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KINETICS OF GENE AND CHROMOSOME MUTATIONS INDUCED BY UV-C IN YEAST *SACCHAROMYCES CEREVISIAE*

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Колтовая Н. и др. E19-2017-5
Кинетика индукции генных и структурных мутаций
УФ-излучением у дрожжей *Saccharomyces cerevisiae*

Систематическое исследование закономерностей индукции генных и структурных мутаций в эукариотических клетках выполнено на модельном организме дрожжей *S. cerevisiae*. В работе использовали разнообразные генетические системы для тестирования всех типов замен пар оснований, сдвига рамки считывания, прямые мутации *canl*, перестройки хромосом и плазмиды в гаплоидных штаммах. Клетки облучали УФ-С-излучением, максимальный флюенс энергии составил 200 Дж/м^2. Кинетика индукции генных и структурных мутаций описывается линейно-квадратичной и экспоненциальной функциями. Наклон прямой в масштабе полного логарифма не являлся постоянной величиной, зависел от интервала доз, и величина его составляла от 2 до 4. Предполагается, что суперпозиция и динамика различных путей мутагенного ответа эукариотической клетки на УФ-облучение определяет форму кривой и является причиной степенных функций высокого порядка.

Работа выполнена в Лаборатории радиационной биологии ОИЯИ.

Препринт Объединенного института ядерных исследований. Дубна, 2017

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Kinetics of Gene and Chromosome Mutations Induced
by UV-C in Yeast *Saccharomyces cerevisiae*

The systematic study of the kinetics of UV-induced gene and structural mutations in eukaryotic cells was carried out on the basis of model yeast *S. cerevisiae.* A variety of genetic assays (all types of base pair substitutions, frameshifts, forward mutations *can1*, chromosomal and plasmid rearrangements) in haploid strains were used. Yeast cells were treated by UV-C light of fluence of energy up to 200 J/m^2. The kinetics of the induced gene and structural mutations is represented by a linear-quadratic and exponential functions. The slope of curves in log-log plots was not constant, had the value 2–4 and depended on the interval of doses. It was suggested that it is the superposition and dynamics of different pathways form the mutagenic responses of eukaryotic cells to UV-C light that cause the high-order curves.

The investigation has been performed at the Laboratory of Radiation Biology, JINR.

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Mathematics is well and good but nature keeps dragging us around by the nose.

A. Einstein

Ultraviolet (UV) light has strong genotoxic effects to produce DNA damage, induce mutations, and, in the worst case, cancerogenesis (*Hoeijmakers 2001*). Several human genetic disorders, including xeroderma pigmentosum and Cockayne syndrome, are characterized by a defect in UV lesion repair (*Kannouche and Stary 2003*). Investigations of mutagenesis are being continued and our knowledge is made more profound (*Friedberg et al. 2006*). But there are unresolved problems and one of them is kinetics of mutagenesis in eukaryotes. Quadratic ("dose-squared") induction curve is typical for UV mutagenesis in bacteria *E. coli* and requires two pyrimidine dimers, one to serve as a premutational lesion, the other to stimulate induction of the error-prone repair system (the "one lesion+SOS induction" hypothesis) (*Radman 1974*). Some experimental evidence suggests that mutagenesis in yeast after treatment with DNA-damaging agents involves inducible functions, but a general-acting error-prone repair activity analogous to the SOS system of *E. coli* has not been demonstrated. However, similar biphasic kinetics was observed in eukaryotic yeast (*Eckardt and Haynes 1977*) and for diploid radiosensitive strains (*Lawrence et al. 1974*; *Lawrence and Christensen 1976*). It's worth mentioning that in cited works ambiguous genetic systems were used. We have reinvestigated the kinetics of different types of mutations induced by UV-C light, using modern unique genetic systems. It was found that the obtained curves were fitted by polynomial functions of high order or exponential function. We suggested that the form of the curves is caused by superposition of several curves reflecting several pathways of UV lesions repair which act at different time of cell cycle and depending on the fluence and lesion.

MATERIAL AND METHODS

Strains

To test gene mutations, the strains of yeast *Saccharomyces cerevisiae* RDKY 3023, a set of six *trp5*-testers (Table 1) and a set of six *cyc1*-testers (Table 2) were used in this study. Strain RDKY 3023 (*MAT*a *his3*Δ*200 ura3-52 leu2*Δ*1 trp1*Δ*63 ade2*Δ*1 ade8 hom3-10 lys2*Δ*1Bgl*) was kindly provided

Table 1. Yeast haploid isogenic *trp5*-strains and their corresponding mutational specificities

Strain	Codons 49–51*	Mutational substitusion	Amino acid 50	Reversion
wt	ATC GAA TTG	G148	Glu	—
1868	ATC AAA TTG	G148A	Lys	AT → GC transition
1756	ATC CAA TTG	G148C	Gln	CG → GC transversion
1862	ATC TAA TTG	G148T	Stop	TA → GC transversion
1675	ATC GCA TTG	A149C	Ala	CG → AT transversion
1663	ATC GGA TTG	A149G	Gly	GC → AT transition
1903	ATC GTA TTG	A149T	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 2. 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.

by Prof. R. D. Kolodner (Institute of Genomic Medicine, University of California School of Medicine, California, USA). Allele *lys2ΔBgl* was obtained by insertion GATC in *BglII*-site at N-terminus of gene *LYS2* and allele *hom3-10* — a +1T insertion in a stretch of 6T's in the *HOM3* gene. Mutations *lys2ΔBgl* and *hom3-10* reverted predominantly as the result of base deletion in stretches 5A or 4C in *LYS2* and 7T in *HOM3* (*Tishkoff et al. 1997*). Various *trp5* mutant strains (*Williams et al. 2005*) were derived from the S288C (*MATα his3Δ200 ura3-52 leu2Δ1*), derivative SJR828a, and were obtained from Dr. G. F. Crouse (Emory University, Atlanta, Georgia, USA). Haploid strains YMH1–YMH7 of *cyc1*-tester

set (*Hampsey 1991*) were isogenic derivatives of strain S260-11B (*MAT*α *cyc1-706::CYH2 cyc7-67 ura3-52 leu2-3,112 cyh2*). Diploid strains YMH51–YMH57 were constructed by crossing strains YMH1–YMH7, respectively, with strain B-7462 (*MAT*a *cyc1-1 cyc7067 ura3-52 his1-1 can1-100*). The strains of *cyc1*-tester set were kindly donated by Dr. M. Hampsey (Louisiana State University Medical Center, Shreveport, Louisiana, USA).

