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Low environmental radiation background impairs biological defence of the yeast *Saccharomyces cerevisiae* to chemical radiomimetic agents

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Abstract

Background radiation is likely to constitute one of the factors involved in biological evolution since radiations are able to affect biological processes. Therefore, it is possible to hypothesize that organisms are adapted to environmental background radiation and that this adaptation could increase their ability to respond to the harmful effects of ionizing radiations. In fact, adaptive responses to alkylating agents and to low doses of ionizing radiation have been found in many organisms. In order to test for effects of adaptation, cell susceptibility to treatments with high doses of radiomimetic chemical agents has been studied by growing them in a reduced environmental radiation background. The experiment has been performed by culturing yeast cells (*Saccharomyces cerevisiae* D7) in parallel in a standard background environment and in the underground Gran Sasso National Laboratory, with reduced environmental background radiation. After a conditioning period, yeast cells were exposed to recombinogenic doses of methyl methanesulfonate. The yeast cells grown in the Gran Sasso Laboratory showed a higher frequency of radiomimetic induced recombination as compared to those grown in the standard environment. This suggests that environmental radiation may act as a conditioning agent.

Keywords: Background radiation; Adaptive response; DNA damage

1. Introduction

Cells ability to adapt to their environment has been widely investigated in recent years. This has been shown by exposing cells grown in the presence of subliminal doses of a harmful agent (DNA alkylating agents or ionizing radiation) to challenge treatments with high doses of the same agent. The conditioned cells were found to be more resistant and less mutable than the unconditioned ones (Samson and Cairns, 1977; Olivieri et al., 1984; Wolf, 1992; Zhou et al., 1993). Moreover, it has been shown that changes in back-

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ground radiation affect cell proliferation (Planel et al., 1987).

Since all organisms evolved in the presence of a background of environmental radiation, the question arises as to whether this background may be considered a conditioning agent, which maximizes cell defence to harmful effects of ionizing radiation. Exposure to a low environmental radiation level represents a possible approach to test this hypothesis.

The Gran Sasso National Laboratory (LNGS) of the National Institute of Nuclear Physics (INFN), located in central Italy, offers an almost unique possibility to study the effects of a low background radiation environment (LBE). It operates under the Gran Sasso d'Italia mountain and it lies under a rock thickness of about 1300 m. Cosmic rays and neutron fluxes are reduced by factors of 10^6 and 10^3, respectively, as compared to a standard environment (The MACRO Collaboration, 1990; Rindi et al., 1988; Belli et al., 1989) and the main constituents of the mountain ensure a low level of natural radioactivity (Fig. 1).

2. Materials and methods

The diploid yeast strain D7 of *Saccharomyces cerevisiae* (Zimmerman et al., 1975) was cultured

