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Interaction between Checkpoint Genes *RAD9*, *RAD17*, *RAD24*, *RAD53*, and Genes *SRM5/CDC28*, *SRM8/NET1*, and *SRM12/HFI1* Involved in the Determination of Yeast *Saccharomyces cerevisiae* Sensitivity to Ionizing Radiation

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Abstract—Analysis of radiosensitivity of double mutants in the yeast *Saccharomyces cerevisiae* revealed that checkpoint genes *RAD9, RAD17*, *RAD24*, and *RAD53* along with genes *CDC28* and *NET1* belong to one epistasis group designated the *RAD9* group. The use of *srm* mutations allowed the demonstration of a branched *RAD9*-dependent pathway of cell radioresistance. Mutation *cdc28-srm* is hypostatic to *rad9Δ*, *rad17Δ*, and *rad24Δ* being additive with *rad53*. Mutation *net1-srm* is hypostatic to *rad9Δ* and *rad53* but additively enhance the effects of mutations *rad17Δ* and *rad24Δ*. Gene *SRM12/HFI1* is not a member of the *RAD9* group. Mutation in gene *hfi1-srm* manifests the additive effect on mutations *rad24Δ* and *rad9Δ*. The analyzed genes can also participate in minor mechanisms of radioresistance that are relatively independent of the above *RAD9*-dependent mechanism.

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INTRODUCTION

Systematic analysis of yeast deletion mutants [1] allowed the replenishment of the list of loci responsible for yeast sensitivity to γ-irradiation [2]. In the meantime, the essential genes may be missed during deletion analysis. Therefore, as applied to this problem, it is still important to analyze viability-compatible (point) mutations, for example, *srm* gene mutations that not only change the maintenance of mitochondrial *rho*-mutability but also cause changes in mitotic stability of chromosomes and radioresistance [3–5]. Some *SRM* genes were identified; in particular, it was shown that genes *SRM5*, *SRM8*, and *SRM12* correspond to *CDC28*, *NET1*, and *HFI1* genes [3, 4, 6, 7].

Since the *CDC28* gene for cyclin-dependent kinase is essential, the influence of *cdc28* mutations on cell sensitivity to the damage agents depends on mutation localization and on the degree of protein disfunction. For instance, as shown previously, mutation *cdc28-1* does not affect UV-sensitivity [8], whereas mutation *cdc28-1N* exerts the influence on yeast cell sensitivity to UV light [9]. The latter mutation represents a base-pair substitution [P250L], and the nature of *cdc28-1N* has not been identified [10]. The mutation *cdc28-1* [G16S] isolated by us has been localized in the conservative glycine-rich G-loop [11], which is significant for protein architecture, manifests pleiotropic effects; particularly, it affects radioresistance [6, 12–14]. The involvement of cyclins, the regulatory kinase subunits, in response to DNA damage was shown in various systems. Thus, the loss of S-phase cyclins Clb5 and Clb6 was shown to increase cell sensitivity to MMS, UV light, and ionizing radiation [15]. Obviously, these data reliably testify to the participation of kinase CDC28 in determining the level of radioresistance.

Gene *NET1*/*SRM8* encodes the structural subunit of the nucleolar RENT complex tethers the Cdc14 phosphatase and deacetylase Sir2 within the nucleus [16, 17]. The involvement of *NET1* in cell radioresistance was shown for the first time in our works using frameshift mutation *net1-srm* [4, 6, 7, 18]. The structural subunit Srm12/Ada1/Hfi1 is a component of the SAGA complex possessing the histone-acetyltransferase activity mediated by the subunit Gen5 [19]. Radioresistance of *HFI1* was shown for the first time in our works with the help of a nonsense mutation *hfi1-srm* [4, 6, 7, 18]. Gene *HFI1* was also identified as a gene for radiosensitivity in the deletion analysis [1].

In this work, the influence of pairwise combinations of checkpoint gene mutations and *srm* mutations on yeast sensitivity to ionizing radiation was studied. The obtained data suggest that genes *RAD9*, *RAD17*, *RAD24*, *RAD53*, *CDC28*, and *NET1* constitute an epistasis group probably maintained by a single major *RAD9*-dependent mechanism of radioresistance in *Sac-*

[†] Deceased.

charomyces cerevisiae. The attempt to identify the epistasis group containing *HFI1* gene failed.

MATERIALS AND METHODS

Saccharomyces cerevisiae used in this study (table) are closely related and were constructed by introducing the mutations listed below in the genotype of isogenic parental strains. Mutations were introduced via crossing the source strains with parental strains 71a or 71α [14], by producing sexual progeny, and by at least three sequential backcrosses with 71a and 71α. Mutants *srm5*/*cdc28-srm* [3], *srm8*/*net1-srm* and *srm12*/*hfi1-srm* [4] were obtained by the authors. As a source of mutation *rad52-1*, we used a strain gl60/2b (*rad52-1*) from the Yeast Genetic Stock Center, Berkeley, United States (further on, ATCC). Strains 7859-7-4a (*rad9::LEU2*), SX46A rad24Δ, SX46A rad17Δ, and CRY1 (*sad1-1* = *rad53*) were used as sources of mutations *rad9Δ*, *rad17Δ*, *rad24Δ*, and *rad53*. The first of these strains was provided by L. Hartwell (University of Washington, Seattle), the others were provided by W. Siede (University of Texas, Dallas).

Media and reagents. We used the standard complete nutrient medium YEPD [20] and BS, PMG, and SMK media described in [3].

Tetrad analysis. Cultures of diploid hybrids were grown on a presporulation medium PMG and transferred onto the sporulation medium SMK. After 3–5 days of incubation at room temperature, ascospores were isolated by a micromanipulator on an agar plug of BS medium and incubated for 4 days at 30°C.

Irradiation. Cells were irradiated with γ-rays UV light under standard conditions by a method described in [21].

RESULTS

Since mutation *cdc28-srm* enhances cell sensitivity to ionizing radiation [13], it was of interest to highlight the epistasis group of radiosensitive genes to which gene *SRM5*/*CDC28* belongs. In *S. cerevisiae*, cell sensitivity to ionizing radiation is mediated by genes of the epistasis *RAD6* and *RAD52* groups. Genes of the latter group are responsible for repair of the major part of γ-radiation-induced DNA damage effected due to the repair of DNA double-strand breaks (DSB) via homologous recombination (HR). Genes of the *RAD6* epistasis group are responsible for replication across DNA damage and recovery of stalled replication forks and postreplication repair of a smaller, although essential, portion of DNA lethal lesions. The action of *cdc28-srm*, *rad6-1* and *cdc28-srm*, *rad52-1* mutation pairs was shown to affect γ-radiation sensitivity in double mutants and manifest the synergistic effect [6, 22]. Thus, mutation *cdc28-srm* could not be assigned to the epistasis *RAD6* or *RAD52* groups. The influence of this mutation on radiosensitivity probably is not confined to a specific disturbance in either corresponding repair pathway. It is known that an important role in mediating radioresistance plays, apart from repair, the checkpoint control that ensures cell cycle arrest sufficient for DNA damage repair. The interaction of gene *CDC28* and checkpoint genes in determining radioresistance is of obvious interest.

