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Thin Is Better！：Ultrathin Cryosection Immunocytochemistry

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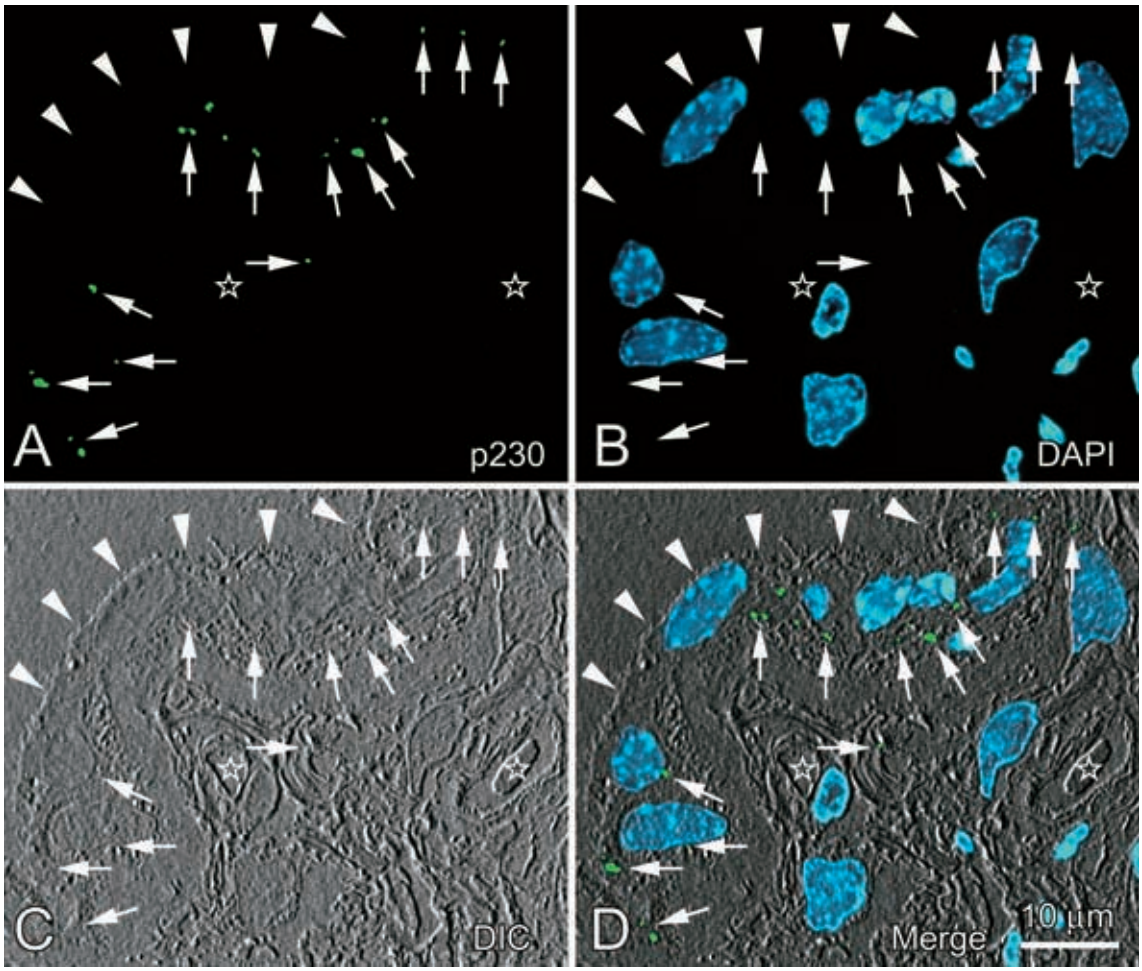

Fig. 1

Abstract

In immunofluorescence microscopy (IFM), the repression of out of focus fluorescence signal is crucial in order to obtain high-resolution images. One option to acquire high vertical resolution (z-axis resolution) is to produce optical sections with a confocal microscope. The z-axis resolution usually obtained with confocal microscopy of biological samples is about 500 nm. Another option is to produce very thin sections with a cryo-ultramicrotome (physical sections). The ultrathin cryosections we employ are about 100 nm in thickness: thus all of the fluorescence must come from within this 100 nm thickness. The use of ultrathin cryosections permits the acquisition of extremely high-quality images and minimizes the possibility for false localization in IFM (**Fig. 1**). Ultrathin cryosections can be applied to immunoelectron microscopy (IEM) as well as IFM (**Fig. 2**). We show new methods of ultrathin cryosection immunocytochemistry[1-3]. Human full-term placentas were fixed with 4% paraformaldehyde, solidified with 10% gelatin, infiltrated with 2.3 M sucrose, and then frozen in liquid nitrogen. Ultrathin cryosections were cut with a cryo-ultramicrotome and then transferred to glass cover slips for IFM or to nickel grids for IEM. Cryosections were incubated with mouse anti-p230, a *trans*-Golgi network marker, and subsequently incubated with Alexa 488-labeled goat anti-mouse IgG or with

