Formation of 'dormant' *Borrelia* stages

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Spirochetes of the genus *Borrelia* are the causative agents of Lyme disease, a tick-borne zoonosis mainly found in North America and in Europe [1]. Even after antibiotic treatment some patients may go on to develop persistent Lyme disease symptoms. The mechanism of bacterial survival in the presence of the hosts' immune system is still poorly understood. One possible explanation for this clinical observation is the formation of Round Bodies (RB), a pleomorphic stage formed when spirochetes are exposed to unfavorable conditions [2]. Here we used GFP-expressing *B. burgdorferi* to characterize this transformation process, exposing the spirochetes to different culture conditions. Using video-microscopy, we were able to follow the conversion of the spirochetes into RBs under unfavorable conditions, with the GFP signal concentrated in a round spot. Cryo-immunolabeling and quantitative fluorescent analysis showed that this is not caused by a leaking of the GFP fluorescence but by a concentration of GFP into a single spot. Although RBs are observed in cultures under normal conditions, their number increased when spirochetes were exposed to adverse conditions. This transformation can be reversed, since bacteria induced to form RBs can be re-transformed back to spirochetes. Live/Dead assay showed that these RBs were viable even after several days under adverse conditions. Negative staining and cryo-tomography [3] observations showed that this transformation led to a detachment of the outer membrane and formation of vesicles that are secreted by the bacteria. When examined through scanning electron microscopy, the bacterial surface was covered with small vesicles. Previously, it was shown that *Borrelia* spirochetes could evade the hosts' immune response via differential expression of outer surface proteins. We applied immunofluorescence and immune-scanning electron microscopy [4] to analyze the pattern of distribution of major surface proteins, i.e. OspA, OspB, OspC, p39 and p83, on the helical forms of spirochetes and RBs. OspA was detected over the whole surface of the spirochetes, while OspB, OspC, p39 and p83 were distributed in a scattered manner. When we observed the RBs after 6 or 24 hours in adverse condition, OspA now presented a scattered distribution, as did OspB. However, we did not detect the presence of OspC, p39 and p83, although the level of protein expression was not altered during RB induction, as analyzed by western blot. Immuno-SEM showed that the bacteria shedded these surface proteins in vesicles, when they are in adverse conditions.

References

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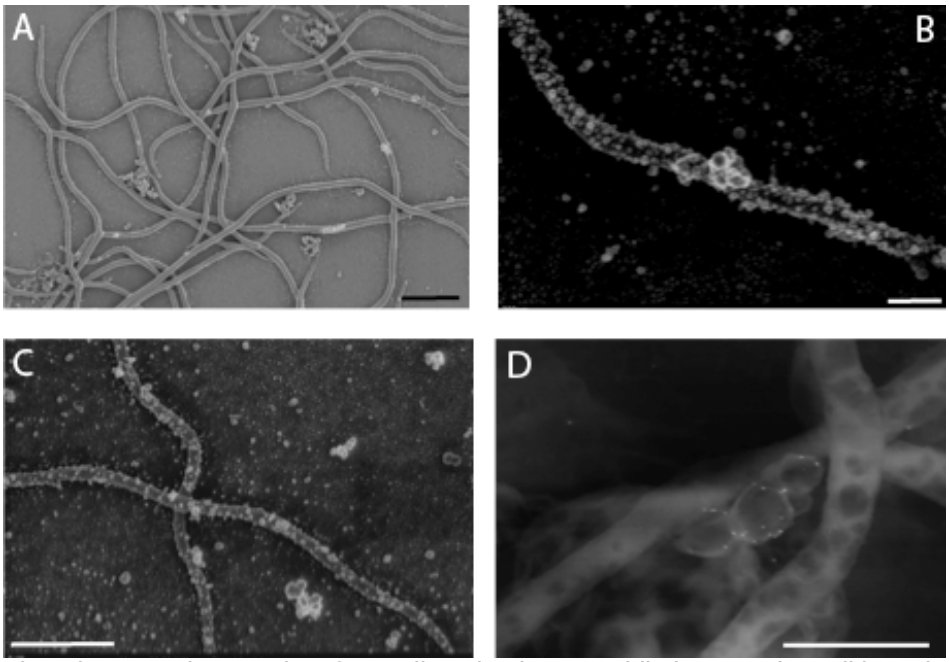

Figure 1. *Scanning electron micrographs of Borrelia spirochetes. While in normal conditions the surface of the bacteria is smooth (A), in adverse conditions (B and C) the surface is covered with a high number of vesicles. Through these vesicles surface proteins are secreted, as observed by immune-scanning electron microscopy (D). Bars: A – 1 µm; B – 200 nm; C – 1 µm; D – 250 nm.*

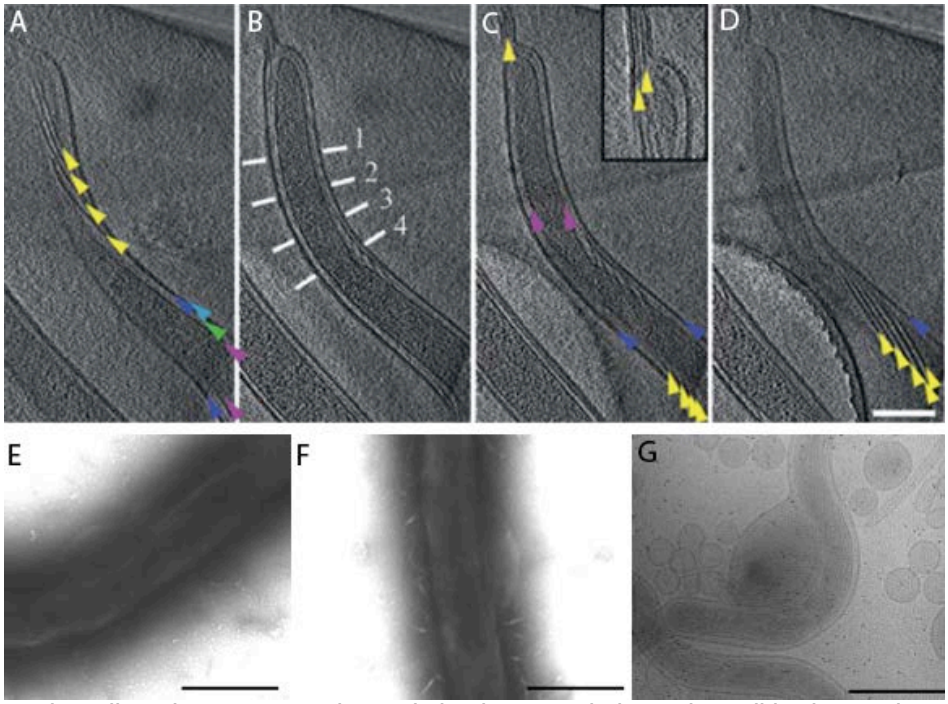

Figure 2. *In normal medium the outer membrane is in close proximity to the cell body, as observed in different z-planes of a cryo-tomogram (A-D) [3]. Arrowheads indicate the flagella and the outer and inner membrane. During the transformation occurs a detachment of the outer membrane of the spirochetes, as observed by negative staining (E and F) and cryo-electron microscopy (G). Bars: A-D = 300 nm, E-G = 200 nm.*