To detect rearrangements of DNA, three systems were used. Interchromosomal recombination strains ALE100 and ALE101 carried allele *lys2::HS-D* which is 658 bp-insertion in *BamH1* site of 3′-termini of *LYS2* gene and the 5′-trancated *lys2*-Δ5′ sequence in nonhomologous chromosomes II and III, respectively. Strains ALE1000 and ALE1001 with an intrachromosomal recombination reporter carried *lys2*-Δ5′ and the *LEU2* gene integrated into chromosome II as a direct repeat with the *lys2::HS-D* allele (*Lobachev et al. 1998*). Strains for chromosome rearrangements ALE1000, 1001 (*MAT*a *ade5-1 leu2-3,112 trp1-289 ura3-52 lys2*-Δ5′ *LEU2 lys2::HS-D*) and ALE100, 101 (*MAT*a *ade5-1 leu2-3,112 trp1-289 ura3-52 lys2*-Δ5′ *lys2::HS-D*) were a gift from Dr. D. A. Gordenin (National Institute of Environmental Health Sciences, North Carolina, USA). Strain CRY1 (*MAT*α *rad53 ade2-1 ura3-1 trp1-1 his3-11,15 leu2-3,112 can1-100*) was obtained from Prof. W. Siede (University of North Texas Health Science Center, Texas, USA) and strain 3D (*MAT*α *ade2-G45 cyh2*) was constructed by the author (*Koltovaya et al. 1998*). Strains R1 and R2 (*MAT*α *ade2-1 ura3-1 trp1-1 his3-11,15 leu2-3,112 can1-100 cyh2*), segregants of hybrid CRY1 x 3D were obtained after several back crosses with CRY1, designated below as RAD, and were transformed by plasmid YCpL2 (*Tsukamoto et al. 1996*). The plasmid YCpL2 [CEN3 ARS1 *TRP1 URA3 CYH2 CAN1 LEU2*] was received from Prof. H. Ikeda (University of Tokyo, Japan).

Media

We used standard complete nutrient medium YEPD and medium with glycerol YEPG (*Sherman et al. 1981*), complete medium (CM) and minimal medium (MM) (*Devin et al. 1990*). Selective media (SM) were obtained by addition of eight from nine appropriate amino acids and bases (ade, arg, his, met, trp, ura — 20 mg/l; tyr, leu, lys — 30 mg/l) to medium MM, for example, SM-lys, SM-leu, SM-trp, SM-arg. Antibiotic canavanine was added at a concentration of 60 mg/l.

Experimental procedure

Night cultures ($\sim 2 \cdot 10^8$ cells/ml) were grown in 5 ml YEPD. Cells were resuspended in water and plated on CM and appropriate selective media to assess cell survival and mutagenesis, respectively. Within 1 hr of plating, cells were exposed to 254 nm UV-C light of varying fluence of energy up to 200 J/m^2 in the dark and put in the tube to avoid photoreactivation. Selective growth was on synthetic complete medium containing 2% glucose (SM) and lacking the appropriate nutrient. Canavanine-resistant mutants in the forward mutation

assay were identified on SM-arg plates supplemented with 60 μg/ml canavanine. Selective medium for *cyc*-assay was YEPG. All growth was at 30°C. Colonies arising on CM and SM plates were counted after 4–5 days of incubation. Each data point corresponds to the mean of 3–4 independent experiments, and error bars represent the standard deviation.

Calculation of mutation frequency

The mutation frequency was calculated as mutants per clone scored suitably corrected for the contribution of spontaneous mutants in the original population. That is, the induced mutation frequency is given by $M(x) = N_{\rm mc}/N_s - N_{m0}/N_0$, where $N_{\rm mc}$ is the number of mutants actually counted at fluence x, N_s is the number of survivors at fluence x and the second term corrects for the number of surviving spontaneous mutants at fluence x. N_{m0} is the number of spontaneous mutants in the unirradiated suspension; N_0 is the original number of viable cells in suspension. The induced mutation frequencies calculated in this way were plotted versus fluence on double-logarithmic axis and fitted by polynomial function to determine the order of curve. The program *OriginPro* was used for plotting and analysis of results.

RESULTS

Spectrum of mutations

To study the kinetics of UV-induced local and structural changes in DNA in the yeast *S. cerevisiae,* several modern genetic assays were used. They included: i) a forward mutation assay that detects mutations inactivating the arginine permease gene ($\rm Can^R$ mutations); ii) frameshift reversion assays (*Tishkoff et al. 1997*) detecting mutations that revert a 4-base insertion in the *LYS2* gene (*lys2ΔBgl*) and $+1$ insertion in the *HOM3* gene (*hom3-10*); iii) a collection of six isogenic *trp5*-strains and 14 isogenic haploid and diploid *cyc1*-strains that are specifically diagnostic for all possible base-pair substitutions (*Williams et al. 2005; Hampsey 1991*); iv) assays for intrachromosome and interchromosome homologous recombination (HR) between two direct repeats of *LYS2* gene ($5'$-truncated *lys2-*$\Delta 5'$ allele and $3'$-termini *lys2::HS-D* allele) localizing in the same chromosome II or in the different chromosomes II and III, respectively (*Lobachev et al. 1998*); v) the [YCpL2]-plasmid assay detects extent deletions including two or more genes (*Tsukamoto et al. 1996*).

A total of 27 strains of *S. cerevisiae* were used. It was shown that haploid and diploid strains have the typical survival curves induced by UV-light irradiation (Fig. 1). It was interesting that the diploid strain YMH51 was more sensitive than mutant strains from the set (Fig. 1, *c*, *d*). It may suggest that they are not completely isogenic and acquired the difference in the processes of strain constructions. The respiration can also influence the sensitivity of diploid strains.

