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RAD9, *RAD17*, *RAD24*, AND *RAD53* CONTROL ONE PATHWAY OF RESISTANCE TO γ IRRADIATION IN *SACCHAROMYCES CEREVISIAE*

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Гены *RAD9*, *RAD17*, *RAD24* и *RAD53* контролируют один путь γ-резистентности дрожжей *Saccharomyces cerevisiae*

Механизмы генетического контроля прохождения цикла деления клетки (чекпойнт-контроля) изучены наиболее полно у дрожжей *Saccharomyces cerevisiae*. Для выявления роли чекпойнт-генов *RAD9*, *RAD17*, *RAD24* и *RAD53* в радиорезистентности клеток проанализирована чувствительность двойных мутантов к ионизирующей радиации. Показан эпистатический характер взаимодействия этих мутациий с мутацией *rad9*Δ. Полученные данные позволяют предположить, что чекпойнт-гены *RAD9*, *RAD17*, *RAD24* и *RAD53* образуют единую эпистатическую группу, обозначенную как *RAD9*-группа, и регулируют один и тот же путь. Причем гены *RAD9* и *RAD53* имеют позитивный, а гены *RAD17* и *RAD24* негативный эффект на чувствительность к γ-излучению. Для гаплоидных штаммов в отношении чувствительности к воздействию γ-лучей и УФ-света взаимодействие мутаций может отличаться, например, для мутаций *rad9*Δ и *rad24*Δ наблюдается аддитивность в первом случае и эпистаз во втором случае. Анализируемые гены могут также участвовать в минорных механизмах радиорезистентности, которые относительно независимы от вышеупомянутого основного механизма.

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RAD9, *RAD17*, *RAD24*, and *RAD53* Control One Pathway of Resistance to γ Irradiation in *Saccharomyces cerevisiae*

Mechanisms for the genetic control of the cell cycle transition (checkpoint control) have been studied in more detail in yeast *Saccharomyces cerevisiae*. To clarify the role of the *RAD9*, *RAD17*, *RAD24*, and *RAD53* checkpoint genes in cell radioresistance, diploid double mutants were analyzed for cell sensitivity to ionizing radiation. All mutations in combination with *rad9*Δ were shown to manifest the epistatic type of interaction. Our results suggest that the *RAD9*, *RAD17*, *RAD24*, and *RAD53* checkpoint genes belong to a single epistasis group called the *RAD9* group and participate in the same pathway. *RAD9* and *RAD53* have a positive effect on sensitivity to γ irradiation, whereas *RAD17* and *RAD24* have a negative effect. For haploid interactions between mutations may differ in the case of γ or UV irradiation, mutations — for example, *rad9*Δ and *rad24*Δ — were shown to have an additive effect in the first case and epistatic — in the second. The analyzed genes can also participate in minor mechanisms of radioresistance that are relatively independent of the above major mechanism.

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

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1. INTRODUCTION

The tolerance of eukaryotic cells to the damage of chromosomal DNA is ensured by processes of lesion recognition and repair and is also mediated by the so-called checkpoint control [1]. The genetic control of the cell division arrest by a DNA break is well studied in yeast *Saccharomyces cerevisiae* [2–4]. Interruptions of the DNA damage checkpoint often, but not always, lead to increased cell sensitivity to inactivation by DNA-tropic agents. A *chk1* mutant deficient in the DNA damage-induced G_2/M-arrest did not show any increase in sensitivity to the lethal action of damaging agents [5]. A helicase *sgs1* mutant with an affected S/M-checkpoint was as sensitive to γ rays or UV light as normal strains [6]. Mutual independence between cell cycle arrest and cell sensitivity to radiation was also demonstrated for human cells deficient in the *ATM* gene [7, 8]. Some data exist suggesting that *post hoc* may not be completely *propter hoc* here. Among these are the results showing that an additional cell cycle arrest fails to completely suppress an increase in sensitivity to damaging agents in checkpoint mutants of *Saccharomyces cerevisiae*, *viz.* in *rad9* [1, 9], *rad53* [10], and *mec1* [11], as well as in checkpoint *rad* mutants of *Schizosaccharomyces pombe* [12, 13]. In the case of increased sensitivity a decreased ability to arrest cell cycle in response to DNA damage does not seem to be the only cause of the increase in cell lethality. Apart from the cell cycle arrest elimination, checkpoint disorders that make cells more sensitive to DNA damage and replication blocks may also interfere with the activation of the repair machinery and/or its proper delivery to damaged sites in DNA [14].

