UDC 551.521.9:582.282.23

MOLECULAR BASES OF THE EFFECT OF LOW DOSES OF RADIATION[*]

© 2020   **V. G. Korolev**

Petersburg Nuclear Physics Institute named by B. P. Konstantinov
of National Research Centre "Kurchatov Institute", Gatchina, Russian Federation
E-mail: *korolev_vg@pnpi.nrcki.ru*

By definition, low doses are minimum doses of a damaging agent, in particular radiation, causing a recorded biological effect. The problem of exposure to low doses of radiation is being discussed in scientific literature for decades, but there is still no generally accepted conclusion concerning the existence of some features of the effect of low doses in contrast to that of acute exposure. This is due to the fact as follows: if being fixed, these effects have a weak expression and can be easily criticized. The second important aspect of this problem is that biological effects are mainly described phenomenologically in literature, without deciphering their molecular causes. In recent years, a number of articles appeared in which the authors, when studying exposure to low doses of DNA-tropic agents, show that postreplication repair (in particular, its error-free branch) plays a key role in these effects. In the laboratory of eukaryotic genetics of Petersburg Nuclear Physics Institute named by B. P. Konstantinov, it was possible to isolate unique yeast mutants with a disrupted branch of error-free postreplication repair. A study of the processes of eliminating DNA damage with minimal deviations of their number from a spontaneous level made it possible to explain at the molecular level the differences in cell response to low doses from acute exposure.

Keywords: low doses, yeast, postreplication repair, tolerance

Cellular genome functions under the constant influence of exogenous and endogenous factors causing DNA damage. It is estimated that in one cell cycle, eukaryotic cells must repair more than 10,000 DNA damage, arising from the effects of endogenous sources only, such as reactive oxygen species, endogenous alkylating agents, and single and double DNA breaks resulting from replication forks collapse. DNA damage number also increases as a result of impact of external factors: chemical mutagens, as well as ultraviolet and ionizing radiation. Unrepaired genetic damage leads to mutations, genetic instability, cancer, and cell death.

DNA damage repair is divided into a number of independent or partially overlapping pathways: nucleotide excision repair (NER); base excision repair (BER); DNA mismatch repair (MMR); postreplication repair (PRR); nonhomologous end joining (NHEJ); and homologous recombination (HR).

---

[*]The materials of the article were presented at the Readings in memory of Academican G. G. Polikarpov "Radiochemoecology: Progress and Prospects" (Sevastopol, IBSS, 2019).

Significant progress has been made in the study of biochemical mechanisms of the main repair pathways, including direct, excisional, recombination, and mismatch. To a lesser extent, this progress affected postreplication repair (hereinafter PRR). This repair type is often included in the system of cell tolerance to DNA damage since DNA damage is not removed but is bypassed in replication process using PRR mechanisms. Such a bypass is not always error-free and is the main source of mutagenesis.

Under normal conditions and under the effect of low doses of mutagens, the key way to combat DNA damage in bacterial and eukaryotic cells is systems of DNA damage tolerance [2 ; 7 ; 8 ; 11 ; 12].

DNA damage tolerance (hereinafter DDT) has historically been called postreplication repair due to the observation that UV treatment of budding yeast cells caused single-stranded gaps in replicating DNA [11]. PRR substrate is replication forks, stopped at DNA damage. UV-induced pyrimidine dimers, causing single-stranded gaps in DNA, were often preserved after "repair"; this indicates that PRR simply bypasses the damage rather than repairs it [3 ; 6].

In all eukaryotic organisms, two different DDT pathways operate: error-prone and error-free ones [10]. In yeast, PRR can also follow two different pathways. The first one is error-prone pathway (translesion synthesis, hereinafter TLS); it involves protein polymerase zeta complex (encoded by *Rev1*, *Rev3*, and *Rev7* genes) and polymerase eta (encoded by *Rad30* gene). These polymerases are conservative in everyone from yeast to human [4]. TLS is controlled by *Rad6*/*Rad18* complex coordinating gap filling by PCNA monoubiquitination. In the second DDT pathway (error-free) one strand (newly synthesized) serves as a matrix for replication of another strand (blocked) [2 ; 12]. The choice between these DDT pathways has serious consequences for genome stability.

The error-free PRR branch, often called recombination one, plays a dominant role in tolerance, since two repair types have a common D-loop formation stage. In the study of relationship between mutagenesis, repair, chromatin dynamics, and cellular cycle, the greatest progress has been made on the example of a unicellular eukaryotic organism: budding yeast *Saccharomyces cerevisiae*. Experiments with yeast have shown that error-free mechanisms are the main PRR pathways under both low and high replication stress [2 ; 7 ; 8 ; 11 ; 12], although the pathway of synthesis through damage (TLS) may also be effective under little number of DNA damage [8].