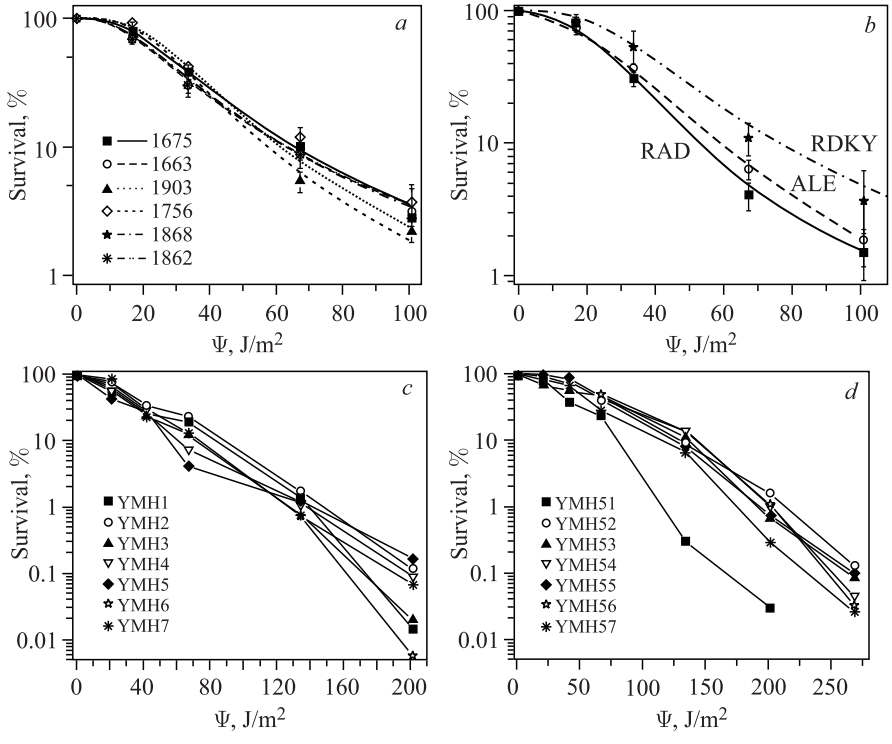

Fig. 1. UV survival curve for *trp5*-tester strains (*a*), RDKY3023, ALE (ALE100-101, 1000-1001) and RAD (R1, R2) (*b*) strains of *S. cerevisiae* used in the studies. The points shown are averages from several independent experiments. Error bars correspond to the standard deviation. Typical UV survival curves for haploid (*c*) and diploid (*d*) *cyc1*-tester strains of *S. cerevisiae* are shown

Strain YMH51 is respiratory sufficient, but strains YMH52–YMH57 are respiratory deficient. It was shown that spontaneous respiration deficient mutants derived from YMH51 didn't completely compensate this difference (data not shown).

The fluence-response curves of gene mutation induction are shown in Fig. 2. The frequency of UV-induced $\mathrm{Can^R}$ mutants was observed with an $\sim$ 600-fold increase at 67 J/m^2 relative to the spontaneous frequency (Fig. 2, *a*) and was $(3.85 \pm 1.12) \cdot 10^{-4}$. For comparison, in work (*Abdulovic and Jinks-Robertson 2006*) the frequency was slightly lower than ours and comprised $\sim 2 \cdot 10^{-5}$ at 60 J/m^2 (survival $\sim$ 10%). It was an $\sim$ 200-fold increase in $\mathrm{Can^R}$ mutant frequency relative to the spontaneous frequency.

A strong induction of the frameshift reversion $\mathrm{Lys^+}$ was evident following UV irradiation (Fig. 2, *b*); the reversion frequency of the *lys2ΔBgl* allele was

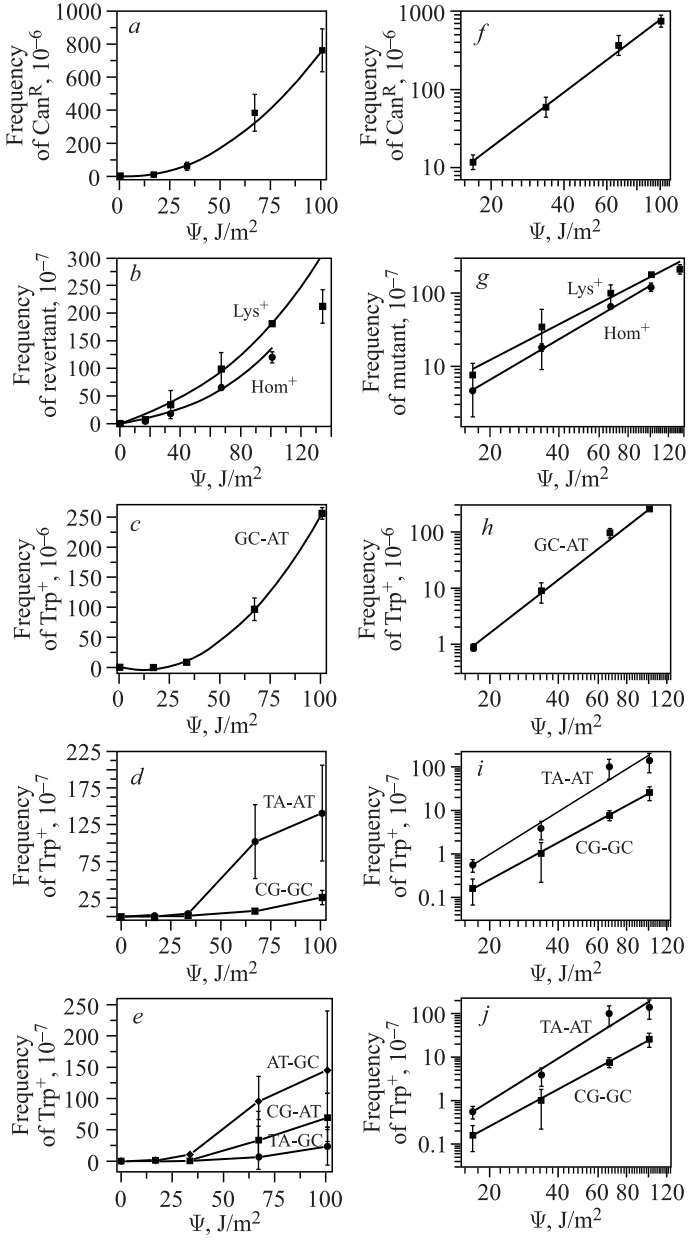

Fig. 2. UV induced mutagenesis. Frequency of forward mutation at gene *CAN1* (*a*), frameshift reversion of *lys2ΔBgl* (*b*) and *trp5*-reversions (base pair substitutions) (*c–e*) induced by UV light. Curves *a–c* were fitted by polynomial function, log-log plots (*f–j*)