Analysis of Changes in Cell Radiosensitivity upon Interaction between Mutation cdc28-srm and Mutations rad9Δ, rad17Δ, rad24Δ, and rad53

The checkpoint gene *RAD9* plays a key role in cell response to DNA damage. In order to examine the interaction between *CDC28* and *RAD9* genes, we employed the impaired *rad9::LEU2* allele and mutation *cdc28-srm*. The following diploid strains (by 4 strains of similar genotype) were constructed: *cdc28-srm*/*cdc28-srm rad9Δ*/*rad9Δ*, *CDC28*/*CDC28 rad9Δ*/*rad9Δ*, *cdc28-srm*/*cdc28-srm RAD9*/*RAD9*, *RAD9*/*RAD9 CDC28*/*CDC28*. Figure 1a presents survival curves of these mutants. It is shown that these mutations are epistatic to each other: the double *rad9Δ cdc28-srm* mutant is not more sensitive to γ-irradiation than the most sensitive single mutant *rad9Δ*. Thus, mutations of genes *CDC28* and *RAD9* belong to a single epistasis group responsible for the determination of cell radiosensitivity to γ-irradiation.

To examine the interaction between gene *CDC28* and checkpoint genes *RAD17*, *RAD24*, and *RAD53*, we constructed strains carrying single and double mutations. Cell sensitivity to γ-irradiation was determined, as previously, in a series of closely related strains (table). Survival curves obtained after irradiation of stationary diploid cultures are shown in Figs. 1b and 1c. Double mutants *cdc28-srm rad17Δ* exhibit radiosensitivity similar to that expected upon additive effect (Fig. 1b). Double mutants *cdc28-srm rad24Δ* exhibit radiosensitivity similar to that of the most sensitive of single mutants *rad24Δ* (Fig. 1c). Thus, mutation *rad24Δ* is epistatic and mutation *rad17Δ* is additive with *cdc28-srm* mutation.

To analyze the interaction between gene *CDC28* and the checkpoint gene *RAD53*, we constructed the corresponding strains carrying single and double mutations. Double mutants *cdc28-srm rad53* exhibit radiosensitivity similar to that expected upon additive effect (Fig. 1d). Thus, mutation *rad53* is additive with *cdc28-srm* mutation, i.e., *CDC28* and *RAD53* protein kinases mediate the control of different pathways of cell radioresistance.

As the pair of genes *CDC28* and *RAD52* control different pathways as well, it was of interest to analyze triple mutants. As seen in Fig. 1e, the triple mutant *rad52-1 rad53 cdc28-srm* is not more sensitive than the double mutant *rad52-1 cdc28-srm*. Although we did not conduct analysis of double mutants *rad52-1 rad53-1*, analysis of radiosensitivity in diploid double mutants *rad52-1 rad53-1* at the stationary growth phase made

Strains used in the study

Strain	Genotype	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*	The same
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*
JG-6	*MAT*a *rad6-1*	The same
71a	*MAT*a *SRM+ ade1*	Constructed by authors [4, 14]
71α	*MAT*α *SRM+ ade1*	The same
R8a	*MAT*a *net1-srm (=srm8) ade2 trp1 ura3*	"
R8α	*MAT*α *net1-srm ade2 trp1 ura3*	"
R12a	*MAT*a *hfi1-srm (=srm12) ade2 trp1 ura3*	"
R12α	*MAT*α *hfi1-srm ade2 trp1 ura3*	"
C50/1–C50/4	*MAT*a/*MAT*α *cdc28-srm/cdc28-srm ade1/ade1*	"
C40/1, C40/2	*MAT*a/*MAT*α *rad52-1/rad52-1 ade1/ade1 cyh2/+ leu1/+ ade6/+*	"
C54/1, C54/2	*MAT*a/*MAT*α *rad52-1/rad52-1 cdc28-srm/cdc28-srm cyh2/+ leu1/+ ade6/+ ade1/ade1*	"
C3/1, C3/2	*MAT*a/*MAT*α *rad52-1/rad52-1 cdc28-srm/cdc28-srm rad53/rad53 ade2/ade2*	"
C59/1–C59/4	*MAT*a/*MAT*α *cdc28-srm/cdc28-srm rad9::LEU2/rad9::LEU2 ade1/ade1 leu2-3,112/leu2-3,112*	"
C09/1–C09/4	*MAT*a/*MAT*α *rad9::LEU2/rad9::LEU2 ade1/ade1 leu2-3,112/leu2-3,112*	"
C05/5–C05/8	*MAT*a/*MAT*α *cdc28-srm/cdc28-srm ade1/ade1 leu2-3,112/leu2-3,112*	"
C00/5–C00/8	*MAT*a/*MAT*α *ade1/ade1 leu2-3,112/leu2-3,112*	"
Series 5/17:		
5/+ 17/+ (1–3)	*MAT*a/*MAT*α *CDC28/cdc28-srm RAD17/rad17::URA3*	Constructed by authors in this work
5/5 17/+ (1–3)	*MAT*a/*MAT*α *cdc28-srm/cdc28-srm RAD17/rad17::URA3*	The same
5/+ 17/17 (1–3)	*MAT*a/*MAT*α *CDC28/cdc28-srm rad17::URA3/rad17::URA3*	"
5/5 17/17 (1–3)	*MAT*a/*MAT*α *cdc28-srm/cdc28-srm rad17::URA3/rad17::URA3*	"
Series 5/24:		
5/+ 24/+ (1–3)	*MAT*a/*MAT*α *CDC28/cdc28-srm RAD24/rad24::URA3*	"
5/5 24/+ (1–3)	*MAT*a/*MAT*α *cdc28-srm/cdc28-srm RAD24/rad24::URA3*	"
5/+ 24/24 (1–3)	*MAT*a/*MAT*α *CDC28/cdc28-srm rad24::URA3/rad24::URA3*	"
5/5 24/24 (1–3)	*MAT*a/*MAT*α *cdc28-srm/cdc28-srm rad24::URA3/rad24::URA3*	"
Series 5/53:		
5/+ 53/+ (1, 2)	*MAT*a/*MAT*α *CDC28/cdc28-srm RAD53/rad53*	"
5/5 53/+ (1, 2)	*MAT*a/*MAT*α *cdc28-srm/cdc28-srm RAD53/rad53*	"
5/+ 53/53 (1–3)	*MAT*a/*MAT*α *CDC28/cdc28-srm rad53/rad53*	"
5/5 53/53 (1–3)	*MAT*a/*MAT*α *cdc28-srm/cdc28-srm rad53/rad53*	"
Series 8/9:		
+/8 +/9 (1–4)	*MAT*a/*MAT*α *NET1/net1-srm RAD9/rad9::URA3*	"

Table (Contd.)

