Direct correlation of cell and structural biology using cryo-correlative light and electron microscopy

Elucidating the structure-function relationship of a pathogen and its interactions with a host cell can provide valuable insight into the mechanism of infection and progression of the virus life cycle, thus guiding the development of vaccines and treatments. In recent years, cryo-electron microscopy (cryo-EM) has become a leading technique in providing high-resolution structural data of biological samples. Since specimens prepared for cryo-EM are quickly converted from liquid to solid phase without the formation of ice crystals (i.e. vitrified), macromolecular interactions are preserved providing a precise snapshot of the native state within a cell. However, locating these interactions during cryo-EM analysis is extremely challenging. To resolve this, a new method called correlative light and electron microscopy (CLEM) was developed.

CLEM combines the advantages of fluorescence microscopy (e.g. ability to monitor processes within the cell over time, fluorescent labeling of specific proteins to observe interactions) with high-resolution imaging characteristic of cryo-electron tomography (cryo-ET) to observe biological processes at the cellular level, while also obtaining structural data, respectively.[1-2] In addition, this approach reduces the amount of cell processing, such as chemical fixation, required for data collection. In a recent study, Wright and co-workers analyze virus infected or transfected mammalian cells using their new cryo-CLEM protocol.[3] First, the authors perform cryo-fluorescence light microscopy (cryo-fLM) to identify a region of interest within a cell followed by cryo-TEM at low magnification. The coordinates of each image, fLM and TEM, are registered to ensure that the regions of interest identified from fLM can be found when switching to high magnification EM. Finally, cryo-ET is performed to collect 3-dimensional structural data. Using this cryo-CLEM approach, Wright and co-workers were able to visualize tethers between HIV-1 virions and the host-cell membrane.[4]

Although there are many advantages to this new technology, the authors do point out two main disadvantages of this imaging technique. Specifically, samples need to be less than 750 nm thick. This limit can make it challenging to analyze thicker regions of whole cells, such as regions around the nucleus, requiring the addition of alternative techniques. The second limitation is the resolution limit of traditional light microscopy (200 nm), but future advances in super-resolution cryo-light microscopy should help mitigate this problem. Despite these factors, cryo-CLEM is an improvement to traditional methods and will prove to be a valuable resource in many areas of structural cell biology, including elucidating processes of virus life cycles.

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References

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