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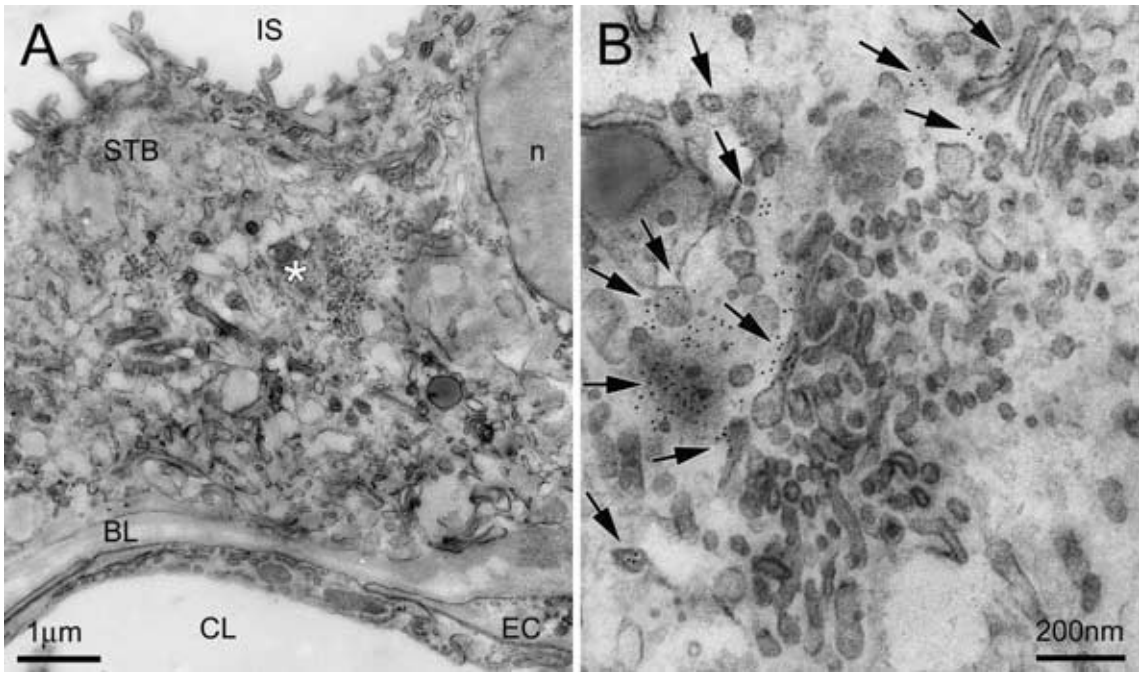

Fig. 2

goat anti-mouse 5-nm colloidal gold particles. For visualization and preservation of ultrastructure of cryosections at the electron microscopic level, the sections on grids were postfixed with ferrocyanide-reduced osmium and then stained with uranyl acetate and lead citrate in polyvinyl alcohol[1]. Ultrathin cryosection immunocytochemistry should be an important technique for functional genomics research, especially for the analysis of the *in situ* expression of target molecules[2,3].

References

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Fig. 1 High-resolution immunofluorescence microscopy detection of p230, a *trans*-Golgi network marker, on an ultrathin cryosection. (A) In a terminal villus of the placenta, fluorescence spots showing p230 (**arrows**) are mainly observed in the syncytiotrophoblast layer (STB). (B) The DAPI image of the same section shown in panel A. (C) The DIC image of the same section shown in panels A illustrates the morphology of the section. The apical portion of the STB (**arrowheads**) and the lumens of the fetal capillaries (**stars**) are evident. (D) The merged image shows the distribution of p230.

Fig. 2 Immunoelectron microscopic localization of p230 in the *trans*-Golgi network in a terminal villus of human placenta. An ultrathin cryosection of tissue fixed in 4% PFA and postfixed in ferrocyanide-reduced osmium before staining with urany acetate and lead citrate in polyvinyl alcohol. (A) A portion of the terminal villus. A Golgi complex (*) , intervillous space (IS) , STB, the nucleus (n) of the STB, basal lamina (BL), an endothelial cell (EC), and a fetal capillary lumen (CL) are evident. (B) Higher-magnification micrograph of the Golgi complex indicated with an asterisk in panel A. Five-nm gold particles demonstrating the distribution of p230 are primarily present in the *trans*-Golgi network (**arrows**). Note the well-preserved and well-contrasted membranes of the Golgi complex.