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Incorporation of Uridine into *Bacillus subtilis* and SPP1 Bacteriophage Deoxyribonucleic Acid

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Tritiated uridine is incorporated into the deoxyribonucleic acid of *Bacillus subtilis* and bacteriophage SPP1; the tritium is recovered in the cytidine moiety of both deoxyribonucleic acids.

In an attempt to study ribonucleic acid (RNA) synthesis in vivo, after infection of *Bacillus subtilis* by the deoxyribonucleic acid (DNA) phage SPP1, it was found that uridine-*5-*^3H was recovered in the mature phages and that the tritium was associated with the nucleic acid. The experiments presented here show that ^{3}H-uridine is not only incorporated into the DNA of the phage chromosome but also into that of *B. subtilis*. Since this conclusion was in disagreement with what was found in a previous report from this laboratory (1), it was decided to further investigate the phenomenon, and the conclusion was that the tritium from uridine could be recovered in the cytidine moiety from both SPP1 and *B. subtilis* DNA species. In the case of the bacterial DNA this conclusion is also true when the bacteria were grown in minimal medium.

The bacterial strain used was *B. subtilis* strain SB 1051 *arg*$^-$, a derivative of the indole-requiring strain 168 M. The growth of SPP1 phage and the extraction of its DNA were done as described by Riva et al. (8).

Uridine-*5-*^3H (8 c/mmole) and thymidine-methyl-^{14}C (52 mc/mmole) were from Schwarz BioResearch Inc., Orangeburg, N.Y. The uridine had a radiochemical purity greater than 97% and the 3% impurity was not due to a contamination of ^{3}H-cytidine.

^{3}H-uridine (15 μc/ml) and ^{14}C-thymidine (0.35 μc/ml) were added to the bacterial suspension at the time of infection with SPP1 in the complex medium PS (8); the Klett reading was 145 at 540 nm.

After 2.5 hr at 37 C, the culture was completely lysed (Klett reading of 30). When SPP1 was produced in a small amount and purified in CsCl gradient, two bands of phage were recovered. The less dense band was stable and infective, whereas the denser one was unstable and not infective. The DNA species extracted from both bands had, on the other hand, the same densities in CsCl gradient. The same was true when the densities of the separated strands were analyzed. The phage DNA species from both bands were recovered labeled with ^{3}H and ^{14}C, and a mixture of the two DNA species was used in the experiments reported here.

The tritium (from ^{3}H-uridine) associated with the DNA was shown to be an integral part of the DNA molecule by following its fate after enzymatic and chemical treatments of the DNA. The doubly labeled DNA was treated with 10 μg/ml of deoxyribonuclease or ribonuclease at 37 C, in 10^{-2} M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (*p*H 7.2), containing 10^{-2} M NaCl and 10^{-2} M $MgCl_2$. The enzymatic digestion was recorded by following the acid-insoluble material, and it was found that both ^{3}H and ^{14}C counts were hydrolyzed by deoxyribonuclease, but were insensitive to ribonuclease. Electrophoretically purified deoxyribonuclease and ribonuclease crystallized three times were from Worthington Biochemical Corp., Freehold, N. J. and were assayed for their specificity.

The possibility of having small (ribonuclease-resistant) RNA fragments hybridized with the DNA was excluded by treating the DNA with 0.5 N NaOH for 15 min at 25 C and by analyzing it in alkaline (10^{-2} M NaOH) sucrose gradient. In this case also, ^{3}H and ^{14}C were both found to be alkali-resistant.

