APPENDIX 2

COO-3419-3

CONF-700610--16

MASTER

CHARACTERIZATION OF GENETIC CODING CHANGES IN BACTERIA PRODUCED BY THE RADIOACTIVE DECAY OF INCORPORATED H^3- AND C^{14}-LABELED COMPOUNDS*

STANLEY PERSON, STEPHEN L. PHILLIPS**, F. ALAN ANDERSEN and HELEN P. NEWTON

The Biophysics Department, The Pennsylvania State University, University Park, Pennsylvania, 16802, U.S.A.

NOTICE
This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

INTRODUCTION

The following studies are based on past work in our laboratory using radioactive decay as a mutagen. As a result of our interest in tritium decay we have developed a method for the determination of the specificity of a mutagen at the level of DNA base substitutions [1]. We use bacterial mutants that are auxotrophic for a particular amino acid. The bacterial mutation is due to the presence of a polypeptide chain-terminating codon (UAG)[2] in the mRNA corresponding to that polypeptide. Bacterial cultures are treated with mutagens, plated in the absence of the growth requirement, and the resulting colonies analyzed. Individual revertants can arise from either of two mechanisms [2]. (1) A base substitution may occur in the DNA triplet corresponding to the mRNA codon, so that it is no longer a chain-terminating codon. These are sometimes referred to as structural gene changes. (2) A base substitution may occur in one of a number of DNA triplets specifying tRNA anticodons so that a tRNA

*Read by W.D. Taylor, Biophysics Department, Penn State University.

**Present address: Department of Biochemistry, University of Pittsburgh Medical School.

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

anticodon one base different from the complement to UAG can become the complement to UAG. In this case the original chain-terminating codon remains, but its terminating effect is partly overcome by the presence of the mutation in the anticodon of the tRNA. These types of mutations are sometimes called by the generic term, suppressor mutations.

METHOD OF MUTATION PRODUCTION

The two mechanisms of mutation production (referred to above) are illustrated in Fig. 1. The mutant genotype is shown in the middle row of the figure and the two revertant genotypes are shown in the top and bottom rows. The original mutant cell is *arg*$^-$ because it contains a UAG chain-terminating codon corresponding to a gene for arginine biosynthesis. Two wild type tRNA's, that normally translate code words for glutamine (CAG, CAA), are also shown in the middle row. Only the anticodon region and the amino acid attached to the 3' terminus of the tRNA are illustrated. The remainder of the tRNA is pictured as dotted lines. Note that the 3' base of the anticodon is written at the left.

Structural gene revertants refer to alterations by single base changes so that the chain-terminating codon is no longer present (top left). The example shown is for the replacement of U by C in the mRNA, a change that could have been caused by a T → C change in the DNA strand specifying the U in UAG. The wild type tRNA's are still present in unaltered form (upper center and right).

Arginine reversion by suppressor mutations is shown at the bottom of the figure. In this case UAG remains unaltered and the DNA information specifying a tRNA anticodon is altered instead. In the example shown, G in a glutaminyl-tRNA anticodon (middle row, center) is changed to an A (bottom, left). This could have occurred by a G → A change in the DNA strand specifying the G in the tRNA anticodon. The altered anticodon now has a sequence of bases that is complementary and anti-parallel to UAG so that UAG can be translated as glutamine. Thus, the

Structural Gene Revertant (su-cell) (arg+)	5' CAG 3'	5' CAG 3' 3' GUC 5' gln	5' CAA 3' 3' GUU 5' gln
MUTANT CELL (arg−)	UAG	CAG GUC gln	CAA GUU gln
UAG suppressor (arg+)	UAG AUC gln	CAG	CAA GUU gln

Fig. 1

chain-terminating effect of UAG is partly overcome and a viable revertant results. A suppressor of this type is referred to as a UAG suppressor.

Genetic code words are often degenerate in their 3' base, mostly due to an extended range of base pairing between codon and anticodon beyond that normally allowed in DNA duplexes. A single base in the 5' position of an anticodon such as U can effectively pair with A or G in the 3' position of a code word. Thus, in the example shown, CAG code words may be translated by $_{3'}GUC_{5'}$ and $_{3'}GUU_{5'}$ anticodons. Therefore the loss of the $_{3'}GUC_{5'}$ species of glutaminyl-tRNA in translating CAG is not necessarily lethal. In addition there might be duplicate, identical genes for the $_{3'}GUC_{5'}$ glutaminyl-tRNA so that one copy could be altered by mutation without a deleterious effect on the cell.

