THE NECESSARY INVOLVEMENT OF BOTH COMPLEMENTARY STRANDS OF DNA IN THE SPECIFICATION OF MESSENGER RNA*

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Studies of the mutagenic action of base analogues have yielded results consistent with the hypothesis of Freese (1959) who has postulated that mutations induced by their incorporation into DNA are the result of transitions of one base pair to another, purines replacing purines and pyrimidines replacing pyrimidines. Induced mutations have been found which continue to arise with DNA replication in the absence of the analogue as would be expected if the analogue had produced an error in replication, as in the case of an A:T to G:C transition where 5-Bromouracil (BU) or 2-aminopurine (AP) were employed as the mutagen. In the case of other mutants, the analogue induced only an initial burst of mutations with no further increase upon additional DNA replication in the absence of the analogue. This would be

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expected if the error was one of incorporation, involving a G:C to A:T transition (Strelzoff, 1961; 1962 a and b).

Auxotrophs of E. coli 15 whose relevant base pairs were identified in this manner, and which seemed to be point-mutants, were further studied in order to determine the number of replications following incorporation of the analogue that were necessary for the expression of a prototrophic mutant phenotype. The number of DNA replications necessary for the expression of BU-induced reversions was determined and compared with the number required for the expression of mutations induced by the purine analogue, AP, in an effort to determine whether the genetic code is read from one or both strands of the DNA molecule. The mutagenic base analogues were incorporated into the DNA during the first replication of synchronous growth. The induced mutants were found to express themselves thereafter in the absence of the analogues according to either of the patterns shown in Figure 1.

Two independent arguments could be drawn from these data, each concluding that both members of a mutant base pair must be modified in order that the new phenotype be imposed on the cell.

In the case of the met_2 auxotroph, which reverts by an error in replication, the mutant phenotype is imposed after the third doubling of DNA, whether the mutagen is BU or AP. Were only one or either strand of DNA capable of yielding the new phenotype, it should have been expressed

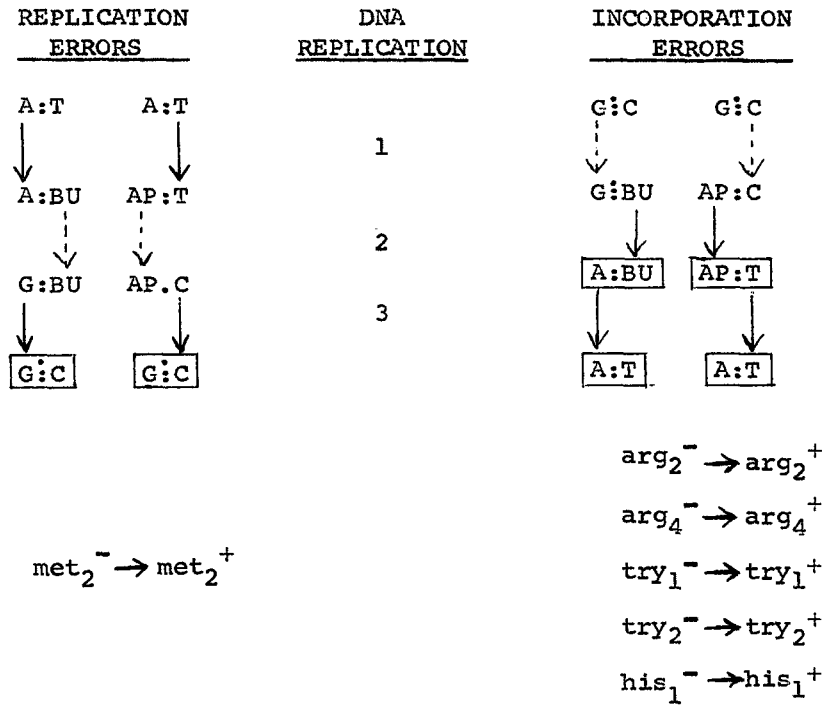

Figure 1. Schemes for the production of mutants in E. coli, based on the transition of single base pairs in DNA in response to the incorporation of 5-bromouracil (BU) or 2-aminopurine (AP) which have not been rejected by experiment (Strelzoff, 1961; 1962). The base pairs in rectangles are those which impose the new phenotype.

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after two replications (the phenotype of a prototroph is expressed almost immediately (Ryan and Wainwright, 1954; Ryan, Fried and Schwartz, 1954; Jacob and Wollman, 1961)). As expected, G:BU and AP:C do not behave as G:C in the cell.

When incorporation errors are made, the mutant phenotype invariably appears after the second replication. Were either strand of DNA sufficient to yield the new message, the novel phenotype should have been imposed after the first, or at the latest, the second replication; were the genetic information in only one strand, half the time the phenotype

should have been imposed after the second and half the time after the third replication, for it was never found after the first. In case the latter hypothesis were true, the chance of obtaining the observed result would be ca. 3% $\left[(1/2)^5\right]$. Apparently the cell reads A:BU and AP:T as A:T, which is consistent with the facts of chemical incorporation (Dunn and Smith, 1954) and the kinetics of the production of mutations (Strelzoff, 1961; 1962 a and b).

The fact that two strands of DNA seem involved in the formation of a single molecule of messenger RNA suggests that either messenger RNA is double-stranded or that, if single-stranded (Shulman and Bonner, 1961), it is not formed by hydrogen bonding with both or either strand of the unravelled DNA helix in the way postulated for the formation of new strands of DNA unless, of course, the alternate DNA strand forms an obligate complement of the message, such as a part of the transfer RNA. In vitro, both single- and double-stranded DNA (Chamberlain and Berg, 1962) seem capable of initiating the synthesis of RNA. The question facing us, however, is not what is necessary for RNA synthesis; rather it asks what is necessary for the formation of an RNA which will enable the cell to synthesize specific proteins. The work of Wood and Berg (1962) indicates that protein synthesis is not enabled by the RNA formed on the template of a single-stranded DNA but only by that formed on double-stranded DNA. On the other hand, single-stranded RNA is sufficient for the in vitro synthesis of abnormal proteins (Nirenberg and

Matthaei, 1961). If a single strand of messenger RNA is formed on the pattern of a DNA double helix, it is possible to understand how it comes to have a base composition complementary to that of the DNA which specifies it (Chamberlain and Berg, 1962) by a mechanism such as that suggested by Zubay (1962). It would be prevented from forming by the histone if both lie in the major groove of DNA (Stedman and Stedman, 1950; Feughelman et al., 1955; Bloch, 1962; Bonner, J., personal communication). Whatever the truth of these propositions it seems that in vivo experimentation must provide the standards by which artifacts can be recognized in experiments performed in vitro (Trautner, Swartz and Kornberg, 1962).

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