A systematic analysis of melanosome structure and distribution in different skin color phenotypes: identification of melanocore clusters as Lysosome Related Organelles.

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Symposium ***BIO : Tomographie cellulaire Nouvelles techniques***

The distribution of melanosomes in keratinocytes was systematically investigated, from highly, moderately and lightly pigmented human skins, classified according to their Individual Typological Angle (ITA), a representative parameter of skin color phenotype. Electron microscopy of skin samples revealed qualitatively and quantitatively that in highly pigmented skins, although melanosomes are mostly isolated and distributed throughout the entire epidermis, clusters can also be observed. In moderately and lightly pigmented skins, melanosomes are mostly present in the first layer of epidermis where they can appear isolated but for most of them, are grouped as clusters of melanocores (melanosomes devoid of membrane), embedded in an electron dense matrix and surrounded by a single limiting membrane. Electron tomography resolving the 3D organization of organelles reveals that clustered melanocores depict contacts with other organelles such as endoplasmic reticulum and mitochondria. Immunogold labeling followed by statistical analysis highlighted that clusters of melanocores do not correspond to autophagosomes or melanophagosomes but rather to non-acidic lysosome related organelles, similar to isolated melanosomes found in melanocytes. These observations enlighten the melanosome structure and fate in keratinocytes and open new avenues to understand the basis of the skin pigmentation in the different skin color phenotypes.

Use of scanning electron tomography of thick sections in the study of the ciliary apparatus in Paramecium

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Paramecium is a unicellular organism, featuring ca. 4000 motile cilia emanating from longitudinal rows of basal bodies anchored in the plasma membrane. The coordinated beating of these cilia allow the cell to swim and to feed. *Paramecium* with its technical facilities is a powerful model to study the basal body/ ciliary system, whose architecture and protein composition is highly conserved throughout the evolution, allowing an understanding of the human ciliopathies

The axoneme, displaying microtubule doublets with dynein arms, required for ciliary beating, is connected to the basal body by a transition zone (TZ) of about 160 nm height. The ultrastructural study of these different components requires the use of 3D analysis methods suitable for thick (at least 500nm) sections. Here we used Scanning transmission tomography (STET), a method of choice for thick biological specimens [1], to study the TZ structure and the distribution of dynein arms along the axoneme. The results on these two structures realised from 500nm epon sections will be presented demonstrating the interest of STET in the study of complex organels.

1. Trepout et al., Micron 77:9-15

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