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Correlative cryo-electron tomography and optical microscopy of cells

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The biological processes occurring in a cell are complex and dynamic, and to achieve a comprehensive understanding of the molecular mechanisms underlying these processes, both temporal and spatial information is required. While cryo-electron tomography (cryoET) provides three-dimensional (3D) still pictures of near-native state cells and organelles at molecular resolution, fluorescence light microscopy (fLM) offers movies of dynamic cellular processes in living cells. Combining and integrating these two commonly used imaging modalities (termed correlative microscopy) provides a powerful means to not only expand the imaging scale and resolution but also to complement the dynamic information available from optical microscopy with the molecular-level, 3D ultrastructure detail provided by cryoET. As such, a correlative approach performed on a given specimen can provide high resolution snapshots of dynamic cellular events. In this article, I review recent advances in correlative light microscopy and cryoET and discuss major findings made available by applying this method.

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Introduction

Both fluorescence light microscopy and electron microscopy have been instrumental for understanding the structure and function of biological processes and have provided a wealth of information regarding protein localization and dynamics and the cellular ultrastructure. Cryo-electron tomography (cryoET) has emerged as a powerful imaging technique that allows three-dimensional (3D) visualization of the molecular landscape within cells, at 2–4 nm resolution, in a close-to physiological state [1]. By applying this method, significant structural detail has been obtained for bacterial cells [2,3], cellular compartments [4], and purified or abundantly budding virus particles [5–7]. With recent developments in subvolume averaging from cellular tomograms, the structures of native protein assemblies, at resolutions below 2 nm, are now possible [8,9]. However, several issues associated with cryoET have limited its potential in advancing cellular structural biology. First, the inherent low contrast of unstained frozen-hydrated specimen in cryoET, combined with specimen radiation sensitivity, makes it difficult to locate areas of interest inside a cell before cryoET analysis. Second, cryoET delivers only still pictures of cells. Third, although providing molecular resolution, cellular tomograms offer a very limited field of view, typically only ~10% of a cell's area and restricted to very thin regions, typically those <300 nm thick. Thus, the vast majority of a eukaryotic cell is not accessible to cryoET alone. Finally, due to the crowded cellular environment, in the absence of EM labels, individual proteins and macromolecular complexes are not readily distinguishable at the current resolution, with the exception of a few, very large complexes [10,11].

On the other hand, fluorescence light microscopy (fLM) offers a complementary set of imaging capabilities. It provides time-resolved, large scale (whole cell) visualization of the dynamics of individual fluorescent protein(s) of interest in living cells. Thus, fluorescence imaging has been employed to investigate the temporal and spatial distribution of specific molecular players in a variety of cellular processes. Recent advances in super-resolution fluorescence microscopy imaging, which apply switchable probes, as in PALM and STORM [12,13], or elegant illumination strategies, as in STED and SIM [14–16], have further improved the resolution of fLM by an order of magnitude and have enabled a better understanding of many cellular processes [17,18]. Yet, the limited spatial resolution of fLM often prevents definitive localization of proteins relative to the cellular ultrastructure or organelles. Further, only a few molecules can be visualized simultaneously with fLM, in contrast to the full repertoire of macromolecules that can be visualized with cryoET.

Therefore, a correlative approach that combines the strength of these two complimentary imaging modalities is highly desirable. In such a way, specific features highlighted by fluorescent signals are identified and located by fLM and then used to guide the acquisition of high resolution 3D structural data by cryoET, which in turn complement the dynamic information obtained by live-cell fLM. Using correlative microscopy, one can not only visualize the dynamics of the cellular process in a whole living cell but can also zoom in to observe the molecular

