Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

Imaging bacteria inside their host by cryo-focused ion beam milling and electron cryotomography

João M Medeiros, Désirée Böck and Martin Pilhofer

Bacterium–host interactions are important for diverse ecological settings including pathogenicity and symbiosis. Electron cryotomography is a powerful method for studying the macromolecular complexes that mediate such interactions *in situ*. The main limitation of electron cryotomography is its restriction to relatively thin samples such as individual bacterial cells. Cryo-focused ion beam milling was recently proposed as a solution to the thickness limitation. This approach allows the artifact-free thinning of biological specimens for subsequent imaging in the transmission electron microscope. By enabling near-native imaging of bacteria inside their eukaryotic host, this combination of techniques promotes the integration of data from structural biology and infection biology. Therefore, electron cryotomography associated with cryo-focused ion beam milling holds great potential for establishing multiscale models of cell–cell interactions from the atomic, to the cellular and to the intercellular scale.

Address
Institute of Molecular Biology & Biophysics, Eidgenössische Technische Hochschule Zürich, CH-8093 Zürich, Switzerland

Corresponding author: Pilhofer, Martin (pilhofer@biol.ethz.ch)

Current Opinion in Microbiology 2018, **43**:62–68

This review comes from a themed issue on **The new microscopy**

Edited by **Stephan Uphoff** and **Ariane Briegel**

https://doi.org/10.1016/j.mib.2017.12.006

1369-5274/© 2017 Elsevier Ltd. All rights reserved.

Introduction

Bacteria only rarely exist as individual cells in the environment. Most often, they interact with other eukaryotic, bacterial or archaeal cells. Well-orchestrated interactions are the basis for ecological settings including pathogenesis, symbiosis, commensalism, biofilm formation, or predation. The ability of the bacteria to manipulate their host is critical for the establishment of such cell–cell interactions. This is frequently accomplished by bacterial effector proteins that act on the target organism. The effectors are typically delivered by specialized macromolecular machines [1]. Besides these secretion systems, macromolecular cell appendages such as pili play an important role in the physical attachment to target cells [2].

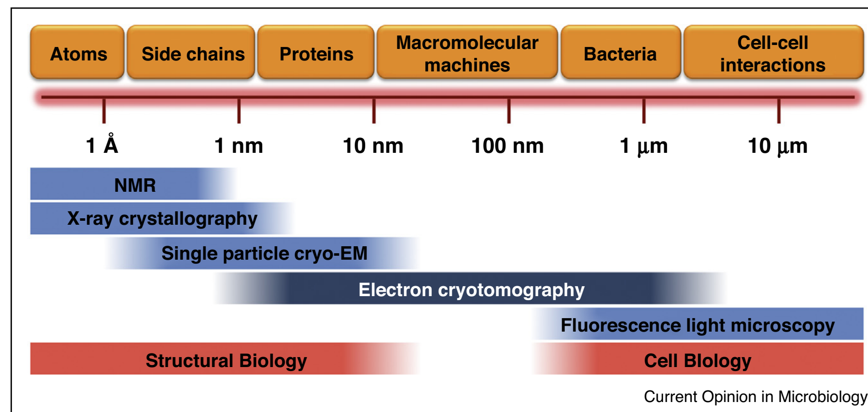
Microbe–host interactions and the involved macromolecular assemblies have been historically studied by two main fields of research. Infection biology successfully addressed significant questions through the use of immunostaining and live cell fluorescence light microscopy, generating data with a resolution of a few hundred nanometers. Structural biology, on the other hand, resolved atomic models of purified complexes using high-resolution methods including X-ray crystallography, nuclear magnetic resonance spectroscopy, or single particle electron microscopy. The different scales of resolution at which both fields operate, present the challenge of generating integrative models of bacterial cell–cell interactions from the atomic to the macromolecular and to the cellular level (Figure 1). A further complication is encountered when addressing cell–cell contact-dependent interactions, involving more than one cell. Here we review the power of *in situ* imaging by electron cryotomography (ECT) and recent advances in overcoming limitations to visualize bacteria inside their host.

