PROCEEDINGS OF THE BALKAN SCIENTIFIC CONFERENCE OF BIOLOGY IN PLOVDIV (BULGARIA) FROM 19TH TILL 21ST OF MAY 2005 (EDS B. GRUEV, M. NIKOLOVA AND A. DONEV), 2005 (P. 283–287)

CRYOBIOLOGICAL METHODS HELP TO UNCOVER THE MOLECULAR BACKGROUND OF A NEW SHORT TERM TEST FOR DETECTION OF CARCINOGENS

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ABSTRACT. Recently, we developed the Ty1 transposition assay as a new short term test for detection of carcinogens. The Ty1 test is based on transposition of a genetically engineered oncogene-like transposon (Ty1). Results obtained till now show that carcinogens (laboratory and environmental) induce the transposition of the oncogene-like transposon. The Ty1 test is very sensitive and is positive with carcinogens undetectable by other short term tests. The carcinogen induced transposition of Ty1 is a novelty in science and its molecular mechanisms are not known. We found that mitochondrial functions participate in the carcinogen induced Ty1 transposition. In order to avoid possible side effect of mutagens/inhibitors used to study mitochondrial function, we developed a principally new method for isolation of mitochondrial mutants based on cryo-treatment of unprotected intact cells. Mitochondrial mutants obtained by cryo-treatment were characterized genetically and studied for carcinogen induced Ty1 transposition. Results obtained show that the mitochondrial mutants lack the carcinogen induced Ty1 transposition typical for cells with preserved mitochondria. It is concluded that mitochondrial function(s) are involved in the induction of a oncogen-like transposon by carcinogens.

KEYWORDS. carcinogens, transposition, mitochondria, cryo-treatment

INTRODUCTION

It is now universally recognized that analysis of biological mechanisms in the yeast *Saccharomyces cerevisiae* contributes to an understanding of analogous mechanisms in mammalian cells (Hieter et al., 1996; Botstein et al., 1997). We used *Saccharomyces cerevisiae* as a model system to study the carcinogen induced transposition of a genetically modified oncogene-like transposon (Ty1). Ty1 transposition was recently used to develop principally new short-term test for detection of carcinogens. Results obtained till now undoubtedly show that different

carcinogens induce Ty1 transposition in yeasts. The carcinogen induced transposition of Ty1 is a novelty in science and its molecular mechanisms are not known.
The Ty1 transposon has a life cycle, nucleotide sequence and functions similar to those of oncoviruses. The integration of Ty1 in new sites of the genome leads to genetical defects similar to those established in carcinogenesis. One of the most common and profound features of cancer cells is their defective mitochondrial function (Wallace D.C., 2001; Bianchi et al., 2001). It is known that cancer cells use glycolysis (instead of oxidative phosphorylation) to obtain ATP. *Saccharomyces cerevisiae* yeasts are an excellent model system in which to study mitochondria, because they keep their viability when mitochondrial mutants occur (rho^- mutants) or even when the whole mitochondrial DNA is lost.
We studied carcinogen induced Ty1 transposition in rho^- mutants obtained by the standard methods with ethidium bromide treatment. To avoid the possible side effects of such chemical agents we obtained rho^- mutants by cryo-treatment. This new method for generation of mitochondrial mutants gives an opportunity to study the role of mitochondria in different processes avoiding the side effects of mutagens and inhibitors.

MATERIAL AND METHODS

The Ty1 transposition assay

The Ty1 assay was performed as already described (Pesheva et al., 2005). *Saccharomyces cerevisiae* strain DG1141ts1 (MAT α ura3-167 his3Δ200:TymHIS3AI ts1) was used. The strain has a Ty1 element marked with the indicator gene HIS3AI (10) which contains the artificial intron AI inserted in antisense orientation relative to HIS3. Each successful transposition of the marked Ty1 gives rise to one histidine prototrophic colony and requires transcription, splicing, reverse transcription and insertion of the resulting Ty1-DNA into new locations of the genome. Histidine prototrophic colonies can not arise by reversion since the indicator HIS3AI gene is inserted in a genome with deleted HIS3 (the his3Δ200). Thus, the number of the HIS^+ colonies is a quantitative measure for the transposition rate of the marked Ty1 transposon.
Standard yeast media were prepared as described (Sherman et al.,1994). Median transposition rates were determined and the "fold increase" of Ty1 transposition was calculated. A "fold increase" of two or more is considered as a positive answer of the Ty1 transposition test.

