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ADA1 AND *NET1* GENES OF YEAST MEDIATE BOTH CHROMOSOME MAINTENANCE AND MITOCHONDRIAL RHO$^-$ MUTAGENESIS

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INTRODUCTION

Haploid cells of *Saccharomyces cerevisiae* are able to maintain, in addition to the components of the hereditary apparatus essential for viability (chromosomes), the facultative genetic structures (FGSs): natural extra chromosomes, the mitochondrial genome, and recombinant plasmids. The fidelity of the mitotic transmission (mitotic stability) of chromosomes of an euploid set is high. The rate (per one cell division) of spontaneous loss of a chromosome is usually 10^{-7} and lower. FGSs manifest much lower mitotic stability as compared with chromosomes indispensable for viability. Among different FGSs, significant variations of their mitotic stability were also found. The rate of spontaneous loss is about $10^{-4} - 10^{-3}$ for an extra chromosome and rarely below 1% for a recombinant plasmid. At even higher rates that may reach, at least in certain laboratory strains, 10% and more cytoplasmic petite mutants arise due to degenerative changes of the yeast mitochondrial genome.

The data of our previous studies suggest that notwithstanding these quantitative differences the same genes designated as *SRM* mediate the maintenance of different FGSs in yeast cells. In particular, a mutation in the *CDC28/SRM5* gene which plays a key role in cell cycle regulation was found to change the mitotic stability of both nuclear and mitochondrial FGSs (Prosvirova and Devin, 1988; Devin *et al.,* 1990).

On the other hand, the *cdc28-srm* mutation was accompanied by an increase in yeast cell sensitivity to lethal effects of ionising radiation (Koltovaya *et al.,* 1998). Thus, interconnection at the genic level was found between FGS maintenance, cell cycle regulation and radiation sensitivity of *S. cerevisiae* cells.

To study this relationship in more detail we isolated additional *srm* mutants with changed mitotic stability of both the mitochondrial genome and a natural extra chromosome. UV mutagenesis and a special selection technique were employed for this purpose and novel *SRM* genes were thus uncovered. Gene cloning and sequencing showed the *SRM8* and *SRM12* genes to be identical with the *NET1* and *ADA1*, respectively. These two genes encode constituents of multiprotein complexes that regulate transcription (Horiuchi *et al.*, 1997) and cell cycle progression (Shou *et al.*, 1999: Visintin *et al.*, 1999). Data concerning their roles in the maintenance of hereditary structures and determination of the yeast sensitivity to ionising radiation are reported below.

MATERIALS AND METHODS

Strains of micro-organisms

The strains of *Saccharomyces cerevisiae* used are listed in Table 1. The *Escherichia coli* strains DH5 and XL-1Blue (Stratagene) (Sambrook *et al.,* 1989) served as a host for plasmid DNA manipulation and a phagemid recipient, respectively. The M13K07 hepler-phage was also employed.

Plasmids and yeast genomic DNA libraries

Standard plasmids YCp50 (Rose *et al.*, 1987), YEp13 (Broach *et al.*, 1979), and YRp12 (Scherer and Davis, 1979) were used in our work as well as the pNGB52 (*SUP110*) plasmid kindly given by Professor T.D. Fox (Cornell University, Ithaca). The pTZ19U phagemid (Sambrook *et al.*, 1989) was used for DNA fragment sequencing. A *S. cerevisiae* genome DNA library designated as 2J351 (Engebrecht *et al.*, 1990) was obtained from Dr. I.P. Arman. The library was produced by partial *Sau3A* hydrolysis of yeast genomic DNA followed by fragment ligation at the *BamHI* site of plasmid YEp351 (Hill *et al.*, 1986) containing the 2μ circle origin of DNA replication and, as a selective marker, the *LEU2* gene. A yeast gene bank in the p366 vector (*CEN4 ARS1 LEU2*) was kindly given by Dr. V. Urakov (All-Russian Cardiology Center, Moscow).

Media and reagents

We used the standard complete nutrient medium YEPD (Difco) (Sherman *et al.*, 1986) and media CM, MM, and LCD described in (Devin *et al.*, 1990), restriction endonucleases and other enzymes (Fermentas, Vilnius), ethidium bromide (Sigma), and cycloheximide (Serva). Plasmid DNA was purified with the Bluesorb sorbent (Clonogene, St. Petersburg).

Transformation of yeast cells with plasmid DNA

was performed by a standard technique (Ito *et al.*, 1983).

DNA sequencing

An approximately 3-kb *Sma-Xba* fragment was cloned in the pTZ19U phagemid which was transformed into a single-stranded form with the use of the M13KO7 helper phage. The XL1-Blue strain was used as a recipient. Single-stranded DNA was isolated according to a conventional procedure (Sambrook *et al.*, 1989). A set of coloured fluorescent terminators (ABIPRISM Dye Terminator Kit with AmpliTaq, Perkin Elmer) and ORF specific primers were employed for determining the nucleotide sequence in a ABIPRISM 3700 sequenator.

Evaluation of yeast cell division rates

Cells were grown overnight in the liquid YEPD medium at 30°C with aeration, diluted in fresh YEPD medium to achieve an initial concentration of 10^5-10^6 cells/ml, and then incubated with agitation at 30°C up to a concentration of 10^7-10^8 cells/ml. The cell concentration in the growing culture was determined at time intervals in a cell counter. Generation times were calculated from the parameters of the exponential parts of the culture growth curves.

Determination of mitotic stability of chromosomes IV and XIV in n+1 disomes

Disomic *ade1* pink colonies grown for 5 days on CM at 30°C were suspended and plated on CM in such a way as to have 100 colonies per dish. In the platings mosaic pink-red colonies with red sectors no smaller than half a colony were then scored. The rate of

spontaneous extra chromosome IV or XIV loss in the first budding after plating was estimated by the ratio of the number of mosaic colonies to the total number of pink and mosaic colonies.

Determination of mitotic stability of chromosome VII

The *ade6* locus is located in the right arm of chromosome VII, the *cyh2* and *leu1* loci are located in the left arm of this chromosome. Mutations at these three loci were used to mark one chromosome VII homolog in diploid cells, whereas the other homolog carried their normal alleles. In addition, cells were homozygous for the recessive *ade2* mutation that caused accumulation of red pigment. In these cells, the loss of nonmarked chromosome VII should lead to simultaneous expression of the *cyh2, ade6*, and *leu1* mutations, i.e. to cycloheximide resistance, the loss of pigmentation, and leucine auxotrophy, respectively (Parry and Zimmermann, 1976). From single diploid cells, in which one of the two chromosome VII homologs was marked with these three mutations, colonies were obtained after growth on the CM medium for 5 days. The colonies were suspended in water, and properly diluted cell suspensions were plated on the CM and CM plus cycloheximide (4 mg/l) media. White (*ade6*) cycloheximide-resistant (*cyh2*) colonies were isolated and tested for leucine auxotrophy (*leu1*) on a selective medium prepared on the basis of the MM medium. Cells possessing all three mutant traits were considered as those that had lost the nonmarked homolog of chromosome VII.

Determination of mitotic stability of recombinant plasmids

Cultures of cells transformed with the YCp50 plasmid (*ARS1 CEN4 URA3*) or the YRp12 plasmid (*ARS1 URA3*) were grown on the nonselective CM medium or a MM-based selective uracil-deficient medium, respectively. Properly diluted samples of grown cultures were plated on CM. The proportion of Ura^+ colonies as a measure of plasmid mitotic stability was determined in these platings. Cultures of YEp13 (2μ *ori LEU2*) transformants were grown on a synthetic medium lacking leucine, plasmid stability was characterised by the proportion of Leu^+ colonies in platings on CM.

Determination of cell sensitivity to γ-irradiation

Aqueous cell suspensions (10^3 to 10^5 cells/ml) of 7-day-old stationary-phase cell cultures grown on the agarised CM medium were irradiated with γ-rays at 0°C on a "Svet" apparatus (^{137}Cs) at the dose rate of 25 Gy/min. Control and irradiated suspensions were plated on the CM medium so as to produce approximately 100 colonies on a Petri dish after incubation for 5 to 7 days at 30°C.

Induction of rho^0 mutants by ethidium bromide

We used the Clark-Walker method with some modifications. A loopful of yeast cells were plated on the surface of LCD medium containing 10 mg/ml ethidium bromide (EthBr). After incubation for 24 h, cells were taken from the margins of the "dead zone" and were sequentially subcloned several times on the LCD medium. Colonies of petite mutants are easily detected on this medium due to their small size. The petite mutants

obtained in this manner were shown to be completely devoid of mitochondrial DNA (mtDNA), i.e., they are rho^0 mutants (Clark-Walker, 1972).

