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All Types of Base Pair Substitutions Induced by γ-Rays in Haploid and Diploid Yeast Cells

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***Abstract*—**We study the biological effects induced by ionizing radiation in view of therapeutic exposure and the idea of space flights beyond Earth's magnetosphere. In particular, we examine the differences between base pair substitution induction by ionizing radiation in model haploid and diploid yeast *Saccharomyces cerevisiae* cells. Such mutations are difficult to study in higher eukaryotic systems. In our research, we have used a collection of six isogenic *trp5*-strains and 14 isogenic haploid and diploid *cyc1*-strains that are specific markers of all possible base-pair substitutions. These strains differ from each other only in single base substitutions within codon-50 of the *trp5* gene or codon-22 of the *cyc1* gene. Different mutation spectra for two different haploid genetic *trp5*- and *cyc1*-assays and different mutation spectra for the same genetic *cyc1*-system in cells with different ploidy — haploid and diploid — have been obtained. It was linear function for dose-dependence in haploid and exponential in diploid cells. We suggest that the differences between haploid yeast strains reflect the dependence on the sequence context, while the differences between haploid and diploid strains reflect the different molecular mechanisms of mutations.

***Keywords*—**Base pair substitutions, γ-rays, haploid and diploid cells, yeast *Saccharomyces cerevisiae*.

I. Introduction

THE biological effect of ionizing radiation has been studied in bacteria, yeast, and animals for many years. Mutagenic lesions are associated with the initiation and progression of human cancer. γ-rays and ion beams are utilized as models of low- and high-linear energy transfer (LET) radiation. There are differences in lethal and mutagenic effects of low- and high-LET radiation. It is necessary to know well the effects of γ-rays with which different types of radiation are compared. In the present work, the special types of mutations, base substitutions, were studied using two genetic assays. We investigate the effects of ploidy and type of assay on spectrum of mutations.

II. Materials and Methods

A. Media

We used standard rich medium YEPD (2 % peptone, 1 % yeast extract, 2 % glucose), medium with glycerol YEPG (2 % peptone, 1 % yeast extract, 3 % glycerol, 3 % ethanol), and YPGD (1% yeast extract, 2% peptone, 3% glycerol, 0.1% glucose). Minimal MM_{300} medium and synthetic complete media (SM) with bases and amino acids (Sigma) are described in [1]. SM contained adenine, arginine, histidine, methionine, tryptophan, uracil at 20 mg/l; tyrosine, leucine, lysine at 30 mg/l and threonine at 200 mg/l supplemented to medium MM_{300}.

B. Trp5-Assay

Various *trp5* mutant strains (*MATα his3-Δ200 ura3-52 leu2-Δ1 trp5-x**) were obtained from Dr. G. F. Crouse (Emory University, Atlanta, Georgia, USA). Various *trp5* mutant strains differ from each other only by single base substitutions within codon-50 of the *trp5* gene (Table I) [2]. Each strain reverts only *via* a true reversion event. Omission media were SM-trp.

C. Cyc1-Assay

Haploid YMH1-YMH7 (*MATα cyc1-x*** *cyc7-67 ura3-52 leu2-3,112 cyh2*) and diploid hemizygous YMH51-YMH57 (*MATα*/*MAT***a** *cyc1-x/cyc1-1 cyc7-67/cyc7-67 ura3-52/ura3-52 leu2-3,112/+ cyh2/+ +/his1-1 +/can1-100*) strains of *cyc1*-tester set [3] were kindly donated by Dr. M. Hampsey (Louisiana State University Medical Center, Shreveport, Louisiana, USA). Haploid strains YMH1-YMH7 (Table 2) differ from each other only by single base substitutions within codon-22 of the *cyc1* gene [3]. The *cyc1-1* and *cyc7-67* alleles are complete deletions of their respective genes, therefore, revertants cannot arise either by mutations that result in overexpression of iso-2-cytochrome *c* or by recombination between the *cyc1-x* and *cyc1-1* or *cyc7-67* genes. Revertants were selected on YEPG medium containing 0.1 % glucose, it is important to obtain efficient and reproducible reversion frequencies.

