

Report on electron microscopy in practice to study proteins

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Biology

The electron microscope has proven to be invaluable in the study of proteins and has enhanced our understanding of cellular biology. The importance to understanding disease and trauma to the body as well as treatments cannot be overstated. Genetic research such as studies of the mechanisms of protein folding is better understood due to electron microscopy.

The image of a sample such as a slice of a fibril or a “ newly synthesized chain of amino acids” (Dobson, 2003, p. 884) created by an electronic beam.

The Transmitting Electron Microscope (TEM) transmits the electron beam through a sample whereas the SEM scans the surface of the sample for secondary electrons. The secondary electrons in Scanning Electron Microscopes (SEM) are caused due to the excitation when the primary beam passes over a sample. Both TEM and SEM offer fine detail and great magnifications.

Ogura (2012, p. e46904) described the importance and the use of SEMs for observing the “ nanometer structures of proteins and viruses.” He was motivated to incorporate the advantages of TEM (high magnification) but used an SEM allowing for low radiation damage to the sample (since the electronic beam did not pass through the sample but instead used the detection of the secondary electrons to make an image). The method was to rely on the high contrast created when samples were prepared for observation under a “ metal-coated insulator films” (Ogura, 2012, e46904). The researcher observed unstained samples of proteins and viruses with an SEM and created high-contrast observations to evaluate the samples. The technique is valuable because staining can damage a sample and electrons

from a beam can cause damaging radiation.

A combination of light microscopy, electron microscopy and a Western-Blot (for testing antibodies) was successfully used to study the signaling of glycogen synthase Kinases-3 β (GSK3 β) by researchers at the University of Birmingham, Alabama. (Perez-Costas et al., 2010) Light microscopy was accomplished by immunostaining sample sections, after appropriate preparations the sections were observed and digital camera with an infrared attachment. The light microscopy step provides a way to assess the sections to be thinly sliced and observed with a TEM coupled with a digital camera. This method allowed for comparing neurons in normal active brains to those with resting cells. The successful use of this methodology will help understand how GSK3 β functions in brain signaling processes. (Perez-Costas et al., 2010)

Amyloid fibril research began in 1854 using stained samples studied under a light microscope and polarizing optics. (Sipe & Cohen, 2000) And then in 1959 research using the electron microscope demonstrated the existence of $\text{A}\beta$; allowing for the identification of “ 20 or more biologically distinct forms of amyloid have been identified throughout the animal kingdom; each is specifically associated with a unique clinical syndrome” (Sipe & Cohen, 2000, p. 88). X-ray diffraction technology has allowed closer inspections of their configurations and properties. More recently comparisons of the protein in connective tissue fibrils are similar to “ in situ, amyloid fibrils are composed of proteoglycans and amyloid P component (AP) as well as amyloid proteins (Sipe & Cohen, 2000, p. 88).

A more sophisticated method, especially for studying the folding

mechanisms of proteins and large proteins in solution is the use of single angle X-ray scattering (SAXS) combined with crystallography and computation. (Putnam et al., 2007, p. 191) Crystallography offers the advantage of “unparalleled detail on structural information critical for mechanistic analyses” (Putnam et al., 2007, p. 191). SAXS offers the capability to compliment the crystallography data with the mechanisms involved in protein folding. The limitation is that the analyses are done at ranges of lower resolution, “but without the size limitations inherent in NMR and electron microscopy studies” (Putnam et al., 2007, p. 191).

References

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