Evaluation of frequency of rho^- mutants induced by ethidium bromide

EthBr (10 μg/ml) was added to yeast cells exponentially growing in liquid CM (10^5-10^6 cells/ml). At definite time intervals, cells were taken and washed, and properly diluted samples were plated on solid CM and incubated for 5 days at 30°C. Colonies of petite mutants are easily detected due to their small size and white colour.

RESULTS

Induction and selection of mutants with coordinate changes in nuclear and mitochondrial genetic stability

Cultures of parental yeast *ade1*-mutant strains 71a and 71α and most of their derivatives employed in this work usually contain up to 50% mitochondrial petite [rho^-] mutants. This high frequency is determined by mutations in several nuclear genes, each of which has only small effect (Devin and Koltovaya, 1987). Cells form colonies of a characteristic starlike shape (*Sta*$^+$ trait), whereas the regular round shape is typical of ordinary yeast colonies (*Sta*$^-$) containing only a few per cent of rho^- cells.

To obtain mutations decreasing both the chromosome stability and the spontaneous rho^- mutability, a special colour assay was employed (Fig. 1). In our strains disomy for chromosome IV markedly suppresses spontaneous rho^- mutability (these disomic cells form regular round colonies instead of starry ones) as well as red colony pigmentation caused by the *ade1* mutation. Chromosome IV loss from disomes is accompanied by restoration of both traits that are characteristic of the initial haploid *ade1* mutant strains, i.e. intense pigmentation and starry colony shape. Mutations simultaneously destabilising extra-chromosome maintenance and suppressing spontaneous rho^- mutability may be selected as they should manifest themselves in frequent spontaneous segregation by strains disomic for chromosome IV of intensely pigmented round-shape colonies and/or sectors. Strain H5 disomic for chromosome IV was grown aerobically for 3 days in a liquid complete medium (CM) up to the stationary phase. Cells were then washed, resuspended in water, irradiated with a UV light dose producing about 10% survival, and then plated on the agarised CM just after the irradiation. After incubation for 4 days colonies of regular shape and intense pigmentation were scored in the platings. 14 uniformly pigmented and 6 "mottled" colonies were thus isolated. By virtue of repeated back-crosses we managed to isolate 10 independent mutations responsible for the above traits (at least 4 successive back-crosses turned out to be necessary for any single mutation). Mutations *srm8, srm12, srm15*, and *srm17* with the most pronounced phenotypic manifestation were analysed further.

Tests for allelism and interactions between srm8, srm12, srm1, and cdc28-srm mutations

As described above, we selected a number of *srm* mutants, producing each a meiotic progeny where the mutant trait (decreased spontaneous rho^- mutability) segregated in the Mendelian (2:2) way. To test whether or not the newly obtained *srm* mutations are

alleles of the *CDC28* or *SRM1* genes obtained and characterized earlier (Devin and Koltovaya, 1981; Devin *et al.*, 1990), the inheritance of the *Sta*$^+$ (high rho$^-$ mutability, "star-like" colonies) and *Sta*$^-$ (lower rho$^-$ mutability, colonies of regular round shape) traits in the meiotic progenies of proper crosses were analysed. Three types of tetrads, viz. parental (P) and non-parental (N) ditypes and tetratype (T), were revealed. The numbers of tetrads belonging to each of the above three types are presented in Table 2. As seen from this Table, for each of the 5 crosses analysed the P:N:T ratio is close to 1:1:4, which is expected for a progeny of a hybrid heterozygous for 2 non-linked nuclear genes. Thus the *srm8*, *srm12*, *srm1* and *cdc28-srm* mutations are in 4 different genes. Similarly, the *srm15* and *srm17* mutations are not allelic to the *srm1* or *cdc28-srm* (data not shown). Double mutant cdc28-*srm srm8* and *srm8 srm12* spores are incapable of forming colonies. This synthetic lethality suggests some functional redundancy or important interactions in the Srm8 and Cdc28 pair of proteins as well as in the Srm8 and Srm12 pair. There is no pronounced phenotypic difference between single *srm8* and double *srm1 srm8* mutants. Also, the phenotypic characteristics of double *srm1 srm12* and *cdc28-srm arm12* mutants do not suggest any dramatic interaction in the respective pairs of mutant proteins.

Morphological changes and characteristics of cell division in srm mutants

The properties of the nonallelic *srm8*, *srm12*, *srm15*, and *srm17* mutants are described below. The *srm15* and *srm17* mutations do not markedly affect morphological cell properties characteristic of *SRM+* cells (Fig. 2a) whereas *srm8* and *srm12* cause marked morphological changes in cells. Most cells in the cultures of the *srm8* strains are elongated (Fig. 2b). In the *srm12*-mutant cultures viewed through a light microscope cells having a changed, non-round, shape as well as the remains of spontaneously lysed cells are frequently encountered (Fig. 2c).

The *srm12* mutation cause intense accumulation of red pigment by *ade2*-cells. Excess of the pigment is observed especially during preparing of DNA from lysed cells.

Many *Ade*$^+$ revertants are accumulated in aged cultures of *srm12* lines carrying the *ochre*-suppressible *ade2-101* mutation. For this reason the *srm12 ade2-101* strains cannot be stored as desiccated cultures in condensed milk: only non-pigmented revertant cells are recovered from these cultures. Parallel to the recovery of adenine prototrophy, morphological changes typical of the original *srm12* mutants disappear in revertants, and cells regain a virtually normal division rate. Apparently, *srm12* is an *ochre*-suppressive mutation. The appearance of phenotypically normal cells was observed in the *srm12* mutant cultures tested for growth at elevated temperatures. This effect can also be caused by suppression of the *srm12* mutation.

On the contrary, cells with the *srm8* mutation reproduce at 37°C much more slowly than at 30°C, and their morphological abnormalities are more pronounced at the higher temperature. Thus, the *srm8* mutation shows a resemblance to temperature-sensitive mutations. Anticipating, we should, however, note that the *SRM8* gene may be disrupted without the loss of cell viability. As already noted, the *srm8* and *srm12* mutations in fact completely block sporulation in homozygous mutant diploids.

Mutations *srm8*, *srm12*, and *srm15* significantly decrease the rate of cell division. In contrast, the *srm17* mutation has a relatively weak effect on the cell division rate (Table 3).

It is known that axial budding (i.e., formation of new buds by a pair consisting of a mother and a daughter cells near the site of junction between the maternal and daughter cells) is typical of round haploid cells, whereas elongated diploid cells manifest bipolar bud formation (Freifelder, 1960). In this connection the budding pattern of the *srm8* and *srm12* cells clearly deviating from the round shape seemed of interest. Studying the initial events of colony formation (i.e. those producing four-cell aggregates, Fig. 2) confirmed that *SRM+* cells formed buds near the junction sites (Fig. 2a). In the *srm12* and *srm8* mutants, unipolar budding (one bud formed near and one opposite to the junction site) was observed at a rather high frequency (about 20%, Fig. 2b and 2c). Apparently, the *SRM8* and *SRM12* genes play a significant role in the mechanism(s) responsible for axial budding of normal haploid cells.

The effect of srm mutations on spontaneous rho⁻ mutability

Two complete tetrads were selected from every cross between each of the *srm8, srm12, srm15*, and *srm17* strains and the *SRM+* strain (71a or 71α). The averaged proportions of spontaneous rho⁻ mutants in the monospore *srm* and *SRM+* clones of these tetrads are presented in Table 4. As seen from this Table, the *srm8, srm12* and *srm15* mutations are like the previously studied *srm* mutations (Devin and Koltovaya, 1981; Devin *et al.*, 1990) in that each of them also decreases the percentage of spontaneous rho⁻ mutants in cell cultures by a factor of several tens. As compared with these tree mutations, the fourth, *srm17*, produces a weaker, although significant effect on the spontaneous rho⁻ mutability.

Induction of rho⁻ mutations by ethidium bromide in cells of different genotypes

From each cross between each of the *srm8, srm12, srm15* and *srm17* mutants and the parental 71a or 71α strains two complete tetrads were isolated. For each monospore clone from these tetrads the sensitivity to mutagenic action of EthBr was determined. Clearly, the *srm8* and *srm12* clones were less sensitive to EthBr than the *SRM+* clones (Fig. 3, a and b). No marked effect of the *srm15* and *srm17* mutations on the EthBr-induced rho⁻ mutability of cells was observed (data not shown).

Effect of srm mutations on the mitotic stability of chromosomes

As already mentioned (see Materials and Methods) colonies of the *ade1* or *ade2* mutant n+1 disomes for chromosome IV or XIV and true n haploid differ in red pigmentation intensity. This difference was used to estimate the mitotic chromosome stability as affected by *srm* mutations. *SRM+* and *srm* disomes were isolated as monospore clones from crosses between *SRM+* disomes and *srm* haploid. Then for these groups of disomic clones the chromosome loss frequencies (i.e. frequencies of half-sectored pink/red colonies) were determined, when possible, and compared.

