Molecular Mechanisms of Mismatch Repair

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Mismatch Repair Deficiency Causes Cancer

Mismatch repair (MMR) deficiency
(Genetic defects or epigenetic modification of MMR genes)

↓

Genomic instability
(e.g., Microsatellite instability)

↓

Cancer
(e.g., HNPCC or Lynch Syndrome)

MMR Genes and Colorectal Cancer

	HNPCC[a]	Sporadic
Population incidence	~1 in 500	1 in 20
Microsatellite instability	>90%	13%
MMR gene mutations	70%	~65% of CRC with MSI
MSH2	~40%	30%
MSH3	0%	
MSH6	~0.5%	
MLH1[b]	~50%	70%
PMS1	<5%	
PMS2		
MLH3		

[a] Hereditary non-polyposis colorectal cancer.
[b] Hypermethylation of the *MLH1* promoter.

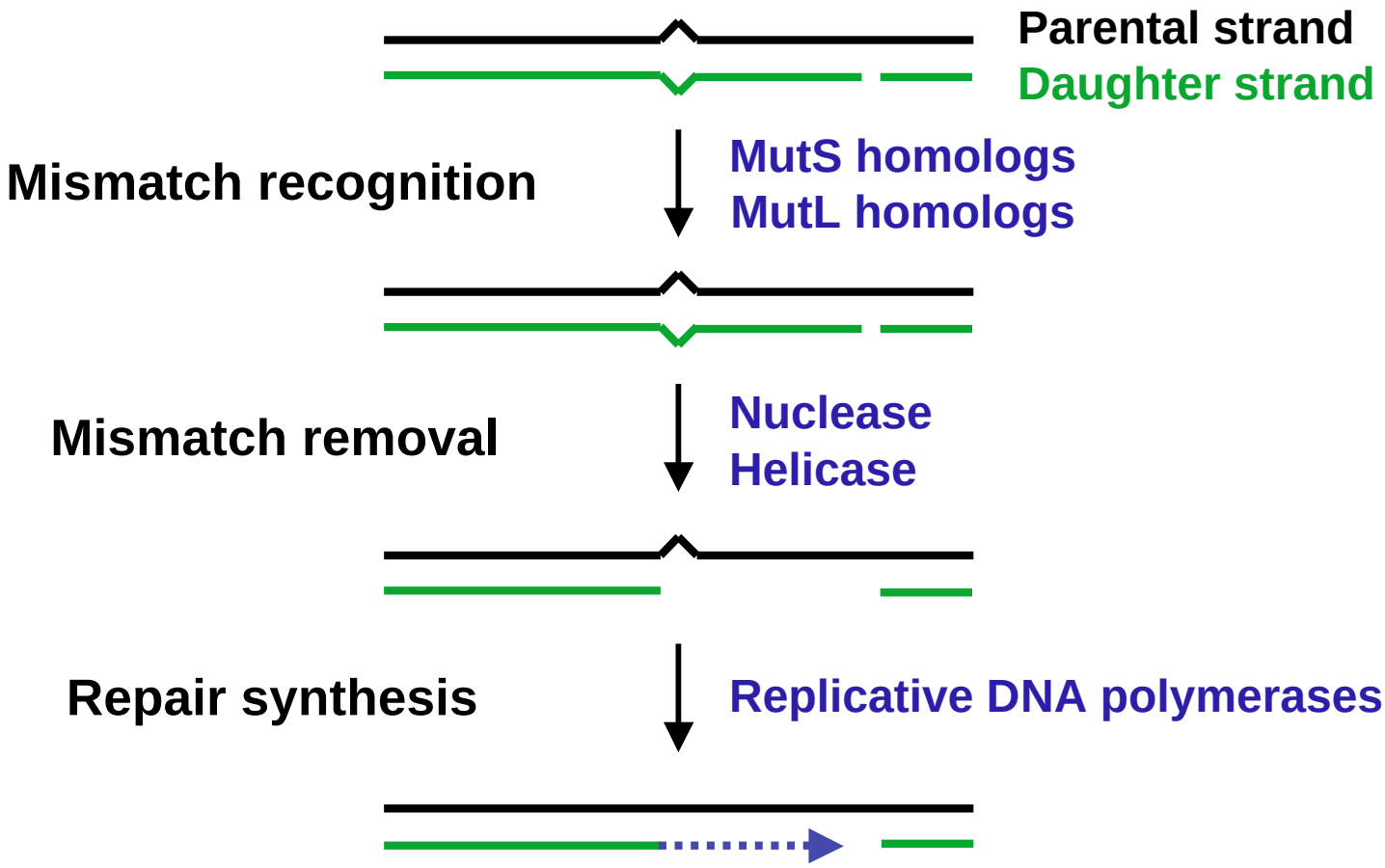
Genome-Maintenance Functions of MMR

I. Correct biosynthetic errors

II. Suppress homeologous recombination

III. Mediate DNA damage response

DNA Mismatch Repair Reaction



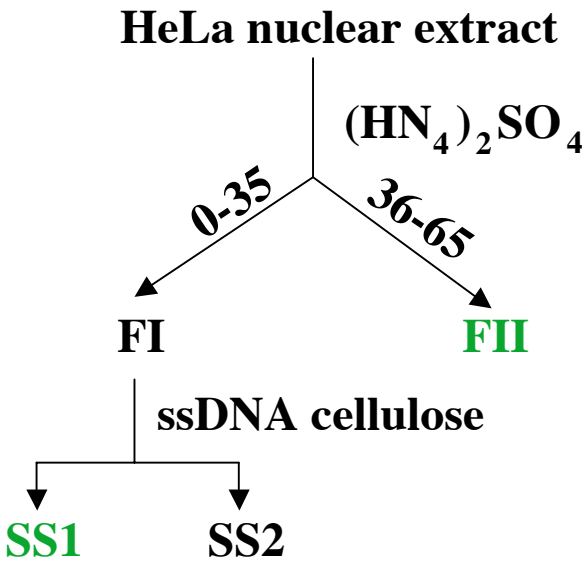
Mismatch Repair Components

E. coli	Human
MutS	MutSα (MSH2-MSH6)
	MutSβ (MSH2-MSH3)
MutL	MutLα (MLH1-PMS2)
MutH	?
UvrD (helicase II)	?
RecJ, ExoI, ExoVII, ExoX	EXO1, ?
SSB	RPA
DNA pol III holoenzyme	Pol δ, PCNA, RFC
DNA ligase	DNA ligase I
	HMGB1

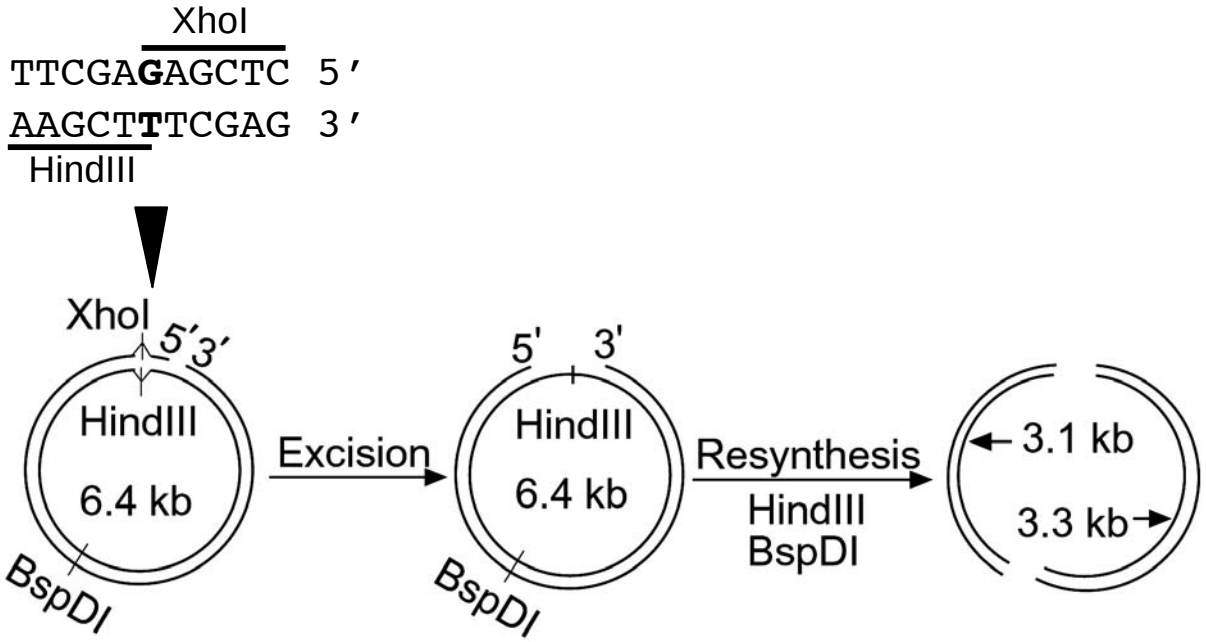
Outlines

- **Identification and characterization of mismatch repair components**
  - **RPA**
  - **HMGB1**
  - **PCNA**
- **Reconstitution of the mismatch repair reaction**

