Table 2. Counting efficiencies for bremsstrahlung produced in rats. Measurements were made at 2 cm. Each source consisted of 100 μc of carbon-14.

Crystal	Surface	Efficiency
	Diffuse	
NaI(Tl)	Dorsal	0.67×10^{-4}
NaI(Tl)	Ventral	1.2×10^{-4}
CsI(Tl)	Dorsal	0.82×10^{-3}
CsI(Tl)	Ventral	1.34×10^{-3}
	Colon	
NaI(Tl)	Dorsal	1.33×10^{-4}
NaI(Tl)	Ventral	8.0×10^{-4}
CsI(Tl)	Dorsal	2.2×10^{-4}
CsI(Tl)	Ventral	6.1×10^{-4}

from the rats are shown in Fig. 3. The control rat was used to measure the background, which was 4.3 count/min for the NaI(Tl) crystal and 104 count/min for the CsI(Tl) crystal. At a 97.5 percent confidence level, the lower limits of detectability for a 30-minute counting period are 1.06 and 5.2 count/min for the NaI(Tl) and CsI(Tl) crystals, respectively (*4*). Dividing these limits by the counting efficiencies, one may determine the amount of isotope that can be detected. The range of activity which may be detected is 60 to 720 nc with the NaI(Tl) crystal and 0.39 to 2.9 nc for the CsI(Tl) crystal.

The average energy of the bremsstrahlung as a percentage of the maximum beta energy differs greatly between the ^{3}H and ^{14}C sources. This is due to the greater absorption of the low-energy end of the spectrum in the tritium source. The difference in hardening of the spectra can be further seen in the absorption curves. Both sources exhibit a rapid decrease in count rate followed by a more gradual decline as the absorber thickness is increased owing to removal of the low-energy components of the spectra with increasing absorber thickness. The carbon source count rate shows a further reduction of 64 percent before becoming exponential in form, while the tritium source rate is reduced only 33 percent and then becomes linear on a semilogarithmic scale.

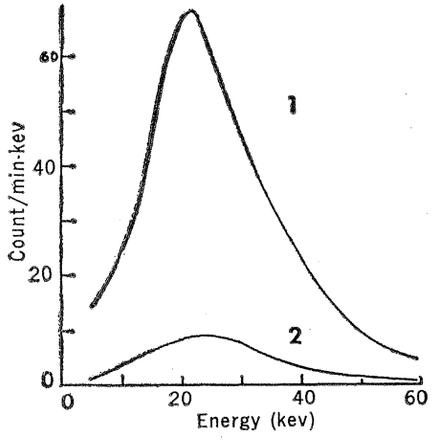

Fig. 3. Selected ^{14}C bremsstrahlung spectra from rats. Curve 1, 100-μc source in colon with the detector over the ventral surface. Curve 2, 100-μc source diffused throughout body; detector over ventral surface.

Based on the low value of the half-value layer for tritium, studies with tritium appear to be limited to the study of surface phenomena or to the first few millimeters of tissue. Absorption of tritiated substances applied to skin could be studied in this way.

With the greater penetration of carbon-14–produced bremsstrahlung, measurements in vivo become feasible in small experimental animals. With suitable collimation, single organs can be counted. Hence, the detectability of carbon-14–produced bremsstrahlung may help to eliminate the need for serial sacrifice of large groups of animals in some biological experiments, and in other cases eliminate lengthy sample preparations.

