thermal denaturation curves of both nucleic acids indicated that they are single stranded; thus, the nucleic acid of SL-TRSV is much smaller than that of TRSV and much smaller than one would have predicted from either the sedimentation coefficient or the apparent nucleic acid content of SL-TRSV (nucleoprotein). Furthermore, if the relation between sedimentation coefficient and molecular weight suggested by Gierer (*6*) holds, a molecular weight of only 86,000 is indicated for the nucleic acid of SL-TRSV compared with a molecular weight of 1.2 million for the nucleic acid from middle component of TRSV. If one considers that the nucleic acid content of SL-TRSV is greater than that of the middle component of TRSV but less than that of bottom component [which is about 2.2 million (*5*)], we must then conclude that each particle of SL contains 14 or more strands of nucleic acid of about the same size, and that some strands, perhaps all, are biologically active in conjunction with TRSV infections.

The SL-TRSV reacted with antiserum to TRSV when SL was diluted to the same concentration as TRSV (in optical density units per milliliter) that was barely detected in microprecipitin tests, or that was barely detected in Ouchterlony double-diffusion tests. In the latter test at higher concentrations of antigen, when SL-TRSV and TRSV were in adjacent wells and opposite the well containing TRSV antiserum, the reaction bands were continuous with no spur formation. Furthermore, when SL-TRSV and TRSV were subjected to electrophoresis for 22 hours in sucrose gradients (0.002*M* phosphate buffer, 0.05*M* NaCl, *p*H 7) (*7*), both apparently migrated to the same position. The SL-TRSV appears to be a nucleoprotein containing many small strands of nucleic acid which are biologically active in the presence of TRSV, but the protein shell which encloses these strands is similar to the protein shell of TRSV.

The SL-TRSV is similar to some plant viruses with functionally incomplete particles in that it is unable to replicate alone. These dependent plant-virus particles relate biologically in their respective systems as symbionts or as parasites. Components of alfalfa mosaic virus, cowpea mosaic virus, or tobacco rattle virus relate symbiotically (*8*). With the exception of the long particle of tobacco rattle virus, no particle of this group of viruses can replicate alone. But the long particle of tobacco rattle virus does not code for its protein shell and depends on the short particle (that cannot replicate alone) for this function.

The parasitic dependency of SL-TRSV on TRSV more closely resembles the parasitic dependency of SV-TNV for TNV, its activator. The SL-TRSV, like SV-TNV becomes the larger part of the virus population in mixed infections with its specific activator. The TRSV and TNV replicate and form complete particles in the absence of their respective satellites. The TRSV, like TNV, codes for its own coat protein.

Although the SL-TRSV–TRSV system resembles the SV-TNV–TNV system, many biological as well as structural differences are evident. The activator (TRSV) is a multicomponent virus; TNV is a single-component virus. The TRSV and SL-TRSV commonly infect hosts systemically; TNV and its satellite are commonly localized in primary infection sites. The SL-TRSV has the same size and shape as TRSV and is serologically indistinguishable from TRSV, and each protein shell of SL-TRSV encloses many small nucleic acid strands. The SV-TNV is smaller than, and serologically unrelated to, TNV, and each shell of SV-TNV contains a single nucleic acid strand. Since the small nucleic acid strands of SL-TRSV can be activated by TRSV or its infectious nucleic acid after extraction, these strands were small before extraction and may occur as subunits within the shell. However, different strands cannot be ruled out at present.

The origin of plant viruses, which are unable to replicate alone, is uncertain. The SV-TNV and the functionally incomplete particles of tobacco rattle virus, alfalfa mosaic virus, and cowpea mosaic virus have been known in virus preparations for many years (*8*). The strain of TRSV used in this study to activate SL-TRSV has been increased and purified routinely in this laboratory since 1963. The ability of SL-TRSV to rapidly become the larger part of the population in mixed infections with TRSV indicates that SL-TRSV probably has not been continuously carried along as an active contaminant with TRSV. Thus it may be that the origin of SL-TRSV as a "contaminant" in our population of TRSV was a recent event.

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9. I thank F. Marshall for technical assistance, F. Jenifer for analytical ultracentrifugation, J. M. Kaper for assistance in the calculation of sedimentation coefficients, H. Scott for TRSV antiserum and assistance in serological tests, R. Lawson for electron microscopy, and R. E. Davis for density-gradient electrophoresis.