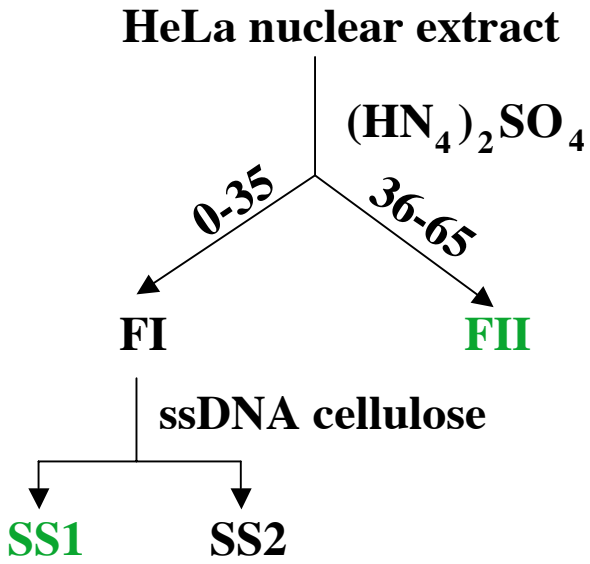
Fractionation

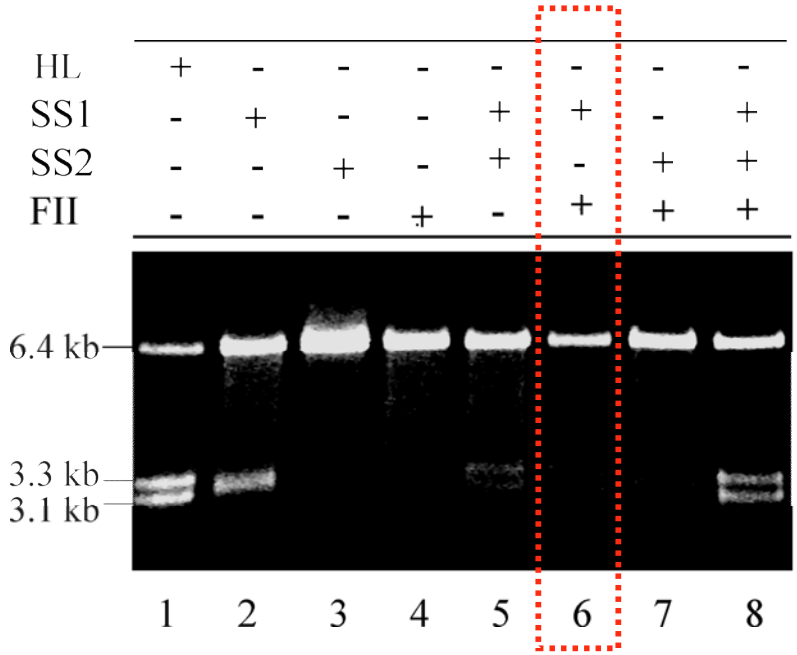


In vitro Mismatch Repair Assay



Fractionation and Reconstitution





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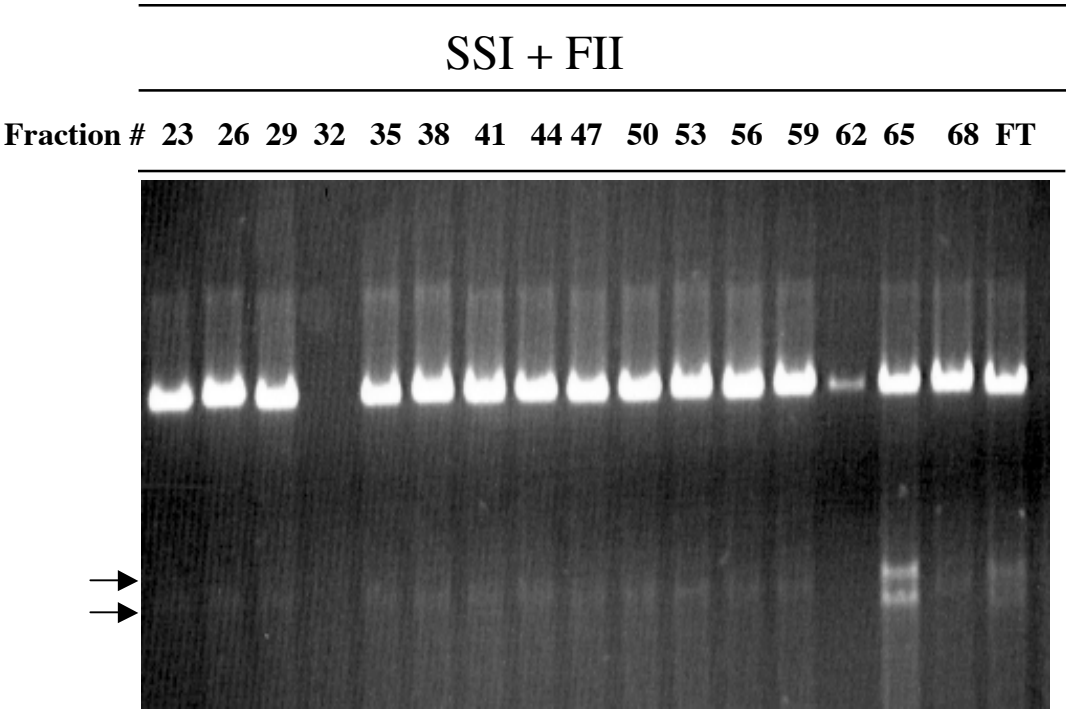
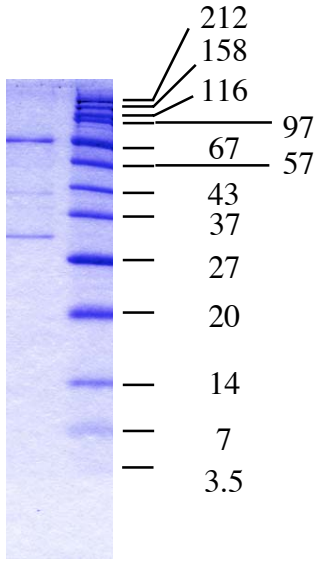
Purification of the SSI/FII-Complementing Activity



SS2 on Mono Q FPLC column



SDS PAGE of fraction 65

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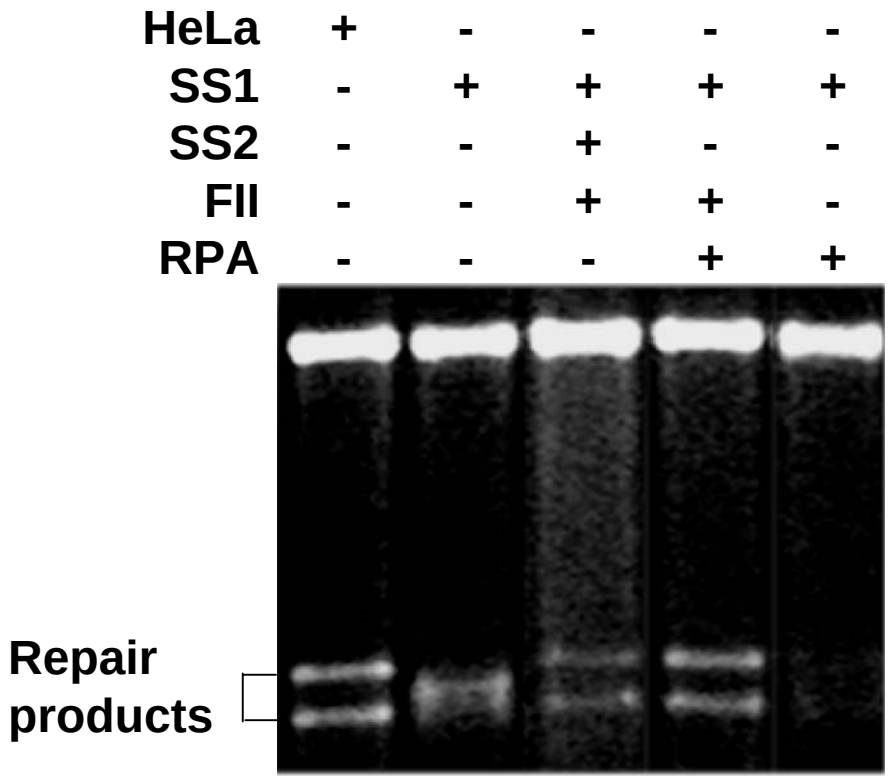
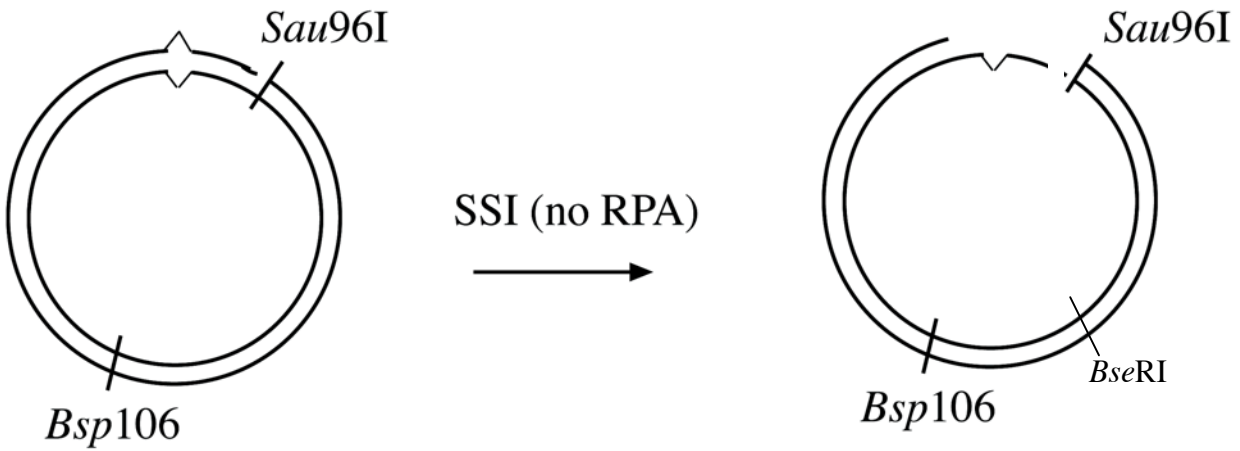
Recombinant RPA Substitutes for SS2



Ramilo et al., *Mol. and Cell. Biol.* 22, 2037-2046, 2002

RPA Protects Nascent ssDNA?



