DNA Content and Nucleoid Distribution in Methanothermobacter thermautotrophicus

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Flow cytometry and epifluorescence microscopy results for the euryarchaeon Methanothermobacter thermautotrophicus were consistent with filaments containing multiple cells. Filaments of one to four cells contained two to eight nucleoids. Single chromosome-containing cells were not observed. Filaments containing multiple genome copies displayed synchronous DNA replication initiation. Chromosome segregation occurred during replication or rapidly after replication termination.

Methanothermobacter thermautotrophicus has become an important model system for biochemical characterization of the archaeal chromosome replication machinery, providing the first functional evidence of helicase activity from an archaeal MCM complex (8, 12, 19) as well as the first structural information concerning these proteins (9, 17). Information concerning the activity and site-specific binding of the two Cdc6 homologues, which are known to play a key role in the control of eukaryotic DNA replication initiation, is also available for this species (7, 10). Insights into the organization of the cell cycle of M. thermautotrophicus will therefore have significant implications for understanding the mechanisms by which the eukaryote-like replication proteins can interface with the bacterial-type cell division proteins found in the euryarchaeota (2) and will allow investigations of the cell cycle control mechanisms of these organisms.

M. thermautotrophicus (DSMZ 1053) cultures were grown in a 3-liter bioreactor under chemoautotrophic conditions (16), with H2 and CO2 as the sole energy and carbon sources. Growth from an initial estimated optical density at 600 nm (OD600) of 0.002 resulted in at least five doublings before exponential growth to an OD600 of about 1 (1,500 min), a transition between 1,500 and 2,100 min, and then a stationary phase, despite a clear plateau in the OD curve. Cation runout was not observed for the entire population in the graph around a DNA content of 12 chromosomes (Fig. 1C). The DNA content gradually decreased with increasing ODs, and the eight-chromosome peak could no longer be detected after the 2,125-min time point. The four-chromosome peak dominated the DNA content distributions between the 1,765- and 2,125-min time points, followed by a dominant two-chromosome peak after the 2,125-min time point. Complete replication runout was not observed for the entire population in stationary phase, despite a clear plateau in the OD curve.

The filament size distributions also displayed changes over the course of cultivation. Samples from exponential-phase cultures produced a broad light-scatter peak (Fig. 1B), consistent with M. thermautotrophicus cells forming filaments of different lengths (23, 24). The distribution was significantly extended to the right, towards high light-scatter values, indicating extensive filamentation in part of the population. At higher OD values, the light scatter decreased and a sharper peak was obtained, indicating that the range of filament lengths and the average filament length decreased. In stationary phase, after the 2,305-min time point, no further changes in either the light scatter or fluorescence distribution occurred. The flow cytometry data were confirmed by examining DAPI (4’,6-diamidino-2-phenylindole)-stained samples by epifluorescence microscopy (Fig. 1F to H). The average number of nucleoids in the filaments gradually decreased during culture growth, in agreement with the decrease in cellular DNA content observed by flow cytometry analysis.

The nucleoids were regularly distributed inside the filaments (Fig. 1F to H), and the number of nucleoids (243 filaments counted) (data not shown) correlated well with the number of genome equivalents determined by flow cytometry. This indicates that a majority of the nucleoids consisted of only a single chromosome and that genome segregation therefore must have occurred rapidly after the termination of chromosome...
FIG. 1. (A) Growth curve of *M. thermautotrophicus* in batch fermentor culture. The log(OD<sub>600</sub>) values of bioreactor samples were plotted against time. The insert shows a linear plot of the same data. (B and C) Flow cytometry filament size (light scatter) and DNA content (fluorescence after staining with a combination of ethidium bromide and mithramycin A) distributions in the same culture at different time points. The time (in minutes) after inoculation of the culture and the OD<sub>600</sub> (average of four independent samples) are indicated to the right. (D) Superimposed DNA fluorescence peak positions from *M. thermautotrophicus* (thick line) and *E. coli* MG1655 seqA::Tn10 treated with rifampin (thin line). The numbers next to the *M. thermautotrophicus* peaks indicate the deduced numbers of genome equivalents. (E) Peak positions plotted versus chromosome sizes of *E. coli* (open circles) and *M. thermautotrophicus* (closed circles) indicate a comparable linear relationship between fluorescence and DNA content for both organisms. (F to H) Epifluorescence micrographs of DAPI-stained *M. thermautotrophicus* filaments demonstrating that filamentation decreases in a fermentor batch culture over time. Bars, 5 μm. (F) Exponentially growing cells from the 1,390-min time point. (G) Cells from the 1,945-min time point. (H) Stationary-phase cells from the 2,665-min time point.
replication or in parallel with replication elongation. This in turn implies a short, or no, G2 phase, in contrast to the case for crenarchaea from the genus *Sulfolobus*, in which the G2 phase is the dominating cell cycle period (4, 18). In *E. coli*, genome segregation occurs concomitantly with chromosome replication, and in this respect, the *M. thermotogae* cell cycle displays a bacterial type of organization. Exponentially growing *M. thermotogae* filaments contained between two and eight copies of the chromosome. These were distributed in a 2n fashion (Fig. 1C), again similar to the situation in replication or in parallel with replication elongation. This in 1858 NOTES J. BACTERIOL. 109:707–715.

Our results demonstrate several differences in DNA content and nucleoid distribution between *M. thermautotrophicus* and *M. jannaschii*, the other methanogen for which these parameters have been investigated (15). There are also significant differences between *M. thermautotrophicus* and the euryarchaeon *Archaeoglobus fulgidus* (14) as well as crenarchaeal *Sulfolobus* species (4), most notably the complete lack of cells containing a single chromosome for *M. thermautotrophicus*. This is also different from the situation in *E. coli*, which otherwise resembles *M. thermautotrophicus* in that the chromosomes are distributed in a 2n fashion and that genome segregation occurs rapidly upon the completion of DNA replication. Despite these differences, it is interesting that both *Sulfolobus* and *Methanothermobacter* maintain a majority of the cell population with at least two chromosomes during exponential growth, possibly as a means of maintaining a backup copy of the chromosome at high temperatures, which are known to result in elevated levels of DNA damage (11).

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