To decide whether the incorporation of ^{3}H-uridine into the phage DNA was a property coded in the phage genome or present also in the host, the recipient bacterial strain SB 1051 was grown for 3 hr (up to a Klett reading of 210 at 540 nm) in a medium of the same composition as that used for the phage preparation and in the

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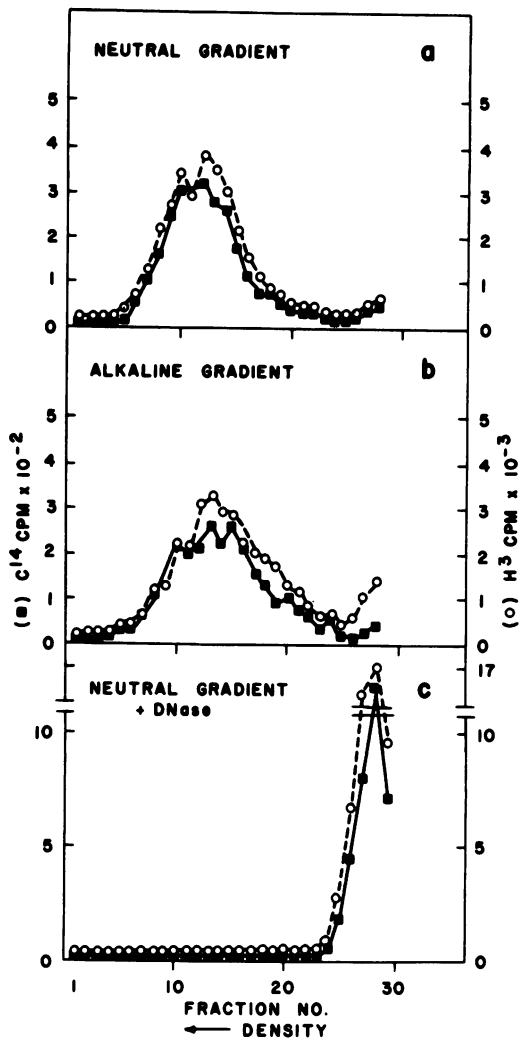

FIG. 1. *Fate of ^3H and ^{14}C after enzymatic and chemical treatment of Bacillus subtilis DNA. Cell growth and DNA preparation were carried out as described in the text. (a) A 2.5-μg amount of DNA was layered on 3.5 ml of 5 to 20% (wt/vol) sucrose gradient made in 10^{-2} M Tris-hydrochloride (pH 7.4), 10^{-3} M EDTA, and 0.8 M NaCl and centrifuged for 2.5 hr at 45,000 rev/min at 15 C in the SB 405 rotor of an International B60 ultracentrifuge. (b) Same as in 1a but with the DNA treated for 15 min at 25 C in 0.5 N NaOH; the sucrose gradient contained in addition 10^{-2} M NaOH. (c) Same as in 1a, but with 20 μg of deoxyribonuclease. For the analysis of the gradients, the bottom of the tube was pierced and eight-drop (approximately 0.12 ml) fractions were collected directly on glass filters. The filters were dried and counted in a scintillation spectrometer. The total counts/min of (○) ^3H and (□) ^{14}C recovered per fraction are reported.*

presence of the same amount of ^3H-uridine and ^{14}C-thymidine. The DNA, extracted by the Marmur procedure (7), was found to be labeled with tritium, and the specific activity was similar to that of the phage DNA. In this case, the bacterial DNA was also treated with deoxyribonuclease or alkali, and it was found that ^3H and ^{14}C were both deoxyribonuclease-sensitive and alkali- or ribonuclease-resistant (Fig. 1).

These results were obtained by growing the bacteria in a complex medium. In a previous report (1), in which minimal medium was used, the conclusion was that uridine was not utilized by *B. subtilis* for DNA synthesis. To decide whether the discrepancy observed was related to the different medium used, *B. subtilis* SB 1051 was grown for 3.5 hr (up to a Klett reading of 168 at 540 nm) in Spizizen minimal medium (9) supplemented with 0.5% glucose, 50 μg/ml of amino acid mixture, 5 μg/ml of uridine-*5-^3H* (3.3 μc/ml), and 1.35 μg/ml of ^{14}C-thymidine (0.063 μc/ml). When the DNA from such cells

