Environmental radiation as the conditioning factor for the survival of yeast

Saccharomyces cerevisiae

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Whether natural radiation can be a conditioning factor for the growth and survival of a living organism was investigated using diploid yeast S. cerevisiae D7. Yeast cells were conditioned by growing them continuously for at least 100 generation in 3 different radiation background such as i) ambient radiation (1.1 mSv/y), ii) sub-ambient radiation (0.44 mSv/y, within a shielded chamber) and iii) an elevated background radiation (88 and 880 mSv/y in a γ-field). At the end, the cells were challenged with 60Co γ, 100 Gy and the viable fractions were determined. Conditioning the cells in 880 mSv/y and in ambient radiation, enabled the cells to reduce the deleterious effect of the challenging dose significantly (P < 0.05) compared to that of sub-ambient radiation. The cellular viability of yeast cultures seems to be influenced by the prevailing radiation background, apart from starvation. Comparatively, a rapid decline in viability was noticed when the cultures were incubated for 60 days in the shielded chamber. The results indicate that some amount of radiation equivalent to background level or little above is needed to confer fitness in biological systems against stress factors, including radiation. The adaptive dose for the diploid yeast was also determined by single exposure. The priming dose ranged from 0.01 to 1.2 Gy. An adaptive dose of 0.25 or 0.4 Gy, almost nullified the deleterious effect of the challenging dose. The adaptive response may have a greater role in the field of cancer therapy and in radiation risk assessment. Understanding the response of an organism at different radiation-background will be helpful for successful space management.

Background radiation is considered to be one of the factors associated with biological evolution and the organisms are best adapted to live with it. It is also hypothesized that such adaptation can increase their ability to withstand the harmful effects of ionizing radiation. A possible approach to test this hypothesis is to subject them under sub-ambient condition. Background radiation and very low dose exposure could have a stimulatory effect in different biological systems; these results illustrate the concept of radiation hormesis.

Human peripheral blood lymphocytes become less sensitive to radiation-induced genetic damage when they are exposed to a low radiation dose, prior to a higher dose. This observation was called 'adaptive response' (AR) by Olivieri et al. when they found that the yield of chromatid aberrations was less than the sum of yields of the aberrations induced by [3H]thymidine and X-rays, separately. The radioadaptive mechanism is quite evident from a recent study conducted on flight engineers who were exposed to cosmic radiation. An in vivo AR has been reported in the lymphocytes of chronically exposed workers and in children exposed to fallout radiation in the Ukraine following the Chernobyl accident. There was no detectable increase in chromosomal aberrations (CA) in lymphocytes of Estonian men who took part in the cleaning up of the Chernobyl nuclear power site and those who did not, in spite of evaluating more than a quarter of million metaphase using FISH. In fact, the translocation frequency was lesser among the exposed workers than the controls, though not significantly. While the phenomenon of AR could be well established by these experimental results, the radiation hormesis can be determined only by end points such as cell survival, cancer index and growth kinetics. Moreover, the relationship between hormesis and natural background radiation or low background exposure can be understood best with the whole-life studies by recruiting the entire physiological processes available for such expression. Since, experimental testing of hormesis with human is not possible, data obtained from population living in high natural background area or those exposed to accidental radiation are considered. The A-bomb survivors exposed to 500-14,900 mSv showed significantly lower mortality from non-cancerous diseases than unmatched controls. But as the exposure to the A-bomb survivors approaches the background, the sample size needed exceeds that available. Most of the investigations, including
A-bomb survivor's study were based on short or intense radiation allowing only part of the wholesome physiological process to operate in. At the same time, people living in high background radiation displayed lower mortality from cancer though it was not statistically significant from the control\textsuperscript{9}. In another study involving large population cohort has consistently shown a significant decrease in lung cancer in those countries where the natural radon concentration in dwellings is slightly elevated\textsuperscript{10}. It should be, however, borne in mind that the above phenomenon quite often loose ground due to the difficulty in producing reproducible results and hence remain a subject of controversy in the filed of radiation protection. This dilemma is partly due to difficulty in getting statistically significant sample size or matching control sample for comparison. Systematic and complete investigations are therefore required to establish horesis or adaptive response in various organisms.