We failed to evaluate the effect of the *srm8* and *srm17* mutations on the maintenance of extra chromosomes due to slow growth of the corresponding mutant

disomes. Similarly, no half-sectored colonies could be found in platings of the *srm12* disomes for chromosome IV due to a great difference in growth rates between the *srm12* haploids and disomes. However great proportions of relatively fast-growing (i.e. non-disomic) colonies found in these platings suggest a decline in mitotic stability of chromosome IV in the *srm12* disomes.

We managed to estimate the effects of mutations *srm12* and *srm15* on chromosome XIV stability (Table 5). As seen from this Table, an approximately 30-times increase in the frequency of extra chromosome XIV loss was observed in the *srm12* disomes as compared with the *SRM+* ones. A considerably smaller, although statistically significant increase in this rate was also caused by the *srm15* mutation.

We also attempted to assess the effect of *srm* mutations on the fidelity of mitotic transmission of chromosomes in euploid cells. For this purpose, a genetic system proposed by Parry and Zimmermann (1976) was used. Employing specially constructed *srm* mutant and *SRM+* diploids *cis*-heterozygous for chromosome VII markers (*ade6/+ cyh2/+ leu1/+*) we could detect the loss of a chromosome VII homologue bearing the normal *ADE6*, *LEU1* and *CYH2* alleles.

As seen from Table 6, the *srm8/srm8* strains lost chromosomes VII spontaneously at a rate about 100 times that for the *SRM+* strains, whereas the *srm12/srm12* strains had a several-fold increase in this rate. These data confirmed participation of the *SRM8* and *SRM12* genes in the maintenance of chromosomes in yeast cells. Mutations *srm15* and *srm17* did not markedly increase the rate of spontaneous loss of chromosome VII (Table 6).

Effect of srm mutations on the mitotic stability of recombinant plasmids

Cells of specially constructed closely related *srm*-mutant and nonmutant uracil-dependent (*ura3*) haploid recipient strains were transformed with circular plasmids YCp50 (*CEN4 ARS1*) and YRp12 (*ARS1*). Note that apart from *srm* mutations *per se*, differences in the genotypic background of the transformed cells possibly make a contribution to differences between transformants in plasmid maintenance. We indicated above that the recipient strains were closely related. However they cannot be regarded as completely identical with respect to the genotypic background. This is especially true because (as shown in the preceding section) *srm* mutations may be responsible for a marked decrease in the genetic stability of cells and, therefore, may promote changes in the genotypic background. Moreover, it cannot be ruled out that changes in the genotypic background also occur upon genetic transformation of cells. Having taken the above reasons into consideration we regularly used two strains, *MAT*a and *MAT*α, bearing a certain *srm* mutation and two *SRM+* strains to obtain transformants. Mitotic plasmid stability was estimated for 2 to 4 independent transformants of each recipient strain, all clones of the same genotype were grouped, the data were averaged within each group of *srm* clones as well as within *SRM+* clones, and the averaged data were compared.

As seen in Table 7A, the mitotic stability of the circular YCp50 minichromosome was lower in each of four groups of the mutant *srm* transformants with the normal mitochondrial genome [rho$^+$] than in the *SRM+* [rho$^+$] transformants. A decrease in the mitotic stability of centromereless plasmid YRp12 was observed on CM in *srm12, srm15*,

and *srm17* mutants (Table 7B). Obviously, the *SRM8, SRM12, SRM15*, and *SRM17* genes, like the *SRM1* and *CDC28/SRM5* (Devin *et al.*, 1990), participate in the maintenance in yeast cells of circular recombinant plasmids carrying a chromosomal *ARS* element.

Lesions in the mitochondrial genome can modify the mitotic stability of plasmids

Some publications demonstrated association between the plasmid mitotic stability and the functioning of the mitochondrial genome of yeast cells (Larionov *et al.*, 1983; Irie *et al.*, 1991). Since *srm* mutations affect the maintenance of the mitochondrial genome and the possible effect of these mutations on its functioning cannot be ruled out, we considered it expedient to estimate mitotic stability of plasmids in respiratory deficient rho$^-$ cells with a damaged mitochondrial genome in parallel with the analysis of plasmid maintenance in *srm* [rho$^+$] cells.

Most spontaneous cytoplasmic petite mutations are rho$^-$ mutations; that is, they are irreversible degenerate rearrangements of the mitochondrial genome. In the groups of transformants obtained, we selected and analysed spontaneous petite mutants retaining plasmids. As seen from Table 7, A and B, we detected at least two cases of modifications of plasmid maintenance in rho$^-$ mutants: formation of rho$^-$ mutations was accompanied by an increase in the mitotic stability of both plasmids in the *srm8* cells and by a decrease in the mitotic stability of YRp12 in the *srm15* cells ($\chi^2 = 21.2$; $P < 0.0001$).

In the original *SRM+* strains (3D and 1B), rho^0 mutants were induced by ethidium bromide (see Materials and Methods); four independent mutants were obtained for each strain. These clones that lacked the mitochondrial genome were transformed with plasmids YCp50 and YRp12. Two to four independent transformants were selected for each strain. We determined the mitotic stability of plasmids in thirteen independent transformants carrying plasmid YCp50 and in eight clones transformed with YRp12 and did not observe any marked effect of mitochondrial genome elimination on the mitotic stability of the examined plasmids in the *SRM+* cells (Table 7).

Effect of srm mutations on sensitivity of yeast cells to the lethal action of γ-radiation

To evaluate this effect, we used the above-mentioned diploid strains that were *cis*-heterozygous for markers of chromosome VII. Typical survival curves of diploid strains C3(VII)x72a, C3xC3(VII), C9xC9(VII), C14xC14(VII), F5xF5(VII) (Table 1) with genotypes *SRM+/srm8, srm8/srm8, srm12/srm12, srm15/srm15*, and *srm17/srm17*, respectively, are shown in Fig. 4. These data indicate that homozygotes for recessive *srm8*, *srm12*, and *srm17* mutations are more sensitive to the lethal effect of γ-irradiation than the heterozygous strain with normal radiation sensitivity. The *srm15* mutation did not markedly affect cell sensitivity to radiation.

Genetic mapping of srm8 mutation

An analysis of crosses between *srm8* mutants and disomes (n+1) for chromosomes II, III, IV, VII, VIII, X, XIV has shown the *srm8* mutation to be in chromosome X. Then crosses between *srm8* strains and closely related strains bearing chromosomes X genetically marked with *arg3* and *ura2* mutations were analysed. The above mutations were derived

from strains STX-9-1A and S1780C (Table 1), respectively, by crossing them and then repeatedly (at least 4 times) backcrossing with strains 71a and 71α isogenic to each other. The data of tetrad analysis (Table 8) showed the *srm8* mutation to be between the centromere and the *arg3* locus at a distance of about 10 cM from the latter. In the close proximity to this site of chromosomes X the *SCP160* gene was previously mapped (Wintersberger *et al.,* 1995). However *SCP160* on a plasmid kindly given to us by Professor U. Wintersberger did not complement the *srm8* mutation. Apparently, *SCP160* and *SRM8* are different genes.

Cloning the SRM8 gene and identification of its nucleotide sequence

To clone the *SRM8* gene, we took advantage of partial temperature sensitivity of *srm8* cells. Strain C3L (*srm8 leu2*) was transformed with DNA from the 2J351 yeast genomic library (see Materials and Methods). Cells treated with transforming DNA were plated on the MM-based selective leucine-deficient medium, grown for 2 days at 30°C, and then incubated at 37°C. After 4 days of incubation, we selected 127 large colonies and subcloned them on the selective medium. Among transformed clones that lost temperature sensitivity typical of C3L, variants with morphologically normal round cells (C3L cells, like cells of other *srm8* strains, are elongated) were then identified. One of the identified clones designated C3L-2J-1 was subjected to further analysis.

From a culture of C3L-2J-1 cells, we isolated a 9-kb plasmid, which was designated as 2J-1. Subtracting the size of the YEp351 vector DNA leaves about 3,5 kb as the size of the cloned DNA fragment.

We retransformed cells of the strain C3L (*srm8*) with plasmid 2J-1. The mitotic stability of plasmid 2J-1 was relatively high (approximately 80%) both in the original C3L-2J-1 transformant and in retransformants (Table 9). As the YEp351 vector, that was original for 2J-1, was not in our collection, the YEp13 (2μm *ori LEU2*) plasmid, structurally similar to YEp351 (2μm *ori LEU2*), was characterised for comparison with 2J-1 and found to manifest a relatively high mitotic stability in 71L (*SRM* +) cells while being much less stable in *srm8* cells (Table 9).

