Structural Exploration of Ex Vivo Erythroblast Differentiation by Serial Block-Face Scanning Electron Microscopy and Electron Spectroscopic Imaging

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The process of erythropoiesis whereby erythrocytes, i.e., red blood cells (RBCs), are produced in the body involves continuous regeneration of erythroblasts from hematopoietic stem cells, and their subsequent differentiation into enucleated RBCs. There has been much recent interest in *ex vivo* erythropoiesis as an approach to tissue engineering of blood, which is one of the reasons why we have explored the structural changes that occur in cultured human erythroblasts during their highly orchestrated differentiation [1]. One important aspect of erythropoiesis is the enormous uptake of iron that is needed to fill the RBCs with hemoglobin, which in mammals constitutes 96% of red blood cells' dry content. Extracellular iron must be transported by transferrin receptors across the plasma membrane, and then to mitochondria for heme synthesis, before being released back to the cytoplasm where it combines with globins. During erythropoiesis, the differentiating erythroblasts must produce large quantities of heme and hemoglobin, while also maintaining sufficient cellular machinery to carry out protein synthesis. It is therefore not surprising that the iron storage protein, ferritin, has a role in erythropoiesis [2].

Here we use serial block-face scanning electron microscopy (SBF-SEM) [3] to study changes in 3-D morphology when human erythroid progenitor cells obtained by leukapheresis, are cultured *ex vivo* and undergo differentiation. We examined these cultured cells at four developmental stages, where the last stage is a erythrocyte-like cell. The SBF-SEM data in conjunction with confocal light microscopy imaging reveal a highly specialized cellular architecture with dynamic clustering of mitochondria (Fig. 1) as well as a coordinated loss of organellar and cellular volumes with hemoglobinization and terminal differentiation. Electron energy loss spectroscopy is utilized to visualize and quantitatively map ferritin iron in iron-containing vesicles, which happen to closely associate with the mitochondria at the centrosomal pole of the cells. These complementary electron microscopy methodologies provide novel and more detailed structural/functional views of erythropoiesis that have previously been possible. [4].

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4. This work was supported by the intramural research programs of NIBIB and NIDDK at the National Institutes of Health, United States.

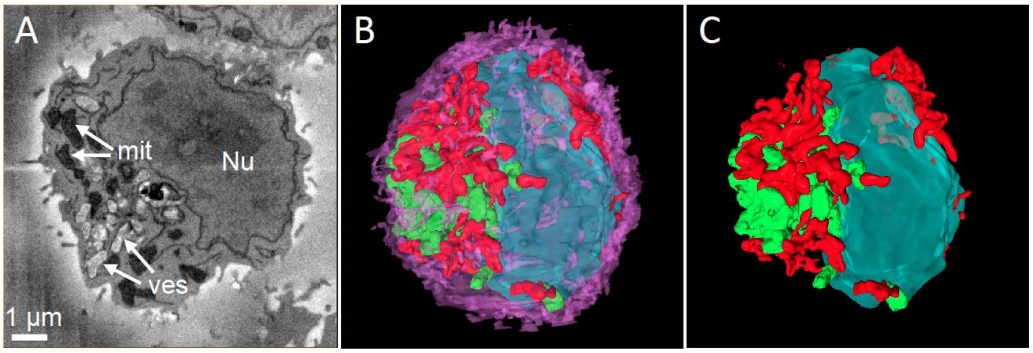

Fig. 1. SBF-SEM data from a CD36$^+$ erythroid progenitor cell after 7 days in culture: (A) an orthoslice of the cell reveals nucleus, mitochondria, and vesicular structures; (B) 3-D visualization of the same cell showing highly ruffled plasma membrane (magenta), nucleus (cyan), mitochondria (red) and vesicles (green); (C) same 3-D visualization as in (B) but with plasma membrane removed, showing tight association of vesicles and mitochondria at two poles of cell.