JOURNAL OF BACTERIOLOGY, July 1976, p. 433-439

Vol. 127, No. 1
Printed in U.S.A.

Segregation of *Bacillus subtilis* Chromosomes Radioactively Labeled During the First Round of Replication After Germination of Spores

R. G. WAKE

Biochemistry Department, University of Sidney, Sydney, N.S.W. 2006, Australia

Received for publication 5 April 1976

Spores of *Bacillus subtilis* W23 *thy his* were allowed to incorporate [^{3}H]thymine for short periods of time either continuously from, or soon after, the start of the first round of replication after germination. They were then transferred to nonradioactive medium to allow growth into microcolonies (up to 12 cells), which were autoradiographed. The relative numbers of various types (major versus minor) of grain clusters associated with individual microcolonies throughout the populations were scored. Analysis of the results showed clearly that, in the majority of spores at least, only one chromosome was undergoing replication soon after the start of deoxyribonucleic acid synthesis. Furthermore, under the conditions used, no evidence for initiation of replication of a second chromosome within 25 min after the first could be obtained. Accepting that *B. subtilis* spores are essentially homogenous in deoxyribonucleic acid content, the results support the conclusion that the spore contains only one copy of the chromosome, not two.

It has been established that the *Bacillus subtilis* spore chromosome, which is circular and accounts for the whole genome (8, 14), is completed in its replication (2, 10). In addition, there is homogeneity in individual spore deoxyribonucleic acid (DNA) content (3), but there is uncertainty as to the number of completed chromosomes present in each spore.

On the basis of early X-ray sensitivity studies, it was suggested that spores of *B. subtilis*, in contrast to some other *Bacillus* species, contain only one copy of the chromosome (15). Supporting evidence for such a conclusion was provided by a comparison of the spore DNA contents of several *Bacillus* species (5).

Since these earlier studies, a number of autoradiographic investigations into the segregation of [^{3}H]thymine-labeled chromosomes after germination of *B. subtilis* spores have been made. Accepting that the spore chromosome is completed in its replication and that there is homogeneity in individual spore DNA content, the following opposite conclusions can be drawn: (i) the spore contains one copy of the chromosome (4, 6, 12); (ii) the spore contains two copies of the chromosome (16).

This paper describes the results of further autoradiographic experiments aimed at distinguishing between the possibilities of one and two copies of the chromosome per spore.

If the single polydeoxynucleotide chains of the *B. subtilis* chromosome were conserved intact from one generation to the next, the chromosome content of the spore could be established readily by the number of such chains segregating from one another during growth into a microcolony. Unfortunately, the interpretation of the results of autoradiographic studies of this type, using [^{3}H]thymine-labeled spores, has been rendered difficult because of "fragmentation" of the labeled chains, resulting in relatively large numbers of grain clusters (seven to ten) in some colonies after a few generations (1, 6, 12, 16). It is almost certain that the difficulty in assessing the extent of such fragmentation has been the major factor leading to the different conclusions with respect to the number of copies of the chromosome (one versus two) in the spore.

In the present work an attempt to overcome this problem has been made by labeling spore chromosomes with [^{3}H]thymine as they undergo the first round of replication after germination over only a minor portion of their length. Fragmentation is probably the result of some recombinational process, and the likelihood of it occurring within a restricted region of the chromosome would be less. Partial labeling of the chromosome has been achieved in two ways: continuously from the start of the first round of replication, and by pulse labeling during the first round. Replication of the circular chromosome is bidirectional (14) and, in the case of continuous labeling, each of the two

daughters from a parent chromosome would contain a single region of [^{3}H]thymine covering the origin of replication. If the labeled region was short, the chances of it remaining intact through subsequent cell divisions would be improved. Pulse labeling, on the other hand, would give rise to two such regions equidistant from and on opposite sides of the origin. The disadvantage in this situation is that the two labeled regions, if separated by a significant portion of the chromosome, could readily separate from one another during subsequent cell divisions through the fragmentation process. However, pulse labeling has the advantage of enabling easier detection of a putative second spore chromosome that might start replicating after the first (see later).