Strain	Genotype	Origin
8/+ 9/9 (1–4)	*MAT*a/*MAT*α *NET1/net1-srm rad9::URA3/rad9::URA3*	"
8/8 9/+ (1–4)	*MAT*a/*MAT*α *net1-srm/net1-srm RAD9/rad9::URA3*	"
9/9 8/8 (1–4)	*MAT*a/*MAT*α *net1-srm/net1-srm rad9::URA3/rad9::URA3*	"
Series 8/17:		
8/+ 17/+ (1–4)	*MAT*a/*MAT*α *NET1/net1-srm RAD17/rad17::URA3*	"
8/8 17/+ (1–4)	*MAT*a/*MAT*α *net1-srm/net1-srm RAD17/rad17::URA3*	"
8/+ 17/17 (1–4)	*MAT*a/*MAT*α *NET1/net1-srm rad17::URA3/rad17::URA3*	"
8/8 17/17 (1–4)	*MAT*a/*MAT*α *net1-srm/net1-srm rad17::URA3/rad17::URA3*	"
Series 8/24:		
8/+ 24/+ (1–3)	*MAT*a/*MAT*α *NET1/net1-srm RAD24/rad24::URA3*	"
8/+ 24/24 (1–3)	*MAT*a/*MAT*α *NET1/net1-srm rad24::URA3/rad24::URA3*	"
8/8 24/+ (1–4)	*MAT*a/*MAT*α *net1-srm/net1-srm RAD24/rad24::URA3*	"
8/8 24/24 (1–3)	*MAT*a/*MAT*α *net1-srm/net1-srm rad24::URA3/rad24::URA3*	"
Series 8/53:		
8/+ 53/+(1–3)	*MAT*a/*MAT*α *NET1/net1-srm RAD53/rad53*	"
8/8 53/+ (1–3)	*MAT*a/*MAT*α *net1-srm/net1-srm RAD53/rad53*	"
8/+ 53/53 (1–3)	*MAT*a/*MAT*α *NET1/net1-srm rad53/rad53*	"
8/8 53/53 (1–3)	*MAT*a/*MAT*α *net1-srm/net1-srm rad53/rad53*	"
Series 5/12:		
5/+ 12/+ (1–4)	*MAT*a/*MAT*α *CDC28/cdc28-srm HFI1/hfi1-srm*	"
5/5 12/+ (1–4)	*MAT*a/*MAT*α *cdc28-srm/cdc28-srm HFI1/hfi1-srm*	"
5/+ 12/12 (1–3)	*MAT*a/*MAT*α *CDC28/cdc28-srm hfi1-srm/hfi1-srm*	"
5/5 12/12 (1–4)	*MAT*a/*MAT*α *cdc28-srm/cdc28-srm hfi1-srm/hfi1-srm*	"
Series 12/9:		
12/+ 9/+ (1.2)	*MAT*a/*MAT*α *HFI1/hfi1-srm RAD9/rad9::LEU2*	"
12/+ 9/9 (1)	*MAT*a/*MAT*α *HFI1/hfi1-srm rad9::LEU2/rad9::LEU2*	"
12/12 9/+ (1–4)	*MAT*a/*MAT*α *hfi1-srm/hfi1-srm RAD9/rad9::LEU2*	"
12/12 9/9 (1–4)	*MAT*a/*MAT*α *hfi1-srm/hfi1-srm rad9::LEU2/rad9::LEU2*	"
Series 12/24:		
12/+ 24/+ (1–4)	*MAT*a/*MAT*α *HFI1/hfi1-srm RAD24/rad24::URA3*	"
12/12 24/+ (1–3)	*MAT*a/*MAT*α *hfi1-srm/hfi1-srm RAD24/rad24::URA3*	"
12/+ 24/24 (1–4)	*MAT*a/*MAT*α *HFI1/hfi1-srm rad24::URA3/rad24::URA3*	"
12/12 24/24 (1–4)	*MAT*a/*MAT*α *hfi1-srm/hfi1-srm rad24::URA3/rad24::URA3*	"
Series 12/53:		
12/+ 53/+ (1.2)	*MAT*a/*MAT*α *HFI1/hfi1-srm RAD53/rad53*	"
12/+ 53/53 (1)	*MAT*a/*MAT*α *HFI1/hfi1-srm rad53/rad53*	"
12/12 53/+ (1–3)	*MAT*a/*MAT*α *hfi1-srm/hfi1-srm RAD53/rad53*	"
12/12 53/53 (1–3)	*MAT*a/*MAT*α *hfi1-srm/hfi1-srm rad53/rad53*	"

* Yeast Genetic Stock Center, Berkeley, United States.

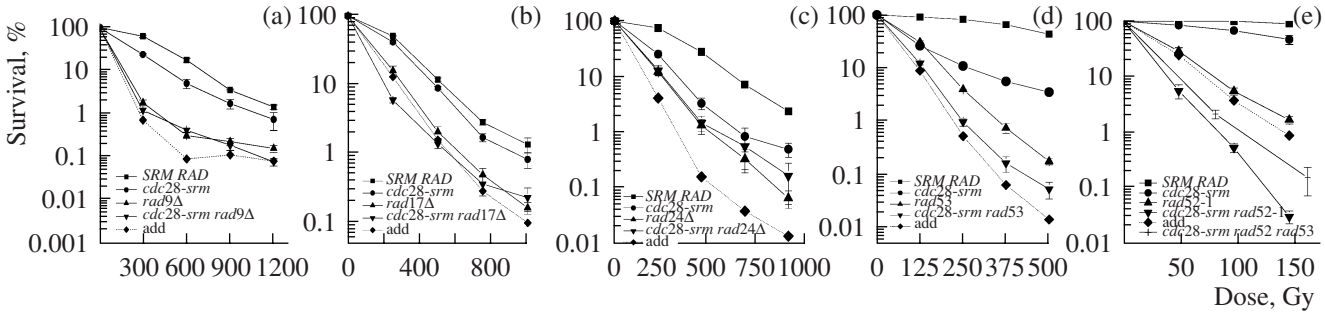

Fig. 1. Survival curves obtained following γ-irradiation of diploid single and double mutants homozygous for mutations *cdc28-srm* and *rad9*Δ (a), *cdc28-srm* and *rad17*Δ (b), *cdc28-srm* and *rad24*Δ (c), *cdc28-srm* and *rad53* (d), single (*cdc28-srm*, *rad52*), double (*rad52 cdc28-srm*), and triple (*rad52 rad53 cdc28-srm*) mutants (e). Strains were irradiated on a Svet γ installation (Cs^{137}, 25 Gy/min), and triple mutant *rad52 rad53 cdc28-srm* was irradiated on a Materialovedcheskaya γ installation (^{60}Co, 180 Gy/min). Each curve corresponds to the averaged data for four or three strains of the same genotype; standard errors are given. For comparison, a curve expected upon additive effect of two mutations on cell radiosensitivity is presented.

