MUTATION AS AN ERROR IN BASE PAIRING*

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Freese (1959) postulated that base analogues, if incorporated into DNA, exert a mutagenic effect by causing "transitions" - replacements of purine by purine or pyrimidine by pyrimidine - changing the base sequence of DNA. Following the incorporation of some base analogues into DNA there is an increased likelihood of error in base pairing, the new stable pair at the mutant site being established only when two replications of DNA succeed the initial incorporation. (Figure 1).

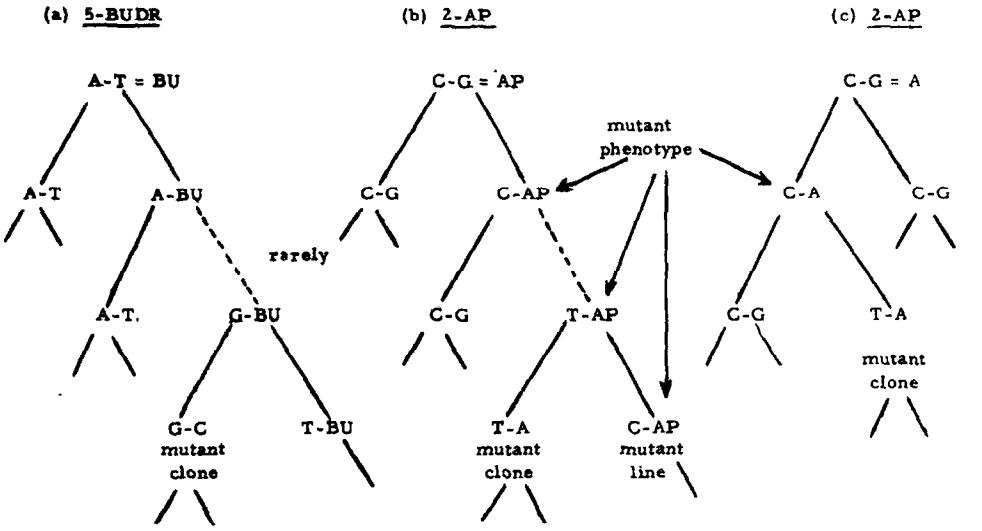

Fig. 1 Models of mutation extending that of Freese in terms of date presented in this note. a) a clone, mutant in phenotype and genotype, may be established two generations after incorporation of BU, b) the incorporation of AP (or of an unusual base in the presence of AP) leads immediately to a phenotypic change and the formation of a mutant line which may at any time give rise to a clone, mutant in genotype, c) a mutant clone is established one generation after the incorporation of a normal base in an unusual site because of the presence of AP.

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Experiments were designed to test whether mutations induced by 5-bromodeoxyuridine (BUDR) and 2-aminopurine (AP) occur in accordance with DNA replication, and how many replications are necessary to obtain a heritable change in the genotype. Tryptophan requiring mutants (Try D-79 and -10 of Salmonella typhimurium, known to incorporate bromouracil (BU), were used in these studies (Rudner and Balbinder, 1960). This system, mutation from tryptophan dependence (try^-) to tryptophan independence (try^+), has the advantage of a low background of spontaneous try^+.

No mutagenic effect or incorporation of BUDR is obtained unless interference with the endogenous methylation of deoxyuridylic acid is achieved. To create artificially conditions of thymidine deficiency, the folic acid inhibitor, aminopterin (Zamenhof et. al., 1958), or 5-fluorodeoxyuridine (FUDR), the inhibitor of thymidylate synthetase (Lorkiewicz and Szybalski, 1960) were employed. The mutant strain (Try D-79 isolated with AP by Dr. Balbinder) was grown in minimal medium supplemented with 20 μg tryptophan/ml and 0.2 per cent glucose. The cells were pretreated for 18 hours in 500 μg aminopterin/ml, a bacteriostatic condition, and then incubated with 250 μg BUDR/ml, a mixture of essential metabolites which contain the one-carbon fragments normally handled by the folic acid-containing coenzymes (Freese, 1950) and 250 μg aminopterin/ml. Aliquots were taken from time to time for the determination of the number of viable parents and mutants and of DNA. In another condition, where mutagenesis was obtained without killing, the cultures were incubated with 10 μg FUDR/ml, 200 μg BUDR/ml and metabolites or 0.2 per cent Bacto Vitamin-free casamino acids. The number of revertants was determined by plating washed cells on minimal agar of two kinds: (a) unsupplemented - MM, (b) supplemented, with 0.03 μg tryptophan/ml which allows 5×10^8 cells to replicate once on the plate - Try^+ MM.