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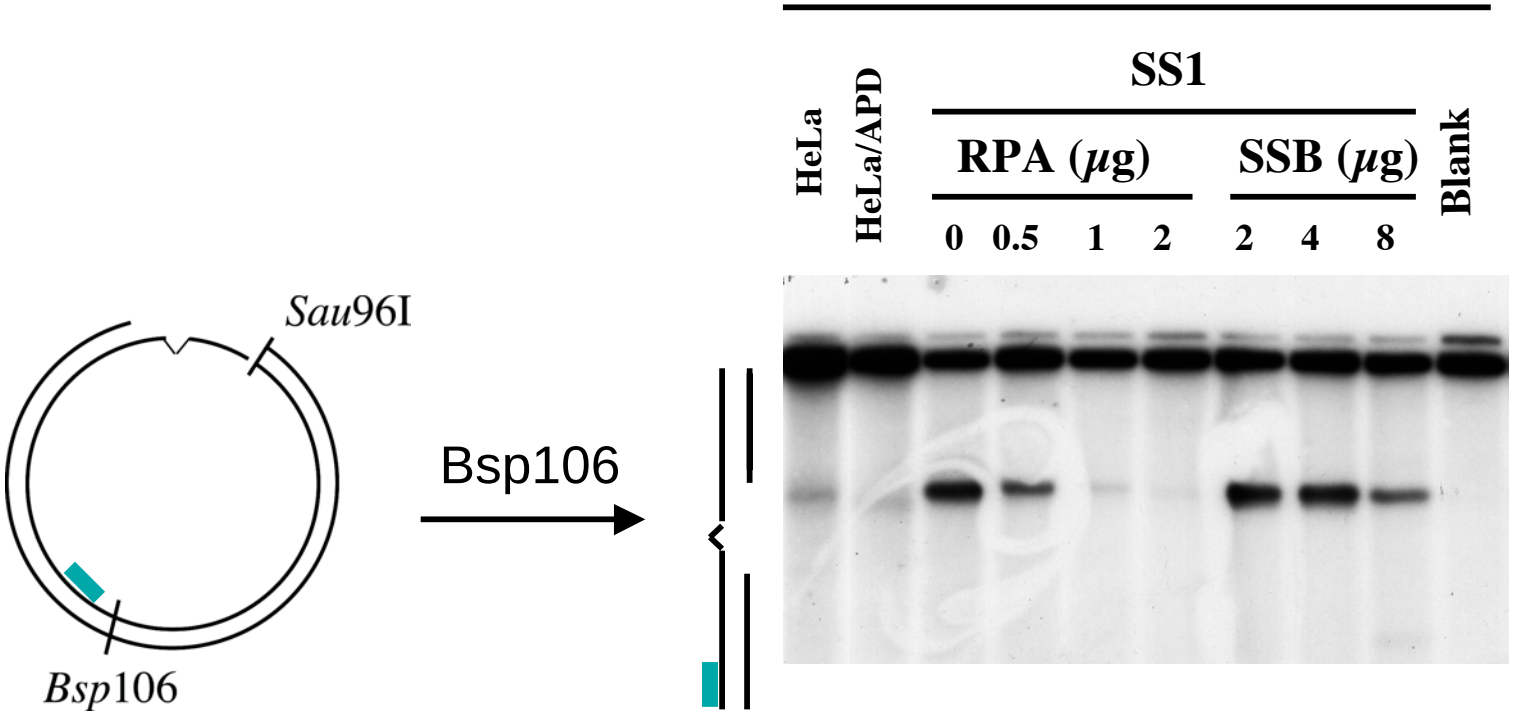
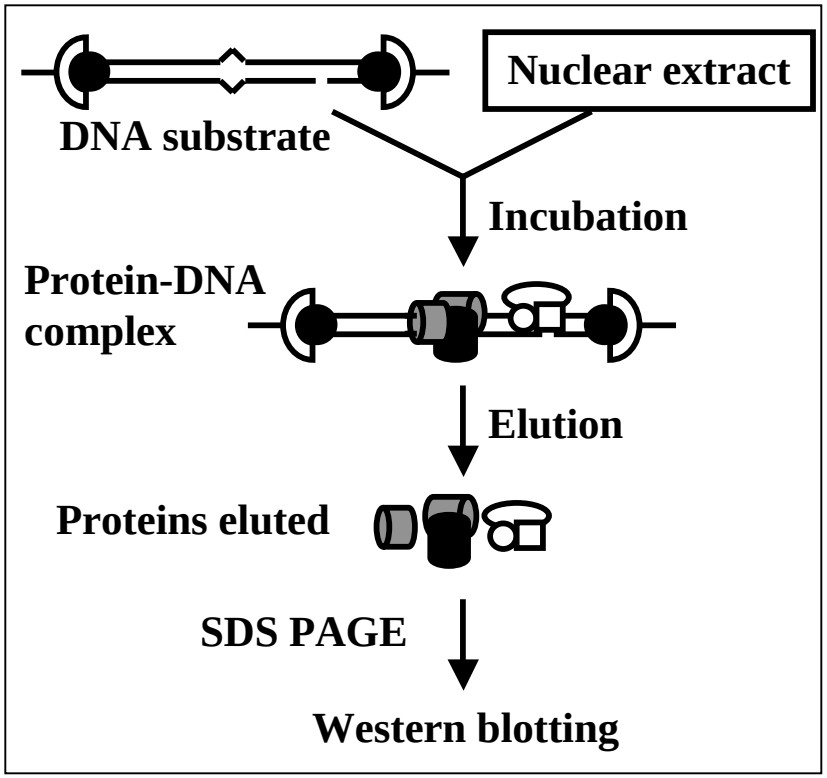
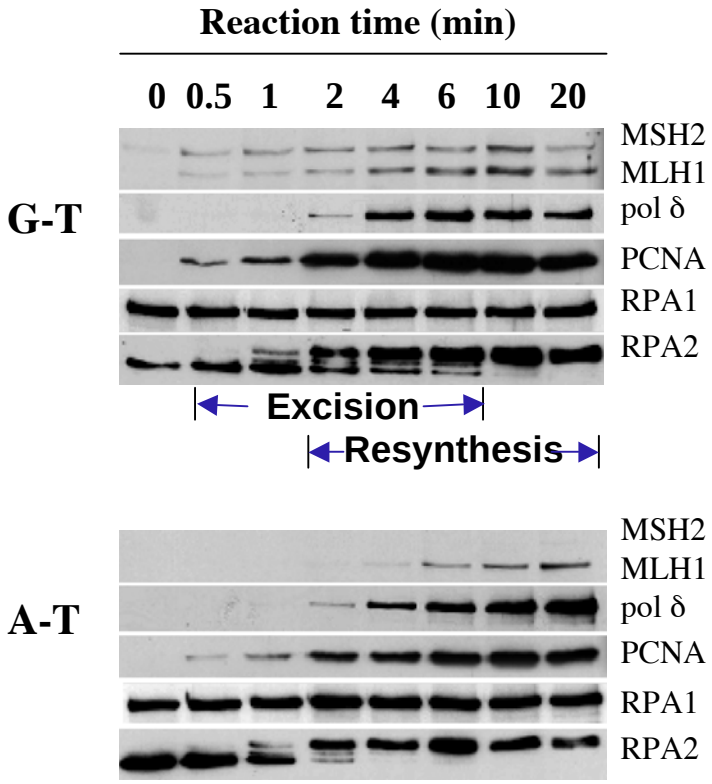