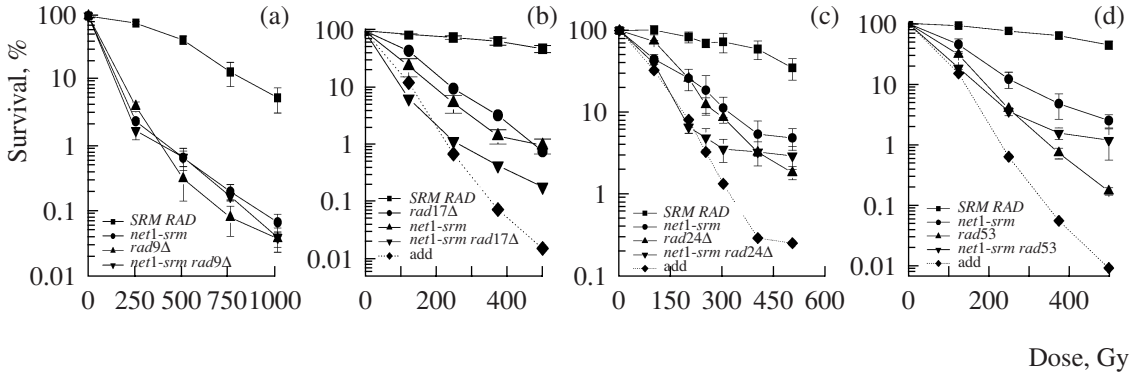

Fig. 2. Relationship between mutation *srm8/net1-srm* and *rad9Δ* (a), *rad17Δ* (b), *rad24Δ* (c), *rad53* (d). Each curve corresponds to the averaged data for four strains of the same genotype; standard errors are given. For comparison, a curve expected upon additive effect of two mutations on cell radiosensitivity is presented.

ducted by Lawrence [23] revealed that radiosensitivity of the double mutant *rad52-1 rad53-1* coincides with radiosensitivity of the single mutant *rad52-1*. Thus, genes *RAD52* or *RAD53* belong to the same epistasis group of radiosensitivity genes. One can assume that genes *CDC28* and *RAD52*/*RAD53* compose two branches of pathway determining sensitivity to γ-irradiation.

Analysis of Changes in Cell Radiosensitivity upon Interaction between Mutation net1-srm and Mutations rad9Δ, rad17Δ, rad24Δ, and rad53

For each of four pairwise combinations of *net1-srm* and checkpoint mutations, we constructed a series of the corresponding single and double mutants along with nonmutant strains (table). Survival curves shown in Fig. 2a indicate that radiosensitivity of double mutants *net1-srm rad9Δ* coincides with radiosensitivity of single mutants. Functions of genes *NET1* and *RAD9* seem to belong to the same pathway involved in determination of cell radioresistance.

Data shown in Fig. 2b suggest that radiosensitivity of the double mutant *net1-srm rad17Δ* revealed on the linear segment of radiosensitivity curve is higher than that of single mutants and coincides with the calculated curve of additivity. Hence, functions of the pair of genes *NET1* and *RAD17* cannot be assigned to the similar nonbranched pathway involved in determination of cell radioresistance. On the basis of the results illustrated in Fig. 2c, we arrived at the analogous conclusion with respect to the pair of functions mediated by genes *NET1* and *RAD24*.

Figure 2d presents survival curves of a series of single and double mutants in *NET1* and *RAD53* along with nonmutant strains. At high doses, the survival curve of double mutants has a "tail" and mainly reflects the behavior of budding cells, for which increased radioresistance is a typical feature [24]. To ensure correctness of the analysis, the initial linear segments of survival curves should be compared. At low doses, radiosensi-

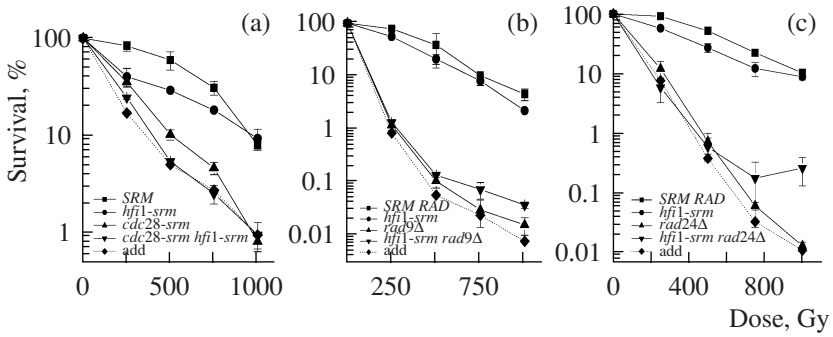

Fig. 3. Relationship between mutation *srm12/hfi1-srm* and *srm5/cdc28-srm* (a), *rad9Δ* (b), *rad24Δ* (c). Each curve corresponds to the mean data for two–four strains of the same genotype; standard errors are given. For comparison, a curve expected upon additive effect of two mutations on cell radiosensitivity is presented.

tivity of double mutants *net1-srm rad53* is higher than that of single mutants and coincides with the calculated additivy curve. Hence, a function of *RAD53* falls out of the nonbranched pathway controlled by genes *NET1* and *RAD9*. Although survival curves for budding cells were linearly approximated, we cannot speak about the epistatic type of interactions between *net1-srm* and *rad53* mutations with respect to radiosensitivity, because a proportion of budding cells was not evaluated in each culture.

Analysis of the interaction between *net1-srm* and *cdc28-srm* mutations with respect to radiosensitivity of double mutants is not available, because double mutants *cdc28-srm net1-srm* are nonviable.

Analysis of Changes in Cell Radiosensitivity upon Interaction between Mutation hfi1-srm and Mutations rad9Δ, rad24Δ, and cdc28-srm

In order to examine effects of pairwise combinations of *hfi1-srm* with checkpoint gene mutations and mutation *cdc28-srm*, we constructed a series of the corresponding single and double mutants along with nonmutant strains (table). The *hfi1-srm* mutation alone causes only relatively slight change in cell sensitivity to γ-irradiation and has the ability to revert. Survival curves shown in Fig. 3 do not allow to determine the interaction between mutation *hfi1-srm* and *rad9Δ* or *rad24Δ* mutations. At small irradiation doses (up to 300 Gy), additive effects of mutations were observed in the above pairs, whereas the additivity disappeared when the dose increased.

