

Autophagy – eating your way out of trouble!

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All eukaryotic cells degrade (or turnover) parts of their internal structure including organelles such as mitochondria by a process called autophagy ("self eating") that occurs in a specialized compartment of cells - the vacuole (in yeast) or the lysosome (in mammals). In yeast, autophagy is mainly involved in cellular homeostasis (removal of damaged organelles) and adaptation to starvation, but in multicellular organisms (mammals) it is involved in a variety of additional processes such as programmed cell death and development of different tissue-specific functions.

Alterations in the levels of autophagy are linked to a growing number of pathological conditions including neurodegenerative diseases such Parkinson's, myopathies such as cardiomyopathic Danon's disease, and some forms of cancer.

Current work

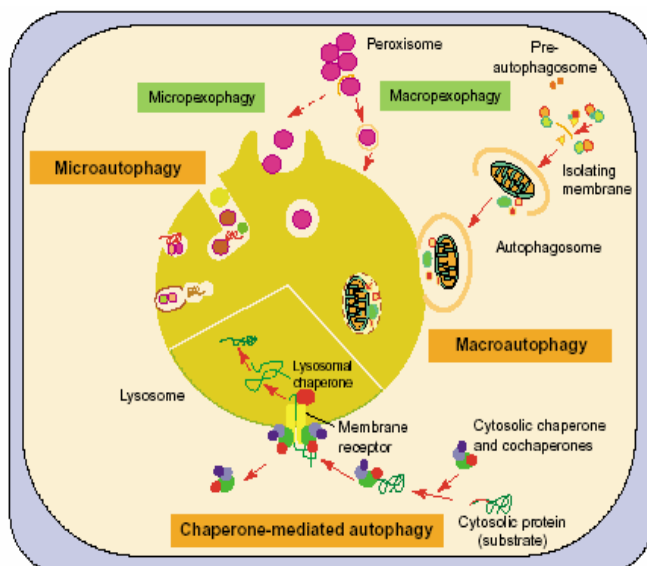
Autophagy as a host-cell response to bacterial infection.

Successful microbial pathogens have evolved strategies to avoid or subvert the autophagy process thereby ensuring their survival within cells. Together with colleagues in the ARC Centre of Excellence in Structural and Functional Microbial Genomics (see <http://www.microbialgenomics.net/People/Devenish.html>) we are looking at the molecular mechanisms by which micro-organism can achieve the avoidance or subversion of autophagy. In this context we will be studying the infection of human cells by the soil bacterium, *Burkholderia pseudomallei*, leading to Melioidosis which is prevalent in tropical regions. Following infection of cells the bacteria persist within the cell in a membrane bound compartment (vacuole) that does not fuse with lysosomes or acidify (and which otherwise could lead to the destruction of the bacteria). Presumably various bacterial proteins act as effectors that interact with host cell trafficking factor(s) and contribute to modulation of normal host cell biology. We are devising screens to identify such effector proteins that alter eukaryotic cell trafficking pathways, by taking advantage of the knowledge of these pathways in yeast.

The turnover of mitochondria by autophagy presumably serves as a means of quality control for mitochondrial function. Mechanistically distinct forms of autophagy have been identified (see figure). However, the molecular details and regulation of these processes and how they relate to organelle turnover are only now becoming understood. We are using fluorescent protein technology, together with a range of other biochemical and molecular techniques, in yeast and mammalian cells to monitor the influence of mitochondrial morphology and bioenergetic function on turnover. This approach will provide new insights into the pathways and molecular mechanisms by which organelle autophagy may occur.

Figure (below). Autophagy comes in different flavours.

Mitochondria (right-hand side) are shown targeted for degradation in the mammalian lysosome by macroautophagy. Other possible pathways are microautophagy, and chaperone-mediated autophagy (in mammalian, but not yeast cells).



Autophagy in human embryonic stem cells.

Human Embryonic Stem cells (hESC's) hold great promise as a renewable source of cells for future use in research and regenerative medicine. They can be grown indefinitely in an undifferentiated state, but are also capable of differentiating into all cell types of the adult body. Autophagy is an essential part of

growth regulation and maintenance of homeostasis in multicellular organisms. Together with collaborators in the Australian Stem Cell Centre we will be investigating autophagy in hESCs and its potential modulation during the differentiation process in vitro.

