Characteristic of the chromosome replication process in predatory bacterium *Bdellovibrio bacteriovorus*

Abstract

B. bacteriovorus is a small Gram-negative bacterium with the distinguishing feature of killing other Gram-negative bacteria, including human pathogens, such as *Helicobacter pylori* or *Pseudomonas aeruginosa*. The intriguing life cycle of *B. bacteriovorus* consists of two phases: a free-living non-replicative attack phase wherein predatory bacterium actively searches for its prey, and an intracellular reproductive phase, wherein *B. bacteriovorus* proliferation leads to the host cell lysis. Inside the prey cell periplasm, *B. bacteriovorus* forms a multinucleoid, elongated, filamentous cell, which synchronously septates to form several (usually three to six) progeny cells. Although the cell biology of *B. bacteriovorus* has gained recently considerable interest, very little is known about one of the key stages of its life cycle - chromosome replication.

The first step in the studies of *B. bacteriovorus* chromosome replication process was to identify *B. bacteriovorus origin of replication* (Bd*oriC* region) and characterize its interaction with the initiator protein (BdDnaA). EMSA (*Electrophoretic mobility shift assay*) and immunoprecipitation assays confirmed the binding of the initiator protein to the *in silico* determined Bd*oriC* region *in vitro* and *in vivo*, respectively. The use of DNA footprinting allowed for precise identification of BdDnaA binding sites within the Bd*oriC* region (BdDnaA-boxes) and determination of the DUE region (*DNA unwinding elements*).

In the second stage of the studies, the TLFM (*Time-lapse fluorescence microscopy*) system was created to observe the dynamics of the *B. bacteriovorus* chromosome replication process at the single cell level in real time. The results showed that the replication of the chromosome starts at the invasive pole inside the bdelloplast, and replication proceeds until several copies of the entire chromosome are completely synthesized. Several replisomes can be observed during *B. bacteriovorus* chromosome replication, suggesting reinitiation of the replication process. This chromosome replication is not coincident with the predator-cell division, and it terminates shortly before synchronous predator-filament septation occurs.

The results of the presented researches allowed the characterization of the *B. bacteriovorus* chromosome replication process and expanded knowledge about the replication of the bacterial chromosome.