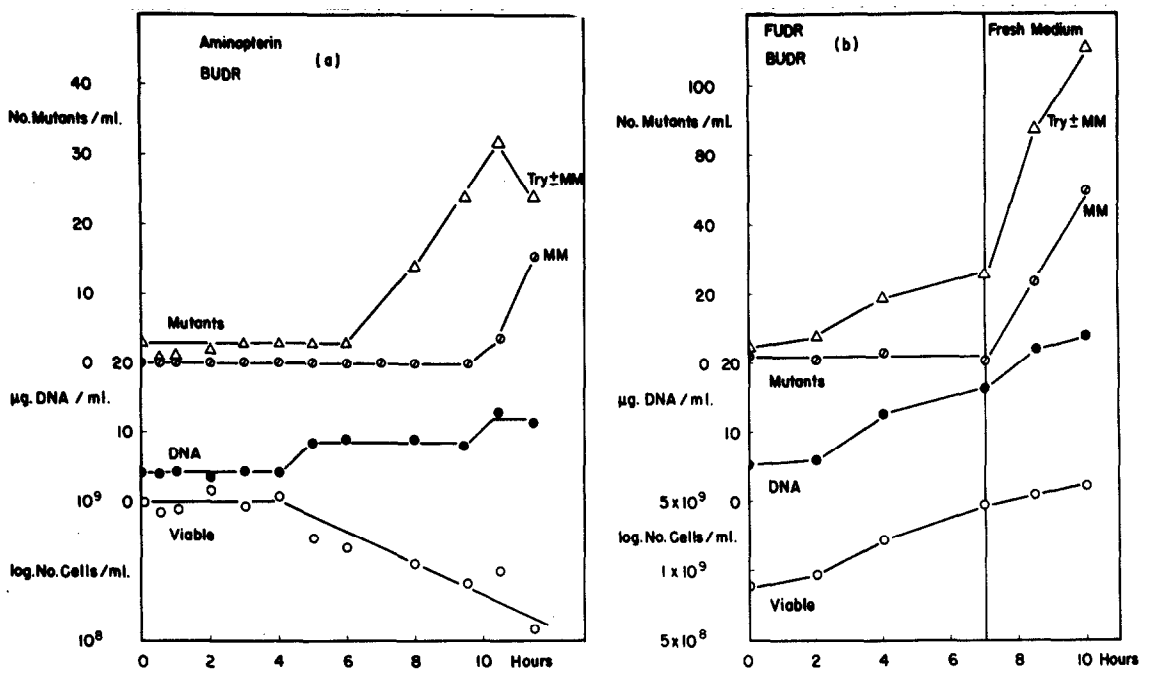

Fig. 2 (a) Aminopterin pretreated cells were resuspended in aminopterin and metabolites. After 9 1/2 hours only the aminopterin was removed from the medium.

(b) Try$^-$ cells were resuspended in FUDR, BUDR and metabolites. After 7 hours both drugs were removed.

Mutations from auxotrophy to prototrophy occur in accordance with DNA replication (Figure 2). Even though the cells begin to die due to the incorporation of BUDR, mutants on the supplemented minimal plages begin to appear after the first replication of DNA. Stable try$^+$ mutants expressed on unsupplemented minimal agar plates appear after two replications of DNA in liquid. Since the detection of try$^+$ mutants involves plating on minimal agar and because another final replication of DNA always occurs on minimal agar (when try$^-$ cells are placed in media devoid of tryptophane, they increase their DNA up to 88 per cent), a total of three replications of DNA take place prior to the occurrence of a heritable change in genotype. (Figure 2a). We can envisage that the first one allows BU to be incorporated in the place of its normal analogous base, thymine. In the

second replication, the incorporated BU may by mistake attach to a guanine in some of the DNA molecules. This ultimately results in the change of a thymine-adenine pair into a guanine-cytosine pair during the third replication. The removal of BUDR from the medium after the cells had undergone one DNA replication and resuspension in fresh media devoid of the mutagen still allowed the appearance of mutants on unsupplemented minimal agar (Figure 2b). This is consistent with the model which predicts that once the base analogue has been incorporated, the further development of the change is independent of its presence or absence in the medium.