stimulated $\sim$ 500-fold and was $(1.81 \pm 0.02) \cdot 10^{-5}$ at the fluence 100 J/m^2. The frequency of frameshift mutation in gene is related to the nature of the target. Frameshift allele *lys2ΔBgl* reverts in the 5A and 4C runs by falling of a compensatory -1 frameshift mutation within a defined 146-bp reversion window (*Tishkoff et al. 1997*). In the *hom3-10* allele there is one 6T's stretch and the frequency was smaller. The frequency of Hom$^+$ was $(1.19 \pm 0.10) \cdot 10^{-5}$ at the fluence 100 J/m^2. These data show a good agreement with investigation of postreplicative mismatch repair (MMR) of frameshift mutations in different runs (*Gragg et al. 2002*) according to which DNA polymerase slippage occurs more often in C/G runs than in A/T runs and MMR repair is preferentially -1 in A/T. A spectrum of mutations at locus *SUP4-o* (*Kunz et al. 1987*) is known. The frequency of frameshift mutations in *SUP4-o* was small. But this gene is small (89 bp) and contains no A/T runs > 3 nt, where most frameshifts were accumulated, for example, in the *lys2ΔA746* assay (*Abdulovic and Jinks-Robertson 2006*). The $+1$-reversion frequency of the *lys2ΔA746* allele was stimulated $\sim$ 800-fold at the fluence 60 J/m^2 and was $\sim 1 \cdot 10^{-5}$. In *Escherichia coli* the majority of UV-induced mutations are base substitutions at dipyrimidine sites, with frameshifts typically comprising $< 25\%$ of the spectra (*Miller 1985; Schaaper et al. 1987*). So, as in bacterial cells, frameshift mutations can compose a sizeable fraction of UV-induced gene mutations in yeast.

UV irradiation induced all types of base substitutions in *trp5*-assay, although transitions GC-AT were predominating (Fig. 2, *c*). We observed more efficient induction of transition GC-AT than of frameshift mutation at the fluence 100 J/m^2 (Table 3). AT-GC and TA-AT are induced by UV light less efficiently (Fig. 2, *d*, e). In the case of UV-induced mutations in the complete gene (*SUP4-o*) it was the same spectrum of mutations; base pair substitutions amounted to 92% with predominating GC-AT (65.9%), AT-GC (18.7%) and TA-AT (8.1%) (*Kunz et al. 1987*).

UV-induced mutations are sequence context dependent. 95% of all substitutions occurred at sites of adjacent pyrimidines with the mutation arising primarily

Table 3. Frequency of mutations induced by UV light (100 J/m^2) in haploid strains

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

at the 3′ position of the pyrimidine (*Kunz et al. 1987*). Similar specificity for UV light has been found at the other yeast loci (*Ivanov et al. 1986; Lee et al. 1988*). Inspection of the sequence context flanking the target base-pairs within codon-22 of *cyc1* reveals that neither of the transition-specific sites was part of a dipyrimidine sequence (Table 2). Each of the four transversion-specific sites was part of a dipyrimidine sequence, although only the sites specific for GC-TA and AT-CG occupied the 3′ position. These were the two most infrequently detected base pair substitutions identified by us and *Kunz et al.* (1987), representing only 3.2% of all UV-induced base substitutions at *SUP4-o*. This information suggests that the *cyc1*-tester strains (Table 2) should exhibit only a weak response to UV. Indeed, none of the six haploid strains was efficiently reverted, even at a UV light fluence that resulted in only 35% cell survival. However, in diploid strains UV-light induced GC-AT, GC-TA, and GC-CG, but not AT-TA, AT-CG, AT-GC (Fig. 3, *a*, data from *Hampsey 1991*). Comparing the survival curves of the same strain, we calculated that 1 s of UV exposure (*Hampsey 1991*) cor-

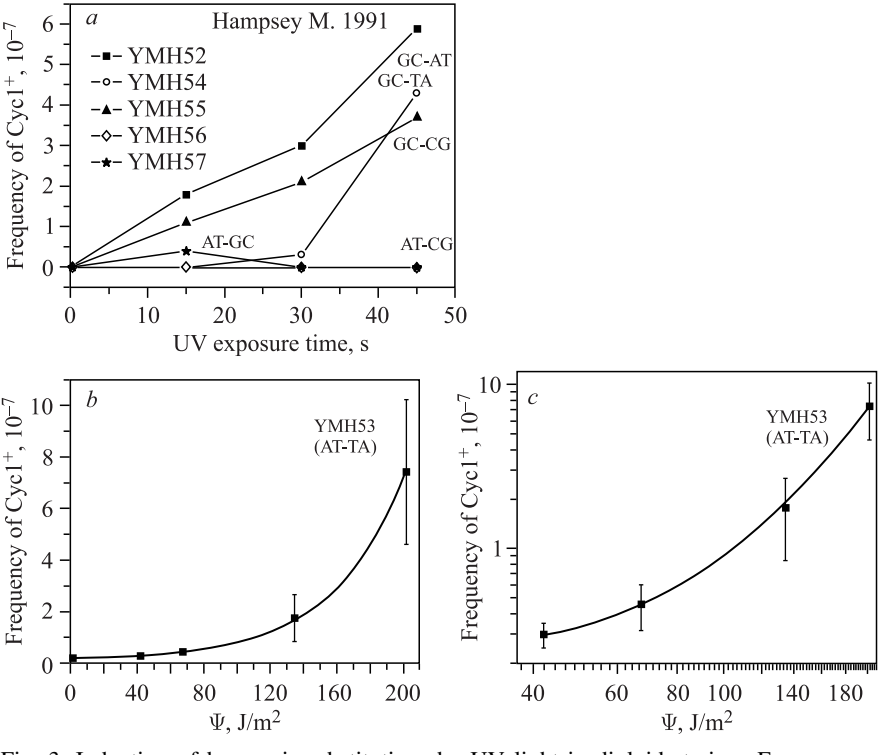

Fig. 3. Induction of base pair substitutions by UV light in diploid strains. Frequency of base pair substitution AT-TA induced by UV light in diploid strain YMH53 (*b*) and log-log plot (*c*). Data reported in *Hampsey 1991* (*a*)

responded to ~ 1 J/m^2. Consequently Dr. Hampsey used UV light fluence up to 45 J/m^2. In our work we used larger fluences of energy and had effects also in the case of AT-TA for strain YMH53 (Fig. 3, *b*). Comparison of base pair substitution spectrum of *trp5* haploid and *cyc1* diploid assays demonstrated the differences of these spectra and frequencies. It may be connected with a different mechanism of mutagenesis.

