INDUCED PHENOTYPIC REVERSION BY 8-AZAGUANINE AND 5-FLUOROURACIL

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Numerous data now exist supporting the hypothesis that certain of the purine and pyrimidine analogues may influence metabolic systems by being incorporated into ribonucleic acid (RNA) (Naono and Gros, 1960a; Hamers and Hamers-Casterman, 1961; Chantrenne, 1961). Naono and Gros (1960a) have shown that cells of Escherichia coli and Bacillus megatherium incorporate 5-fluorouracil (5FU) into RNA and subsequently synthesize proteins modified in their amino acid composition. More specifically, RNA analogues induce the production of altered forms of at least three enzymes, alkaline phosphatase (Naono and Gros, 1960b), catalase (Chantrenne, 1961), and ß-galactosidase (Bussard et al., 1960; Hamers and Hamers-Casterman, 1961). The above results have led these authors to propose that the presence of the unnatural base in RNA interferes with the precise translation of genetic information from DNA to RNA to protein.

Benzer and Champe (1961) have shown that certain rII mutants of phage T4 are capable of multiplication in E. coli strain KB in the presence but not in the absence of 5FU. If, as these and other authors (Hamers and Hamers-Casterman, 1961) have suggested, analogue incorporation causes errors in information translation by RNA, one might predict that many

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mutant systems differing from normal by subtle changes in information content would respond to RNA base analogues. This report shows that approximately 50% of the nitrous acid-induced, leaky, adenine-3 mutants of Neurospora crassa exhibit partial phenotypic reversion when grown in the presence of either 5FU or 8-azaguanine (8AG).

Materials and Methods. — All mutants used were derived from nitrous acid-treated conidia of wild-type strain 74A by Drs. F. J. de Serres and H. G. Kølmark by the direct method (de Serres and Kølmark, 1958). The mutants selected for these tests are blocked in the conversion of 5-amino-1-ribosylimidazole 5'-phosphate to N-(5-amino-1-ribosyl-4-imidazole-carbonyl)-L-aspartic acid 5'-phosphate (Bernstein, 1961) and all are leaky, i.e., produce sparse and usually delayed growth in the absence of exogenously supplied adenine. For a description of the ad-3 locus, see de Serres (1956).

All growth assays were performed in Westergaard's basal medium (Westergaard and Mitchell, 1947) containing 1.0% glucose. This medium was supplemented with 1.5% Difco agar for assaying linear growth rate. The 8-azaguanine was purchased from the California Corporation for Biochemical Research, and the 5-fluorouracil was kindly donated by Mr. R. Duschinsky of the Hoffman-LaRoche Company.

A series of test tubes, each containing 3 ml of medium, were supplemented with 10, 1, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 0 μg/ml of 5FU or 8AG and inoculated with 0.1 ml of conidial suspension. All concentrations were in duplicate, incubated at 30°C, and scored over a 21-day period. A positive response was recorded when the amount of growth in duplicate tubes containing some level of analogue was at least two-fold greater than that in the tubes containing no analogue.

Results. — Two groups of 25 leaky mutants were tested, the first with only 8AG and the second with both 8AG and 5FU individually. It was necessary to eliminate one mutant from each group due to high spontaneous back mutation rates. Fourteen of 24 strains in the first group showed a marked growth stimulation in the presence of 8AG. The results of testing

the second group are shown in Table 1. It is worthy of note that 10 of the 13 mutants showing a response to 8AG are also stimulated by 5FU.

TABLE 1

Response of 24 mutants to 8AG and 5FU

Number responding:			
Both to 8AG and to 5FU	to 8AG only	to 5FU only	to neither
10	3	0	11

Since the possibility existed that the observed responses were due to back mutation, conidia were harvested from eight of the mutant strains growing on 8AG and from six on 5FU, washed by centrifugation, and retested. In each case the conidia were clearly mutant and again were stimulated in the presence of the analogue. These results demonstrate that analogue-induced growth is a result of phenotypic rather than genotypic change.

In order to obtain a more quantitative measure of the effect of 8AG, a series of growth tubes was inoculated with one of the mutants to determine the linear growth rate in the presence of various concentrations of analogue (Table 2).

TABLE 2

Effect of 8AG concentration on linear growth rate

Concentration (μg/ml)	Growth (mm/hour)
0	0.44
10^{-3}	0.63
10^{-2}	2.63
10^{-1}	3.13
1	3.13
10	2.88

The maximal growth rate achieved in this mutant in the presence of 8AG is approximately 75% of that in wild type. The level of analogue required for maximal stimulation differs

among the strains, usually ranging from 10^{-3} - 1 μg/ml with higher levels being inhibitory. Some strains do, however, give maximal response at 10 μg/ml and the possibility exists, therefore, that some mutants might respond to higher levels of 8AG than have been tested.

Guanosine and uridine eliminate the responses to 8AG and 5FU, respectively, when present in 10-fold excess over the analogue. Furthermore the normal bases, at concentrations stimulatory in the case of the analogues, did not stimulate any of the strains tested.

Discussion. — The mutants used in these studies were all induced by nitrous acid and theoretically should be transition mutants, i.e., differ from normal by a single DNA base pair substitution of the purine-for-purine, pyrimidine-for-pyrimidine type (Freese, 1959). Evidence that nitrous acid mutation results in equally subtle modifications in protein structure has been reported by Wittman (1961). This investigator has found that nitrous acid-induced mutants of tobacco mosaic virus contain single amino acid changes in their protein. In the light of these observations and the known effects of analogue incorporation into RNA, it is highly probable that the observed phenotypic reversion is a result of altered RNA information content or translation. The properties of 8AG and 5FU may be such that their substitution for the normal purine or pyrimidine in a coding sequence of bases changes the amino acid this code imparts to a peptide sequence in a protein molecule. If the analogue-induced amino acid change happens to be the reverse of the change resulting from the mutational phenomenon, an enzyme molecule with normal function would be produced. Such a mechanism could account for the growth response of certain nitrous acid-induced ad-3 mutants to these analogues.

Several other hypotheses are also compatible with the observations reported here, notably that originally proposed as a possible mechanism for suppressor mutation by Yanofsky and St. Lawrence (1960) and discussed by Benzer and Champe (1961).

Summary.— A number of ad-3 mutants of Neurospora are shown to revert in phenotype but not in genotype when exposed to either 8AG or 5FU. These observations may be interpreted on the basis of a modification of RNA information content by analogue incorporation.

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