Mechanisms in *E. coli* & human mismatch repair

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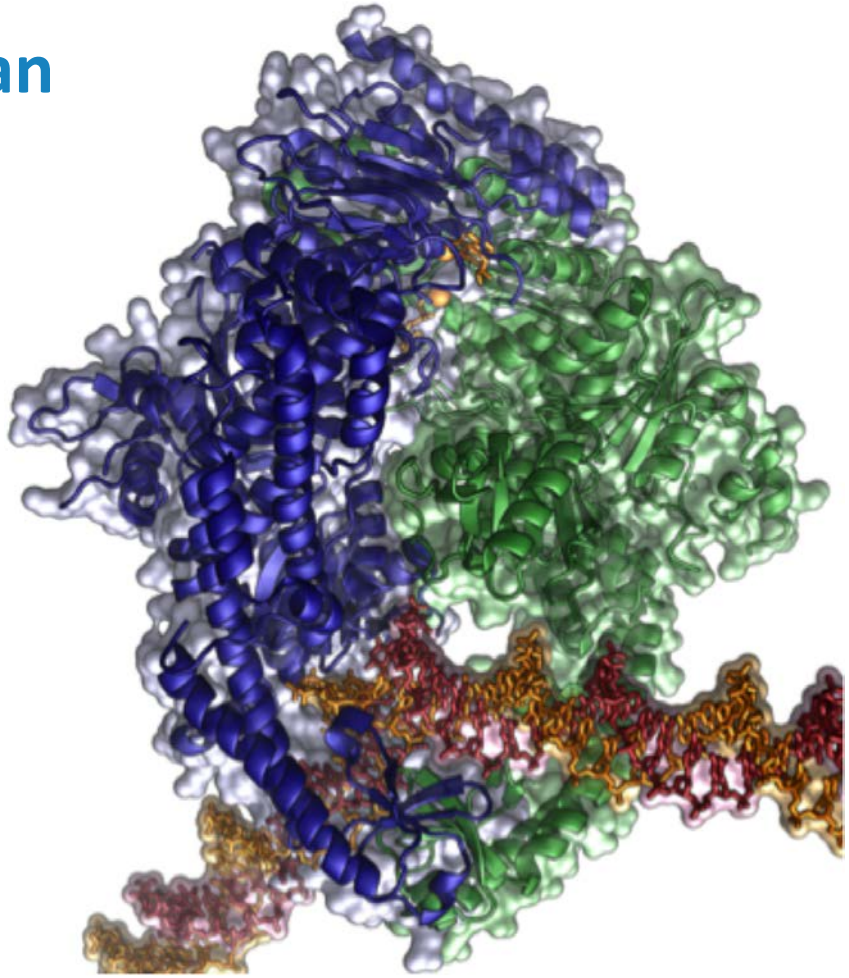
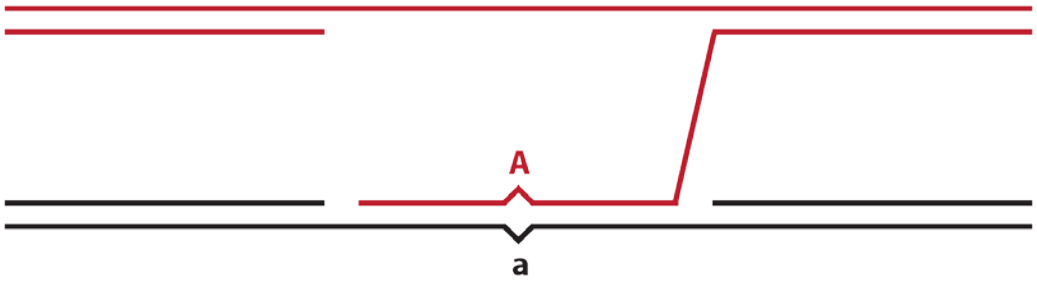


human MutSα•heteroduplex complex
image courtesy of Lorena Beese

Early history of mismatch repair: genetic recombination



Robin Holliday (1964) Genet. Res. Camb. 5, 282-304

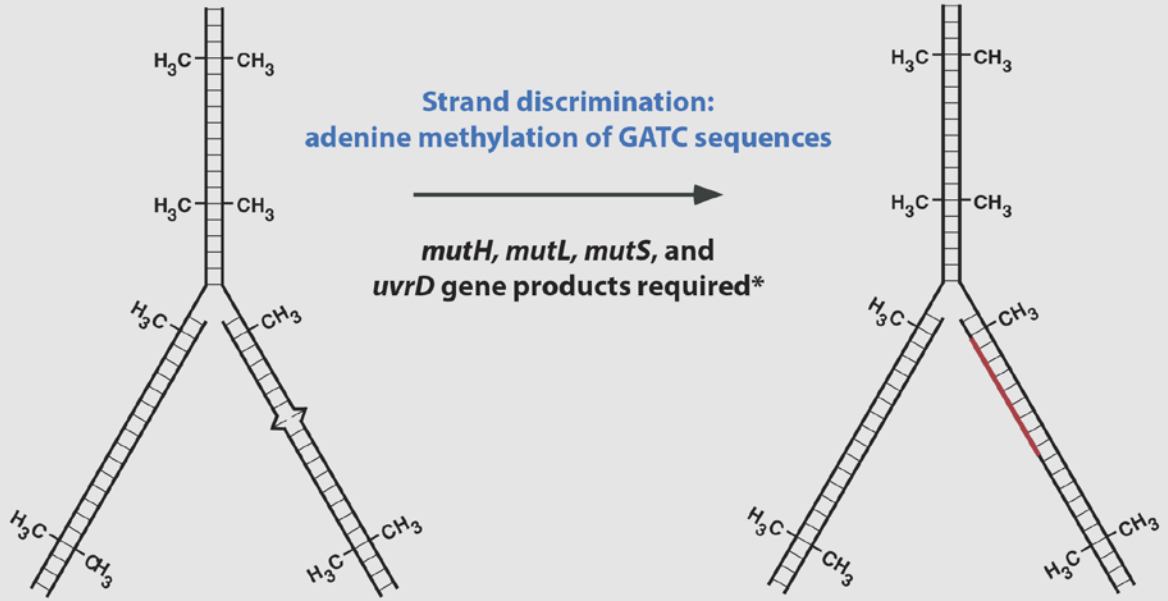
"If there are enzymes which can repair points of damage in DNA, it would seem possible that the same enzymes could recognize the abnormality of base pairing, and by exchange reactions rectify this."

Early history of mismatch repair: *E. coli* replication fidelity & mutation avoidance

R Wagner, Jr. and M Meselson (1976) Proc. Natl. Acad. Sci. USA 73, 4135-4139

"....mismatch repair may act to correct mutations that arise as replication errors. If so, it may be that mismatch repair acts in a directed manner in conjunction with sister chromatid exhange or that it occurs with particularly high efficiency on newly synthesized DNA strands, possibly because of their undermethylation or because of a special relation to the replication complex."