In response to DNA damage, cells use a net of signal carriers related both to cell cycle passing (the checkpoint) and to repair implementation. It has been noted as follows: in yeast, zero mutants by the checkpoint are surplus in TLS, but partially defective in gap filling [10]. It is still not clear how do these effects relate to the role of the replication checkpoint in maintaining the stability of stopped replication forks or in regulating factors, providing tolerance [5]. In any case, the checkpoint machine obviously modulates cell response to DNA damage.

Earlier, for the first time in the world, using direct screening, yeast mutants, characterized by increased induced mutagenesis and practically unchanged sensitivity to mutagens lethal action, were isolated by us, as well as spontaneous mutators [1 ; 9]. Epistatic analysis of these mutants showed that they belong to three groups; mutants of *HSM3* epistatic group belong to the error-free PRR branch. Further study of these mutants (with the most pronounced mutator phenotype) showed that the products of these genes are related to control of polymerases involved in gap filling in DNA. Replacement of accurate replicative polymerases with inaccurate polymerase Polη often occurs in mutant cells, and this significantly increases mutation rate. The study of molecular mechanisms of biological action of ultra-small number of DNA damage is very convenient if using methods of accounting for spontaneous mutagenesis in yeast (Table 1).

Table 1. Spontaneous mutagenesis in repair mutants

Strain	Mutation frequency per generation, $\cdot 10^{-7}$ (replicative)	Mutation frequency per generation, $\cdot 10^{-7}$ (reparative)
Wild type	3.2 ± 0.3	3.2 ± 0.6
rad1	10 ± 1.4	28 ± 4.0
rad2	2.4 ± 0.5	18 ± 3.9
rad14	3.3 ± 0.2	31 ± 3.5
pol3	80.2 ± 7.2	75 ± 4.5

Table 1 shows our results for measuring mutation rate by two methods. The first one is Lea – Coulson method. It measures mutation rate in cells, growing under the most favorable conditions. The generation lasts for less than 2 hours. During this time, the number of spontaneous DNA damages in cells is minimal, and most of the mutagenesis is a consequence of replication errors. The second one is method of ordered seeding; it has been developed in the Leningrad State University. This method is simpler and more convenient in execution, but, as we have shown, it is applicable only for strains with an undamaged repair system. This method differs from the previous one: cell cycle is artificially stretched many times (it lasts for several days). In this case, a significant number of spontaneous damages accumulate in DNA, which are effectively removed in cells with normally functioning repair. In cells with disrupted repair system, some of these damages remain and get into the replication fork.

As it can be seen from the Table 1, wild type cells show the same mutation rate in both tests. In polymerase mutant, where all the increased mutagenesis is defined by errors of damaged polymerase, two tests also give identical mutation rate. At the same time, all repair mutants show a significantly higher mutation rate in the Leningrad Test (see Table 1).

The data in Fig. 1 are a good illustration of the effects of low doses of spontaneous damage. This figure shows the effect of adaptive mutagenesis, controlled by *HSM3* gene we have discovered. In the upper row, there are dishes with antibiotic, sown with wild type cells. Colonies of antibiotic-resistant mutants can be seen, which grew after 3 days (dish on the left) and after 15 days (the same dish on the right). Dishes of the lower row were sown with *hsm3* mutant cells. It is noticeable that in the upper row, the difference in colonies number after 3 and 15 days is little. In the lower row, this difference reaches 2 orders of magnitude.
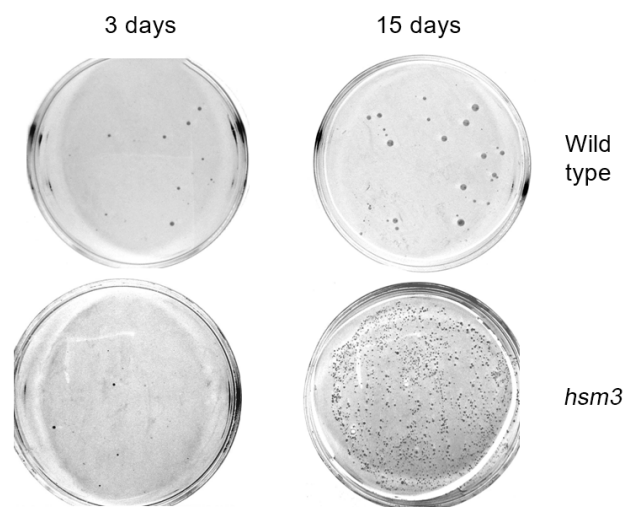
Fig. 1. Adaptive response of *hsm3* mutant with a disrupted error-free branch of postreplication repair

