Analysis of Plasmid Deletion Induced by Ionizing Radiation in Yeast *Saccharomyces cerevisiae*

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I. INTRODUCTION

The objective of this study is to determine the mutagenic effects of ionizing radiation. As a model [1] we used a plasmid system for quantitative analysis of deletion formation. A *can1 cyh2* cell on a YCp plasmid (with two negative markers: the *CAN1* and *CYH2* genes) is sensitive to canavanine and cycloheximide. This cell becomes resistant to both drugs when the plasmid has a deletion over the *CAN1* and *CYH2* genes. The structure of centromeric plasmid YCpL2 [*ARS1 CEN3 URA3 TRP1 LEU2 CAN1 CYH2*] with length 13.8 kbp are shown in Figure 1.

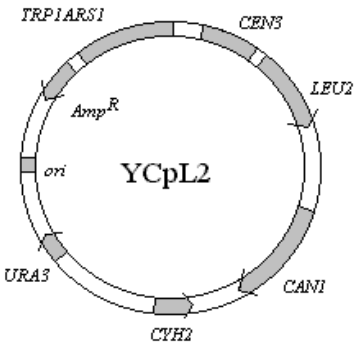

Figure 1. Scheme of the plasmid YCpL2.

II. DATA ANALYSIS

The genetic analysis of selected mutants induced by ionizing radiation is shown in Figure 2. In the cell's population before irradiation the majority of deletions (~70%) was formed by the smallest deletions covering two markers (*CYN2* and *CAN*). The rest part of mutants (~10%) had large deletions covering four markers (*CYN2, CAN1, LEU2, TRP1*). With the radiation dose the portion of mutants with large deletions increases up to 30%. Induction of the large deletions was less effective under irradiation by heavy ions.

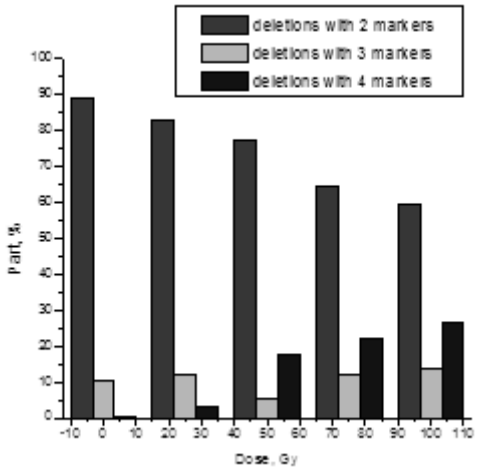

Figure 2. Genetic analysis of mutants induced by γ-rays with the flux 0.7 Gy/min and energy 1.3 MeV in strain R1-1 (*RAD*$^+$).

The plasmids rescued from the Can^R Cyh^R mutant cells (eight clones) induced by radiations were introduced into *E. coli* strain and analyzed by agarose gel electrophoresis. The strain *E. coli* TG1 served as hosts for plasmids. Restriction analysis of plasmid DNA allows localizing deletion on the plasmid map (see Table 1). Restriction fragments of plasmid DNA prepared from two mutants GammaK-4 and YB100-2-2 are shown in Figures 3.

All eight rescued plasmids were found to be smaller than the parental plasmid YCpL2. Therefore, resistance to canavanine and cycloheximide appeared is not due to plasmid loss coupled with Ura$^+$ reversion but more likely to a deletion in the *CAN1-CYH2* region of plasmid. Restriction analysis of the eight recombinant plasmids shows that they have various sizes of deletion in the *CAN1-CYH2* region of the YCpL2. The size of plasmid deletion from mutant GammaK-4 is about 2600 bp, and the size of plasmid deletion from mutant YB100-2-2 is about 1000 bp.