It's well known that photoproducts can be a source of not only the mutations but also a sister chromatid recombination in diploid (*Haynes et al. 1984*; *Kadyk and Hartwell 1993*). We investigated ectopic recombination in haploid strains. Structural rearrangements were induced by UV light more efficiently than mutations (Fig. 4, *a*). The frequency of chromosome recombination in the results of HR was 10^{-1} at the fluence 100 J/m^2. UV light induced intrachromosome rearrangements more efficiently than interchromosome recombination.

We also studied large deletions in recombined DNA (*Tsukamoto et al. 1996*). The plasmid YCpL2 has size 13.8 kb and contains five genes. Deletion

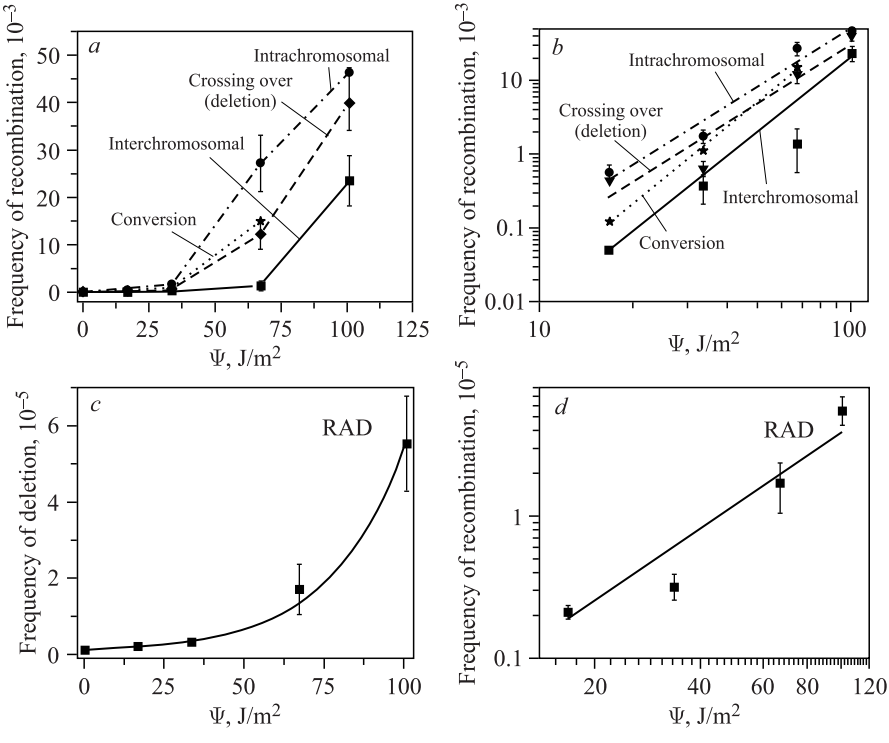

Fig. 4. Rearrangements induced by UV light: (*a*) intrachromosomal and interchromosomal recombinations; (*c*) deletions in plasmid. Log-log plots of induced mutation frequency, correspondingly (*b*, *d*)

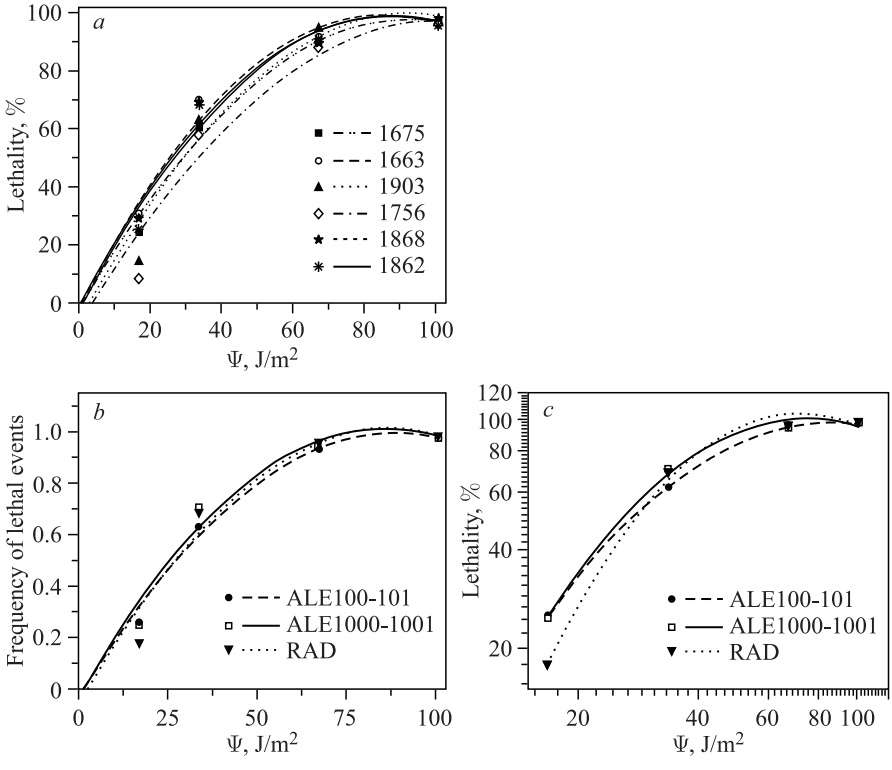

Fig. 5. Lethal effects after exposure to UV light: haploid *trp5*-tester (*a*); ALE100, 101, 1000, 1001, RAD strains (*b*) and their fitting in log-log plot (*c*)

of 2–4 plasmid genes occurred with the frequency $(5.5 \pm 1.25) \cdot 10^{-4}$ at fluence 100 J/m^2 (Fig. 4, *c*).

Formally using survival curves, we can calculate UV-induced lethal events besides lesions leading to mutation. The survival curves were typical for exposure to UV light and close for using strains. In Fig. 5 reciprocal curves of lethality for haploid strains are represented. It is interesting to compare complete spectrum of mutations including lethality (Table 3). The ratio $1 : 10 = \text{HR} : \text{lethal}$ was observed at 100 J/m^2.