A very subtle tool for assessing the effect of a little number of DNA damage on cell survival is the measurement of spontaneous death of mutant cells along certain repair pathways. For example, disabling of recombination repair blocks DNA repair from double-strand breaks, which rarely occur in normally growing yeast cells (less than 1 break per generation). Nevertheless, we can see a significant increase in the proportion of dead cells in population with blocked recombination repair: wild type – (3.6 ± 1.2) %; *rad52* mutant – (10.1 ± 3.2) %.

Low doses of DNA damage do not activate the checkpoint induced by DNA damage. The checkpoint may not be essential for survival under these conditions [5]. Therefore, in cells with blocked nucleotide excision repair after low-dose irradiation, almost all UV-induced damages get into the replication fork and are exposed to PRR. In our experiments, nucleotide excision repair mutants were used for studying the characteristics of this PRR type. We injected into *rad2* mutant an additional *hsm3* mutation, disrupting the main pathway of the error-free repair branch. As it can be seen from Fig. 2, the double mutant showed significantly higher UV-resistance than the single *rad2* and very high induced mutagenesis. Thus, disabling of error-free repair branch directs DNA damage to an erroneous repair pathway being less cytotoxic.
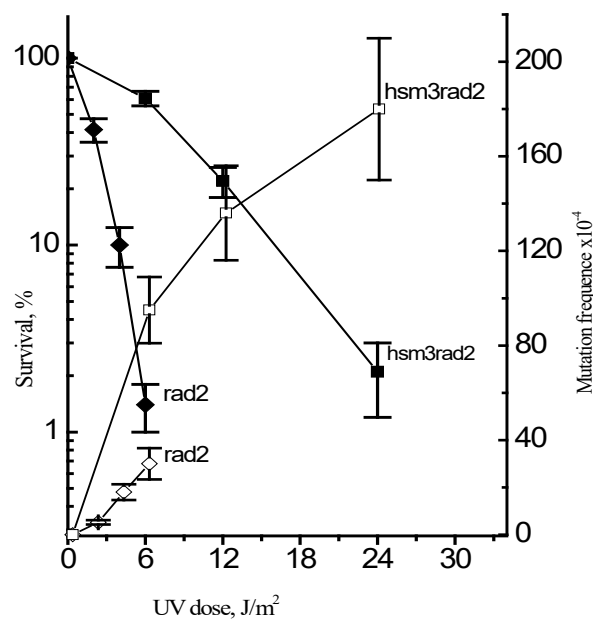