Double mutants *hfi1-srm rad53* and *hfi1-srm net1-srm* [4] are virtually nonviable. In contrast, *hfi1-srm cdc28-srm* cells retain viability. Survival curves shown in Fig. 3a testify in favor of additive effects of mutations *hfi1-srm* and *cdc28-srm* that cause changes in cell radiosensitivity. Ultimately, we cannot establish with confidence *HF11*-dependent pathway of yeast radiosensitivity.

DISCUSSION

There are many works devoted to genetic control of yeast sensitivity to ionizing radiation [2]. epistasis group s of genes corresponding to various pathways of DNA DSB repair have been established. A large group of genes controlling cell cycle arrest upon DNA damage and enhancing repair capacity has been isolated [25]. The results of studying checkpoint genes *RAD9*, *RAD17*, *RAD24*, and *RAD53* showed that these genes could be assigned to a single epistasis group seemingly corresponding to *RAD9*-dependent pathway involved in the determination of cell radioresistance [21]. However, the ultimate identification of radioresistance genes is not completed [1, 26]. In particular, we identified for the first time *CDC28/SRM5*, *NET1/SRM8*, and *HFI1/SRM12* as radioresistance genes [22, 6]. In this work, effects caused by combinations of mutations in these genes and mutations in other radioresistance genes were studied.

Analysis of epistatic relationships between mutations revealed that mutation *rad9Δ* is epistatic with *cdc28-srm* and *net1-srm* mutations. Meanwhile, the effects of *srm* mutation and of at least one of mutations *rad17Δ*, *rad24Δ*, and *rad53* (in combinations *cdc28-srm* and *rad53*; *cdc28-srm* and *rad17Δ*; *net1-srm* and *rad17Δ*; *net1-srm* and *rad24Δ*; *net1-srm* and *rad53*; *hfi1-srm* and *rad9Δ*; *hfi1-srm* and *rad24Δ*; *hfi1-srm* and *cdc28-srm*) were additive. Thus, all analyzed genes belong to a single though branched, *RAD9* epistasis, group; for instance, *RAD53* and *CDC28* kinases are attributed to different branches of the *RAD9*-dependent pathway involved in the determination of yeast radioresistance.

Our data indicate that the CDC28 kinase is involved in interchromosomal recombination [14]; its mutants are characterized by an essential enhancement of sensitivity to ionizing radiation in diploids and by only a slight increase in haploid mutant cells. These properties are characteristic of, for instance, Srs2 DNA helicase [27, 28], whose activity depends on kinase CDC28 [29]. The influence of CDC28 on the frequency of spontaneous and induced mitotic recombination has

been detected [14]. The rate of spontaneous gene conversion in mutant *cdc28-srm* is increased by a factor of 10, compared to the wild-type cells, the frequency of induced mitotic recombination being inhibited in cells of this mutant and accompanied by a decrease in the frequency of gene conversion and crossingover. Gene *CDC28* does not belong to the *RAD6* or *RAD52* epistasis groups mediating the repair of a major portion of DNA radiation lesions via postreplication repair and HR. Mutations confererring disturbance in minor pathways of DNA DSB repair, for example NHEJ, virtually do not change sensitivity to ionizing radiations in *RAD52* cells with competent HR but exert quite noticeable effect in mutant *rad52* cells [30, 31]. Radiosensitivity of these double mutants, for one, *sir2 rad52* cells [32] is close to that of double mutants *cdc28-srm rad52*. Gene *CDC28* can be assumed to also regulate the minor repair pathway (pathways).

However, the effect on radiosensitivity of double *cdc28-srm rad52* mutants may be related to the involvement of gene *CDC28* in the checkpoint control [33]. This is also confirmed by indirect data, such as the epistatic type of mutation in checkpoint genes *RAD9*, *RAD17*, and *RAD24* with respect to *cdc28-srm* mutation. The additivity of *cdc28-srm* and *rad53* effects points in this case to the branched pathway of checkpoint control. The introduction of *rad53* in the genotype of double mutant *cdc28-srm rad52* does not additionally enhance γ-sensitivity. Nevertheless, mutation *rad53* decreases the effectiveness of NHEJ [34] and, in contrast to mutations *rad9Δ*, *rad24Δ*, and *rad17Δ*, decreases the effectiveness of HR [35]. Apparently, exactly this is the reason for the additivity of *rad53* and *cdc28-srm* effects.

The results obtained by us about the role of gene *CDC28* in repair and checkpoint control agree with data of the other authors [36]. CDC28 kinase activity is required for efficient resection of DSB ends depending on the MRX (*MRE11/RAD59/XRS2*) comlex and for DNA damage checkpoint activation by a DSB. The functional interaction between kinase Cdc28/Gib2 and the MRX complex during the cell cycle transition, recombination, and repair has been shown in [37, 38]. Although some proteins required for DNA recombination (including Mre11p, Rad50p, Srs2p, and Rad9p) are targets of the CDC28 kinase [29, 39], the functional significance of phosphorylation is still unclear. In nonirradiated cells, the Mre11 protein forms a complex with Srs2 and Sgs1 helicases. The irradiation leads to the inducible CDC28-dependent Srs2 helicase phosphorylation and Mec1p-dependent formation of two Sgs1p–Mre11p and Srs2p–Mre11p complexes [40]. The Mre11 protein having DNA-nuclease and DNA-helicase activities may be potentially phosphorylated with the CDC28 kinase at several sites. However, it was shown that mutations at these sites do not affect the checkpoint control activation and, probably, the resection of DSBs [36]. Moreover, the possibility of CDC28 involvement in a minor BIR repair pathway is not excluded. Dihybrid interaction was shown between Clb2 and the Mus81p protein involved in BIR repair [41].

It should be noted that HR is not fully inhibited in G1-arrested cells (the low activity level of kinase) or in Cdc28-inhibited cells, in which resection of DNA DSB ends, which is possibly accomplished by Cdc28-independent exonuclease, was observed. Nevertheless, for cells irradiated with UV light at G1 phase, it is known that radiation-induced phosphorylation of Rad53 kinase depends on kinase activity of Cdc28/Clb [42]. Correspondingly, in tests of cell sensitivity to UV light we will expect that mutations *cdc28* and *rad53* should be epistatic to each other. In experiments with γ-irradiation, we found additive effects of these mutations, suggesting that there is an additional *CDC28* dependent pathway of postradiation recovery. Note that *cdc28-srm rad53* cells are more sensitive to γ-irradiation than the corresponding single mutants and double mutants *rad9Δ cdc28-srm* and *rad9Δ rad53*, Apparently, the noticeable role in determining yeast radioresistance may be attributed to the *RAD9*-independent regulatory mechanism mediated by *CDC28* and *RAD53* genes. When these two genes are simultaneously damaged, the mechanism of radioresistance seems to be inactivated.