Electron (cryo-)microscopy

The invention of transmission electron microscopy (TEM) equipped scientists with a technique capable of overcoming the resolution limit inherent to conventional light microscopy [3]. In order to withstand the vacuum in the microscope's column, biological samples were conventionally prepared by chemical fixation, plastic embedding, thin-sectioning, and heavy metal staining. For decades, such conventional room temperature TEM approaches have delivered a tremendous amount of insight into the cell biology of eukaryotic cells, as well as the fate of intracellular bacteria. The harsh sample preparation procedures, however, limited insights into the molecular mechanisms of macromolecular machines. Furthermore, conventional TEM was shown to introduce artifacts and sometimes fails to resolve entire classes of cellular components [4].

In pioneering work, Dubochet and coworkers founded the era of electron cryomicroscopy (cryoEM) by exploring the preservation and imaging of biological specimens in a frozen-hydrated life-like state [5]. Typically, a suspension of cell culture or purified complex is spread across a TEM grid, excess liquid is removed by blotting with filter paper, and the grid is then plunged into a cryogen such as liquid ethane-propane [6]. This leads to artifact-free

Figure 1



Multiscale approach. Electron cryotomography bridges the gap in resolution between techniques used in Structural Biology and Cell Biology. Applied in an integrative approach, electron cryotomography generates models ranging from the atomic to the multicellular scale.

preservation of the sample in a glass-like amorphous ice (vitrification) [5] and provides significant protection against beam-induced damage [7]. Vitrified specimens are then imaged in a dedicated TEM at cryogenic temperature. Cryopreservation combined with significant advances in cryoEM instrumentation and image processing software, have led to tremendous scientific advances in both structural and cell biology [8]. Sample type and imaging mode classify cryoEM into three fundamentally different modalities. Single particle cryoEM and electron crystallography aim at generating atomic models by averaging information obtained from many thousands of copies of a purified particle. The third cryoEM modality, electron cryotomography (ECT), aims at resolving three-dimensional images of *unique* objects.

Electron cryotomography and limitation by sample thickness

In ECT, projection images of the target, an individual cell for instance, are collected across a range of angles (typically from −60 to +60 degrees). The resulting 'tilt-series' is then computationally reconstructed into a three-dimensional image — the tomogram [9]. In contrast to near-atomic resolution structural techniques, this approach allows the visualization of individual macromolecular complexes in their cellular context. Individual tomograms of bacterial cells for instance, can be extremely rich in detail and reveal ultrastructures such as membranes, cell envelope layers, cytoskeletal filaments, secretion systems, storage compartments, flagella motors, chemoreceptor arrays, cellular appendages, phages, or ribosomes. The resolution of individual tomograms is in the range of a few nanometers. 'Subtomogram averaging' improves the contrast-to-noise ratio by computationally extracting, aligning, and averaging identical subtomograms [10]. For an ideal sample, this approach can resolve macromolecular complexes down to sub-nanometer and even atomic resolution [11,12].

CryoEM images of increasingly thicker samples suffer a sharp decrease in contrast and signal-to-noise ratio. This is due to the physics of image formation in TEM. Here, only the interference of unscattered with elastically scattered electrons generates meaningful signal. However, the number of inelastic scattering events increases with the length of the path the electrons take to travel through the sample, effectively restricting the application of ECT to cells with a thickness of well below 800 nm. As such, ECT imaging has had a tremendous impact on our mechanistic understanding of relatively thin bacterial cells (reviewed in [13]). Also the thin peripheral regions of intact eukaryotic cells are amenable to direct ECT imaging [14]. A few studies used this approach to investigate the invasion of host cells by *Chlamydia trachomatis* (Figure 2a) [15•,16], the *in situ* structure of invading adenoviruses [17], the life cycle of vaccinia virus [18], and the maturation of HIV-1 particles [19]. Most problems in infection biology, however, require a sample-thinning step to be able to target all subcellular areas of interest for the acquisition of high-quality ECT data.

Cryosectioning

Cryosectioning is the traditional approach to thinning a vitreous sample to a thickness that is suitable for ECT imaging. 60–80 nm thick sections are cut using a diamond knife inside an ultramicrotome that is operated at cryogenic temperature [20]. Samples for this approach are typically vitrified by high-pressure freezing, a cryo-fixation technique that allows for vitrifying samples with tens of microns in thickness [21]. Cryosectioning allowed for the first time to image bacterial cells inside their host by ECT, revealing the recruitment of host cell

Download English Version:

https://daneshyari.com/en/article/8745067

Download Persian Version:

https://daneshyari.com/article/8745067

Daneshyari.com