Ramilo et al., *Mol. and Cell. Biol.* 22, 2037-2046, 2002

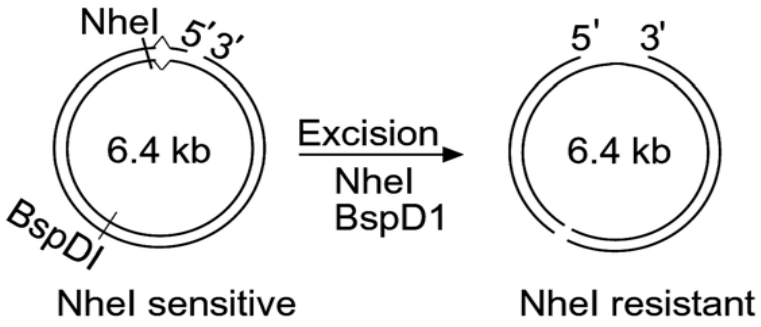
RPA Is Phosphorylated during MMR

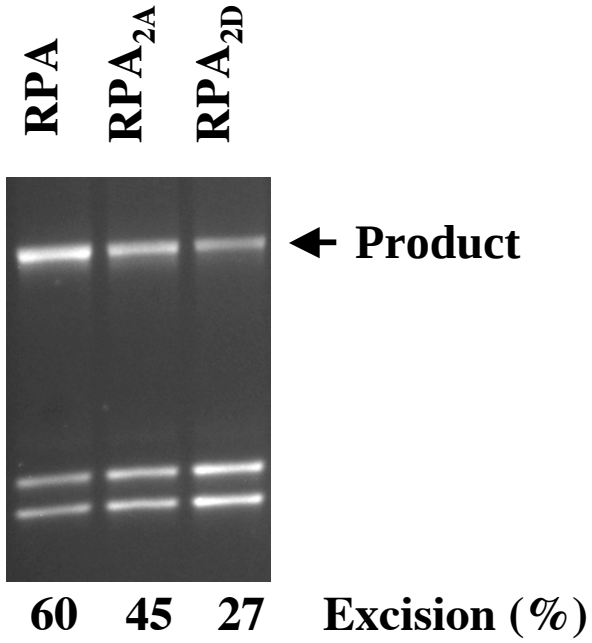




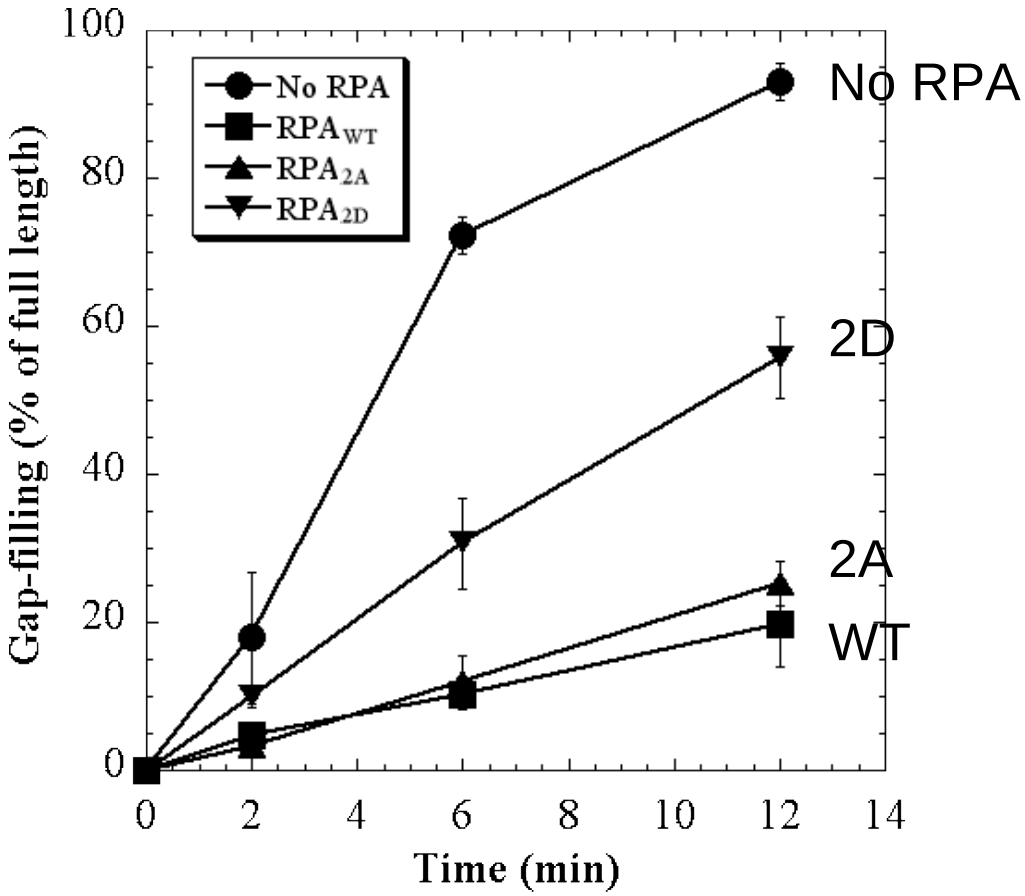
Unphosphorylated RPA for excision?
Phosphorylated RPA for resynthesis?