The impact of the nucleolar protein Net1 on radiosensitivity is likely to be mediated by its participation in regulating the localization of deacetylase Sir2. Deacetylase Sir2 is involved in processes affecting cell radiosensitivity, namely, in silencing the sex expression loci [43, 44] and in histone deacetylation [45]. Diploid cells that express both alleles of the mating type locus, *MAT*a and *MAT*α, manifested a decreased repair of NHEJ compared to haploids that express only *MAT*a or *MAT*α allele [43, 44, 46]. Disturbance in genes *SIR2, SIR3*, and *SIR4* involved in transcriptional gene silencing decreases the effectiveness of NHEJ in haploids [32] mainly, as a consequence of derepression and simultaneous expression of *HML*a and *HML*α loci [44, 45]. Disturbance in the distribution of silencing proteins in diploid cells of mutant *net1-srm* may lead to a change in NHEJ effectiveness at the expense of an incomplete expression of *MAT* loci or, more likely, to the enhancement of NHEJ effectiveness.

In our case, the enhancement of radiosensitivity in *net1-srm* mutants is apparently related to modification, deacetylation of histones, and/or other proteins at DNA break rather than to silencing at *MAT* loci. Activation of DNA repair and checkpoint regulation of cell cycle are coupled with changes in chromatin structure related, in particular, to chemical modification of histones. Deacetylases Rpd3, Hst1, and Sir2 involved in deacetylation of N-tails of H4 and H3 histones are shown to come together in the DSB region during HR [45].

In the course of HR, histone-acetyltransferases Gcn5 and Esa1, components of the corresponding SAGA and NuA4 complexes that accomplish the acetylation of histones H3 and H4, respectively, also were detected near DSB [45]. Deletion of the N-tail of his-

tone H3 causes more pronounced enhancement of sensitivity upon DSB induction, compared to disturbance in H4-tailΔ [45]. The acetylation of N-tails of H4 histones is essential for the regulation of minor pathways in DNA repair, NHEJ and BIR [47]. Mutations affecting acetylated lysine residues in positions 14 and 23 of histone H3 [48] and mutation of *gcn5* histone-acetyltransferase [49] enhance cell sensitivity to the agents that induce DNA DSB. Mutation *hfi1-srm* of the structural subunit of the SAGA complex Ada1/Hfi1 also enhances sensitivity to ionizing radiation [6, 4]. The interaction between acetyltransferase hGCN5 and the Ku70 protein [50], which, as a heteromer, together with the Ku70 protein, regulates binding of DNA-dependent proteinkinase to DNA, was detected in human cells. As shown in [51–53], the acetylation by the STAGA complex (components *hADA3* and *hGCN5*) and NAD-dependent deacetylation of Sir2α histones mediated by a modified p53 protein are involved in apoptose and determination of radioresistance level in human cells.

The results obtained in this work with respect to radioresistance showed that gene *NET1* is epistatic with the *RAD9* gene but additive with genes *RAD17*, *RAD24*, and *RAD53*. Gene *HFI1* manifests the additive type of interaction with *RAD9* and *RAD24*. Apparently, gene *NET1* functions via the same pathway as gene *RAD9*, differing from *RAD24*-dependent pathway, whereas the *HFI1* pathway differs from *RAD9*- and *RAD24*-dependent pathways. Genetic data testify to the interaction between genes *CDC28*, *NET1*, and *HFI1/ADA1*. Double mutants *cdc28-srm net1-srm*, and *net1-srm hfi1-srm* are nonviable [4]. Mutations *hfi1-srm* and *cdc28-srm* manifest additive effect following exposure to γ-ray radiation.

Interactions between mutation *cdc28-srm* and *rad17Δ* or *rad24Δ* are puzzling. It is possible that the Rad17–Mec3–Ddcl complex is loaded onto the DNA DSB with the aid of the Rad24–Rfc1-5 complex [54]. According to our data, mutation *rad24Δ* is epistatic with *cdc28-srm*, and mutation *rad17Δ* is additive with *cdc28-srm*. These results suggest that the Rad17 complex might be engaged in an unknown *CDC28*-independent process. As shown previously, there are at least two REC-like complexes, Rad24–RFC and Chl12/Ctf18–RFC, which are involved in checkpoint regulation when replication fork is stalled [55]. These complexes are likely to control two branches, one of which is independent of CDC28. We demonstrated earlier that kinase CDC28 has no influence on the checkpoint control upon replication block [56]. Note that Rad9p is not the only activator protein for kinase Rad53. In response to replication block, the Mre1 protein mediates the Rad53 kinase activation. The additive interaction between mutations in genes *RAD24* and *RAD53* with respect to irradiation with UV light and epistatic effect upon γ-irradiation also do not correspond to the scheme of checkpoint regulation pathways. The study of radioresistance pathways requires further analysis and consideration of additional genes.