Thus, the *srm8* mutation is accompanied by a decrease in the mitotic stability of not only plasmid YCp50 (*ARS1 CEN4*) but also plasmid YEp13 (2 μm-*ori* DNA). The *SRM8* gene is quite probably involved in the maintenance of plasmids containing the 2 μm-*ori*, including YEp351, the original plasmid of 2J-1. Correspondingly, the *srm8* mutation must decrease the mitotic stability of these plasmids. Relatively high mitotic stability of plasmid 2J-1 in *srm8* cells is probably caused by cloned fragment present in this plasmid and complementing the *srm8* mutation. In other words, these data point to possible presence of the cloned *SRM8* gene sequence within 2J-1.

In support of this assumption the C3LD (*srm8/srm8*) cells transformed with plasmid 2J-1: were (1) less sensitive to γ-radiation than transformants of the same recipient carrying plasmid YEp13 (similar to YEp351, the original vector for 2J-1) (Fig. 5) and (2) able to sporulate (data not shown).

Thus, the presence of plasmid 2J-1 in mutant *srm8* cells is accompanied by the elimination of morphological changes in cells and by the restoration of normal radiation

resistance and sporulation ability in homozygous diploids. In addition, the DNA fragment cloned in 2J-1 seems to ensure a relatively high (compared to YEp13) mitotic stability of this plasmid in mutant *srm8* cells, which corresponds quantitatively to the mitotic stability of YEp13 in 71L (*SRM+*) cells. The above properties of 2J-1 clearly indicate functional complementation of the *srm8* mutation by the DNA fragment cloned in this plasmid.

Note that the mitotic stability of plasmid 2J-1 in 71L (*SRM+*) transformants was unexpectedly low (Table 9). The nature of this effect demands special investigation. It may be caused by the overexpression of *SRM8* gene present in a cell in multiple copies.

We determined nucleotide sequences (760 bp) at both sides of the cloned fragment. Analysis of the nucleotide sequence using the SGD (BLAST) database revealed in the cloned fragment two divergently transcribed open reading frames (ORFs) located at chromosome X (as was expected from the results of genetic mapping) and situated at a distance of less than 200 bp from each other. One of these frames, YJL076w, is 3566 nucleotides long and the other, YJL077c, contains 392 nucleotides. Our cloned DNA sequence contains a fragment of YJL076w encoding 80% of the corresponding protein sequence beginning from its N-end and only 38% of the relatively short (nine times shorter than YJLO76w) YJLO77c sequence.

The null mutant for the YJLO76w ORF did not differ phenotypically from and was allelic to *srm8* mutants: diploids derived from crosses between this null mutant and *srm8* mutants were like *srm8/srm8* homozygotes in that they divided at a markedly decreased rate, manifested specific morphological changes and did not sporulate.

Thus the *SRM8* gene, corresponds to the open reading frame YJL076w that encodes the 128,5 kDa Net1 protein of 1189 amino acids (Visintin *et al.*, 1999; Shou *et al.*, 1999). Sequencing the *srm8* allele revealed a single AA insertion (frameshift) at the position 861 which results in ORF shift after 286 aa codon (T287 is changed to K) and then terminated by stop codon in position 300 (thus truncating gene product).

Cloning the SRM12 gene and identification of its nucleotide sequence

A 11,6-kb DNA fragment containing the *SRM12* gene was cloned with the use of the p366 yeast gene bank. Restriction mapping and partial sequencing showed the cloned fragment to originate from chromosome XVI. This fragment includes several reading frames. Subcloning data suggested that *SRM12* was identical to the *SUP110/HFI1/ADA1* gene isolated in Professor T.D. Fox's Laboratory at Cornell University as a gene probably encoding a component of yeast chromatin or transcription apparatus (Brown, 1994).

Professor Fox kindly sent us the NGB121 strain (*SUP110::LEU2*), as well as the pNGB52 plasmid containing the cloned *SUP110* gene. Crossing NGB121 with a *srm12* mutant gave slowly growing diploids that did not sporulate. On the other hand, transformation of *srm12* cells with pNGB52 restored normal cell growth, sporulating ability and normal radiation resistance (data not shown). Thus *SRM12* and *SUP110* are identical.

Sequencing revealed two single nucleotide substitutions, T→C and A→T, at positions 587 and 589, respectively, of the mutant allele *srm12* of *ADA1* gene. The first substitution gives a proline (CCU) codone instead of the initial leucine (CUU) one. The second substitution makes a nonsense codone immediately after the mutated leucine

codone. Thus a double missense plus nonsense mutation [L196P; K197Stop] was found in the *srm12* sequence.

DISCUSSION

Hierarchy of SRM genes

The yeast mitochondrial genome is constituted by numerous copies (20-50 in a cell) of a circular mtDNA molecule containing eight canonical sites of replication initiation (de Zamaroczy *et al.*, 1984). The formation of cytoplasmic petite [rho$^-$] mutants in which some mtDNA sequences are lost and some are amplified may result in mutant mtDNA molecules containing each many more sites of replication initiation as compared with normal mtDNA and thus probably having a replicative advantage (Bernardi, 1979). Mutations in many nuclear genes modulate the spontaneous rho$^-$ mutagenesis (Contamine and Picard, 2000). This modulation is mainly unidirectional and usually results in increased spontaneous rho$^-$ mutability that may probably be of adaptive significance. Most probably, the rho$^-$ mutagenesis begins with one single mtDNA copy while other copies in a cell remain unchanged and the cell becomes transiently heteroplasmic. Experiments with genetically marked mtDNA molecules revealed a rapid segregation by heteroplasmic cells of homoplasmic cells each containing only a set of identical mtDNA copies (Treat and Birky, 1980). Genetic modifications of this heteroplasmy eliminating process would probably modify the yield of rho$^-$ mutants as well.

We identified two kinds of genotypic factors, viz mutations in the *SRM* genes (Devin, Koltovaya, 1981) and the disomy for single chromosomes (Devin *et al.*, 1987; Smirnova *et al.*, 1994), that decreased the rho$^-$ mutability.

Our data show that certain *srm* mutations determining decreased spontaneous rho$^-$ mutability (probably a result of less efficient amplification of certain mtDNA fragments in *srm* mutants as compared with normal *SRM+* cells), also affect the maintenance of natural chromosomes as well as recombinant plasmids (Devin *et al.*, 1987; 1990). The decreased mitotic stability of nuclear hereditary structures may reflect their less efficient and/or accurate replication. Thus some *SRM* genes might probably be involved in mechanisms that mediate the replication efficiency of both nuclear and mitochondrial genomes.

In this work mutations of a few more yeast chromosomal *SRM* genes are characterised that changed, in a co-ordinate way, the fidelity of mitotic transmission of both nuclear and mitochondrial genetic determinants.

Phenotypic tests revealed differences between the *srm8, srm12, srm5*, and *srm17* mutants. On the other hand, some phenotypic resemblance between the previously characterised *srm5* (*cdc28-srm*) mutant (Devin *et al.*, 1990) and the *srm8* and *srm12* strains was also observed. In addition to a decline in both the spontaneous rho$^-$ mutability and mitotic stability of natural and/or recombinant nuclear genetic structures each of these three mutations was accompanied by morphological cell changes, a decreased cell division rate, and also by an increase in cell sensitivity to the lethal action of γ-rays.

Note that cell radiation sensitivity was practically unchanged in the *srm15* mutant as well as in the *srm1* mutant studied before (Devin and Koltovaya, 1981; Koltovaya *et al.*,

1998). Other mutant alleles of these two genes could presumably change the radiation sensitivity as well, still this differential effect of the *srm* mutations on cell radiation sensitivity suggests some hierarchy of genes that control maintenance of hereditary structures in yeast cells.

This hypothesis places the *CDC28/SRM5, SRM8/NET1*, and *SRM12/ADA1* genes at higher steps of the hierarchical scale above the *SRM1, SRM15*, and *SRM17* genes. Correspondingly pleiotropy is more pronounced in mutants for the former three genes as compared with other *srm* mutants.

The present work shows that the *srm8* and *srm12* are mutant alleles of the *NET1* and *ADA1* genes, respectively. We were the first to report the isolation of these mutants (Devin *et al.*, 1994) further designated as *net1-srm* and *ada1-srm*, and recently the two genes became objects of thorough studying in other laboratories.

NET1 gene and function

The Net1 protein is involved in regulating Cdc14 protein phosphatase (Shou *et al.*, 1999; Visintin *et al.*, 1999; Traverso *et al.*, 2001). Data available suggest that during interphase and early mitosis Net1 holds Cdc14 within the nucleolus where its activity is suppressed. During exit from mitosis Cdc14 is freed from the nucleolus and allowed to promote inactivation of Clb/Cdc28 protein kinase by dephosphorylating targets in the nucleus and cytoplasm. An analysis of truncation mutants indicates that the Cdc14-binding site is located within a segment of Net1 containing residues 1-341 (Traverso *et al.*, 2001). Evidence was presented suggesting the Cdc14 active site occlusion by the Net1 inhibitor.

