Nucleoside diphosphate kinase escalates A to C mutations in MutT deficient strains of *Escherichia coli*

Running Title: Role of NDK in genome instability

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Supplementary Methods

DNA oligomers

Sequences of DNA oligomers are provided in Table S1.

Generation of strains

E. coli CC101 Δ*mutT*Δ*ndk*

E. coli CC101 Δ*mutT::kan* strain was used as the parent to generate additional Δ*ndk::kan* mutation. The kanamycin marker cassette was excised (cured) from CC101 Δ*mutT*::*kan* strain using pCP20 plasmid. The plasmid harbors the gene sequence for FLP recombinase, which recognizes the FRT sites present in the sequence flanking the marker cassette and removes the DNA region between the FRT sites (1). The CC101 Δ*mutT* cured strain was selected by its sensitivity to kanamycin and verified by colony PCR using the primers *Eco_mutT*_Up and *Eco_mutT*_Dn, flanking *mutT* locus. To obtain CC101 Δ*ndk*::*kan* Δ*mutT* strain, P1 phage lysate was raised on JW2502-1 Δ*ndk*::*kan* strain and used to transduce *E. coli* CC101 Δ*mutT* cured strain followed by plating on LB Kan plate. Transductants were confirmed by colony PCR using the primers *Eco_ndk*_KO_Fp and *Eco_ndk*_KO_Rp, flanking the *ndk* locus.

E. coli CC101 Δ*mutT*Δ*ribA*

Using *E. coli* CC101 Δ*mutT* strain cured for the antibiotic marker as a parent, *ribA* locus was disrupted by pKD46 mediated recombination (2). The linear fragment corresponding to Δ*ribA::cm* was created by amplification of chloramphenicol cassette from pKD3 using *Eco_ribA*_KO_Fp and *Eco_ribA*_KO_Rp primers containing 36 nucleotides flanking sequence of *ribA* gene. *E. coli* CC101 Δ*mutT*/ pKD46 was grown at 30 ˚C in the presence of 0.2% arabinose and processed at O.D. 0.6 for electrocompetent cell preparation. Cells were electroporated with ~300ng of linear fragment at 1.8 kV, 200 Ω and 25 μF in a 0.1 cm cuvette, incubated for recovery at 37 ˚C in the

LB medium supplemented with 0.25 mg/ml riboflavin and selected for the knockouts on LB Cm plate containing 0.25 mg/ml riboflavin. Knockouts were verified by PCR amplification of *ribA* locus using flanking primers (*Eco_ribA*_Up and *Eco_ribA*_Dn) and internal primers (*Eco_ribA*_IN_Fp and *Eco_ribA*_IN_Rp).

Supplementary References

1. Cherepanov PP, Wackernagel W. 1995. Gene disruption in Escherichia coli: Tc^R and Km^R cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance determinant. Gene 158:9-14. PMID: 7789817
2. Datsenko KA, Wanner BL. 2000. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc Natl Acad Sci U S A. 97:6640-6645. PMID: 10829079

Table S1. List of DNA oligomers used in the study.

DNA oligomer	Sequence (5' to 3'), restriction site is underlined
*Eco_ndk*_NcoI_Fp	TGTAACCATGGCTATTGAACG
*Eco_ndk*_BglII_Rp	AAAAGATCTACGGGTGCGCGG
*Eco_ndk*_BglII_Rp_stop	ATTAGATCTTTAACGGGTGCGCG
*Eco_ndk*_KO_Fp	ATGGATGACGTGGGGCAAATCG
*Eco_ndk*_KO_Rp	CAGGTGTGACTAATTGTTCAGACATATGCTATTC
*Eco_mutT*_KO_Up	TACAAGCAGTGCCATGGCCCCTG
*Eco_mutT*_KO_Dn	GATGCGGCGAAAACGCCTTATCTG
*Eco_ribA*_KO_Fp	TAAACGTGTGGCAGAAGCCAAACTGCCAACCCCATGTGTAGGCTGGAGCTGCTTCG
*Eco_ribA*_KO_Rp	TTATTTGTTCAGCAAATGGCCCATTTTCTCGGCTTTCCATATGAATATCCTCCTTA
*Eco_ribA*_Up	TGTGCCATTCCGTGAACGAT
*Eco_ribA*_Dn	GCTTGCCGGTTATTTTGCTT
*Eco_ribA*_IN_Fp	TGACGCCCTGTTCAGCTTGC
*Eco_ribA*_IN_Rp	CATTGACGCCAAGGAGTTTG

Table S2: Mutation frequency (Rif ^{r}) values and analysis for Fig. 3.

