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# Jim Haber's Lecture Part 2:
# Details of DNA Repair in Budding Yeast

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## 1. Keywords and Terms

HO endonuclease, MAT switching, Break induced replication (BIR), Gene conversion (GC), Synthesis Dependent Strand Annealing (SDSA), Holliday Junction (HJ) dissolution, Holliday Junction resolution, conservative DNA synthesis, semiconservative DNA synthesis, resection, RPA, Rad51, Rad55-57, BRCA2, Sgs1, Mph1, Srs2, Top3, ChiP, nucleosomes, Sir2, MM-BIR

## 2. Lecture Notes

Specific examples of homologous recombination in budding yeast are presented.

## 3. Review Questions

1. Explain 3 key features of BIR that distinguishes it from GC.
2. Explain how BIR could lead to LOH as opposed to SDSA.
3. Name one experimental technique used to examine the interaction between protein and DNA?
4. Describe one key advancement that allowed us to control the *MAT*-switching system in yeast and to study DSB repair in detail.
5. What are the functions of the following proteins in DSB repair? RPA, Rad52 and Rad55-57.
6. At what step do Rad1/Rad10 and Msh2/Msh3 protein complexes come into play in DSB repair?
7. Which key aspect of DSB repair was demonstrated using a *CDC-7* variant allele (*CDC-7 as*)?
8. Based on the experiments with the temperature-sensitive alleles of replicative polymerases, what major conclusion can be drawn regarding the need for replicative polymerases in DSB repair?
9. List three techniques mentioned in the lecture that were used to monitor DSB repair *in vivo*.
10. If both *SGS1* and *EXO1* are deleted, what do you imagine will happen to *MAT* switching?
11. Irradiated chicken DT40 cells exhibit RAD51 repair “foci”. What was observed for cells in which BRCA2 was deleted? Based on the observation, discuss the possible function of *BRCA2*.

## 4. Answers to Review Questions

1. Invasion from only one end of the DSB, leading and lagging strand synthesis, and a requirement for pol32.

2. In BIR, invasion is only from one end of the DSB and therefore markers present on the non-invading end are lost resulting in LOH.
3. Chromatin immunoprecipitation (ChiP)
4. Placing HO endonuclease under the control of a galactose-inducible promoter.
5. RPA coats ssDNA and functions to smooth out the secondary structures. Rad52 facilitates RPA displacement and Rad51 loading on ssDNA. Rad55-57 is an additional facilitator for Rad51 loading.
6. Rad52 facilitates RPA displacement and Rad51 loading on ssDNA.
7. Rad55-57 is an additional facilitator for Rad51 loading.
8. Clipping of the non-homologous 3' tail.
9. DNA synthesis during DSB repair does not require the loading of normal replication machinery.
10. Either Pol-delta OR Pol-epsilon is sufficient for repair synthesis. Absence of either one did not significantly affect repair synthesis.
11. Southern blot, ChiP and fluorescence microscopy.
12. No resection and therefore no generation of ssDNA. Therefore, no loading of Rad51, no homology search and no *MAT* switching.
13. There were no Rad51 foci in the absence of BRCA2. BRCA2 functions to facilitate loading of Rad51 onto ssDNA.

## 5. Discussion Questions

1. Three helicases were described in the lecture: Srs2, Sgs1, and Mph1. Discuss how the absence of each of these impacted the DSB repair crossover outcomes.
2. Describe the function of Srs2 in DSB repair? Speculate why some of the outcomes of SDSA were not recoverable?
3. Which step of the DSB repair process is impaired in the absence of Rad54?

## 6. Answers to Discussion Questions

1. Because of a reduction in the recovery of SDSA intermediates, absence of Srs2 resulted in an apparent increase in crossover outcomes.
2. Because of its role in unwinding in D-loops to facilitate SDSA, absence of Mph1 resulted in an increase in crossovers.
3. Because of the failure to dissolve dHJs, absence of Sgs1 resulted in an increase in crossovers.
4. Srs2 catalyzes displacement of Rad51 from ssDNA. In the absence of Srs2, the SDSA intermediates are likely “stuck” subsequent to the invasion step.
5. The DNA synthesis step.

## 7. Explain or Teach These Concepts to a Friend

1. Conservative vs. semi-conservative synthesis.
2. Recombination Execution Checkpoint (REC): Possible functions and likely consequences of its absence.
3. Various steps involved in DSB repair.
4. Exonuclease vs. endonuclease action in DSB repair.
5. Discuss the implication of the finding that mutation frequencies are ~1000x higher during DSB repair than during standard DNA replication.

## 8. Research the Literature on Your Own

1. Role of Rad1/Rad10 and Msh2/Msh3 in DSB repair. (Suggestion, see following paper).
2. Sugawara, N., Ivanov, E. l., Fishman-Lobell, J., Ray, B. l., Wu, X., et al. 1995. DNA structure-dependent requirements for yeast RAD genes in gene conversion. Nature 373: 84–86.

3. Role of Pol epsilon and Pol delta in DSB repair and replication. (Suggestion, see following papers)
4. Lydeard, J.R., Jain, S., Yamaguchi, M., and Haber, J.E. 2007. Break-induced replication and telomerase-independent telomere maintenance require Pol32. Nature 448(7155): 820-823.
5. Lydeard, J.R., Lipkin-Moore, Z., Sheu, Y.J., Stillman, B., Burgers, P.M., and Haber, J.E. 2010b. Break-induced replication requires all essential DNA replication factors except those specific for pre-RC assembly. Genes Dev 24(11): 1133-1144
6. DNA damage checkpoint
7. Role of Chromatin remodelers in DSB repair: Asf1, Caf1

## 9. Papers for Journal Club

1. Jain, S., Sugawara, N., Lydeard, J., Vaze, M., Tanguy Le Gac, N., and Haber, J.E. 2009. A recombination execution checkpoint regulates the choice of homologous recombination pathway during DNA double- strand break repair. Genes Dev 23(3): 291-303. *Detailed experiments that revealed competition between GC and BIR and the existence of REC*
2. Hicks WM, Kim M, Haber JE. (2010) Increased mutagenesis and unique mutation signature associated with mitotic gene conversion. Science. 2;329(5987):82-5, 22;330(6003):448. *Discusses the discovery of increased mutagenesis associated with DSB repair.*