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Supplemental Information

Seeing Mutations in Living Cells

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Induction of eGFP-MutL^{wt} Expression by Arabinose

Addition of arabinose to growth medium induces the expression of T7 RNA polymerase gene located on the chromosome, which is under control of the arabinose promoter. T7 RNA polymerase gene induction results in eGFP-MutL^{wt} induction from T7 RNA polymerase promoter, which is located on plasmid. In the absence of arabinose, the basal level of T7 RNA polymerase gene expression allows production of the eGFP-MutL^{wt}, which is sufficient for complementation of chromosomal *mutL* gene inactivation. The overproduction of eGFP-MutL^{wt} does not change the number of eGFP-MutL^{wt} fluorescent foci (Supplementary Figure 1).

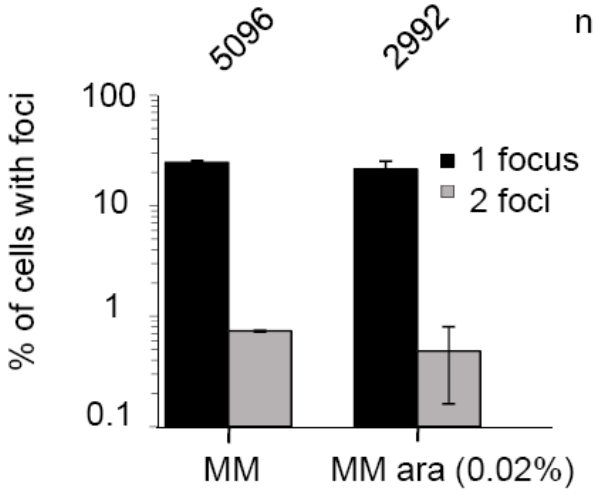

Figure S1, Related to Figure 2. Frequency of eGFP-MutL^{wt} Foci with or without Induction of eGFP-MutL^{wt} Expression
Percentage of *mutL mutH* cells with eGFP-MutL^{wt} foci grown in minimal medium (MM) or MM supplemented by 0.02% arabinose (ara). Error bars indicate standard error of the mean. n indicates the number of cells examined.

Table S1, Related to Figure 2. MutS^{wt} and MutL^{wt} Fusion Proteins Are Functional

Strain		Phenotype	Frequency of Rif^{R} Mutants		
Chromosome	Plasmid		x10^{-8}		
wild-type	pBR322	WT	1.8	±	0.7
mutL	peGFP-MutL^{wt}	WT	1.7	±	1.0
mutS	peGFP-MutS^{wt}	WT	3.3	±	1.5
mutL	peGFP-MutLK159E	MutL^{-}	234	±	125
mutS	peGFP-MutSF36A	MutS^{-}	391	±	57.9
mutL mutH	peGFP-MutL^{wt}	MutH^{-}	208	±	53.6
mutL mutH	peGFP-MutL^{wt} MutH^{wt}	WT	1.1	±	0.5

Mutation frequency was determined from three to six independent cultures grown in minimal medium. Mean and standard errors are indicated. Rif^{R}, rifampicin-resistant.