JERRY C. ROSEN
GERARD R. LAURER
MERRIL EISENBUD

Institute of Environmental Medicine, New York University Medical Center, 550 First Avenue, New York 10016

References and Notes

1. International Atomic Energy Agency, *Tritium in the Physical and Biological Sciences* (Gerin, Vienna, 1962), vols. 1 and 2; D. E. Watt and D. Ramsden, *High Sensitivity Counting Techniques* (Macmillan, New York, 1964), pp. 113–261.
2. R. D. Evans, *The Atomic Nucleus* (McGraw-Hill, New York, 1955), pp. 600–620; K. Liden, in *Progress in Nuclear Energy, Series XII*, W. G. Marley and K. Z. Morgan, Eds. (Pergamon Press, Oxford, 1959), vol. 1, p. 307; J. F. Cameron, J. R. Rhodes, P. F. Berry, *Tritium Bremsstrahlung and its Applications* (AERE-R 3086, United Kingdom Atomic Energy Authority Research Group, London, 1963); R. S. Pressly, *Low-Energy Gamma and X-Ray from Radioactive Sources* (ORNL-P 1928) (Oak Ridge National Laboratories, Oak Ridge, Tennessee, 1966).
3. The average energy is determined from multichannel analyzer data. The crystal window is neglected, and, for low energy photons, it is assumed that 100 percent of the photon energy is absorbed. Average energy $\bar{E}$ is then obtained from the equation:

$$\bar{E} = \frac{\Sigma \text{ channel No.} \times \text{count}}{\Sigma \text{ counts}} \times \frac{\text{kev}}{\text{channel}}$$

4. These minimum counting rates were obtained as follows:

$$\text{minimum count rate} = 1.96(2 \times B)/t,$$

where t is the total counting time and B is the total background count for this counting time.

5. Supported in part by Merck and Co., Inc., the Division of Radiological Health of the U.S. Public Health Service, and is part of a program supported by a core grant from the USPHS grants ES 00014 and CA 06989.

3 May 1967.

Genetic Mapping of Phenylalanyl-sRNA Synthetase in Escherichia coli

Abstract. *Genetic mapping of the structural gene for a phenylalanyl-sRNA synthetase in* Escherichia coli *was accomplished by the use of mutants with temperature-sensitive or* p-*fluorophenylalanine-resistant enzymes. The structural gene is located at minute 33 of the Taylor-Thoman map of the* E. coli *chromosome, closely linked to a structural gene* (aroD) *for one of the enzymes involved in the biosynthesis of the aromatic amino acids, and distant from the known locations of other aminoacyl-sRNA synthetase genes.*

During the search for mutants of *Escherichia coli* with conditionally expressed lesions in indispensable enzymes, a number of mutants were obtained with alterations in phenylalanyl-sRNA synthetase. One class of mutants was isolated by selection for the ability to grow in the presence of the phenyl-

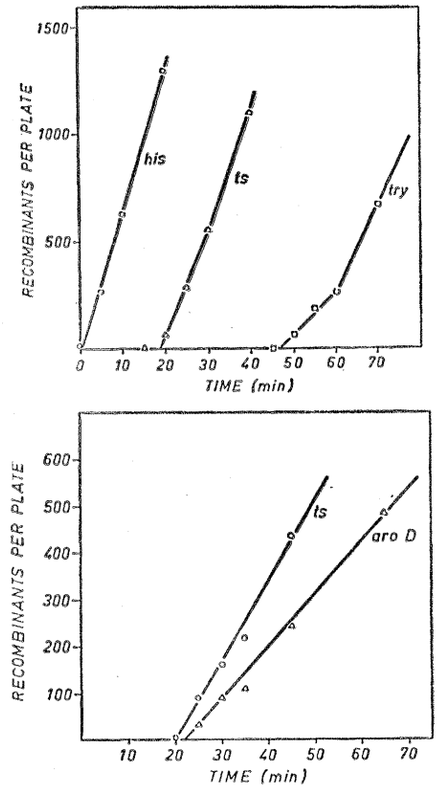

Fig. 1. Appearance of several kinds of recombinants in an interrupted mating experiment between Hfr AT2572a and either (top) NP37021, or (bottom) NP37022. Donor cells were counter-selected by streptomycin. Recombinants for nutritional markers were selected on appropriate mediums at 30°C. Recombinants for temperature-resistant growth were selected on plates incubated at 30°C for 6 hours before shifting them to 40°C.