Unphosphorylated RPA Facilitates Excision

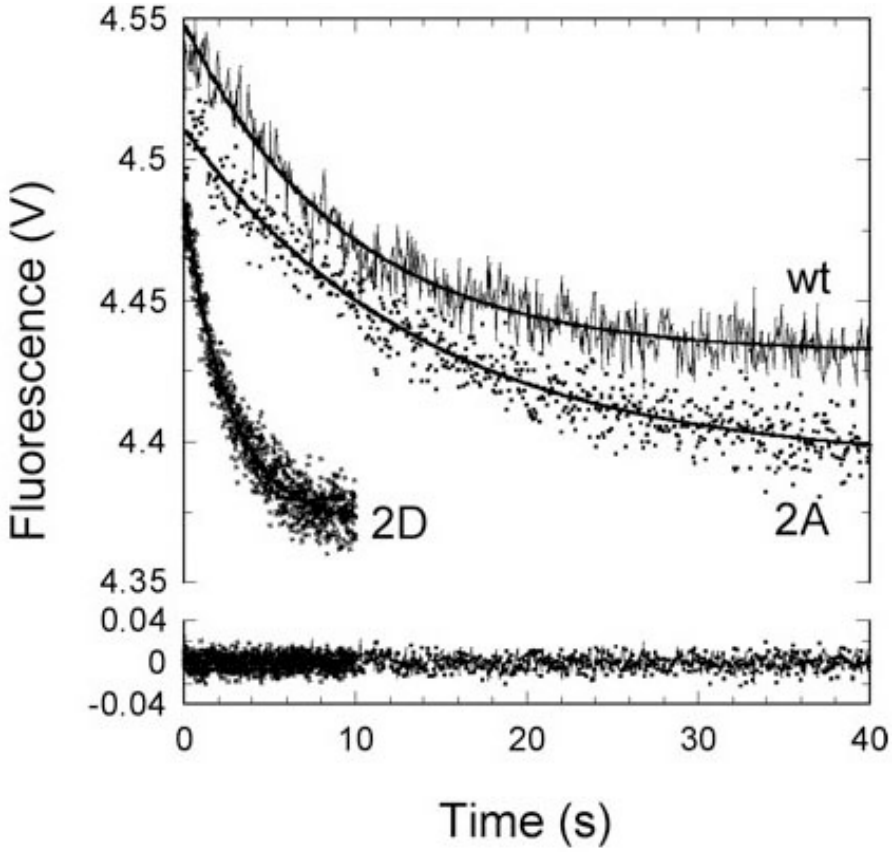




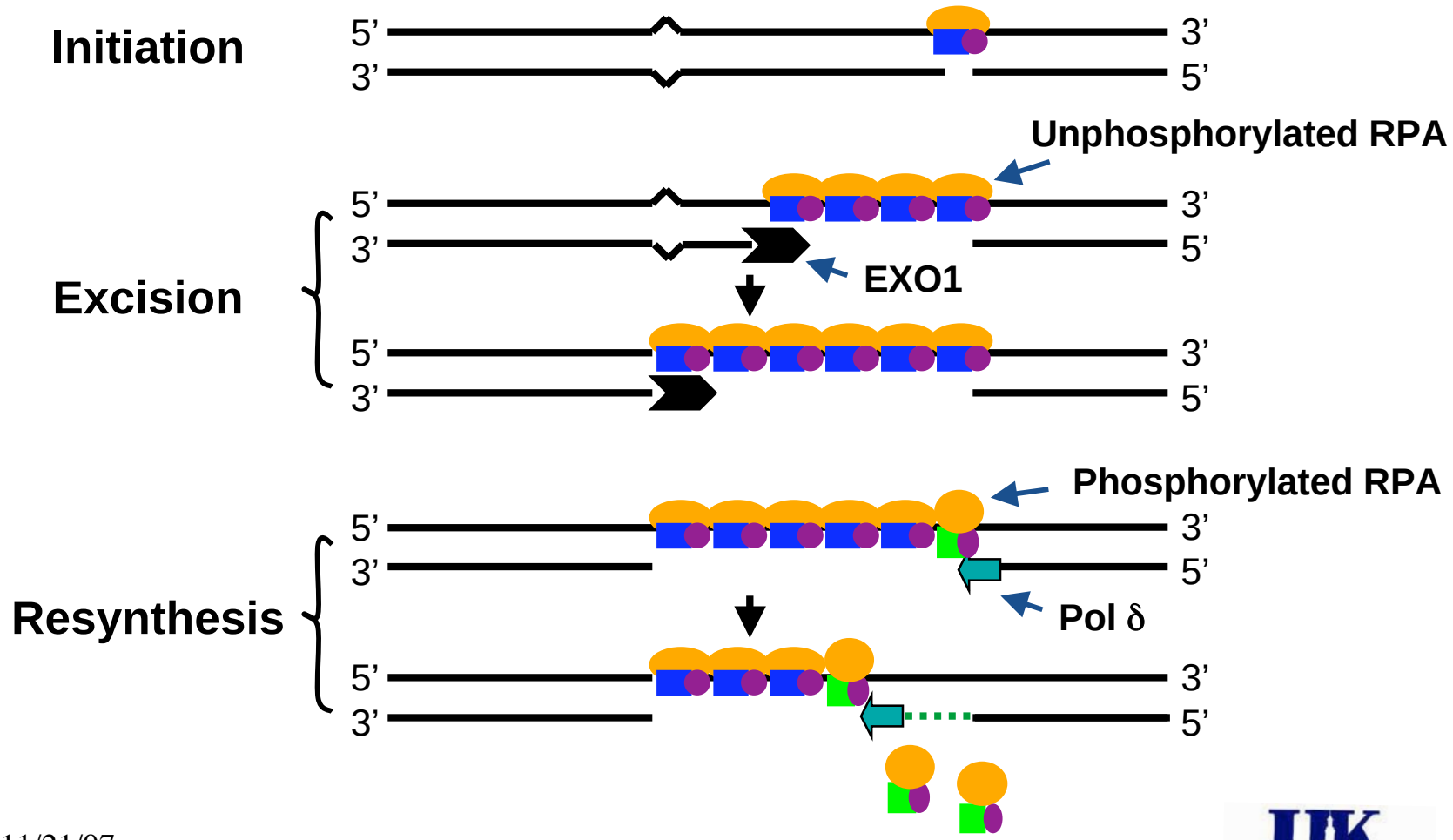
Phosphorylated RPA Promotes Resynthesis



Phosphorylation Reduces RPA DNA Binding



Phosphorylation Regulates RPA Functions?



Role of RPA in MMR

1. Involved in all steps of MMR
2. Protect the nascent ssDNA from degradation
3. Phosphorylation may regulate RPA functions in MMR

Role of HMGB1 and PCNA in MMR

HMGB1:

Involved in the excision step in MMR

PCNA:

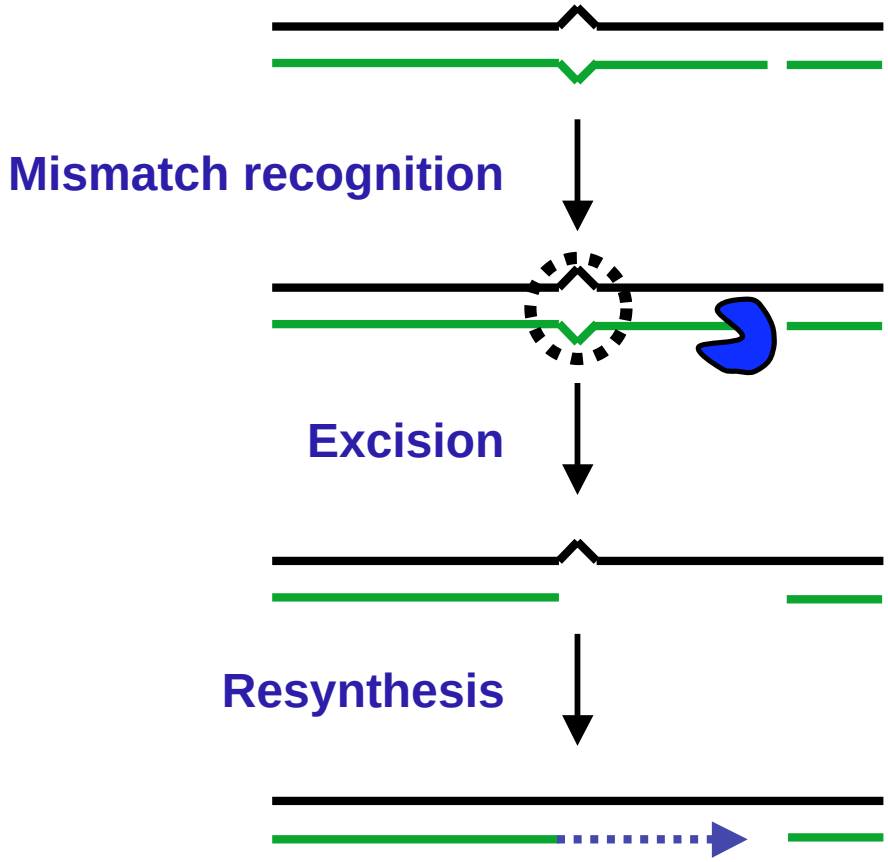
1. **Co-IP with MSH2 and MLH1**
2. **Involved in both the steps of excision and resynthesis in MMR**
3. **Differentially required for 3' and 5' directed MMR**

Yuan et al., J. Biol. Chem. 279, 20935-20940, 2004
Guo et al., J. Biol. Chem. 279, 16912-7, 2004.
Gu et al., Nucleic Acids Res. 26, 1173-1178, 1998

Outlines

- **Identification and characterization of mismatch repair components**
  - **RPA**
  - **HMGB1**
  - **PCNA**
- **Reconstitution of the mismatch repair reaction**

Unsolved Fundamental Problems



How does mismatch recognition by MutS homologs trigger the downstream repair events?

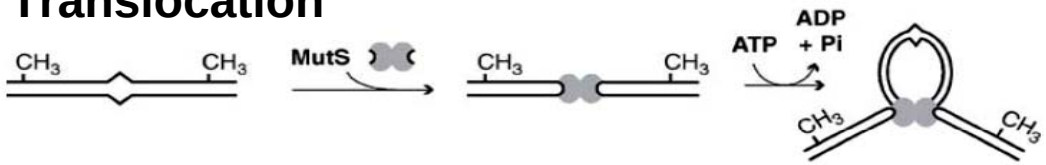
How is mismatch-provoked excision terminated upon mismatch removal?