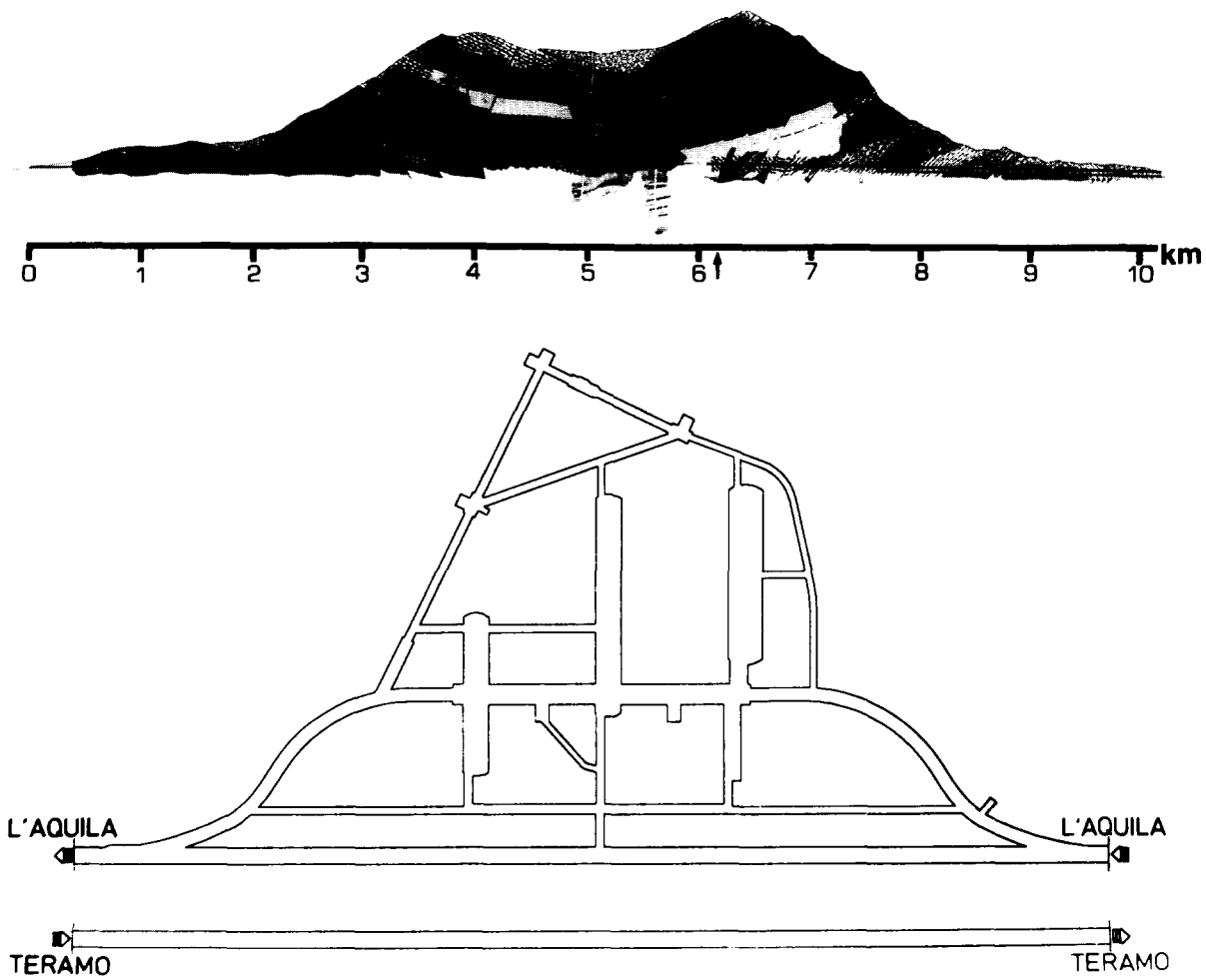

Fig. 1. Geologic profile of the Gran Sasso d'Italia mountain. The arrow indicates the location of the underground Gran Sasso Laboratory (upper section). A plan of the underground laboratory is shown in the lower section.

at 30°C in a Dubnoff bath (160 periods/min) in complete liquid medium YEPD (1% yeast extract, 2% peptone, 2% glucose) for at least 120 generations, both in the Gran Sasso Laboratory and in the standard background environment (SBE) (Dipartimento di Biologia Cellulare e dello Sviluppo, University of Rome 'La Sapienza'). The natural background radiation was measured with Harshaw calcium fluoride thermoluminescent dosimeters. The integrated dose was 4 μSv/day in the University of Rome laboratory and 0.6 μSv/day in the LNGS.

After this conditioning period, both LBE and SBE cultured cells were subjected to challenge experiments with methyl methanesulfonate (MMS). Three different subcultures of the D7 strain were checked for spontaneous recombination frequency and then conditioned in different periods (July and December 1992, and April 1993). Each subculture was used for at least four repetitions of an experiment, each consisting of an untreated control and three samples treated with 2.5, 5 and 10 mM MMS, respectively.

Treated cells and untreated controls were plated on complete solid medium (YEPD + 2% Bacto-Agar) and their survival was measured as the ability to form colonies and expressed as percentage of untreated controls. In the used D7 strain, a single event of crossing-over at the *Ade-2* locus provokes the formation of a twin-spot red-pink colony (Zimmerman et al., 1975), while all the other recombinational rearrangements of the same locus resulted in red, pink or sectored colonies. The percentage of the red-pink twin-spot colonies is reported here as '*Ade-2* reciprocal recombinants', that of all aberrant colonies as '*Ade-2* total aberrants'. All experiments were carried out in parallel at the LNGS and at the laboratories of the University of Rome under the same experimental conditions. In detail, the same types of baths and incubators were used and their temperature was periodically checked. Liquid media were prepared at the University of Rome, divided into 50 ml aliquots and randomly distributed between Rome and LNGS. Plates were prepared 3 days before each experiment in amounts suitable for one experiment in parallel, kept at room temperature and randomly divided into two parts the same day of each experiment. The same MMS stock solution was split into two parts the day before the experiment. Air circulation in the LNGS is ensured by a forced ventilation system and it is subjected to periodical tests. The LNGS air showed levels of CO, CO_2 and hydrocarbons (possibly originating from organic solvents used in the laboratories) similar to those of the external environment (Leoni, 1992).

