Recruitment Mechanism of DNA Polymerases during Homologous Recombination Repair

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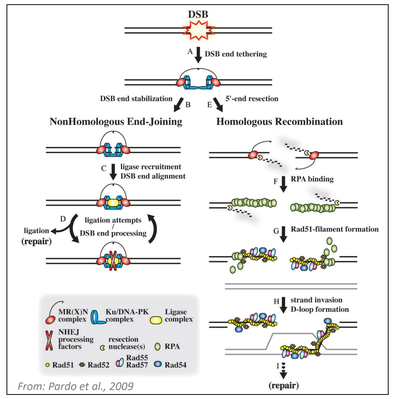
DNA Damage is Dangerous

- A cell's DNA is damaged from both internal and external sources threatening genomic integrity.
- Proper DNA repair prevents mutation accumulation and the development of diseases and disorders.

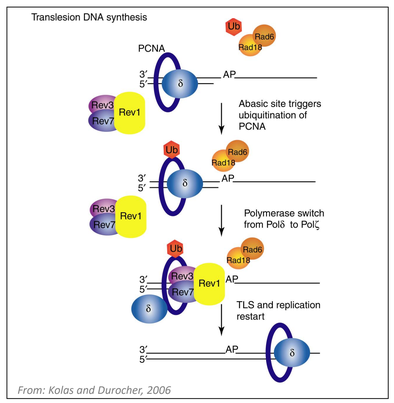
- Double-strand breaks (DSBs) are particularly dangerous as large amounts of information may be deleted.
- DSBs can be repaired by two pathways: non-homologous end joining (NHEJ) or homologous recombination (HR). HR provides accurate repair while NHEJ is error-prone.
- Steps of HR initiation are well known, but how new DNA synthesis is coordinated and performed is still unclear.
- DNA synthesis requires a DNA polymerase.



From: Pardo et al., 2009

- Humans have 15 DNA polymerases.
- Two broad categories of polymerases: replicative polymerases, which replicate the bulk of DNA during S-phase, and translesion (TLS) polymerases, which replicate through damaged DNA templates.
- Rev1 is a TLS polymerase that replicates across abasic (AP) sites. The replicative polymerase delta (δ) is replaced with TLS polymerases when its clamp (PCNA) is modified (Ub).
- Rev1 can interact with multiple polymerases through its C-terminal domain (CTD).

- Clamps are used to increase the processivity of polymerases during DNA synthesis.
- The 9-1-1 clamp (made of subunits: Rad9, Rad1 and Hus1) is used in signaling in response to DNA damage.



From: Kolas and Durocher, 2006

- Are Rev1 and 9-1-1 (Hus1) coordinating DNA polymerase recruitment and synthesis during HR repair?

Previous Experiments with Flies

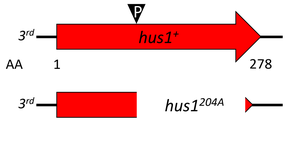
- Replicative polymerase delta is involved in long distance DNA repair synthesis.
- TLS polymerases act redundantly and are involved in short amounts of HR repair synthesis.

Mutants: A Genetic Approach

Polymerase *rev1* is on chromosome 3

- Rev1-ΔCTD lacks the last 100 amino acids required to interact with other TLS DNA polymerases.

Clamp *hus1* is on chromosome 3



- *hus1*^{204A} was created by transposon (P) excision and is a full null (no functional protein).

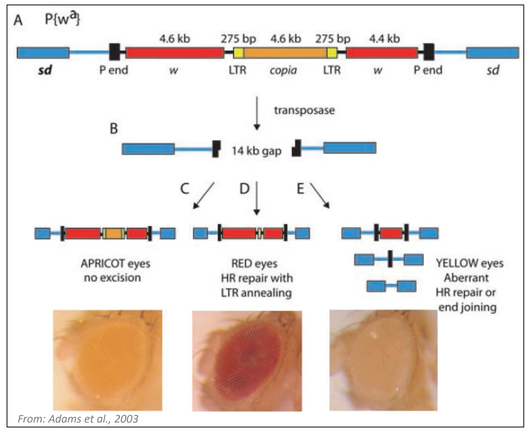
Flies lacking Rev1-CTD or Hus1 will be tested in genetic assays assessing DNA repair capabilities. If defects are seen, then Rev1-CTD and Hus1 must be involved in these important processes.

The *P{wᵃ}* Assay: Involvement in HR Repair

P{wᵃ} assay creates a single DSB and allows for distinguishing NHEJ from HR repair based on eye color.

