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MODIFICATION OF MUTATION FREQUENCY IN *SACCHAROMYCES CEREVISIAE*

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In a reverse mutation system, using haploid, histidine-requiring strain of *Saccharomyces cerevisiae*, the frequency of uv-induced prototrophs increased if the post-irradiation minimal medium was supplemented with limited amounts of histidine. Addition of natural amino acids or RNA bases in the post-irradiation minimal medium, with or without histidine, also increased the uv-induced mutation frequency. Thus, post-irradiation conditions favouring protein and RNA synthesis, are effective in increasing uv-induced mutations in yeast.
As compared to uv light, nitrous acid was more effective in inducing reversions in this strain and the frequency increased if the treated cells were plated on minimal medium supplemented with limited amounts of histidine. However, the addition of amino acids or RNA bases decreased the number of revertants. An additional inclusion of histidine reversed the suppressive effect of these metabolites. The mutation induction processes are thus different or differently modifiable in uv and nitrous acid.

INTRODUCTION

The mutation induction process after treatment with ionizing radiations and ultraviolet light (uv) takes some time for completion and certain metabolic conditions within a critical period markedly affect the frequency of mutations. The frequency of uv-induced prototrophic reversions in *Escherichia coli* and *Salmonella typhimurium* was found dependent upon the presence of a pool of amino acids in the incubation medium before the first post-irradiation cell division (Witkin, 1956). Later studies indicated the importance of both protein and ribonucleic acid (RNA) synthesis in induction and expression of bacterial mutants (Haas & Doudney 1957, 1959; Doudney & Haas , 1958, 1959, 1960; Doudney , 1963). The mutation induction process is thus considered to proceed in several steps and involves premutational damage, mutation stabilization, fixation and expression of altered phenotype.

How does the chemical mutagenesis differ from the radiation mutagenesis? The information on this can be obtained indirectly by comparing the modifying effects of various treatments on radiation and chemically induced mutagenesis. The present study describes the results of such an approach to answer the above question.

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Materials and Methods

A haploid, histidine-requiring strain of *Saccharomyces cerevisiae,* 2081-2A, *his* 3 *canar*, obtained from Dr D. C. Hawthorne of the University of Washington was used in these studies.

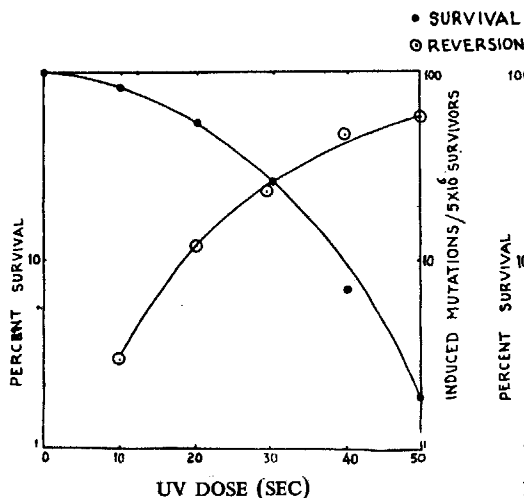
A heavy inoculation of cells was grown under aeration at 30° for 36 hr in a liquid medium containing 1 % yeast extract, 2 % peptone and 2 % dextrose. Before further use, the cells were starved for 12 hr to exhaust endogenous metabolites. For scoring revertants a synthetic minimal medium was used, which was supplemented with various metabolites to see their effect on induced mutation frequency (Wickerham, 1946).

For reversion studies with uv-irradiation, approximately 5×10^6 cells/ml were plated on the supplemented or unsupplemented minimal plates. For survival about 500 cells were plated on minimal plates supplemented with 10 μg/ml histidine. After plating, the cells were exposed to uv-light for different periods of time from a 30W Philips germicidal lamp at a distance of 45 cm from the source. The plates were incubated in dark at 25°. Survival and reversion frequencies were estimated from the number of colonies growing after 7 days of incubation.

