Mismatch repair proteins in homologous recombination and genome stability

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Mismatch repair proteins & cancer

Overview

- Mismatch repair proteins correct DNA polymerase misincorporation errors (**Figure 5**), and have a high mutational burden as a result.
- A subset of the mismatch repair proteins regulate the homologous recombination DNA repair pathway (**Figure 6**), but the contribution of loss of this regulation to tumorigenesis is unknown.
- Project goal is (1) to define the mechanism of regulation of homologous recombination by the mismatch repair proteins, and (2) to determine the contribution of loss of regulation of this pathway to carcinogenesis.

Therapeutic potential

- Most testing for mismatch repair deficiency in tumors involves antibody staining for the presence/absence of MSH2, MSH6, MLH1, and PMS1 (**Figure 5**).
- Database analysis (**Figure 1**) indicates that the majority of tumors with abnormalities in *MSH2* or *MSH6* have mutations or amplifications, which may not be detected *via* this method.
- MSH3 is another component of the mismatch repair pathway, and a regulator of homologous recombination. Though a significant fraction of certain tumor types have *MSH3* abnormalities (**Figure 1**), antibody testing for MSH3 is not routine.

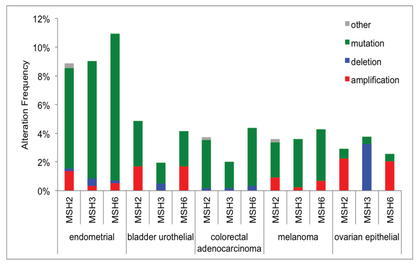


Figure 1. Frequency of MSH2, MSH3, and MSH6 alterations across five of the most commonly affected cancer subtypes. Data from TCGA PanCancer Atlas Studies.

D-loop capture (DLC) & extension (DLE) assays

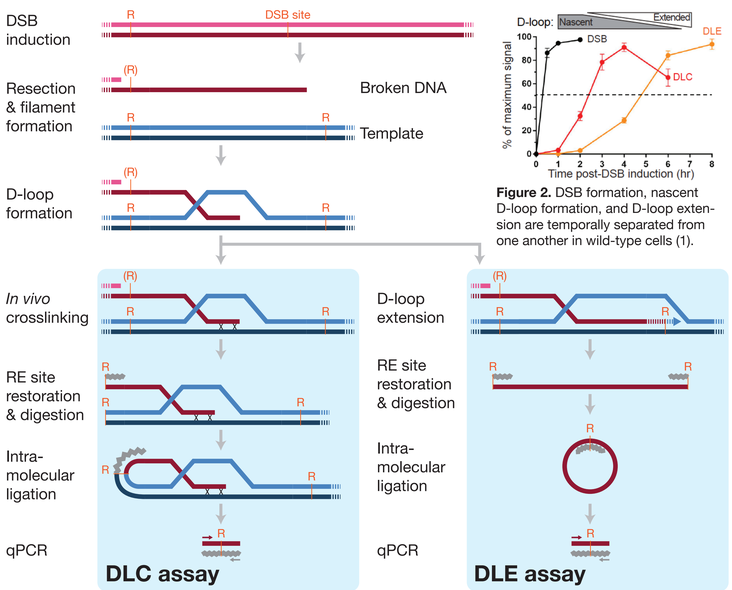


Figure 2. DSB formation, nascent D-loop formation, and D-loop extension are temporally separated from one another in wild-type cells (1).

Figure 3. The DLC and DLE assays are proximity ligation-based assays that quantify nascent D-loop formation and extension, respectively, independent of cell viability. R, restriction enzyme (RE) site; (R), a RE site that cannot be cut.

Long-read sequencing

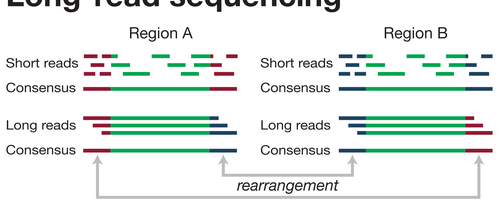


Figure 4. Short-read sequencing gives typical read lengths of ~300 nucleotides. In contrast, long-read sequencing can give continuous reads of more than 100 kbp (2). Due to their differences in average read lengths, long-read sequncing is capable of capturing rearrangements between repetitive elements (shown in green) in the genome that are impossible to detect by short-read sequencing.

