The Photoreceptor Ciliary Rootlet Interacts and Provides a Support for the Endoplasmic Reticulum to Branch and Make Contact with Mitochondria

Bernardo S. Mendes[1], Michael E Cheetham[2], Thomas Burgoyne[2]

1. University College London (UCL), Gower St, London WC1E 6BT
2. UCL Institute of Ophthalmology, 11-43 Bath St, London EC1V 9EL

Background

The rootlet of the photoreceptor connecting cilium spans the length of the metabolically active inner segment (IS) and anchors the light-sensitive outer segment (OS) to the remainder of the photoreceptor. We have discovered that the endoplasmic reticulum (ER) surrounds the rootlet and hypothesise that the rootlet acts as scaffold for the ER to run through the IS and network with other organelles.

Methods

- Immunofluorescence antibody labelling of the ER, mitochondria, and rootlet was performed to investigate associations between these structures.
- Electron tomography based high resolution 3D imaging, alongside immunoelectron microscopy labelling with an anti-KDEL antibody was used to assess association of ER with the rootlet.
- Transmission electron microscopy (TEM) and serial block-face scanning electron microscopy (SBFSEM) was carried out to evaluate the branching of the ER and its contacts with the rootlet and mitochondria.
- Electron tomography of human retina was conducted to identify whether these interactions existed in human photoreceptors.
- A portion of the SBFSEM data was used to train a 3D U-Net deep learning model in Microscopy Image Browser (University of Helsinki) to allow automated segmentation of ER and mitochondria within a larger dataset.

Results

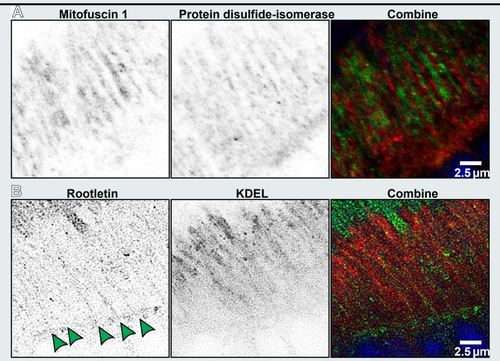


Figure 1. A. Immunofluorescence labelling of ER marker Protein Disulfide-Isomerase and mitochondria marker Mitofusin. **B.** Immunofluorescence labelling of the ER marker KDEL and rootlet marker Rootletin.

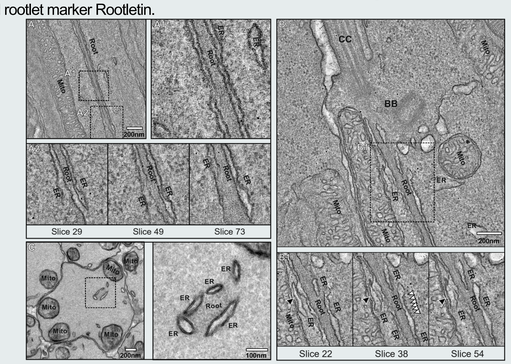


Figure 2. A & B. Electron tomography of mouse retina resolved ribosome-like structures (white arrowheads) on membranes running alongside the rootlet. **C.** Conventional TEM images of mouse retina indicating the mitochondria (mito), rootlet (root), connecting cilium (CC) and basal body (BB).

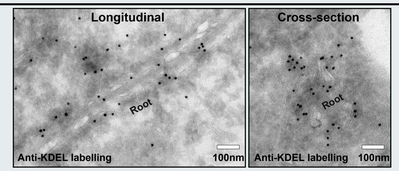


Figure 3. Immunoelectron microscopy labelling with an anti-KDEL antibody showing association of the ER with the rootlet.

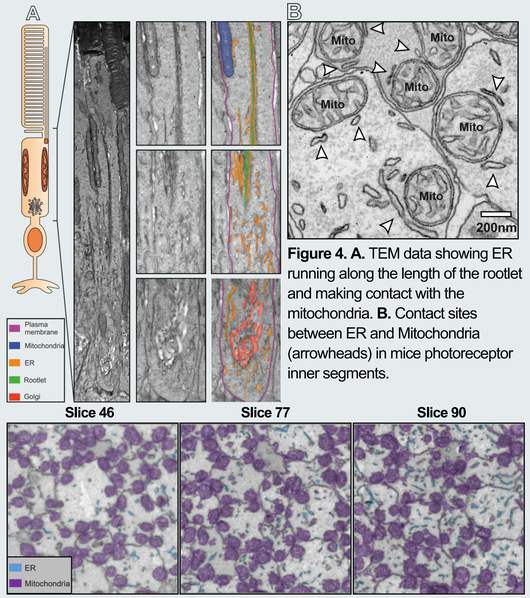


Figure 4. A. TEM data showing ER running along the length of the rootlet and making contact with the mitochondria. **B.** Contact sites between ER and Mitochondria (arrowheads) in mice photoreceptor inner segments.

Figure 5. Automated deep learning segmentation of ER and mitochondria within mouse photoreceptors imaged by SBFSEM.

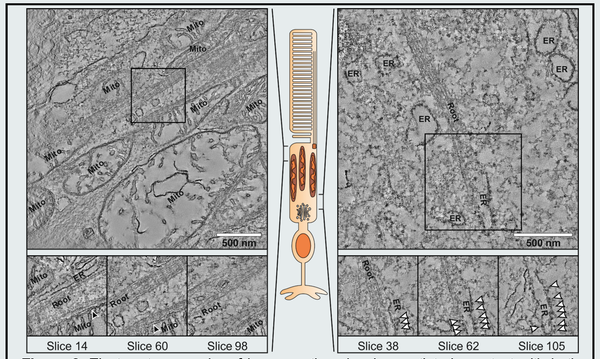


Figure 6. Electron tomography of human retina showing rootlets in contact with both mitochondria (distal inner segment) and ER (proximal inner segment).

Conclusions

- We show that the ER is associated with the rootlet running the length of the photoreceptor inner segment where it interacts with mitochondria.
- We are developing our deep learning model to enable us to determine the extent of these interactions in 3D within multiple photoreceptor IS.
- We have confirmed the presence of these interactions in the IS of photoreceptors of the human retina.
- These interactions likely exist to allow inter-organelle communication for healthy vision.
- We plan to investigate the interactions in greater depth with further animal, human and disease models.

Acknowledgments

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