Although special investigations by other authors first failed to detect in *net1* mutants, as compared with normal strains, any marked changes in UV-sensitivity, spontaneous mutability of nuclear genes, or in the mitotic stability of minichromosomes (Visintin *et al.*, 1999; Entian *et al.*, 1999; Straight *et al.*, 1999) high rate of mini-chromosome loss by *net1* cells was reported quite recently (Shou and Deshaies, 2002). Still the data presented here include some additional information on the phenotypic manifestation of mutations in the *NET1/SRM8* gene.

Net1 has also a central role in nucleolar Sir2 localization. Sir2 is very important for silencing HM mating-type loci as well as telomeric silencing and rDNA silencing and recombionation, being also involved in cell ageing control (Kaeberlein *et al.*, 1999; Guarente, 2000; Gartenberg, 2000; Gottschling, 2000). Sir2 binding site is located within a segment of Net1 containing residues 566-801 (Cuperus *et al.*, 2000).

Large-scale analysis of multiprotein complexes in *S. cerevisiae* showed that Net1 might be associated with the nuclear Cdc14, Sir2, and Sir4, cytoplasmic Adh1, Tdh3, and Yef3, membrane Bgl2 and cytoskeletal Hsp42 proteins (Gavin *et al.*, 2002; http://yeast.cellzome.com).

In our *net1-srm* frameshift mutant (missense from 287 codon and truncated in 300) both Cdc14-binding and Sir2-bindng sites are damaged. The mutant cells frequently become inviable, their shape, genetic stability and radiation sensitivity are changed and sporulation is blocked. A deletion analysis of *NET1* is indicated to reveal its regions specifically responsible for the above effects.

NET1 inactivation leaves the cells viable although changed in their shape, frequently forming cell chains and growing slowly on both fermentable and non-fermentable substrates (Straight *et al.*, 1999; Entian *et al.*, 1999) just like *net1-srm* cells in our work. In *net1* cells entering mitosis, replication and bud formation are delayed (Visintin *et al.*, 1999) and cell cycle regulation is also changed (Entian *et al.*, 1999). Mitochondrial abnormalities, for instance a reduced mitochondrial membrane potential, were also found in cells with disrupted *NET1* (Entian *et al.*, 1999) and might be the cause of a decrease in EthBr-induced rho^- mutability reported in this paper.

Mutants resistant to the mitochondrial mutagen ethidium bromide (EthBr) (Slonimski *et al.*, 1968) were detected in the yeast *Kluyveromyces lactis* (Brunner *et al.*, 1973) and in *S. cerevisiae* (Bech-Hansen and Rank, 1972; 1973; Gouhier and Mounolou, 1973). A *K. lactis* mutant for the *PMA1* gene encoding the plasma membrane H^+-ATPase was resistant to EthBr and deficient in K^+ uptake (Miranda *et al.*, 1995). In general, a sizable proportion (10 to 20%) of EthBr-resistant mutants of *K. lactis* are deficient in both EthBr transport and the transport of monovalent cations, especially K^+.

The transport of EthBr molecules and K^+ cations depends on the membrane potential and is probably accomplished via the same carriers (Brunner *et al.*, 1982; Pena and Ramirez, 1975; 1991). Resistance of *net1-srm* and *ada1/srm12* mutants to EthBr detected in our work is probably associated with a decrease in the permeability of cells and/or mitochondria for the dye.

ADA1 gene and function

The mutation first designated as *srm12* [L196K; K197Stop] is in the *ADA1* gene (*ada1-srm*). In other studies the *ada1* mutants manifested severe slow-growth defects, dramatically shortened lifespan, and strain-specific morphological changes (Horiuchi *et al.*, 1997; Marcus *et al.*, 1994; Sinclar *et al.*, 1997; Shore, 1998). *ada1* mutations produce synthetic lethals when in combination with mutations *net1-srm* (this work), *swi1* (Pollard and Peterson, 1997) and *pho85* (Lenburg and O'Shea, 2001).

The *SUP110/ADA1* gene was initially cloned by its ability to suppress cell respiratory incompetence caused by the *pet494* mutation in a gene encoding an activator of mitochondrial *COX3* transcript translation (Brown, 1994). As no sequence changes in the *pet494* mutant gene inserted into a plasmid were found the Sup110 protein seemed to suppress the cold-sensitive *pet494* mutation through stimulation of the *PET494* transcription. As the *sup110* mutation itself determined a temperature-sensitive respiratory deficiency, the *SUP110* gene might be directly involved in mitochondrial gene expression as well.

A few years later the *ADA1* gene was shown to encode a component of the histone acetyltransferase complexes playing important roles in transcriptional regulation of various genes.

It is well known that RNA polymerase II (Pol II)-dependent transcription in eukaryotes requires general transcriptional factors (Pol II itself, TBP, TFIIB etc.) required for preinitiation complex formation at promoters as well as gene-specific transcriptional activators binding to upstream activating sequences (UAS) or enhancers and stimulating

transcription through their activation domains. Transcription factors of a third type referred to as mediators, coactivators, or adaptors have also been isolated.

A number of transcriptional coactivators have been shown to possess histone acetyltransferase (HAT) activity, providing a direct molecular link between histone acetylation and gene activation (Sterner and Berger, 2000). In *S. cerevisiae* the coactivator protein Gcn5 has HAT activity and is involved in the regulation of various genes (Georgakopoulos and Thireos, 1992; Welihinda *et al.*, 1997). Gcn5 is associated with other proteins in two native yeast complexes, 0.8-MDa Ada/Gcn5 and 1.8-MDa SAGA (Spt-Ada-Gcn5-acetyltransferase). Ada/Gcn5 is a distinct complex and not a subcomplex or artifactual fragment of SAGA (Eberhârter *et al.*, 1999) although the former is required for the overall structural integrity of the latter (Sterner *et al.*, 1999). Ada/Gcn5 and SAGA have overlapping, yet distinct, patterns of acetylation (Grant *et al.*, 1999).

The 488-amino-acid protein encoded by *ADA1* is a member of the Ada/Gcn5 and also SAGA complexes (Horiuchi *et al.*, 1997; Grant *et al.*, 1999). There are at least five proteins in the Ada/Gcn5 complex. Proposedly *ADA2*, *ADA3* and *GCN5* function together to acetylate nucleosomes, opening up some promotors while *ADA1* and *ADA5* recruit TBP and possibly other basal factors to bind to the TATA box. The pair yTAFII68-yADA1 is a critical structural element in the SAGA complex (Gangloff *et al.*, 2000). The Ada1 protein contains a high percentage of charged residues, its first third being basic and the C-terminal part more acidic. Given a codon bias index of ~-0.04, one should expect *ADA1* expression index to be very low (Soussi-Boudekou and Andre, 1999). The sequence features led to suspect that Ada1 might contribute to activating gene expression by protein-protein interactions rather than by direct binding to DNA.

SAGA has global roles in transcription. SAGA and TFIID were recently shown to be involved in the expression of around 70% of yeast genes (Lee *et al.*, 2000). By the way, SAGA may co-regulate an interface between phosphate metabolism and cell-cycle control, as genes involved in uptake of extracellular phosphate and regulators of the Pho80-Pho85 cyclin-dependent kinase complex are dependent on SAGA components.

So the mutations determining co-ordinate changes in the fidelity of mitotic transmission of chromosomes, plasmids and mtDNA molecules most probably mark genes that are at high positions in the hierarchy of general genetic regulation of a yeast cell and thus are highly pleiotropic. This hierarchy of yeast genes as such as well as in relation to gene hierarchies of higher eucaryotes is a matter of an undoubted interest.

Genes NET1 and ADA1 and the maintenance of optional genetic structures

Some although not many publications suggest that the maintenance of recombinant plasmids in a yeast cell depends on the functioning of its mitochondrial genome. The mitotic stability of plasmid Rcp-CEN3 (*rDNA ARS CEN3*) was markedly higher in rho$^-$ mutants induced by ethidium bromide than in respiratory-competent cells of the original strain (Larionov *et al.*, 1983). A strong stabilising effect of rho^0 and rho$^-$ mutations on the maintenance in *S. cerevisiae* cells of a *Zygosaccharomyces rouxii* plasmid with two *ARS* sequences was also reported (Irie *et al.*, 1991). This plasmid stabilisation was not observed in nuclear *pet* mutants, which, although respiratory-deficient like rho$^-$ mutants, retained the

intact mitochondrial genome. For *S. cerevisiae* cells incompatibility of their mtDNA with a killer plasmid from *Kluyveromyces lactis* was shown (Gunge and Yamane, 1984).