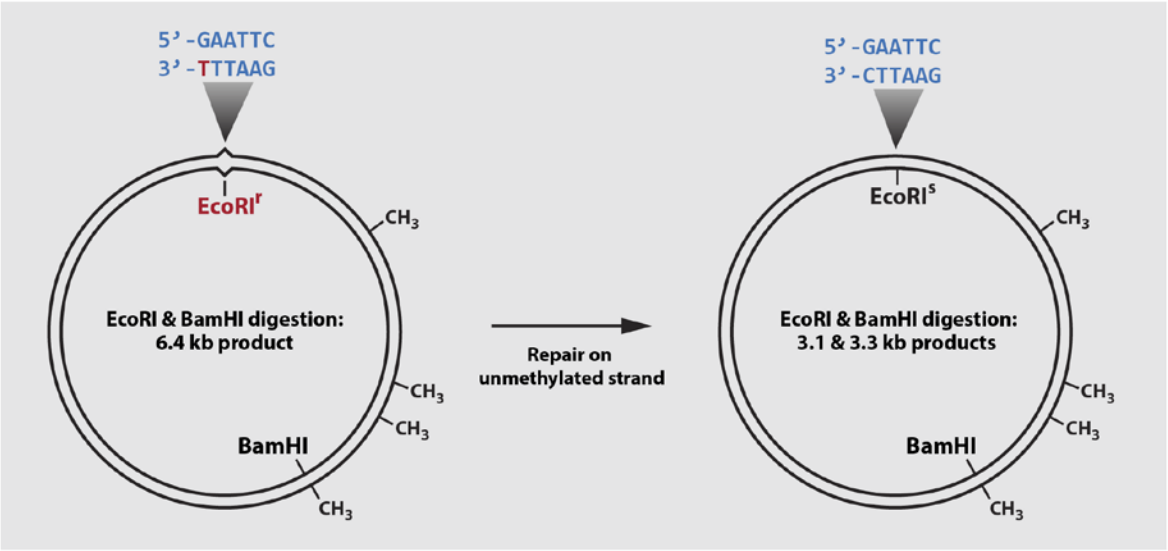
PJ Pukkila et al (1983) Genetics 104, 571-582
BW Glickman and M Radman (1980) Proc. Natl. Acad. Sci. USA 77, 1063-1067



*inactivation of *mutH, mutL, mutS* or *uvrD* increases the mutation rate 50- to 100-fold

Methyl-directed repair of DNA base-pair mismatches *in vitro*

A-L Lu et al (1983, 1984)



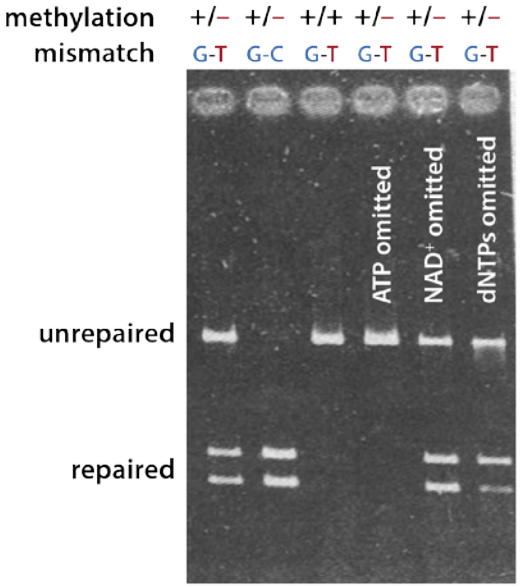


FIG. 2. Mismatch repair *in vitro*.

Table 1. Extracts of Mutator Strains Are Defective in Mismatch Repair

Source of fraction I	*Eco*RI sites repaired (fmoles/hr·mg protein)
KMBL 3752 (*mut*$^+$)	42 ± 9
KMBL 3773 (*mutH101*)	4
KMBL 3775 (*mutS101*)	< 4
KMBL 3789 (*uvrE502*)	< 4
KMBL 3774 (*mutL101*)	< 4
D6432 (*mutL*::Tn*10*)	< 4
mutH101 + *mutS101*	45
mutH101 + *uvrE502*	42
mutS101 + *uvrE502*	36
mutH101 + *mutL101*	11
mutH101 + *mutL*::Tn*10*	36
mutS101 + *mutL101*	21
mutS101 + *mutL*::Tn*10*	50
uvrE502 + *mutL101*	10
uvrE502 + *mutL*::Tn*10*	42

Features of methyl-directed mismatch repair in *E. coli* extracts

Mismatch specificity

- *repairs all base-base mismatches except C-C*
- *repair of each depends on mutH, mutL, & mutS gene products*

S-S Su et al (1988)

GATC site involvement

- *at least one hemimethylated (or unmethylated) GATC sequence required*
- *GATC site can direct repair of a mismatch several kb distant, but proximal sites are more effective*
- *repair of unmethylated DNA occurs on either strand*

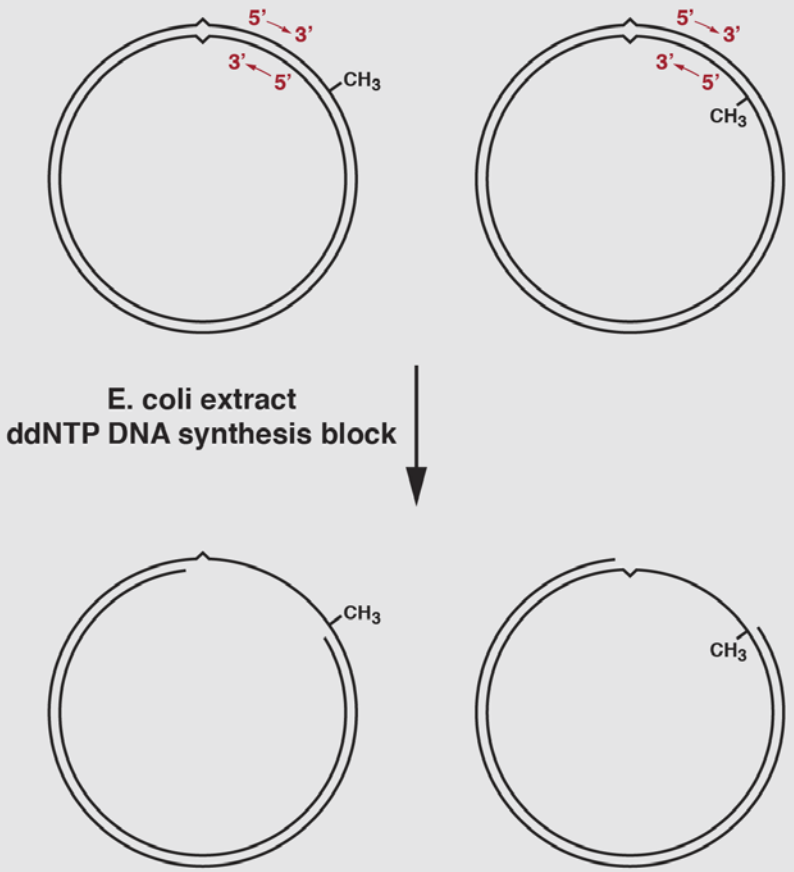
RS Lahue et al (1987)

Repair DNA synthesis

- *repair associated with DNA synthesis that is strongly biased to unmethylated strand*

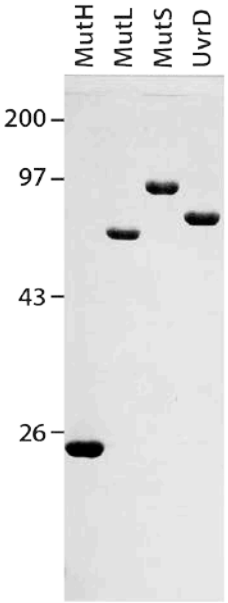
A-L Lu et al (1983, 1984)

Methyl-directed MMR supports bidirectional excision



S-S Su et al (1989), M Grilley et al (1993)
Collaboration with Jack Griffith, UNC

MutH, MutL, MutS, and UvrD proteins



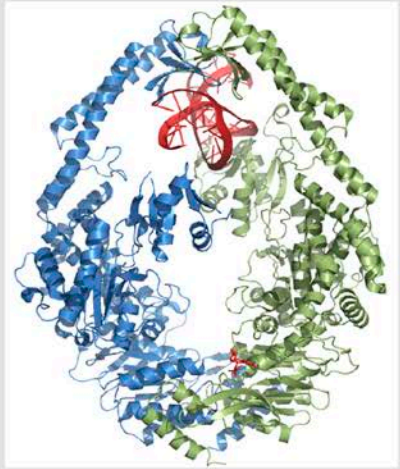
MutS

Recognizes mismatched base pairs

S-S Su et al (1986, 1988)

Apparent affinities of mutS protein for base pair mismatches

Mismatch	Apparent dissociation constant
	nM
G-T	39 ± 4
A-C	53 ± 4
A-A	110 ± 7
T-T	140 ± 9
G-G	150 ± 10
A-G	270 ± 30
C-T	370 ± 40
C-C	480 ± 50



