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A Progress Report

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BIOLOGICAL AND CHEMICAL STUDIES ON PHYSICAL MUTAGENS

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Submitted by

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Progress Report

Part I. Mutagen Specificity

During the past year several studies on mutagen specificity have been completed, and several others are nearing completion. A derivative of E. coli K12, designated PS2, is now routinely used in our laboratory for these studies. It appears to offer the best system yet for assigning genetic coding changes at the level of single base substitutions. Its characteristics are described in Appendix 1. *Removed*

A. Ionizing Radiation. This physical mutagen produces a variety of base substitutions, with no striking specificity. Mutagenicity is not random, however, because approximately three-fourths of the base substitutions originate as GC pairs. Transitions and transversions both occur. Two papers have been published on this work, and are included as Appendices 2 and 3. *Reprints (Removed)*

B. UV Mutagenesis and Repair. We have studied the specificity of UV-induced mutations, as well as their repair by both photoreactivation and dark repair processes. When dark repair is inhibited, 99% of the mutations are due to a GC $\rightarrow$ AT genetic coding change, so the UV lesion is extremely specific. Repair processes are also specific and, interestingly, both dark repair and photoreactivation are specific for the same classes of mutations. All our results are consistent with pyrimidine dimers being the initial UV lesion which leads to mutagenesis. Appendix 4 is a preprint of our paper which describes and discusses this work. *Reprints (Removed)*

C. Radioactive Phosphorous. These studies are not yet complete, but the data we have so far indicate that P^{32} decay produces mutations in excess of those expected on a radiation basis, whereas P^{33} does not. These

mutations are distributed among several classes, so P^{32} is not a very specific mutagen. We need to analyze more mutants produced by P^{32} decay before any definite conclusions can be drawn. This could be a very interesting broad spectrum mutagen since the decays are not associated with one particular base.

D. C^{14} -Labeled Thymidine. We now have some data for thymidine- $2C^{14}$ and thymidine-methyl- C^{14} , and it appears that a local mutagenic effect occurs for decay of the former but not the latter. These studies are of necessity extended over many months due to the long half-life of C^{14} . As for P^{32} we are still analyzing revertants to determine the specificity of mutagenesis. As expected a majority of mutants originate as AT base pairs.

E. Nitrogen Mustard. This chemical mutagen was found to have a very interesting specificity, in that it produces almost exclusively transversions. This is in contrast to most specific mutagens. Nitrogen mustard is a bifunctional alkylating agent, and chemical studies have shown that it reacts with N(7) on guanine. This is consistent with our results showing that nitrogen mustard produces $GC \rightarrow CG$, $GC \rightarrow TA$, and to a small extent $GC \rightarrow AT$, base alterations. This compound may be very useful as a somewhat specific producer of transversions in other studies. A manuscript of this work is in progress.

Part II. Suppressor Mutations

A. Duplicate tRNA Genes. Our bacterial system for studying mutagen specificity involves mutations in the anticodon region for genes specifying tRNA molecules. For quantitative studies, it is necessary therefore to know the number of gene copies specifying these tRNAs. Furthermore, the problem of duplicate genes is a fundamental one in molecular biology. We have

evidence for E. coli WU36-10, a derivative of B/r, that there are a minimum of two copies of the gene specifying a glutamyl tRNA with anticodon complementary to CAG that can be mutated to form a suppressor tRNA. This evidence is summarized in Appendix 4. For PS2 we have evidence that there is only one copy of this same glutamyl tRNA gene. There is only one copy of the gene specifying the seryl tRNA molecule with anticodon complementary to UCG that can be mutated to form a suppressor tRNA.

B. Altered UAA Suppressors. We observed earlier that derivatives of WU36-10 containing a UAA suppressor can undergo, with a rather high frequency, a genetic change which leads to increased suppressor activity. We have found this same phenomenon in derivatives of PS2. In this latter case, we are in a position to determine whether or not the altered suppressors arise by recombination. The recA56 mutation was introduced into PS2, giving a cell designated as PS7. We now find that, for PS7, altered suppressors are formed as readily as for PS2. Although we still do not know the mechanism for generating the increased suppressor activity, we know that it does not require a totally functional recombination system. The bacterial selection used would select for mutations to an increased UAA suppressor activity. There are a number of sites that could produce this increased activity: (1) alterations in the DNA information specifying precursor nucleotides for the tRNA suppressors. Some of these nucleotides are parts of recognition sites for the endonuclease(s) that cleave the mature tRNA from the larger precursor molecules; (2) alterations in the DNA information for the endonuclease that forms the mature tRNA from precursor; (3) alterations in information specifying either certain regions of the tRNA or the amino acyl synthetases. These mechanisms would give rise to increased suppression by

increasing the quantity of suppressor tRNA formed such as in (1) or (2) or by increasing the specific activity of charged suppressor tRNA as in (3).

C. Identification of Amino Acids Inserted by Suppressors. A system for identification of the amino acids inserted by various suppressor classes, in response to a nonsense codon was outlined in our renewal proposal last year. It involved the use of RNA from a nonsense mutant of f2 bacteriophage as messenger in an in vitro polypeptide synthesizing system to which tRNAs from suppressor cells were added. This system has become operational during the past year, and is described in detail in the Ph.D. thesis of F. A. Andersen. We have confirmed that the class 1 UAG suppressor of PS2 inserts serine, and the class 2 UAG suppressor inserts glutamine, in response to a UAG codon. The system has also been used to identify glutamine as the amino acid inserted by an unusual suppressor that we have been studying for some time. However, we are not yet ready to describe this latter work in detail.

Personnel on the project:

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