Because of the pairing of 3' terminal G code letters with 5' terminal U anticode letters, UAA suppressors

(complementary and anti-parallel to UAA) can also cause the translation of the UAG chain-terminating codon. This is not shown in the figure, but it can be visualized that a G to A change in the $_{3'}GUU_{5'}$ glutaminyl-tRNA would produce such a suppressor. In this case identical tRNA genes must be postulated to allow for continued translation of CAA code words.

In summary, there are two general types of revertants possible, a structural gene revertant modifying UAG by a single base, or UAG- or UAA- suppressors formed by changing a single base in a tRNA anticodon. The UAG suppressor is a tRNA with an anticodon altered so as to be complementary and anti-parallel to UAG, and the UAA suppressor is visualized as a tRNA with an anticodon altered so as to be complementary and anti-parallel to UAA. Because of the degeneracy of the genetic code, the latter suppressor also causes the effective translation of UAG. The evidence for this mechanism of production of suppressors can be found in references 2, 4, and 5. For UAG suppressors the evidence rests on genetic and chemical evidence (see reference 2), while for UAA suppressors the evidence is entirely genetic [4,5].

By using cultures of bacteria, each derived from an individual colony, as hosts for a set of T4 bacteriophage chain-termination mutants, one can quickly split a group of revertant colonies into two categories because those that arise by an alteration in the original chain-terminating codon do not support the growth of any of the phage mutants, and those that arise by an alteration in a tRNA anticodon support the growth of some or all of the phage mutants.

The different patterns of phage growth for revertants arising as UAG- or UAA- suppressors reflect the insertion of different amino acids. For example, if an anticodon in a seryl-tRNA is altered so as to be complementary to UAG, the pattern of phage growth may be different from that obtained if an anticodon corresponding to a glutaminyl-tRNA is altered. By comparing patterns of phage growth with those for bacteria in which the amino acid insertion by the altered tRNA has been

determined chemically [2], it is possible to infer the amino acid inserted in some cases. Using a set of chain-terminating mutants of T4 we are able to divide individual revertant colonies into a total of eight classes: class 1, 2, and 3 UAG suppressors shown in the last slide; class 1, 2, 3, and 5 UAA suppressors; and structural gene revertants that contain no suppressor [4]. Class 1 suppressors are inferred to insert serine; class 2, glutamine; and class 3, tyrosine. The class 5 UAA suppressor could be a multiple class and their amino acid insertion is unknown.

MATERIALS AND METHODS

E. coli WWU was grown in supplemented minimal medium [3]. Cells were labeled with one of the radioactive precursors, either thymidine-methyl-H^3, uracil-6H^3, or histidine-H^3, and stored at 4°C to accumulate radioactive decay of incorporated tritium. Procedures and specific activities used were as described previously [3]. Samples of these cultures were plated for arginine revertants and survivors when the bacteria had accumulated a number of decays that was sufficient to give approximately a maximum number of revertants per ml. Spontaneous revertants were isolated by growing a culture without radioactivity and storing it for a similar time at 4°C prior to plating. Samples of other cultures, grown as described, but in the absence of radioactivity, were frozen in a dry ice-acetone mixture and exposed to ionizing radiation from a Co^{60} γ-ray source (Gammacell 200; Atomic Energy of Canada, Ltd.). After each culture received a dose of 8700 rads, the suspension was thawed and plated immediately for arginine revertants and survivors.

RESULTS

The results of dividing revertants, produced by decay from incorporated tritium and by Co^{60} γ-rays, into classes as just described are shown in Tables 1 and 2. The net number distributions of revertants into classes are shown in Table 1 for revertants produced by thymidine-methyl-H^3, uracil-6H^3, and histidine-H^3 decay or by

TABLE 1. The Net Number Distributions Into Classes For *E. Coli* WWU Arginine Revertants Occurring By The Mutagens Listed

