BBSRC Research Project Rotations for 2013-2014

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Contributions of lysosomes in cytosolic calcium signalling

Lysosomes are best known for their roles in degrading cellular contents, but it is becoming increasingly clear that they can also contribute to intracellular calcium signalling. Calcium-permeable channels are expressed in lysosomal membranes and they probably contribute to both regulation of cytosolic calcium signals and trafficking within endo-lysosomal pathways. We have recently shown that lysosomes may also selectively sequester calcium released from the endoplasmic reticulum (ER) via inositol 1,4,5-trisphosphate receptors. The physiological significance of this calcium sequestration, the identity of the lysosomal calcium uptake pathway and the nature of the association between ER and lysosomes that allows them selectively to sequester only calcium released by the ER are entirely unknown. During this rotation project, you will use cells loaded with fluorescent calcium indicators to address these questions in single cells and cell populations.

Brailoiu, E, Rahman, T, Churamani, D. Prole, DL, Brailoiu, GC, Taylor, CW & Patel, S (2010) An NAADP-gated two-pore channel targeted to the plasma membrane uncouples triggering from amplifying Ca^{2+} signals. *J. Biol. Chem*. **285**, 38511-38516.

Learning outcomes
You will gain practical experience with cell culture and use of fluorescent indicators in both single-cell and high-throughput formats. Through background reading, lab meetings, and interactions with others in the lab, you will be exposed to a variety of advanced techniques applied to calcium signalling and be expected to become familiar with the basic features of lysosomes and calcium signalling pathways.

The role of IP_3 receptors in controlling migration of glial cells

Gliomas are the commonest brain tumour and among the most challenging to treat, partly due to their invasion of local white matter. Migration is critical for glioma cell invasion. However, the molecular mechanisms are poorly understood. In other cells, localized Ca^{2+} signals at the leading edge of the cell triggered by transient receptor potential melastatin 7 (TRPM7) and amplified by type 2 IP_3 receptors (IP_3R2) are involved in directed cell migration (Wei *et el.*, 2009). Our work with rat C6 glioma cells suggests a role for local Ca^{2+} transients at the leading edge as glioma cells move towards epidermal growth factor (EGF). C6 cells express all three IP_3R subtypes, but IP_3R2 comprises only ~2% of all IP_3Rs. IP_3R2s are concentrated at the leading edge of polarised C6 cells, where they rapidly move in vesicles. Reducing the expression of IP_3R2 using siRNA reduces the speed of migration. You will use quantitative western blot and qPCR to quantify expression of IP_3R subtypes in human glioma cells (U87MG) and to establish the effectiveness of siRNAs. You will use chemotaxis and invasion assays to assess the effects of each IP_3R subtype on migration.

Giese, A, Bjerkvig, R, Berens, ME & Westphal, M (2003) Cost of migration: invasion of malignant gliomas and implications for treatment. *J Clin Oncol* **21**, 1624-1636.
Wei, C. *et al.* (2009) Calcium flickers steer cell migration. *Nature* **457**, 901-905.

Learning outcomes
You will gain practical experience with cell culture, molecular biology, transfection and assays for cell migration. Through background reading, lab meetings, and interactions with others in the lab, you will be exposed to a variety of advanced techniques applied to calcium signalling and migration, and be expected to become familiar with the basic features of migration and calcium signalling pathways.