Fig. 2. Effect of blocking the error-free branch of postreplication repair

Japanese scientists have obtained interesting data on the effect of low doses of UV rays on yeast cells [7]. They showed (Fig. 3) that *rad14* mutant, which blocks nucleotide excision repair, grows under conditions of chronic irradiation at about the same rate as wild type cells, while cells of *rad18* mutant (the one that blocks PRR) show high sensitivity to this impact. At the same time, according to our studies, behavior of *rad14* and *rad18* mutants in the experiment with usual dose commitments (acute irradiation) has a completely different character (Fig. 4). In this case, *rad14* mutant is much more sensitive than *rad18*. There are two main reasons for this paradoxical difference. Firstly, with a little number of DNA damage, the checkpoint is not activated; as a consequence, there is no induction of repair systems being under control of the checkpoint. Secondly, at low doses, most of the resulting DNA damage avoids the action of non-activated repair systems due to difficulties in detecting them and gets into the replication fork. Replication forks, stopped at DNA damage, are PRR substrate.
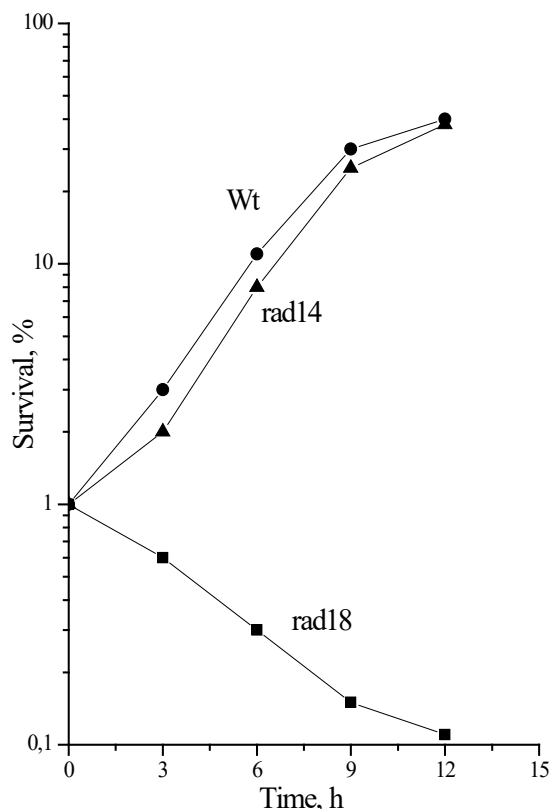
Fig. 3. Dependence of yeast strains survival on exposure to chronic UV with a low dose rate
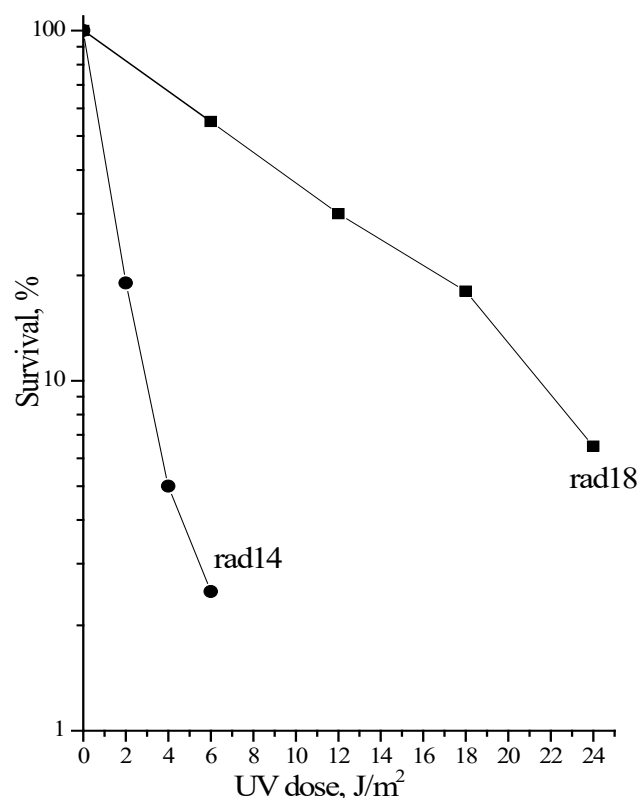
Fig. 4. Survival of *rad18* and *rad14* mutants in an acute experiment

The checkpoint activation has a threshold character and occurs when a certain number of single-stranded DNA accumulates, arising during damage repair. Thus, when a threshold level of DNA damage is exceeded as a result of induction, the efficiency of repair systems increases dramatically, and that allows the cells to get rid of the overwhelming number of DNA damage and to reduce the load on postreplication repair. It follows that the efficiency of DNA damage repair, not reaching a threshold level, will be much lower than in case of its exceeding, and the biological significance of the former will be higher than that of the latter.

REFERENCES

1. Ivanov E. L., Fedorova I. V., Kovaltzova S. V. Isolation and characterization of new mutants of the yeast *Saccharomyces cerevisiae* with increased spontaneous mutability. *Genetika*, 1992, vol. 28, pp. 47–55. (in Russ.)

2. Baynton K., Bresson-Roy A., Fuchs R. P. P. Analysis of damage tolerance pathways in *Saccharomyces cerevisiae*: A requirement for *Rev3* DNA polymerase in translesion synthesis. *Molecular and Cellular Biology*, 1998, vol. 18, iss. 2, pp. 960–966. https://doi.org/10.1128/MCB.18.2.960

3. Bridges B. A., Munson R. J. Mutagenesis in *Escherichia coli*: Evidence for the mechanism of base change mutation by ultraviolet radiation in a strain deficient in excision-repair. *Proceedings of the Royal Society B: Biological Sciences*, 1968, vol. 171, iss. 1023, pp. 213–226. https://doi.org/10.1098/rspb.1968.0065

4. Friedberg E. C. Suffering in silence: The tolerance of DNA damage. *Nature Reviews Molecular Cell Biology*, 2005, vol. 6, iss. 12, pp. 943–953. https://doi.org/10.1038/nrm1781