Published data mainly concern division arrest and survival following cell exposure to UV light [15, 16]. The study of interactions of checkpoint genes that modulate yeast γ-ray radioresistance is not sufficiently advanced [17]. Moreover, little is known of interactions between genes for the repair *per se* and genes responsible for the checkpoint control and of the role of these gene interactions in determining cell radioresistance [2]. Although in the studies of the ramified system of checkpoint regulation, effects on both the cell cycle arrest and cell sensitivity to damaging agents have usually been analyzed, caution is still needed in interpreting the data on radiation sensitivity in terms of checkpoint pathway branches. On the other hand, it is expedient and interesting to try and com-

pare checkpoint pathway schemes based on radiation sensitivity data with those deduced from the analysis of cell cycle arrest modifications.

In this work, the influence of pairwise combinations of checkpoint mutations in genes functioning at different stages of checkpoint regulation on yeast sensitivity to ionizing radiation was analyzed. The *RAD9*, *RAD17*, and *RAD24* genes are believed to act at the initial steps of damage recognition; the *RAD53* protein kinase is involved in a signal transduction cascade. The obtained data suggest that the genes *RAD9*, *RAD17*, *RAD24*, and *RAD53* constitute one epistasis group. The analyzed genes can also participate in minor mechanisms of radioresistance that are relatively independent of the above major mechanism.

2. MATERIALS AND METHODS

2.1. Yeast strains. The genotypes of the strains of *Saccharomyces cerevisiae* are given in the Table. All strains were constructed in the 71a background [18]. As sources of the *rad9*Δ, *rad17*Δ, *rad24*Δ, and *rad53* mutations, the strain 7859-7-4a (*rad9::LEU2*) from Prof. L. H. Hartwell (University of Washington, Seattle) and the strains SX46A rad24Δ (*rad24::URA3*), SX46A rad17Δ (*rad17::URA3*), and CRY1 (*sad1-1(=rad53)*) from Prof. W. Siede (University of Texas, Dallas) were used, respectively.

2.2. Media. YEPD — 1% yeast extract (Difco), 2% bacto-pepton (Difco), 2% glucose. Complete medium (CM) — 0.5% yeast extract, 0.5% pepton, 2% glucose, 2% agar. Presporulation (PM) and sporulation (SM) media were described [19].

2.3. Tetrad Analysis. Diploid hybrids were grown on PM for 1 d and then transferred onto SM. After 3–5 d at room temperature, asci were dissected with the aid of a micro-manipulator. Asco-spores were isolated on CM surface and incubated at 30 °C for 4 d.

2.4. Irradiation. *γ rays.* Seven-day-old cultures grown on solid CM and then resuspended (10^3 to 10^7 cells in 1 ml) in water were irradiated at 4 °C on a «Svet» γ installation (25 Gy/min, ^{137}Cs) of the Joint Institute for Nuclear Research (Dubna) and in special cases on a «Materialovedcheskaya» γ installation (180 Gy/min, ^{60}Co) of the Kurchatov Institute (a Federal Research Center, Moscow). The control and irradiated suspensions were diluted immediately after irradiation, plated on CM at approximately 100 surviving cells per plate, and incubated for 5–7 d at 30 °C.

UV light. Cells were grown on the CM plates at 30 °C for 7 d and resuspended in water. Suspensions were plated on CM at approximately 100 surviving cells per plate and irradiated with UV lamp (0.28 J/m$^2\cdot$ s). The irradiated plates were kept in the dark. To provide exponential cell irradiation, the yeast strains were grown overnight to an early logarithmic phase (2–5·10^6 cells in ml) in liquid YPD at 30 °C and UV-irradiated as described above.

Strains used in the study

Strain	Genotype	Source of origin
7859-7-4a	*MAT*a *rad9::LEU2 leu2-3,112 trp1-289 ura3-52 his7*	L.H.Hartwell (University of Washington, Seattle)
SX46A rad24Δ	*MAT*a *rad24::URA3 ade2 his3-532 trp1-289 ura3-52*	W.Siede (University of Texas, Dallas)
SX46A rad17Δ	*MAT*a *rad17::URA3 ade2 his3-532 trp1-289 ura3-52*	”
CRY1	*MAT*a *rad53* (=*sad1-1*) *ade2-1 ura3-1 trp1-1 his3-11,15 leu2-3,112 can1-100*	“
g160/2b	*MAT*a *rad52-1 ade2-1 arg4 arg9 trp1 his5 lys1-1 ilv3 leu pet*	*YGSC*[*]
71a	*MAT*a *SRM+ ade1*	Constructed by authors [19]
71α	*MAT*α *SRM+ ade1*	“
+	*(7D*α, 11Da, 2456-7B, 1224-1C)	Constructed in this work
rad9	(4α *rad9 ade2*, 5a *rad9 ade2*, from series 9/53 (25,26))	“
rad24	(6A α *rad24 ade2*,10D a *rad24 ade2*, 2453-9B, 1224-7C)	“
rad9 rad24	(8α, 21α, 31α, 41a)	“
9/+ 17/+ (1-4)	*MAT*a/*MAT*α *RAD9/rad9::LEU2 RAD17/rad17::URA3*	“
9/+ 17/17 (1-3)	*MAT*a/*MAT*α *RAD9/rad9::LEU2 rad17::URA3/rad17::URA3*	“
9/9 17/+ (1-4)	*MAT*a/*MAT*α *rad9::LEU2/rad9::LEU2 RAD17/rad17::URA3*	“
9/9 17/17 (1-4)	*MAT*a/*MAT*α *rad9::LEU2/rad9::LEU2 rad17::URA3/rad17::URA3*	“
17/+ 24/+ (IbxVC, IBxXIID, XIBx89-10, XIBx89-29)	*MAT*a/*MAT*α *RAD17/rad17::URA3 RAD24/rad24::URA3*	“
17/+ 24/24 (VD x a rad24, VIB x a rad24, XIIIA x a rad24, XIVD x a rad24)	*MAT*a/*MAT*α *RAD17/rad17::URA3 rad24::URA3/rad24::URA3*	“
17/17 24/+ (VD x a rad17, VIB x a rad17, VIIIA x a rad17, XIVD x a rad17)	*MAT*a/*MAT*α *rad17::URA3/rad17::URA3 RAD24/rad24::URA3*	“
17/17 24/24 (VDxIA, VIBxIA, VIIIAxIA, XIVDxIA)	*MAT*a/*MAT*α *rad17::URA3/rad17::URA3 rad24::URA3/rad24::URA3*	“

