RADIATION&APPLICATIONS
ISSN 2466-4294 (online) | rad-journal.org
Vol. 2 | Issue 1 | pp. 10 – 13
doi: 10.21175/RadJ.2017.01.003
Original research paper

KINETICS OF UV-INDUCED GENE AND CHROMOSOME MUTATIONS*

Natalia Koltovaya, Alexandra Kokoreva, Natalia Shvaneva, Nadya Zhuchkina**

Joint Institute for Nuclear Research, Dubna, Russia

Abstract. *To study the kinetics of UV-induced gene and chromosome mutations in the yeast S. cerevisiae, several genetic assays were used. We treated yeast cells with UV light of up to 130 J/m². UV irradiation induced all types of base substitutions, although transitions — in particular, GC-AT events — were predominant. Frameshift mutations were induced at the same frequency as the base pair substitution GC-AT, while forward mutations in the CAN1 gene exceeded the more expressive base pair substitutions by about an order of magnitude. Chromosome mutations were the most efficient. The kinetic of the induced gene and chromosome mutations is represented by a linear-quadratic function. Such curves have been reported for UV mutagenesis in bacteria and they have been explained by the induction of SOS error-prone repair. Similar biphasic kinetics was described for yeast in our work. These data suggest the occurrence of several factors forming the mutagenic response of eukaryotic cells to UV light.*

Key words: *UV irradiation, frameshift mutation, base pair substitution, forward mutation, rearrangement of chromosome and plasmid DNA, yeast*

Ultraviolet (UV) light has strong genotoxic effects – it can induce DNA damage, mutations, and, in the worst case, cancerogenesis. Several human genetic disorders, including xeroderma pigmentosum and Cockayne syndrome, are characterized by a defect in UV lesion repair. Investigations of the mechanisms of mutagenesis are continued and our knowledge is made more profound. To study the kinetics of the UV-induced gene and structural mutations on the bases of the model of the yeast *S. cerevisiae,* several new interesting genetic assays were used. They included a forward mutation rate assay that detects mutations inactivating the arginine permease gene (Can^R mutations), frameshift reversion assays [1] detecting mutation that reverts a 4-base insertion in the *LYS2* gene (*lys2ΔBgl*), and a collection of six isogenic *trp5*- strains, specifically diagnostic for all possible base-pair substitutions [2]. Assays for intrachromosome homologous recombination (HR repair) based on the 5'trancated *lys2* sequence and the *LEU2* gene integrated into chromosome II as a direct repeat with the *lys2:HS-D* allele which is 658 bp-insertion in *BamH1* site of 3'-termini of *LYS2* gene are shown in [3]. The [YCpL2]-plasmid assay was used to detect the extent of deletions including two or more genes which arise during NHEJ repair [4].

Mutant strains were grown nonselectively in YEPD. Selective growth was on a synthetic complete medium containing 2 % glucose (SM) [5] and lacking the appropriate nutrient [6]. Canavanine-resistant mutants in the forward mutation assay were identified on SM-Arg plates supplemented with 60 μg/ml canavanine. All growth was at 30° C. Overnight cultures (~$2x10^8$ cells/ml) were grown in 5 ml YEPD. Cells were resuspended in water and plated on YEPD and appropriate SM-based selective media to assess cell survival and mutagenesis, respectively. Within 1 hr of plating, cells were exposed to 254 nm UV light and varying doses up to 130 J/m² in the dark and put in the tube to avoid photoreactivation. Colonies that arose on YEPD and SM plates were counted after 4-5 days of incubation, respectively. Each data point corresponds to the mean of 3-4 independent experiments, and error bar represents the standard deviation.

The dose-response curves of gene mutation induction are shown in Fig. 1. The frequency of UV-induced Can^R mutants was observed with an ~600-fold increase in Can^R mutant frequency at the highest UV dose relative to the spontaneous frequency (Fig. 1a).

A strong induction of Lys^+ revertants was evident following UV irradiation (Fig, 1b); at the same dose of 100 J/m², the reversion frequency of the *lys2ΔBgl* allele was stimulated ~500-fold. The frequency of frameshift mutation in genes is related to the nature of the target. Frameshift allele *lys2ΔBgl* reverts in the 5A and 4C tracks by falling of a compensatory -1 frameshift mutation within a defined 146-bp reversion window [1], although the +1-reversion frequency of the *lys2ΔA746* allele was stimulated ~800-fold at a dose of 60 J/m² [7]. In *Escherihia coli* the majority of UV-induced mutations are base substitutions at dipyrimidine sites, with frameshifts typically composing <25% of the spectra [8, 9]. So, as in bacterial cells, frameshift mutations can compose a sizeable fraction of UV-induced gene mutations in yeast.