D. Mutation Assays

The method of ordered plating [4] was used to measure spontaneous mutation rates. The tested yeast cultures were grown on plates with YEPD medium for 2 days. 5 ml of a suspension (10^7 cells/ml) was prepared. A special 220-stamp replicator was dipped into this suspension and inverted on lavsan filter covering a solid medium in the Petri plate. The replicator places 220 equal drops of yeast suspension at equal distances from each other. It was about 2 μl each and contained ~ 2000 cells. They were growing up to $2 \cdot 10^6$ cells. After two days, the filter was replaced on selective medium where the mutants arising during growth have faster growth that shows up as papillae on the spots with limited growth of the tested culture. After five days of incubation, the papillae and the total viable cells were counted. The later was done after washing the cells from entire plate or from a number of

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single-drop replicas lacking visible papillae. The yeast cells grow uniformly when plated in this manner. The mutation rate was determined by dividing the number of papillae by the total number of viable cells on the plate.

TABLE I
YEAST HAPLOID ISOGENIC *TRP5*-STRAINS AND THEIR CORRESPONDING MUTATIONAL SPECIFICITIES

Strain	Codons 49–51*	Amino acid 50	Mutational specificity
wt	ATC GAA TTG	Glu	-
1868	ATC AAA TTG	Lys	AT → GC transition
1756	ATC CAA TTG	Gln	CG → GC transversion
1862	ATC TAA TTG	Stop	TA → GC transversion
1675	ATC GCA TTG	Ala	CG → AT transversion
1663	ATC GGA TTG	Gly	GC → AT transition
1903	ATC GTA TTG	Val	TA → AT transversion

*DNA sequence changes in codon-50 are underlined. Codons 49 and 51 are included to depict the immediate sequence context flanking the mutational targets.

TABLE II
YEAST ISOGENIC *CYC1*-TESTER STRAINS AND THEIR CORRESPONDING MUTATIONAL SPECIFICITIES

Haploid strain	Diploid strain	Codons 21–23*	Amino acid 22	Mutational specificity
YMH1	YMH51	CAA TGC CAC	Cys	-
YMH2	YMH52	CAA CGC CAC	Arg	GC→AT
YMH3	YMH53	CAA AGC CAC	Ser	AT→TA
YMH4	YMH54	CAA GGC CAC	Gly	GC→TA
YMH5	YMH55	CAA TCC CAC	Ser	GC→CG
YMH6	YMH56	CAA TTC CAC	Phe	AT→CG
YMH7	YMH57	CAA TAC CAC	Tyr	AT→GC

*DNA sequence changes in codon-22 are underlined. Codons 21 and 23 are included to depict the immediate sequence context flanking the mutational targets.

E. Irradiation

The source of γ-rays was ^{60}Co (the dose rate 0.7 Gy/min, a LET of 0.25 keV/μm) at therapeutic equipment "Rokus" (JINR, Dubna). Cells were irradiated in Eppendorf tubes and keeping in ice for prevention of DSB repair. Irradiated suspension after serial dilutions plated on appropriate selective media (SM-trp in *trp5*-assay and YPGD in *cyc1*-assay) or on YEPD to assess mutagenesis and cell survival, respectively.

III. RESULTS AND DISCUSSION

We analyzed mutation spectra induced by γ-rays using two genetic assays (*trp5*- and *cyc1*-) (Tables I and II). Haploid and diploid strains have the typical survival curves after γ-irradiation (Figs. 1 (a), 2 (a) and 3 (a)). Spontaneous frequencies of base pair substitutions were very low. No revertants of the *trp5*-strains were seen in multiple cultures ($<10^{-10}$). But, for haploid and diploid *cyc1*-strains, spontaneous frequency was 10^{-10}-10^{-9} per cell per generation (see Table III). The G-T and G-C transversions were more efficient in haploid strains, and G-T transversion and G-A transition were frequent in diploid strains.

In control cultures, the frequency of Trp^+ revertants was about 10^{-10}-10^{-9} ($6.5\cdot10^{-10}$ for 1868, $<8\cdot10^{-10}$ for 1756, $<7\cdot10^{-10}$ for 1862, $<5\cdot10^{-10}$ for 1675, $<4.8\cdot10^{-10}$ for 1663, $<4\cdot10^{-10}$ for 1903 strains). Irradiation by γ-rays induced efficiently all types of base substitutions in *trp5*-assay, although GC–AT transitions were predominating (Fig. 1 (b)). AT–GC and CG-AT were induced by γ-rays less efficiently. The third group consists of mutations which induced with small efficiency (TA-AT, CG-GC, TA-GC). Linear dose dependence was observed.