Project Areas

1. How autophagy can be avoided or subverted in microbial infection of mammalian cells.
2. Autophagy in disease, focusing on mitochondrial turnover.
3. Autophagy in embryonic stem cells.

Bringing light and colour to research in the life-sciences. Fluorescent proteins and chromoproteins.

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Coral reefs are vital global ecosystems. There is increasing concern about the increased frequency of coral bleaching events occurring in coral reef ecosystems around the world. Bleaching involves the loss of endosymbiotic photosynthetic microalgae from the coral host tissue. Given global climate trends it can be predicted that reef-building corals (scleractinian corals) will not survive into future decades, with significant economic and environmental consequences.

The vivid and diverse colours for which reef-building corals are renowned result from host-based pigmentation. These pigments or chromoproteins (CPs) are generally found in the branch tips or surfaces of coral colonies where light levels are the highest. The intensely coloured pink and blue pigments of two families of scleractinian corals have previously been partially characterised and described as pocilloporins. A study of pocilloporins revealed they exhibit in different species a broad range of spectral properties and possess multiple photo-protective functions. Recent studies demonstrate they protect the photosystems of their resident microalgae from high amplitude light fluctuations that can lead to severe photoinhibition.

In addition to their role in vivo CPs and their close homologues, fluorescent proteins (FPs) also found in coral and other marine organisms represent important biotechnological tools whose use has revolutionized research in the life-sciences. FP technology allows a vast range of different events inside the living cell to be visualised in a way that cannot be achieved with any other currently available technologies. The fluorescence properties of particular proteins can be reversibly 'switched' on and off with light making them useful as 'optical highlighters' and the basis for optical data storage systems. Access to FPs with novel properties, therefore, is of considerable interest to the scientific community in general.

The chromophore responsible for light absorption and the fluorescence properties in CPs and FPs arises from an extended conjugated π -system that consists of a cyclic tripeptide structure. This chromophore forms inside the characteristic 11-stranded β -barrel structure of CPs and FPs (see figure below left) as a result of the covalent rearrangement of three consecutive amino acids.

Our aim is twofold: (a) to understand the structure and formation of the protein chromophore in relation to the many different and unique spectral properties of this family of proteins (see picture below right, showing some of the colours available), and (b) and to engineer proteins for novel biotechnology applications. Thus, we are investigating several aspects of these colourful and intriguing proteins using a powerful interdisciplinary approach.

1. With our collaborators (*Dr Sophie Dove and Professor Ove Hoegh-Guldberg, Centre for*

Marine Studies, University of Queensland) we are studying how these light absorbing pigments regulate light conditions *in vivo* under constantly changing lighting conditions to which corals are subjected. (2) In conjunction with *Dr. Jamie Rossjohn of the Departmental Protein Crystallography unit* we are determining the 3D structure of a range of CPs and FPs. Fluorescent protein crystals used to determine X-ray crystal structures are shown below. The characteristic light-absorbing and light-emitting chromophore is located inside the protein 'barrel'. This important structural information allows us to understand the formation of the all-protein chromophore and their resultant spectral (absorption and fluorescence) properties. (3) In conjunction with *Prof. Sean Smith from the Centre for Computational Molecular Science, University of Queensland* we are using supercomputers to help model the properties of chromophores in CPs and FPs from first principles. This fundamental approach will help in the rational design of novel proteins with useful properties. (4) We are investigating the use of certain CPs as a basis for developing FPs with novel properties. This involves the isolation of new pigments from corals and/or alteration the spectral properties of these proteins using mutagenesis.

Project Areas

1. Engineering and characterization of CPs and FPs having novel and useful properties.
3. Crystallization and structure determination of fluorescent protein variants.
3. Isolation from natural sources of CPs and FPs with novel properties.
4. The application of CPs and FPs in Fluorescence Life Time Imaging to study protein-protein interactions in live cells.
5. Investigating the photo-protection role of coral pigments.

