Ultrastructural & Analytical Methods in Life Sciences

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Interaction between mammalian cells and bacterial inclusion bodies through light and electron microscopy techniques

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Bacterial Inclusion Bodies (IBs) are protein aggregates commonly observed during recombinant protein production processes in microbial hosts. These sub-micron particles, firstly regarded as by-products, have been shown to be highly pure, mechanically stable and biocompatible protein deposits able to retain biological activity [1, 2]. In this scenario, several potential applications for bacterial IBs have recently emerged, being particularly appealing the use of this particulate material in biomedicine [3]. In recent years, it has been also demonstrated that these protein deposits, when formed by protein with a therapeutic interest can actually rescue challenged cells in culture [4]. Nevertheless, despite the clear recovery of the IB treated cells, the mechanism by which the targeted cells obtain functional protein from IBs remains unsolved.

To clarify this fact, a complete structural and ultrastructural evaluation of eukaryotic cell-IBs interaction at 0, 0.5, 1, 3, 8 and 24h, after the addition of the IBs, was performed using light (CLSM) and electron microscopy (SEM and TEM) techniques. The evaluation evidenced the fine architecture of IBs (Figure 1) and its interaction with mammalian cells: when placed on cell surface, filopodia of HeLa cells rapidly contact with IBs at early times of the study (Figure 1) being subsequently uptaken via endocytosis at latter times (Figure 2).

In summary, this study reports for the first time the ultrastructure of IBs and the interaction with eukaryotic cells characterized with conventional and non-conventional microscopy techniques.

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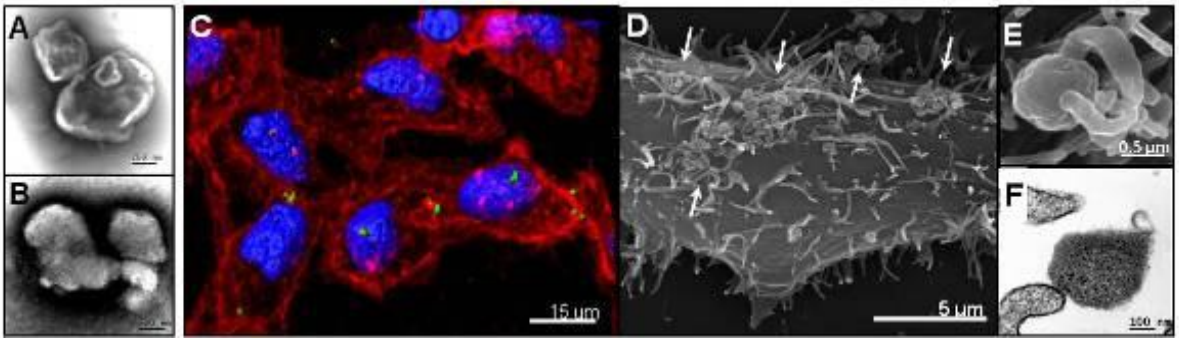

Figure 1. Representative micrographs of IBs and Hela interacting cells. A-B) Negative staining of ultrastructure and immunolocalization of IBs; C-D) General views of IBs on HeLa cells and E-F) Contact of IBs-filopodia at early times of the study (0-3h).

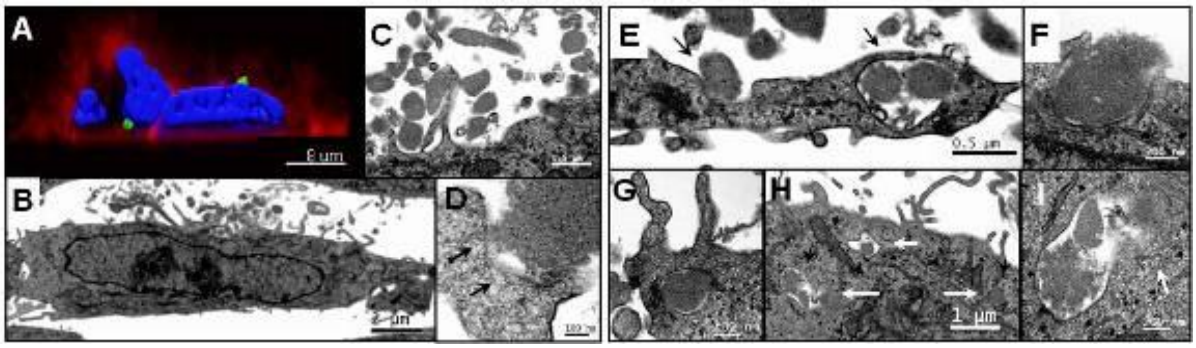

Figure 2. Representative micrographs of IBs and Hela interacting cells. A-C) Structural and ultrastructural details of IBs on HeLa cell surface; D) Detail of filopodia cell membrane disorganization after IB contact at latter times (8h-24h). At these times, several IBs were internalized via endocytosis (E-I) producing membrane disorganization also in endosomes (I).