

Membrane Traffic in Disease and Novel Therapies

Research Project

Among the critical players in defining membrane identity and function are Rab GTPases. More than 60 Rabs have been identified in mammalian cells and each one exhibits a specific subcellular localisation. Upon activation by binding GTP, Rabs recruit effector proteins such as molecular motors, enzymes (e.g. PI-3 kinase) and membrane fusion factors, thus conferring specific functions to their target organelles. We are interested in membrane traffic and in particular in the role of Rab GTPases and their interacting partners in the control of vesicle trafficking and organelle motility.

These processes are relevant to many diseases, genetic and acquired. Our approach is to combine fundamental and pathogenesis studies as we believe that each aspect reinforces the other. Therefore, we work on cellular pathogenetic processes that involve dysfunction of intracellular membrane traffic pathways as follows:

1) Host/pathogen interactions in malaria

We are interested in understanding the molecular mechanisms of *Plasmodium* parasite invasion in host cells. The first important host-cell interaction in malaria infection is between the sporozoite and liver cells. We plan to dissect the mechanism by which the sporozoite induces the formation of a parasitophorous vacuole membrane, where the sporozoite develops into thousands of merozoites which eventually rupture the infected cell. Important questions to investigate include: Where does the parasitophorous vacuolar membrane derive from? The host, the parasite, or both? What are the components (proteins and lipids) present in this membrane? How is the vacuole transported to the centre of the cell? What are the optimal conditions inside the vacuole that allow for parasite growth? How can the process of parasite invasion/growth and vacuole maturation be interrupted?

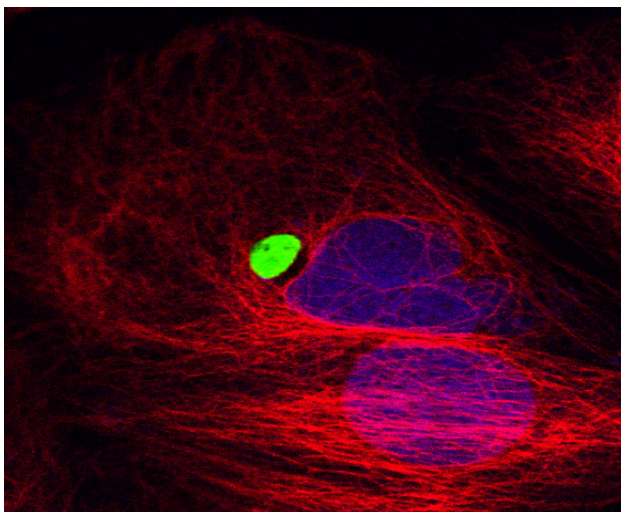


Figure 1 *Plasmodium bergeri* sporozoite inside a liver cell, 24 hours after infection, showing the parasite (green), the host microtubules (red) and the host nucleus (blue).

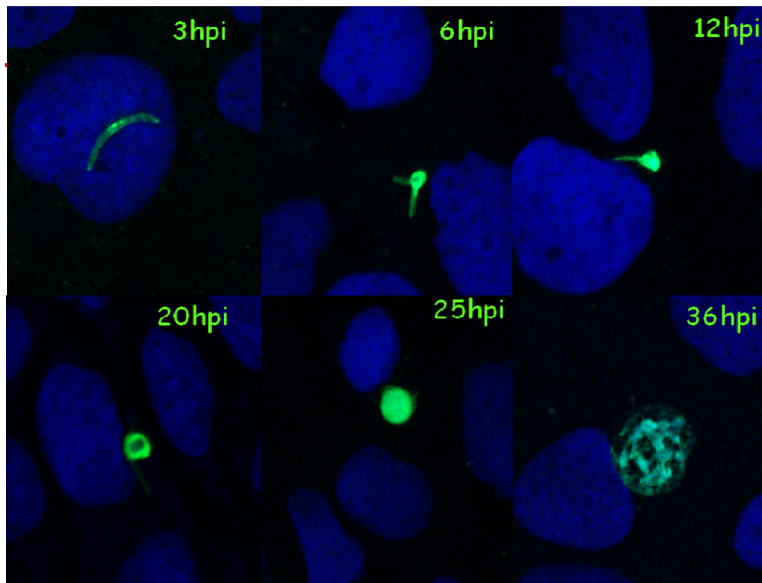


Figure 2 *Plasmodium bergi* sporozoites inside liver cells. Series of images showing the morphological modifications and maturation process associated with growth during this life stage of the parasite (green) inside the liver host cell (host cell nucleus in blue). (hpi= hours post injection).

2) Membrane traffic, retinal pigment epithelium and retinal degeneration

wild type

CHM model

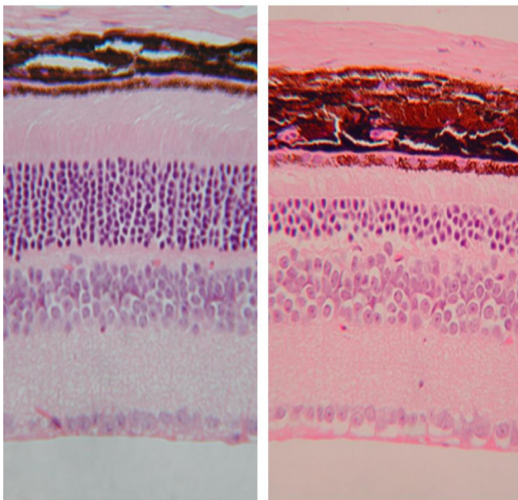


Figure 3. Immunohistochemical analysis of retina taken from wild type and CHM model mice.