* The paper was presented at the Fourth International Conference on Radiation and Applications in Various Fields of Research (RAD 2016), Niš, Serbia, 2016.

** koltovaya@jinr.ru

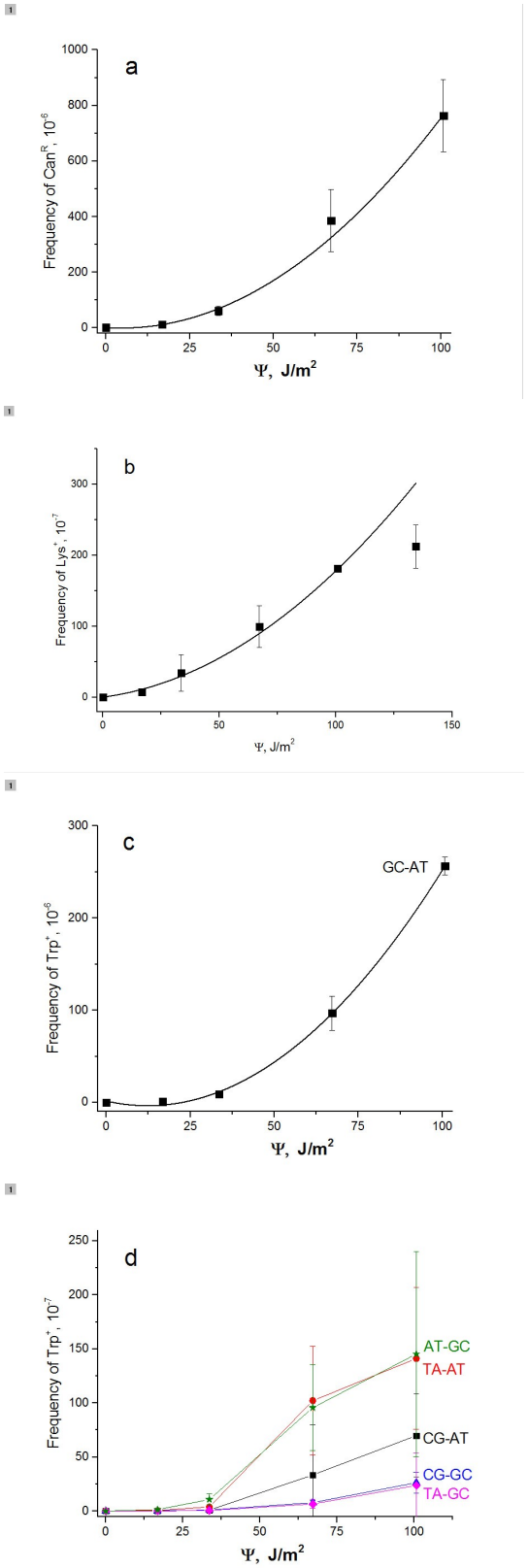

Figure 1. UV induced mutagenesis. Frequency of UV-induced forward mutation at *CAN1* (a), *lys2ΔBgl* reversion (frameshift) (b) and *trp5* reversion (base pair substitution) (c, d).

Table 1. Frequency of mutation induced by UV light (100 J/m²) in haploid strains

Event	Frequency
Lethal damage	~$9.7 \cdot 10^{-1}$
Rearrangement	
recombination (HR)	10^{-1}
deletion (NHEJ)	$(1.8 \pm 1.19) \cdot 10^{-4}$
Gene mutation	
Can^R	$(0.76 \pm 0.13) \cdot 10^{-3}$
GC-AT	$(2.56 \pm 0.10) \cdot 10^{-4}$
-1 nt	$(1.8 \pm 0.2) \cdot 10^{-5}$

UV irradiation induced all types of base substitutions, although transitions — in particular, GC-AT events — were predominant (Fig. 1c). We observed the stronger induction of GC-AT transition than of frameshift mutants at the dose of 100 J/m² (see Table 1). AT-GC and TA-AT were induced by UV light less efficiently (Fig. 1d). In the case of UV-induced mutations in the complete gene (*SUP4-o*), it was the same spectrum of mutations; base pair substitutions composed 92 % with predominantly GC-AT (65.9 %), then, AT-GC (18.7 %) and TA-AT (8.1 %) [10].

Chromosome mutations were induced by UV light more efficiently (Fig. 2). At the dose of 100 J/m² the frequency of chromosome recombination in the results of HR was 10^{-1} and the deletion of 2-4 plasmid genes in the results of NHEJ was $1.8 \cdot 10^{-4}$. The size of plasmid YCpL2 is 13.8 kb.