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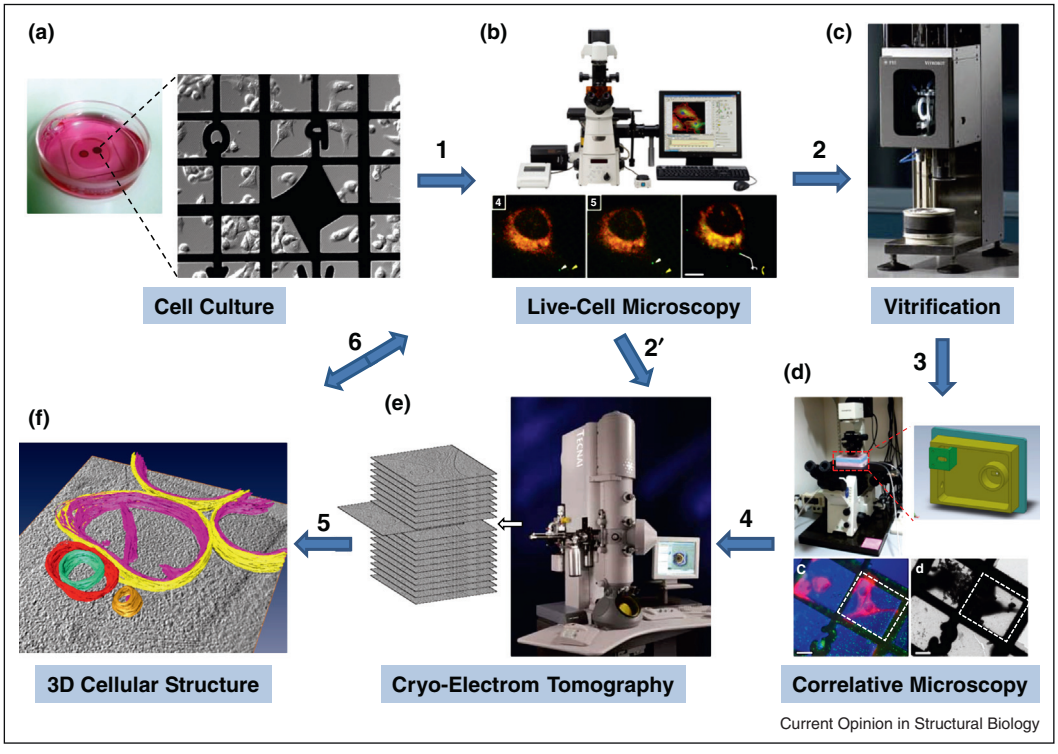
details of the cellular context of the process to gain novel insights into the process.

Correlative light and electron microscopy (CLEM) has become a powerful approach for structural and functional analysis of cellular processes in a single experiment. CLEM was achieved primarily using either chemically or cryo-fixed, resin embedded and sectioned specimen at room temperature, typically after immunolabeling [19,20] or photoconversion of diaminobenzadine into an electron-dense marker, such as miniSOG [21•]. Abundant application of this approach has yielded many significant findings and has provided insights into some fundamental biological processes and their underlying molecular mechanisms [19,22•]. Here, I focus on CLEM studies performed at cryogenic temperature (cryo-CLEM), which is more challenging, but allows fluorescent signals to be examined in frozen-hydrated cells preserved at near-native condition for subsequent cryoET analysis [23]. In this review, I outline the workflow for cryo-CLEM, highlight recent technical advances and the major findings obtained using this approach, and discuss the future prospects for using correlative microscopy in conjunction with super-resolution, cryoFIB milling and/or genetically expressed EM markers, to advance our imaging capability for investigating cell signaling and cellular processes.

Cryo-CLEM implementation

Cryo-correlative light and electron microscopy (i.e. cryo-CLEM) is a multistep experiment involving protein fluorescent-labeling, live-cell confocal microscopy, sample vitrification, cryo-fluorescent light microscopy (cryo-fLM), and cryoET data collection and analysis. A typical workflow for cryo-CLEM is illustrated in Figure 1 (steps 1 through 6). Cells grown directly on indexed, gold EM grids (Figure 1a) are typically labeled with one or more fluorescent dyes and examined with time lapse, live-cell confocal imaging (Figure 1b) to assess the dynamic behavior of the labeled protein(s) of interest. At a specific dynamic stage of interest, the cells are preserved at near-native condition by rapid vitrification (Figure 1c). Cryo-fLM is performed to identify the area of interest using a cryo-light microscopy (cryo-LM) stage that maintains the sample in the frozen-hydrated state (Figure 1d). Specific features highlighted by fluorescent

Figure 1



An illustrative workflow of correlative light microscopy and cryo-electron tomography of cells. The steps include: **(a)** culturing of eukaryotic cells on carbon-coated gold EM index grids in a glass-bottom petri dish; **(b)** time-lapse, confocal live-cell imaging of a dynamic cellular process, such as viral infection, directly performed on cells grown on EM grids; **(c)** arresting the cellular process at a particular state by vitrification (plunge-freezing); **(d)** localizing the target of interest using fluorescence signals by cryo-fLM for subsequent cryoET; **(e)** cryoET data acquisition and 3D reconstruction of the identified target; and **(f)** 3D structural analysis and interpretation, as well as correlating the high resolution cellular structure with the dynamics of the fluorescently labeled proteins (step 6). For stable and static structures, live cell fluorescence images can also be used to guide cryoEM localization (step 2′), without cryo-fLM.

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