DOI: 10.1017/S1431927605502368
Microsc Microanal 11(Suppl 2), 2005

Correlative Immunolabeling on Etched Epon Samples

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Correlative immunolabeling provides a method to observe one sample using reporters both for light (LM) and electron microscopy (EM); for example fluorophores and colloidal metal particles, respectively. The technique enables collection of complementary sets of data using a single labeling step [1]. It is also useful as a screen to determine the success of the labeling prior to continuing on with subsequent preparation and EM observation.

Epon is widely used as an embedding medium for EM to study morphological structure of biological samples [2]. The material is easily sectioned and the specimen can be stained without major difficulty. Thin Epon sections resist heat and strong vacuum [3]. However, Epon is usually avoided for immunolabeling application due to problems with antigenic masking. Therefore, surface etching employing potassium ethoxide followed by heat treatment is carried out in order to retrieve antigenic sites, thus allowing antibody (Ab) access, while still preserving sample structure. Thin sections, necessary for EM, also contain limited numbers of antigenic sites hence it is important to maximize the efficiency of labeling and detection of label.

We performed correlative double immunolabeling on 70nm etched Epon sections of skeletal muscle tissue. The EM labels consisted of 6nm colloidal gold (cAu_6) particles conjugated directly to mouse anti myosin primary Ab along with 6nm colloidal palladium (cPd_6) particles conjugated directly to goat anti alpha actinin primary Ab [4]. The colloidal metal labels were distinguished by Electron Spectroscopic Imaging (ESI). For detection by LM, the sections were labeled with the primary Ab-cAu_6 conjugates followed by donkey anti mouse IgG conjugated to Cy3, which has an emission at 552 nm, along with donkey anti goat IgG conjugated to Cy2, which has an emission maximum at 492 nm. The conjugation of the small gold markers to primary Ab provides the highest level of spatial and quantitative resolution relative to epitope localization by EM. Fluorescent secondary Ab amplifies the fluorescent signal and separates the fluorophores a sufficient distance from the cAu particles to minimize quenching, yet still provide resolution well within LM limits. Sections were observed via LM using a Zeiss Axiovert 200M with an Axiocam CCD camera and either Cy2 (ex: 470±20nm) or Cy3 (ex: 546±12nm) filters.

In LM, the broad band pattern of the Cy3 signal reflects the distribution of myosin in the A band of muscle tissue (Fig.2A). In contrast, a narrow band pattern of the Cy2 signal corresponds to the well known presence of alpha actinin in the Z lines (Fig.2B). In EM, two different metals (cAu and cPd) were distinguished by ESI using a Leo 912 energy filtering TEM equipped with an Omega filter [5]. The images were formed using inelastically scattered electrons of specific energy losses which associated with certain elemental compositions. The three window method was used for extrapolation of background, with two images taken at energy losses before and one image at the maxima of Au and Pd.

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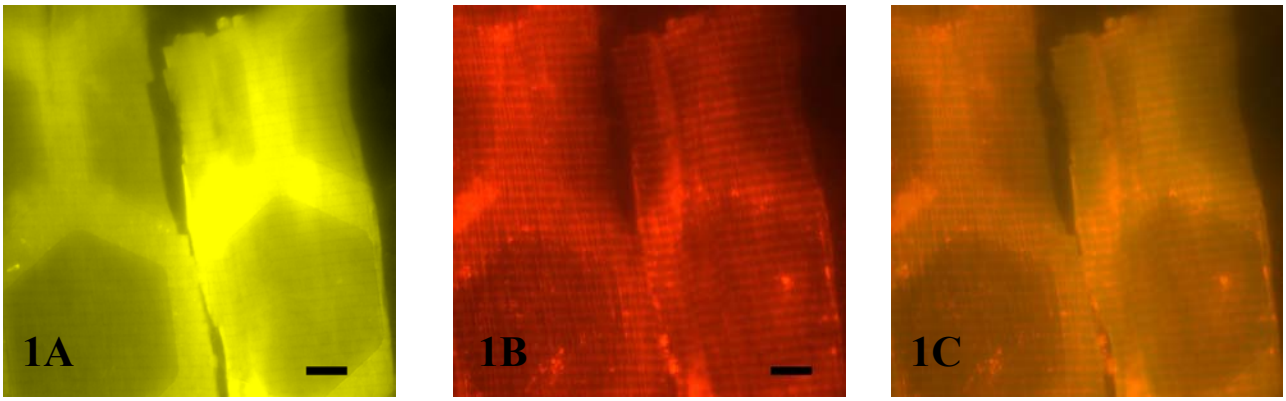

Figure1. Light microscopy studies of rat skeleton muscle tissue Epon sections incubated by two different labels. On the same area, **1A** shows fluorescent signal from mouse anti myosin-cAu_6 labeled by secondary antibody conjugated toCy3, **1B** shows fluorescent signal from goat anti alpha actinin-cPd_6 labeled by secondary antibody conjugated to Cy2 and **1C** shows merged images from 1A and 1B. Scale bar represents 10 μm.

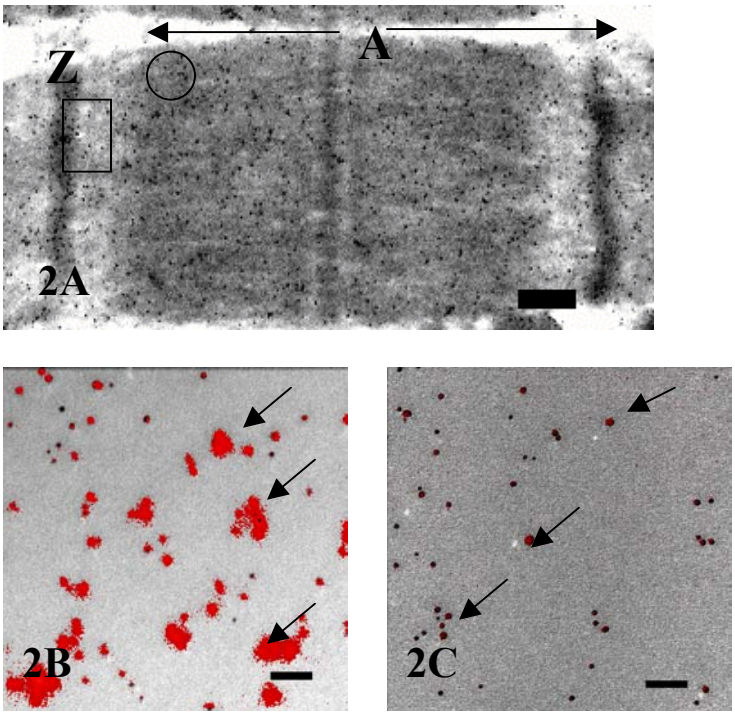

Figure **2A**. Electron micrographs of rat skeleton muscle tissue embedded in Epon labeled by cPd conjugated to anti alpha actinin and cAu conjugated to anti myosin. Scale bar represents 100nm. **2B** Elemental map showing Au distribution (arrows, taken from circle area of figure 2A) using three window method in which images were taken at 65 eV, $Au_{O2,3}$ specific energy loss maximum, subtracted by the background extrapolated from energy losses at 35 and 46eV. Scale bar represents 50nm. **2C**. Elemental distribution of cPd over Z line (taken from rectangular area of figure 2A) in which images were taken at 420eV, Pd $_{M4,5}$ specific energy loss maximum, subtracted by the background extrapolated from energy loss at 293 and 324 eV. Scale bar represents 50nm.

https://doi.org/10.1017/S1431927605502368 Published online by Cambridge University Press