Table (continuation)

Strain	Genotype	Source of origin
+/9 +/24 (IICxIIA, VIIICxIVA)	*MAT*a/*MAT*α *RAD9/rad9::LEU2 RAD24/rad24::URA3*	"
+/9 24/24 (2Ax4D rad24, 4Ax6D rad24, 6Ax4D rad24)	*MAT*a/*MAT*α *RAD9/rad9::LEU2 rad24::URA3/rad24::URA3*	"
9/9 +/24 (4Axa rad9, 5Bx 3a rad9, 6Ax11rad9)	*MAT*a/*MAT*α *rad9::LEU2/rad9::LEU2 RAD24/rad24:URA3*	"
9/9 24/24 (2Ax4A, 7Ax6A, 5Bx2A, 5Bx6A)	*MAT*a/*MAT*α *rad9::LEU2/rad9::LEU2 rad24::URA3/rad24::URA3*	"
+/9 +/53 (10κρx7, 13κρx28, 14κρx23)	*MAT*a/*MAT*α *RAD9/rad9::LEU2 RAD53/rad53*	"
+/9 53/53 (26x28, 16x20, 4x8, 22x23)	*MAT*a/*MAT*α *RAD9/rad9::LEU2 rad53/rad53*	"
9/9 +/53 (6κρx7, 1κρx20, 6κρx20, 19κρx28)	*MAT*a/*MAT*α *rad9::LEU2/rad9::LEU2 RAD53/rad53*	"
9/9 53/53 (15x28, 26x28, 7x28, 8x28)	*MAT*a/*MAT*α *rad9::LEU2/rad9::LEU2 rad53/rad53*	"
17/+ 53/+ (55x11Da, 78x7Dα, 80x11Da)	*MAT*a/*MAT*α *RAD17/rad17::URA3 RAD53/rad53*	"
17/17 53/+ (55x1a rad17, 78x 1a rad17, 80x 2α rad17)	*MAT*a/*MAT*α *rad17::URA3/rad17::URA3 RAD53/rad53*	"
17/+ 53/53 (61x 26 rad53, 80x 16 rad53, 55x 16 rad53)	*MAT*a/*MAT*α *RAD17/rad17::URA3 rad53/rad53*	"
17/17 53/53	*MAT*a/*MAT*α *rad17::URA3/rad17::URA3 rad53/rad53*	"
24/+ 53/+ (19Bx7A, 7Bx7D, 7Cx19A)	*MAT*a/*MAT*α *RAD24/rad24::URA3 RAD53/rad53*	"
24/24 53/+ (7Ax9B,22Bx7D,24Bx19A)	*MAT*a/*MAT*α *rad24::URA3/rad24::URA3 RAD53/rad53*	"
24/+ 53/53 (9Dx7A, 9Ax7D, 19Ax22A)	*MAT*a/*MAT*α *RAD24/rad24::URA3 rad53/rad53*	"
24/24 53/53 (7Ax7D,19Ax7D,19Dx7D)	*MAT*a/*MAT*α *rad24::URA3/rad24::URA3 rad53/rad53*	"

* Yeast Genetic Stock Center, Berkeley, USA

The theoretical additive effect was calculated using the following formula: $lgS_{\rm add} = lgS_1 + lgS_2 - lgS_{wt}$, where S_1 and S_2 are the survival of single mutants and S_w is the wild type survival.