Generation of rho^- strains by ethidium bromide

Strains with mitochondrial mutations (rho^-) were generated with ethidium bromide from DG1141ts1 strain (Sherman et al.,1994). Briefly, ethidium bromide was added to a concentration of 10μg/ml and the cells were incubated at 30^0C with agitation for 24h in YPD medium. Cells were diluted in water and plated on YPD to obtain single colonies. The rho^- mutants were selected as cells unable to form colonies on rich medium with glycerol as the sole carbon source (YPG medium). These mutants lack

segments of mitochondrial DNA (mtDNA deletions) but maintain the same nuclear markers, and thus are isogenic to the parental wild type strain (Sherman et al.,1994).

Generation of rho⁻ strains by cryo-treatment

Yeasts are cultivated in YPD medium to reach exponential stage (5 x 10^7 cells/ml). Cells are washed twice by centrifugation and the precipitate is suspended in distilled water. The suspension is gradually iced: up to 4ºC for 2h, up to -10ºC for 1h, up to -20ºC for 16h, and gradually deiced in reversed order. Cells were diluted in water and plated on YPD to obtain single colonies. The rho⁻ mutants were selected as cells unable to form colonies on rich medium with glycerol as a sole carbon source (YPG medium).

Materials

Nutritional media components were from Difco Chem.Co. All tested carcinogenic substances were purchased from Sigma (USA).

RESULTS AND DISCUSSION

Mitochondria participate in carcinogen induced Ty1 transposition

Results showing that mitochondria participate in carcinogen induced Ty1 transposition were obtained by treating rho⁺ and rho⁻ cells (DG1141ts1 strain) with carcinogens. Table 1 shows the results with ethidium bromide induced rho⁻ mutants and their initial strain rho⁺.

Табл. 1. *Канцероген-индуцирана Ty1 транспозиция в rho⁺ и rho⁻ клетки*

Carcinogen[a]	Concentration (mM)	Phenotype	Survival (%)	Median rate[b] of transposition x10^{-7}	Fold Increase
H_2O (Control)		rho⁺	100	2.89 ± 0.21	1,00
		rho⁻	100	6.12 ± 0.33	1,00
EMS	0.080	rho⁺	48	18.81 ± 1.10	7,60
	0.080	rho⁻	56	6.10 ± 1.42	0,90
MMS	0.050	rho⁺	63	19.68 ± 0.35	8,56
	0.050	rho⁻	73	8.01 ± 0.41	0,81
AC	340.0	rho⁺	60	22.80 ± 3.51	7,13
	340.0	rho⁻	75	6.10 ± 1.05	1,82
TAC	130.0	rho⁺	82	23.61 ± 3.41	7,38
	130.0	rho⁻	77	11.01 ± 2.83	2,02
THF	600.0	rho⁺	66	36.22 ± 1.11	15,06
	600.0	rho⁻	62	7.20 ± 0.91	1,39
DCM	100.0	rho⁺	58	24.41 ± 2.10	7,63
	100.0	rho⁻	62	7.81 ± 1.80	1,21

[a] EMS – ehylmetanesulfonate; MMS – mthylmetanesulfonate; AC - aetamide; TAC - tioacetamide; THF – tetrahydrofuran; DCM – dichloromehtane.
[b] Average values from 3-5 experiments.