Initiation Models

# of Protein complex

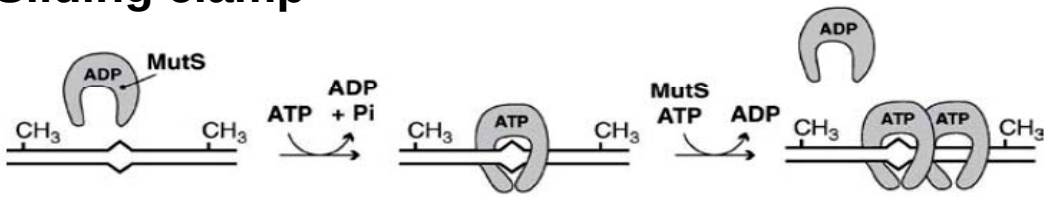
Moving or Cis

Translocation



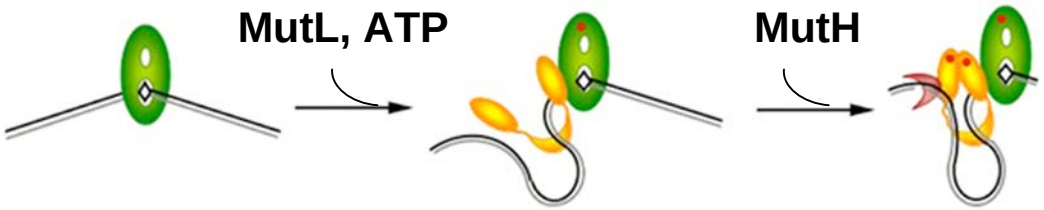
1

Sliding clamp



More than 1

Stationary or Trans



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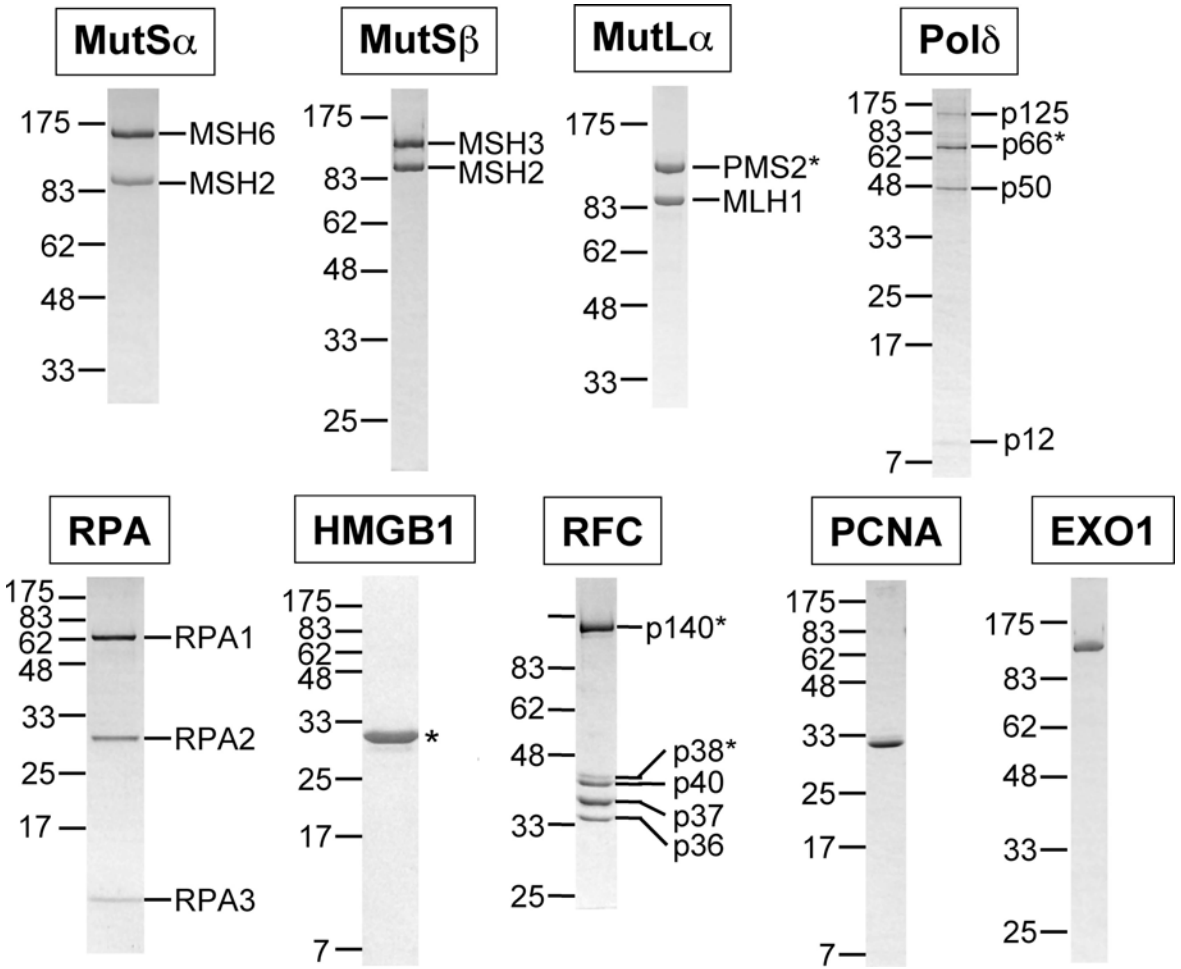
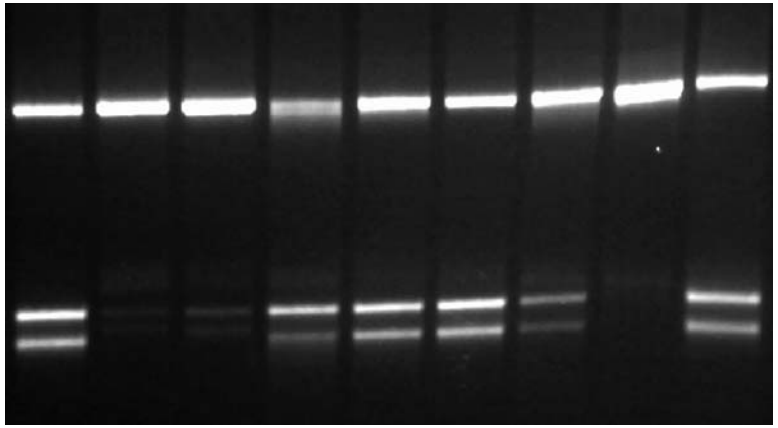
Purification of MMR Proteins



Reconstitution of MMR Reaction

+	+	+	+	+	+	+	–	+	Pol δ
+	+	+	+	+	+	+	+	–	PCNA
+	+	+	+	+	+	+	+	–	RFC
+	+	+	+	+	–	–	+	+	HMGB1
+	+	+	+	–	+	–	+	+	RPA
+	+	+	–	+	+	+	+	+	MutLα
+	+	–	+	+	+	+	+	+	MutSα
+	–	+	+	+	+	+	+	+	EXO1



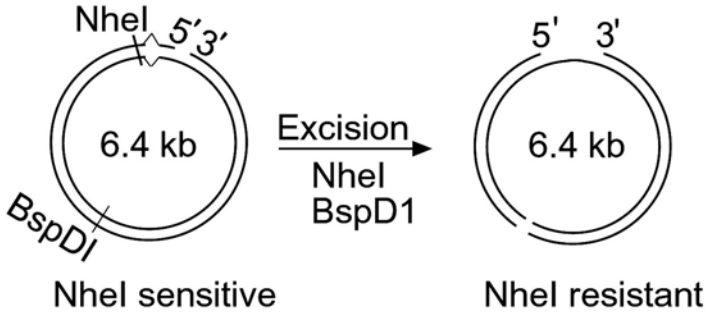
3.3 kb
3.1 kb

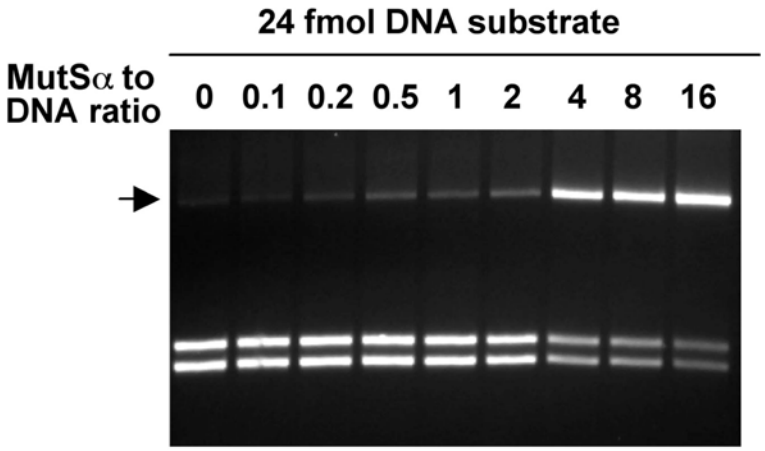
Stoichiometry of MMR Reaction

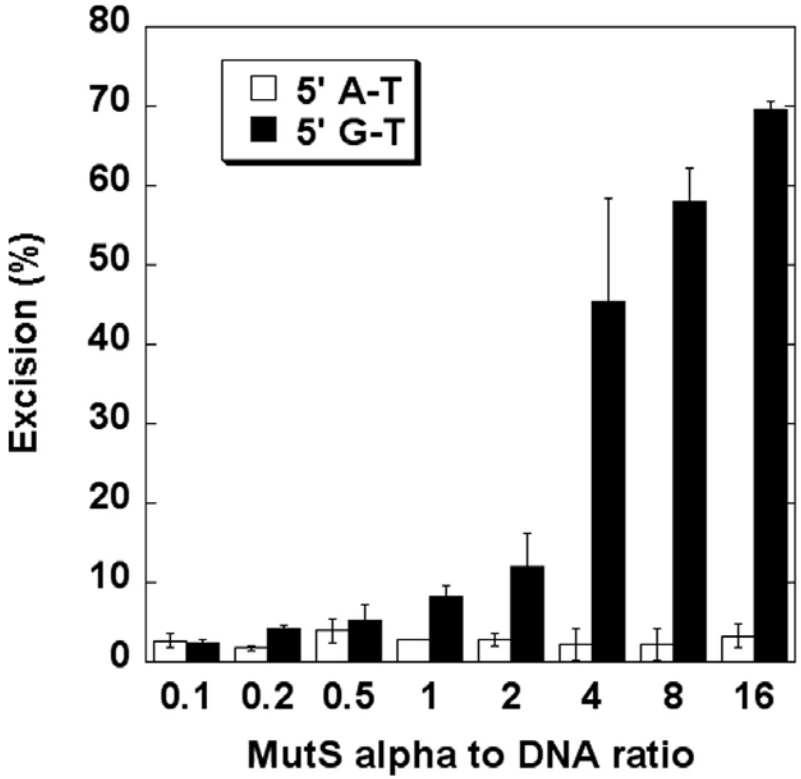
- **Protein concentrations were based on the amount of individual proteins in 50 μg of HeLa nuclear extracts:**
  - **400 fmol MutSα**
  - **300 fmol MutLα**
  - **800 fmol RPA**
  - **1.2 pmol HMGB1**
  - **5 fmol EXO1**

- **24 fmol heteroduplexes**

Multiple MutSα/MutLα Proteins Are Required for Repair of a Single Mismatch



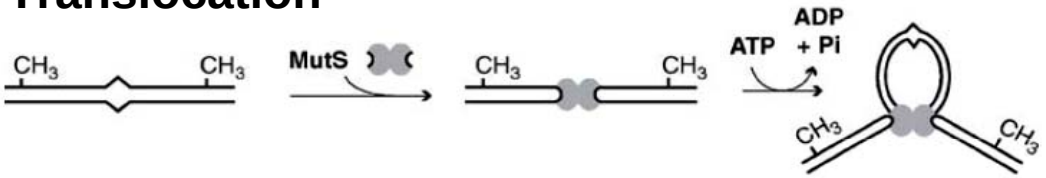




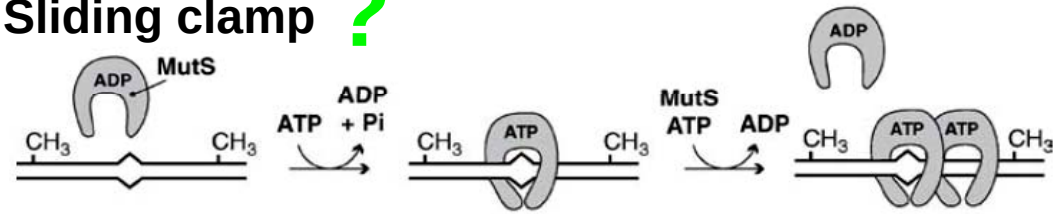
Initiation Models

Moving

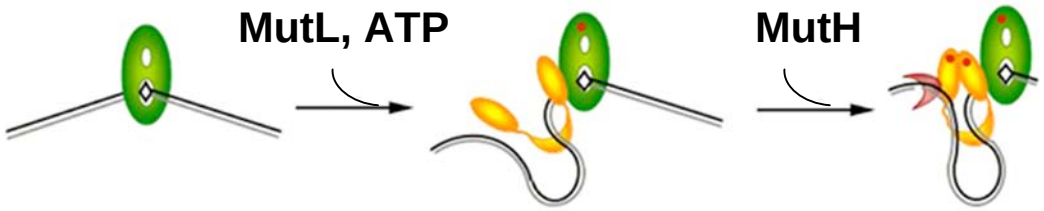
Translocation



Sliding clamp ?