3. RESULTS

To determine how the *RAD9, RAD17, RAD24,* and *RAD53* checkpoint genes of budding yeast interact, we generated a panel of closely related single and double mutants. The diploid strains were constructed with the aid of a micromanipulator by crossing the initial strains bearing mutations in the checkpoint genes with mutually isogenic normal strains 71a and 71α and then repeatedly backcrossing (at least four times) the progeny with the 71a and 71α parents. Cell sensitivity to γ radiation was determined for groups of closely related strains (3–4 strains of the same genotype in a group). For irradiation, we have used cultures in a late stationary phase — practically synchronized non-growing cultures.

3.1. Interaction among *RAD9, RAD17*, and *RAD24*. Survival curves for the double *rad9*Δ *rad17*Δ and *rad17*Δ *rad24*Δ mutants seem to be in an intermediate position between the curves for the respective single mutants (Fig. 1). As the differences in the radiation sensitivity between *rad9*Δ and *rad17*Δ strains as well as between *rad17*Δ and *rad24*Δ cells are small and not significant, one may conclude that these mutations interact in an epistatic way.

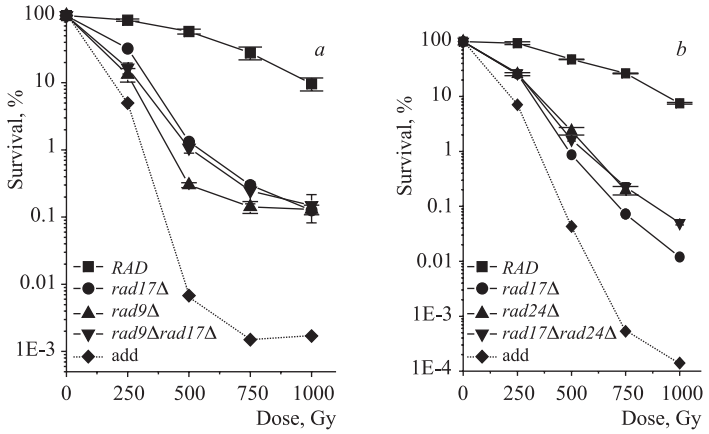

Fig. 1. Interactions of *RAD17* with the *RAD9* and *RAD24* genes. Survival curves of single and double mutants after γ irradiation of stationary diploid cultures. All survival curves are the mean of at least three or four independent experiments mostly performed on independent segregants; the range or SD is shown for each data point: *a*) mutations (*rad9*Δ, *rad17*Δ), *b*) mutations (*rad17*Δ, *rad24*Δ)