Yeast is ideal for this study as it is short-lived, rapidly breeding eukaryotic organism and its large cell population can be screened with its well-tested genetic markers. The present investigation has been carried out with the diploid Saccharomyces cerevisiae D7 to elucidate the influence of low environmental background radiation and to determine the optimal dose at which the phenomenon of AR is operational in the organism.

Materials and Methods

Yeast strain—The diploid yeast Saccharomyces cerevisiae D7 strain\textsuperscript{11}, kindly supplied by Dr. B.S. Rao, was used. This strain has complementing alleles in the trp locus and reverts to tryptophan independence by the mechanism of gene conversion. The convertants can easily be scored as individual colonies after 3-5 days of growth on tryptophanless medium. The spontaneous gene-conversion frequency was 7-14 convertants per million survivors.

Media—Cells were routinely cultured in YEPD broth which contains yeast extract 1%, peptone 2% and dextrose 2% (W/V). A liter of synthetic-complete (SC) medium contains yeast-nitrogen base without amino acids and ammonium sulphate (Hi-media, India) 1.5 g, ammonium sulphate 5 g, dextrose 20 g, adenine 5 mg, arginine 10 mg, histidine 10 mg, isoleucine 60 mg, leucine 60 mg, lysine 10 mg, methionine 10 mg, tryptophan 10 mg and uracil 10 mg. Tryptophanless medium (trp\textsuperscript{-}) was prepared in the same way but for the omission of tryptophan. Solid media were prepared by adding 2% agar to the liquid media. Further details of the media are described elsewhere\textsuperscript{12}.

Conditioning of yeast strain in different radiation background—Yeast cells were conditioned by growing them continuously for at least 100 generations in: 1) ambient radiation (normal environmental background radiation; 1.1 mSv/y), 2) sub-ambient radiation (within a shielded chamber containing the layers of 200 mm mild steel, 3 mm Pb, 2mm Cd and 1 mm Cu; ~0.44 mSv/y), 3) elevated radiation background (in a source room containing \textsuperscript{60}Co-\gamma source; 0.08 and 0.88 Sv/y at different distance). Radiation Environmental Monitor (Scintillometer, SM141D, ECIL) was used to monitor the radiation background. For each time of sub-culturing, the YEPD broth was inoculated with 100 cells/ml at final volume. Cell numbers were determined by haemocytometer counting. In another set of experiment, the cultures were not subcultured but remained incubated for 60 days in nutritionally starved condition under different radiation backgrounds. At the end, cells were washed thrice and suspended in distilled water to the cell titre of $5 \times 10^7$/ml before plating or for further exposure to 100 Gy of \textsuperscript{60}Co-\gamma (19.6 Gy/min) as challenging dose. The viable cells and convertants were observed as colony-forming units (cfu) on SC and trp\textsuperscript{-} medium, respectively. The gene-conversion frequency was assessed for $10^6$ survivors. All cultures were maintained at 30±1°C to avoid variations in growth rates arising from temperature fluctuations.

Adaptive response (single dose) study—Yeast cells, grown up to stationary phase for 48 hr in YEPD broth, were washed and suspended in distilled water to the titre of $5 \times 10^7$ cells/ml. The cells were then exposed to priming doses varying between 0.01 to 1.2 Gy of \textsuperscript{137}Cs-\gamma (0.43 Gy/h) and after an interval of 19 hr, they were further challenged with 100 Gy of \textsuperscript{60}Co-\gamma (19.6 Gyl/min). During the interval, cells were incubated at 30°C. Control cultures received no irradiation but only sham treatment. Immediately after challenging dose, cells were transferred on ice and plated on SC medium to score surviving population after suitable dilutions.