MATERIALS AND METHODS

Bacterial strains and spore preparation. *B. subtilis* strain W23 *thy his* was obtained from N. Sueoka. Spores were prepared on plates of tryptose-blood-agar base (Difco) supplemented with thymine (20 μg/ml) and agar (0.5%), as described by others (11).

Media. GM-11 is the germination medium of Sueoka and Quinn (11). Penassay agar consisted of antibiotic medium 3 (Difco) plus 1% agar.

Labeling of spores with [^{3}H]thymine during the first round of replication after germination and preparation of slides for autoradiography. In all cases, germination was at 37 C with a density of 3×10^8 to 4×10^8 spores/ml in GM-11 plus uracil (20 μg/ml). For continuous labeling from the start of germination, [*methyl*-^{3}H]thymine (20 Ci/mmol, Radiochemical Centre, Amersham) was present at 2.5 μg/ml. At appropriate times, samples (0.10 ml) were filtered (Millipore Corp., VMWP, 0.05 μm), washed with 2 to 3 ml of GM-11 plus uracil (20 μg/ml) plus thymine (100 μg/ml) at room temperature, and suspended by vortexing in the same medium. After diluting to various extents, 0.01- to 0.02-ml volumes were spread on Penassay agar plus thymine (20 μg/ml) for growth into microcolonies (four to six or six to twelve cells) at 37 C, as already described (6).

For continuous labeling after a period of thymine starvation, the germination mixture (no thymine) was diluted at the appropriate time into an equal volume of [^{3}H]thymine medium at 37 C to give a final level of [^{3}H]thymine of 1.25 μg/ml at 20 Ci/mmol. For pulse labeling during the first round of replication (no thymine starvation) the germination mixture containing unlabeled thymine (0.8 μg/ml) was diluted at the appropriate time into an equal volume of [^{3}H]thymine medium at 37 C to give a final specific activity of 15 Ci/mmol at 1.7 μg/ml. For pulse labeling after an initial period of thymine starvation, the germination mixture (no thymine) was first mixed with unlabeled thymine (0.8 to 0.9 μg/ml) at 37 C and, after a further interval, diluted with an equal volume of [^{3}H]thymine medium to give a final specific activity of 15 Ci/mmol at 1.7 μg/ml. In all cases the radioactivity was removed by filtration at the appropriate time, and microcolonies were allowed to develop as described above.

Autoradiography. This was carried out by using Koday AR10 stripping film, as described previously (6). The time of exposure varied between 3 and 8 weeks, depending upon the particular experiment.

Scoring of grain clusters and presentation of results. A more refined procedure than that used previously for scoring grain clusters (4, 6) was devised. The colonies were examined under bright-field illumination at a magnification of ×1,000 in a Zeiss photomicroscope. Grain clusters were identified and classified as major or minor according to the following rules. (i) Single grains were ignored, and when the total number of grains per colony was more than 20, groups of two grains were ignored. (Thus, for a colony containing about 15 grains, a group of two grains would be scored as a cluster but a single grain would not). (ii) Groups of two grains were ignored when the individuals were more than 1 cell width (about 1.5 μm) apart. (iii) A cluster was classified as minor when judged to contain less than half the number of grains associated with the largest cluster (a major one).

The results are presented as histograms in which the colonies are grouped into classes defined by the number of major clusters (1, 2, 3, 4) present; those containing five major clusters (very rare) are included under the ambiguous category. Each class is shown as being made up of a number of smaller ones, defined by the number of minor clusters (*0, 1, 2, 3)* in addition to the major ones. In all histograms the $2 + 0$ and $2 + 1$ classes are shaded, and the $2 + 2$ is specifically identified.

Only colonies unambiguously derived from single spores were scored. Their size (four to six or six to twelve cells) was defined by the number of cells, clearly separated by septa.

RESULTS

Continuous labeling. The conditions of germination used in all of the experiments described in this paper are the same as those of Quinn and Sueoka (11), except for differences in the levels of thymine. By using spores of a bromouracil-tolerant derivative of W23 *thy his*, these workers found that the first round of replication commenced sometime before 85 min. By 140 min the majority (more than 80%) of chromosomes had started a second round of replication.