MH Lamers et al (2000)
courtesy of Titia Sixma

UvrD

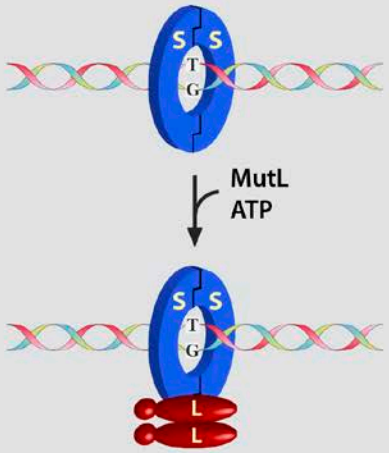
DNA helicase II - unwinds helix in ATP-dependent fashion by tracking 3′ to 5′ along the strand to which it is bound

ID Hickson et al (1983)

MutL

Recruited to MutS-mismatch complex in an ATP-dependent fashion

M Grilley et al (1989)



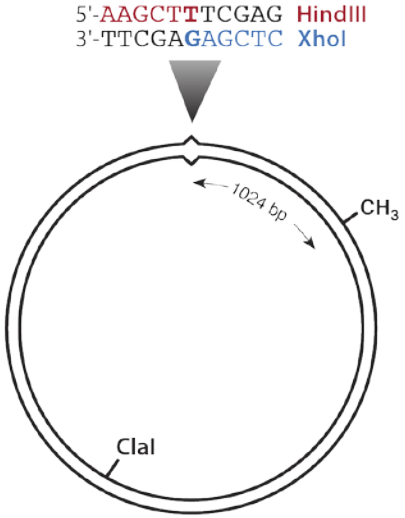
MutH

Has tightly associated, but nearly dead endonuclease (< 1 turnover/hr) specific for unmethylated GATC sequences

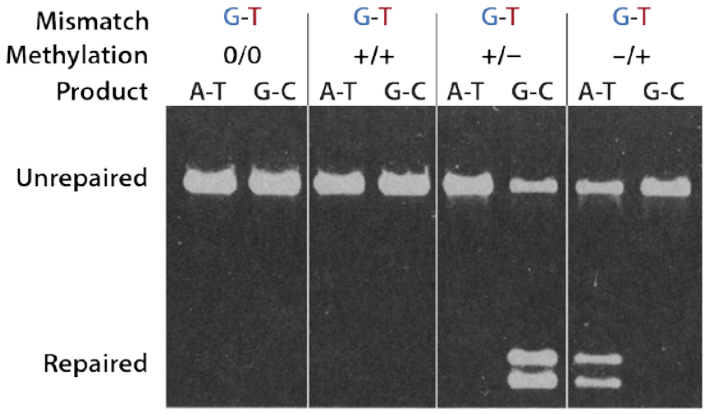
KM Welsh et al (1987)

Methyl-directed mismatch repair in a defined system

RS Lahue et al (1989): MutH, MutL, MutS, UvrD (DNA helicase II), ExoI, SSB, DNA polymerase III holoenzyme & ligase are "sufficient" to reconstitute methyl-directed repair in a purified system



Reaction conditions	Repair (fmol/20 min)
Complete	15
Minus MutH	<1
Minus MutL	<1
Minus MutS	<1
Minus DNA polymerase III holoenzyme	<1
Minus SSB	2
Minus exonuclease I	2
Minus DNA helicase II	16
Minus helicase II, plus immune serum	<1
Minus helicase II, plus preimmune serum	14
Minus ligase/NAD^+	14
Minus $MgCl_2$	<1
Minus ATP	<1
Minus dNTP's	<1



Corrects all base-base mismatches except C-C

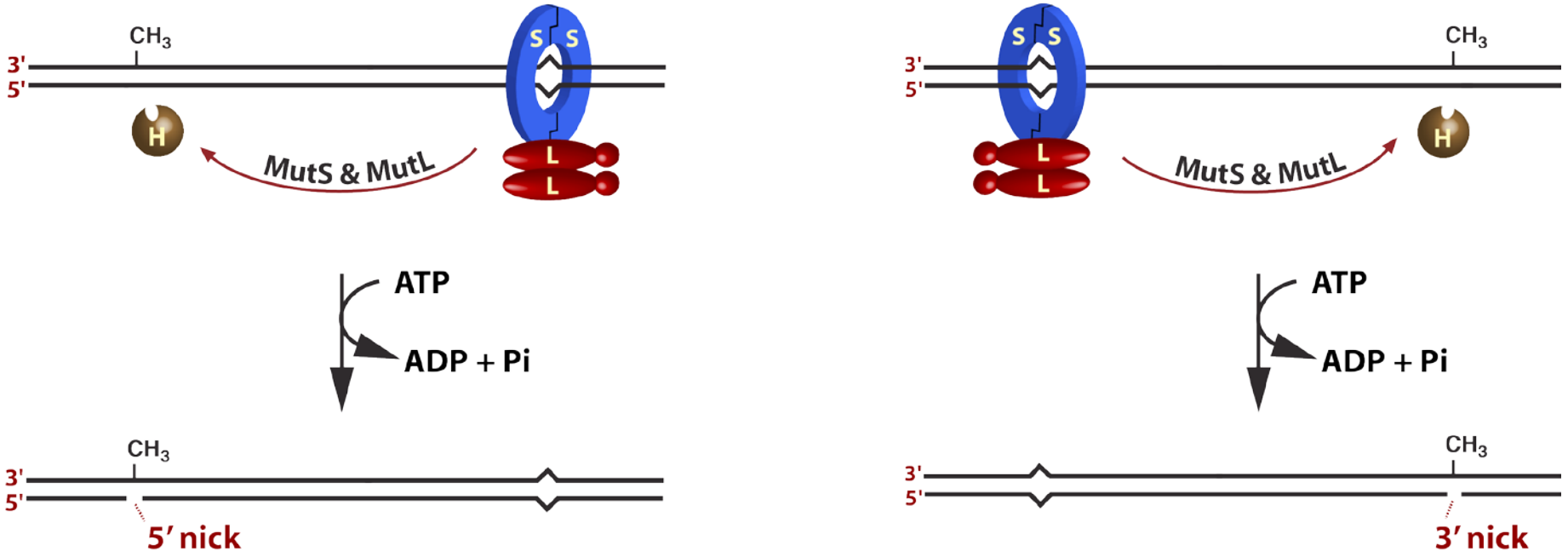
The exonuclease question

- *5'-directed repair requires a 5' to 3' single-strand exonuclease: ExoVII or RecJ exo*
- *3'-directed repair requires a 3' to 5' single-strand exonuclease: ExoI or ExoX* (ExoVII may play a very minor role)*
- *genetic inactivation of ExoI, ExoVII, ExoX & RecJ leads to mismatch-, MutH-, MutL-, MutS-, & UvrD-dependent cell death*

DL Cooper et al (1988), M Viswanathan et al (2001), V Burdett et al (2001)
*Collaboration with S Lovett

Initiation of methyl-directed repair: MutH GATC endonuclease is activated in a mismatch-, MutS-, and MutL-dependent fashion