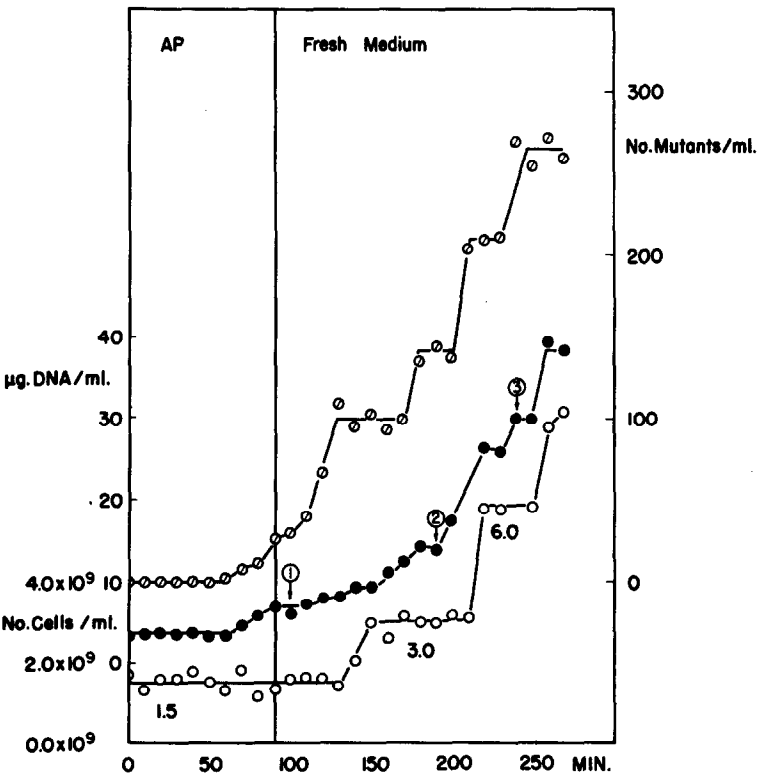

Fig. 3 The small cell fraction obtained by filtration was resuspended in AP. After 90 min. the drug was removed.
O = viable No., ● = µg DNA/ml.
⊘ = No. mutants/ml on MM.

In contrast to what was found with BUDR, AP-induced mutants appeared only after a single DNA replication. This has been tested in populations of TryD-10 bacteria whose division has been synchronized by fractional filtration (Maruyama and Yanagita, 1956). The small cell fraction was exposed to 250 μg AP/ml and was followed every 10 minutes for 3-4 hours. The mutants as determined on unsupplemented minimal agar appeared as soon as the DNA began to replicate and not after two subsequent replications as the model predicts (Figure 3). This was found whether AP was present continuously or only during the first replication. It was also observed when the cells were allowed to replicate their DNA without cell division in the presence of inhibitory concentrations of AP or when chloramphenicol was added (Nakada, Strelzoff, Rudner and Ryan, 1960).

Since the bacteria did not synthesize as much DNA as was thought to be needed, it was postulated that (a), the try^+ character is dominant and (b), the expression of the phenotype does not require further replication. Cells which have incorporated AP (or some other base as a consequence of its presence) are capable of tryptophane synthesis and hence can undergo the extra divisions necessary for the establishment of the try^+ genotype (Figure 1b). This may indicate that one strand of DNA is sufficient to carry genetic information. (Marmur and Lane, 1960).

To date the chemical incorporation of AP into DNA has not been reported; nevertheless, biological evidences indicate that the mutagenesis observed is due to its incorporation (or some unusual base formed only in its presence) and not to the incorporation of a normally synthesized base at an unusual site. It was postulated earlier that cells which have incorporated AP in the correct site can synthesize tryptophane and undergo divisions on the plate. They will as a consequence give rise to try^+ and

try$^-$ cells as daughters and form a line with mutant clones rising from time to time on minimal agar as a result of base-pairing errors. A series of minimal plates on which try$^-$ cells were plated after treatment with AP were observed microscopically. The cells began to divide very slowly with a generation time of ca. 5 1/2 hours, eventually forming snaky micro-colonies. When the increase in cell number was plotted against time, it was found that the cells increased arithmetically and not exponentially. (This process, although much later and with a generation time of 23 hours, also took place on the control plates). In the case of BUDR, the mutant phenotype and genotype arise at the same replication and after a total of three DNA replications. Hence no try$^+$ lines should be formed (Figure 1a); indeed, none were observed microscopically. To conclude, although the two mutagens appear to act with different kinetics, basically their mutagenic effect involves their incorporation followed by base-pairing errors during subsequent DNA replications.

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