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Cytosine to Thymine Transitions from Decay of Cytosine-5-^{3}H in Bacteriophage S13

Abstract. *Decay of cytosine-5-^{3}H incorporated into bacteriophage S13 DNA causes a molecular rearrangement of the cytosine molecule undergoing the decay. The molecular rearrangement produces a cytosine to thymine coding change with an efficiency approaching one. Decay of either thymidine-(methyl)-^{3}H or cytosine-6-^{3}H is less than 1 percent as effective in causing either cytosine to thymine or thymine to cytosine transitions.*

Radioactive decay of uracil-5-^{3}H incorporated into *Escherichia coli* (*1*) is more mutagenic than that of other tritiated precursors (*2, 3*). *Escherichia coli* efficiently interconverts uracil and cytosine (*4*) so that cells grown in the presence of uracil-^{3}H will have uridine-^{3}H and cytidine-^{3}H incorporated into their RNA and cytidine-^{3}H incorporated into their DNA (*5*). For each disintegration, the mutation frequency is threefold greater when bacteria are labeled with uracil-5-^{3}H than when labeled with thymidine-(methyl)-^{3}H (*2*).

Later experiments indicated that most mutations produced by uracil-5-^{3}H are the result of a molecular rearrangement occurring in the bases labeled by uracil-^{3}H since the mutation frequency due to decay of uracil-6-^{3}H is sixfold less than that observed for uracil-5-^{3}H (*3*). The mutations produced in bacteria by these ^{3}H-induced molecular rearrangements are due to decays of cytidine-5-^{3}H in DNA (*5*, *6*) and are probably due to C → T transitions (*7*).

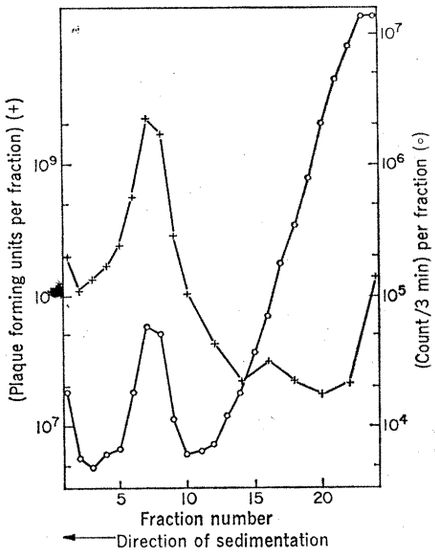

Fig. 1. Sucrose sedimentation of H_l1 labeled with cytosine-5-^{3}H. The H_l1 mutant was grown in *Escherichia coli* C, and th39 was grown in *E. coli* WWU (*2*). The phage were first adsorbed on cells starved for glucose. After adsorption, glucose and casamino acids were added to make a complete medium (*9*), and the infected cells were added to tritium precursors (dry). The specific activities of the precursors were 10 to 24 c/mmole at 4 μg/ml. Average burst sizes were 200. After the cells were lysed, the lysate was cleared by low-speed centrifugation, and treated with pancreatic deoxyribonuclease and ribonuclease (50 μg/ml) each and $5 \times 10^{-2}M$ $MgSO_4$ for 20 minutes at 37°C; EDTA was then added to make $2.5 \times 10^{-2}M$. A portion was diluted 1:100 in storage medium ($10^{-1}M$ NaCl, $10^{-2}M$ tris, *p*H 8.0) and stored at 5°C to be used for biological assays. Portions (0.2 ml) of the remainder were layered on a sucrose gradient (5 to 20 percent) made up with storage medium and sedimented (Spinco SW39L, 70 minutes, 39,000 rev/min). Two fractions of the phage band were mixed, and a portion was placed in another sucrose gradient to determine the amount of residual radioactive contamination. The remainder was assayed for radioactivity in a liquid-scintillation counter. Biological recovery from the sucrose gradient was 50 to 95 percent, and we assumed that no phage particles were lost during purification. The concentration of phage particles was obtained by extrapolation of the survival curves to zero disintegration and the assumption of a plating efficiency of one.

We used two S13 bacteriophage mutants to determine the efficiency with which decays of cytosine-5-^{3}H, cytosine-6-^{3}H, and thymidine-(methyl)-^{3}H produce C → T and T → C transitions. Randomly occurring decays of cytosine-5-^{3}H produce base alterations only at the sites of the decays. If the alteration of a single unique base is required for reversion, the mutation frequency from decays of cytosine-5-^{3}H will be inversely proportional to the number of cytosine bases in the DNA of the organism studied. Bacteriophage S13 which contains less than 1/1000 the number of DNA bases found in *E. coli* was used to amplify the mutagenic response. This phage has the additional advantage of containing single-stranded DNA which permits determination of the specific base changes produced by each tritiated precursor as compared to *E. coli* where only base pair changes can be determined.

