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Fate of Transforming Deoxyribonucleic Acid After Uptake by Competent *Bacillus subtilis*: Nonrequirement of Deoxyribonucleic Acid Replication for Uptake and Integration of Transforming Deoxyribonucleic Acid

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Studies on transformation of *Bacillus subtilis* using the inhibitor 6-(*p*-hydroxyphenylazo)-uracil show that deoxyribonucleic acid (DNA) replication is not required for the uptake and integration of donor DNA and genetic markers.

It has been proposed that deoxyribonucleic acid (DNA) replication may be required for transformation in *Bacillus subtillis*. One model suggests that integration occurs at the replication point and that uptake proceeds concomitantly with integration, the process presumably being driven by the movement of the growing point relative to the membrane entry sites for donor DNA (9, 10). This notion is contradicted by the low level of DNA synthesis in competent and newly transformed *B. subtilis* cells (5, 10, 14) and by the results of Bodmer (1, 2) using 5-bromouracil incorporation and buoyant density analysis. We have approached the problem by determining the effect of 6-(*p*-hydroxyphenylazo)-uracil (HPU) on the rate and extent of DNA and genetic marker integration during transformation of *B. subtilis*. This inhibitor has been shown to specifically and reversibly inhibit DNA replication in *B. subtilis* but to have little effect on repair synthesis following ultraviolet irradiation (3).

Figure 1b shows that HPU (300 μM) inhibits incorporation of ^{3}H-thymidine into both the competent and noncompetent fractions of *B. subtilis* BD170 (*trp-2 thr-5*). (Some residual HPU-resistant incorporation occurs in both fractions, but at levels below 0.5% of the control.) Figure 1a demonstrates that the noncompetent fraction is far more active in DNA synthesis than is the competent fraction, in accord with the results of other investigators (5, 10). Figure 2a shows the absence of any detectable effect of HPU on ^{3}H-BD204 DNA uptake by a competent culture. A portion of the same culture was incubated with ^{14}C-thymidine, with and without inhibitor, and HPU was found to reduce the incorporation rate markedly (2b). Samples of the incubation mixtures

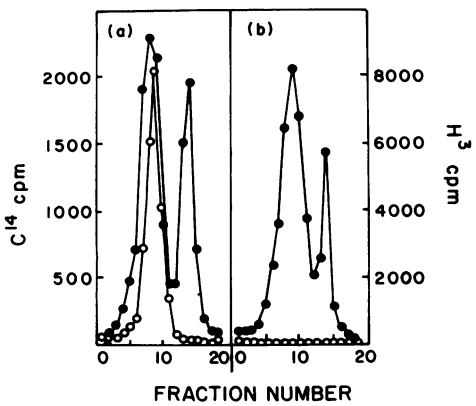

FIG. 1. *Incorporation of ^{3}H-thymidine into competent ^{14}C-thymine-labeled B. subtilis BD170 in absence (a) and presence (b) of 300 μM HPU. Competent BD170 cultures grown in the presence of ^{14}C-2-thymine (10μCi/ml), 30 μg of thymine per ml, and 200 μg of deoxyadenosine per ml were incubated for 30 min at 37 C with B. subtilis transforming DNA (5 μg/ml), deoxyadenosine (250 μg/ml), and ^{3}H-thymidine (20 μCi/ml, with a specific activity of 60 Ci/mol). The samples were layered on linear gradients of renograffin-76 (η = 1.360 to 1.390) and centrifuged for 20 min at 20 C in an SW27 rotor (4, 12). Samples of 0.75 ml were collected from the bottom of the tubes, and the trichloroacetic acid-precipitable ^{14}C (●) and ^{3}H (○) in each fraction was determined. The smaller light bands in (a) and (b) contained about 90% of the total Trp$^+$ transformants.*

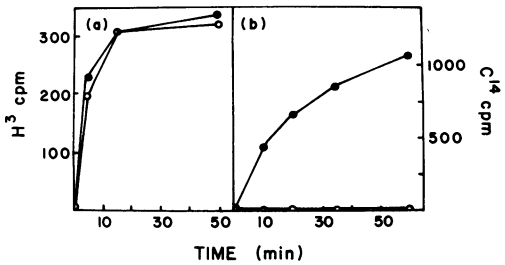

FIG. 2. *Effect of HPU (300 μM) on uptake of ^{3}H-thymidine-labeled BD204 DNA (a) and on incorporation of ^{14}C-thymine (b). Competent B. subtilis BD170 was incubated at 37 C with radioactive transforming DNA (1 μg/ml), and uptake was determined on washed samples as described previously (6, 7, 8). Another sample of the same culture was incubated with transforming DNA (5 μg/ml), deoxyadenosine (250 μg/ml), and ^{14}C-2-thymine (0.77 μCi/ml, with a specific activity of 56 mCi/mmol). Samples were removed and counted for trichloroacetic acid-precipitable counts per minute. Symbols: ●, no HPU; ○, plus HPU.*