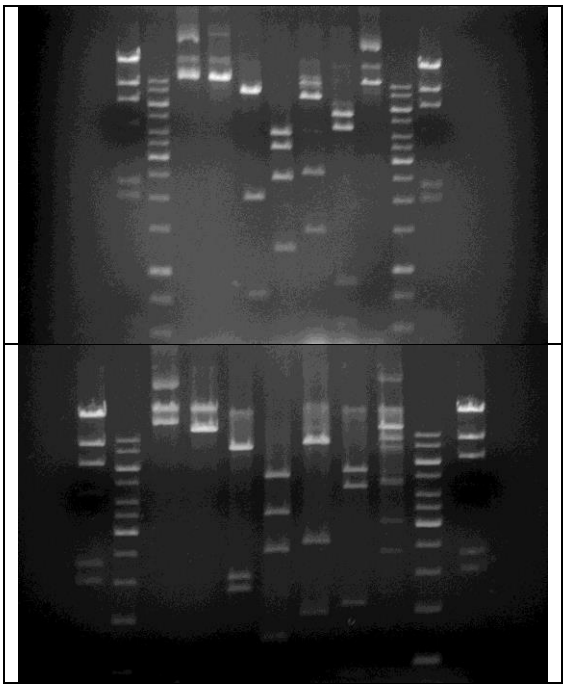

Figure 3. Restriction analysis of plasmid DNA from mutant GammaK-4 (top) and YB100-2-2 (bottom).

TABLE 1. Restriction fragments of plasmid DNA from mutants GammaK-4 and YB100-2-2

Enzymes	YCpL2			Gamma K-4	YB100-2-2
	actual	calculated using marker λ/HindIII leader			
XbaI	13800	15700	10300	12000	12000
HpaI	13800	14300 9800	9900 8300		
KpnI	8100 3600 2100	9100 3400 2100	8100 4000 2000	8300 2100 1500	8300 3100 2000
EcoRV	6900 3500 2200 1300	7600 3200 2400	7300 3800 2600	3800 3100 2400 1700	5100 3200 2400 1700
EcoRI	6000 2500 2300 1600 1500	6400 2500 1900 1800	6600 2700 1600 1400	6900 2500 1800	9500 2600 1800
HindIII	5400 4300 2000 1400 800	5800 4200 2100 1800 1700	6200 5000 2100 1200 850	6600 5100 4000 1600	5800 4400 1800

Our data were compared with the results of the structural analysis of eight spontaneous mutants from strain *RAD*$^+$, eight mutants from strain *rad53* and five mutants from strain *hdf1* [1, 2] (see Table 2). Restriction analysis of the recombinant plasmids showed that the plasmids had deletions at various sites of the *CAN1-CYN2* region. 85% of deletions were covered two genes (*CYN2, CAN*), while more large deletions covering tree genes (*CAN1*, *CYN2* and *LEU2*) composed only 5%. At the same time, authors note that another short genetics changes in both genes *CAN1* and *CYN2* appear (10%). The size of deletion, which covered two genes *CAN* and *CYN2*, does not exceed 5.1 kbp and ranged from 0.3 to 5.1 kbp.

TABLE 2. Size of spontaneous deletions of strains *RAD*$^+$, *rad52* and *hdf1* [1, 2]

Strain	Number of mutants	Point mutations	Deletion	
			CAN1-CYN2	*CAN1-CYN2-LEU2*
			Size of deletion (kb)	Size of deletion (kb)
RAD$^+$	8	-	2.5; 3.1; 2.9; 4.1; 3.1; 2.4; 4.9; 1.6	-
rad 52	8	1	3.1; 4.0; 3.2; 0.3; 4.5; 2.0	5.9
hdf1	5	1	5.1; 3.3; 0.6; 1.2	-

III. CONCLUSION

1. Genetic analysis shows that mutation is more likely due to a deletion in the two genes *CAN1-CYN2* then large deletion of four genes (*CAN1, CYN2, LEU2, TRP1)*.
2. It was found that the size of all analyzed mutants less then size of initial plasmid YCpL2.
3. Deletion of plasmids from two analyzed mutants GammaK-4 and YB100-2-2 localized in the *CAN1-CYN2* region, their sizes are about 2600 and 1000 kbp respectively.

This work just has started. In future we plan to make detailed analysis a collection of mutants selected after irradiation of gamma ray and heavy ions.

REFERENCES

[1] Tsukamoto Y., Kato J., Ikeda H. 1996. Genetics 142: 383.
[2] Tsukamoto Y., Kato J., Ikeda H. 1996. Nucleic Acids Research. 24: 2067.