Figure 1 shows autoradiographs of microcolonies growing out from W23 *thy his* spores which had germinated in the presence of [^{3}H]thymine for 70 min before being replaced by unlabeled thymine. All colonies shown are seen to contain two major grain clusters, and in the two at the bottom there is also a minor cluster present; i.e., they fall into either the $2 + 0$ or $2 + 1$ class. The $2 + 0$ class would have resulted from the labeling of a single replicating chromosome,

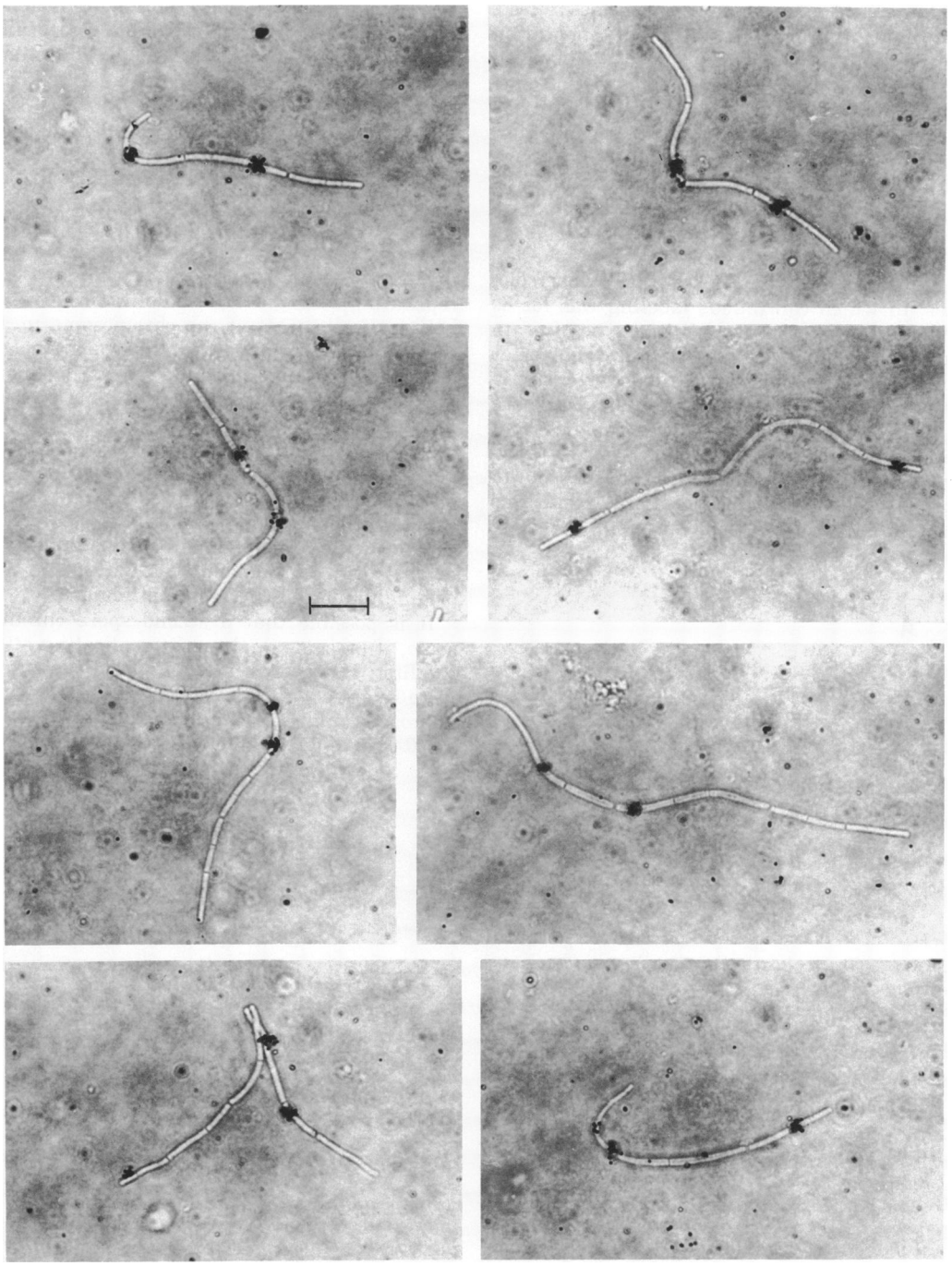

FIG. 1. *Autoradiographs of colonies developing from spores of W23 thy his germinated, from 0 min, in the presence of [^{3}H]thymine. At 70 min the radioactivity was replaced by unlabeled thymine to allow growth of the germinated spores into microcolonies. The photographs were taken under bright-field illumination; the scale shows 10 μm.*

and it is most likely that the 2 + *1* class reflects the same situation, followed by fragmentation at some later time within one of the labeled regions to yield an additional and minor cluster. The combined (2 + *0*, 2 + *1*) category has therefore been used as an estimate of the minimum number of colonies resulting from the situation in which just one chromosome was replicating during the period of radioactive labeling; and it is represented by the shaded area in the various histograms.

Figure 2 shows the results of analyzing colonies, in which continuous labeling was for 60 and 70 min, for the number and type of grain clusters present. The colonies from the shorter labeling had been allowed inadvertently to grow out to only four to six cells, and these are compared with six- to twelve-cell colonies from the longer labeling. (In the latter case, however, colonies that had grown out to only four to six cells were scored, and the overall result was very similar for the two size classes.) By 60 min, 40% of all colonies had incorporated radioactivity to the extent of having at least 15 grains (mostly more than 20). Figure 2A shows that, in this case, 50% of all colonies analyzed contain two major clusters exclusively, with 66% falling into the combined (2 + *0*, 2 + *1*) category. After 70 min of continuous labeling (Fig. 2B) there is a slight decrease, to 58%, in this combined category.