The survival curves were typical for exposure by UV light and close for using strains. In Fig. 3 we represented the reciprocal curve of lethality. It is shown that at 100 J/m² on 10 lesions only 1 was repaired by HR and 9 were lethal.

The kinetic of the induced gene and chromosome mutations is represented by a linear-quadratic function. Curves of lethality presented in Fig. 3 were also linear-quadratic polynomial fits).

Quadratic ("dose-squared") induction curves are typical for UV mutagenesis in bacteria *E. coli* and require two pyrimidine dimmers, one to serve as a premutational lesion, the other to stimulate the induction of the error-prone repair system (the "one lesion+SOS induction" hypothesis) [11]. Similar biphasic kinetic was observed in eukaryotic yeast. These data suggest the occurrence of several factors forming the mutagenic response of eukaryotic cells to UV light. In the G1 phase, the damages are repaired by NER. NER is error-free. Unrepaired DNA lesions in the template strand block synthesis by replicative DNA polymerases. But there is a replication through DNA lesions via translesion synthesis (TLS) by specialized DNA polymerases (error-prone) and by template switching (error-free). As in bacteria, pyrimidine dimers stimulate induction of the error-prone TLS repair pathway, particularly polymerase Polη (Rad30). The UV induction of *RAD30* transcription [12, 13], *RAD6* [14], and *RAD18* [15] is known. Polη is an error-free polymerases but it is necessary for the work of the second error-prone polymerases Polζ [7]. The mechanisms of mutagenesis are needed in the following investigations.

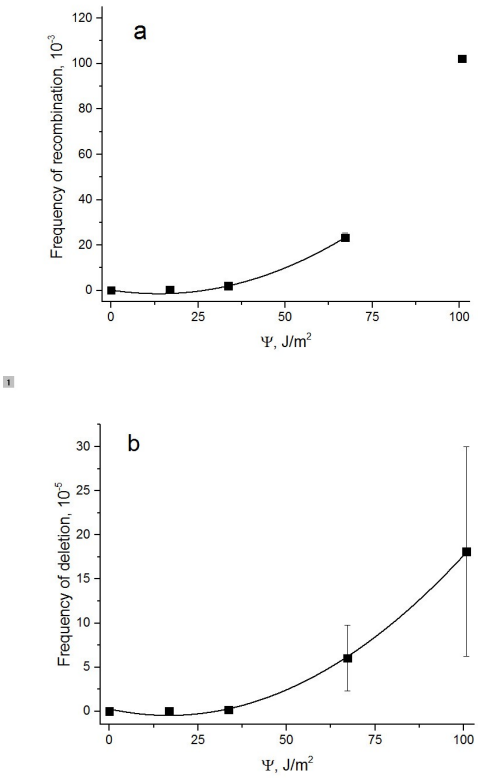

Figure 2. UV induced mutagenesis. Frequency of rearrangements: intrachromosome recombination (a), deletion in plasmid (b).