Similar situation may take place for the plasmid from *Z. rouxii.* On the other hand, respiratory-deficient mutations can promote the expression of nuclear genes (Parikh *et al.,* 1987; Kaisho *et al.,* 1989; Puglisi and Algeri, 1971), possibly including genes involved in the maintenance of this plasmid. In our experiments, rho$^-$ mutations proved to be able to modify the maintenance of plasmids YCp50 and YRp12 in certain *srm* mutants being without any appreciable effect in normal *SRM+* cells. These data suggest some interaction or functional redundancy between the nuclear *SRM* genes and certain mitochondrial genetic determinants important for the maintenance of recombinant genetic structures in yeast cells. This interaction or functional redundancy seems worth further studying.

In recent years the relationship between the cell cycle regulation and DNA replication and repair has been extensively studied. DNA underreplication or its structural disorders induced by external factors, in particular, by ionizing radiation, were shown to determine a cell cycle delay or arrest. Genes responsible for DNA repair and cell radiation sensitivity as well as genes regulating the cell division cycle participate in this negative feedback control termed DNA damage checkpoint (Weinert and Hartwell, 1988; 1990). Disturbances in checkpoint control often increase cell radiation sensitivity and decrease the fidelity of mitotic transmission of hereditary structures; resembling conspicuously the effects typical for the *srm5, srm8,* and *srm12* mutants examined here.

Data suggesting the involvement of *CDC28* (*SRM5*), the central gene of yeast cell cycle regulation, in the checkpoint control have been obtained (Li and Cai, 1997; Koltovaya *et al.*, 1998).

The DNA damage response might control the efficiency of individual repair pathways by post-transcriptional means. For example, re-localization of Ku and Sir proteins from telomeres to double strand breaks is under checkpoint control (Martin *et al.*, 1999; Mills *et al.*, 1999; McAinsh *et al.*, 1999). Ku and Sir proteins are involved in illegitimate reconbinational repair of DSBs by NHEJ (Featherstone, Jackson, 1999;1999).

Acetyltransferase activity participate in transcriptional regulation different proteins, including checkpoint-proteins. Recently it has been found that in human hGCN5 associates with Ku70 (Barlev *et al.*, 1998), which as a heterodimer with Ku80 regulates the DNA binding of DNA-PK, and hADA3 associates with p53 (Wang *et al.*, 2001), transcriptional factor involved in G1-arrest and apoptosis. Acetylation of p53 by PCAF/yGCN5 regulates the activity of p53 as a part of the pathway of DNA damage response (Gu and Roeder, 1997; Liu *et al.*, 1998; Sakaguchi *et al.*, 1998; Barlev *et al.*, 2001). p53 regulatory pathway is also mediated by NAD-dependent histone deacetylation by mammalian Sir2α (Luo *et al.*, 2001; Vaziri *et al.*, 2001). Expression of the catalitically inactive hSir2 protein potentiates p53-dependent apoptosis and radiosensitivity.

We may suggest that yeast acetyltransferase Ada/Gcn5-complex and Net1-dependent localization of deacetylase Sir2 also participate in response to DNA damage.In further studies, we intend to examine the involvement of CDC28/SRM5, NET1/SRM8, and ADA1/SRM12 genes in the checkpoint regulation in yeast.

TABLES

Table 1. Strains used in the study

Strain	Genotype	Source of origin
71a	*MAT*a *SRM+ ade1*	Constructed by authors (Devin *et al.*,1990)
71α	*MAT*α *SRM+ ade1*	The same
72a	*MAT*a *SRM+ ade2*	"
71L	MATa *SRM+ ade1 leu2*	Constructed in this work*
H5	*MAT*a *ade1* (n+1) IV	The same
3D	*MAT*a *SRM+ ade2 trp1 ura3*	"
1B	*MAT*α *SRM+ ade2 trp1 ura3*	"
a srm8	*MAT*a *srm8 ade2 trp1 ura3*	"
α srm8	*MAT*α *srm8 ade2 trp1 ura3*	"
a srm12	*MAT*a *srm12 ade2 trp1 ura3*	"
α srm12	*MAT*α *srm12 ade2 trp1 ura3*	"
a srm15	*MAT*a *srm15 ade2 trp1 ura3*	"
α srm15	*MAT*α *srm15 ade2 trp1 ura3*	"
a srm17	*MAT*a *srm17 ade2 trp1 ura3*	"
α srm17	*MAT*α *srm17 ade2 trp1 ura3*	"
C3 L	*MAT*a *srm8 ade2 leu2*	"
C3LD	*MAT*a/*MAT*α *srm8/srm8 ade2/ade2 leu2/leu2*	"
C3x72a (VII)	*MAT*a/*MAT*α *SRM+/srm8 ade2/ade2 ade6/+ leu1/+ cyh2/+*	"
C3xC3 (VII)	*MAT*a/*MAT*α *srm8/srm8 ade2/ade2 ade6/+ leu1/+ cyh2/+*	"
C9x72a (VII)	MA*T*a/*MAT*α *SRM+/srm12 ade2/ade2 ade6/+ leu1/+ cyh2/+*	"
C9xC9 (VII)	*MAT*a/*MAT*α *srm12/srm12 ade2/ade2 ade6/+ leu1/+ cyh2/+*	"
C14xC14 (VII)	*MAT*a/*MAT*α *srm15/srm15 ade2/ade2 ade6/+leu1/+ cyh2/+*	"
F5xF5 (VII)	*MAT*a/*MAT*α *srm17/srm17 ade2/ade2 ade6/+ leu1/+ cyh2/+*	"
srm15/srm15	MATa/*MAT*α *srm15/srm15 ade1/ade1*	"
srm17/srm17	*MAT*a/*MAT*α *srm17/srm17 ade1/ade1*	"

Table 1. Continuation

Strain	Genotype	Source of origin
6a	*MAT*a *ade1 (n+1)XIV*	Constructed in this work
6α	*MATα ade1* (n+1) XIV	The same
STX-9-1A	*MAT*a *arg3 ade2 gal2*	U. Wintersberger (University of Vienna)
S1780C	*MATα ura2 his6 arg4 thr1 met1 gal2*	YGSC**
NGB121	*MATα SUP110:LEU2 ade2-101 ura3052 leu2Δ*	T.D. Fox (Cornell University)

* To obtain strains listed in the table, the genetically marked strains previosly constructed by the authors (Devin *et al.*, 1990)
** Yeast Genetic Stock Center, Berkeley, USA

Table 2. Test of allelism between the *srm8*, *srm12*, *srm1*, and *srm5* mutations

Cross	Total No. of tetrads	No of tetrads of the type			$X^2_{f=2}$
		P	***N***	***T***	
srm8 x *srm1*	18	5	2	11	2.25
srm8 x *srm5*	31	3	5	23	1.20
srm12 x *srm1*	21	4	3	14	0.14
srm12 x *srm5*	19	5	3	11	1.25
srm8 x *srm12*	14	2	1	11	4.72

Table 3. Effect of *srm* mutations on the growth rate of diploid cells

Strain	Genotype	Number of tested clones	Number of culture generations	Generation time, min
C3x72a(VII)	*srm8/+*	2	3 - 5	75.9 ± 3.7
C3xC3(VII)	*srm8/srm8*	3	2	200.7 ± 9.0
C9xC9(VII)	*srm12/srm12*	4	3 - 6	126.4 ± 3.2
srm15xsrm15	*srm15/srm15*	3	7 - 9	113.0 ± 12.0
srm17xsrm17	*srm17/srm17*	2	4	78.7 ± 3.3

Table 4. Proportions of cytoplasmic petite mutants in monospore clones as affected by *srm* muations

Genotype	No. of clones	No. of clones scored	Per cent *petite* mutants
SRM+	4	14782	74,6±6,5
srm8	4	7373	3,4±1,1
SRM+	4	4167	82,9±5,6
srm12	4	1879	1,8±0,8
SRM+	3	11017	48,2±4,0
srm15	3	8892	2,7±0,9
SRM+	4	11678	71,5±10,7
srm17	4	5969	23,7±18,4

Table 5. Mitotic stability of extra chromosome XIV in disome haploid strains

Genotype	Number of tested clones	Total number of colonies	Frequency of the spontaneous loss of chromosomes,%
srm12 (XIV)	4	396	5.6±0.8
SRM+ (XIV)	4	943	0.2±0.03
srm15 (XIV)	4	20695	0.16±0.03
SRM+ (XIV)	4	25172	0.07±0.03

Table 6. Mitotic stability of chromosome VII in diploids

Strain	Genotype	Number of tested clones	Frequency of the spontaneous loss of chromosomes
C3xC3(VII)	*srm8/srm8*	3	$(4.2\pm2.1) \times 10^{-5}$
C3x72a(VII)	*srm8/+*	2	$(1.5\pm0.6) \times 10^{-7}$
C9xC9(VII)	*srm12/srm12*	4	$(6.2\pm3.9) \times 10^{-7}$
C9x72a(VII)	*srm12/+*	1	0.9×10^{-7}
C14xC14(VII)	*srm15/srm15*	1	1.6×10^{-7}
F5xF5(VII)	*srm17/srm17*	1	2.9×10^{-7}

Table 7. Mitotic stability of plasmids YCp50 (A) and YRp12 (B) in haploid strains with various genotypes

A.