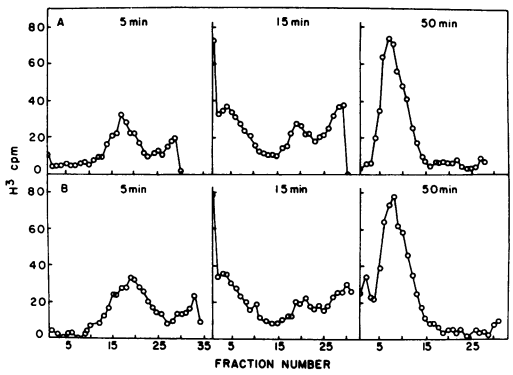

FIG. 3. *Sucrose gradient centrifugation analysis of lysates prepared from competent BD170 cultures at various times during incubation with ^{3}H-thymidine-labeled BD204 transforming DNA in the presence (A) and absence (B) of HPU (300 μM). The samples were layered on 4.2-ml linear gradients of 5 to 20% sucrose, resting on 0.8-ml 60% sucrose cushions. Centrifugation in an SW50.1 rotor was at 20 C for 105 min at 44,000 rpm.*

described in Fig. 2a were removed at 5, 15, and 50 min and washed. The lysates were prepared as described previously (6) and were analyzed for the presence of donor-recipient complex (DRC) and double-strand fragments by sedimentation through sucrose gradients (6, 7, 8). In addition, the lysates were assayed for donor (Trp^+), recombinant (Trp^+, His^+) and recipient (His^+) transforming activity by using BD55 (*trp-2 hisB2*) as the recipient strain. Figure 3 shows that the sucrose gradient profiles are very similar for lysates prepared with or without prior treatment with HPU. DRC (fractions 1 to 15) is barely present at 5 min and accumulates with time. Double-strand fragments (fractions 11 to 25), precursors of DRC (7; Davidoff-Abelson and Dubnau, manuscript in preparation), are the major components at 5 min and then decrease in amount. In addition, a characteristic peak at the meniscus consisting of single-strand fragments and acid-soluble products is present (6). Table 1 shows that HPU also has no significant effect on the extent or rate of donor marker recovery or on the appearance of the recombinant marker configuration. We conclude that DNA replication is not required for the uptake and integration of transforming DNA. It is possible that a small amount of localized HPU-resistant DNA synthesis is required for integration. The present data exclude models which involve successive integration events at the growing point as it sweeps continuously through the genome (9, 10). This conclusion is strengthened by the well-established observation that integration is virtually complete by 30 min at 37 C (7, 8, 11, 15) and that massive replacement of recipient by donor DNA occurs under the conditions used in the present experiments (7). Our interpretation is also in agreement with the results of Bodmer (1, 2).

Bodmer (1, 2) has suggested that integration

TABLE 1. *Transforming activity in lysates from transformed cultures: effect of HPU*

Time of incubation (min)	− HPU				+ HPU			
	Trp^+/ml	Trp^+His^+/ml	Trp^+/His^+	Trp^+His^+/His^+	Trp^+/ml	Trp^+His^+/ml	Trp^+/His^+	Trp^+His^+/His^+
5	3.40×10^3	25	2.40×10^{-4}	1.76×10^{-6}	1.55×10^3	30	5.46×10^{-5}	1.06×10^{-6}
15	9.50×10^3	1.66×10^3	3.56×10^{-4}	6.22×10^{-5}	7.00×10^3	1.15×10^3	2.95×10^{-4}	4.83×10^{-5}
50	6.47×10^4	1.63×10^4	4.98×10^{-3}	1.25×10^{-3}	3.63×10^4	8.5×10^3	5.33×10^{-3}	1.25×10^{-3}

occurs at a stationary replication point. This attractive model, which is consistent with the present data, was rendered unlikely by the later density transfer experiments of Laird, Wang, and Bodmer (13). The latter authors, however, suggested that the integration sites may become the points of initiation for post-recombinational replication. This possibility is under investigation.

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