MG1655 Δ*mutT*			
	pBAD	pBAD_ *Eco_mutT*	pBAD_ *Eco_ndk*
1.	4.31e-005	1.50e-006	1.89e004
2.	3.80e-005	1.08e-006	1.47e-004
3.	1.77e-005	7.63e-007	1.10e-004
4.	1.21e-005	5.88e-007	1.00e-004
Analysis (95% CI of median)			
Actual confidence level	100%	100%	100%
Lower confidence limit	1.21e-005	5.88e-007	1.00e-004
Median	2.78e-005	9.21e-007	1.28e-004
Upper confidence limit	4.31e-005	1.50e-006	1.89e-004

Table S3: Mutation frequency (A to C) values and analysis for Fig. 4.

CC101 Δ*mutT*			
	pBAD	pBAD_ *Eco_mutT*	pBAD_ *Eco_ndk*
1.	8.91e-006	2.38e-008	8.33e-006
2.	7.54e-006	0.00e+000	8.00e-006
3.	5.94e-006	0.00e+000	7.25e-006
4.	5.70e-006	0.00e+000	6.05e-006
5.	5.26e-006	0.00e+000	5.71e-006
6.	5.16e-006	0.00e+000	5.69e-006
7.	4.20e-006	0.00e+000	5.09e-006
8.	4.07e-006	0.00e+000	4.62e-006
9.	3.29e-006	0.00e+000	4.58e-006
10.	2.12e-006	0.00e+000	3.16e-006
Analysis (95% CI of median)			
Actual confidence level	97.85%	97.85%	97.85%
Lower confidence limit	3.290e-006	0.0	4.580e-006
Median	5.210e-006	0.0	5.70e-06
Upper confidence limit	7.540e-006	0.0	8.000e-006

Table S4: Lac$^+$ reversion frequency values and analysis for Figs. 5A and B.

	Experiment 1		Experiment 2	
	CC101 Δ*mutT*	CC101 Δ*mutT* Δ*ndk*	CC101 Δ*mutT*	CC101 Δ*mutT* Δ*ndk*
1.	8.46e-006	2.11e-006	2.25e-005	4.93e-006
2.	7.63e-006	2.00e-006	1.67e-005	4.64e-006
3.	6.86e-006	1.26e-006	8.89e-006	4.59e-006
4.	6.84e-006	1.13e-006	7.50e-006	3.60e-006
5.	3.46e-006	1.03e-006	6.23e-006	2.81e-006
6.	3.37e-006	9.82e-007	6.15e-006	2.09e-006
7.	2.86e-006	9.34e-007	6.14e-006	1.87e-006
8.	2.79e-006	7.41e-007	5.23e-006	1.77e-006
9.	2.77e-006	7.03e-007	4.87e-006	1.71e-006
10.	2.13e-006	6.62e-007	4.68e-006	1.19e-006
Analysis (95% CI of median)				
Actual confidence level	97.85%	97.85%	97.85%	97.85%
Lower confidence limit	2.77e-006	7.03e-007	4.87e-006	1.71e-006
Median	3.41e-006	1.00e-006	6.19e-006	2.45e-006
Upper confidence limit	7.63e-006	2.00e-006	1.67e-005	4.64e-006

Table S5: Lac$^+$ reversion frequency values and analysis for Figs. 6A and B.