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REFERENCES

1. Bennett, C.B., Lewis, L.K., Karthikeyan, G., et al., Genes Required for Ionizing Radiation Resistance in Yeast, *Nat. Genet.*, 2001, vol. 29, pp. 426–434.
2. Game, J.C., The *Saccharomyces* Repair Genes at the End of the Century, *Mutat. Res.*, 2000, vol. 451, pp. 277–293.
3. Devin, A.B., Prosvirova, T.Yu., Peshekhonov, V.T., et al., The Start Gene *CDC28* and the Genetic Stability of Yeast, *Yeast*, 1990, vol. 6, pp. 231–243.
4. Koltovaya, N.A., Guerasimova, A.S., Tchekhouta, I.A., and Devin, A.B., *NET1* and *HFI1* Genes of Yeast Mediate Both Chromosome Maintenance and Mitochondrial rho$^-$ Mutagenesis, *Yeast*, 2003, vol. 20, pp. 955–971.
5. Devin, A.B., Koltovaya, N.A., Gavrilov, B.V., and Arman, I.P., Isolation and Characterization of New Nuclear *srm* Gene Mutations that Change the Maintenance of Both Nuclear and Mitochondrial Genetic Structures in the Yeast *Saccharomyces, Russ. J. Genet.*, 1994, vol. 30, no. 9, pp. 1036–1042.
6. Koltovaya, N.A., Karviga, T.D., Lyubimova, K.A., et al., Radiation Sensitivity of the Yeast *Saccharomyces* and *SRM* Genes: Effects of *srm1* and *srm5* Mutations, *Russ. J. Genet.*, 1998, vol. 34, no. 5, pp. 493–506.
7. Koltovaya, N.A., Maiorova, E.S., Rzyanina, A.V., et al., New mutations of *SRM* genes in *Saccharomyces cerevisiae* and Certain Characteristics of Their Phenotypic Effects, *Russ. J. Genet.*, 2001, vol. 37, no. 9, pp. 1014–1024.
8. Baranowska, H., Swietlinska, Z., Zaborowska, D., and Zuk, J., *Cdc* and *prt* Mutants of *Saccharomyces cerevisiae* with Increased Sensitivity to Diepoxybutane and Ultraviolet, *Acta Microbiol. Pol. Safetyn.*, 1982, vol. 31, pp. 119–128.
9. Ostroff, R.M. and Sclafani, R.A., Cell Cycle Regulation of Induced Mutagenesis in Yeast, *Mutat. Res.*, 1995, vol. 329, pp. 143–152.
10. Lorincz, A.T. and Reed, S.I., Sequence Analysis of Temperature-Sensitive Mutations in the *Saccharomyces cerevisiae* Gene *CDC28*, *Mol. Cell. Biol.*, 1986, vol. 6, pp. 4099–4103.
11. Kholmurodov, Kh.T., Kretov, D.A., Guerasimova, A.S., and Koltovaya, N.A., Molecular Dynamics Modeling of the Substitution of Serine for the Conservative Glycine in the G-Loop in the Yeast *cdc28-srm* Mutant Using the Crystalline Lattice of Human Kinase CDK2, *Biofizika*, 2006, vol. 51, pp. 679–691.
12. Koltovaya, N.A. and Devin, A.B., *srm* Mutations and γ-Sensitivity of Yeast *Saccharomyces*, in *Trudy rabochego soveshchaniya po geneticheskomu deistviyu korpuskulyarnykh izluchenii* (Proceedings of Workshop on Genetic Effect of Corpuscular Radiation), Dubna, 1989, pp. 145–149.
13. Koltovaya, N.A. and Devin, A.B., Coordination of Mitotic Stability of Genetic Structures and the Radiation

Sensitivity of Yeast *Saccharomyces*, *Dokl. Akad. Nauk SSSR*, 1990, vol. 315, pp. 986–990.

14. Koltovaya, N.A., Arman, I.P., and Devin, A.B., Mutation of the *CDC28* Gene and the Radiation Sensitivity of *Saccharomyces cerevisiae*, *Yeast*, 1998, vol. 14, pp. 133–146.
15. Meyn, M.A. and Holloway, S.L., S-Phase Cyclins Are Required for a Stable Arrest at Metaphase, *Curr. Biol.*, 2000, vol. 10, pp. 1599–1602.
16. Cuperus, G., Shafaatian, R., Shore, D., Locus Specificity Determinants in the Multifunctional Yeast Silencing Protein Sir2, *EMBO J.*, 2000, vol. 19, pp. 2641–2651.
17. Traverso, E.E., Baskerville, C., Liu, Y., et al., Characterization of the Net1 Cell Cycle-Dependent Regulator of the Cdc14 Phosphatase from Budding Yeast, *J. Biol. Chem.*, 2001, vol. 276, pp. 21924–21931.
18. Koltovaya, N.A. and Devin, A.B., New Nuclear Gene Mutations That Cause Coordinate Changes in Mitotic Stability of Various Genetic Structures in *Saccharomyces cerevisiae*, *Yeast,* 1995, vol. 11, p. 72.
19. Horiuchi, J., Silverman, N., Pina, B., et al., ADA1, a Novel Component of the *ADA/GCN5* Complex, Has Broader Effects Than *GCN5, ADA2,* or *ADA3*, *Mol. Cell. Biol.*, 1997, vol. 17, pp. 3220–3228.
20. Sherman, F., Fink, G.R., and Hicks, J.B., *Laboratory Course Manual for Methods in Yeast Genetics*, New York: Cold Spring Harbor Lab., 1986.
21. Koltovaya, N.A., Nikulushkina, Yu.V., Roshina, M.P., and Devin, A.B., Interactions of Chekpoint-Genes *RAD9*, RAD17, *RAD24* and *RAD53* in Determining Radioresistance of Yeast *Saccharomyces*, *Russ. J. Genet.*, 2008, vol. 44, no. 6, pp. 659–668.
22. Koltovaya, N.A. and Kadyshevskaya, E.Yu., *CDC28* Gene and Chekpoint Control in Yeast *Saccharomyces*, *Dokl. Ros. Akad. Nauk*, 1997, vol. 357, pp. 710–712.
23. McKee, R.H. and Lawrence, C.W., Genetic Analysis of γ-Ray Mutagenesis in Yeast: III. Double-Mutant Strains, *Mutat. Res.*, 1980, vol. 70, pp. 37–48.
24. Kadyk, L.C. and Hartwell, L.H., Sister Chromatids Are Preferred over Homologs as Substrates for Recombinational Repair in *Saccharomyces cerevisiae, Genetics*, 1992, vol. 132, pp. 387–402.
25. Elledge, S.J., Cell Cycle Checkpoints: Preventing an Identity Crisis, *Science*, 1996, vol. 274, pp. 1664–1672.
26. Game, J.C., Birrell, G.W., Brown, J.A., et al., Use of a Genome-Wide Approach to Identify New Genes That Control Resistance of *Saccharomyces cerevisiae* to Ionizing Radiation, *Radiation Res.*, 2003, vol. 160, pp. 14–24.
27. Aboussekhra, A., Chanet, R., Zgaga, Z., et al., *RADH*, a Gene of *Saccharomyces cerevisiae* Encoding a Putative DNA Helicase Involved in DNA Repair: Characteristics of *radH* Mutants and Sequence of the Gene, *Nucleic Acids Res.*, 1989, vol. 17, pp. 7211–7219.
28. Aboussekhra, A., Chanet, R., Adjiri, A., and Fabre, F., Semidominant Suppressors of Srs2 Helicase Mutations of *Saccharomyces cerevisiae* Map in the *RAD51* Gene, Whose Sequence Predicts a Protein with Similarities to Prokaryotic RecA Proteins, *Mol. Cell Biol.*, 1992, vol. 12, pp. 3224–3234.
29. Ubersax, J.A., Woodbury, E.L., Quang, P.N., et al., Targets of the Cyclin-Dependent Kinase Cdk1, *Nature*, 2003, vol. 425, pp. 859–864.
30. Boulton, S.J. and Jackson, S.P., *Saccharomyces cerevisiae* Ku70 Potentiates Illigitimate DNA Double-Strand Break Repair and Serves as a Barrier to Error-Prone DNA Repair Pathways, *EMBO J.*, 1996, vol. 15, pp. 5093–5103.
31. Boulton, S.J. and Jackson, S.P., Identification of a *Saccharomyces cerevisiae* Ku80 Homologue: Roles in DNA Double-Strand Break Rejoining and in Telomeric Maintenance, *Nucleic Acids Res.*, 1996, vol. 24, pp. 4639–4648.
32. Tsukamoto, Y., Kato, J.-I., and Ikeda, H., Silencing Factors Participate in DNA Repair and Recombination in *Saccharomyces cerevisiae*, *Nature*, 1997, vol. 388, pp. 900–903.
33. Koltovaya, N.A. and Devin, A.B., Yeast Genes Involved in Both Cell Cycle Regulation at Checkpoints and Maintenance of Various Genetic Structures, *Biochem. Soc. Trans.*, 1996, vol. 24, p. 516.
34. De la Torre-Ruiz, M.A. and Lowndes, N.F., *DUN1* Defines One Branch Downstream of *RAD53* for Transcription and DNA Damage Repair in *Saccharomyces cerevisiae*, *FEBS Lett.*, 2000, vol. 485, pp. 205–206.
35. Glaser, V.M., Glasunov, A.V., Tevzadze, G.G., et al., Genetic Control of Plasmid DNA Double-Strand Gap Repair in Yeast *Saccharomyces cerevisiae*, *Curr. Genet.*, 1990, vol. 18, pp. 1–5.
36. Ira, G., Pellicioli, A., Balijja, A., et al., DNA End Resection, Homologous Recombination and DNA Damage Checkpoint Activation Require CDK1, *Nature*, 2004, vol. 431, pp. 1011–1017.
37. Grandin, N. and Charbonneau, M., Mitotic Cyclins Regulate Telomeric Recombination in Telomerase-Deficient Yeast Cells, *Mol. Cell. Biol.*, 2003, vol. 23, pp. 9162–9177.
38. Aylon, Y. and Kupiec, M., Cell Cycle-Dependent Regulation of Double-Strand Break Repair, *Cell Cycle*, 2005, vol. 4, e61–e63.
39. Liberi, G., Chiolo, I., Pellicioli, A., et al., Srs2 DNA Helicase Is Involved in Checkpoint Response and Its Regulation Requires a Functional Mec1-Dependent Pathway and Cdk1 Activity, *EMBO J.*, 2000, vol. 19, pp. 5027–5038.
40. Chiolo, I., Carotenuto, W., Maffioletti, G., et al., Srs2 and Sgs1 DNA Helicases Associate with Mre11 in Different Subcomplexes Following Checkpoint Activation and CDK1-Mediated Srs2 Phosphorylation, *Mol. Cell. Biol.*, 2005, vol. 25, pp. 5738–5751.
41. Uetz, P., Giot, L., Cagney, G., et al., A Comprehensive Analysis of Protein–Protein Interactions in *Saccharomyces cerevisiae*, *Nature*, 2000, vol. 403, pp. 623–627.
42. Clerici, M., Baldo, V., Mantiero, D., et al., A Tel1/MRX-Dependent Checkpoint Inhibits the Methaphase-to-Anaphase Transition after UV Irradiation in the Absence of Mec1, *Mol. Cell. Biol.*, 2004, vol. 24, pp. 10126–10144.
43. Astrom, S.U., Okamura, S.M., and Rine, J., Yeast Cell-Type Regulation of DNA Repair, *Nature*, 1999, vol. 397, no. 6717, p. 310.
44. Lee, S.E., Paques, F., Sylvan, J., and Haber, J.E., Role of Yeast *SIR* Genes and Mating Type in Channeling Double-Strand Breaks to Homologous and Nonhomologous