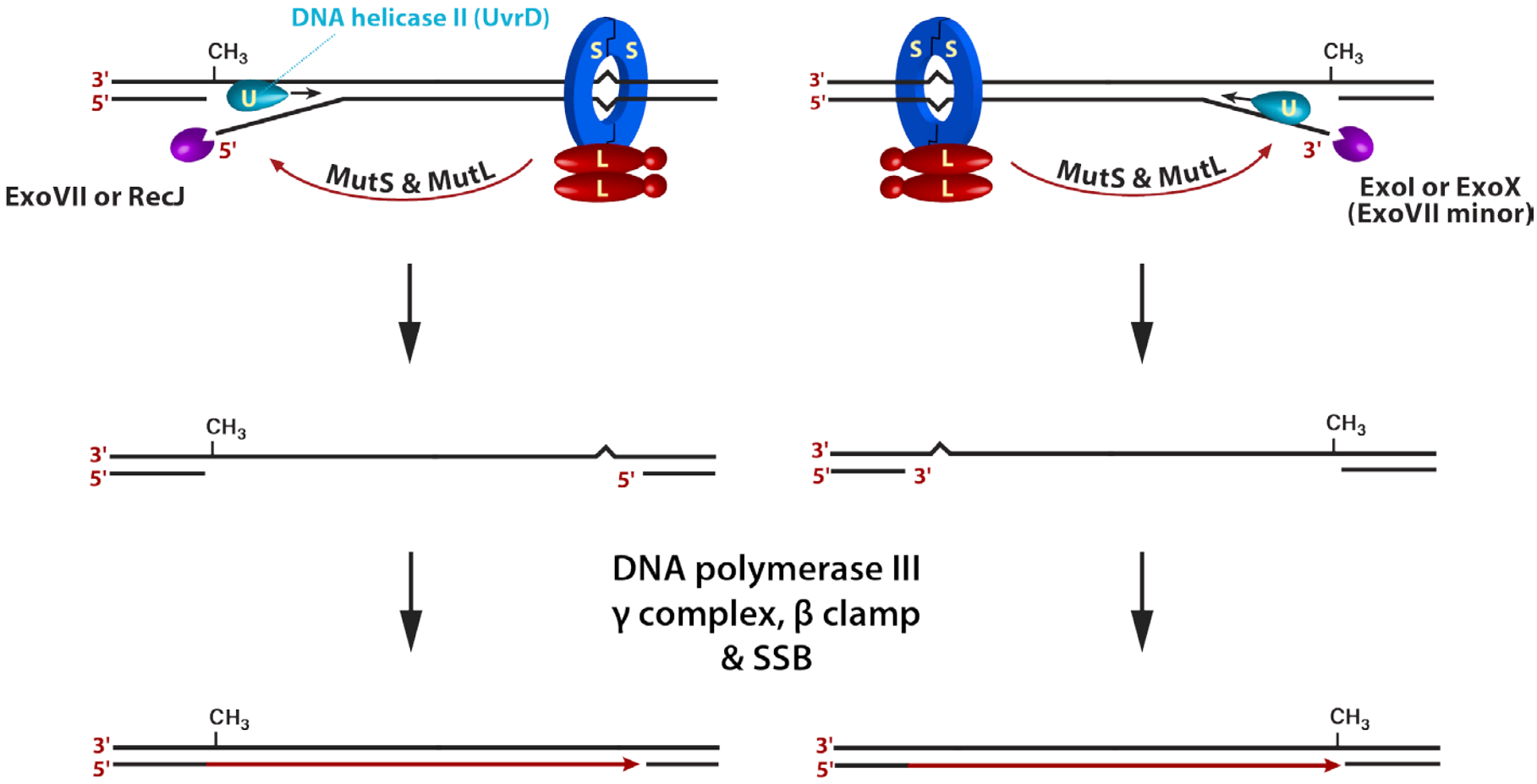
KG Au et al (1992)



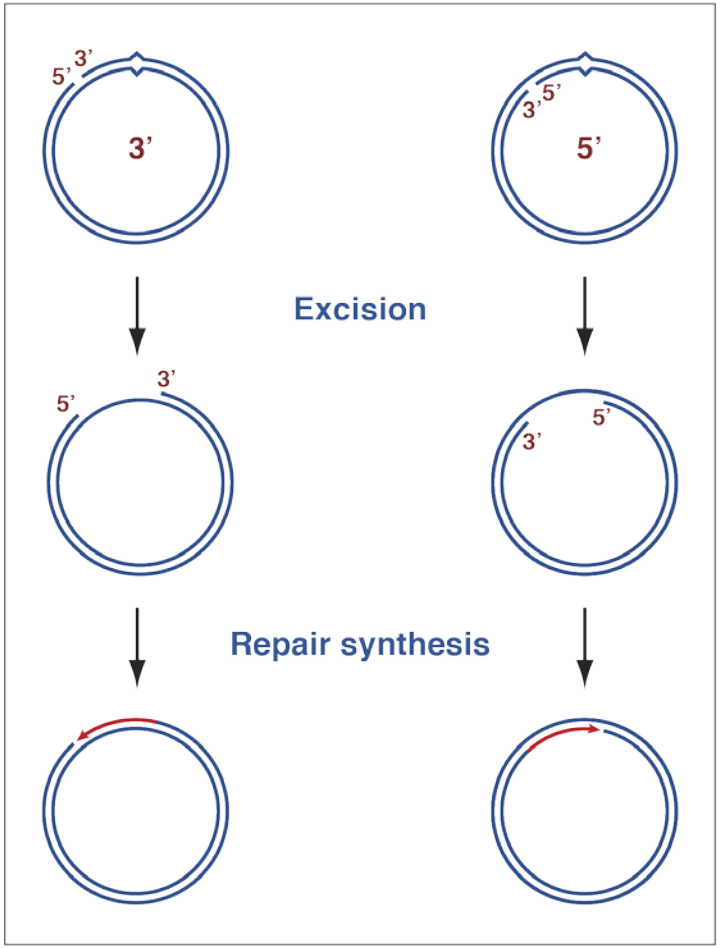
- Nick introduced 5′ or 3′ to mismatch on unmethylated strand
- Nick is the actual signal that directs repair

MutS, MutL and a mismatch activate orientation-dependent excision at the MutH strand break

V Dao et al (1998), M Yamaguchi et al (1998), A Pluciennik et al (2009)

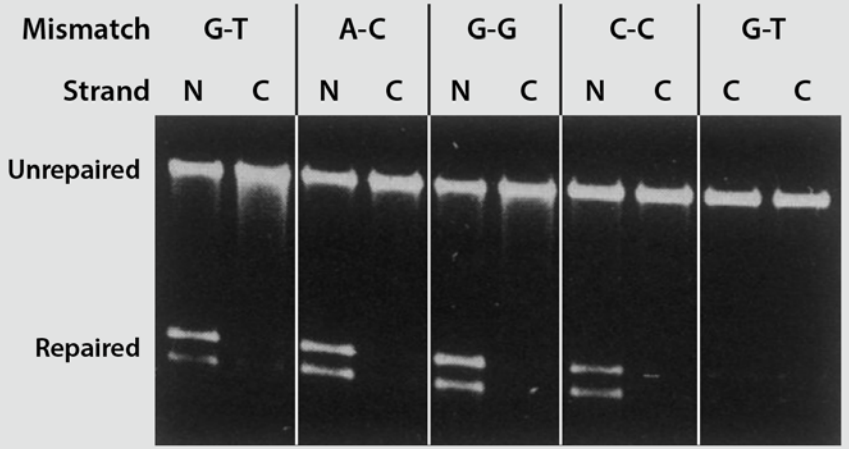


Nick-directed mismatch repair in human cell extracts



Mismatch repair in HeLa cell nuclear extract

J Holmes et al (1990)



Human mismatch repair is bidirectional

W-h Fang et al (1993)

- *the nick that directs repair can be located 3′ or 5′ to the mismatch*
- *excision tract endpoints indicate that DNA hydrolytic events are restricted to the shorter path in the circular heteroduplex*
- *the nick-mismatch separation distance can be as much as 1000 bp*

Microsatellite instability in tumor cells

LA Aaltonen et al (1993), Y Ionov et al (1993)

Frequent mutations in $(CA)_n$ and $(A)_n$ microsatellite repeat sequences are a characteristic of:

- tumors in Lynch syndrome patients
  (colon, endometrial, ovarian & gastric cancers;
  accounts for ≈ 5% of total colon cancer burden)