The S13 mutants H_l1 and th39 have been characterized by Tessman, Poddar, Kumar (*8*); H_l1 reverts by C → T transitions, and th39 reverts by T → C transitions. The phages were labeled with either cytosine-5-^{3}H, uracil-6-^{3}H or thymidine-(methyl)-^{3}H as described for T4 (*9*). Uracil-6-^{3}H is incorporated in S13 as cytosine-6-^{3}H. For H_l1 the number of disintegrations occurring per phage per unit of time was determined by measuring the rate of decay and the number of plaque-forming units in a stored sample of purified labeled phage. The number of plaque-forming units was normalized for any decrease that occurred during purification. The phage were purified by sedimentation in a sucrose gradient (5 to 20 percent) (Fig. 1). The th39 mutant, although stable in storage media, was not stable under the conditions of purification. Therefore, the disintegrations per phage per unit time were calculated from the specific activity of the tritiated precursor in the medium and the nucleotide composition of øX174, assumed to be the same as that of S13 (*10*).

Like that of T4, the kinetics of inactivation for S13 are single-hit kinetics (Fig. 2). The slope is the probability of inactivation per decay α, and for this experiment is 0.5.

The slopes or k values of the curves in Fig. 3 represent the probability of producing a revertant per disintegration per phage. Hence for decays of

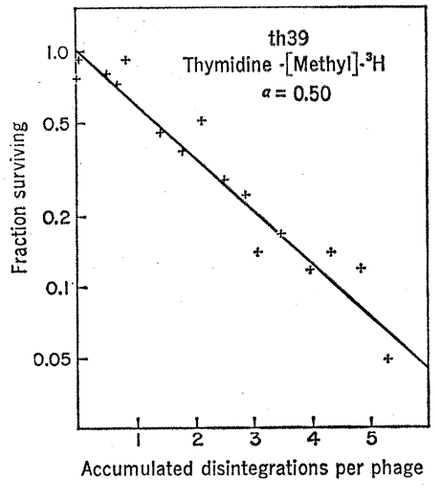

Fig. 2. Inactivation of th39 from decay of thymidine-(methyl)-^{3}H. A portion of the clarified lysate was diluted 1:100 in a solution consisting of $10^{-1}M$ NaCl and $10^{-2}M$ tris (*p*H 8.0) and stored at 5°C. Loss of plaque-forming ability and the production of revertants was followed as a function of time.

cytosine-5-^{3}H in H_l1 (+) this probability is 7.9×10^{-4} or over 400 times greater than that for decays of thymidine-(methyl)-^{3}H (○). Therefore, cytosine-5-^{3}H is much more effective in reverting the H_l1 mutant, and hence in causing C → T transitions than thymidine-(methyl)-^{3}H.

The average value of k for decays of cytosine-5-^{3}H in the H_l1 mutant is 8×10^{-4} (Table 1). Hence decays of cytosine-5-^{3}H produce C → T transitions very efficiently. All of the other k values in Table 1 are about 1 to 2 $\times 10^{-6}$. These lower values indicate very inefficient induction, since the spontaneous reversion frequency, already subtracted, is about 10^{-6}. Since this level of induction is similar to that produced in the th39 revertant by external ionizing radiation (*11*), it is probably due to the nonspecific ionizations of the β-particle released during the disintegration (*2*). Hence decays of thymidine-(methyl)-^{3}H do not cause T → C transitions nor do decays of cytosine-6-^{3}H cause C → T transitions with a detectable frequency due to molecular rearrangement at the site of the decay. Production of transitions by a molecular rearrangement of the parent molecule with an efficiency of about 1 percent that observed for C → T transitions from decays of cytosine-5-^{3}H would have been detectable.

The efficiency with which cytosine-5-^{3}H produces, per decay, transitions of C → T can be estimated by com-

paring the maximum expected reversion frequency with the experimental value. In calculating the maximum possible reversion frequency, we assumed that the transition to thymidine of only one unique cytosine residue in the H_l1 genome could produce a revertant phage, and that the number of cytosines per phage DNA is about 1080 as it is for the closely related phage øX174 (*10*). The probability that any one of a set of randomly occurring disintegrations will occur in the cytosine residue for which reversion can occur is then 1/1080 or 9×10^{-4}. Hence 9×10^{-4} is the reversion frequency one would expect if every cytosine-5-^{3}H disintegration produced a C → T transition. The average observed value of k is 8×10^{-4}, indicating that the probability of producing a C → T transition per cytosine-5-^{3}H decay is about 0.8. This efficiency would be reduced if the assumptions were not valid; for example, if there were two or more cytosine residues per genome at which reversion could occur.