Table S2. Bacterial Strains

Strain	Genotype	Source, Reference, or Construction	
MG1655			Lab stock
FR535	As GM6494, but *mutL*::Tn5		Lab stock
BL21-AI	As BL21, but *araB*::T7RNAP-tetA		Invitrogen
IB11	As MG1655, but *mutS*::Spec/Strep		Lab stock
KM55	As AB1157, but *mutH*::Cam		[5]
FR680	As MG1655, but *mutD5 zae13*::Tn10		Lab stock
JW0233	As BW25113, but *proB*::Kan		[6]
JW2799	As BW25113, but *mutH*::Kan		[6]
KM52	As AB1157, but *mutL*::Cam		M. Marinus
ME001	As MG1655, but *mutL*::Tn5	MG1655 X P1 (FR535)	This work
ME002	As ME001, but *araB*::T7RNAP-tetA	ME001 X P1 (BL21-AI)	This work
ME003	As ME002, but peGFP-MutL^{wt}	ME002 X peGFP-MutL^{wt}	This work
ME004	As ME001, but *mutS*::Spec/Strep	ME001 X P1 (IB11)	This work
ME005	As ME004, but *araB*::T7RNAP-tetA	ME004 X P1 (BL21-AI)	This work
ME006	As ME005, but peGFP-MutL^{wt}	ME005 X peGFP-MutL^{wt}	This work
ME007	As ME003, but *mutH*::Cam	ME003 X P1 (KM55)	This work
ME008	As ME006, but *mutH*::Cam	ME006 X P1 (KM55)	This work
ME009	As ME002, but peGFP-MutLK159E	ME002 X peGFP-MutLK159E	This work
ME010	As ME002, but *mutH*::Cam	ME002 X P1 (KM55)	This work
ME011	As ME010, but peGFP-MutLK159E	ME010 X peGFP-MutLK159E	This work
ME012	As IB11, but *araB*::T7RNAP-tetA	IB11 X P1 (BL21-AI)	This work
ME013	As ME012, but peGFP-MutS^{wt}	ME012 X peGFP-MutS^{wt}	This work
ME014	As ME012, but peGFP-MutSF36A	ME012 X peGFP-MutSF36A	This work
ME015	As MG1655, but *mutH*::Kan	MG1655 X P1 (JW2799)	This work
ME016	As ME015, but *araB*::T7RNAP-tetA	ME015 X P1 (BL21-AI)	This work
ME017	As ME016, but *mutS*::Spec/Strep	ME016 X P1 (IB11)	This work
ME018	As ME017, but peGFP-MutS^{wt}	ME017 X peGFP-MutS^{wt}	This work
ME019	As ME017, but peGFP-MutSF36A	ME017 X peGFP-MutSF36A	This work
ME020	As FR680, but *proB*::Kan	FR680 X P1 (JW0233)	This work
ME021	As MG1655, but *araB*::T7RNAP-tetA	MG1655 X P1 (BL21-AI)	This work
ME022	As ME021, but *mutL*::Cam	ME021 X P1 (KM52)	This work
ME023	As ME022, but peGFP-MutL^{wt}	ME022 X peGFP-MutL^{wt}	This work
ME024	As ME023 but, *mutD5 zae13*::Tn10 *proB*::Kan	ME023 X P1 (ME020)	This work

Table S3. Plasmids

Plasmid	Source, Reference, or Construction
pWY1076	[2]
pWY1170	[2]
peGFP-MutL^{wt}	This work
peGFP-MutLK159E	This work
peGFP-MutS^{wt}	[1]
peGFP-MutSF36A	This work

Table S4, Related to Figure 4. *mutD5* Mutator Effect in *mutL* Cells Producing eGFP-MutL^{wt}

Strain		Frequency of Rif^{R} mutants
		x10^{-8}
wild-type	pBR322	1.8 ± 0.7
mutL mutH	peGFP-MutL^{wt}	255 ± 42.9
mutL mutD5	peGFP-MutL^{wt}	578 ± 185

Mutation frequency was determined from three to six independent cultures. Mean and standard errors are indicated. Rif^{R}, rifampicin-resistant.

Supplemental Experimental Procedures

Fusion of Fluorescent Reporters to Mismatch Repair Proteins

Plasmids peGFP-MutS^{wt} and peGFP-MutL^{wt} were constructed by *Li-Jun Bi & al.*, as described previously for MutS [1]. Briefly, pET-32a plasmid *(Novagen)* was modified by restriction digestion and ligation to include DNA fragments encoding MutS or MutL, a $(Ser\text{-}Gly)_6$ linker, and the eGFP (enhanced Green Fluorescent Protein), generating peGFP-MutS^{wt} and peGFP-MutL^{wt}, respectively. eGFP-tagged MutS and MutL cloned on pET-32a are expressed from LacI repressible T7 RNA polymerase promoter.

peGFP-MutS^{wt} and peGFP-MutL^{wt} were modified to encode mutant versions of MutL or MutS fused to eGFP as described below. The plasmid expressing MutLK159E mutant (deficient for DNA binding and ATP hydrolysis) fused to eGFP, was made using the plasmid pWY1076 [2] expressing the MutLK159E. Briefly, the fragment encoding part of the MutLK159E was digested with *HindIII* and *SpeI*, and cloned into peGFP-MutL^{wt} to yield peGFP-MutLK159E. The plasmid expressing MutSF36A mutant (deficient for mismatch binding) fused to eGFP was made by using the primers 5'-*CCGAATTCATGAGTGCAATAGAAAATTTC*-3' and 5'-*CGGTAATGCGAATGCCCGGTT*-3' spanning MutS N terminal and including *EcoRI* and *BsmI* restriction sites at N and C termini, respectively. With those primers, the fragment encoding part of the MutSF36A mutant was amplified by PCR from template pWY1170 [2]. PCR product was digested by *EcoRI* and *BsmI* and cloned into vector peGFP-MutS^{wt} to yield peGFP-MutF36A.