Statistics—Statistical analyses were carried out using the INSTAT GRAPH-PAD program. The values shown in graphics are the mean ± SE of the 3 independent sets of experiments. Statistical significance of difference was calculated on the basis of Student's t test.
Results

Growth kinetics of conditioned yeast cells in different radiation background—Cells, conditioned for 100 generations in different radiation background (1.1 mSv/y; 0.44 mSv/y; 88 mSv/y and 880 mSv/y) yielded different survival fractions in response to acute challenging dose (Fig. 1). The challenging dose chosen fell in the shoulder region of the survival curve which allows an accurate estimation of survival fraction or gene conversion. Radiation at low doses or in normal background level influences the deleterious consequences of high dose acute exposure. Cells, conditioned in 880 mSv/y and in ambient radiation, exhibited significant survival against the challenging dose compared to sub-ambient radiation (Table 1).

![Radiation-background used for conditioning (dose mSv/hr)](image)

Fig. 1 — Survival fraction of conditioned-yeast after a challenging dose of 100 Gy. (Error bar represents the standard deviation of the mean value)

The influence of different radiation background on the cellular viability of yeast under nutritionally starved condition was checked by incubating the culture for 60 days without adding any fresh nutrients. In this way, most of the dose delivered was at non-cycling stage. The cellular viability was reduced to 54% when the culture was incubated in shielded chamber (the normal background radiation was reduced by ~60%). At the same time, cultures incubated in elevated radiation background, exhibited either insignificant increase (in 880 mSv/y) or decrease in viability (in 88 mSv/y) compared to that of ambient radiation (Fig. 2). No co-relation could be made between number of surviving fraction and the trp- convertants, at the end of incubation (Table 2).

Adaptive response (single dose) study—Stationary phase-grown yeast D7 strain exhibited radioadaptive response when exposed to a low acute radiation. Cells were exposed to priming dose ranging from 0.01 to 1.2 Gy. A priming dose of 0.25 or 0.4 Gy almost nullified the effect of the challenging dose (100 Gy, γ). The response become insignificant as the dose approaches the extreme sides of the narrow adaptive window-dose (Fig. 3).

Discussion

During biological evolution, adaptation to physical factors such as radiation was necessary. Changes in the level of background radiation affect cell proliferation. These results reveal that the yeast cells conditioned in natural background and in elevated radiation background responded to subsequent challenge dose as if they developed resistance than those conditioned in sub-ambient level. It indicates that some amount of radiation equivalent to background level, or little above, is needed to confer fitness in biological systems against stress factors, including radiation. Interestingly, similar results were obtained with Saccharomyces cerevisiae D7, earlier; when the yeast cells were grown ~7 times lower the dose level (25 μSv/h) of natural background radiation, the genetic recombi-

<table>
<thead>
<tr>
<th>Type of Experiment</th>
<th>P value</th>
<th>Significant (S)/ non-significant (NS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 mSv/y + 100 Gy vs 88 mSv/y + 100 Gy</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>1.1 mSv/y + 100 Gy vs 880 mSv/y + 100 Gy</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>1.1 mSv/y + 100 Gy vs 0.44 mSv/y + 100 Gy</td>
<td>&lt;0.05</td>
<td>S</td>
</tr>
<tr>
<td>88 mSv/y + 100 Gy vs 880 mSv/y + 100 Gy</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>88 mSv/y + 100 Gy vs 0.44 mSv/y + 100 Gy</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td>880 mSv/y + 100 Gy vs 0.44 mSv/y + 100 Gy</td>
<td>&lt;0.05</td>
<td>S</td>
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Table 2 — The gene-conversion frequency of nutritionally starved yeast after 60 days of conditioning in various radiation background

<table>
<thead>
<tr>
<th>Radiation background (mSv/y)</th>
<th># Cells counted on trp + medium</th>
<th>Convertants/ml</th>
<th>Convertants/10^9 survivors</th>
</tr>
</thead>
<tbody>
<tr>
<td>880</td>
<td>999</td>
<td>5.95 × 10^5</td>
<td>9.67 × 10^4 (7.1 × 10^3)</td>
</tr>
<tr>
<td>80</td>
<td>827</td>
<td>7.41 × 10^5</td>
<td>12.89 × 10^4 (0.89 × 10^3)</td>
</tr>
<tr>
<td>0.44 (sub-ambient)</td>
<td>721</td>
<td>6.31 × 10^5</td>
<td>19.3 × 10^4 (1.1 × 10^2)</td>
</tr>
<tr>
<td>1.1 (ambient)</td>
<td>933</td>
<td>2.91 × 10^5</td>
<td>4.81 × 10^2 (3 × 10^1)</td>
</tr>
</tbody>
</table>