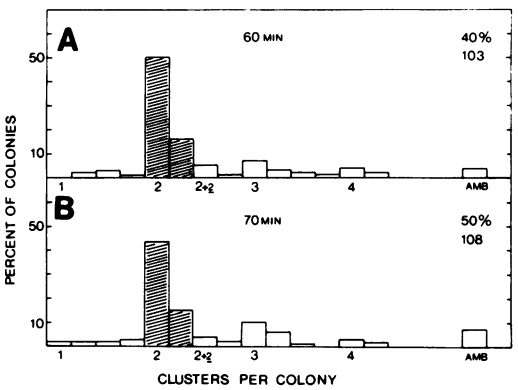

FIG. 2. *Grain cluster distribution within colonies developing from spores of W23 thy his germinated, from 0 min, in the presence of [³H]thymine. Separate samples were taken at 60 and 70 min, and in each case the radioactivity was replaced by unlabeled thymine to allow growth of the germinated spores into colonies of suitable size for examination by autoradiography. For the 60-min sample, colonies containing 15 grains or more were scored; for the 70-min sample colonies containing 20 grains or more were scored. The numbers (1 to 4) on the abscissa identify those colonies containing 1, 2, 3, or 4 major clusters exclusively. The classes immediately to the right of these numbers contain, in addition, 1, 2, or 3 minor clusters. Colonies showing an ambiguous cluster distribution are scored on the extreme right (amb). The percentages of colonies having the defined number of grains (40% or 50%) and therefore scored are shown in the top right of each panel; the actual number scored is shown immediately below this. The exposure times for the 60- and 70-min samples were 8 and 4 weeks, respectively.*

Clearly, in both cases, the majority of labeled chromosomes must have arisen from germinating spores in which only one chromosome was replicating during the period of radioactive labeling. If all copies of the chromosome in an individual spore had started replicating at exactly the same time, it would follow that there must have been only one chromosome present in the majority of spores that gave rise to the colonies analyzed. Knowing that there is homogeneity in individual spore DNA content (3), one would then have to conclude that all spores contain just one copy of the chromosome. The colonies with larger numbers of clusters would be the result of fragmentation within the two originally labeled chromosomal regions.

