

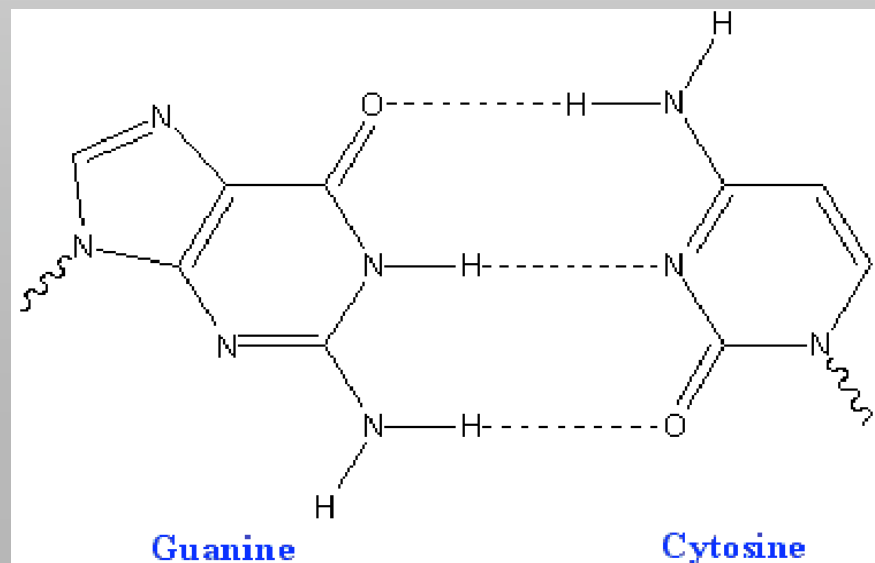
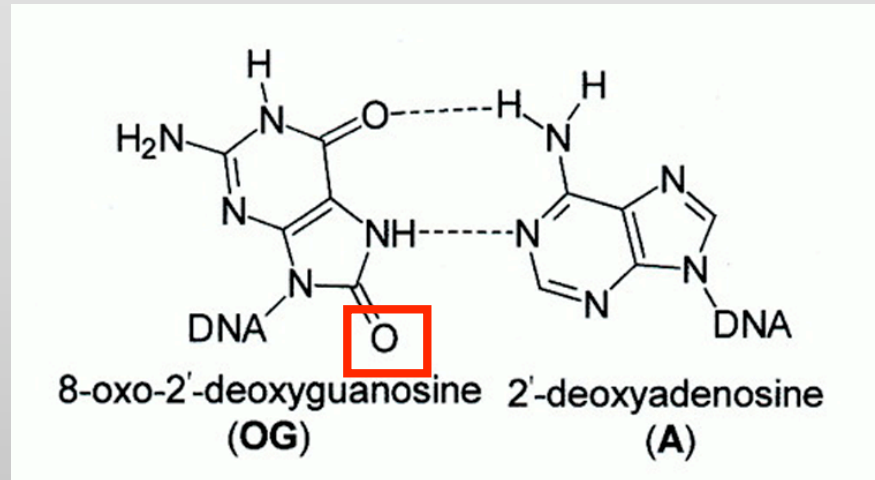
MB Journal Club

4/2/07

Bryan Troxell

Background

- Guanine sensitive to oxidation
- 8-oxoG may result in G:A base pairing and ultimately a transversion event if unrepaired

