iBiology.org Teaching Tools
Jim Haber's Lecture Part 1:
Mechanisms of DNA repair by recombination

Teaching Tools were prepared by Ranjith Anand with Jim Haber.

Contents

1. Keywords and Terms
2. Lecture Notes
3. Recommended Reading
4. Review Questions
5. Answers to Review Questions
6. Discussion Questions
7. Answers to Discussion Questions
8. Explain or Teach These Concepts to a Friend
9. Research the Literature on Your Own

---

1. Keywords and Terms

DNA repair, DNA damage, Genome instability, DNA Double Strand Breaks (DSBs), homologous recombination (HR), Non- Homologous End Joining (NHEJ), Single Strand Annealing (SSA), Break induced replication (BIR), Holliday Junctions (HJs), Cross-Over (CO), Non-Cross over (NCO), Holliday Junction resolution, Resolvases, RecA, Rad51, Sgs1, Top3, BLM, Pol32, Exonuclease, Endonuclease, Fork Stall, Fork Collapse, Fork regression, "Chicken foot", Branch migration, strand invasion, homology search, Telomerase, sister chromatid exchange (SCE), DNA damage checkpoint, Loss of Heterozygosity (LOH), fragile sites, Gene conversion (GC).

2. Lecture Notes

Genome instability in tumor cells

Genome instability – a hallmark of tumor cells – is characterized by chromosome rearrangements including translocations, inversions, deletions, truncations, copy number changes etc. Some of the rearrangements are directly implicated in the etiology of cancer (for example in leukemia). Rearrangements that result in loss of heterozygosity (LOH) expose the effect of recessive alleles (for example in retinoblastoma). A major motivation for studying DNA repair is to understand how genome instability arises from the background of normal cells. From yeast to humans there is high degree of conservation of DNA repair pathways. Defects in repair of chromosome breaks (BRCA2, BLM defects) or DNA damage checkpoint (P53, ATM defects) are associated with genome instability.

Replication of single stranded DNA lesions can lead to double strand breaks (DSBs).

Even without the addition of extraneous DNA damaging agents, depletion of the recombinase Rad51 was shown to result in unrepaired chromosome breaks. Replication-stress in pre-cancerous and cancerous cells is ultimately thought to lead to DSBs and genome rearrangements. Other factors that can lead to fork breakage are replication of UV damaged DNA, DNA secondary structures, camptothecin-Topoisomerase I complex, replication blocking chemicals such as aphidicolin etc. Recombination functions to resume replication from broken forks. Chromosomal fragile sites are regions that are particularly prone to breakage under replication stress.

Pathways for repairing DSBs

Homology based: Homologous recombination utilizes homologous pairing catalyzed by recombinases. Involves various sub pathways including Synthesis Dependent Strand Annealing (SDSA), Single Strand Annealing (SSA), Break Induced Replication (BIR) and so on.

Non-homologous end joining (NHEJ): Largely error free. End joining of DNA ends regardless of sequence identity, inherently mutagenic.

Addition of telomeres at DNA ends: Telomerase dependent and independent pathways (ALT pathway; dependent on Pol32).

Holliday junctions

Four-way branched, fully paired, DNA strands. An important intermediate in homologous recombination. Specialized enzymes catalyze branch migration or resolution of Holliday junctions. Depending on the configuration of cleavage and re-ligation, resolution of HJs results in cross over or non-cross over outcomes. Segregation of same alleles result in generation of LOH.

Replication restart by Break-Induced Replication.

Fork regression is a mechanism to replicate past a fork-blocking lesion. Fork regression involves unwinding, re-pairing of newly synthesized strands and generation of a Holiday junction ("chicken foot") structure. Holliday junctions can be branch migrated or resolved by specialized enzymes. Resolution of chicken foot structure leads to one broken and one intact DNA molecule. Replication is re-established/resumed by strand invasion of the broken DNA into the intact sister.

Molecular biology of homologous recombination.

RecA and Rad51: Prokaryotic and eukaryotic homologues. Each protein molecule binds to 3 DNA bases. Results in stretching and unwinding the B-DNA helix by ~1.5 fold. The 3' end of the ssDNA-RecA/Rad51 nucleofilament searches homology in a duplex DNA molecule. Successful homologous pairing is followed by strand exchange – a process involving expenditure of ATPs. An intermediate in this reaction is called the D-loop (displacement loop). Extensive (in vitro) biochemical experiments and X-ray crystal structures have helped elucidate the mechanism of strand exchange in more detail.

Immortal cells:

Immortal cells have either reactivated telomerase or undergone alternative lengthening of telomeres (ALT) by recombination. ALT involves two mechanisms: Rad51 dependent or independent, but both dependent on Pol32.

SSA

Single Strand Annealing between repeats. Exonuclease digestion followed by strand annealing. Rad52 dependent, but Rad51 independent. Results in deletion. SSA between ALU repeats in separate chromosome can lead to chromosome rearrangements.

Gene conversion (GC):

GC can be between sister chromatid, homologous chromosomes or between ectopic loci. One mechanism involves SDSA. Key features are: short tract of DNA synthesis, only leading strand synthesis involved that is primed from the 3' invading end as opposed to leading and lagging strand synthesis in BIR. D-loops and dHJs are important intermediates. HJs are dissolved by Sgs1/Top3 complex or resolved by resolvases. Resolution can lead to either cross over or non-cross over. Unless Sgs1/Top3 (BLM) is absent, very little cross overs in mitosis. In meiosis however, dHJs are almost always resolved as cross overs.

