IDENTIFICATION OF BASE PAIRS INVOLVED IN MUTATIONS INDUCED BY BASE ANALOGUES*

Eunice Strelzoff**

Department of Zoology
Columbia University
New York City

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Mutations induced by the incorporation of base analogues into DNA have been postulated by Freese (1959) to be the result of "transitions" of one base pair to another base pair, a purine replacing a purine and a pyrimidine replacing a pyrimidine. Further, the mutation may either result from a base pairing error occurring at some subsequent DNA duplication after an analogue is incorporated into the site of the natural base which it usually replaces or the mutation may be the direct result of an analogue substituted for a base which it rarely replaces. Mutations can be predicted to arise in a specific manner depending on which of these two alternatives has taken place. Thus, if it is known which natural base is usually replaced by an analogue, a tentative identification of the base pair changed by the induced mutation is possible.

It is known that 5-bromouracil (BU) quantitatively replaces thymine (T) in the DNA of thymine-requiring organisms (Dunn and Smith, 1954). It is therefore considered to be an analogue of thymine. When BU is incorporated opposite adenine (A) there is a certain small chance that with each additional DNA replication this BU will make an error and pair with guanine (G). Guanine on the next DNA replication will pair with cytosine (C) and the transition of

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AT to GC has taken place. This has been termed an error in "replication". There is also a small chance that BU will immediately replace cytosine. Upon the next replication this BU would be expected to act as thymine and pair with adenine leading to a CG to TA transition by an error in incorporation. In both cases three replications are necessary for the establishment of stable genotypic mutations. (Fig. 1).

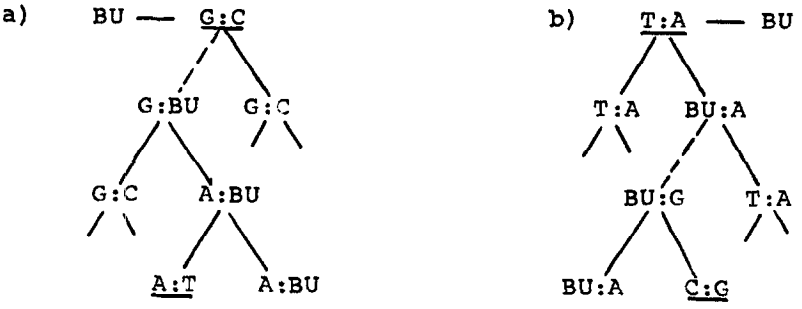

Fig. 1. Models of mutation as postulated by Freese: a) an error in incorporation takes place when BU replaces cytosine; b) an error of replication takes place as a result of BU replacing thymine and pairing with guanine at some later replication of the BU containing strand.

The fundamental difference between these two events is that a replication error does not occur until some time subsequent to the incorporation of the analogue. Once BU has replaced thymine there is a constant probability with each additional DNA replication that this BU will pair with guanine thus making the error that will result upon two more DNA replications in the AT to GC transition. On the other hand on every occasion in which a BU replaces a cytosine a stable transition will result after two additional DNA replications. In other words if all mutants could be removed at the time of the first mutant burst no more would appear upon additional DNA replications in the case of incorporation error but mutations caused by replication errors would continue to arise with additional DNA increase.

EXPERIMENTAL. -- Experiments have been carried out dividing the treated culture into many small tubes making it possible to isolate mutants resulting

from original incorporation errors or from errors resulting from additional DNA replications. The cells used were the thymineless mutant of Escherichia coli 15 t^- (Barner and Cohen, 1954) with additional amino acid requirements of arginine and methionine ($thy^-arg^-_2$ and $thy^-met^-_2$). The amino acid requiring mutants were isolated using 2-amino purine (AP). These mutants had the advantage of readily controlled incorporation of BU and in both cases the amino acid requirement had a spontaneous back reversion rate too low to interfere with the induced back mutations.

Washed exponentially-growing cells were suspended in minimal medium supplemented with 25 μg. required amino acid/ml. and .1 percent glucose for 1/2 hour in the absence of thymine in order to obtain synchrony (Barner and Cohen, 1956). Ten μg. of BU/ml. were then added along with 1 μg. thymine/ml. and the cells were allowed to undergo one DNA replication. Under these conditions no death took place. Cells were then washed and allowed to undergo one additional duplication in the absence of the analogue. These cells were then washed and distributed in aliquots of 2 ml. at a concentration below visibility (1×10^6/ml. for $thy^-met^-_2$ and 5×10^5/ml. for $thy^-arg^-_2$) to three groups of small tubes in which they underwent 1, 2, and 3 additional divisions. The additional divisions in the tubes were controlled by a limiting amount of required amino acid, this being 0.015, 0.025, and 0.05 μg./ml. for $thy^-met^-_2$ and 0.05, 0.1 and 0.2 μg./ml. in the case of $thy^-arg^-_2$. In the tubes where a mutation to amino acid independance occurred the mutants overgrew and caused a visible turbidity. From the fraction of tubes without revertants (P_o) and the zero term of the Poisson distribution,

$$P_o = e^{-m},$$

m, the mean number of mutations/tube, could be calculated. Thus, it was possible to determine whether cells once treated with analogue continued to

mutate with each additional replication in the absence of analogue or if all induced mutations occurred as a result of the initial analogue incorporation independant of further replications.

RESULTS AND CONCLUSIONS. -- In the case of the methionine requiring mutant, mutations from auxotrophy to protrophy was found to be dependant upon cell increase suggesting reversions at that site involved an AT to GC transition. In the case of the arginine requiring mutant the number of turbid tubes did not appear to be a function of further cell divisions after the initial induced mutations were expressed. This implied that a CG to TA transition had taken place. (Fig. 2).

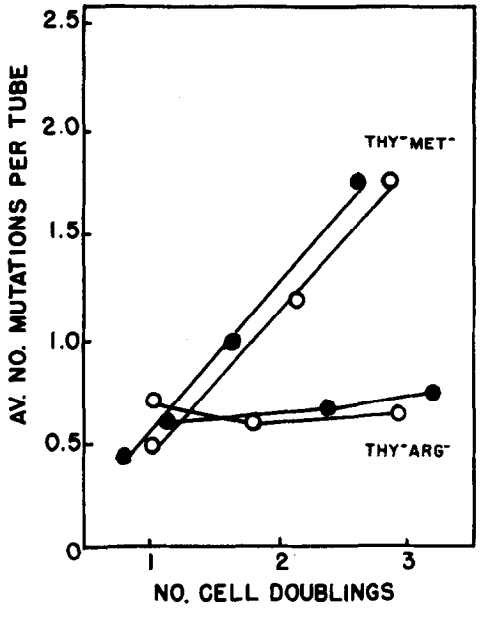

Fig. 2. The appearance of mutants with cell doublings after treatment with analogue.
o = cells treated with BU (x 10).
● = cells treated with AP.

Results obtained with the use of BU were compared with results using AP. In the case of the latter analogue incorporation is so slight that the natural base usually replaced by this purine can not be determined by chemical means (Rudner, 1961). However the pattern of mutant induction was identical to that obtained using BU (Fig. 2).

In summary, these studies show that base analogues may induce mutations which continue to arise with DNA replications in the absence of the analogue as would be expected if the analogue had produced an error in replication. For other mutants the analogue will only induce an initial burst of mutations with no further increase upon additional DNA replications in the absence of the analogue. This would be expected if the error was one of incorporation. As it is known that BU is an analogue of thymine this technique allows tentative identification of the base pair involved in the induced mutation. Further the patterns of induction were found to be the same regardless of the analogue (BU or AP) used. This implies that AP preferentially replaces adenine.

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