The shape of the dose–effect relations

The kinetics of mutations induced in the interval of fluence from 16 up to 100 J/m^2 was represented in Figs. 2–5. Using *OriginPro* we have fitted them by polynomial functions (Fig. 2, *a–c*) or exponential (Fig. 3, *b*; Fig. 4, *c*) and calculated the slope (n) for log-log plots (Fig. 2, *f–j*; Fig. 3, *c*; Fig. 4, *b*, *d*). The coefficient of determination, denoted R^2, a number that indicates how well data

Table 4. The curve slopes for different mutations fitting by polynomial function in log-log plot

Strain/type of mutation	Linear fitting	
	Slope (n)	R^2
Base pair substitution:		
Haploid:		
1663 (GC-AT)	2.0 ± 0.1 (< 12 J/m^2)	0.9896
	1.9 ± 0.1 (< 25 J/m^2)	0.99592
	3.1 ± 0.1 (< 100 J/m^2)	0.9972
1903 (TA-AT)	3.3 ± 0.3	0.97144
1756 (CG-GC)	2.8 ± 0.1	0.9989
1675 (CG-AT)	3.4 ± 0.5	0.92545
1862 (TA-GC)	3.2 ± 0.1	0.99794
1868 (AT-GC)	2.7 ± 0.3	0.97318
Diploid:		
YMH53 (AT-TA)	1.9 ± 0.3	0.93372
Frameshift:		
lys2ΔBgl $\rightarrow$ Lys$^+$	1.6 ± 0.1	0.97373
hom3-10 $\rightarrow$ Hom$^+$	1.8 ± 0.1	0.99654
Gene mutation:		
CAN1 $\rightarrow$ Can^R	2.3 ± 0.1	0.99666
Rearrangements:		
intrachromosome	2.6 ± 0.4	0.94837
conversion	3.5 ± 0.2	0.99555
crossing-over (+deletion)	2.7 ± 0.6	0.87209
interchromosome	3.4 ± 0.2	0.99246
deletion in plasmid	1.7 ± 0.3	0.89322

fit a statistical model, is presented in Table 4. An R^2 of 1 indicates that the regression line perfectly fits the data. As we can see, it was linear-quadratic ($n \sim 2$) for forward (Can$^{\rm R}$) and frameshift (Lys$^+$, Hom$^+$) mutations and n was more than 2 (2.7–3.4) for base pair substitutions ($R^2 > 0.97$). In the case CG-AT coefficient R^2 was smaller (0.93) and it is possible that induced frequency was determined with insufficient accuracy.

Siede and *Eckardt* (1984) have measured the frequency of UV-induced reversions (locus plus suppressor) for the *ochre* allele *his5-2* in the *rev2-ts* strain at permissive temperature and found the linear-quadratic induction kinetics. It was linear up to 10 J/m^2. We also have measured the frequency of base pair substitution GC-AT induced by smaller doses (Fig. 6). But we didn't find linearity at small fluences of energy from 3 up to 12 J/m^2 although value of slope was decreased. It was 2.0 instead of 3.1. Thus, n was not constant but depended on

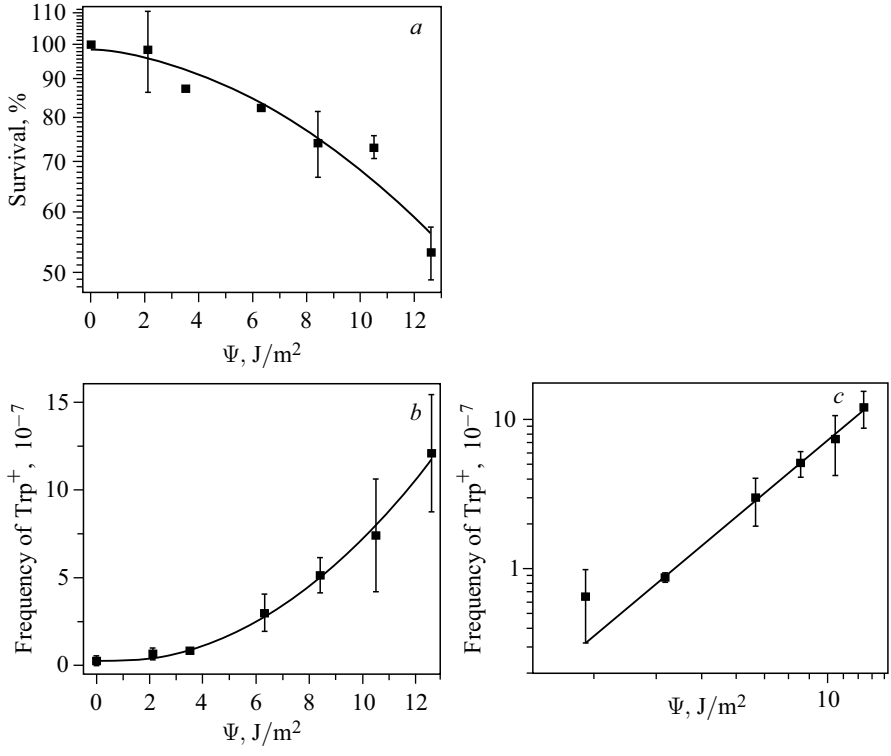

Fig. 6. Lethal (*a*) and mutagenic (*b*) effects after irradiation of strain 1663 by UV light with fluences of energy up to 12.5 J/m^2 and fitting in log-log plot (*c*)

the interval of fluence. Maybe it was possible to find linearity at smaller value of fluence (< 3 J/m^2) but it couldn't exclude that the form of curve depends on nature of mutations. We have measured the frequency of base pair substitutions GC-AT, but *Siede* and *Eckardt* (1984) measured reversion *ochre* mutation (locus+suppressor).

Mutagenesis in the yeast was investigated intensively and authors used variant assays. For example, *Abdulovic* and *Jinks-Robertson* (2006) and *Pages et al.* (2009) have investigated the dependence of forward mutations Can$^{\mathrm{R}}$, and *Harfe* and *Jinks-Robertson* (1999), reversion of frameshift allele *lys2*Δ*A746*, which specifically detects net $+1$ frameshift mutations. Cells were irradiated with varying doses of UV. Using the curves from these articles, we have calculated the slope in log-log plots and obtained approximately the same values as ours, ~ 2 (Table 5). So in repair-competent wild-type yeast we obtained polynomial function from fluence of energy for different types of gene mutations.