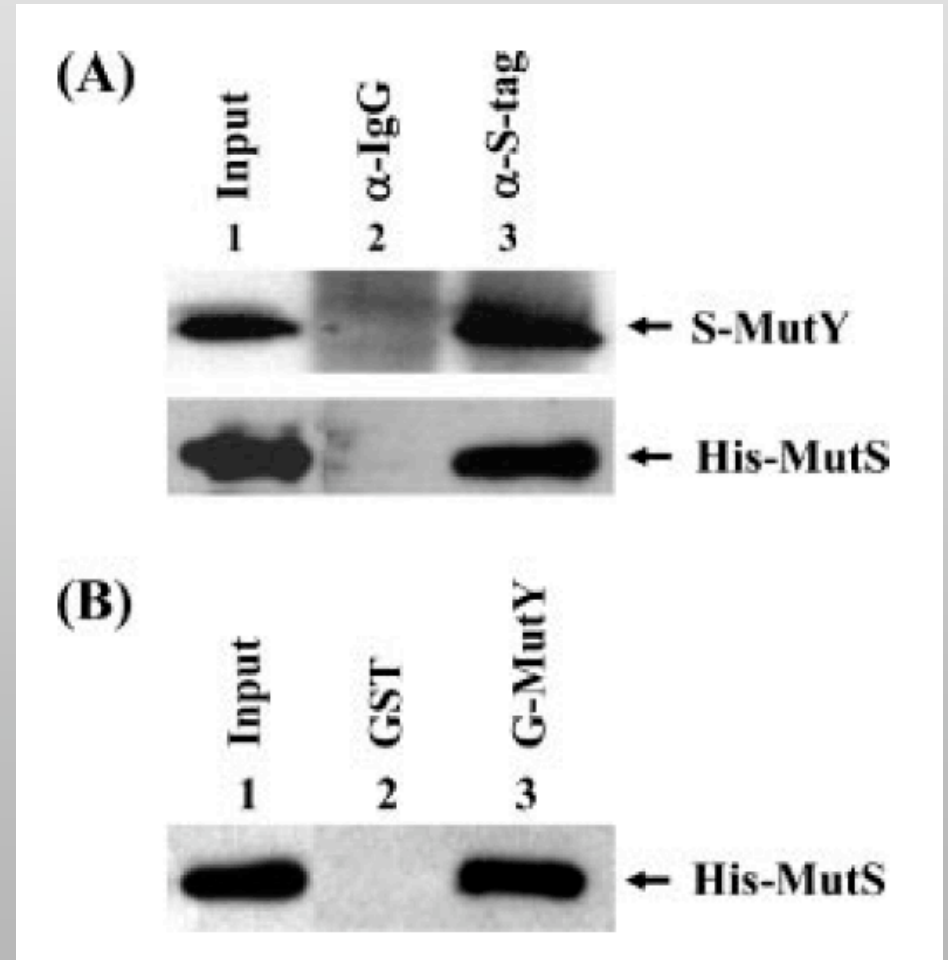


Background

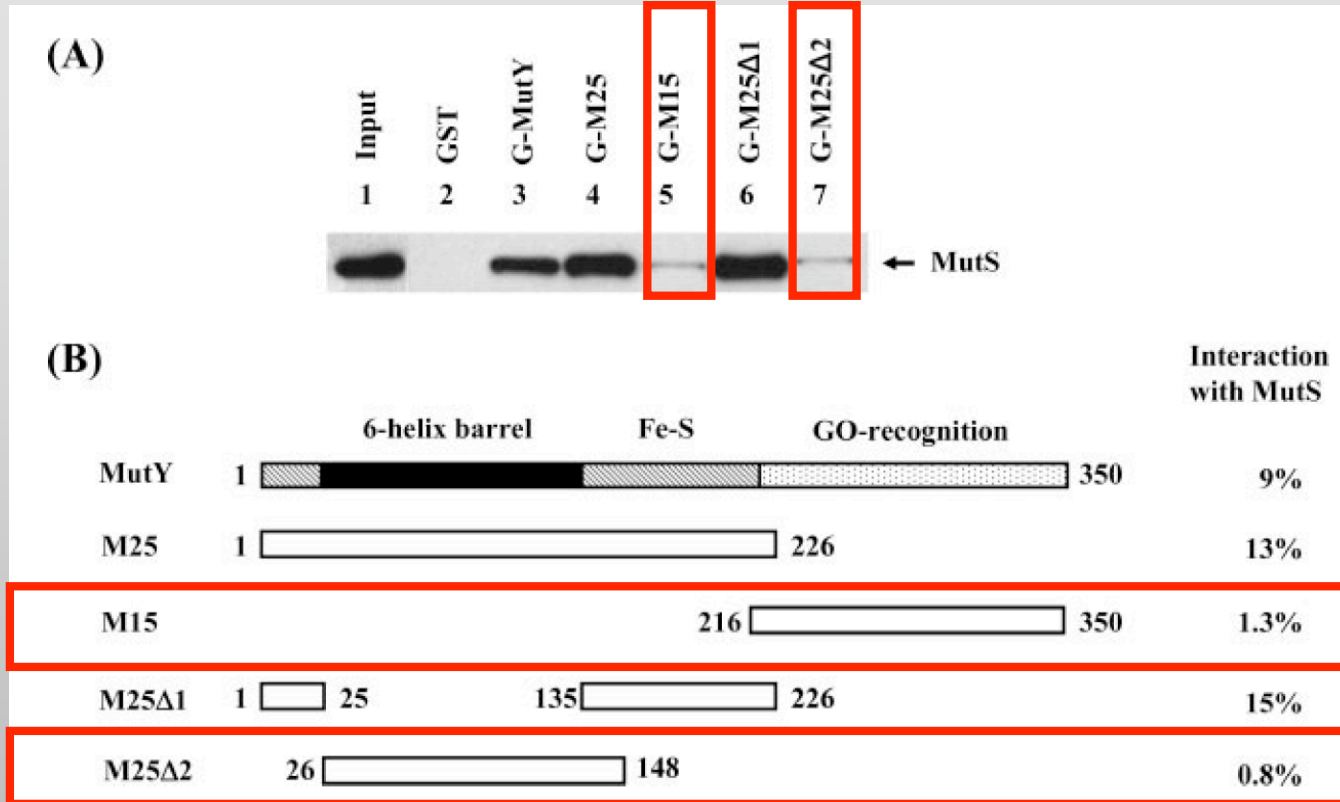
- Repair systems
 - MutT – hydrolyzes 8-oxo-GTP to 8-oxo-GMP
 - MutM – removes 8-oxoG
 - Nei (endonuclease VIII) – removes 8-oxoG when opposite C or A
- Methyl-dependent mismatch repair (MMR)
 - MutS, MutH, DNA helicase II, SSBP, Exonucleases, and DNA Pol III
 - MutS recognizes mispairings and has ATPase activity
- MutY
 - Removal of adenines that are misincorporated opposite 8-oxoG during DNA synthesis