Recombination Pathways, *Curr. Biol.*, 1999, vol. 9, pp. 767–770.

45. Tamburini, B.A. and Tyler, J.K., Localized Histone Acetylation and Deacetylation Triggered by the Homologous Recombination Pathway of Double-Strand DNA Repair, *Mol. Cell. Biol.*, 2005, vol. 25, pp. 4903–4913.
46. Clikeman, J., Khalsa, G., Barton, S., and Nickoloff, J., Homologous Recombinational Repair of Double-Strand Breaks in Yeast Is Enhanced by *MAT* Heterozygosity Through yKU-Dependent and -Independent Mechanisms, *Genetics*, 2001, vol. 157, pp. 579–589.
47. Bird, A.W., Yu, D.Y., Pray-Grant, M.G., et al., Acetylation of Histone H4 by Esa1 Is Required for DNA Double-Strand Break Repair, *Nature*, 2002, vol. 419, pp. 411–415.
48. Qin, S. and Parthun, M.R., Histone H3 and the Histone Acetyltransferase Hat1p Contribute to DNA Double-Strand Break Repair, *Mol. Cell. Biol.*, 2002, vol. 22, pp. 8353–8365.
49. Choy, J.S. and Kron, S.J., NuA4 Subunit Yng2 Function in Intra-S-Phase DNA Damage Response, *Mol. Cell. Biol.*, 2002, vol. 22, pp. 8215–8225.
50. Barlev, N.A., Poltoratsky, V., Owen-Hughes, T., et al., Repression of GCN5 Histone Acetyltransferase Activity via Bromodomain-Mediated Binding and Phosphorylation by the Ku/DNA-PK Complex, *Mol. Cell. Biol.*, 1998, vol. 18, pp. 1349–1358.
51. Barlev, N.A., Liu, L., Chehab, N.H., et al., Acetylation of p53 Activates Transcription through Recruitment of Coactivators/Histone Acetytransferases, *Mol. Cell*, 2001, vol. 8, pp. 1243–1254.
52. Luo, J., Nikolaev, A.Y., Imai, S., et al., Negative Control of p53 by Sir2 Promotes Cell Survival under Stress, *Cell*, 2001, vol. 107, pp. 137–148.
53. Vaziri, H., Dessain, S.K., Ng, Eaton.E., et al., *hSIR2* (*SIRT1*) Functions as an NAD-Dependent p53 Deacetylase, *Cell*, 2001, vol. 107, pp. 149–159.
54. Kondo, T., Matsumoto, K., and Sugimoto, K., Role of a Complex Containing Rad17, Mec3, and Ddc1 in the Yeast DNA Damage Checkpoint Pathway, *Mol. Cell. Biol.*, 1999, vol. 19, pp. 1136–1143.
55. Naiki, T., Kondo, T., Nakada, D., et al., Chl12 (Ctf18) Forms a Novel Replication Factor C-Related Complex and Functions Redundantly with Rad24 in the DNA Replication Checkpoint Pathway, *Mol. Cell. Biol.*, 2001, vol. 21, pp. 5838–5845.
56. Koltovaya, N.A. and Kadyshevskaya, E.Yu., Checkpoint Control in Yeast *Saccharomyces cerevisiae* in *"Problemy radiatsionnoi genetiki na rubezhe vekov"* (Problems of Radiation Genetics at the Turn of Centuries), Proc. Int. Conf., Moscow, 2000, pp. 130–131.