOMOLOGOUS RECOMBINATION


Gene Conversion w/o Crossover


Gene Conversion w/ Crossover

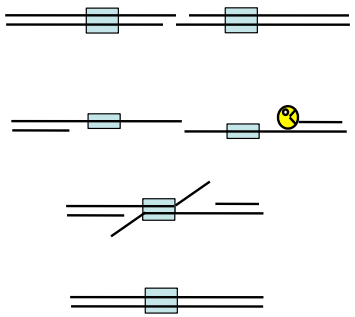

Break-Induced Replication


Nonreciprocal translocation


Single-strand annealing


Gene amplification

Single-Strand Annealing

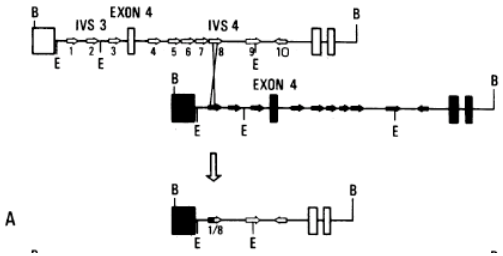


The human genome contains >500,000 short interspersed repeated sequences such as Alu and many longer repeats.

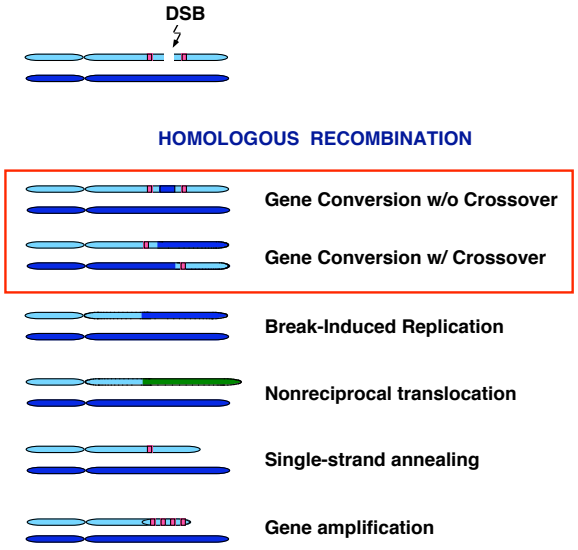
SSA can produce deletions between repeats as far apart as 100 kb.

Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 1551–1555, February 1990
Genetics

Clusters of intragenic *Alu* repeats predispose the human C1 inhibitor locus to deleterious rearrangements

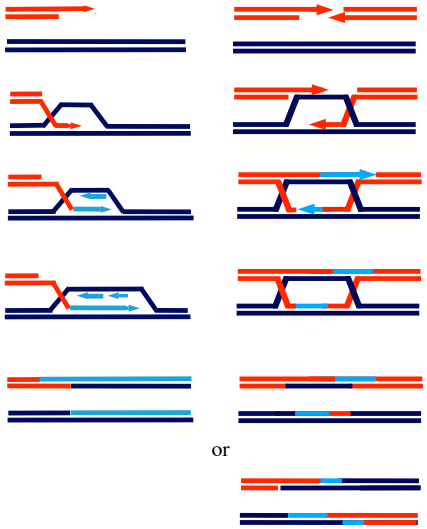


Often interpreted as unequal SCE but more likely SSA



BIR

Double Holliday Junction mechanism
Gene conversion

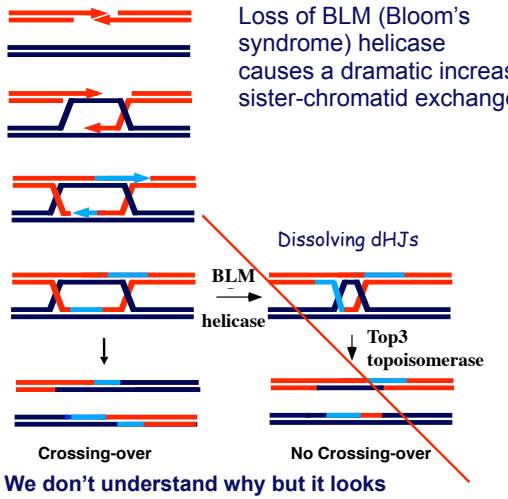


apparently similar initial steps: resection and strand invasion

Noncrossover

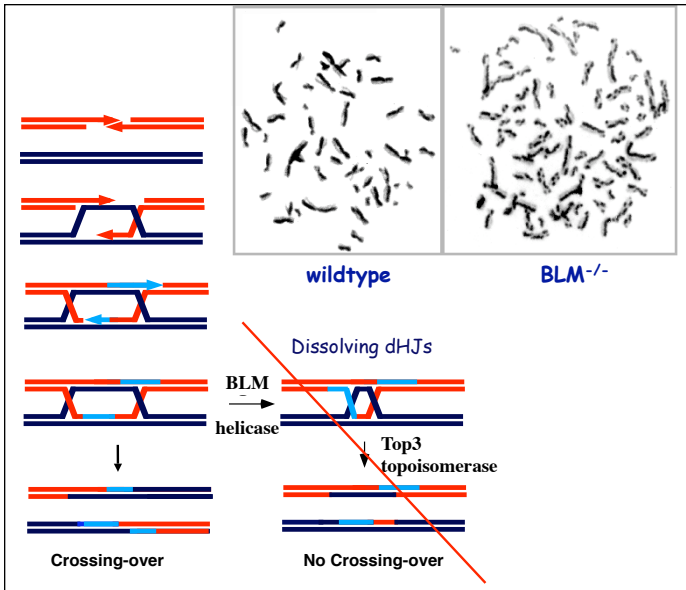
or

Crossover



We don't understand why but it looks as if dHJs are always resolved as crossovers (at least in meiosis)

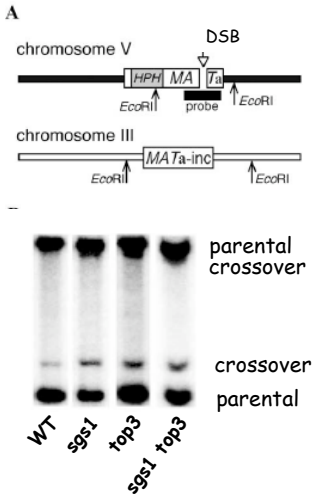
http://atlasgeneticsoncology.org/Kprones/BLO10002.html

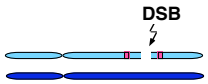


http://atlasgeneticsoncology.org/Kprones/BLO10002.html

Srs2 and Sgs1–Top3 Suppress Crossovers during Double-Strand Break Repair in Yeast

Grzegorz Ira,[1,4] Anna Malkova,[1,5] Giordano Liberi,[2,3] Marco Foiani,[2,3] and James E. Haber[1,*]





HOMOLOGOUS RECOMBINATION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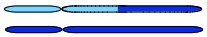
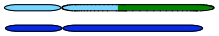
Gene Conversion w/ Crossover


Break-Induced Replication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