The second haploid *cyc1*-assay was irradiated up to 1000 Gy, and linearity of dose dependence was also observed (Fig. 2 (b)). But, in this case, GC-CG and GC-TA transversions and GC-AT transition were induced more efficiently. Since the dose interval for two haploid assays was different, we have compared the slopes of linear functions. Comparison of two haploid assays showed that base substitutions were induced more efficiently in *trp5*-assay with factor 2-10 (Table IV).

TABLE III
FREQUENCY OF SPONTANEOUS BASE PAIR SUBSTITUTIONS IN DIPLOID *CYC1*-STRAINS

Mutation	Frequency	
	haploid	diploid
Transition		
GC→AT	$6\cdot10^{-10}$	$13\cdot10^{-10}$
AT→GC	$4\cdot10^{-10}$	$3\cdot10^{-10}$
Transversion		
AT→TA	$10\cdot10^{-10}$	$3\cdot10^{-10}$
GC→TA	$28\cdot10^{-10}$	$10\cdot10^{-10}$
GC→CG	$16\cdot10^{-10}$	$1\cdot10^{-10}$
AT→CG	$<2.8\cdot10^{-10}$	$3\cdot10^{-10}$

In diploid *cyc1*-strains, GC–AT transition and GC–TA transversion induced more efficiently after γ-irradiation (Fig. 3 (b)). The curves were fitted by exponential or sigmoidal functions. Earlier non-linear function of mutagenesis was also observed by Mortimer et al. [5] in diploid cells.

Comparison of base pair substitution spectrum of haploid and diploid *cyc1*-assays demonstrated the differences of these spectra (Fig. 4) and frequencies (Table V). Induced reversion frequency in diploid strains was higher. The frequencies of GC-AT and GC-TA in diploid hemyzigote strains were approximately three times more than in haploid strains at 1000 Gy. The spectra of spontaneous and induced mutations were similar for the same ploidy. Spectrum did not depend on dose (Fig. 4).

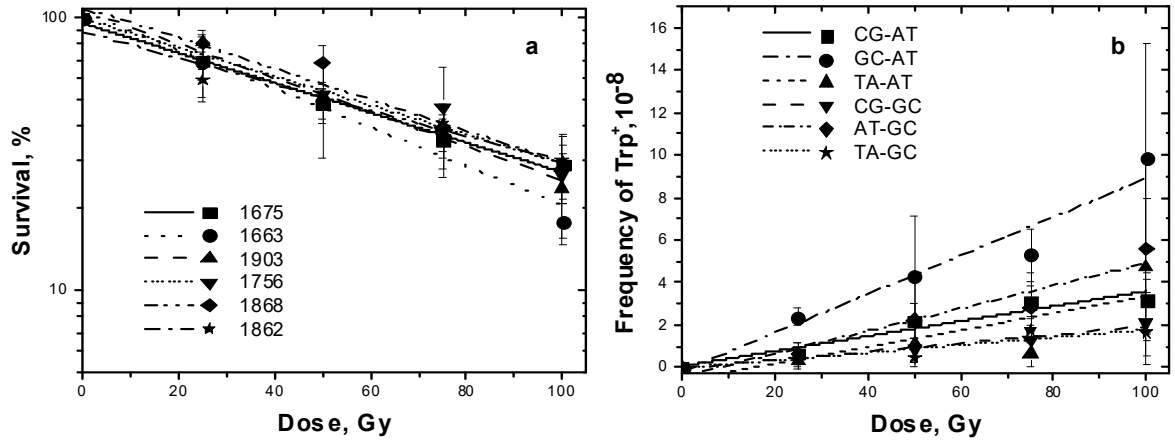

Fig. 1 Cell killing (a) and mutagenesis (b) of haploid trp5-strains after γ-irradiation