Table 5. The curve slopes of log-log plot at fitting by polynomial function. The data were taken from literature

Genotype	Assay	Fluence, J/m^2	Slope (n)	Resource
wt	*his5-2 (ochre)* $\rightarrow$ His$^+$	< 10	~ 1.1 (R^2 0.895)	*Siede, Eckhardt, 1984*
	(locus+suppressor)	10–120	~ 1.6 (R^2 0.96)	
		< 120	~ 1.6 (R^2 0.98)	
rad2-20	*ade2-1 (ochre)* $\rightarrow$ Ade$^+$	< 1	~ 1	*Eckhardt, Haynes, 1977*
		< 5	3.5	
	lys2-1 (ochre) $\rightarrow$ Lys$^+$	< 1	~ 1	
		< 5	2.9	
	ade2 $\rightarrow$ *ade2 adeX*	< 5	Max 1.4	
wt	*CAN1* $\rightarrow$ Can^R	0–60	~ 1.5	*Abdulovic, Jinks-Robertson, 2006*
	lys2Δ*A746* (frameshift) $\rightarrow$ Lys$^+$		~ 2.0	
rev3Δ	*CAN1* $\rightarrow$ Can^R		~ 0.03	
	lys2Δ*A746* $\rightarrow$ Lys$^+$		~ 1.2	
rad30Δ	*CAN1* $\rightarrow$ Can^R		~ 1.2	
	lys2Δ*A746* $\rightarrow$ Lys$^+$		~ 1.9	
wt	*CAN1* $\rightarrow$ Can^R	0–15	~ 1.6	*Pages et al., 2009*
rev3Δ	*CAN1* $\rightarrow$ Can^R		~ 2.0	
rad30Δ	*CAN1* $\rightarrow$ Can^R		~ 1.1	
pol30-119	*CAN1* $\rightarrow$ Can^R		—	
*rev1*S31A	*CAN1* $\rightarrow$ Can^R		~ 0.8	

It was shown that the UV-induced mutation rates (*ade2*, *lys2*; locus + suppressor) of haploid and diploid strains were almost identical (*Eckardt et al. 1975*). The frequencies for suppressor mutations were lower than for locus mutations ($>$ factor 10) in haploid strains. They had different forms — polynomial for locus and linear for suppressor *lys2* $\rightarrow$ Lys^+ mutations. We obtained a different form of curve for diploid cells. In diploid strain YMH53 the curve of substitution AT-TA was fitted by exponential function (Fig. 3, Table 4) and a good fitting by linear-quadratic function in log-log plots ($R^2 = 0.999$) was shown.

In the case of chromosome rearrangements the curves were fitted better by linear-quadratic function for ectopic intrachromosome recombination or exponential for interchromosome recombination and plasmid deletion (Fig. 4). As we can see from Table 4 in log-log plots there was a good linear fitting for interchromosome recombination and intrachromosome conversion ($R^2 > 0.99$); the plasmid deletion was fitted better by linear-quadratic function ($R^2 = 0.99848$). The nonlinear dose–response curves for UV-induced mitotic crossing-over and gene

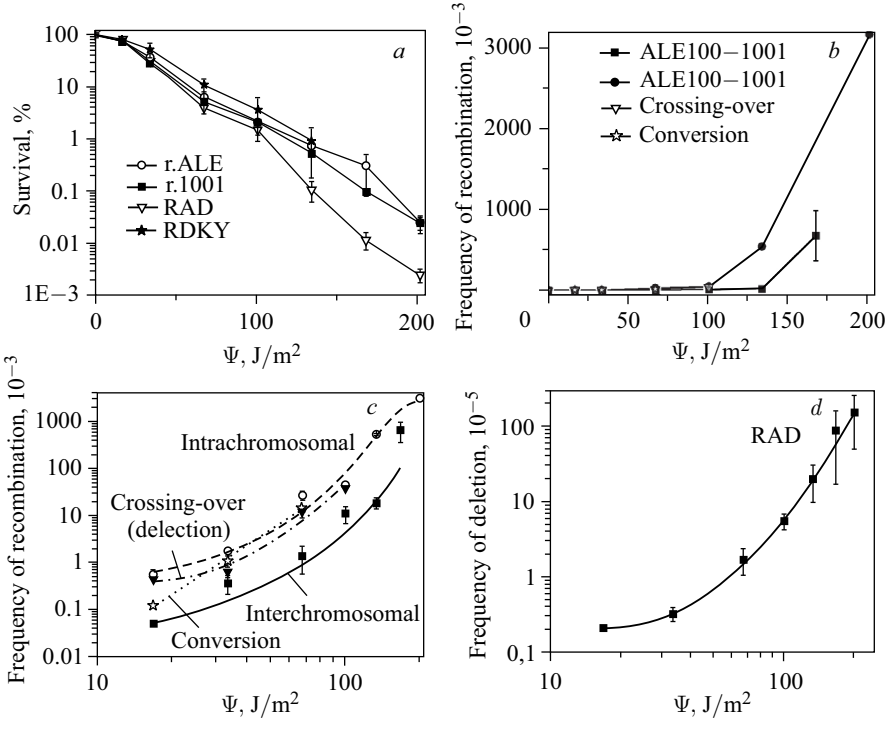

Fig. 7. Survival (*a*), frequency of intrachromosomal and interchromosomal recombination (*b*) induced by UV light with fluence up to 200 J/m^2 and fitting of curves in log-log plot (*c*, *d*)

conversion in wild-type diploid cells were obtained earlier (*Kunz et al. 1985*). Frequency curves of UV-induced mitotic HR were often linear at low fluence of energy. As fluence increased, these curves either increased at higher powers of fluence or/and reached a maximum induced frequency and then declined. The frequency of crossing-over at the *ADE2* locus was higher than frequency of conversion at the *TRP5* locus in diploid strains of yeast. The maximum was achieved at 40 and 80 J/m^2, respectively. Studying chromosome rearrangements in haploid cells, we specially examined fluencies up to 200 J/m^2 but didn't observe any maximum (Fig. 7).