	Experiment 1		Experiment 2	
	CC101 Δ*mutT* Δ*ribA*	CC101 Δ*mutT* Δ*ribA* Δ*ndk*	CC101 Δ*mutT* Δ*ribA*	CC101 Δ*mutT* Δ*ribA* Δ*ndk*
1.	5.17e-005	6.50e-006	3.66e-005	6.96e-006
2.	4.67e-005	5.44e-006	3.65e-005	6.78e-006
3.	3.65e-005	5.38e-006	2.24e-005	3.96e-006
4.	3.21e-005	5.19e-006	1.81e-005	3.96e-006
5.	3.09e-005	4.60e-006	1.39e-005	3.92e-006
6.	1.85e-005	3.39e-006	1.28e-005	3.55e-006
7.	1.57e-005	3.30e-006	1.08e-005	3.32e-006
8.	1.20e-005	2.84e-006	7.46e-006	2.88e-006
9.	8.15e-006	2.68e-006	7.38e-006	2.86e-006
10.	7.41e-006	2.66e-006	3.95e-006	1.52e-006
11.	4.37e-006	2.12e-006		
Analysis (95% CI of median)				
Actual confidence level	98.83%	98.83%	97.85%	97.85%
Lower confidence limit	7.41e-006	2.66e-006	7.38e-006	2.86e-006
Median	1.85e-005	3.39e-006	1.33e-005	3.73e-006
Upper confidence limit	4.67e-005	5.44e-006	3.65e-005	6.78e-006

Table S6: Lac$^+$ reversion rates and analysis for Fig. 6C.

	CC101 Δ*mutT* Δ*ribA*	CC101 Δ*mutT* Δ*ribA* Δ*ndk*
1.	1.00e-006	4.13e-007
2.	8.44e-007	2.56e-007
3.	7.24e-007	2.36e-007
4.	6.36e-007	1.82e-007
5.	5.04e-007	1.57e-007
6.	4.32e-007	1.41e-007
7.	3.89e-007	1.40e-007
8.	3.55e-007	1.17e-007
9.	3.43e-007	9.63e-008
10.	3.32e-007	8.73e-008
11.	2.43e-007	3.83e-008
Analysis (95% CI of median)		
Actual confidence level	98.83%	98.83%
Lower confidence limit	3.32e-007	8.73e-008
Median	4.32e-007	1.41e-007
Upper confidence limit	8.44e-007	2.56e-007

Figure S1

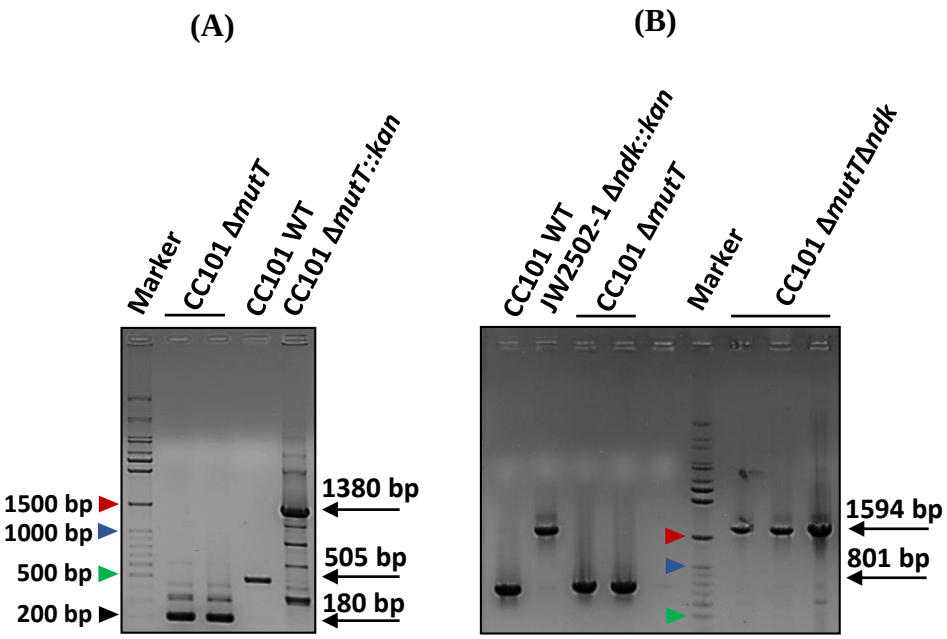

Fig. S1: Verification of *ndk* deletion in CC101 Δ*mutT strain.* (A) Unmarked deletion of *mutT* gene was confirmed by the amplification of *mutT* locus, using the flanking primers, which amplifies 180 bp DNA from the CC101Δ*mutT* strain as opposed to the 1380 bp DNA from the parent (Δ*mutT::kan*) strain. **(B)** Deletion of *ndk* by transducing Δ*ndk::kan* allele in CC101 Δ*mutT* strain was verified by the amplification of *ndk* locus, using the flanking primers, where the wild type and *ndk*::*kan* alleles resulted in 801 bp and 1594 bp long amplicons, respectively.