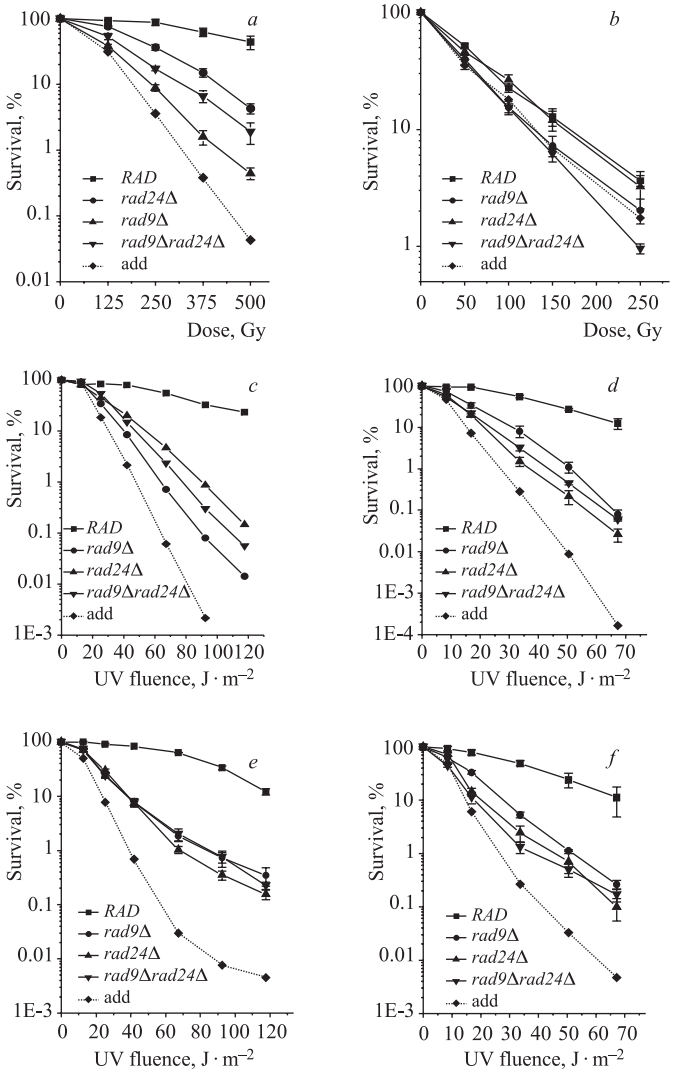

Fig. 2. Interactions of the *RAD9* and *RAD24* genes. Survival curves of single and double mutants *rad9*Δ and *rad24*Δ after irradiation by γ ray (a, b) and UV light (c–f). Stationary (a–d) and exponential (e, f) phase populations of yeast diploid (a, c, e) and haploid (b, d, f) cells were irradiated. All survival curves are the mean of at least three or four independent experiments mostly performed on independent segregants; the range or SD is shown for each data point: *a*) γ ray, diploids, stationary phase; *b*) γ ray, haploids, stationary phase; *c*) UV, diploids, stationary phase; *d*) UV, haploids, stationary phase; *e*) UV, diploids, exponential phase; *f*) UV, haploids, exponential phase

On the other hand, there is a more pronounced difference between the *rad9*Δ and *rad24*Δ mutants as regards their radiation sensitivity; the diploid strains *rad9*Δ were more sensitive than the *rad24*Δ strains. The survival curve of double mutant strains, which is intermediate between the curves of the single mutants, differs from both of them significantly (Fig. 2, *a*). Most probably, the studied genes act in the same epistatic way, and their mutations interact in a somewhat compensatory way.

The published data on the sensitivity of the yeast *rad9* and *rad24* mutants to DNA-damaging agents seem contradictory [20–23]. To fill this gap, we have analyzed the γ and UV sensitivity of haploid and diploid cell cultures irradiated in various growth phases.

As regards the diploid stationary-phase cultures, similar effects of the two mutations (taken apart and combined) on the cell survival were observed after exposure to γ rays (Fig. 2, *a*) or UV light (Fig. 2, *c*). On the other hand, an analysis of the UV-irradiated log-phase cultures failed to reveal any significant difference between the single and double mutants (Fig. 2, *e*).

Figure 2, *b* shows the single *rad9*Δ and double *rad9*Δ *rad24*Δ mutant haploid strains are slightly γ-ray sensitive, whereas the *rad24*Δ strains were just like the wild-type strains. For the haploid strain, the additive effect of mutations could be expected. Stationary-phase cultures of the haploid *rad9*Δ cells were less UV-sensitive as compared with the haploid *rad24*Δ cultures, whereas the double mutant *rad9*Δ *rad24*Δ cells manifested an intermediate UV-sensitivity (Fig. 2, *d*). Comparing the linear parts of the survival curves, one may conclude that the exponential haploid single mutant *rad9*Δ was less UV-sensitive then *rad24*Δ mutant, and double-mutant cultures were more UV-sensitive than the single mutant *rad24*Δ cultures, although the difference was less than could be expected for the additive effect of mutations (Fig. 2, *f*).

We conclude that the *RAD9* and *RAD24* act in the same pathway determining the sensitivity of yeast diploid cells to radiation. Still the mechanism of the interaction between these two genes may be modified in accordance with the type of DNA damage, ploidy and phase of culture.

3.2. Interaction of *RAD53* with *RAD9, RAD17*, and *RAD24*. A comparison of the survival curves (Fig. 3) did not reveal any significant differences in γ-radiation sensitivity between the double mutant *rad9*Δ *rad53, rad17*Δ *rad53* or *rad24*Δ *rad53* strains and their single-mutant counterparts. Thus, as far as the mutational effects on the γ-ray sensitivity are concerned, the mutations *rad9*Δ, *rad17*Δ, *rad24*Δ were epistatic to the *rad53* mutation. Apparently, *RAD24*, as *RAD9* and *RAD17* belong to the same epistasis group with *RAD53*. This assumption is confirmed by some published data concerned with other mutational effects [24, 25].

For the *rad24*Δ and *rad53* mutations, Fig. 3, *d* presents the results obtained following exposure of the same diploid cultures in the stationary growth phase