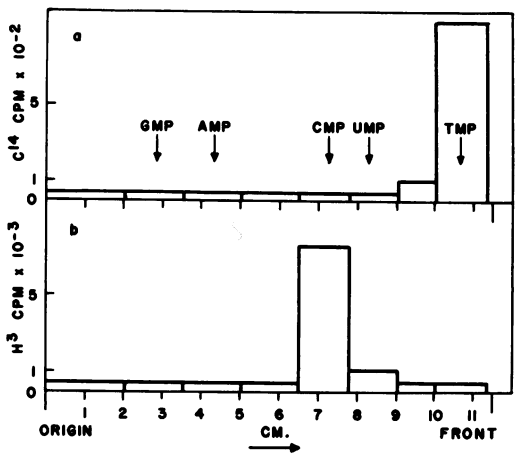

FIG. 2. *Chromatography of ^3H-^{14}C DNA hydrolysate from SPP1 or SB 1051 DNA. About 80 nmoles of hydrolyzed DNA were spotted in a 20 μliter sample at the origin of a precoated (20 by 20 cm) plastic foil (MN Polygram 300 Cellulose made by Brinkman) and the solvent used was made of isopropanol, HCl, water (65:16.7:18.3; vol/vol). 5'-Mononucleotides were used as standards. After approximately 5 hr, the chromatogram was dried, and the position of the ultraviolet-absorbing material was marked. The strips of the chromatogram containing the label were cut into small pieces and distributed in vials containing the scintillation fluid. Each sample was directly counted on a Tri-Carb scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). Figures 2a and 2b are, respectively, the distribution of ^{14}C and ^3H, along the chromatogram, of the hydrolysate of phage or bacterial DNA prepared as described in the text.*

TABLE 1. *Specific activities of phage and bacterial deoxyribonucleic acid (DNA)*

DNA	^{3}H-Cytidine	^{14}C-Thymidine
Phage[a] SPP1[b]	3,902	399
Bacillus subtilis SB 1051[b]	4,242	432
B. subtilis SB 1051[c]	5,886	153

[a] Values shown refer to the mixture of DNA species from the two phage bands recovered during purification in CsCl gradient. Expressed in counts per microgram of DNA.

[b] Grown in infection medium in the presence of 15 μc/ml of ^{3}H-uridine and 0.35 μc/ml of ^{14}C-thymidine.

[c] Grown in minimal medium in the presence of 5 μg/ml of ^{3}H-uridine (3.3 μc/ml) and 1.35 μg/ml of ^{14}C-thymidine (0.063 μc/ml).

was extracted and analyzed, it was again found that the tritium from the uridine was recovered as integral part of the bacterial DNA. These data indicate that the ability to utilize uridine for DNA synthesis is coded by the bacterial genome and that this property is not dependent on some factor present in the complex medium used for phage infection.

The DNA species from the above preparations were hydrolyzed, by using deoxyribonuclease and snake venom phosphodiesterase, down to 5'-mononucleotides as described by Lehman et al. (4), and the hydrolysate was chromatographed on thin layer chromatography. It was found that all the tritium was localized in the cytidine moiety of the DNA (Fig. 2).

In Table 1 are summarized the specific activities of the DNA species from the various preparations. When minimal medium was used, it was possible to compare the efficiency of incorporation of ^{3}H-uridine with that of thymidine. The ratio of ^{3}H to ^{14}C (counts/min) in the medium was about 8, whereas that recovered in the DNA was about 38. This means that uridine was incorporated with an efficiency four- to fivefold higher than was thymidine. The limited incorporation of thymidine could be explained by its rapid conversion to thymine by thymidine phosphorylase (1). The data presented indicate that, in *B. subtilis*, a biosynthetic pathway for the transformation of uridine monophosphate to deoxycytidine triphosphate might exist similar to that described in *Escherichia coli* (2, 3, 5, 6, 10) and that it functions at a level sufficient to interfere with the use of uridine as a specific label for RNA in experiments in which the DNA is not eliminated.

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