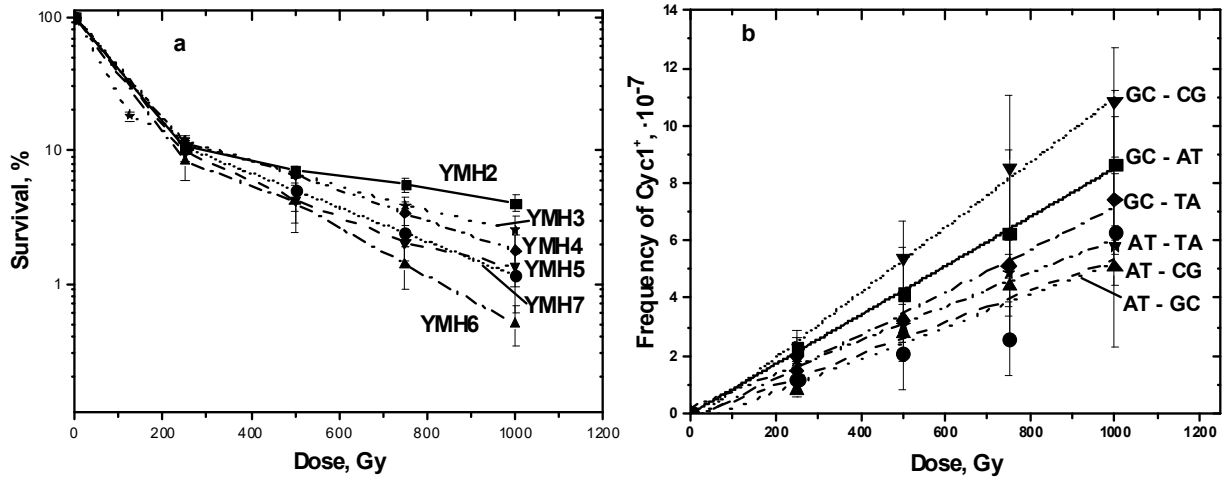

Fig. 2 Cell killing (a) and mutagenesis (b) for *cyc1*-strains of haploid yeast after γ-irradiation. Represent a mean of 3-6 experiments and standard errors

TABLE IV
SLOPE OF LINEAR FUNCTION OF MUTATIONS FOR TWO HAPLOID ASSAYS

Mutation	Slope		F (*trp5*/*cyc1*)
	trp5-assay	*cyc1*-assay	
Transition			
G→A	0.0908±0.01228	0.00853±0.00017	10.6
A→G	0.0533±0.00836	0.0056±0.00125	9.5
Transversion			
G→T	0.03533±0.00554	0.0074±0.0003	4.8
G→C	0.01968±0.00169	0.01132±0.00058	1.7
A→T	0.03929±0.01684	0.00587±0.00035	6.7
A→C	0.01807±0.00464	0.00548±0.000498	3.3

Molecular mechanisms of mutagenesis are known well, but their regulation and choice are not studied. Ionizing radiation induces single-strand breaks, double-strand breaks, AP-sites, and also has an indirect effect. Radicals from water radiolysis mainly induce oxidative damage to DNA which causes mispairing during DNA replication [7]. Most of cells reversions occur in the results of translesion synthesis (TLS) across damage by different DNA polymerases. Oxidative modifications of DNA may decrease the accuracy of replicative DNA polymerases, as was observed for DNA polymerase β in mammalian [8]. The GC-AT transition is known to be induced by various types of base lesions. Cytosine glycol, a form of oxidized cytosine, is a possible candidate lesion that induced GC-AT transitions. Cytosine glycol often changes to 5-hydroxy-uracil by dehydration and deamination, which is mispaired with adenine and induces the GC-AT transition. The GC-TA and AT-CG transversions are mainly induced by guanine oxidation and subsequent 8-oxoguanine and adenine mispairing during DNA replication. The response of DNA polymerases to DNA damage is lesion-specific and depends on the specific interaction between the lesion and the polymerases. The yeast DNA polymerase η efficiently bypassed 8-oxoguanine, incorporating C, A, and G opposite to the lesion with a relative efficiency of ~100:56:14, respectively [9]. The Rev1 transferase efficiently incorporates C opposite G [10].