Other sources of DSBs:

Ionizing radiations, failed Topoisomerase II action such as that induced by the cancer drug etoposide.

Various mechanisms in place to repair DNA DSBs.

Some precise and some not so. Defective or imprecise repair leads to genome rearrangements such as that seen in cancer cells.

Visualization of sister chromatid exchange in replicating cells.

BrdU experiments. Increased Sister Chromatid Exchanges (SCE) seen in cells treated with DNA damaging agents. Also seen in cells deficient for BLM helicase.

3. Recommended Reading

1. Pâques, F. and Haber, J.E. 1999. Multiple pathways of recombination induced by double-strand breaks in Saccharomyces cerevisiae. Microbiol Mol Biol Rev 63(2): 349-404.
2. Haber, J.E. 2012. Mating-type genes and MAT switching in Saccharomyces cerevisiae. Genetics.
3. Krogh BO, Symington LS. 2004. Recombination proteins in yeast. Annu Rev Genet. 38:233-71.

4. Review Questions

1. With respect to a normal genome, describe a characteristic of a cancer/tumor genome?
2. Describe a major source for endogenous DNA DSBs.
3. Describe two exogenous sources of DNA DSBs.
4. Mention the proposed molecular steps involved that lead to DSBs during replication in response to thymine dimers.
5. Name two mechanisms and the corresponding enzymatic complexes involved in the processing of double Holliday junctions.
6. Give an example for how loss of heterozygosity (LOH) can lead to cancer development.
7. With regards to HJ resolution, how do mitotic cells differ from meiotic cells?
8. Describe why *Alu* repeats present in the human genome pose a threat to genome integrity? What mechanism(s) are likely responsible?

9. What experimental technique was used to visualize sister chromatid exchange (SCE) in vertebrate cells?

5. Answers to Review Questions

1. Chromosomal rearrangements, including translocations, inversions, truncations etc., in other words, genome instability.
2. Replication fork-collapse at single strand lesions. For example, nicks, abasic site, damaged base, etc.
3. Ionizing radiation, drug-induced failed topoisomerase reactions.
4. Fork stalling, fork regression, HJ resolution.
5. HJ dissolution: Sgs1/Top3:
6. HJ branch resolution: Resolvase complex, for example, RuvAB/RuvC.
7. Rb/rb is heterozygous and cell cycle regulation is normal, whereas loss of the dominant Rb allele leads to the homozygous recessive rb/rb genotype and misregulated cell cycle progression.
8. In mitotic cells, HJs are largely resolved as non-crossovers whereas in meiotic cells, HJs are largely resolved as crossovers.
9. Recombination between dispersed Alu repeats could lead to gross chromosome rearrangements. Single Strand Annealing, Sister chromatid recombination.
10. BrdU staining/labeling. Cells were grown in BrdU containing medium and then grown for two generations without BrdU. Discontinuous BrdU staining in one of the sisters reflects SCE.

6. Discussion Questions

1. Based on the use of sequence identity, describe two major mechanisms that cells use to repair DNA DSBs?

2. Name two replication-blocking drugs described in the lecture and for each discuss what was the outcome when these agents were added to cells?
3. Starting from a DSB, describe the major steps involved in homology search? Mention the proteins involved in the process.
4. With respect to the use of replication machinery, mention the difference between GC and BIR?
5. Explain why Rad51, a recombinase by action, is essential even for normal replication in vertebrate cells?
6. BLM syndrome patients are cancer prone (because of defective BLM). Describe the observed phenotype of BLM cells with respect to the known action of BLM protein.

7. Answers to Discussion Questions

1. Homologous recombination and non-homologous end joining.
2. Aphidicolin and Hydroxyurea. Aphidicolin increased expression of fragile sites. HU treated cells exhibited regressed forks.
3. Sgs1 and Exo1 mediated DNA end resection and generation of ssDNA:
4. Rad51 coating of ssDNA.
5. Rad51 mediated strand invasion into homologous duplex DNA
6. GC uses only leading strand synthesis, whereas BIR involves both leading and lagging strand synthesis.
7. Large genomes encounter DSBs during replication that have to repaired by means of BIR requiring Rad51 or by sister-chromatid recombination.
8. BLM protein dissolves dHJs leading to non-cross overs. In the absence of BLM, dHJs could be resolved as cross overs resulting in increased SCE phenotype (visualized by BrdU staining).

8. Explain or Teach These Concepts to a Friend

1. Steps involved in homologous recombination.
2. Experiments that elucidated the strand exchange activity of RecA.
3. BrdU experiments to visualize SCE.
4. Cross overs vs. non cross overs.
5. HJ dissolution vs. resolution.
6. How SSA between repeats can lead to a large deletion.
7. Mechanisms by which LOH can be generated.

9. Research the Literature on Your Own

1. Oncogene induced DNA replication stress model for the development of sporadic cancers.
2. Hereditary vs. sporadic cancers.
3. The role of exonucleases, helicases and topoisomerases in recombination.
4. Mechanism of action of the cancer drugs camptothecin and etoposide.