Strain	Genotype of clones		Number of tested clones	Colonies examined	Percentage of colonies that retained plasmid
3D, 1B	*SRM*$^+$	[*rho*$^+$]	3	916	35.6±7.0
		[*rho*$^-$]	6	1827	47.1±16.2
		[*rho*^{o}]	13	4407	38.6±20.1
a, α srm8	*srm8*	[*rho*$^+$]	4	1240	21.8±9.1
		[*rho*$^-$]	5	1414	43.5±19.0
a, α srm12	*srm12*	[*rho*$^+$]	7	2586	20.7±3.6
		[*rho*$^-$]	4	359	20.1±7.0
a, α srm15	*srm15*	[*rho*$^+$]	4	1202	24.5±8.0
		[*rho*$^-$]	4	854	18.4±7.0
a, α srm17	*srm17*	[*rho*$^+$]	8	1944	28.7±5.6

B.

Strain	Genotype of clones		Number of tested clones	Colonies examined	Percentage of colonies that retained plasmid
3D, 1B	*SRM*$^+$	[*rho*$^+$]	4	3393	35.9±7.2
		[*rho*$^-$]	6	2667	41.2±8.1
		[*rho*^{o}]	8	1939	28.6±4.7
a, α srm8	*srm8*	[*rho*$^+$]	6	1956	29.7±14.0
		[*rho*$^-$]	5	2103	50.3±7.4
a, α srm12	*srm12*	[*rho*$^+$]	7	3390	7.1±5.8
a, α srm15	*srm15*	[*rho*$^+$]	7	2024	10.5±2.6
		[*rho*$^-$]	3	957	6.5±2.7
a, α srm17	*srm17*	[*rho*$^+$]	8	1938	16.8±2.5

Table 8. Genetic mapping of the *srm8* mutation

Pair of markers	Tetrads examined	Number of tetrads of type			Genetic distance, cM
		P	N	T	
ura2 – arg3	93	28	0	65	35.0
ura2 – srm8	124	33	6	85	48.8
arg3 – srm8	209	163	0	46	10.6

Table 9. Mitotic stability of plasmids with the ori 2 μm DNA sequence

	Plasmid					
	YEp13			2J-1		
Recipient strain	Number of tested clones	Colonies examined	Percentage of colonies that retained plasmid	Number of tested clones	Colonies examined	Percentage of colonies that retained plasmid-
71L(*SRM+*)	5	796	57.5±8.3	4	648	10.6±00.0
C3 (*srm8*)	4	970	23.0±5.3	1	243	77.8
C3L(*srm8*) (retrans-formants)	-	-	-	5	890	82.0±16.1

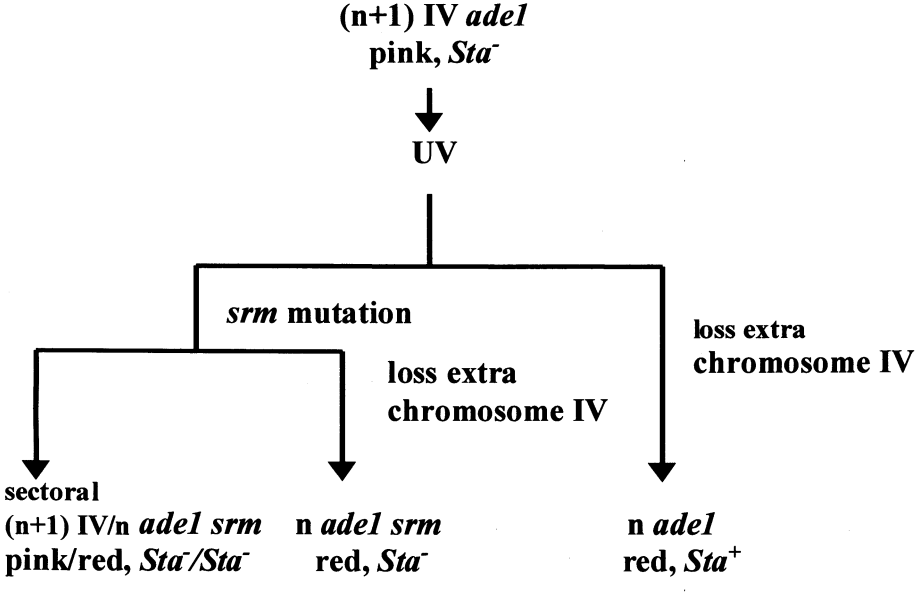

Figure 1. Scheme for isolating *srm (spontaneous rho⁻ mutability)* mutants. See text for detail.

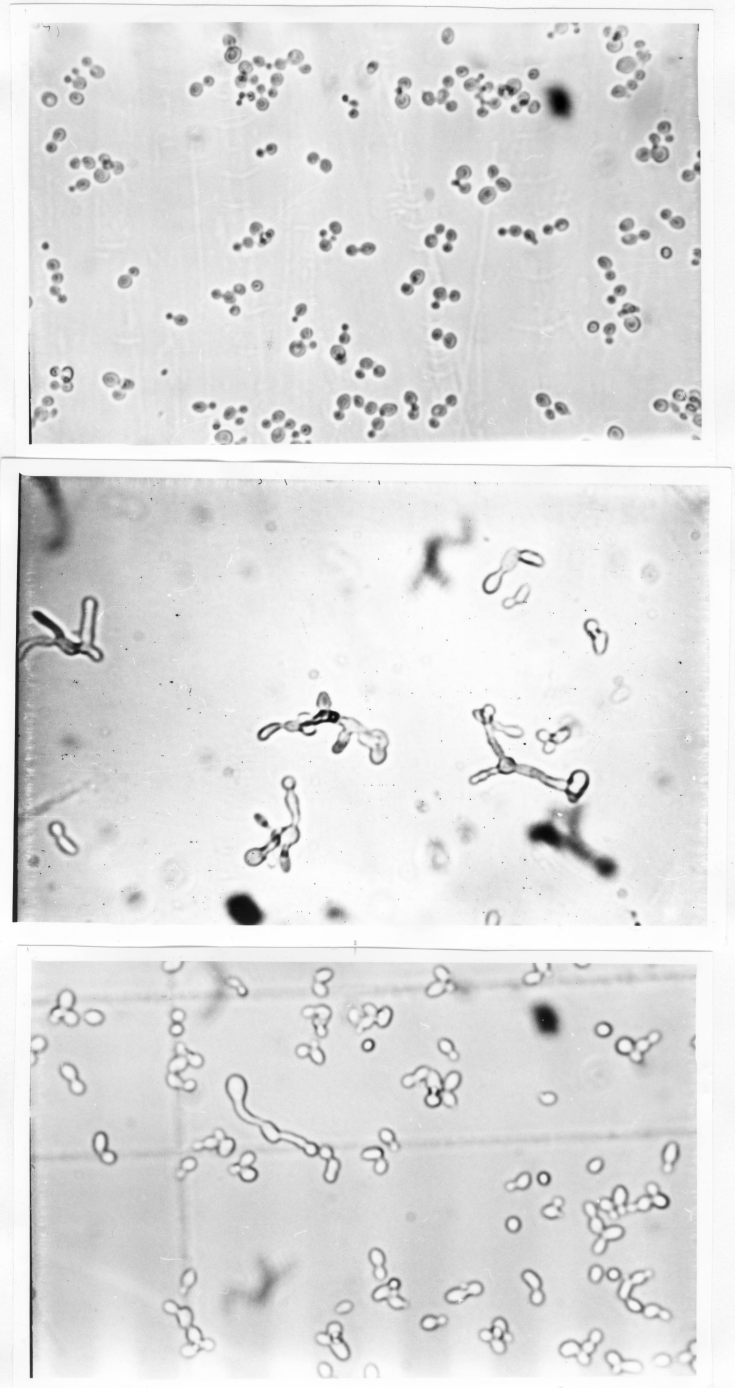

Figure 2. Budding haploid cells: (a) *SRM+*; (b) *srm8*; and (c) *srm12*.