- ≈ 15% of Lynch-like sporadic cancers

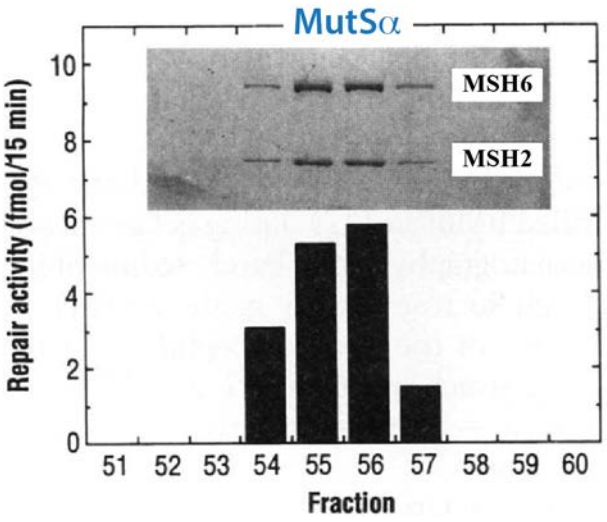
Such mutations are also common in *E. coli* mismatch repair mutants (Levinson and Gutman (1987))

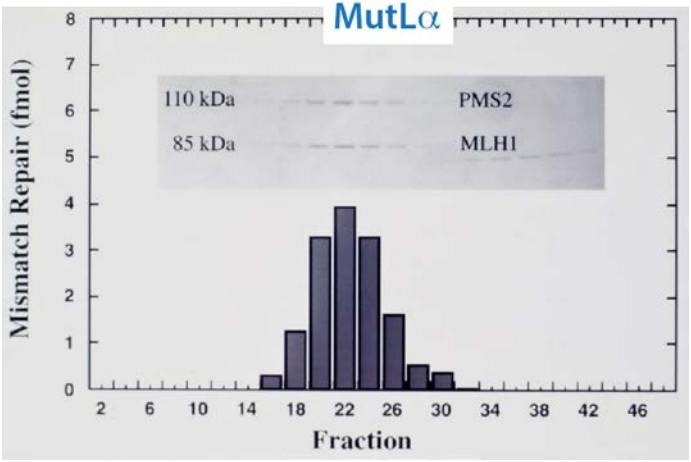
Cell lines derived from tumors with microsatellite instability are deficient in MutLα or MutSα

R Parsons et al (1993), J Drummond et al (1995), G-M Li et al (1995), ML Veigl et al (1998)

Tumor cell line	Microsatellite instability	3' G-T heteroduplex repair (fmol)	Defect
HeLa (cervix)	No	9	--
SW480 (colon)	No	13	--
Vaco 410 (colon)	No	7.4	--
LoVo (colon)	Yes	< 0.3	MutSα
HCT-15 (colon)	Yes	< 0.3	MutSα
HEC-59 (endometrial)	Yes	< 0.3	MutSα
HCT 116 (colon)	Yes	< 0.3	MutLα
Vaco 481 (colon)	Yes	< 0.3	MutLα
RKO (colon)*	Yes	< 0.3	MutLα
Vaco 5 (colon)*	Yes	0.4	MutLα
Vaco 6 (colon)*	Yes	< 0.3	MutLα
AN3CA (endometrial)*	Yes	< 0.3	MutLα

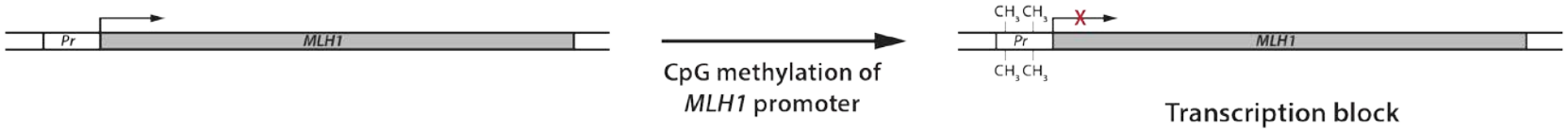
*sporadic cancers with *MLH1* epigenetically silenced



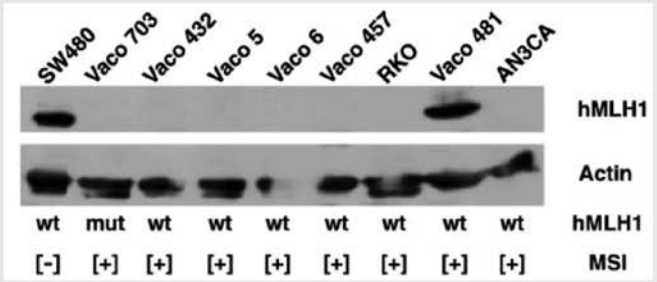


Biallelic epigenetic silencing of the *MLH1* promoter in MSI$^+$ sporadic cancers

ML Veigl et al (1998)

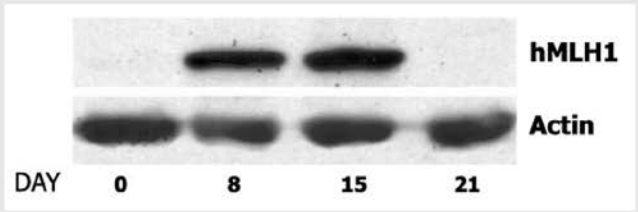


Despite wild type gene sequence, MLH1 absent in MSI$^+$ sporadic cancers

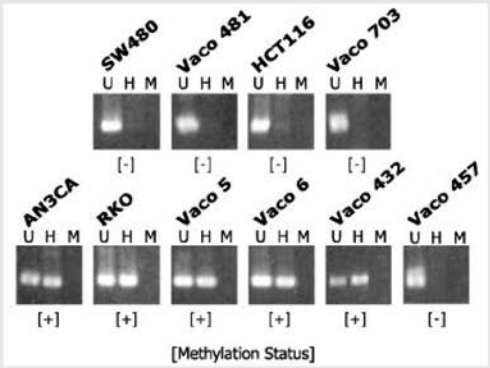


5-azacytidine expsure transiently rescues MLH1 expression in AN3CA cells

24 hr azacytidine exposure on days 1 and 8



Resistance to HpaII (H) cleavage indicates *MLH1* promoter methylation in MSI$^+$ sporadic cancers

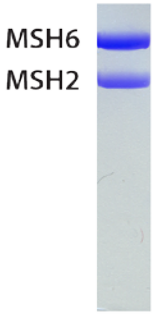


This effect is believed to account for ≈ 15% of colon cancers.

Minimal protein set sufficient to reconstitute human strand-directed mismatch repair *in vitro*

MJ Longley et al (1997), J Genschel et al (1998, 2002, 2009), L Dzantiev (2004), N Constantin et al (2005)