3. Results and discussion

No significant differences in MMS induced cytotoxicity were observed between the cells

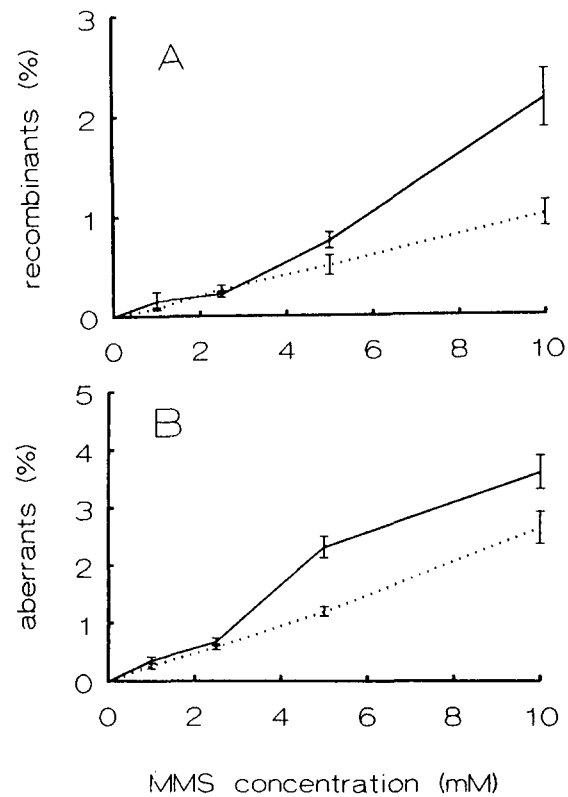

Fig. 2. Induction by different methyl methanesulfonate (MMS) concentrations of *Ade-2* reciprocal recombinants (A) and of *Ade-2* total aberrants (B) in *Saccharomyces cerevisiae* D7 cells grown either in a low (solid line) or in a standard (dotted line) background radiation environment. Each point is the mean ± SEM of at least four independent experimental observations. Data refer to the experiments carried out using the subculture conditioned in July 1992.

Table 1
Results and statistical analysis of the pooled data from the three independent sets of experiments carried out in July and December 1992 and in April 1993

MMS (mM)	*Ade-2* reciprocal recombinants			*Ade-2* total aberrants		
	SBE	LBE	*P*	SBE	LBE	*P*
2.5	0.285 ± 0.045 (13)	0.4 ± 0.089 (13)	NS	1.042 ± 0.148 (12)	1.025 ± 0.192 (12)	NS
5	0.814 ± 0.127 (14)	0.98 ± 0.123 (15)	NS	1.786 ± 0.2 (14)	2.285 ± 0.211 (13)	NS
10	1.447 ± 0.151 (13)	2.107 ± 0.147 (14)	< 0.01	3.577 ± 0.275 (13)	4.929 ± 0.333 (14)	< 0.01

The values shown are the mean ± SEM; statistical significance of difference was calculated on the basis of Student's *t*-test; number of observations in parentheses; NS, not significant; MMS, methyl methanesulfonate.

grown in LBE and those grown in SBE. The MMS concentration chosen fell in the shoulder region of the survival curve (data not shown), which allows an accurate determination of the intragenic recombination (Zimmerman et al., 1975). Fig. 2 shows the results of the experiments carried out using the subculture conditioned in July 1992. It can be observed that, for the highest MMS doses used, the frequencies of both recombinants and aberrants were significantly higher in the cell cultures grown in LBE than in those grown in SBE. A similar trend was recorded in each group of experiments, and the statistical analysis of the pooled data from the three sets of experiments, reported in Table 1, confirms the significance of the difference observed in each experiment.

Mitotic intergenic recombination is an early index of DNA damage induction (Boreham and Mitchell, 1991; Cundari et al., 1986) and at a high survival level its frequency increases with increasing doses of genotoxic treatments. Our data show that, in spite of the same exposure to the DNA damaging agent used, the yeast cells grown in LBE behaved as if they were more heavily damaged than those grown in SBE. This is consistent with the hypothesis that the environmental radiation background might contribute to the maintenance or induction of mechanisms involved either in protection (catalase, SOD, etc.) (Morichetti et al., 1989) or in repair against the induction of DNA damage (Demple et al., 1983; Rigaud and Moustacchi, 1994) or in the expression of those genes known to be modulated by the exposure to ionizing radiation (Fornace, 1992).

The experimental model and procedure used offered for the first time the opportunity to investigate the role that background environmental radioactivity might have played in the genesis and maintenance of the cellular mechanisms responsible for adaptation to the environment.

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