Two temperature-sensitive mutants of øX174 which revert by C → T transitions give reversion frequencies of about

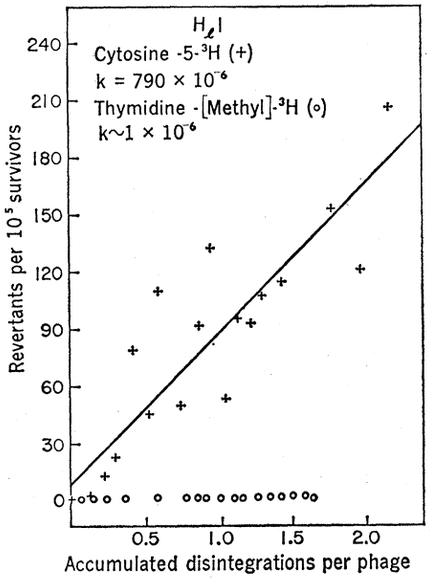

Fig. 3. Rate of production of revertants of H_l1 resulting from decay of cytosine-5-^{3}H (+) and thymidine-(methyl)-^{3}H (○). Revertants of H_l1, an inverted host range mutant, were assayed on a mixture of *Escherichia coli* S26 and *E. coli* C (1:1) to allow expression of the DNA mutations. Since this procedure allows a significant number of spontaneous revertants to be formed on the plate, the equivalent number of plaque-forming units were always seeded on the control plates (*8*). The th39 revertants were assayed on *E. coli* C at 42°C.

Table 1. Summary of the killing efficiencies (α) and mutagen frequencies (k). All values shown for th39 were obtained from values of decays per phage per unit time calculated from the specific activity of the medium; α represents the mean of the number of lethal hits per decay. All curves for the determination of values for k and α were fitted to the data by the method of least squares; k represents the number of revertants per survivor per decay. The precursor, uracil-6-^{3}H actually used in the experiments labeled as cytosine-6-^{3}H, is incorporated into the S13 DNA as cytosine-6-^{3}H.

Tritium precursors	H_l1 (C → T)		th39 (T → C)	
	α	$(10^{-6} \times k)$	α	$(10^{-6} \times k)$
Cytidine-5-^{3}H	0.52	750		
Cytosine-5-^{3}H	0.31	770		3.0
	0.35	790		
	0.33	930		
Cytosine-6-^{3}H	0.65	2.2	0.78	2.1
	0.53	1.3	0.79	1.7
Thymidine-^{3}H	0.38	0.8	0.43	1.3
			0.50	1.3
			0.49	1.2
			0.48	0.9

2×10^{-4} for decays of cytosine-5-^{3}H (*12*). The difference between the reversion frequencies for S13 and øX174 remains unexplained, but for either case the probability of producing a C → T transition per decay is very high.

The observed values of α were from 0.3 to 0.8 (*13*). The values for th39 were uniformly larger than those for H_l1, which may reflect the different methods used to determine the number of accumulated disintegrations per phage for the two mutants. Since most of the curves were not followed to low survival values, no conclusion as to the relative efficiencies of inactivation per decay for different precursors can be drawn. For T4 phage 5-hydroxymethylcytosine-6-^{3}H and thymidine-(methyl)-^{3}H decays produce equivalent loss of plaque-forming ability (*9*). It is doubtful if the occurrence of C → T transitions, even at an efficiency of one per decay of cytosine-5-^{3}H, would produce a detectable increase in the killing efficiency since C → T transitions lead mainly to codons specifying either the same amino acid or an amino acid which is chemically and physically similar to the original (*14*).

This mutagen has the unique advantage of being both specific in its effect and in the localization of the effect. Only those cytidines in the DNA in which a disintegration has occurred will undergo a C → T transition.

The localized effect of the decay of cytosine-5-^{3}H is especially useful where one is studying the effects of foreign DNA introduced into the cell as in a phage infection. In these cases DNA newly labeled with cytosine-5-^{3}H can be introduced, and at some later time the complex can be frozen to allow the mutagenic decays to occur. This procedure effectively mutagenizes a highly selected fraction of the cellular DNA at a specific period of development, a result not now feasible by any other method.

Accidental ingestion by laboratory personnel of large quantities of cytosine-5-^{3}H could be a health hazard, as with any mutagen. Hence, in those cases where the location of the tritium atom at position 5 is not essential, routine use of cytosine-^{3}H and uracil-^{3}H should be restricted to cytosine-6-^{3}H and uracil-6-^{3}H.

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1. The number refers to the carbon atom on the pyrimidine ring to which the tritium atom is bonded, in this case the number 5 carbon atom. The abbreviations used are C, cytosine; T, thymine; tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.
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13. By reference to earlier work (*9*), we have estimated that the killing efficiency of S13 due to the nonspecific ionizing effects of the tritium β-particles that accompany decay should be about 0.5.
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