Mutagen	UAG suppressors			UAA suppressors			Structural gene revertants (su^-)	Net no. of revertants tested
	1	2	3	2	3	5		
Thymidine-methyl-H^3	36.5*	101.2	2.9	8.6	124.0	56.6	125.4	459.9
Uracil-6H^3	107.9	122.5	11.9	23.0	93.1	55.3	216.4	629.9
Histidine-H^3	57.0	98.0	35.9	14.5	124.3	76.0	161.9	593.7
Ionizing radiation	94.6	123.9	29.0	38.2	240.9	204.5	258.0	985.1

*The net number of revertants in each class was determined by subtracting the number of revertants in a particular class that can be attributed to the control, from the total number of revertants assigned to that class. The correction for revertants of spontaneous origin was the source of non-integral numbers. In the case of the revertants produced by radioactive decay, a separate culture was used as a control so that corrections were made by using the control percentages in classes averaged for many experiments. The number of revertants produced by each mutagen was a sum of at least three independent isolations. Class 1 revertants containing an ochre suppressor occur with a low frequency (<1%) for the mutagens used and are not listed.

TABLE 2. The Percentage Distributions Into Classes For *E. Coli* WWU Arginine Revertants Occurring By The Mutagens Used. Figures Are Given To The Nearest 1%

Mutagen	UAG suppressors			UAA suppressors			Structural gene revertants (su^-)
	1	2	3	2	3	5	
Thymidine-methyl-H^3	8	22	1	2	27	12	27
Uracil-$6H^3$	17	20	2	4	15	9	34
Histidine-H^3	10	16	6	2	21	13	27
Ionizing radiation	10	13	3	4	24	21	26
Uracil-$5H^3$	1	81	1	15	0	0	2
Thymidine-2-C^{14}	4	8	2	3	6	20	57

ionizing radiation from Co^{60} γ-rays (first column).

The net number of revertants in each of the classes is listed in succeeding columns. The net number is obtained by subtracting the number of revertants in a particular class that can be attributed to spontaneous origin. It is this correction that is the source of non-integral numbers. The main reason for including Table 1 is to show the net number of revertants tested for each of the mutagens used (final column). These numbers are the sums of numbers for at least three independent experiments. The relatively large size of the number reflects the fact that the method used to analyze progeny from individual revertant colonies is a relatively rapid screening technique. Table 2 shows the distribution of revertants into classes on the basis of percentage. This table shows that the percentage distributions of revertants into classes for thymidine-methyl-H^3, uracil-$6H^3$, histidine-H^3 are mostly similar to those found for external ionizing radiation.

For example, the percentage of structural gene revertants (su^-) is similar to ionizing radiation for all tritium-labeled compounds. Differences do exist and some of these may be significant (note, for example, the percentage of class 1 UAG suppressors produced by thymidine-methyl-H^3, and the percentage of class 5 UAA suppressors produced by uracil-6H^3).

Results for uracil-5H^3 decay [1], a highly specific mutagen, are shown in the next to the last row, for comparison.

A net number of 459 revertants produced by thymidine-2C^{14} decay were analyzed in a similar manner. The percentage distribution of revertants into classes is shown in the bottom row of the table. This distribution seems significantly different from those produced by tritium decay and external ionizing radiation. The percentage of class 1 and 2 UAG suppressors (columns 1 and 2) is about one-half the value of the lowest figure found in the top four rows. Similarly for class 3 UAA suppressors (column 5). Finally, the percentage class 5 UAA suppressors is higher than the average (column 6) and structural gene revertants (last column) account for a majority of the revertants tested (57%).

DISCUSSION

Since a complete table of the genetic code words exists, it is possible to convert the amino acid insertions into the DNA base substitutions that lead to those insertions. The results of doing this are shown in Table 3. The base changes are listed in the first column and the percentages of mutations produced by these changes, for the mutagens used, are listed in succeeding columns. Since the mechanism for the production of class 5 UAA suppressors is unknown, the percentages of revertants for this suppressor class are not listed in the table (about 15% for thymidine-methyl-H^3, uracil-6H^3, histidine-H^3, ionizing radiation, thymidine-2C^{14}, and 0 for uracil-5H^3).

The reader is referred to ref. 1 where this is shown in detail.