It is possible that a second spore chromosome, if present, might start replicating after the first. However, if the delay between starts were relatively short, the continuous labeling approach could result in colonies of the 2 + *2* or 4 + *0* type. Figure 3 (top panel) shows diagram-

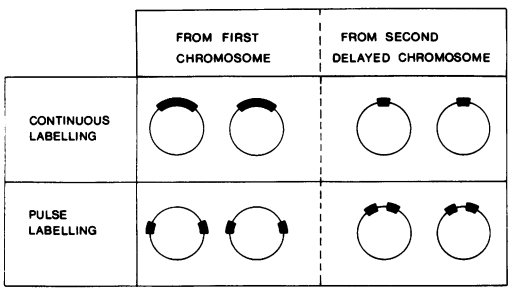

FIG. 3. *Diagrammatic representation showing expected patterns of radioactive labeling within the circular daughter DNA chains segregating intact from a parent spore chromosome after its replication in the presence of [³H]thymine for a restricted period of time during the first round of replication. The labeled regions are represented by the thick lines overlaying portions of the circles, the origin of replication being at 12 o'clock. For continuous labeling from the start of the first round (top panel), one would expect a single-labeled region, covering a continuous portion of each circle and including the origin. In the case of pulse labeling (lower panel), one would expect to have, in each circle, two labeled regions separated from one another and on opposite sides of the origin.*

matically how the 2 + 2 pattern could arise. It is significant that the percentages of the 2 + 2 and 4 + 0 classes of colony in Fig. 2 are small (less than 10% in both cases). Furthermore, even though 80% of the colonies analyzed at 70 min would have been actively replicating DNA at 60 min, there is no increase in either of these classes at the later time. Both these facts argue against the presence of a second chromosome starting to replicate after the first, but before 70 min.

Some further comment on the heterogeneity in numbers of grains over colonies in experiments of the type just described and possible bias in the scoring procedure is warranted. Variations in the number of silver grains can arise in two ways. (i) The asynchrony in initiation of replication will mean that individual spores will have incorporated variable amounts of radioactivity (in some cases none) by the time it is removed. This has been assumed to be the major factor resulting in the presence of colonies with no or very few grains. (ii) The Poisson nature of the distribution of silver grains produced over a radioactive source of fixed size will add to the spread in grain numbers resulting from the variable amounts of incorporated radioactivity. The scoring of colonies with a minimum number of grains ensures that, on the average, spores furthest advanced in replication at the end of labeling are included in the analysis. Also, it means that there is no bias against colonies with larger numbers of clusters because, for a fixed amount of radioactivity giving a Poisson distribution of grains, there is greater opportunity for detecting more clusters when the number of grains is higher.

Some comment on the possible origin of colonies with grain cluster distributions other than those specifically considered in the analysis is also relevant. One might wonder, for example, how a 3 + 0 type colony could arise. It may have been the result of one of two labeled regions fragmenting into two smaller ones of approximately equal size. The subjective nature of the scoring in conjunction with the expected variation in the number of grains over a radioactive source of fixed size could have resulted in such a colony classified as being of either the 1 + 2, 2 + 1, or 3 + 0 class. Alternatively, it could represent a colony containing four clusters of equal size, two of which were very close to one another. These types of uncertainty exist with respect to the origin of most of the minor classes of colony, but it is not a serious problem in drawing conclusions on the basis of the relative number of all types present.

With respect to the opportunity for chromosome segregation in the present experiments, nuclear staining showed that a six- to twelve-cell colony, the size analyzed in Fig. 2B and all subsequent experiments, contained about 20 to 40 clearly distinguishable nuclear bodies. Thus, four labeled nuclear bodies should generally separate well from one another in such a situation.