Besides UV-induced mutations we also have UV-induced lethal events. Curves of lethality presented in Fig. 5 were fitted by polynomial (linear-quadratic) function. In log-log plots R^2 was small for linear fitting but good (0.98–0.999) for linear-quadratic fitting.

DISCUSSION

It is interesting to understand the molecular basis of observed dependences. Dimers are the major lethal and mutagenic photoproducts of UV-irradiation. In the presence of light (365–385 nm) photoreactivation of cyclobutane pyrimidine dimers (CPD), but not 6,4 photoproducts (6-4 PP) goes. In the absence of photoreactivation immediately after irradiation nucleotide excision repair (NER) is started. Usually, a nongrowing culture of cells was irradiated in the experiments. In the G1-phase UV-induced lesions are repaired by NER controlling by *RAD2*-epistatic group of genes. NER is preferentially error-free. In phase S unrepaired DNA lesions in the template strand block DNA synthesis by replicative DNA polymerases. Post-replicative repair (*RAD6*-epistatic group) operates in phase G2. In eukaryotic yeast the Rad6–Rad18 ubiquitin-conjugating enzyme complex recognizes and monoubiquitinates replicative complex PCNA and then follows recruitment of DNA helicase/ubiquitin ligase Rad5 (Rev2) to the lesion by the way of interaction with Rad18. Rad5 regulates passing across lesion via interaction with Rev1 for translesion synthesis (TLS) by specialized DNA polymerases or template switching via interaction with Ubc13-Mms2 and following PCNA polyubiquitination (*Kuang et al. 2013*). A Rad6–Rad18-independent template-switching pathway dependent on Rad51, Rad52, and Rad54 proteins can also restore the continuity of newly synthesized DNA from UV-damaged DNA templates (*Prakash 1981; Gangavarapu et al. 2007*). In yeast TLS is a general mechanism of base damage-induced mutagenesis (*Zhou et al. 2010*). The data demonstrate that TLS is also a general mechanism of base damage-induced mutagenesis in higher eukaryote (*Gibbs et al. 1998; 2000; Poltoratsky et al. 2005*). In parallel the MMR repair goes. MMR promotes Polζ-dependent mutagenesis by inhibiting an alternative, error-free pathway that

depends on HR (*Lehner and Jinks-Robertson 2009*). MMR removes mistakes made by replicative DNA polymerases, but fails to efficiently correct errors introduced by Polζ (Rev3).

We suggested that the form of the curve is a result of superposition of several curves corresponding to different repair pathways. How do the genes participating in the different pathways of UV-induced lesion repair influence this dependence? The frequency of induced CPD raises linearity with increases in fluence of UV light energy in haploid and diploid cells (*Unrau et al. 1973; Fäth and Brendel 1975*). About 200 pyrimidine dimers/1 J/m^2 occurs after irradiation of yeast cells by UV light (254 nm) (*Resnick and Setlow 1972*). It being known that the fluence is higher, the occurrence of dimer is more and their excision is less (*Waters and Moustacchi 1975*). The curve of survival at fluence up to 200 J/m^2 rather accurately reflect intensity of repair by dimer excision. Deviations from this curve at higher fluence of UV light are caused by other repair pathways.

In earlier works the quadratic component of mutagenesis has been interpreted as the consequence of inducible mutagenic processes, while the linear component (which predominates at low doses) suggests the operation of a constitutive mutagenic mechanism (*Haynes et al. 1985*). *Eckardt* and *Haynes* (1977) have measured the frequency of UV-induced reversions (locus plus suppressor) for the *ochre*-alleles *ade2-1* and *lys2-1* and forward mutations (*ade2 adeX* double auxotrophs) for strain *rad2-20*, in which NER is disrupted and a large number of lesions occur. Disruptions of *RAD2* completely block all branches of NER. They reported that for very low fluence up to 9 ergs/mm^2 (0.9 J/m^2), both mutational systems exhibited linear induction kinetics. At higher doses up to 50 ergs/mm^2 the curves rose even more rapidly and attained values of n 3.5 and 2.9, respectively. The locus and suppressor mutants independently followed similar kinetics. The curve of forward mutations began linearly and went through a maximum near 14 ergs/mm^2 (1.4 J/m^2). In this case NER didn't work but the mutation frequency had the same dependence, nonlinearity maintained. But linearity at small doses may be causes of NER. As in bacteria, pyrimidine dimers stimulate induction of the error-prone TLS repair pathway by the way of transcription and chemical modifications. The UV induction of *RAD30* (*McDonalds et al. 1997; Roush et al. 1998*), *RAD6* (*Madura et al. 1990*) and *RAD18* (*Jones and Prakash 1991*) transcriptions is known. This interpretation was supported by the observation that extended postirradiation incubation under nongrowth conditions in the presence of the protein synthesis inhibitor cycloheximide reduced the quadratic component (*Kunz et al. 1985*).

Additionally, a *rev2/rad5* mutant that was potentially deficient in an inducible (regulated) component of the mutagenic processing of UV-radiation lesions abolished the nonlinear component of the dose-response curve (*Siede and*

Eckardt 1984). These data suggest the existence of inducible pathways of UV repair and nonlinearity in mutation frequency curves (biphasic, linear-quadratic or higher-order) response. The nonlinearity can arise from higher-order effects inherent in the molecular mechanisms of mutagenesis and/or from "δ-effects" (*Eckardt and Haynes 1977*), i. e., differential probabilities of clone formation for mutant and nonmutant cells.

Using the plots from paper by *Pages* and coworkers (2009), we could estimate the slope of curves of frequency of UV-induced forward mutations Can^R in yeast cells carrying deletion mutations of the various TLS polymerase genes (Table 5). Cells were irradiated with fluence of energies up to 15 J/m^2. Mutation *pol30-119,* where PCNA-Lys164 has been changed to arginine and therefore cannot be ubiquitinated, demonstrated a drastic reduction in UV-mutagenesis. Absence of polymerases Rev1 and Polη but not Polζ cause linearity of mutagenesis. Polη (*RAD30*) is an error-free polymerase but necessary for work of the second error-prone polymerase Polζ (*REV3*) (*Abdulovic and Jinks-Robertson 2006*). So, quadratic effect may be caused by TLS polymerases and inducibility of some repair components by UV-irradiation. The mechanisms of mutagenesis are needed in the following investigations.

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