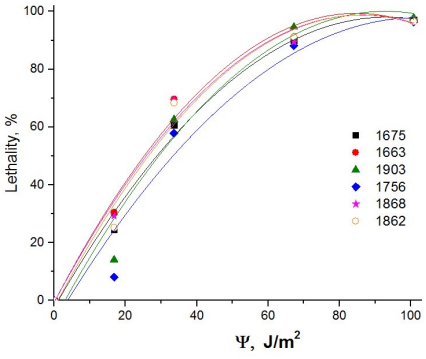

Figure 3. Lethality of haploid *trp5*-tester strains after exposure to UV light

References

1. D. X. Tishkoff, N. Filosi, G. M. Gaida, R. D. Kolodner, "A novel mutation avoidance mechanism dependent on *S. cerevsiaie RAD27* is distinct from DNA mismatch repair," *Cell,* vol. 88, pp. 253-263, 1997.
   DOI: 10.1016/S0092-8674(00)81846-2
2. T-M. Williams, R. M. Fabbri, J. W. Reeves, G. F. Crouse, "A new reversion assay for measuring all possible base pair substitutions in *Saccharomyces cerevisiae,*" *Genetics,* vol. 170, pp. 1423-1426, 2005.
   DOI: 10.1534/genetics.105.042697
   PMid: 15911571
   PMCid: PMC1451166
3. K. S. Lobachev, B. M. Shor, H. T. Tran, W. Taylor, J. D. Keen, M. Resnick, D. A. Gordenin, "Factors affecting inverted repeat stimulation of recombination and deletion in *Saccharomyces cerevisiae,*" *Genetics,* vol. 148, pp. 1507-1524, 1998.
   PMid: 9560370
   PMCid: PMC1460095
4. Y. Tsukamoto, J. Kato, H. Ikeda, "Effects of mutations of *RAD50, RAD51, RAD52*, and related genes on illegitimate recombination in *Saccharomyces cerevisiae,*" *Genetics,* vol. 142, pp. 383-391, 1996.
   PMid: 8852838
   PMCid: PMC1206973
5. A. B. Devin, T. Yu. Prosvirova, V. T. Peshekhonov, O. V. Chepurnaya, M. Ye. Smirnova, N. A. Koltovaya, E. N. Troitskaya, I. P. Arman, "The start gene *CDC28* and the genetic stability of yeast," *Yeast,* vol. 6, pp. 231-243, 1990.
   DOI: 10.1002/yea.320060308
   PMid: 2190433
6. F. Sherman, G. R. Fink, J. B. Hicks, *Laboratory Course Manual for Methods in Yeast Genetics*, Cold Spring Harbor Laboratory New York, 1981.
7. A. L. Abdulovic, S. Jinks-Robertson, "The in vivo characterization of translesion synthesis across UV-induced lesions in *Saccharomyces cerevisiae*: insights into Polζ and Polη-dependent frameshift mutagenesis," *Genetics,* vol. 172, pp. 1487-1498, 2006.
   DOI: 10.1534/genetics.105.052480
   PMid: 16387871
   PMCid: PMC1456278
8. J. H. Miller, "Mutagenic specificity of ultraviolet light," *J. Mol. Biol.*, vol. 182, pp. 45-65, 1985.
   DOI: 10.1016/0022-2836(85)90026-9
9. R. M. Schaaper, R. L. Dunn, B. W. Glickman, "Mechanisms of ultraviolet-induced mutation. Mutational spectra in the *Escherihia coli lacI* gene for a wild-type and an excision-repair-deficient strain," *J. Mol. Biol.,* vol. 198, pp. 187-202, 1987.
   DOI: 10.1016/0022-2836(87)90305-6
10. B. A. Kunz, M. K. Pierce, J. R. Mis, C. N. Giroux, "DNA sequence analysis of the mutational specificity of u.v. light in the *SUP4-o* gene of yeast," *Mutagenesis,* vol. 2, pp. 445-453, 1987.
    DOI: 10.1093/mutage/2.6.445
    PMid: 2832698
11. M. Radman, "Phenomenology of an inducible mutagenic DNA-repair pathway in *Escherihia coli*: SOS repair hypothesis," in: *Molecular and Environmental Aspects of Mutagenesis*, M. Miller, ed., Springfield (IL), USA: Thomas, 1974, pp. 128-142.
12. J. P. McDonald, A. S. Levin, R. Woodgate, "The *Saccharomyces cerevisiae RAD30* gene, a homologue of *Eschrihia coli dinB* and *umuC*, is DNA damage inducible and functions in a novel error-free postreplication repair mechanism," *Genetics,* vol. 147, pp. 1557-1568, 1997.
    PMid: 9409821
    PMCid: PMC1208331
13. A. A. Roush, E. C. Suarez, E. C. Friedberg, M. Radman, W. Siede, "Deletion of the *Saccharomyces cerevisiae* gene *RAD30* encoding *an Escherihia coli DinB* homolog confers UV radiation sensitivity and altered mutability," *Mol. Gen. Genet.*, vol. 257, pp. 686-692, 1998.
    DOI: 10.1007/s004380050698
    PMid: 9604893
14. K. Madura, S. Prakash, L. Prakash, "Expression of the *Saccharomyces cerevisiae* DNA repair gene *RAD6* that encodes a ubiquitin conjugating enzyme, increases in response to DNA damage and in meiosis but remains constant during the mitotic cell cycle," *Nucleic Acids Res.*, vol. 18, pp. 771-778, 1990.
    DOI: 10.1093/nar/18.4.771

DOI: 10.1093/nar/18.16.4737
PMid: 2179869
PMCid: PMC330326

15. J. S. Jones, L. Prakash, "Transcript levels of the *Saccharomyces cerevisiae* DNA repair gene *RAD18* increase in UV irradiated cells and during meiosis but not during the mitotic cell cycle," *Nucleic Acids Res.*, vol. 19, pp. 893-898, 1991.
DOI: 10.1093/nar/19.4.893
PMid: 2017370
PMCid: PMC333727