PENETRATION OF THYMINE-STARVED BACTERIAL DNA DURING TRANSFORMATION OF B. subtilis 168 T⁻

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Summary

The decrease of transforming activity of bacterial DNA after thymine starvation is not due to an abnormal penetration but, more likely, to a failure in genetic recombination after uptake.

In the absence of thymine, cell viability of all thymine requiring mutants decreases. The occurence of episome induction has been already demonstrated as an important factor of lethality but the base line lethal effect which is always observed even in non episomic strains is still obscure (1-2). It seems however that DNA is altered. In previous experiments we have shown that there is a loss in the transforming activity of the DNA of bacteria undergoing thymineless death (3).

This inactivation could result from a difference in the uptake or a discrimination after uptake of the DNA particules into the recipient cells. We have therefore studied the penetration during transformation of radioactive DNA derived from starved and unstarved cultures of Bacillus subtilis 168 T⁻.

No differences are observed in the amount of radioactive material which penetrates into the recipient cells. Although DNA from starved cells has a transforming activity reduced to less than a half of its initial value, its uptake is the same as for DNA from unstarved cells.

EXPERIMENTAL

a) Preparation of radioactive DNA from starved and unstarved cultures

B. subtilis 168 T⁻ strain is inoculated into 40 ml of minimal medium

containing 1 mc of ^{3}H thymidine(specific activity 3000 mc/mM). Cells grown exponentially (10^{8} cells/ml) are centrifuged, washed and one part is resuspended into the same medium without thymidine. Samples are taken at different times to determine the fraction of cells surviving thymine starvation. After 3 hours of incubation the cells are collected, washed, lysed and used without further treatment or treated with phenol in the presence of para aminosalicylate according to a modification of Kirby's method (4) to extract purified DNA. Lysates are precipitated by alcohol, centrifuged and the pellet is resuspended in Saline citrate solution (0.15 M NaCl, 0.015 M trisodium citrate pH = 7).

b) Transformation procedure and uptake measurement.

The radioactive material is used for the transformation of a tyrosine deficient strain. The method has already been described (4).

After 45 mn contact of donor DNA (0.10 to 0.15 µg/ml) with competent cells, samples are taken to determine the number of tyr^{+} recombinants and calculate the transforming activity of the DNA. Then DNase is added to the culture and after 5 mn incubation, the cells are collected on a millipore membrane. The filter is washed, dried and the retained radioactivity measured in a scintillating counter. The radioactivity of the washing fluid are also estimated to calculate the input radioactivity of the donor DNA. The uptake is given by the ratio of the amount of radioactivity retained on the filter to the input radioactivity.

RESULTS AND DISCUSSION

The viability of a culture of *B. subtilis* 168 T^{-} is reduced to 5 to 15.% after 3 hours of thymine starvation.

DNA preparations, crude or purified, were made from 3 hours starved and unstarved cells previously grown in radioactive thymidine. The two batches are used to transform a tyrosine strain in order to evaluate their transforming activity. The amount of radioactivity retained by recipient cells is taken as a measure for the penetration of the radioactive donor DNA. The results are given in the table.

The biological activity of transforming DNA decreases during thymine starvation while the penetration remains almost constant (Part a of the table). On the other hand, when DNA is purified by phenol treatment, its transforming activity is quite similar irrespective of being isolated from starved or unstarved cultures (Part b of the table).

It looks as if the inactive DNA was discarded by purification procedure. This can explain why physico-chemical properties of DNA from starved cells seem unaltered (5-6-7-8) while sometimes some differences are found (9-10) which disappear

a) DNA from lysates : for each experiment duplicates are made

	Source of DNA	dpm's retained on filter	transformation % ($\times 10^{-2}$)	Transf. efficiency: trans. % / dpm's retained on filter ($\times 10^{-6}$)	Uptake: dpm's retained on filter / input dpm's ($\times 10^{-6}$)
experiment 1	+	459	0,43	9,36	0,54
	+	633	0,53	8,37	0,42
	-	506	0,13	2,56	0,40
	-	510	0,14	2,74	0,42
experiment 2	+	7006	1,81	2,58	1,33
	+	9556	1,70	1,77	1,86
	-	13132	1,70	1,29	1,50
	-	9616	0,92	0,96	1,28

b) Purified DNA

		($\times 10^{-3}$)	($\times 10^{-6}$)	($\times 10^{-2}$)
+	604	0,33	0,54	1,4
-	604	0,3	0,5	1,3

TRANSFORMING EFFICIENCY AND UPTAKE OF DNA FROM CULTURES OF B.subtilis 168 T^- GROWN WITH (+) OR WITHOUT (-) THYMINE

after purification of the DNA (11-12).

We have already shown that the transforming activity decreases after thymine starvation, using crude preparations (3). The results of the experiments presented here suggest that this is not due to a decrease in uptake of the DNA but to events occuring after uptake.

Okamoto et al mentioned that 15 to 20 % of the host genome can be incorporated into phage particules when DNA synthesis is inhibited by mitomycine (13). By thymine starvation (14-15-1-16) or after addition of mitomycine (17), phages are induced and it is possible that the decrease in transforming activity should be partial ly related to the conversion of bacterial DNA into phage DNA. However this could not easily explain the loss of 90 % of transforming activity obtained after thymine starvation (3).

Still, it has not yet been possible to eliminate the role of prophage induction in studying biological activity of DNA during thymineless death.

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