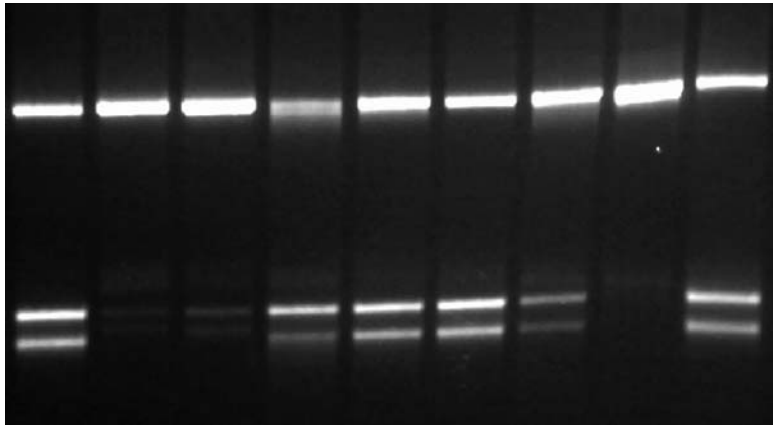


Stationary



Role of MutLα in MMR

+	+	+	+	+	+	+	–	+	Pol δ
+	+	+	+	+	+	+	+	–	PCNA
+	+	+	+	+	+	+	+	–	RFC
+	+	+	+	+	–	–	+	+	HMGB1
+	+	+	+	–	+	–	+	+	RPA
+	+	+	–	+	+	+	+	+	MutLα
+	+	–	+	+	+	+	+	+	MutSα
+	–	+	+	+	+	+	+	+	EXO1



3.3 kb
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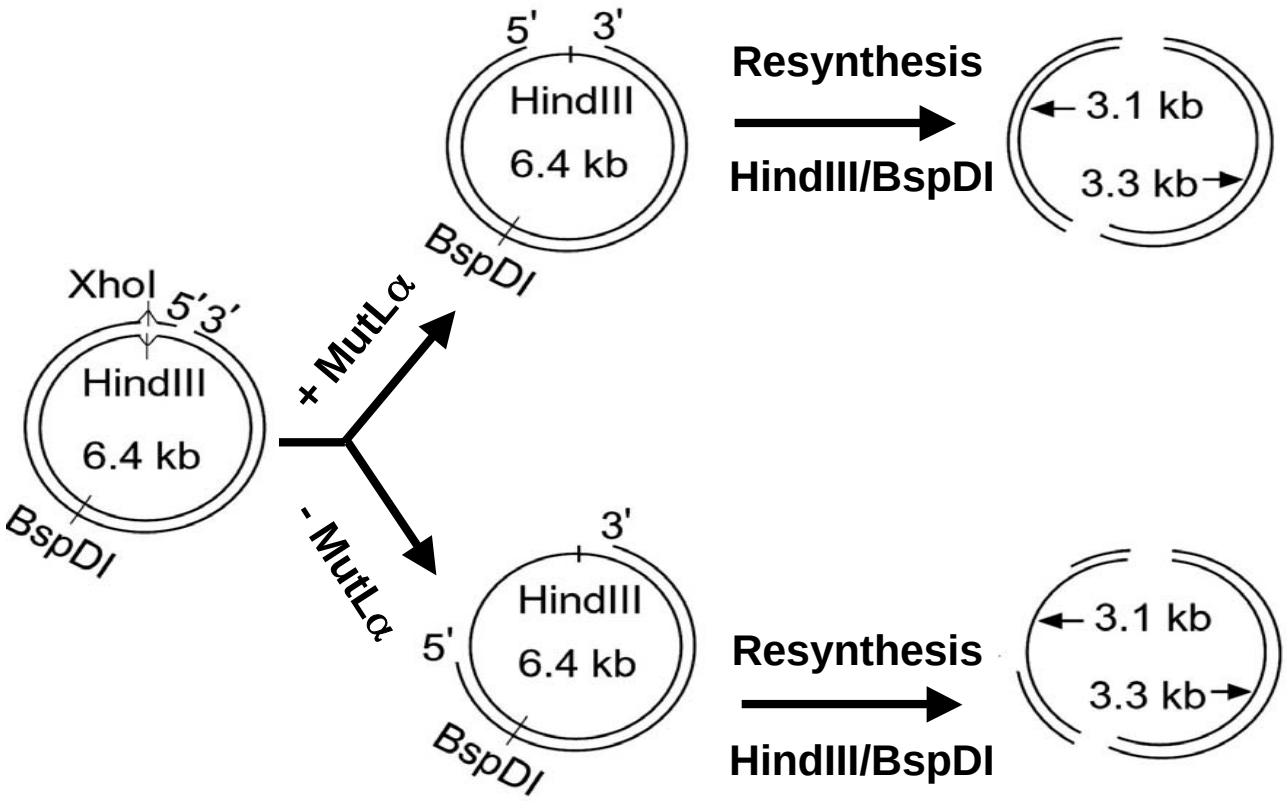
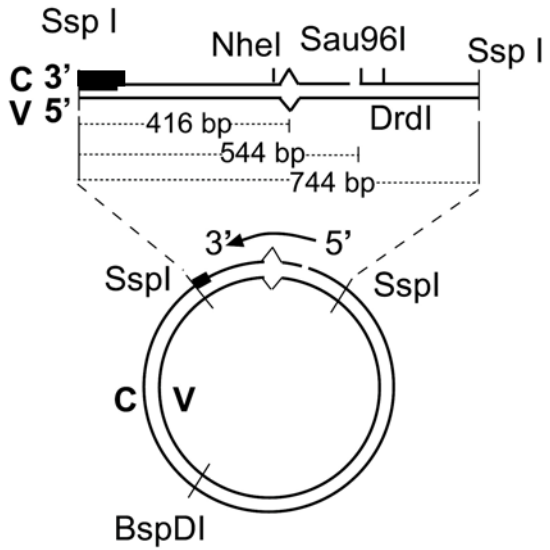
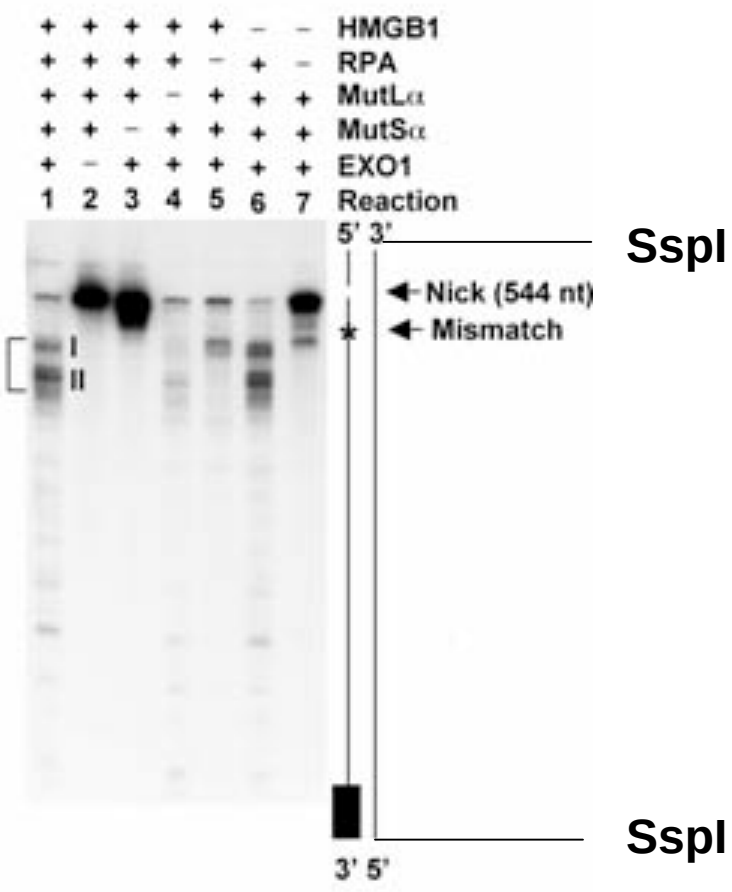
Role of MutLα in MMR