Physical Interaction of MutY and MutS

- Coimmunoprecipitation of MutY with MutS
- MutY pulldown with MutY and MutS probing
 - MutY appears associated with MutS *in vivo* (lane 3)
- Immobilize MutY with MutS probing
 - MutY associates with MutS *in vitro* (lane 3)

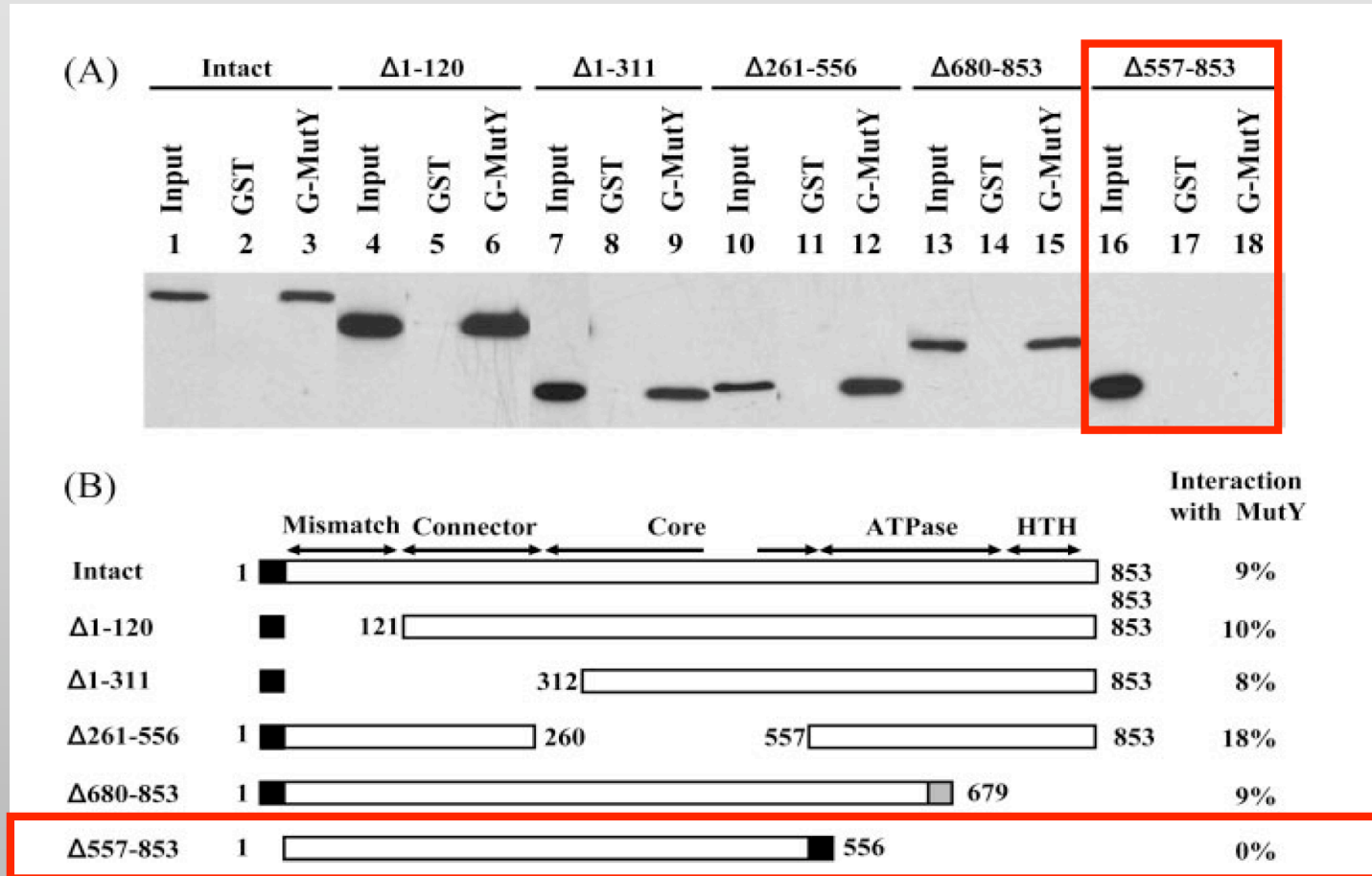


Regions of MutY Interacting with MutS



- Pulled down and immobilized MutY with MutS probing
- Fe-S region of MutY appears to be interaction site with MutS (similar region present in human MutY homolog)
- Previously shown that heterodimer hMutS₂ interacts with hMYH through binding of one of the subunits

Regions of MutS Interacting with MutY

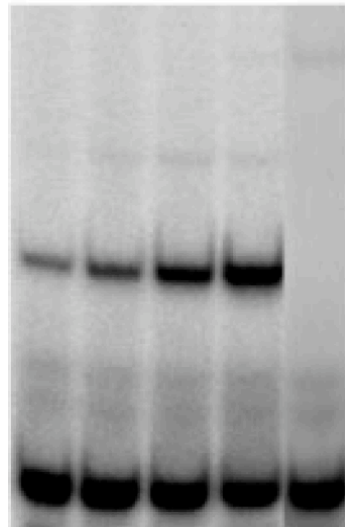


- Pulled down and immobilized MutY with MutS probing
- ATPase region appears to be region of interaction of MutS with MutY within residues 557 and 679