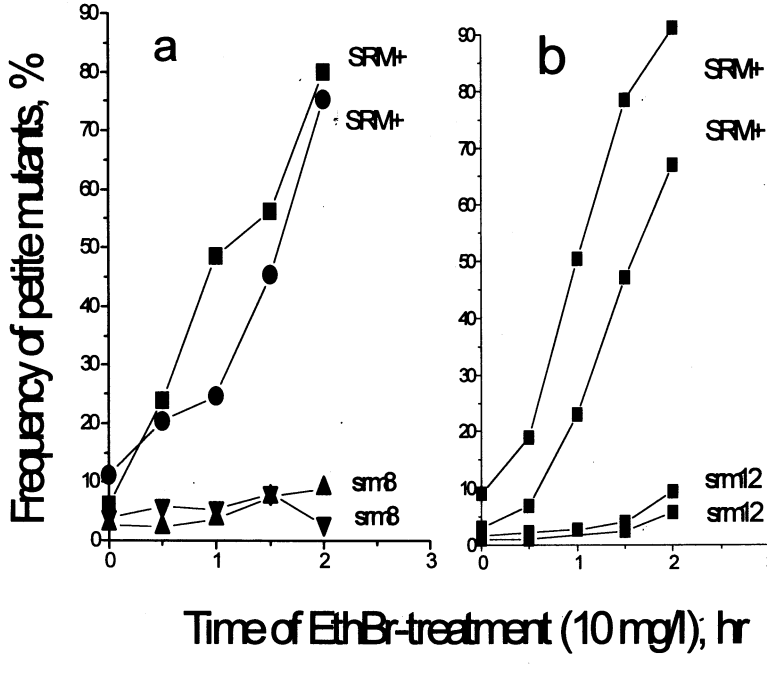

Figure 3. Relationship between the yield of induced rho⁻ mutations and the time of ethidium bromide treatment (10 μg/ml). The data obtained for monosporous clones *SRM+* and *srm8* (a) and *SRM+* and *srm12* (b) originated from one tetrad for each mutation are presented.

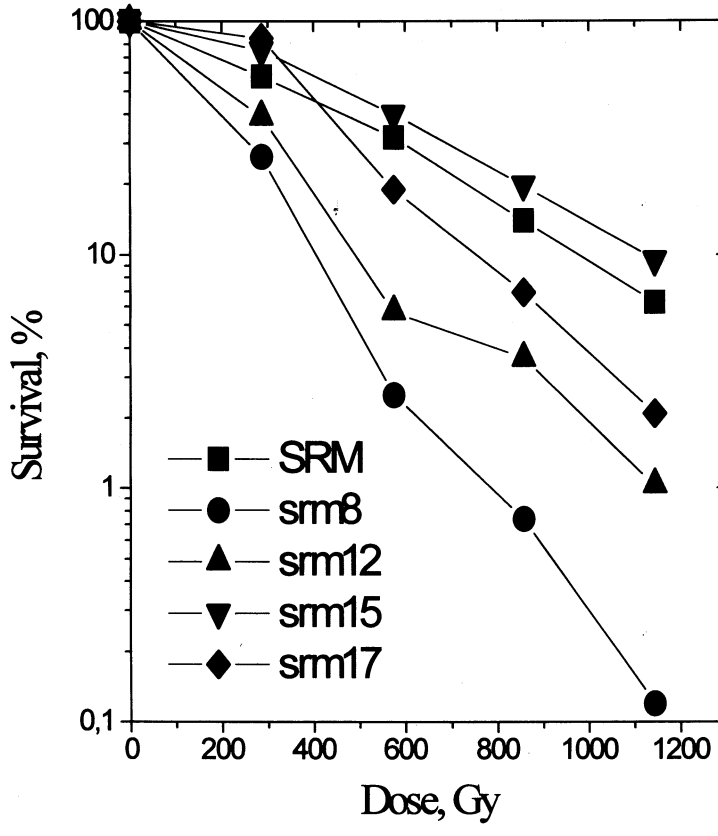

Figure 4. Typical survival curves of diploid strains with various genotypes: C3(VII)x72a (*srm8/+*), C3xC3(VII) (*srm8/srm8*), C9xC9(VII) (*srm12/srm12*), C14xC14(VII) (*srm15/srm15*), F5x F5(VII) (*srm17/srm17*).

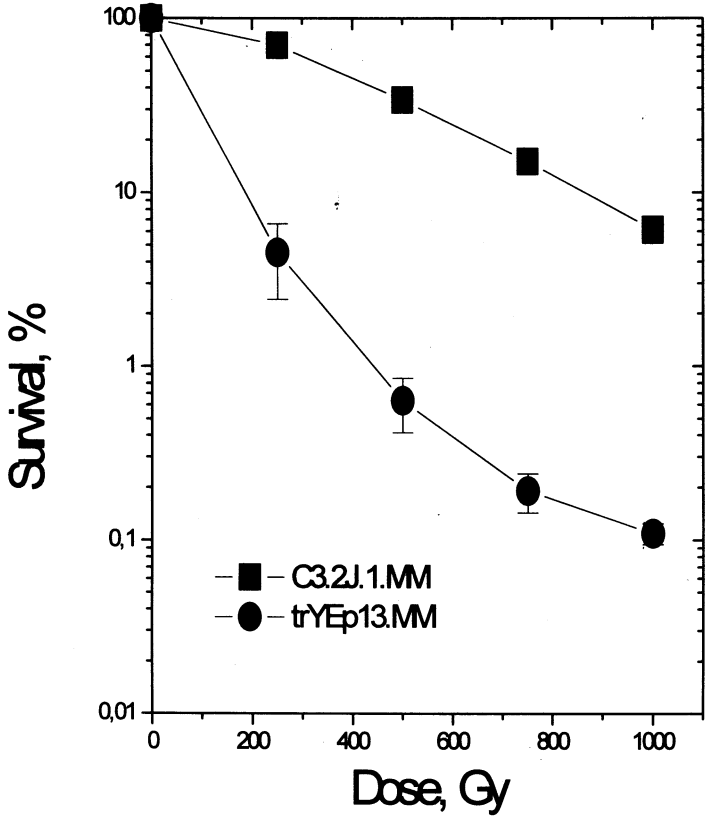

Figure 5. Survival curves of transformants of the diploid *srm8/srm8* strain that carry plasmid 2J-1 or YEp13. Irradiated cultures were plated on a leucine-deficient synthetic medium. Each curve corresponds to the mean data for four independent transformants.

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Гены *ADA1* и *NET1* дрожжей играют роль как в поддержании хромосом, так и в изменчивости митохондриального генома

Хорошо известно, что мутации в многочисленных ядерных генах, а также различного рода стрессовые ситуации приводят к увеличению митохондриального rho$^-$ мутагенеза. Несмотря на интенсивные исследования последних лет биологическое значение этой реакции еще недостаточно ясно. Генетические подходы для решения этой задачи включают в себя изучение генов, которые необходимы для поддержания высокой спонтанной rho$^-$ мутабильности. Ранее мы обнаружили, что мутации в некоторых ядерных генах, включая центральный регуляторный ген *CDC28*, могут снижать спонтанную rho$^-$ мутабильность и одновременно влияют на поддержание дрожжевых хромосом и плазмид. Настоящая работа представляет данные по идентификации еще двух генов, сходных в этом аспекте с *CDC28*. Эти гены, *NET1* и *ADA1*, опосредуют важные белок-белковые взаимодействия в клетках дрожжей. В работе описывается влияние мутаций *net1* и *ada1* на поддержание митохондриального генома, хромосом и плазмид, а также на чувствительность к ионизирующей радиации.

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ADA1 and *NET1* Genes of Yeast Mediate Both Chromosome Maintenance and Mitochondrial rho$^-$ Mutagenesis

An increase in the mitochondrial (mt) rho$^-$ mutagenesis is a well-known respose of yeast cells to mutations in the numerous nuclear genes as well as to various kinds of stress. Notwithstanding the extensive studies during several decades the biological significance of this response is not yet fully understood. The genetic approach to solution of this subject includes the study of genes that are required for the high incidence of spontaneous rho$^-$ mutants. Previously we found that mutations in certain nuclear genes including *CDC28*, the central cell-cycle regulation gene, may decrease the spontaneous rho$^-$ mutability and simultaneously affect maintenance of the yeast chromosomes and plasmids. The present work provides data on identification of two more genes, resembling *CDC28* in this respect. These genes *NET1* and *ADA1* mediate important regulatory protein-protein interactions in the yeast cell. The effects of *net1* and *ada1* mutations on the maintenance of yeast mt genome, chromosomes and plasmids as well as on cell sensitivity to ionising radiation are also described.

The investigation has been performed at the Division of Radiation and Radiobiological Research, JINR.

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