Continuous labeling after thymine starvation. The drawback in extending the time of continuous labeling in the hope of detecting a second and later replicating chromosome, if it were present, is that a longer region of the first chromosome would become labeled. The probability of fragmentation within this labeled region during subsequent divisions would then be greater, and the amounts of radioactivity in the daughters arising from each of the two chromosomes could be so disparate as to impair the sensitivity of detecting the second. The start of DNA replication can be held back, even though the requirements for initiation have been completed, by withholding thymine from the medium. Subsequent labeling with [^{3}H]thymine can then be extended to later times with less danger of encountering the problems just mentioned. Figure 4 shows the distribution of grain clusters within colonies derived from spores germinated in the absence of thymine for 60 min and then labeled with [^{3}H]thymine over the periods of 60 to 75 and 60 to 90 min. In the former case (Fig. 4A) most colonies (56%) still fall into the combined (2 + 0, 2 + 1) category. There is no significant increase in the 2 + 2 class to indicate a second spore chromosome starting to replicate during the second half of the 60- to 75-min period.

After the longer period of labeling (Fig. 4B), the 2 + 2 class increases slightly, but it is still minor at less than 10%. At least 55% of the colonies analyzed in this case would have incor-

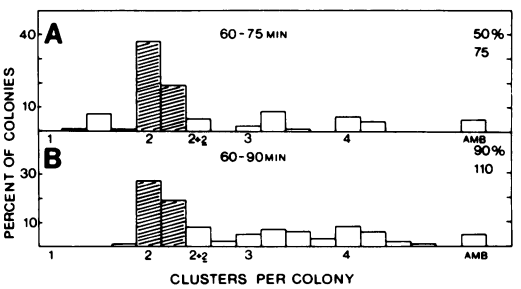

FIG. 4. *Grain cluster distribution within colonies from spores germinated in the absence of thymine for 60 min followed by labeling with [^{3}H]thymine over the periods of 60 to 75 min and 60 to 90 min. The colonies were six to twelve cells in size and those containing 15 grains or more were scored. The exposure time was 3 weeks in both cases.*

porated a significant amount of radioactivity by 75 min, and there is thus no evidence of a second spore chromosome commencing replication during the 75- to 90-min interval. There is a significant increase in the percentage of colonies with larger numbers of clusters, but these would be expected because of the increased opportunity for fragmentation within the relatively long chromosomal regions that would have incorporated [^{3}H]thymine over the 30-min period.

Pulse labeling. Pulse labeling during the first round of replication after germination should enable easier detection of a second spore chromosome starting shortly after the first. Figure 3 (bottom panel) shows how the daughters from both chromosomes would all be labeled to the same extent, provided the second chromosome began replication before the introduction of the pulse and that the rates of DNA chain growth in both chromosomes were the same. 4 + *0* type colonies would result. Figure 5 shows the grain cluster distribution in colonies growing out from spores and pulse labeled over the period of 75 to 85 min. In this experiment 80% of the colonies, those containing a certain minimum number of grains, were scored. Knowing that at least 40% of all colonies would have started replication before 60 min, it follows that at least 50% of the colonies scored in Fig. 5 would have commenced replication before 60 min. If a second chromosome started to replicate up to 15 min after the first, one would expect to see a dramatic increase in the proportion of the 4 + *0* type of colony, but this has not occurred. In addition, it is significant that the 2 + *2* class of colony also comprises only a small proportion (less than 5%) of all types found. This argues against the presence of a second chromosome starting to replicate sometime during the 75- to 85-min interval, i.e., 15 to 25 min after the first.

Pulse labeling after thymine starvation. Figure 6 shows the grain cluster distribution among colonies developing from spores germinated in the absence of thymine for 60 min and

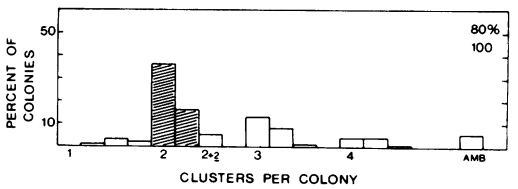

FIG. 5. *Grain cluster distribution within colonies pulse labeled with [^{3}H]thymine over the 75- to 85-min period. Colonies of 6 to 12 cells in size, containing 10 to 20 grains, were scored (2% contained more than 20 grains). Exposure time was 4 weeks.*