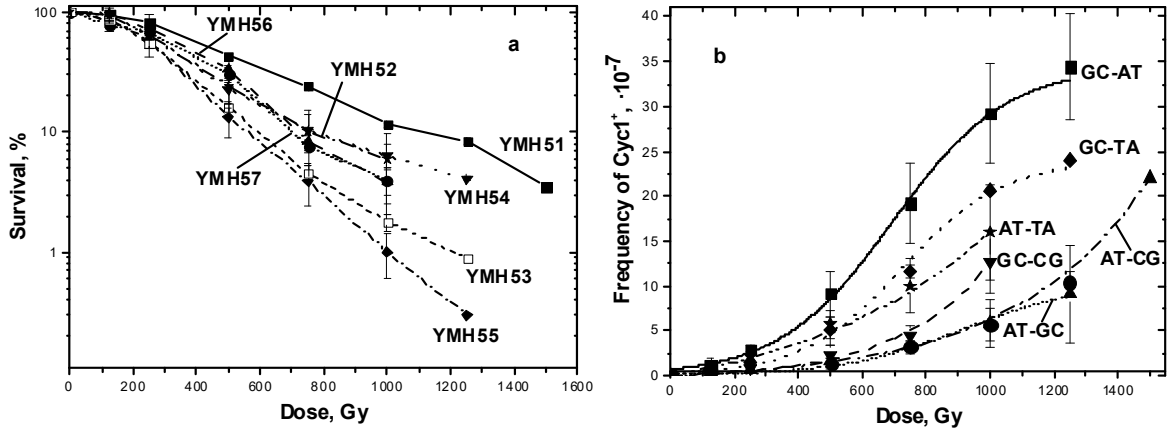

Fig. 3 Cell killing (a) and mutagenesis (b) of diploid *cyc1*-strains after γ-irradiation [6]. Represent a mean of 3-6 experiments and standard errors

TABLE V
MUTATION SPECTRA OF HAPLOID AND DIPLOID YEAST *SACCHAROMYCES CEREVISIAE* AFTER Γ-RAY IRRADIATION (1000 GY)

Mutation	Haploid strain		Diploid strain		F (diploid/haploid)
	0 Gy	1000 Gy	0 Gy	1000 Gy	
Transition					
G→A	$4 \cdot 10^{-9}$	$(8.7 \pm 2.6) \cdot 10^{-7}$	$12 \cdot 10^{-9}$	$(29.2 \pm 5.5) \cdot 10^{-7}$	3
A→G	$6 \cdot 10^{-9}$	$(6.3 \pm 4.0) \cdot 10^{-7}$	$15 \cdot 10^{-9}$	$(5.8 \pm 2.7) \cdot 10^{-7}$	0.9
Transversion					
G→T	$4.5 \cdot 10^{-9}$	$(7.4 \pm 0.9) \cdot 10^{-7}$	$4.3 \cdot 10^{-9}$	$20.6 \cdot 10^{-7}$	2.8
G→C	$10 \cdot 10^{-9}$	$(10.8 \pm 1.9) \cdot 10^{-7}$	$7.4 \cdot 10^{-9}$	$(12.6 \pm 3.5) \cdot 10^{-7}$	1.2
A→T	$<10^{-8}$	$(5.8 \pm 0.3) \cdot 10^{-7}$	$5 \cdot 10^{-9}$	$(16.0 \pm 5.3) \cdot 10^{-7}$	2.8
A→C	$4.5 \cdot 10^{-9}$	$(5.1 \pm 0.6) \cdot 10^{-7}$	$<10^{-8}$	$(5.6 \pm 1.9) \cdot 10^{-7}$	1.1

AP sites are significant spontaneous and induced DNA lesions. In yeast, the error-prone bypass of AP sites is realized by Rev1 and Rev3-Rev7 proteins [10], [11]. An important role of the Rev1 protein is to insert a dCMP opposite to the template AP site such that the Polζ activity encoded by *REV3* and *REV7* genes could continue the primer extension further downstream of the AP site [10]. Moreover, the yeast Polη is able to insert efficiently a nucleotide opposite the template AP site *in vitro* [9]. Synthesis product may be subsequently extended by another DNA polymerase, for example Polζ, to achieve a complete AP site bypass. Since the Polη is DNA damage-inducible [12], [13], the putative contribution of this polymerase to AP site bypass, if any, may only be important when cellular DNA is damaged by exogenous agents. Also, Polα alone is able to bypass efficiently a template AP site [9].

The mutation analysis in the present work showed an increase in the total number of base substitutions after γ-ray irradiation, but it was observed differences for three genetic models. The frequency of GC-AT transitions increased significantly following γ-ray exposure for all assays. In haploid assays, there were more frequent G-A and A-G transitions for *trp5*-assays and G-C and G-T transversions for *cyc1*-assay. In diploid *cyc1*-assay, G-A transition and G-T transversion were more frequent. The mutation spectrums may differ for bacteria, yeast, and mammalian. Sequence analysis showed that more than 78 % changes in damage sites induced by radiation in bacteria and mammalian cells were transversion [14]. We have observed the predominating of GC-TA transversion (20-30%) and transition GC-AT (20-50%).

The reason of differences in the spectra of *trp5*- and *cyc1*-assays is not known, but it may be suggested that they differ depending on the sequence context. The differences in dose dependence functions of haploid and diploid *cyc1* reversions could be due to their rise by fundamentally different mechanisms. It is known that reversion in the diploid occurs during heteroallelic recombination with the homolog. It was shown that double-strand breaks DNA, and their repair can be important sources of mutations which occur wherever breaks located [15], [16]. Although we use hemizygous diploid strains and they have only one allele for *cyc1* gene but double-strand break may occur near *cyc1* gene, and this region is resized by recombination repair in G2 phase. In any case, the regulation and choice of pathway of mutagenesis is unknown and is required for the further study.