Table 1. Description of strains used. Strain designations have recently been changed to conform with the suggestions of Demerec *et al.* (*9*). Strain numbers in parenthesis give former designations. Abbreviations: (S) sensitive; (R) resistant; (Sm) streptomycin; (Hist) histidine; (Tryp) trytophan; (Thr) threonine; (*pheS*) phenylalanyl-RNA synthetase locus; (ts) temperature-sensitivity; (pFP) *p*-fluorophenylalanine; (*aroD*) locus governing shikimic acid biosynthesis.

Strain	Genotype		Phenotype					
	pheS	*aroD*	ts	pFP	Sm	Hist	Tryp	Thr
NP37021 (IV-4 deriv.)	5	+	−	S	R	−	−	−
NP37022 (IV-4 deriv.)	5	1	−	S	R	+	+	−
NP21 (PFP-10)	1	+	+	R	S	+	+	+
AT2572a	+	+	+		S	+	+	

alanine analog, *p*-fluorophenylalanine; the phenylalanyl-sRNA synthetase of these strains was damaged in such a manner that the natural amino acid could still serve as substrate, but the analog was only poorly activated (*1*). The second class of mutants consists of strains that are able to grow almost normally at temperatures up to 30°C, but cannot grow at all above 37°C. Examination of some of these strains revealed that they possess an altered phenylalanyl-sRNA synthetase that is rapidly inactivated upon preparation of cell-free extracts (*2, 3*). Because of the diverse regulatory functions of amino acid activating enzymes (*3, 4*), their possible function as suppressor molecules, and the numerous unsolved problems regarding their function, we have mapped the structural gene for the phenylalanyl-sRNA synthetase, using at first one of the temperature-sensitive mutants. The several strains used in this study are described in Table 1.

For the mating experiments about 2×10^8 Hfr cells in the log phase of growth and 5×10^9 cells of the recipient (F^- phenocopies) were mixed on a sterile membrane filter (*5*); the filter was immediately placed on plates containing warmed nutrient soft agar, incubated for 5 minutes at 30°C, and then put into 250-ml erlenmeyer flasks containing 20 ml of warmed L-broth (*6*). After the cells were suspended by gentle agitation, 1-ml samples were removed at intervals and pipetted into 1 ml of ice-cold L-broth containing 500 μg of streptomycin. The chilled samples were mixed vigorously for 1 minute with the aid of a Vortex mixer, and 0.1-ml portions were spread on appropriate selective plates.

The results of a cross between Hfr AT2572a and NP37021 are shown in Fig. 1, top. In this cross, the gene conferring ability to grow at high temperature entered the recipients between the histidine and the tryptophan biosynthetic markers, permitting its localization at approximately minute 33 on the *E. coli* chromosome map presented by Taylor and Thoman (*7*).

To sharpen the location, use was made of one of the few loci known in this area of the linkage map, the *aroD* locus governing formation of shikimic acid (*7*). A derivative of NP37 with a shikimic acid requirement was isolated and mated with Hfr AT2572a. The results (Fig. 1, bottom) indicate that the temperature-sensitive marker entered the recipient slightly before, but closely linked to, the *aroD* locus. In all the matings the speed of chromosome transfer by Hfr AT2572a at 30°C was approximately one-third the rate at 37°C.

Since the results with matings suggested a location of the temperature-sensitive marker about 0.5 minute removed from the *aroD* locus, cotransduction of these genes was attempted. For this purpose the ability to grow at high temperature was transduced into NP37022 by way of phage P1kc that had been grown in strain NP21. The use of NP21 was designed to explore the genetic relation between the mutational sites *pheS1* which confers analog resistance and *pheS5* which confers temperature sensitivity (Table 2). All transductants selected for growth at 40°C had an active phenylalanyl-sRNA synthetase in vitro. (The original temperature-sensitive enzyme in the recipients, is denatured in vitro.) In addition, approximately 97 percent of these transductants had received an enzyme which, like that in the donor strain, has a greatly reduced ability to activate *p*-fluorophenylalanine and attach it to sRNA. Cotransduction of the temperature-sensitive marker and *aroD* also occurred; approximately 33 percent of the transductants received the *aroD* marker of the donor strain.