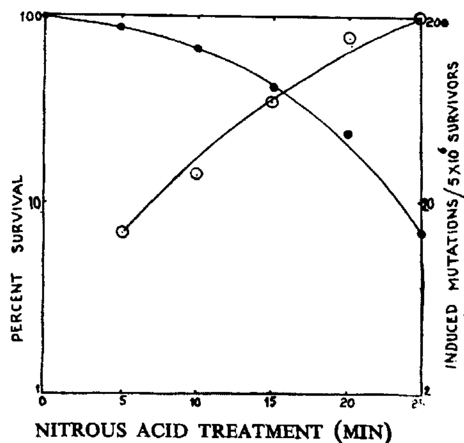
For treatment with nitrous acid (HNO_2), the cells were suspended in 0.1M sodium citrate-HCl buffer (*p*H 4.5) at a density of 10^8 cells/ml. To a 10 ml sample, 30 mg of sodium nitrite was added. Samples of 1 ml were withdrawn at various times and added to 4 ml of 1/15 M phosphate buffer (*p*H 7.0) to stop the reaction. After treatment the cells were washed and appropriate dilutions plated for reversion and survival.

Results

Dose survival and reversion — Fraction of surviving cells and reversion frequencies after uv and HNO_2 treatments are shown in Figs. 1 and 2. With increase in dose, the survival decreased while the number of revertants amongst survivors increased in both cases. Further experiments were carried out using irradiation periods of 10, 25 and 40 sec and HNO_2 treatments of 6, 15 and 25 min, both of which gave around 80, 40 and 6 % survival values at the three doses.



UV DOSE (SEC)
Fig. 1



NITROUS ACID TREATMENT (MIN)
Fig. 2

Effect of histidine on induced reversion—The addition of limited amounts of histidine to the post-irradiation minimal medium enhanced the uv-induced mutation frequency slightly, response increasing with increase in dose (Table I). Maximum histidine effect was seen at a supplementation of 2 μg/ml. In contrast, in nitrous acid treatment, supplementation with histidine considerably increased the revertants, response being more with increase in treatment time. The maximum effect of histidine was at 1 μg to 3 μg/ml supplementation.

TABLE I

Effect of histidine supplementation on mutation frequency

Media	Number of revertants per 5 x 10^6 survivors — UV treatment time (sec)				Nitrous acid treatment time (min)			
	0	10	25	40	0	6	15	25
Minimal (M)	0	5	19	28	0	17	66	279
M + 0.5 μg histidine/ml	1	7	31	53	1	257	1504	2534
M + 1 μg histidine/ml	2	8	34	64	1	312	1757	2947
M + 2 μg histidine/ml	2	10	51	71	2	395	1873	2871
M + 3 μg histidine/ml	2	10	42	64	3	387	2446	2582
M + 4 μg histidine/ml	4	11	44	66	5	313	1907	2361

Effect of amino acids and RNA bases on induced reversion—When the uv-irradiated cells were plated on minimal medium supplemented with 10 μg/ml each of the natural amino acids except histidine or 10 μg/ml each of the RNA bases, there was a slight increase in the number of uv-induced prototrophs but less than that with 2 μg/ml supplementation with histidine, the increase being more apparent at highest dose, and the RNA bases having more effect than the amino acids pool (Table II). In contrast, when the nitrous acid-treated cells were incubated on the above mentioned media, the number of prototrophs decreased considerably as compared to that in the control, the reduction being more marked with amino acids supplementation.

TABLE II

Effect of amino acids or RNA bases supplementation on mutation frequency

Media	Number of revertants per 5 × 10^6 survivors — UV treatment time (sec)				Nitrous acid treatment time (min)			
	0	10	25	40	0	6	15	25
Minimal (M)	0	3	14	28	1	17	64	250
M + Amino acids*	0	4	21	51	1	5	10	30
M + RNA bases*	0	6	22	67	0	9	21	80

*Medium supplemented with 10 μg/ml of each of the natural amino acids except histidine or 10 μg/ml of each of the RNA bases.