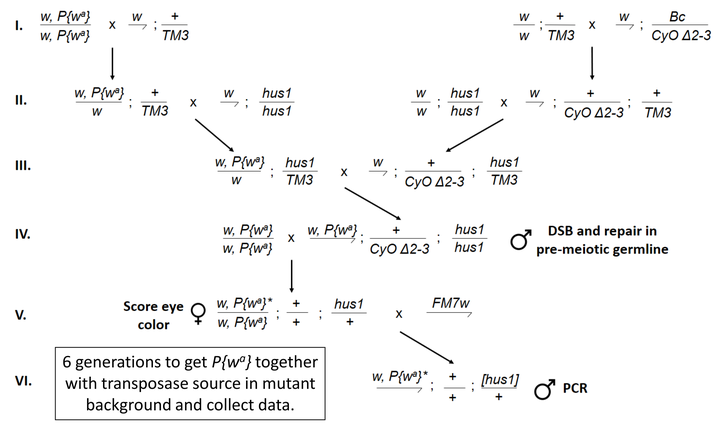
- *P{wᵃ}* is a transposable element located in the *scalloped* (*sd*) gene on the X chromosome.
- *P{wᵃ}* contains the *white* (*w*) gene interrupted by a *copia* retrotransposon insertion.
- With the introduction of a transposase source (*Δ2-3*), *P{wᵃ}* is excised, creating a single DSB gap of 14 kilobases.

- Different types of repair lead to different fly eye colors:
  - Apricot: no excision;
  - Red: Full HR repair; DNA synthesis of 9kb up to the long terminal repeats (LTRs) of the *copia* retrotransposon;
  - Yellow: aborted HR repair.



From: Adams et al., 2003

The *P{wᵃ}* genetic cross scheme:

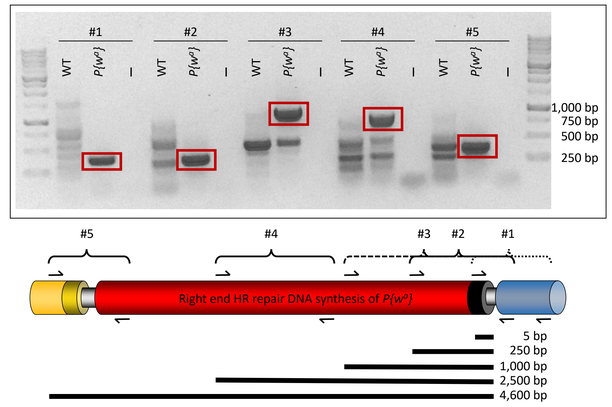


Screen of HR repair by fly eye color (red eyes = full HR; yellow = aborted HR) in female flies in generation V:

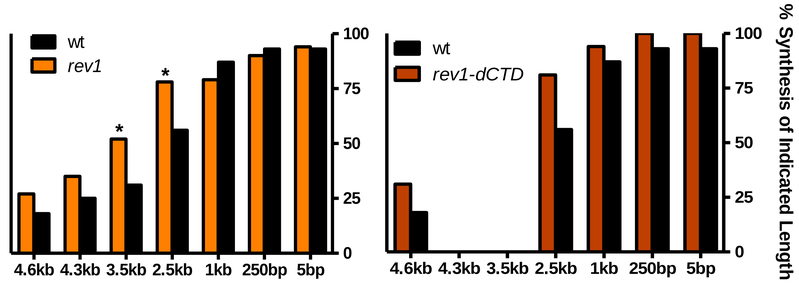
*P<0.05, Mann-Whitney test; Standard errors; Sample sizes: wildtype = 119, *rev1-ΔCTD* = 31, *hus1*= 159.

***rev1-ΔCTD* phenotype matches *rev1* phenotype (Kane et al., 2012);**
***hus1* phenotype appears distinct from checkpoint role as compared to *ATR* phenotype (LaRocque et al., 2007)**

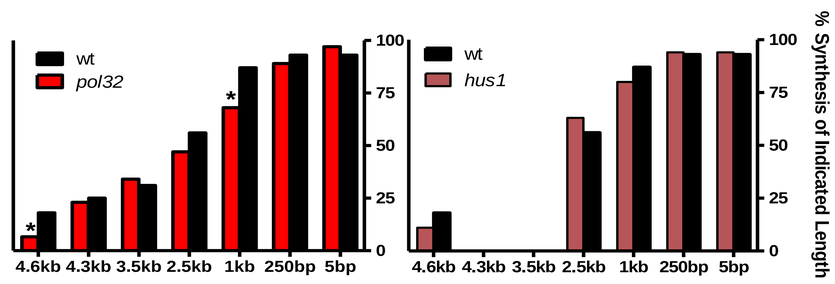
PCR of yellow-eyed (aborted HR) events recovered in generation VI determines repair synthesis capabilities:



Repair synthesis tract length capabilities in polymerase and clamp mutants (*rev1* and *pol32* data from Kane et al., 2012; *P<0.05, Fisher's exact test; *rev1-ΔCTD* and *hus1* data is subset of samples, PCRs in progress):



***rev1-ΔCTD* phenotype continues to match *rev1* phenotype (Kane et al., 2012);**
Is Rev1 (through CTD) used to recruit TLS DNA polymerases for short distance synthesis?



If *hus1* phenotype doesn't match *ATR* phenotype does it have a checkpoint-independent role in HR gap repair?
Is Hus1 used to recruit replicative DNA polymerases for long distance synthesis?

Future Directions and Acknowledgements

We will continue to run PCRs and investigate mechanism of recruitment and coordination of new DNA synthesis during HR repair.

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