PYRIMIDINE ANALOGS and the MUTATION of Neurospora crassa

William M. Scott

Department of Microbiology, Wayne State University
Detroit, Michigan

Received February 3, 1964

Failure to observe mutants among the progeny of Neurospora crassa cultures that had been grown in the presence of purine or pyrimidine analogs, and the lack of reports of mutation in molds following treatment with such analogs, indicated the need for investigation of the problem. This study showed that, in Neurospora, the simple pyrimidine base, 5-bromouracil (BU), is incorporated into DNA too inefficiently to cause detectable mutation, while 5-bromodeoxyuridine (BUDR) is incorporated more readily and causes mutation.

N. crassa was grown in 13 x 75 mm. tubes on Medium N (Vogel, 1956) or on Fries medium which yields a higher proportion of uninucleate conidia. Radioisotope labelled analogs were added to the growth medium. Incorporation of the label into cellular fractions was measured by counting the disintegrations of isotopes in the washed 48-hour mycelial pads, extracting with fat solvents and cold 5% trichloroacetic acid and counting again, extracting RNA with 10% perchloric acid at room temperature for 2 hours and simmering 5% trichloroacetic acid for 15 minutes (or with ribonuclease from Worthington, Freehold, N. J.), and after counting the pads again, DNA was extracted with simmering 10% perchloric acid or with Worthington's DNase. The acid extractions and nucleases gave comparable results in parallel experiments.

When the leucine-requiring mutant B 273-1-49a was grown in triplicate tubes of Fries medium plus leucine and a pyrimidine analog, BU was not mutagenic (as measured by phenotypic reversion of the mutant to growth on minimal medium), but BUDR was mutagenic. Table 1 shows the results.

TABLE 1

Reversion Frequencies Observed After Growth In Pyrimidine Analogs

Analog	Reversions per million conidia: Replicates (3)	Average reversion frequency	Maximum reversion frequency
Control	3.1 0.3 0.5	1.3	3.1
BU	0.9 0.6 0.6	0.7	0.9
BUDR	1.0 16 1.3	6.1	16

The cultures were grown at 30°C and conidia were plated on minimal Fries medium to determine the number of mutant colonies.

The use of C^{14}-labelled thymine or 5-bromouracil showed that the incorporation of BU into DNA was only about 15% as efficient as the incorporation of thymine into the DNA of wild type Neurospora. Calculations showed that only 0.4 to 0.8% of the thymine was replaced by bromouracil.

Although C^{14}-labelled BUDR was not available, 5-iododeoxyuridine labelled with I^{131} (I^{131}-UDR) was kindly donated by Dr. A. D. Welch. Competitive incorporation experiments showed that the incorporation of the labelled IUDR into DNA was more strongly antagonized

by BUDR than by BU. Thus it seemed likely that insufficient BU was incorporated into DNA to cause detectable mutation. Figure 1 shows, graphically, the incorporation of I^{131}UDR into DNA, and the antagonism resulting from addition of a number of other pyrimidines and their nucleotides in concentrations equimolar with the labelled IUDR. It should be noted that more I^{131} is incorporated into RNA and other cellular fractions, but mutagenesis is correlated with incorporation into DNA, and not with incorporation of analogs into RNA.

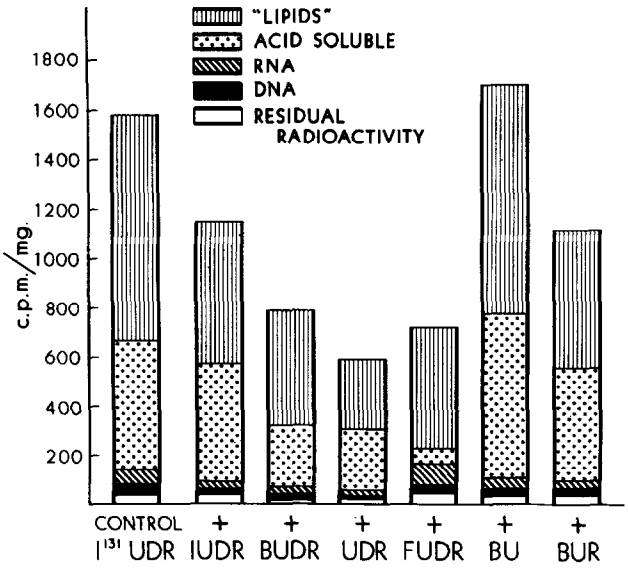

FIGURE 1

Radioactivity of Mycelium in I^{131}UDR and Analogs

The control medium contained 1.4×10^{-3}M I^{131}UDR, equivalent to 8.7×10^{6} d.p.m. All other tubes contained the same amount of I^{131}UDR plus the following non-radioactive analogs:
8×10^{-4}M IUDR, 8×10^{-4}M BUDR, 1×10^{-3}M deoxyuridine (UDR), 5×10^{-5}M FUDR, 8×10^{-4}M BU, 8×10^{-4}M bromouridine (BUR).

The foregoing observations suggested that BUDR might cause mutation of wild type Neurospora. Apparently, Mary Case (personal communication) was the first person to obtain mutants of Neurospora with BUDR. She used both BUDR and 5-fluorodeoxyuridine (FUDR). The present work did not indicate that FUDR is needed, and the mold was simply grown in the presence of BUDR. The conidia were subjected to the filtration enrichment of Woodward et al. (1954) and plated on enriched media. In three such experiments, mutants were isolated that required leucine, isoleucine, valine, adenine or hypoxanthine, and tryptophan (or indol or anthranilic acid). Many similar experiments with BU failed to produce any mutants.

Thus, three lines of investigation: reverse mutation experiments, forward mutation, and incorporation of radiotracers, all support the idea that mutation results when a pyrimidine is incorporated into DNA, and that the mutation frequency is, at least roughly, proportional to the extent of incorporation of the analog.

Acknowledgement: Most of the work reported herein was done in the laboratory of Professor David M. Bonner, at Yale University. The author wishes to express his gratitude to Dr. Bonner for his help, and also to Dr. Marko Zalokar for advice and encouragement.

REFERENCES

Vogel, H. J. 1956 A convenient growth medium for Neurospora. Microbial Genetics Bull. 13, 42.

Woodward, V. W., DeZeeuw, J. R., and Srb, A. M. 1954 The separation and isolation of particular biochemical mutants of Neurospora by differential germination of conidia followed by filtration and selective plating. Proc. Nat. Acad. Sci. (U.S.) 40, 192-200.