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Correlative EFTEM, STEM and Fluorescence Microscopy as a Tool for Chromatin Biology

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Understanding mechanisms that regulate gene activity is essential for developing tools to help prevent and treat human disease. In eukaryotes, expression of genes is highly coordinated and requires sophisticated levels of regulation. One such mechanism involves the spatial and temporal organization of genes and their associated regulatory sequences in higher-order chromatin domains. Chromatin insulators [1], specific gene regulatory elements, form large nucleoprotein complexes known as insulator bodies and are thought to influence the organization of higher-order chromatin domains. In order to test current models of insulator function and provide ultrastructural information about these chromatin based domains, we use a correlative microscopy approach based on light microscopy and energy filtered transmission electron microscopy (EFTEM) [2], and further introduce scanning transmission electron microscopy (STEM) to localize specific protein complexes on a nanoscale.

We explore the ultrastructure of the well-studied *Drosophila melanogaster gypsy* chromatin insulator by immunolabeling a key insulator protein CP190 using a fluoronanogold conjugated antibody probe. First the cells are chemically fixed and embedded in plastic. Then thin sections of 50 nm to 100 nm are collected on locator copper grids to be examined by all three microscopy techniques. In our correlative method, fluorescent imaging is initially performed to identify nuclei that contain insulator bodies, which are rare within thin sections. A comparison of low-magnification EM image of a whole cell with the corresponding fluorescent image reveals the approximate location of the structure of interest (arrow in Fig. 1a). The fluorescence signal observed by light microscope guarantees the presence of the conjugated nanogold, which can be visualized using STEM [3], and used to locate precisely the labeled CP190 proteins. EFTEM [4] is then performed to image the distribution of nitrogen and phosphorus and thus map the distributions of protein and nucleic acid. Figures 1b and 1c show overlays of N (green) and P (red) elemental distributions with the STEM image (outlined with a dotted line in Fig. 1a), respectively, in which the nanogold particles labeling the insulator body complex are highlighted in white (arrows). It is evident from these two elemental maps that the insulator body contains an abundance of protein but a small quantity of nucleic acid. A clearer depiction of this is seen in Fig. 1d, in which both net N and net P maps are overlaid together with the STEM image. Even though dense chromatin surrounds the insulator body, it is difficult to determine whether the low levels of phosphorus within the insulator body structures correspond to DNA or RNA, which requires further investigation.

Our approach of correlative fluorescence, EFTEM and STEM imaging has the capacity to map labeled protein and nucleic acid complexes that are transiently or are sparsely distributed with in nuclei. We have demonstrated that this technique can provide helpful structural information about specific gene regulatory processes. We are currently extending this approach to three dimensions.

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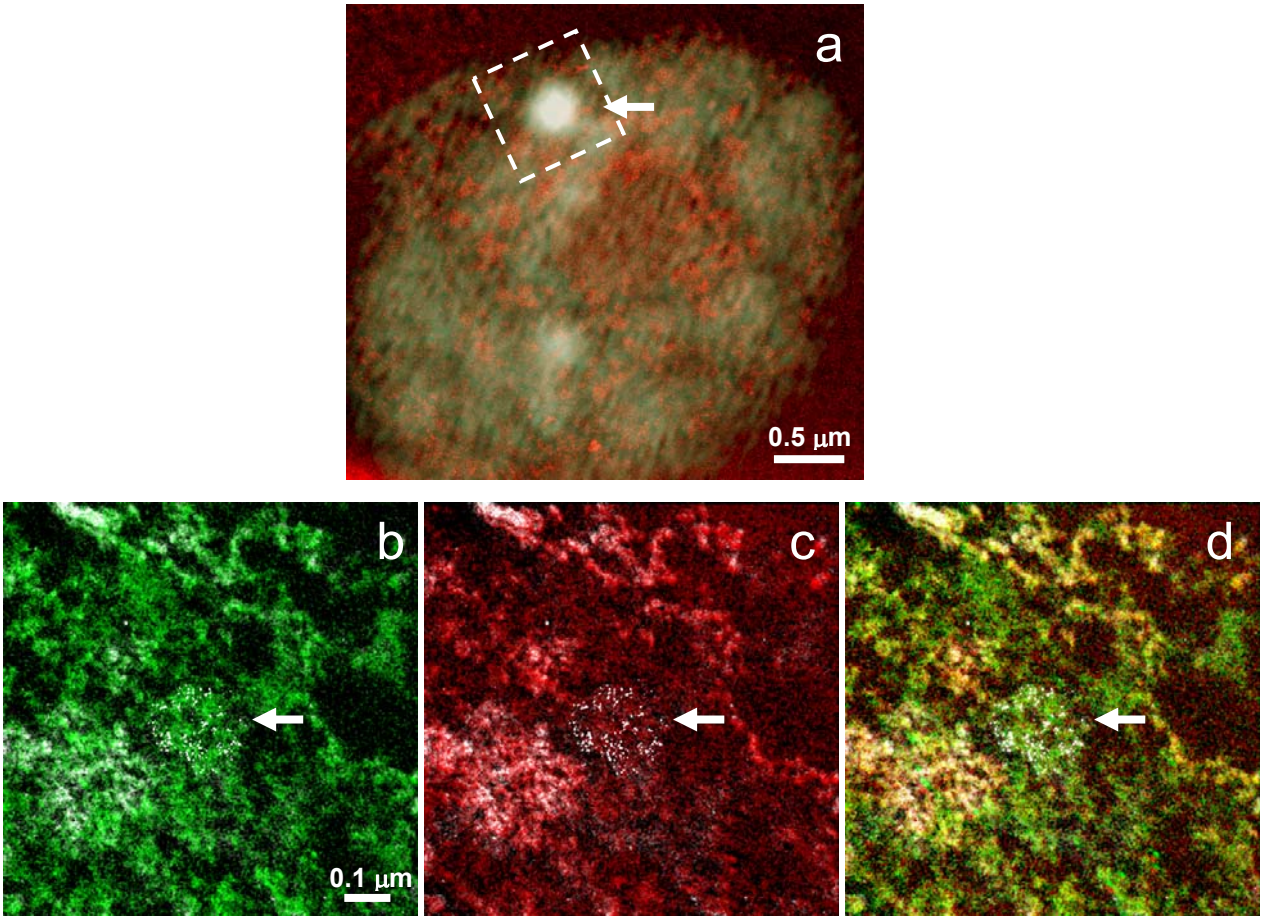

Figure 1. Correlative electron and light microscopy technique for imaging chromatin insulator bodies (white arrow) in *Drosophila* cells. (a) An overlay of fluorescent and EM images is used to approximate the location the labeled body. STEM image (white) overlaid with false colored (green) nitrogen map (b) and (red) phosphorus map (c) with flouronanogold labels localizes the labeled CP190 protein component of the insulator body. (d) P and N maps together with STEM image show that the body is primarily made of proteins and is surrounded by densely packed chromatin in the periphery of the cell nucleus. The images in (b), (c) and (d) correspond to the area outlined by the dashed line in (a).

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