Effect of amino acids, RNA bases and histidine on induced reversion—When a limited amount of histidine (1 μg/ml of the medium) was also included in the medium in addition to supplementation with amino acids or bases, the reversion frequencies were enhanced slightly in case of uv-irradiation, the effect being synergistic, more pronounced at high dose of irradiation and greater with RNA bases than with amino acids (Table III). In the case of nitrous acid, however, the inclusion of histidine with the above-mentioned metabolites tremendously increased the number of HNO_2-induced revertants. The inclusion of histidine reversed the suppressive effect of these metabolites in induction of prototrophs.

TABLE III

Effect of amino acids or RNA bases and histidine supple nentation on mutation frequency

Media	Number of revertants per 5×10^6 survivors							
	UV treatment time (sec)				Nitrous acid treatment time (min)			
	0	10	25	40	0	6	15	25
Minimal (M)	0	5	15	35	0	13	48	220
M + 1.0 μg histidine/ml	2	8	26	77	2	351	1232	2370
M + 1.0 μg histidine/ml + amino acids*	1	8	34	93	1	534	1325	2707
M + 10. μg histidine/ml + RNA bases*	1	10	34	139	2	498	1636	2460

*Medium was supplemented with 10 μg/ml of each of the remaining natural amino acids or 10 μg/ml of each of the RNA bases.

DISCUSSION

The results of present experiments indicate the existence of a relationship between mutation induction process and post-mutagen cellular metabolism in yeast. The results with uv-irradiation are similar in some respects to the ones reported in *Neurospora* and *E.coli.* Whereas in *Neurospora* the presence of required growth factor in the post irradiation medium increased uv-induced reversion after a high dose of irradiation (Vaharu, 1961), in yeast this increase was observed at all doses of irradiation, though the increase was comparatively more at high dose. Bockrath & Cheung (1973) also observed an increase in uv-induced reversion in *E.coli* under similar circumstances.

Presence of histidine either permits the cells to go through a few divisions and helps in the incorporation of "potential mutation" in the genome by suppressing spontaneous reversal of pre-mutational damage or increases the induced mutation frequency by increasing the post-irradiation protein synthesis. This is also suggested by the fact that the presence of the amino acids pool, with or without histidine, increased uv-induced mutation frequency, the effect of histidine and other amino acids being additive. Similar modifying effects of amino acids pool have also been reported in *E.coli* (Witkin, 1956; Haas & Doudney, 1957, 1959; Doudney & Haas, 1958; Schwartz & Strauss, 1958) and in *Streptomyces* (Philips, 1961).

Similarly, the presence of RNA bases and the required growth factor in the post-irradiation incubation medium increased the number of uv-induced prototrophs in *Neurospora* at low doses (Vaharu, 1961), whereas in yeast this effect was observed at all doses.

These results suggest that in yeast the uv-induced mutation frequency can be increased by the post-irradiation conditions favouring protein and RNA synthesis.

When nitrous acid treated cells were plated on minimal medium supplemented with small amounts of histidine, the induced mutation frequency was tremendously increased as also in an adenine auxotroph (Zimmermann *et al.*, 1966). When the nitrous acid treated cells were plated on minimal medium supplemented with amino acids or RNA bases, the mutation frequency was reduced. These results are different from the ones obtained with uv-irradiation where similar conditions slightly increased the mutation frequency. However, when in addition to the above supplements, small amounts of histidine were also included, the number of revertants increased considerably.

Although it is not possible from the present studies to speculate on the mutation induction process for nitrous acid, one thing is clear that the process is rather complex, is subject to modification by post-mutagen treatments and involves cellular metabolism.

Thus it is clear that identical circumstances give very different results in uv and nitrous acid induced mutations. The processes leading from the time of action of mutagens on DNA to the final expression of mutation are either different or are differently modifiable in the two cases.

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