5. Gangavarapu V., Santa Maria S. R., Prakash S., Prakash L. Requirement of replication checkpoint protein kinases Mec1/Rad53 for postreplication repair in yeast. *mBio*, 2011, vol. 2, iss. 3, e00079-11. https://dx.doi.org/10.1128/mBio.00079-11

6. Ganesan A. K. Persistence of pyrimidine dimers during post-replication repair in ultraviolet light-irradiated *Escherichia coli* K12. *Journal of Molecular Biology*, 1974, vol. 87, iss. 1, pp. 103–119. https://doi.org/10.1016/0022-2836(74)90563-4

7. Hishida T., Kubota Y., Carr A. V., Iwasaki H. *RAD6–RAD18–RAD5*-pathway-dependent tolerance to chronic low-dose ultraviolet light. *Nature*, 2009, vol. 457, pp. 612–615. https://doi.org/10.1038/nature07580

8. Huang D., Piening B. D., Paulovich A. G. The preference for error-free postreplication repair in *Saccharomyces cerevisiae* exposed to low-dose methyl methanesulfonate is cell cycle dependent. *Molecular and Cellular Biology*, 2013, vol. 33, iss. 8, pp. 1515–1527. https://doi.org/10.1128/MCB.01392-12

9. Ivanov E. L., Kovaltzova S. V., Korolev V. G. *Saccharomyces cerevisiae* mutants with enhanced induced mutation and altered mitotic gene conversion. *Mutation Research / Fundamental and Molecular Mechanisms of Mutagenesis*, 1989, vol. 213, iss. 2, pp. 105–115. https://doi.org/10.1016/0027-5107(89)90141-3

10. Pages V., Santa Maria S. R., Prakash L., Prakash S. Role of DNA damage-induced replication checkpoint in promoting lesion bypass by translesion synthesis in yeast. *Genes & Development*, 2009, vol. 23, iss. 12, pp. 1438–1449. https://doi.org/10.1101/gad.1793409

11. Prakash L. Characterization of postreplication repair in *Saccharomyces cerevisiae* and effects of *rad6*, *rad18*, *rev3*, and *rad52* mutations. *Molecular and General Genetics MGG*, 1981, vol. 184, iss. 3, pp. 471–478. https://doi.org/10.1007/bf00352525

12. Zhang H., Lawrence C. W. The error-free component of the *RAD6/RAD18* DNA damage tolerance pathway of budding yeast employs sister-strand recombination. *Proceedings of the National Academy of Sciences of the United States of America*, 2005, vol. 102, iss. 44, pp. 15954–15959. https://doi.org/10.1073/pnas.0504586102

МОЛЕКУЛЯРНЫЕ ОСНОВЫ ЭФФЕКТА МАЛЫХ ДОЗ РАДИАЦИИ[*]

В. Г. Королев

Петербургский институт ядерной физики имени Б. П. Константинова

Национального исследовательского центра «Курчатовский институт», Гатчина, Российская Федерация

E-mail: *korolev_vg@pnpi.nrcki.ru*

По определению, малые дозы — это минимальные дозы повреждающего агента, в частности радиации, вызывающие регистрируемый биологический эффект. Проблема воздействия малых доз радиации обсуждается в научной литературе в течение десятилетий, но прийти к общему выводу о наличии каких-то особенностей их воздействия, в отличие от таковых острого облучения, не удаётся. Это связано с тем, что эффекты, если они фиксируются, имеют слабое выражение и легко могут быть подвергнуты критике. Другой важный аспект проблемы — то, что биологические эффекты в основном описаны в научной литературе феноменологически, без расшифровки их молекулярных причин. В последние годы появился ряд статей, в которых авторы, изучая действие малых доз ДНК-тропных агентов, показывают, что ключевую роль в этих эффектах играет пострепликативная репарация, в частности её безошибочная ветвь. В лаборатории генетики эукариот Петербургского института ядерной физики имени Б. П. Константинова удалось выделить уникальных мутантов дрожжей с нарушенной ветвью безошибочной пострепликативной репарации. Исследование процессов ликвидации повреждений ДНК при минимальных отклонениях их количества от спонтанного уровня позволило на молекулярном уровне объяснить различия в клеточном ответе на малые дозы от острого облучения.

Ключевые слова: малые дозы, дрожжи, пострепликативная репарация, толерантность

---

[*]Материалы статьи были представлены на Чтениях памяти академика Г. Г. Поликарпова «Радиоэкология: успехи и перспективы» (Севастополь, ИнБЮМ, 2019 г.).