MutSα

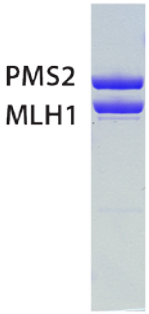


base-base &
1 ≈ 3 extrahelical
I/D mismatches

MutSβ

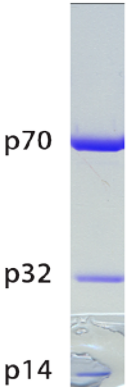
2 ≈ 10 extrahelical
I/D mismatches

MutLα



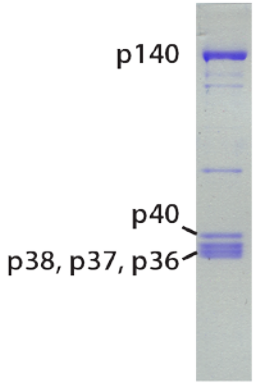
Exo1b

RPA



PCNA

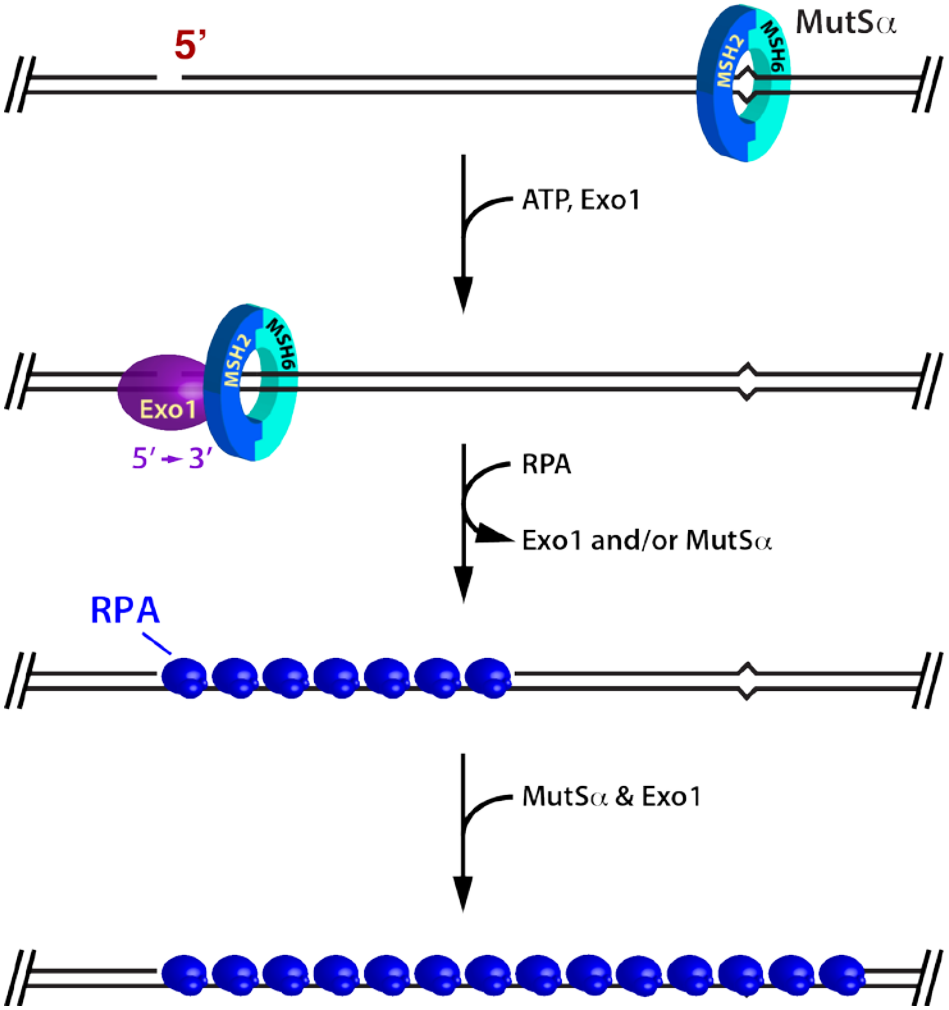
RFC



Pol δ

5′ → 3′ mismatch-provoked excision by MutSα-activated Exo1

J Geschel et al (2002, 2003, 2009), Y Liu et al (2011)

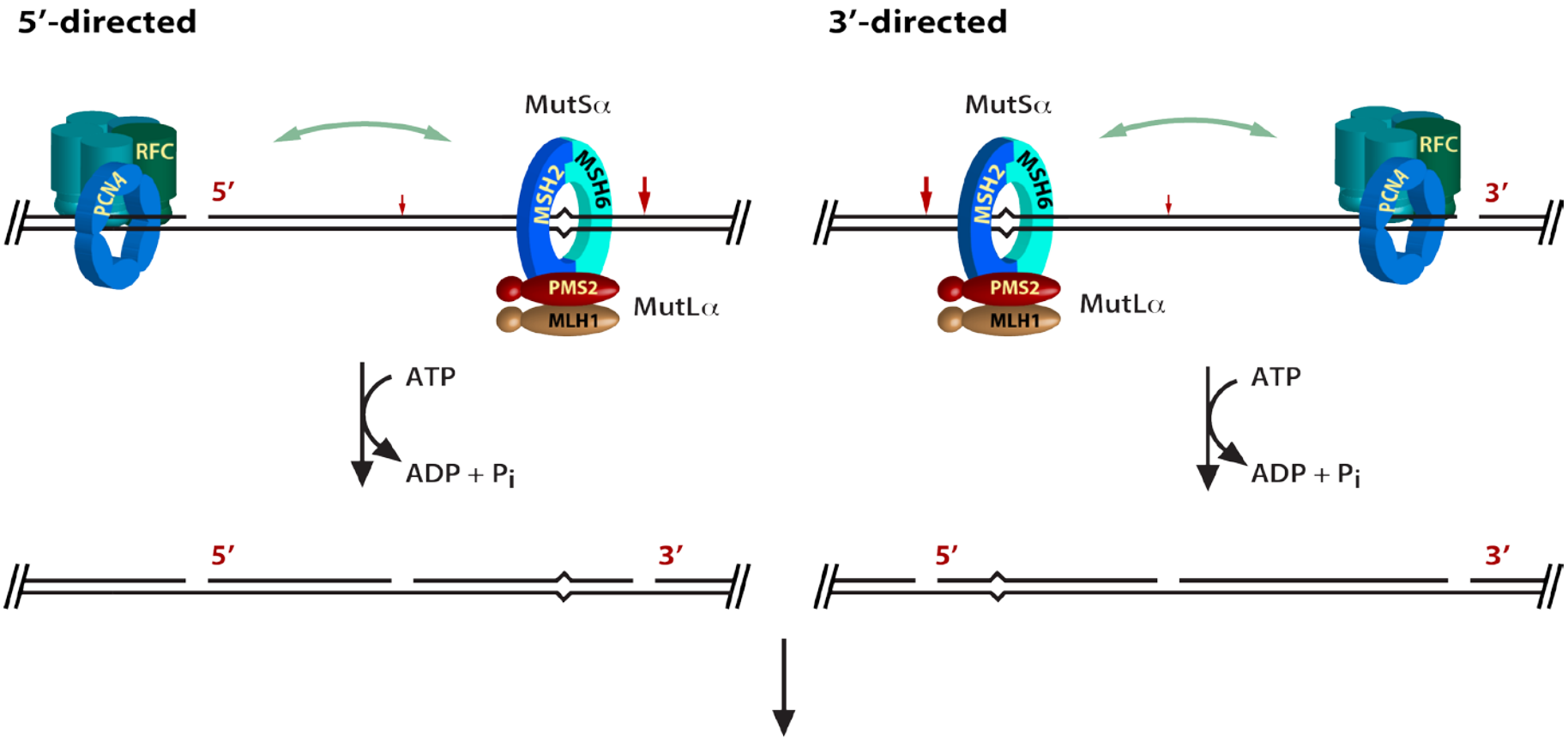


Features of the reaction

- *MutSα renders Exo1 highly processive.*
- *RPA displaces processive complex after removal of about 200 nucleotides.*
- *An RPA-filled gap is a poor substrate for Exo1 loading. Efficient Exo1 reloading requires the mismatch-dependent assistance of MutSα.*
- *Excision greatly attenuated upon mismatch removal.*
- *Although not required for the reaction, MutLa and PARP-1 enhance the mismatch-dependence of excision by suppressing hydrolysis on mismatch-free DNA.*
- *May preferentially function on lagging strand at the fork.* *SE Liberti et al (2013)*

MutLα is a strand-directed endonuclease that requires a mismatch, a strand break, MutSα, RFC, & PCNA for activation

F Kadyrov et al (2006, 2007)