Figure S2

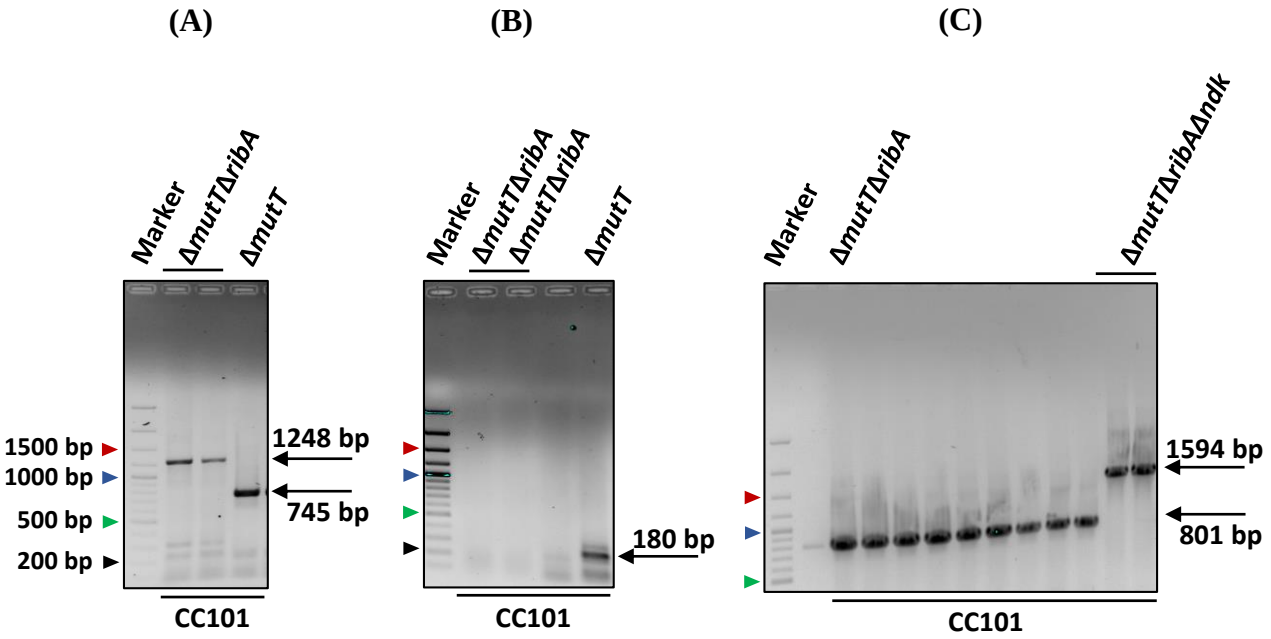

Fig. S2: Validation of CC101 Δ*mutT*Δ*ribA* and CC101 Δ*mutT*Δ*ribA*Δ*ndk* strains. Disruption of *ribA* gene was checked by the amplification of the locus, **(A)** using the flanking primers (amplifies 1248 bp and 745 bp amplicons from the knockout and the parent strains, respectively), **(B)** using the internal primers (results in no amplification versus an amplicon of 180 bp from the knockout and the parent strains, respectively). **(C)** Deletion of *ndk* gene in CC101 Δ*mutT*Δ*ribA strain* was examined by the amplification of *ndk* locus, using the flanking primers (801 and 1594 bp long amplicons for the wild type and *ndk*::*kan* alleles resulted in 801 bp and 1594 bp long amplicons for the wild type and *ndk*::*kan* alleles, respectively.