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Patterns of Protein Localization Observed using Transmission Electron Microscopy do not Always Correlate with Patterns Observed using Light Microscopy

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It is gratifying when attempts to localize a particular protein of interest at the transmission electron microscopic level (TEM) correlate with the localization pattern previously observed in extensive, perhaps published, light microscopic studies (LM). However, many of us have experienced the peculiar anxiety that occurs when we first view the image formed on the screen of the TEM and witness a completely different pattern of localization. This case study examines possible reasons why patterns of protein localization at TEM and LM levels do not always match. BRD1 is a putative transcription factor that we previously localized at the LM level within the nuclei of epidermal cells of human skin using lightly fixed, cryoprotected, 6 µm thick cryostat sections employing specific antibodies against BRD1 followed by species-specific fluorescence secondary detection. In our attempts to correlate this localization pattern at the TEM level we used a close approximation of the LM protocol with lightly fixed, cryoprotected, ultrathin cryosections of the same human skin and subsequent immunogold detection [1, 2]. We found, however, that intercellular adhesion structures called desmosomes were the unexpected dominant site of gold decoration with minor labeling within nuclei. This study established that while tissues prepared solely for LM cryostat sectioning and immunolabeling consistently showed signal only in the nuclei, we could duplicate the dominant desmosomal labeling pattern at the LM level by cutting semithin (1 µm) sections of tissue prepared for ultrathin cryomicrotomy, place them on glass slides and perform routine immunofluorescence. This indicated that the change in pattern was the result of differences between the LM and TEM protocols exclusive of the mode of secondary detection. Therefore, despite our close approximation of protocols, the variables that may influence localization included: 1. Fixation chemistry (paraformaldehyde, glutaraldehyde, picric acid). 2. Cryoprotection chemistry (OCT vs. sucrose, polyvinyl pirrolidone [3]). 3. Preservative chemistry (sodium azide). 4. Section thickness (100nm, 1µm, 6µm. 5. Blocking steps (normal serum, bovine serum albumin, glycine). 6. Incubation times (1hr vs. 17hr). 7. Temperature of tissue storage and cutting (-70/-17°C vs. -196/-110°C). While we found that some variables were not testable due to technical barriers (# 4, 7), the variables we could test (# 1, 2, 3, 5, 6) revealed no evidence that would lead us to discount the TEM results as aberrant and suggested that the original LM results may be missing a key component of localization. These studies demonstrate how slight differences in methods can significantly affect immunolocalization findings and emphasize the importance of optimizing experimental conditions for the particular antigen and antibody being studied.