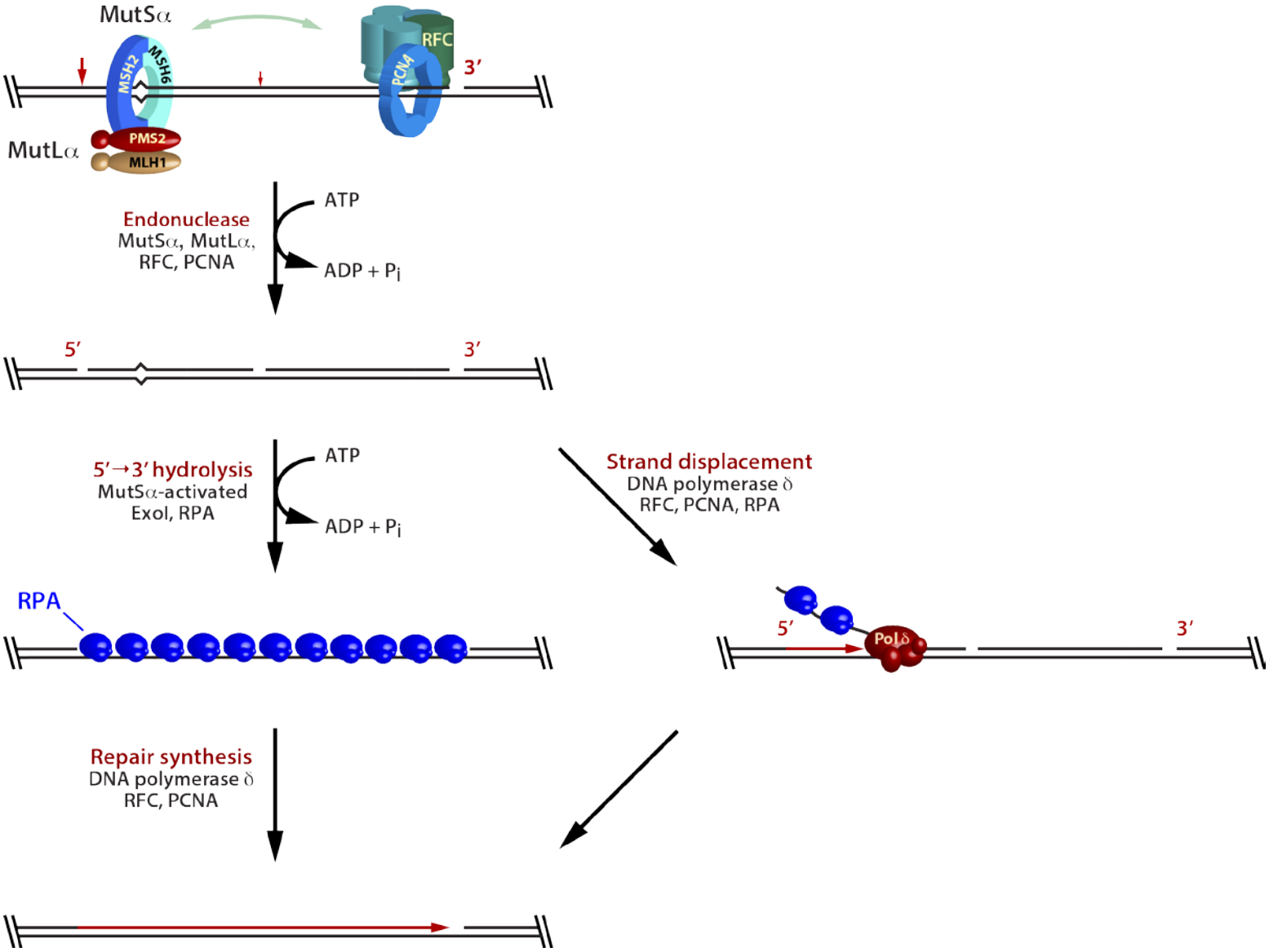


Repair:

- 5'→3' excision by MutSα-activated Exo1; DNA pol δ repair synthesis
- synthesis-driven strand displacement by DNA pol δ

Exo1-dependent & independent modes of mismatch repair

F Kadyrov et al (2006, 2009)



PMS2 metal binding motif DQHA(X)$_2$E(X)$_4$E is required for MutLα endonuclease function and mismatch repair

Conservation of PMS2 metal binding site

			N ↑ K ↑	
H. sapiens	PMS2	696	FIVDQHATDEKYNFEM	711
H. sapiens	MLH3	1220	VLVDQHAAHERIRLEQ	1235
D. melanogaster	PMS2	715	FIVDQHATDEKYNFET	730
C. elegans	PMS2	639	FIVDQHASDEKYNFER	654
A. thaliana	PMS1	725	FIVDQHAADEKFNFEH	740
S. cerevisiae	MLH3	520	VLVDQHACDERIRLEE	535
S. cerevisiae	PMS1	729	FIVDQHASDEKYNFET	744
N. pharaonis	MutL	533	VLIDQHAADERINYER	548
M. mazei	MutL	510	VIIDQHAAHERILYEQ	538
B. subtilis	MutL	459	YIIDQHAAQERIKYEY	474
L. innocua	MutL	418	YIIDQHAAQERIKYEF	450
L. casei	MutL	473	YILDQHAAQERVNYEY	488
S. aureus	MutL	501	YMIDQHAAQERIKYEY	516
T. aquaticus	MutL	360	YIVDQHAAHERILFEE	375
H. sapiens	MLH1	304	DVNVHPTKHEVHFLHE	319
H. sapiens	PMS1	36	GATSVDVKLENYGFDK	51
S. cerevisiae	MLH2	243	IVEENFVIDEKINLDL	258
E. coli	MutL	300	DVNVHPAKHEVRFHQS	315
S. typhimurium	MutL	300	DVNVHPAKHEVRFHQS	315

Amino acid substitution mutants

- Human *in vitro*
  - MutLα endonuclease dead
  - defective in repair

  F Kadyrov et al (2006)
- S. cerevisiae
  - endonuclease dead *in vitro*
  - repair null *in vivo*

  F Kadyrov et al (2007)
  (collaboration with T Kunkel)
- Mouse
  - repair defective *in vivo* & *in vitro*
  - strong cancer predisposition
  - partial defect in class switch recombination

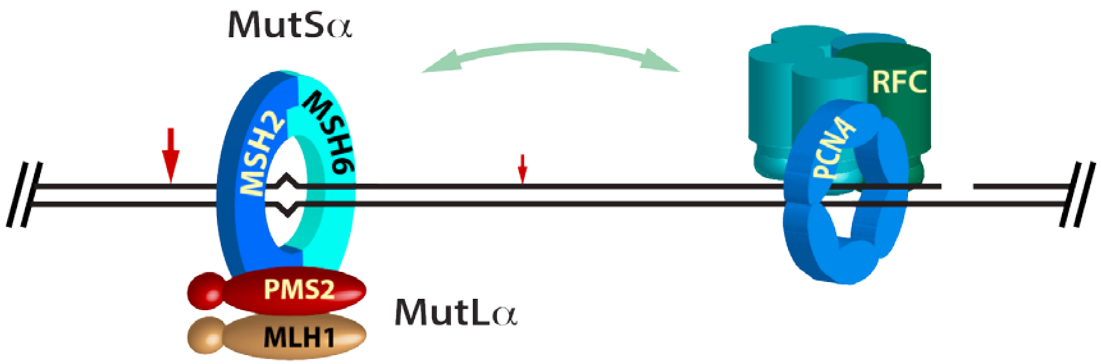
  van Oers et al (2010)
  (collaboration with W Edelmann)

PMS2 substitution mutant properties:

- stable MLH1•PMS2 heterodimers
- fully functional MuLα ATPase
- fully active in MuLα•MutSα•heteroduplex assembly

Activation of MutLα endonuclease - how does it work?