Binding Affinity With MutY Substrate is Enhanced by MutS

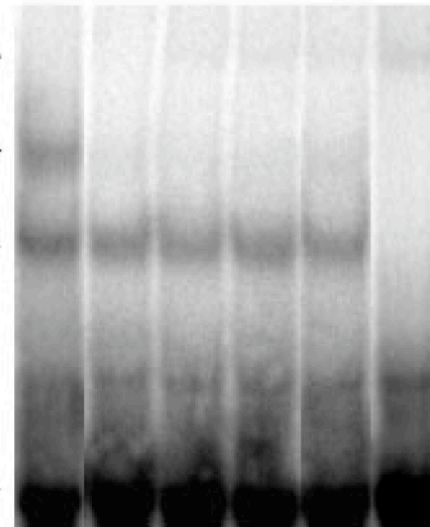
(A) A/GO binding

0	.05	.1	.4	.4
.05	.05	.05	.05	0
1	2	3	4	5



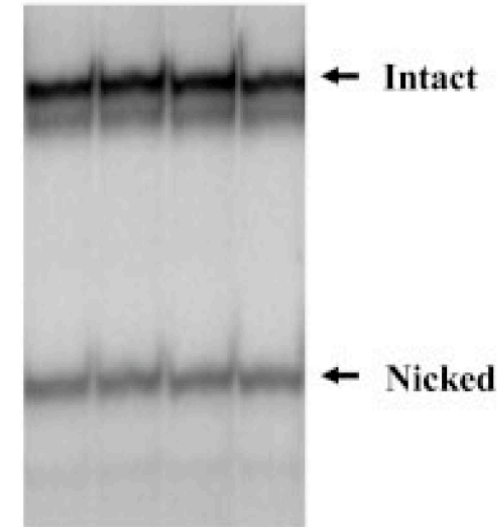
(B) C:G binding

0	0	4	8	32	32
30	4	4	4	4	0
1	2	3	4	5	6



(C) DNA glycosylase

0	.05	.1	.4	MutS (nM)
.05	.05	.05	.05	MutY (nM)
1	2	3	4	

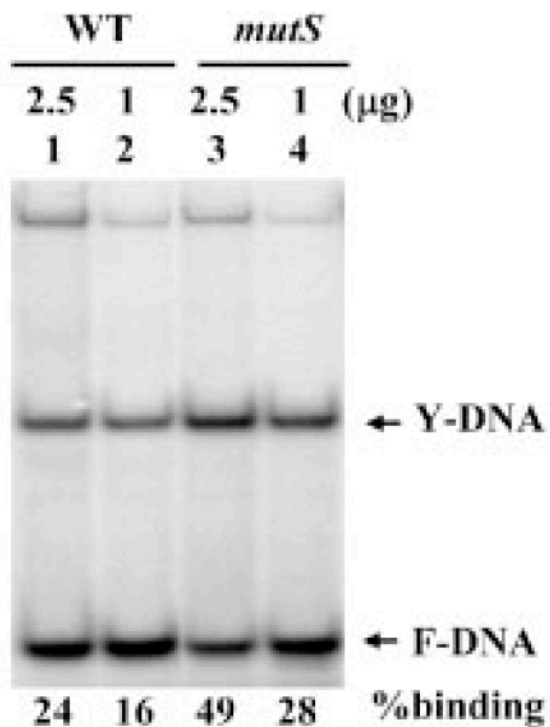


- MutY affinity for A:GO binding enhanced with MutS
- MutY and MutS show poor binding for C:G
- Activity of MutY with substrate is not enhanced by MutS

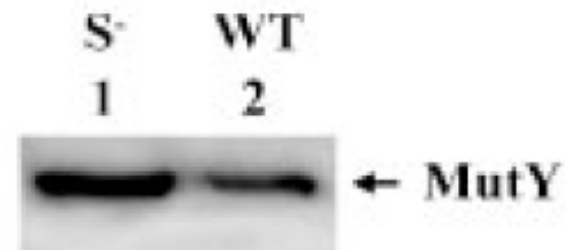
MutY Expression is Impacted by MutS

- MutY binding increased in *mutS* strain roughly 2 fold

(A) MutY DNA binding activity

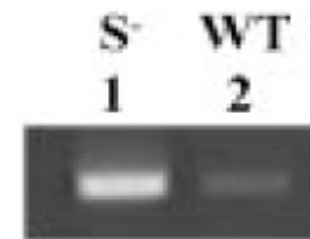


(B) MutY level in soluble extracts



- MutY expression increased roughly 6 fold in *mutS* strain
- MutY specific binding activity is roughly 3 fold less in *mutS* strain




(E) RT-PCR of MutY RNA



Mutation Frequencies and Distribution in Mutants

- Measured mutation frequency by rifampin resistance in *mutS*, *mutY*, and *mutS mutY* strains

TABLE 1. Mutation frequencies of *mutS*, *mutY*, and *mutS mutY* mutants

Strain	No. of Rif ^r cells/ 10 ⁸ cells	Increase (fold)
AB1157 (wild type)	0.6 ± 0.7	1
KM75 (<i>mutS</i>)	299 ± 64	498 
GM7724 (<i>mutY</i>)	14 ± 13	23
GM7726 (<i>mutS mutY</i>)	128 ± 24	213
AB1157 plus pET-MYW1	2.5 ± 0.8	4.2
KM75 plus pET-MYW1	359 ± 53	598 
GM7724 plus pET-MYW1	2.1 ± 2.3	3.5
GM7726 plus pET-MYW1	357 ± 41	595 

- Overexpression of MutY without MutS leads to increased mutations

Mutation Frequencies and Distribution in Mutants (cont)

- Measured mutation distribution with sequencing of *rpoB* in *mutS*, *mutY*, and *mutS mutY* strains

TABLE 2. Mutation distribution of *rpoB* in *mutS* and *mutS mutY* mutants


Mutation	No. of clones with <i>rpoB</i> mutation (% ^a) in mutant type			
	<i>mutS</i>		<i>mutS mutY</i>	
	This study	Kim et al. (27)	This study	Kim et al. (27)
A:T→G:C	26 (87)	72 (84)	21 (70)	35 (94)
G:C→A:T	3 (10)	14 (16)	8 (27)	1 (3)
G:C→T:A	1 (3)	0 (0)	0 (0)	1 (3)
A:T→T:A	0 (0)	0 (0)	1 (3)	0 (0)
Total	30	86	30	37