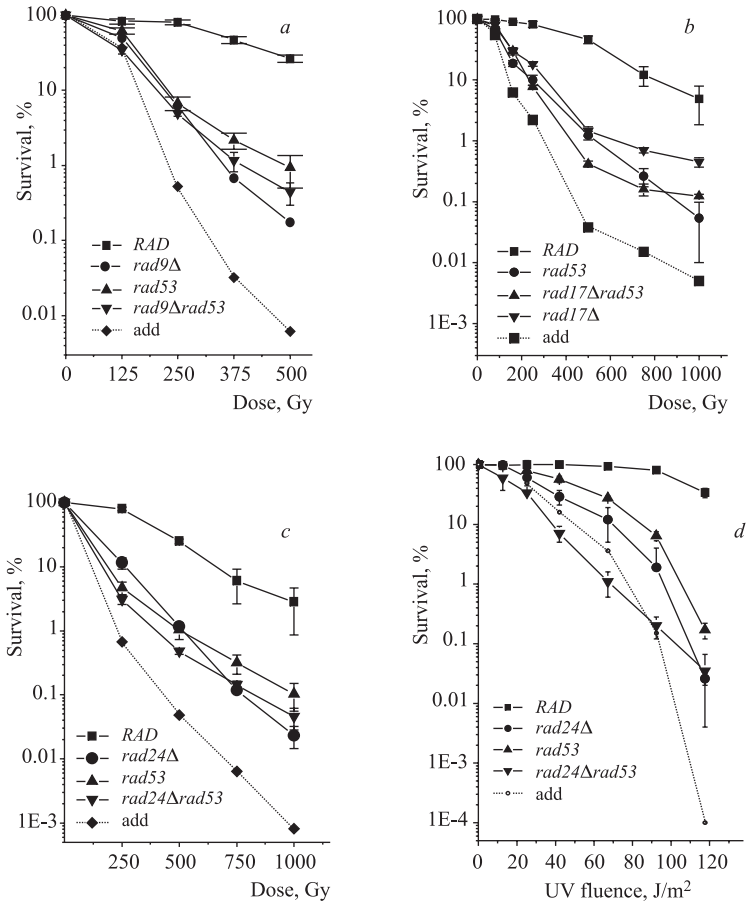

Fig. 3. Interactions of *RAD53* with the *RAD9, RAD17,* and *RAD24* genes. Survival curves of single and double mutants after γ (*a–c*) and UV (*d*) irradiation. Stationary phase cultures of yeast diploid cells were irradiated. All survival curves are the mean of at least three or four independent experiments mostly performed on independent segregants; the range or SD is shown for each data point: *a*) strains (*rad9*Δ, *rad53*); *b*) strains (*rad17*Δ, *rad53*); *c*) strains (*rad24*Δ, *rad53*); *d*) strains (*rad24*Δ, *rad53*)

to UV light. We found that the sensitivity of the double mutants is significantly higher than that of each single mutant. In this case, one can speak about additive interactions between *RAD24* and *RAD53* with respect to UV light.

4. DISCUSSION

A study of a group of genes responsible for radioresistance to ionizing radiation allowed us to attribute the checkpoint genes *RAD9*, *RAD17*, *RAD24*, and *RAD53* to a single epistasis group denoted by us the *RAD9*-dependent pathway involved in the determination of cell radioresistance. However, these interactions do not correspond to the checkpoint control scheme following from the study of various damage agents [26]. In the regulation of the checkpoint control, the genes *RAD9* and *RAD17*/*RAD24* are additive [20–22], whereas the genes *RAD9* and *RAD53* are epistatic [25, 27]. The pathways of activation and checkpoint regulation may differ depending on various types of damage. Moreover, the effect of checkpoint genes on radioresistance is likely to be mediated not only by the regulation of cell cycle arrest, but also by the participation of genes in other processes, such as repair through the induction of repaired gene transcription or posttranscriptional regulation of certain repair pathways [25, 9].

We have analyzed radiosensitivity of diploid strains in the stationary growth phase. In diploid strains with the double set of chromosomes, HR is possible in all phases of the cell cycle. HR is accomplished by a mechanism of interchromosomal recombination in diploids in the stationary phase and in G_1, and by a mechanism of interchromatid recombination in haploids and diploids in G_2. In diploid strains, the minor repair pathway via NHEJ is suppressed, because heterozygote for mating type repressed the *NEJ1* gene involved in NHEJ and due to repair via HR pathway [28, 29]. No influence of the mutation *rad9*Δ on mitotic homologous recombination, gene conversion, and crossing-over was observed [15]. *RAD9* is not related to the repair of an induced break or plasmid integration resulting from homologous recombination [30–32]. Thus, it was unreasonable to suggest the direct involvement of the checkpoint gene *RAD9* in DNA DSB repair via homologous recombination. *RAD9*, *RAD24*, and *RAD17* participate in minor repair pathways (NHEJ) [31–33] inhibited in diploids [28, 29]. Apparently, their effect is primarily mediated by the cell cycle arrest regulation [9].

Genes can be placed into three groups with respect to their radiosensitivity. The published data on the sensitivity of the yeast *rad9* and *rad24* mutants to DNA-damaging agents seem contradictory [20–23]. A comparison of the radiosensitivity of single *rad9*Δ and *rad24*Δ mutants showed all possible variants: equal [23] or greater [20, 21] sensitivity to UV or lower sensitivity for γ ray [23] and MMS [22]. These data were mostly obtained for haploid exponential cultures. In our experiments with diploid strains, the mutation *rad9*Δ had a stronger modifying effect on cell sensitivity to γ irradiation compared to the mutations *rad24*Δ and *rad17*Δ which decreased the effect of *rad9*Δ on radiosensitivity. Obviously, the genes *RAD9*, *RAD24*, and *RAD17* are epistatic in the cell cycle arrest regulation and it is exactly this fact that is responsible for the determination

of the radiosensitivity level upon γ and UV irradiation of diploid strains in the stationary growth phase.

