SEQUENTIAL REPLICATION OF DNA IN SYNCHRONOUSLY GERMINATING *BACILLUS SUBTILIS* SPORES*

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Cairns (1963) has shown by autoradiography that when the *E. coli* chromosome, consisting of a single piece of DNA, replicates it does so by forming a "fork" which then moves along its length until the entire structure is doubled. Furthermore the results of Nagata (1963) and Yoshikawa and Sueoka (1963) show that in *E. coli* K12(Hfr)and *B. subtilis* the starting point of replication must always be at the same position, with replication then proceeding in always the same direction along the length of the chromosome.

This report describes the change in relative frequency of two unlinked genetic markers during one replication of the DNA in synchronously germinating *B. subtilis* spores. The results provide independent evidence for the general scheme suggested above.

The synchrony in chromosome replication which accompanies emergence of the vegetative cell makes the germinating spore an attractive system for studying the events leading up to the initiation of DNA replication in bacteria.

EXPERIMENTAL

The *B. subtilis* strains, SB19 (prototroph) and SB26 (ind^- $meth^-$) were obtained from the Genetics Department, Stanford

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University School of Medicine, Palo Alto, Calif. U.S.A. The indol and methionine markers are unlinked (Nester and Lederberg, 1961).

Highly refractile SB19 spores were prepared by growth at 30° in an effectively aerated medium similar to that described by Stewart and Halvorson (1953). After the final washing they were heat activated at 65° for 2½ hours. The final preparation contained less than 1% dark spores.

Germination of the spores was carried out by aeration at 30° in the medium described by Woese and Forro (1960). Samples were taken at various times and heated immediately at 60° for 10 min. DNA was estimated in a manner similar to that described by Woese and Forro (1960). Lysates for transformation assays were prepared by the method of Marmur (1961). After one deproteinization the lysates were dialyzed against the standard saline-citrate. Transformation assays were done by a modification of the procedure of Anagnostopoulos and Spizizen (1961) using SB26 as the recipient cell.

RESULTS

The synchrony of germination is considerably better than that of Woese and Forro (1960). Using the phase-contrast microscope 90% of the spores are dark after 15 min (this is reduced to 10 min at 37°). The cells emerge at 160 - 170 min, but the division septum is not clearly visible until 360 min.

Fig. 1a shows the course of DNA synthesis. There is no DNA synthesis during the first 160 min. It then starts abruptly at approximately the same time as the emergence of the cell, and it takes about 90 min for the DNA to double. The overall result is similar to that of Woese and Forro (1960). Under standard germination conditions, using the same batch of spores, the time of emergence of the cells can be reproduced to within 10 min.

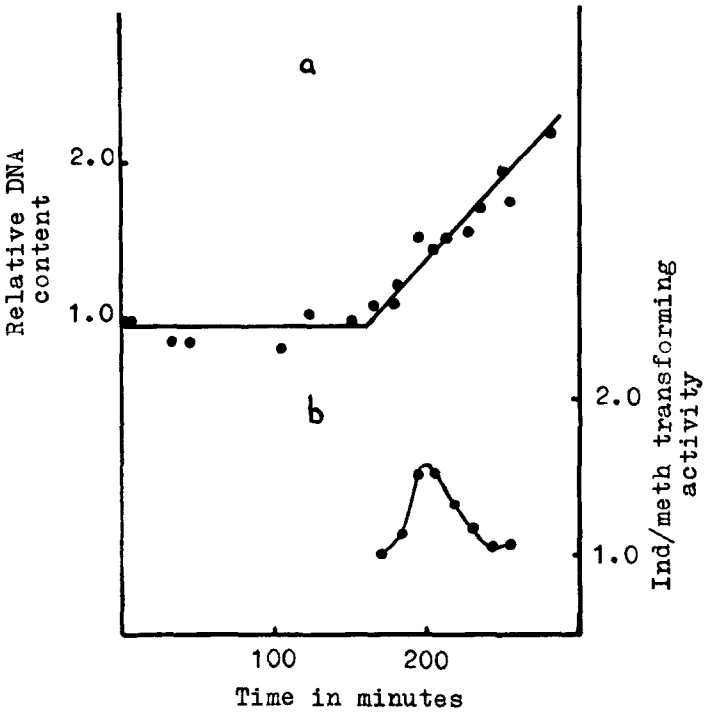

Fig. 1.a. Relative DNA content as a function of time in the germinating B. subtilis spore suspension.

b. Ind/meth transformation ratios in the DNA lysates obtained at various times from the germinating spore suspension. For analysis the lysates, containing approx. 10 ug DNA/ml, were diluted 1:1000 into the recipient cell suspension (2×10^8 cells/ml). An experiment using a range of dilutions of the lysate showed a linear relationship between the number of transformed cells and the total amount of lysate in this dilution range. The ind/meth ratio for the first sample has been set at 1.0 and subsequent ratios have been normalized to this.

In a separate experiment samples were taken at eight 12 min intervals beginning at 170 min. Over this period the DNA would have doubled. Fig. 1b shows the ratio of transforming activities for the indole and methionine markers in the DNA lysates. Clearly, the indole marker is copied before the methionine marker.

Assuming that there is only one replicating "fork" per chromosome as in E. coli (Cairns, 1963) these results clearly show that DNA replication must always start at the same fixed point on the bacterial chromosome and then proceed in only one and always the same direction. With an "open" chromosome the starting point of replication would probably be at one end.

Obviously the indole marker is closer to the starting point of replication than the methionine marker. This is the same as shown on the genetic map of Yoshikawa and Sueoka (1963) who used a different mutant. The ratio of activity of the two markers should reach 2.0 if one were completely copied before the other. Experimentally, this will depend upon the relationship between the degree of synchrony and the chromosomal distance separating the markers. If the markers are separated by more than 50% of the chromosomal length as in Yoshikawa and Sueoka's case it is obvious that the synchrony here could be improved considerably. As well, further work is needed to fix more accurately the time of initiation of DNA replication. It will then be possible to obtain an exact correlation between the amount of DNA replicated and the doubling of a marker.

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