- Overproduction of MutY in absence of MutS leads to transition mutations

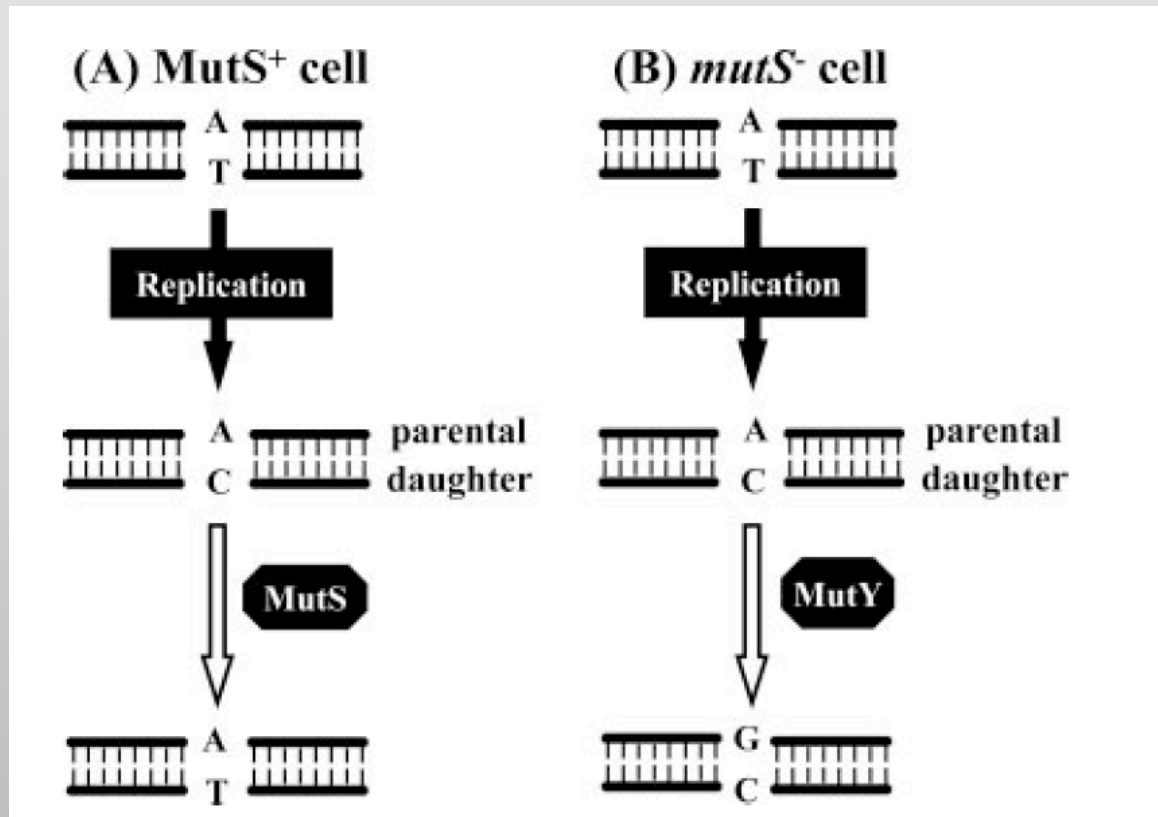
Transition Frequencies of Mutants Using a Lac⁺ Reversion Assay

TABLE 3. A:T→G:C transition frequencies of *mutS*, *mutY*, and *mutS mutY* mutants

Strain	No. of Lac ⁺ cells/ 10 ⁸ cells	Increase (fold)
CC106 (wild type)	0.05 ± 0.10	1
CC106 (<i>mutS</i>)	38.2 ± 16.8	 751
CC106 (<i>mutY</i>)	0.06 ± 0.12	1
CC106 (<i>mutS mutY</i>)	13.0 ± 1.1	256

- Data confirms sequencing of *rpoB* mutations

Model For MutY/MutS Interaction



- Misincorporation of C across from A by Pol III during replication normally repaired by MutS
- However, overproduction of MutY without MutS leads to decrease in substrate specificity for MutY with removal of A from A:C pairing, resulting in transitions