Mismatch repair pathway

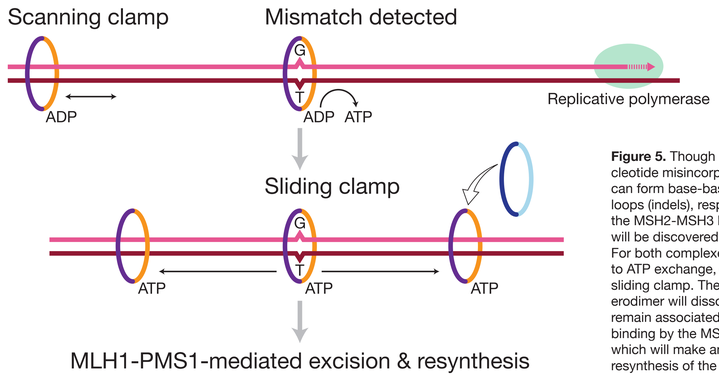


Figure 5. Though rare, replication fork slippage or nucleotide misincorporation errors during DNA replication can form base-base mismatches and insertion/deletion loops (indels), respectively. Indels will be recognized by the MSH2-MSH3 heterodimer, whereas mismatches will be discovered by the MSH2-MSH6 heterodimer. For both complexes, mismatch detection triggers ADP to ATP exchange, converting the heterodimer into a sliding clamp. The MSH2-MSH3 or MSH2-MSH6 heterodimer will dissociate from the indel or mismatch, but remain associated with the dsDNA, encircling it. ATP binding by the MSH complex will recruit MLH1-PMS1, which will make an excision to promote removal and resynthesis of the mispaired bases.