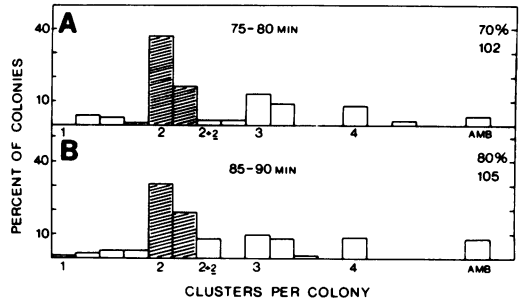

FIG. 6. *Grain cluster distribution within colonies from spores germinated in the absence of thymine for 60 min followed by pulse labeling with [^{3}H]thymine over periods of 75 to 80 min and 85 to 90 min. The colonies were six to twelve cells in size and those containing 10 to 20 grains were scored (less than 1% contained more than 20 grains). Exposure time was 4.5 weeks in both cases.*

pulse labeled with [^{3}H]thymine over the periods 75 to 80 and 85 to 90 min. In the former situation (Fig. 6A) at least 60% of the colonies analyzed would have been ready to start replication at 60 min. The relatively small number (less than 10%) of the 4 + *0* class of colony argues strongly against the presence of a second chromosome starting to replicate up to 15 min after the first. The similar result for the later pulse labeling, where at least 50% of the colonies would have started replication at 60 min, argues against the presence of a second chromosome starting to replicate up to 25 min after the first. It is significant that, even with this relatively late labeling over the short 5-min period, 50% still fall into the combined (2 + *0*, 2 + *1*) category. However, it should be pointed out that, when four to six colonies were analyzed, the value for this category was somewhat less, 40%, with slightly more, 15%, being of the 4 + *0* or 4 + *1* type. In every other experiment four to six cell colonies likewise were scored and, without exception, the combined (2 + *0*, 2 + *1*) category was the same as or slightly greater than in the case of six- to twelve-cell colonies. The reason for the unexpected behavior after the 85- to 90-min labeling in this particular experiment is not obvious. But certainly there was not the dramatic shift in the pattern of the histograms for either the smaller colonies or those analyzed in Fig. 6, when compared with the earlier ones, to indicate a majority of colonies with larger numbers of grain clusters. Such a result would have been expected had there been additional replications in second chromosomes along with fragmentation within the daughters of a single-labeled

chromosome, which could occur more readily in these cases of short pulse labeling.

DISCUSSION

In the present series of experiments it was hoped that, by labeling only a small portion of the germinating spore chromosome with [^{3}H]thymine, the probability of fragmentation within the labeled regions during subsequent growth into a microcolony would be reduced. Certainly, where [^{3}H]thymine was present right from the start of germination and up until shortly after the beginning of the first round of replication, the approach has been successful. The major class of colony (more than 40%) was that containing two grain clusters exclusively (see Fig. 2). This is very different from the situation arising from the use of fully labeled spores, where usually much less than 20% of colonies of similar size were of this type (1, 16). Comparison with the previous results of our own with fully labeled spores (6) do not appear to show this difference. However, in scoring grain clusters in that case, minor clusters were ignored.

The experiments utilizing continuous labeling up to 70 min without prior thymine starvation show clearly that, in the majority of spores at least, only one chromosome is undergoing replication soon after the initiation of DNA synthesis. In the other experiments, later times of labeling, continuous and pulse, were used in an attempt to detect a putative second spore chromosome beginning replication after the first. Certainly, there was no positive indication of a second chromosome starting within 25 min of the first. The results of other workers strongly suggest that heterogeneity to such an extent in the time of initiation of DNA replication within individual spores is most unlikely (see references 7 and 10). Thus, considered altogether, the results of the present experiments, designed to distinguish between the alternatives of one and two chromosomes per *B. subtilis* spore, clearly favor the former situation.

Of the previous autoradiographic studies suggesting the presence of just one chromsome in the majority of spores, those of Ryter and Jacob (12) are the most convincing. Complications due to fragmentation were minimized here by examining spore-derived microcolonies after just two rounds of replication by high-resolution autoradiography under the electron microsope. But, there is one particular aspect of Yoshikawa's (16) results, suggesting two chromosomes, that is difficult to explain. It is that the four segregating units produced during the early generations in his experiments were of similar size. It is most unlikely that the different strain used by Yoshikawa would be of any consequence as the amount of DNA per *B. subtilis* spore has been found to be the same for various strains (1, 4, 5).