Fig. 2 — Survival fraction of nutritionally-starved yeast after 60 days of continuous exposure in different radiation background and after a challenging dose [■ viable cell population at the end of conditioning; □ conditioned cell after a challenge dose]

Fig. 3 — Adaptive response of diploid yeast S. cerevisiae [■ viable cell population after a challenging dose; □ viability after adaptive dose + challenge dose]

nation induced by radiomimetic agent (MMS) in them was higher compared to that of natural background environment. It was concluded that natural radiation may activate the defense mechanisms of yeast. Similarly, Planal et al. observed that the growth of unicellular protozoa Paramecium tetraurelia was reduced to 2/3 when the background radiation 1.75 mSv/y was lowered to 0.3 mSv/y using lead shield. The population of P. bursaria decreased in proportion to lead shielding. Similar results were also obtained with algae Synechococcus. All these support the paradigm that sub-ambient radiation levels are deleterious because of radiation deficiency.

Though sufficient investigations have been made with growing organisms under sub-ambient condition, their growth kinetics were not compared with elevated background radiation. Background radiation generally prevails at 2-2.5 mSv/y but ranges up to 10 times higher in certain region of the world. Also, biological systems do come across high background-radiation exposure due to man-made nuclear fall-outs and intrinsic bioaccumulation of heavy metals, including naturally occurring uranium series. In the present study, the cellular viability of yeast cultures seems to be influenced by the natural background radiation and other factors. Increased survival was observed with elevated background radiation (880 mSv/y) or in ambient condition when compared to sub-ambient level. It is in consistent with the hypothesis that some amount of radiation equivalent to background environmental level or other stress factors contributes to the cell survival by undefined mechanism. At the same time, prolonged incubation of yeast cultures in different radiation background under nutritional starvation, brought down their sensitivity-differences against challenging dose. The elevated gene-conversion observed in those cultures incubated for a long duration of 60 days, suggests low-dose as well as other factors during starvation, induced genetic damage may trigger the over all defense mechanism. Such enhancement of repair observed for two different stresses, alone or together, are roughly the same. The enhanced background convertants observed in long-culture-duration as well as after low-dose-radiation, has already been reported. The radiosensitivity of S. cerevisiae has been shown to be modified
The radiobiology of yeast has been extensively studied but its radioadaptive window is not well defined. The present results reveal that the adaptive response in yeast lies in a narrow window range of 0.25-0.4 Gy (single dose) which is at higher side for most mammalian cells (0.5 cGy to 20cGy). However, AR is highly variable depending upon the species, dose and experimental conditions. Deorukhakar and Rao observed AR to chronic radiation in yeast D7, whereas no such response could be detected when the cells were challenged with acute high dose exposure (20 Gy of 60Co-γ).

The exact mechanism of how the environmental radiation background contributes to the AR or hormesis is not clearly understood. It could be the resultant from the formation of free radicals to reactive oxygen species to altered molecular signaling to differential gene expression leading to DNA repair, or the failure of it leading to apoptosis.

The enhanced survival may have a greater role in tumour therapy and in radiation risk assessment. Colonizing Moon and Mars may take priority in the coming millennium. The stimulatory effect of cosmic radiation in paramecia during space flight-Salyut 6, was reported. The radiobiological knowledge of individual organisms will be helpful for successful space management.

Acknowledgements

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References

1 Parsons P A, Radiation hormesis: an evolutionary expectation based upon exposure to background radiation, BULE Newsletter, 3 (1994) 9.
5 Tuschi H, Kovac R & Altmann H, UDS and SCE in lymphocytes of persons occupationally exposed to low levels of ionizing radiation, Health Phys, 45 (1983) 1.

11 Zimmermann F K, Kern R & Rasenberger H. A yeast strain for simultaneous detection of induced mitotic crossing over, mitotic gene conversion and reverse mutation, Mutat Res, 28 (1975) 381.