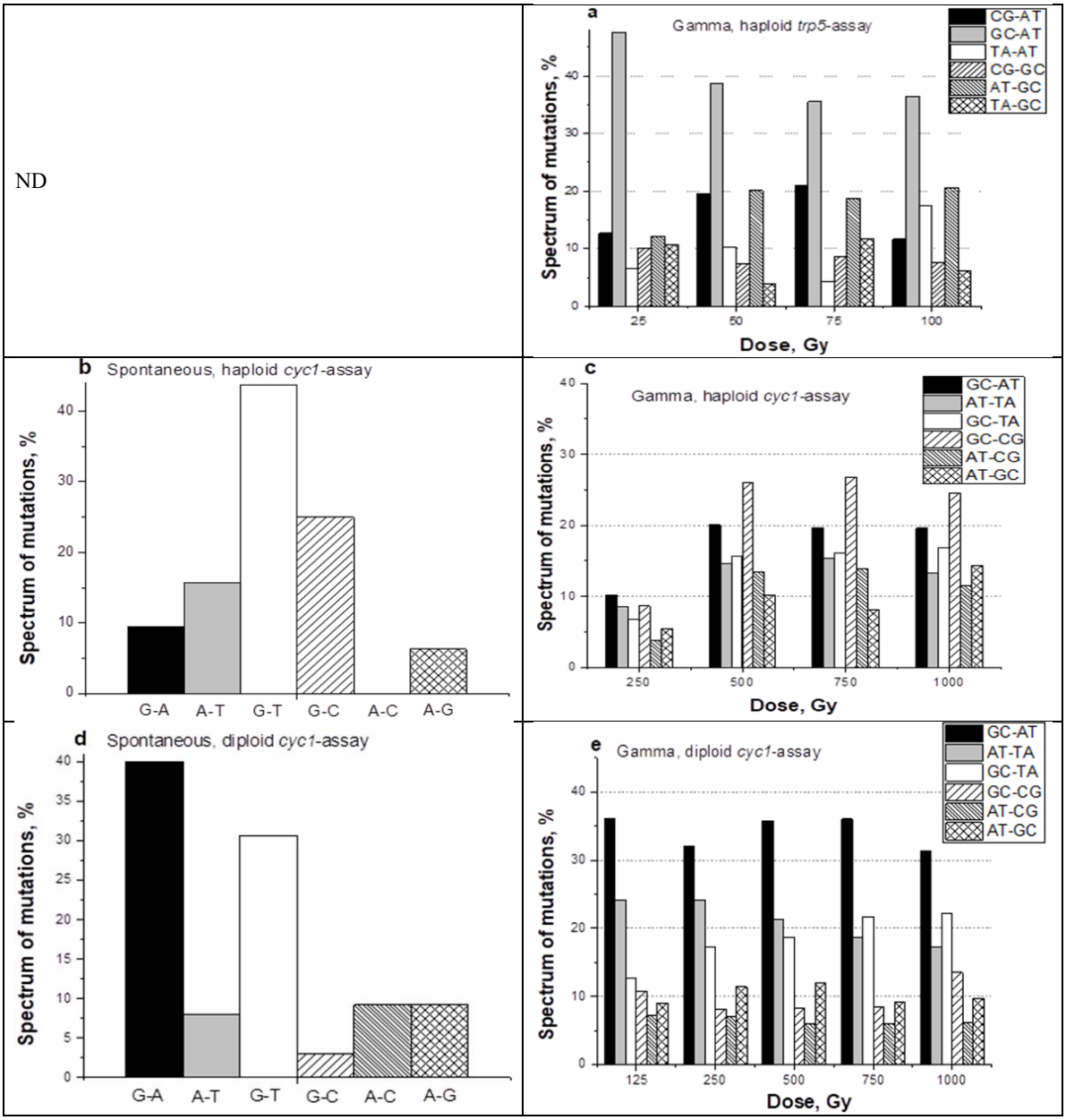

Fig. 4 Spectrum of base pair substitutions in haplod *trp5*- (a) and *cyc1*- (b, c) strains and diploid *cyc1*-strains (d, e) spontaneous (b, d) and induced by γ-ray (a, c, e). ND – nondetermined

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