The radiosensitivity of *rad53* mutants is higher than that of *rad17*Δ and *rad24*Δ but lower than the radiosensitivity of *rad9*Δ on the linear segment of the curve, i.e., in the G_1 phase (Fig. 3). It may be assumed that *RAD9* is engaged in an additional *RAD53*-independent pathway involved in the determination of radiosensitivity, whereas the gene *RAD53* controls additional pathways apart from the *RAD17*/*RAD24*-dependent pathway. The *RAD9*-branch plays more important role in the determination of radioresistance, probably, at the expense of the *CHK1*-pathway. These branches are not completely independent. For the activation of the checkpoint control, the activated phosphorylation of Rad9 and Rad53 is needed. The phosphorylation of Rad9 in G_2 is associated with Mec1, and also with Rad24, Rad17-Ddc1-Mec3 in G_1 [27]. The DNA damage-induced modification of Rad53 in cells blocked in G_1 and G_2 depends on intact *RAD9*- and *RAD24*-branches of the checkpoint control [25]. The overproduction of Rad9 in *rad24*Δ or Rad24 in *rad9*Δ restores the G_1/S arrest, G_2/M arrest, transcriptional response, and cell survival.

Upon irradiation of diploid cultures with UV light, the suppressive effect of *rad17*Δ and *rad24*Δ with respect to the mutation *rad9*Δ was observed. It is known that upon UV irradiation, cell cycle arrest led to a slight increase in the survival of mutant *rad9*Δ [9]. *RAD9* and *RAD24* were shown to participate in inducible nucleotide excision repair (NER) of pyrimidine dimers in transcribed and non-transcribed regions [33]. However, the type of interaction of these genes was not determined. Moreover, *RAD9*, *RAD17*, and *RAD24* are indispensable for the replication across non-repaired DNA damage [15]. The analysis revealed that *RAD9* and *RAD52* positively regulate sister-chromatid exchange, whereas *RAD24* and *RAD17* down-regulate this process, and these mutations are epistatic with each other. The analogous pattern was observed upon UV irradiation of stationary diploid cultures. Thus, it cannot be ruled out that mutations involved in the determination of radiosensitivity interact epistatically not only in the process of the cell cycle arrest but also in some repair processes.

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REFERENCES

1. *Weinert T. A., Hartwell L. H.* The *RAD9* gene controls the cell cycle response to DNA damage in *Saccharomyces cerevisiae* // Science. 1988. V. 241. P. 317–322.

2. *Weinert T.* DNA damage checkpoints update: getting molecular // Curr. Opin. Genet. Dev. 1998. V. 8. P. 185–193.

3. *Weinert T.* DNA damage and checkpoint pathways: molecular anatomy and interactions with repair // Cell. 1998. V. 94. P. 555–558.

4. *Foiani M. et al.* DNA damage checkpoints and replication controls in *Saccharomyces cerevisiae* // Mutation Res. 2000. V. 451. P. 187–196.

5. *Sanchez Y. et al.* Control of the DNA damage checkpoint by chk1 and rad53 protein kinases through distinct mechanisms // Science. 1999. V. 286. P. 1166–1171.

6. *Frai C., Gasser S. M.* The yeast Sgs1p helicase acts upstream of Rad53p in the DNA replication checkpoint and colocalizes with Rad53p in S-phase-specific foci // Genes Dev. 2000. V. 14. P. 81–96.

7. *Shiloh Y.* Ataxia-telangiectasia and Nijmegen breakage syndrome: related disorders but genes apart // Ann. Rev. Genet. 1997. V. 31. P. 635–662.

8. *Jeggo P. A., Carr A. M., Lehmann A. R.* Splitting the ATM: distinct repair and checkpoint defects in ataxia-telangiectasia // Trends Genet. 1998. V. 14. P. 312–316.

9. *Aboussekhra A. et al.* A novel role for the budding yeast *RAD9* checkpoint gene in DNA damage-dependent transcription // EMBO J. 1996. V. 15. P. 3912–3922.

10. *Allen J. B. et al.* The SAD1/RAD53 protein kinase controls multiple checkpoints and DNA damage-induced transcription in yeast // Genes Dev. 1994. V. 8. P. 2401–2415.

11. *Bashkirov V. I. et al.* DNA repair protein Rad55 is a terminal substrate of the DNA damage checkpoints // Mol. Cell. Biol. 2000. V. 20. P. 4393–4404.

12. *Al-Khodairy F., Carr A. M.* DNA repair mutants defining G_2 checkpoint pathways in *Schizosaccharomyces pombe* // EMBO J. 1992. V. 11. P. 1343–1350.

13. *Rowley R., Subramani S., Young P.G.* Checkpoint controls in *Schizosaccharomyces pombe*: rad1 // EMBO J. 1992. V. 11. P. 1335–1342.