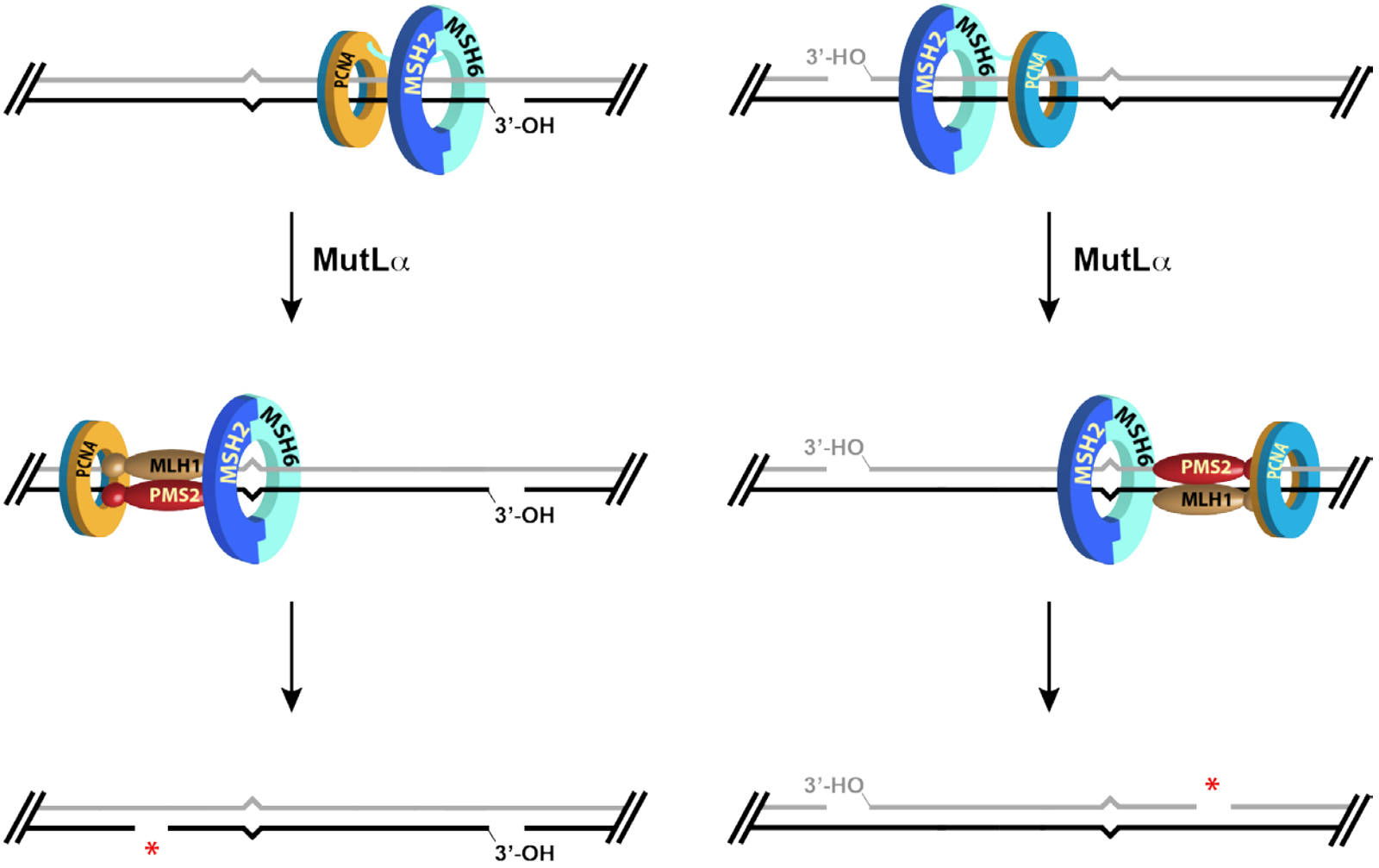
A Pluciennik et al (2010)



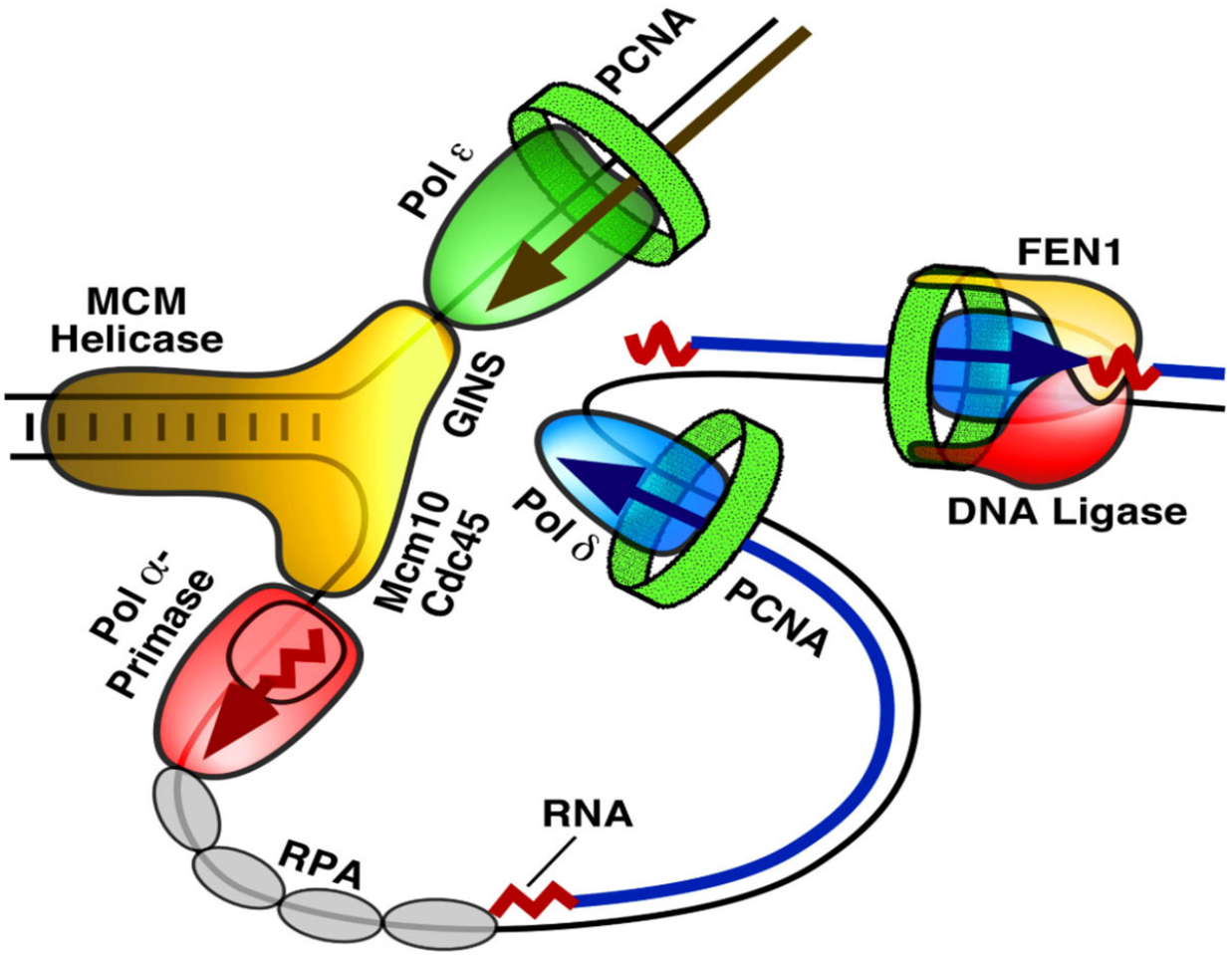
- Strand break provides a PCNA loading site.
- RFC function is restricted to loading of the PCNA clamp onto the helix.
- Physical interaction of MutLα with loaded PCNA is required for activation (1:1 complex in solution).
- Strand direction of MutLα incision is dictated by PCNA loading orientation

Model: MutLα interaction with PCNA orients endonuclease active site with respect to the two DNA strands

A Pluciennik et al (2010, 2013)



Do 5’- and 3’-termini at the fork function as default strand signals to direct mismatch repair?



Graphic courtesy of Peter Burgers

Current and some former laboratory colleagues
Duke, October 12, 2015

In order of appearance

Laboratory Colleagues

Gail Herman
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Robert Lahue
Deani Cooper
Beverly Yashar
Karin Au
Michelle Grilley
Jude Holmes
Woei-horng Fang
Leroy Worth
Dwayne Allen
John Taylor
Guo-Min Li
Miyuki Yamaguchi
Marc Prudhomme
Matthew Longley
Jane Smith
Vivian Dao
Wendy Bedale

James Drummond
Elisabeth Penland
Celia Baitinger
Susan Littman
Dawn Chandrasekhar
Andrew Pierce
Derek Duckett
Jochen Genschel
Leonard Blackwell
Diana Martik
Maynard Bronstein
Greg Runyon
Yizhong Sha
Rochelle Bazemore
Keith Bjornson
Shuntai Wang
Vickers Burdett
Kent Christiansen
Claudia Spampinato
Sihong Chen
Nicoleta Constantin

Leonid Dzantiev
Ravi Iyer
Anna Pluciennik
Sally York
Lored Asllani
Farid Kadyrov
Yanan Fang
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Hongbing Shao
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