Strain Construction and Media

Strains and plasmids are listed in Supplementary Tables 2 and 3. All strains were derived from wild-type sequenced *E. coli* MG1655 by P1 transduction and transformation. Strain genotypes were verified by testing the UV resistance, capacity to generate mutations conferring resistance to rifampicin, or ability to use arabinose in McConkey plates supplemented by arabinose. Cells were grown on standard M9 minimal medium [3] supplemented by 2 mM MgSO4, 0.003% vitamin B1, 0.001% uracile, 0.2 % casamino acids, 0.01 % glycerol and ampicillin (100 μg/ml).

Microscopy

Supplemented minimal medium (see above) overnight cultures of the strains expressing fluorescent reporter(s) were diluted 250-fold and grown to early exponential phase. Cells were concentrated and spread on agarose supplemented by minimal medium, in a cavity slide to obtain a cell monolayer, as described previously [4]. The slide was mounted on Metamorph software (Universal Imaging) driven temperature controlled (Life Imaging Services) Zeiss 200M (Zeiss) inverted microscope. Images were recorded at 100-fold magnification using CoolSNAP HQ camera (Princeton Instruments), in phase contrast and in fluorescence [50% neutral density filter on a 100 W Fluo-Arc Hg-vapor lamp (Zeiss) regulated to 100% power] at wavelength of 500 nm during 20 seconds of exposure time. For time-lapse microscopy excitation was limited to 50% of output of 100 W Fluo-Arc Hg-vapor lamp and cells were exposed during 6 seconds to avoid photo-bleaching.

All experiments were repeated at least 3 times. Data from all experiments are plotted.

Image Analysis

For all foci, the region delimiting each focus and one control region in the cell, were defined using Metamorph region tool. Fluorescence of the control and focus regions were measured for each cell. Focus fluorescence is calculated by subtracting the fluorescence of the control region from the maximal pixel intensity of the focus region. The fluorescence background of the agarose was recorded for each image. Cell fluorescence was determined by subtracting the average fluorescence of the agarose from the average cell fluorescence.

Spontaneous Mutation Frequency Assay

Strains expressing fluorescent reporter(s) fused to wild-type or mutant MutS and MutL were grown overnight in supplemented minimal medium (see above). The overnight cultures were then diluted 10^7-fold and grown to saturation. Dilutions of overnight cultures were plated on selective medium (LB containing 100 μg/ml rifampicin and 100 μg/ml ampicillin) to select rifampicin-resistant (Rif^R) colonies and on LB (containing 100 μg/ml ampicillin) to determine the total number of colony forming units. Colonies were scored after 24h of incubation at 37°C. The average mutation frequency of each strain was determined from three to six independent experiments.

Estimating the Mutation Frequency

We need to go from a measured number of eGFP-MutL foci per cell to an estimate of the mutation frequency per base pair per generation. If each cell had exactly one copy of the genome, this would be a simple calculation, but we must deal with three complexities: cells at different stages of the cell cycle have different numbers of genomes, rapidly growing *E. coli* are born with partially replicated genomes, and, in an exponentially growing population, younger cells are more frequent than older ones.

To deal with the first two problems, we used the measured cell division time of 40 minutes and two pieces of information about the *E. coli* cell division cycle: it takes 40 minutes to replicate the chromosome and there is a minimum interval of 20 minutes between the end of replication and cell division. Using this information we conclude that the replication forks in a newly born cell must be at least half way to the terminus. Thus these cells already have 1.5 genomes (0.5 in single copy + 0.5 in two copies). From birth until twenty minutes, the forks move linearly until this round of replication terminates, meaning that the DNA content of the cell rises linearly from 1.5 to 2 genomes over this period. At twenty minutes, one round of replication finishes and the next starts, with the DNA content increasing twice as rapidly since there are now four forks instead of two. This means that over the second half of the cell cycle, the DNA content increases linearly from 2 to 3 genomes.

The last problem is that cell ages are distributed non-uniformly. The standard age distribution for exponentially growing cells is $N(x) = 2^{(1-x)}$ where N is the number of cells of age x and x varies from 0 (newly born cells) to 1 (cells about to divide). To find the average number of genomes we

compute the number of genomes at each point of the cell cycle and multiply it by the fraction of cells that are at each point in the cell cycle. This exercise gives a mean number of genomes per cell of 2.0. To calculate the genome wide mutation rate, we use the following formula:
Genomic mutation rate = eGFP-MutL focus frequency/(Mean Genome Number × Genome Length)

Genomic mutation rate = $0.0057/(2.0 \times 4.5 \times 10^{6}) = 6.3 \times 10^{-10}$.

Author Contributions

M.R., I.M and M.E. conceived the experiments. M.E. performed the experiments. M.R., I.M, A.M and M.E. analyzed results, interpreted results, and wrote the paper. L.B and X.Z. constructed the plasmids peGFP-MutL^{wt} and eGFP-MutS^{wt}.

Supplemental References

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