14. *Longhese M. P., Foiani M., Muzi-Falconi M., Lucchini G., Plevani P.* DNA damage checkpoint in budding yeast // EMBO J. 1998. V. 17. P. 5525–5528.

15. *Paulovich A. G., Armour C. D., Hartwell L. H.* The *Saccharomyces cerevisiae RAD9*, *RAD17*, *RAD24*, and *MEC3* genes are required for tolerating, Ultraviolet-induced DNA damage // Genetics. 1998. V. 150. P. 75–93.

16. *Gardner R., Putnam C. W., Weinert T. RAD53*, *DUN1*, and *PDS1* define two parallel G_2/M checkpoint pathways in budding yeast // EMBO J. 1999. V. 18. P. 3173–3185.

17. *King W. R., Rowley R., Schroeder A. L.* Ionizing irradiation effects on S-phase in checkpoint mutants of the yeast *Saccharomyces cerevisiae* // Curr. Genet. 2003. V. 42. P. 313–321.

18. *Koltovaya N. A., Arman I. P., Devin A. B.* Mutation of the *CDC28* gene and the radiation sensitivity of *Saccharomyces cerevisiae* // Yeast. 1998. V. 14. P. 133–146.

19. *Devin A. B. et al.* The Start gene *CDC28* and the genetic stability of yeast // Yeast. 1990. V. 6. P. 231–243.

20. *Eckhardt-Schupp F., Siede W., Game J. C.* The *RAD24* (=R_1^s) gene product of *Saccharomyces cerevisiae* participates in two different pathways of DNA repair // Genetics. 1987. V. 115. P. 83–90.

21. *Lydall D., Weinert T.* Yeast checkpoint genes in DNA damage processing: implications for repair and arrest // Science. 1995. V. 270. P. 1488–1491.

22. *Paulovich A. G. et al. RAD9*, *RAD17*, and *RAD24* are required for S phase regulation in *Saccharomyces cerevisiae* in response to DNA damage // Genetics. 1997. V. 145. P. 45–62.

23. *Corda Y. et al.* Interaction between Set1p and checkpoint protein Mec3p in DNA repair and telomere functions // Nature Genetics. 1999. V. 21. P. 204–208.

24. *Navas T. A., Sanchez Y., Elledge S. J. RAD9* and DNA polymerase ε form parallel sensory branches for transducing the DNA damage checkpoint signal in *Saccharomyces cerevisiae* // Genes Dev. 1996. V. 10. P. 2632–2643.

25. *de la Torre Ruiz M. A., Green C. M., Lowndes N. F. RAD9* and *RAD24* define two additives, interacting branches of the DNA damage checkpoint pathway in budding yeast normally required for Rad53 modification and activation // EMBO J. 1998. V. 17. P. 2687–2698.

26. *Rouse J., Jackson S. P.* Interfaces between the detection, signaling, and repair of DNA damage // Science. 2002. V. 297. P. 547–551.

27. *Vialard J. E., Gilbert C. S., Green C. M., Lowndes N. F.* The budding yeast Rad9 checkpoint protein is subjected to Mec1/Tel1-dependent hyperphosphorylation and interacts with Rad53 after DNA damage // EMBO J. 1998. V. 17. P. 5679–5688.

28. *Frank-Vaillant M., Marcand S.* Nonhomologous end joining regulation by mating type is exercised through a novel protein, Lif2p, essential to the ligase IV pathway // Genes Deversion. 2001. V. 15. P. 3005–3012.

29. *Valencia M. et al. NEJ1* controls non-homologous end joining in *Saccharomyces cerevisiae* // Nature. 2001. V. 414. P. 666–669.

30. *Schiestl R. H., Zhu J., Petes T. D.* Effect of mutations in genes affecting homologous recombination on restriction enzyme-mediated and illegitimate recombination in *Saccharomyces cerevisiae* // Mol. Cell. Biol. 1994. V. 14. P. 4493–4500.

31. *Sugawara N., Ivanov E. L., Fishman-Lobell J., Ray B. L., Wu X., Haber J. E.* DNA structure-dependent requirements for yeast *RAD* genes in gene conversion // Nature. 1995. V. 373. P. 84–86.

32. *de la Torre-Ruiz M. A., Lowndes N. F. DUN1* defines one branch downstream of *RAD53* for transcription and DNA damage repair in *Saccharomyces cerevisiae* // FEBS Lett. 2000. V. 485. P. 206–206.

33. *Yu S., Teng Y., Lowndes N. F., Waters R. RAD9*, *RAD24*, *RAD16*, and *RAD26* are required for the inducible nucleotide excision repair of UV-induced cyclobutane pyrimidine dimers from the transcribed and non-transcribed regions of the *Saccharomyces cerevisiae MFA2* gene // Mutation Res. 2001. V. 485. P. 229–236.

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