Cells with functional mitochondria (rho^+) respond to the carcinogen treatment by a 7 to 15 fold increase of Ty1 transposition, while the cells with compromised mitochondrial function (rho^-) remained silent and did not induced Ty1 transposition. It should be noted that survival rates of rho^+ and rho^- cells were comparable and the observed responses in the Ty1 transposition test can not be explained with different cell toxicity and has to be attributed to the functional state of mitochondria in the otherwise isogenic rho^+ and rho^- cells.

Generation of rho^- strains by cryo-treatment

The generation of rho^- mutants using ethidium bromide is a standard method but the ethidum bromide side effects are not studied. We suppose that such side effects could cause the lack of carcinogen induced Ty1 transposition in rho^- cells. Therefore we developed a principally new cryo-method for generating mitochondrial mutants from *Saccharomyces cerevisiae*, which does not use mutagens and avoids the possible side effect. To obtain rho^- mutants by cryo-treatment we gradually iced and deiced unprotected cells from two strains. These rho^- mutants are induced by the changes following the cryo-treatment and are not spontaneous. The frequency of the obtained rho^- mutants after cryo-treatment is much higher (about 20 %) than the spontaneous rate (1,5%) (Table2).

Table 2. Generation of rho^- mutants in twostrains *Saccharomyces cerevisiae* yeasts

Strain	Variant	Number of colonies	Survival (%)	% rho^- cells
DG1141	Control	1632	100	1,9
	Cryo-treatment	75	4,6	21,3
DG1141ts 1	Control	4104	100	1,5
	Cryo-treatment	101	2,4	20,0

The reasons for generating rho^- mutants by cryo-treatment are not known yet and the present experiments aim to reveal the mechanism by which the low temperatures cause mutations in mitochondrial DNA.

The rho^- mutants obtained by cryo-treatment were studied for carcinogen induced Ty1 transposition. The results obtained (Fig.1) are similar to the data about the ethidium bromide induced rho^- mutants (Table1).

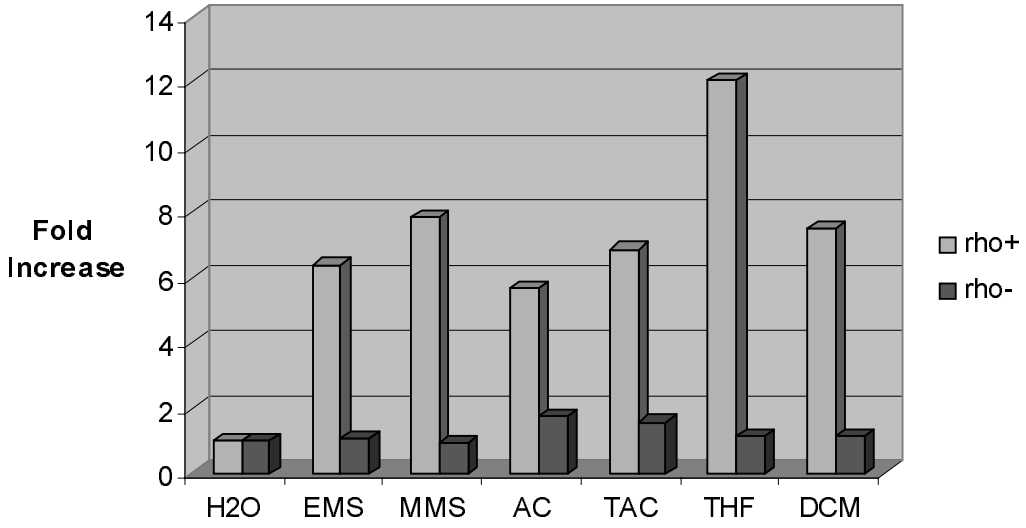

Fig. 1 . *Carcinogen induced Ty1 transposition in rho$^+$ and rho$^-$ cells*

CONCLUSOIN

The results obtained evidence that carcinogen induced Ty1 transposition depends on the functional state of mitochondria and cannot take place in cells with compromised function of mitochondria (rho$^-$).

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