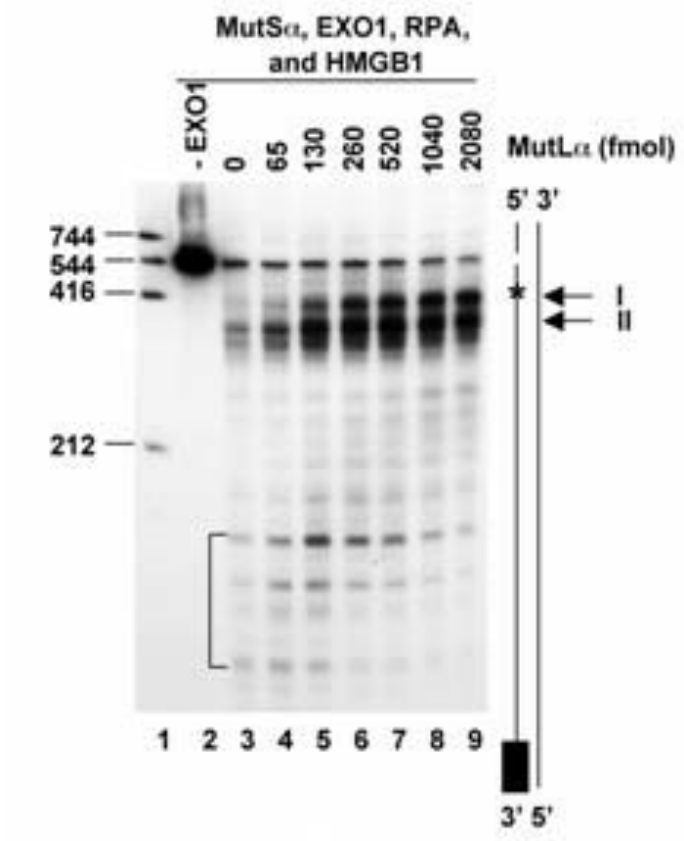


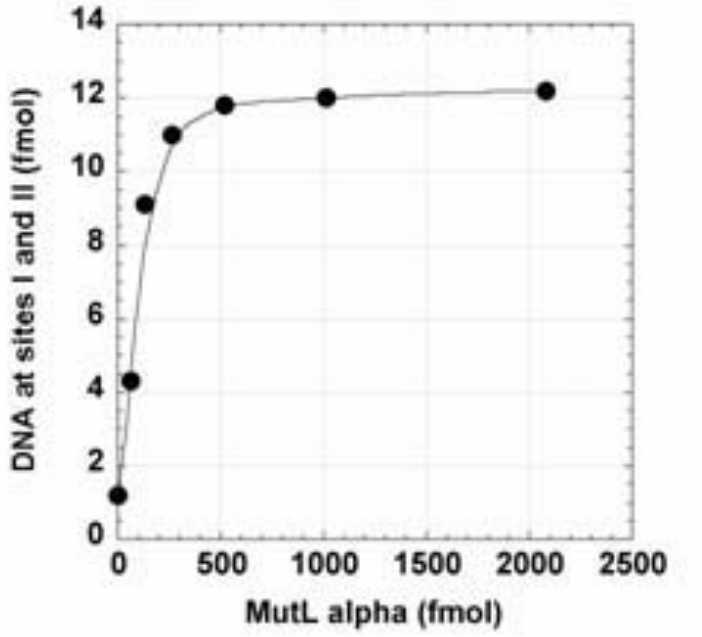
Extensive Excision without MutLα



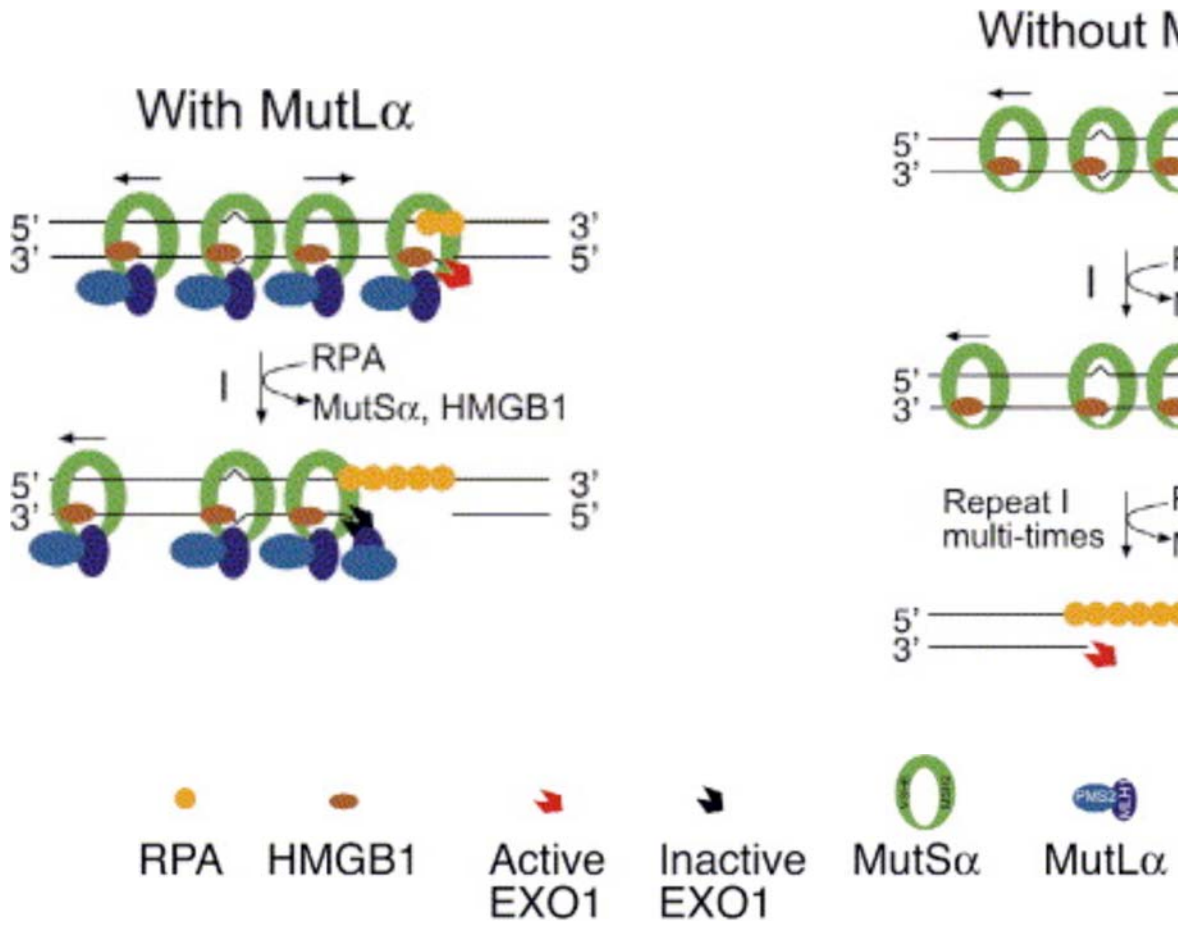


MutLα Terminates Excision upon Mismatch Removal





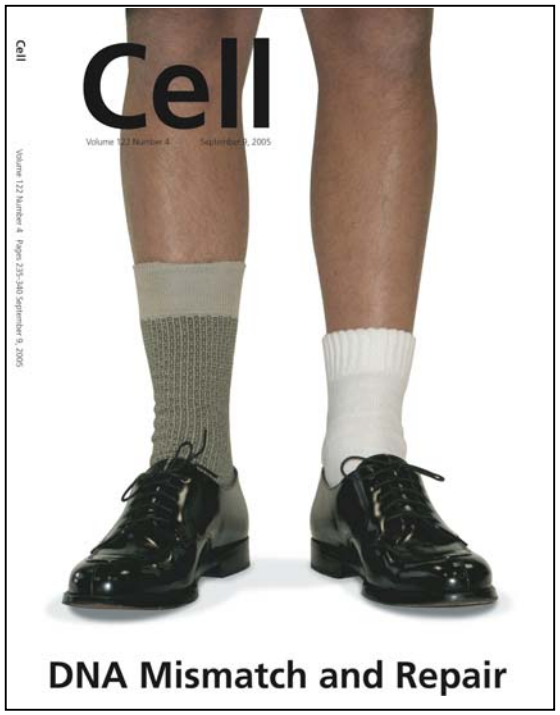
Model for Mismatch-Provoked Excision



Summary

- **Reconstituted the 5' nick-directed mismatch repair using 10 purified human proteins (MutSα, MutSβ, MutLα, EXO1, RPA, HMGB1, pol δ, PCNA, RFC, and DNA ligase I).**
- **MutLα acts to terminate mismatch-provoked excision upon mismatch removal.**
- **More than one MutSα-MutLα complex is required for the repair of a single mismatch**.

Reconstitution of Human MMR



Zhang et al., Cell, 122, 693-705, 2005

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