We are interested in characterising intracellular trafficking pathways of the retinal pigment epithelium (RPE). The RPE is a fascinating polarized epithelial layer that remains poorly understood despite its great importance to retinal function and its involvement in common blinding diseases, such as age-related macular degeneration. On the fundamental side, we wish to understand the involvement of Rab proteins in growth factor secretion, namely VEGF (Vascular Endothelium Growth Factor) by the RPE. VEGF and other growth factors are important players regulating the interactions between cellular layers in the retina. Another objective is to study RPE phagocytosis and endocytosis in collaboration with Clare Futter at the Institute of Ophthalmology in London. We plan to study these topics in vitro using

primary cell culture of mouse RPE and in vivo using mouse models of Rab dysfunction (see below).

Several inherited human disorders have been associated with defects in Rab protein activity, either directly or indirectly. One example is Choroideremia (CHM), an X-linked late-onset retinal degeneration characterised by progressive dystrophy of photoreceptors, RPE and the choroid. Choroideremia is due to a defect in Rab Escort Protein 1 (REP1), a cofactor required for the prenylation of Rab proteins (see below).

3) Novel Therapies

We have recently generated a mouse model for Choroideremia using conditional gene knockout (cre-lox) technology. We have shown that the disease is cell autonomous, with independent degeneration of photoreceptors and retinal pigment epithelial cells. We are using the mouse model to further investigate the pathogenesis of the disease and also to perform pre-clinical gene therapy studies that could lead to a possible cure for Choroideremia patients.

4) Rabs and membrane traffic

Every cell uses constitutive exocytosis or secretion to constantly deliver synthesized plasma membrane proteins to the cell surface or to release proteins to the extracellular space where needed. Exocytic protein traffic in exocrine, endocrine and hematopoietic cells further includes one or more regulated pathways. Here, synthesized mediators such as proteins, peptides and neurotransmitters are stored in specific organelles, and cargo release is usually triggered by external stimuli. Besides, basal component release from the storage organelles, i.e. release in the absence of stimulus, may occur. In addition to these classical secretory pathways, other routes of cellular protein export - e.g. exocytic traffic through recycling endosomes - are described.

Rab GTPases regulate intracellular membrane traffic. A decade ago, we discovered Rab27 as a protein implicated in diseases of membrane traffic (see more below). We found that Rab27 associates with regulated secretory granules in endocrine, exocrine and lysosome-related organelles in melanocytes and hematopoietic cells. Rab27a appears to behave as a maturation sensor, associating only with mature granules and regulating their motility and tethering with the plasma membrane during exocytosis. In melanocytes, Rab27 associates with Melanophilin and MyosinVa and tethers melanosomes to the peripheral actin cytoskeleton. In addition, Rab27 appears to play other roles, such as recruiting Munc13-4, a protein involved in membrane fusion. We are currently investigating the precise function of Rab27a in a variety of medically-relevant secretory cells, such as RPE, mast cells and platelets. Involvement of Rabs in constitutive secretion remains less well investigated. Therefore, we are currently investigating the role Rab proteins play in constitutive secretion and the interplay with regulated secretion in more detail.

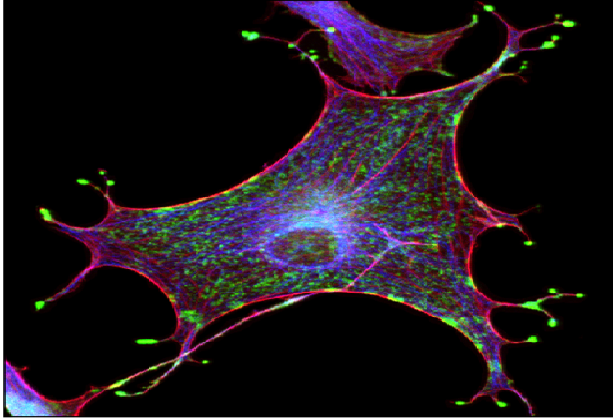


Figure 4. Confocal image of a mouse melanocyte. Rab27a is labelled in green, actin in red and tubulin in blue.

Many intracellular bacteria, fungal and protozoan parasites like *Plasmodium* are engulfed by their host cell, and reside within a membrane-bound vacuole or phagosome. As part of the host defence against pathogens, these organelles are acidified and fuse with lysosomes. Rab proteins, which are GTPase regulators of the endocytic and exocytic pathways, play an important role in host responses to parasites and simultaneously might be targeted by the pathogen as part of its strategy to subvert the host responses. Controlling Rab function through pharmacological or genetic modulation could result in important new therapies. In addition to mediating the uptake of nutrients, endo/phagosomes are also essential for switching on the adaptive immune response towards extracellular and intracellular pathogens. Extracellular antigens are taken up by antigen presenting cells (APCs) (B cells, macrophages and dendritic cells) and processed into peptides within the so called MHC class II compartment. Another important and unique feature is how highly regulated the traffic of MHC-II molecules is from lysosomal compartments to the plasma membrane. Intracellular trafficking of membrane proteins requires a complex of proteins that include members of the Rab family. To date little has been done to examine the function of Rab proteins in the endocytic pathway of APCs. We are interested in the study of the role of Rab proteins on parasite uptake (see also above) and also their involvement on the process of antigen presentation by APCs.

5) Molecular Basis of Membrane Identity: Lipid Modifications of Rab GTPases and Membrane Targeting

Lipid modification is essential for proper membrane association and function of Rab proteins. Rab proteins are prenylated by the enzyme Rab geranylgeranyl transferase (RGGT), this reaction requiring an accessory molecule named Rab Escort Protein (REP). We are studying the biochemistry and structural determinants of complex formation in the prenylation reaction, structural determinants for the Rab interaction with effectors and the mechanisms of membrane targeting and delivery of Rab proteins using *in vitro* assays and cellular studies.