ACKNOWLEDGMENTS

This work has been supported by the Australian Research Grants Committee and the University of Sydney Cancer Research Fund.

LITERATURE CITED

1. **Aubert, J. P., A. Ryter, and P. Schaeffer.** 1969. Fate of spore deoxyribonucleic acid during a new spore cycle in *Bacillus subtilis*, p. 148-158. *In* L. L. Campbell (ed.), Spores IV. American Society for Microbiology, Bethesda, Md.
2. **Callister, H., and R. G. Wake.** 1974. Completed chromosomes in thymine-requiring *Bacillus subtilis* spores. J. Bacteriol. 120:579-582.
3. **Callister, H., and R. G. Wake.** 1976. Homogeneity in *Bacillus subtilis* spore DNA content. J. Mol. Biol. 102:367-371.
4. **Dennis, E. S., and R. G. Wake.** 1968. The *Bacillus subtilis* genome—studies on its size and structure, p. 61-70. *In* W. J. Peacock and R. D. Brock (ed.), Replication and recombination of genetic material. Australian Academy of Science, Canberra.
5. **Fitz-James, P. C., and I. E. Young.** 1959. Comparison of species and varieties of the genus *Bacillus*: structure and nucleic acid content of spores. J. Bacteriol. 78:743-754.
6. **Goldring, E. S., and R. G. Wake.** 1968. A comparison of the segregation of chromosomes within microcolonies developing from single *Bacillus subtilis* and *Bacillus megaterium* spores. J. Mol. Biol. 35:647-650.
7. **Huang, P. C., H. Eberle, L. B. Boice, and W. R. Romig.** 1968. Replication of *Bacillus subtilis* DNA during germination in 5-bromouracil containing medium and marker mapping. Genetics 60:661-672.
8. **Kejzlarova-Lepesant, J., N. Harford, J. A. Lepesant, and R. Dedoner.** 1975. Revised genetic map for *Bacillus subtilis* 168, p. 592-595. *In* P. Gerhardt, H. L. Sadoff, and R. N. Costilow (ed.), Spores VI. American Society for Microbiology, Washington, D. C.
9. **Oishi, M., and N. Sueoka.** 1965. Location of genetic loci of ribosomal RNA on *Bacillus subtilis* chromosome. Proc. Natl. Acad. Sci. U.S.A. 54:483-491.
10. **Oishi, M., H. Yoshikawa, and N. Sueoka.** 1964. Synchronous and dichotomous replication of the *Bacillus subtilis* chromosome during spore germination. Nature (London) 204:1069-1073.
11. **Quinn, W. G., and N. Sueoka.** 1970. Symmetric replication of the *Bacillus subtilis* chromosome. Proc. Natl. Acad. Sci. U.S.A. 67:717-723.
12. **Ryter, A., and F. Jacob.** 1966. Ségrégation des noyaux chex *Bacillus subtilis* au cours de la germination de spores. C. R. Acad. Sci. 263:1176-1179.
13. **Sueoka, N., and W. G. Quinn.** 1968. Membrane attachment of the chromosome replication origin in *Bacillus subtilis*. Cold Spring Harbor Symp. Quant. Biol. 33:695-705.
14. **Wake, R. G.** 1975. Bidirectional replication in *Bacillus subtilis*, p. 650-676. *In* M. M. Goulian, P. C. Hanawalt, and C. F. Fox (ed.), DNA synthesis and its regulation. W. A. Benjamin, Inc., Menlo Park, Calif.
15. **Woese, C. R.** 1958. Comparison of the X-ray sensitivity of bacterial spores. J. Bacteriol. 75:5-8.
16. **Yoshikawa, H.** 1968. Chromosomes in *Bacillus subtilis* spores and their segregation during germination. J. Bacteriol. 95:2282-2292.