Table 2. Three-factor cross between NP21 and NP37022 mediated by P1 transduction. P1kc was grown over two passages on strain NP21 and used at a multiplicity of infection of about 1:1 to transduce temperature-resistant growth into mutant NP37022. A possible phenotypic lag of 6 hours was allowed by incubating the plates at 30°C before shifting them to 40°C. All transductants were still Thr⁻ Sm-R. Crude extracts of the purified recombinants were assayed for their ability to attach ^{14}C-phenylalanine and ^{14}C-*p*-fluorophenylalanine to *E. coli* KB sRNA. Abbreviations: pFP-S and pFP-R, recombinants with a phenylalanyl-RNA synthetase able and unable, respectively, to attach *p*-fluorophenylalanine to sRNA; ts, temperature-sensitivity.

ts⁺ recombinants scored (No.)	pFP-R *aroD*⁺	pFP-R *aroD1*	pFP-S *aroD*⁺	pFP-S *aroD1*
64	21	41	0	2

Thus, our results (i) confirm the notion that the temperature-sensitive growth behavior of strain NP37 and its derivatives is a result of the same genetic change that has modified the activity of the phenylalanyl-sRNA synthetase in vitro; (ii) indicate that single mutations in *E. coli* can render its phenylalanyl-sRNA synthetase either temperature-sensitive or analog-resistant and that these mutational sites are closely linked; (iii) permit the designation of a structural gene for this enzyme at minute 33 on the *E. coli* chromosome map, unlinked to other known loci of aminoacyl-sRNA synthestases (*8*); and (iv) provide the first example of a structural gene for an amino acid–activating enzyme located on a transducible fragment with one of the genes governing a biosynthetic enzyme for that amino acid.

A. Böck
Botanisches Institut der Universität
8 Munchen 19, Germany
F. C. Neidhardt
Department of Biological Sciences,
Purdue University,
Lafayette, Indiana 47907

References and Notes

1. W. L. Fangman and F. C. Neidhardt, *J. Biol. Chem.* **239**, 1839 (1964); W. L. Fangman, G. Nass, F. C. Neidhardt, *J. Mol. Biol.* **13**, 202 (1965).
2. L. Eidlic and F. C. Neidhardt, *J. Bacteriol.* **89**, 706 (1965).
3. F. C. Neidhardt, *Bacteriol. Rev.* **30**, 701 (1966).
4. L. Eidlic and F. C. Neidhardt, *Proc. Natl. Acad. Sci. U.S.* **53**, 539 (1965); S. Schlesinger and B. Magasanik, *J. Mol. Biol.* **9**, 670 (1964); J. R. Roth and B. N. Ames, *ibid.* **22**, 325 (1966).
5. T. S. Matney and N. E. Achenbach, *Biochem. Biophys. Res. Commun.* **9**, 285 (1962).
6. E. S. Lennox, *Virology* **1**, 190 (1955).
7. A. L. Taylor and M. S. Thoman, *Genetics* **50**, 659 (1964).
8. M. Yaniv and F. Gros, *Proc. Fed. Europ. Biochem. Soc., 3rd Congr. Warsaw, April 1966*, in press; A. Böck, L. Eidlic Faiman, F. C. Neidhardt, *J. Bacteriol.* **92**, 1076 (1965); A. Böck and F. C. Neidhardt, *Z. Vererbung.* **98**, 187 (1967).
9. M. Demerec, E. A. Adelberg, A. J. Clark, P. E. Hartman, *Genetics* **54**, 61 (1966).
10. Supported by a grant from the Deutsche Forschungsgemeinschaft and by NSF grant GB 2094. We thank Dr. A. L. Taylor for providing *E. coli* strain AT2572a.

6 April 1967