References

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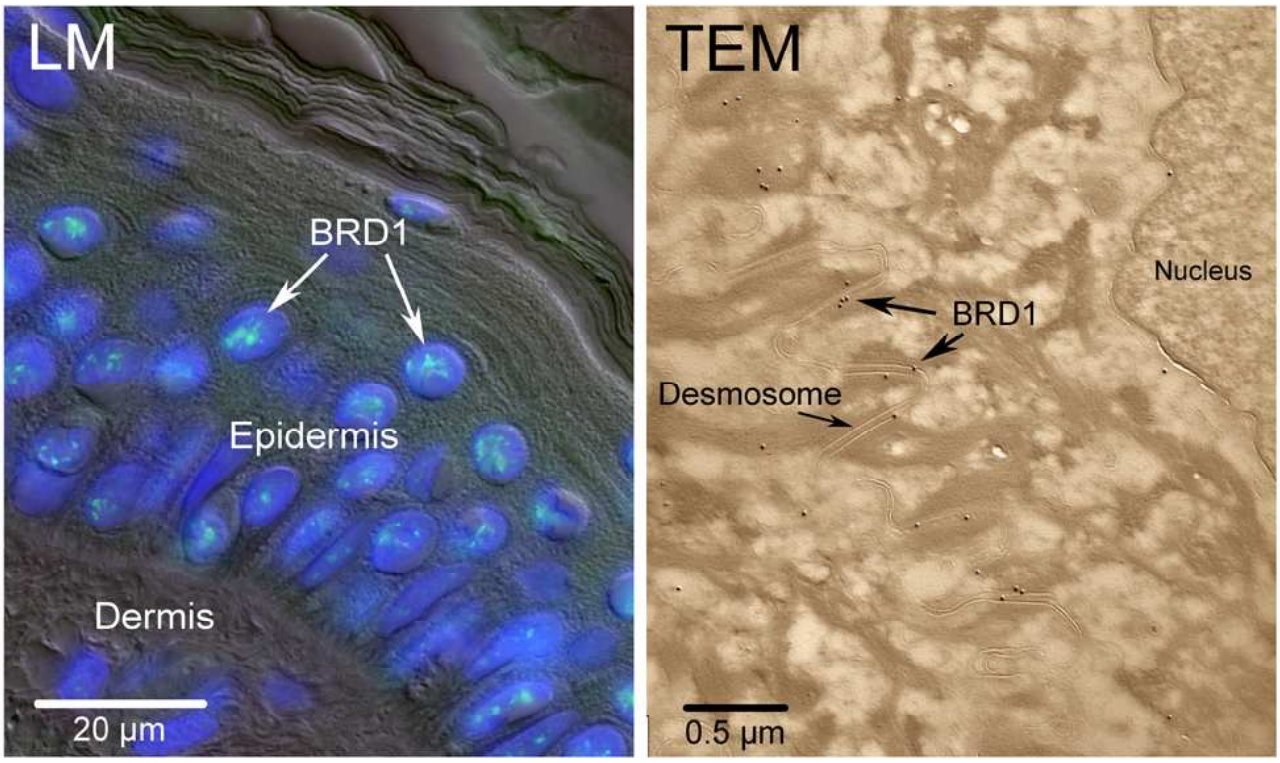
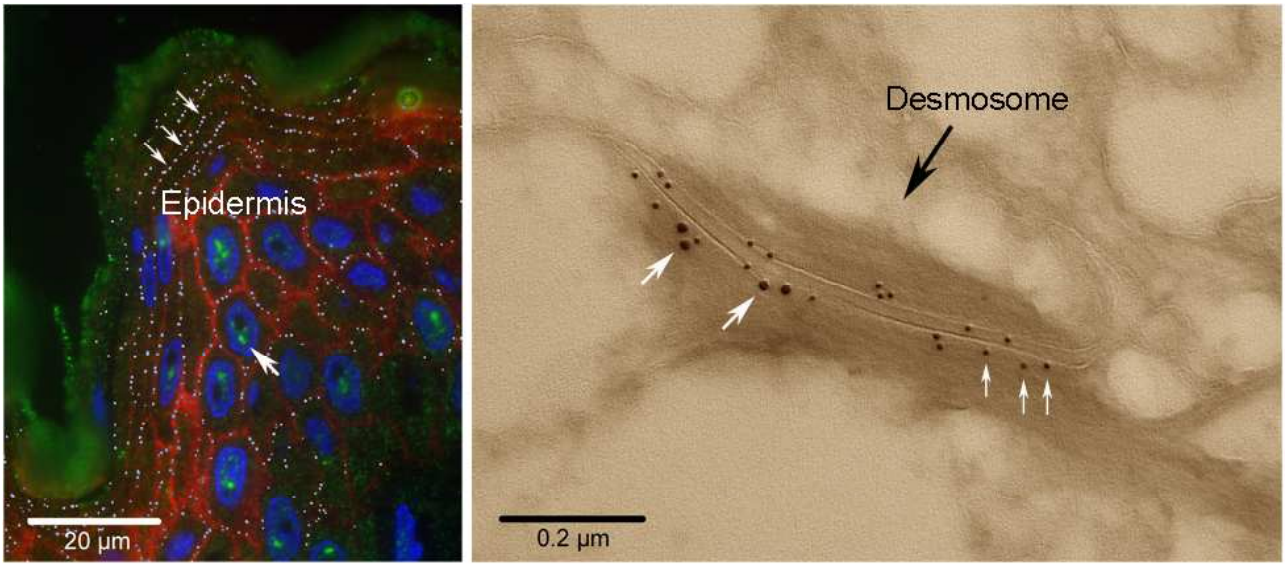


FIG. 1. Left) 6µm thick cryostat section of paraformaldehyde fixed human skin immunofluorescently labeled for BRD1 (arrows). Nuclei are visualized using DAPI blue fluorescence. Underlay of differential interference helps visualize tissue morphology. Right) 100nm thick ultrathin cryosection of paraformaldehyde fixed human skin immunogold labeled for BRD1 showing the desmosomal adhesions along the intercellular border between two epithelial cells.

Figure 2. Left) 1µm semithin section from human skin processed for ultrathin cryomicrotomy and



immunofluorescently labeled for BRD1 (green) localized both intranuclear (large arrow) and desmosomal and the desmosomal protein plakoglobin (red). Sites of maximum colocalization are shown in white (small arrows). Right) Ultrathin cryosection of same human skin showing a desmosome double labeled for BRD1 (15nm gold) and plakoglobin (10nm gold).

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