TABLE 3. Percentage Of Single Base Substitutions Produced By The Mutagens Listed*

Base substitution	Mutagen					
	Thymidine-methyl-H^3	Uracil-6H^3	Histidine-H^3	Ionizing Radiation	Uracil-5H^3	Thymidine-2C^{14}
GC → AT	24	24	18	17	96	11
GC → CG	2	4	12	6	2	4
GC → TA	45	43	41	45	1	13
AT → GC AT → CG AT → TA	9	15	9	6	1	50

*The base change assignments listed in Table 3 may be in error for a number of reasons: (1) Some base changes that produce suppressors also produce structural gene revertants. (2) Class 5 UAA suppressors occur at a significant frequency for some mutagens, and the mechanism of mutation production for this class is uncertain. (3) The number of tRNA genes that can give rise to a particular suppressor is taken as one in all cases. There is some evidence that this may not be valid for all cases [10]. (4) Mutation expression is taken as a constant for all classes of revertants. While none of these assumptions are particularly relevant when assigning base changes for specific mutagens, they could alter the assignments of base changes for a non-specific mutagen, perhaps by as much as a factor of two.

With regard to thymidine-methyl-H^3, histidine-H^3, uracil-6H^3, and ionizing radiation from Co^{60} γ-rays, the following conclusions seem warranted: (1) The percentage distribution according to base substitution produced by these mutagens is very similar. This reflects a similarity of mechanism of mutation production, which, in the case of these tritium labeled compounds, is due to β-ionizations. Since thymidine-methyl-H^3 and histidine-H^3 produce the same single base substitutions with similar frequencies, and since these frequencies are also similar to those found for external radiation, one must conclude that the mechanism by which these tritium compounds produce mutations is by β-particle ionizations. There is no evidence either for lethality or mutation production [6-9], that points to any other mechanism of action for these agents other than intracellular irradiation by the β-particles associated with radioactive decay. (2) Approximately three-fourths of the mutations produced by single base substitutions originate as GC pairs, a finding that could be interpreted in a number of ways. (3) Purine to pyrimidine changes, and *vice versa*, occur nearly as often as pyrimidine to pyrimidine or purine to purine changes.

Essentially all of the revertants produced by uracil-5H^3 decay are caused by GC → AT changes (96%). This conclusion has been reported previously [1, 9].

The revertants produced by thymidine-2C^{14} decay are mainly caused by molecular rearrangements associated with decay. The distribution of revertants produced by the base substitutions listed is noticeably different from those for ionizing radiation, or uracil-5H^3 decay. If there is to be a genetic coding change due to molecular rearrangement, these changes would have to originate as T. An inspection of Table 3 shows that about one-half of the revertants produced by thymidine-2C^{14} originate as AT pairs. Additional work on C^{14} radioactive decay will be reported elsewhere.

ACKNOWLEDGEMENTS

This research was primarily supported by the Atomic

Energy Commission Grant AT(30-1)-4080. Partial support was also provided by a NASA grant to the department (NGR 39-009-008). F.A.A. was a National Science Foundation predoctoral trainee. S.L.P. was a National Aeronautics and Space Administration trainee.

REFERENCES

1. OSBORN, MARY, PERSON, S., PHILLIPS, S. and FUNK, F., *J. Mol. Biol.*, *26*, 437-447, 1967.

2. For a review of research on chain-terminating mutants and their suppressors see GAREN, A., *Science*, *160*, 149, 1968.

3. PERSON, S. and BOCKRATH, Jr., R.C., *Biophys. J.*, *4*, 355-365, 1964.

4. PERSON, S. and OSBORN, MARY, *Proc. Natl. Acad. Sci.*, *60*, 1030-1037, 1968.

5. OHLSSON, B.H., STRIGINI, P.F. and BECKWITH, J.R., *J. Mol. Biol.*, *36*, 209, 1968.

6. BOCKRATH, R.C., PERSON, S. and FUNK, F., *Biophys. J.*, *8*, 1027-1036, 1968.

7. FUNK, F., PERSON, S. and BOCKRATH, R.C., *Biophys. J.*, *8*, 1037-1050, 1968.

8. PERSON, S. In: *Biological Effects of Transmutation and Decay of Incorporated Radioisotopes*, pp. 29-64. IAEA, Vienna, Austria, 1968.

9. FUNK, F. and PERSON, S., *Science*, *166*, 1629-1631, 1969.

10. RUSSELL, R.L., ABELSON, J.N., LAND, A., GEFTER, M.L., BRENNER, S. and SMITH, J.D., *J. Mol. Biol.*, *47*, 1, 1970.