Homologous & homeologous recombination pathways

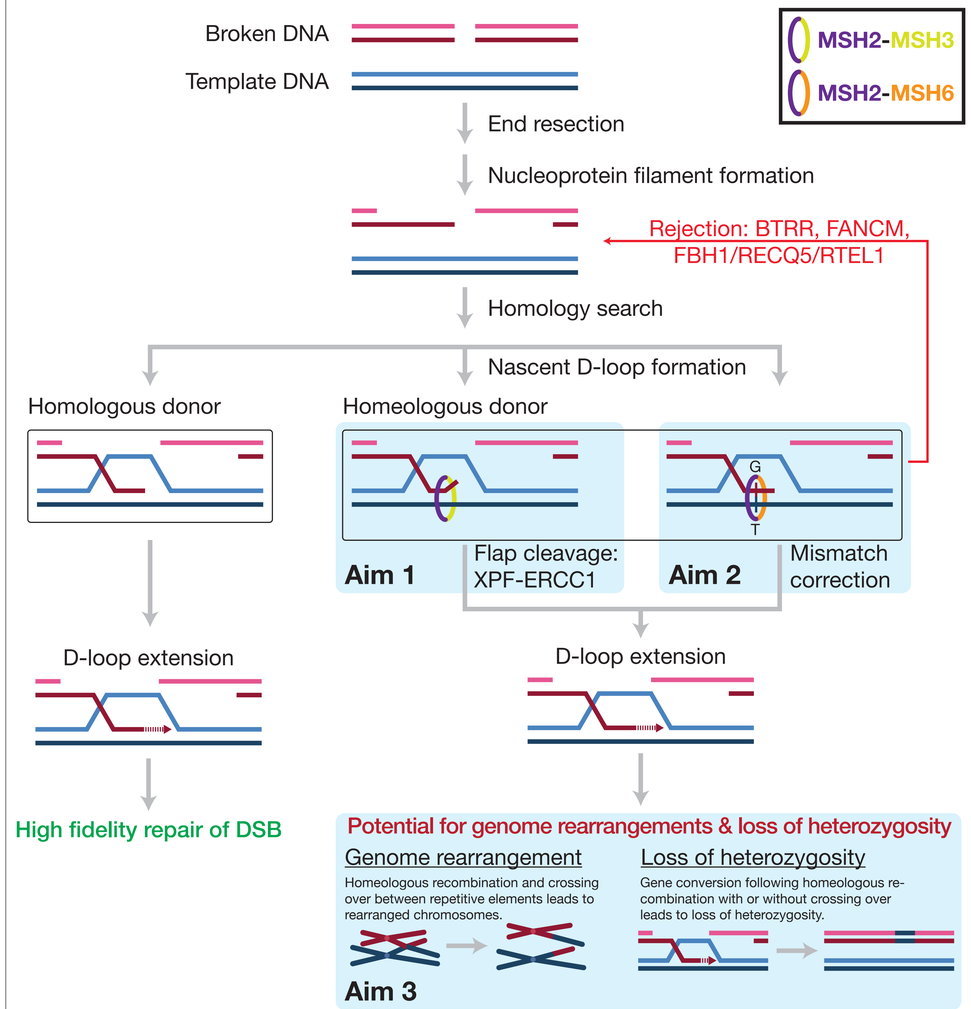


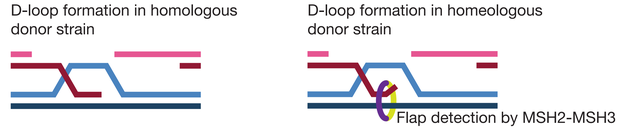
Figure 6. The mismatch detecting heterodimers Msh2-Msh3 and Msh2-Msh6 have additional roles in regulating recombination. Homologous recombination generates a recombination intermediate without heterologies (left) within the heteroduplex DNA. In contrast, recombination between a mismatched, or homeologous donor, forms a recombination intermediate either containing a 3' heterologous flap (middle) or mismatches (right). Msh2-Msh3 and Msh2-Msh6 are thought to recognize these homeologous recombination intermediates, then recruit effectors to disassemble them and allow recombination to begin anew (heteroduplex rejection). Alternatively, Msh2-Msh3 and Msh2-Msh6 can promote repair *via* recruiting factors involved in flap cleavage and mismatch correction, respectively. The molecular mechanism through which heteroduplex rejection occurs, and what controls the balance between rejection and repair, are currently unknown.

Specific aims

(1) Investigate the effect of heterologous flaps on the efficiency and timing of D-loop formation and extension.

- A 3-base pair flap decreased HR efficiency in a break-induced replication assay (3), but the effect of flaps on the canonical recombination pathway is unknown.
- MSH2-MSH3 promotes shorter D-loop extension tracts in response to flaps, preventing potential rearrangements (4, 5). How MSH2-MSH3 achieve this effect is unknown.

A) Evaluate the effect of heterologous flaps on D-loop formation and extension *in vivo* in matched budding yeast strains using the DLC and DLE assays (**Figure 3**).

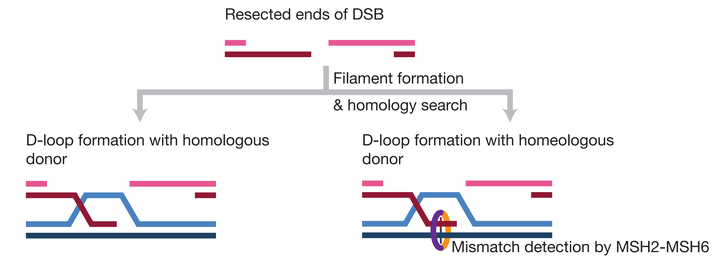


B) Use purified proteins to show that MSH2-MSH3 recognizes D-loops with terminal flaps *in vitro*, and reconstitute the mechanism of MSH2-MSH3 heteroduplex rejection.

(2) Define the mechanism of MSH2-MSH6-dependent heteroduplex rejection.

- Most studies on MSH2-MSH6 and homeologous recombination examine recombination between a broken strand and a mismatched donor (6-9) (mismatch tolerance).
- Only study to examine recombination in the context of both a mismatched and a homologous donor found <2X difference (10). However, their assay only detected very rare crossover products.

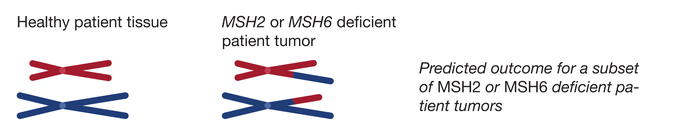
A) In a competitive system in which recombination can engage a mismatched or a homologous donor, determine how MSH-MSH6 promotes use of the homologous donor.



B) Use purified proteins to reconstitute MSH2-MSH6 heteroduplex rejection.

(3) Use long-read sequencing to determine whether *MSH2* and *MSH6* deficient tumors exhibit a mutational signature consistent with homeologous recombination.

- Site-specific studies in *Msh2-/-* and *Msh6-/-* mice found increased homeologous recombination in these backgrounds (11, 12).
- Mouse fibroblasts deficient in *Rtel1* (**Figure 6**) show increased structural variant breakpoints relative to wild-type cells (13).
- Findings suggest that heteroduplex rejection deficient cells harbor large-scale genome rearrangements.



References & funding

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