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SUSTAINABILITY FROM A CELL PERSPECTIVE

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ABSTRACT: Sustainability is a worldwide problem, for cells as it is for human beings and our whole planet. The key to solving the sustainability problem is recycling. In cells, this is accomplished within the lysosome. All biological macromolecules, such as proteins and nucleic acids, which are past their use-by-date, are transported into the lysosome, where enzymes degrade them into their monomeric building blocks. These enzymes are only active at the low pH of ~4.7 of the lysosome lumen. After degradation, the building blocks are transported out of the lysosome into the cytoplasm, where they are used for the biosynthesis of new proteins and nucleic acids. Because of the need to encapsulate the degrading enzymes within the lysosome to avoid damage to functionally active proteins and nucleic acids, the lysosome membrane is a powerhouse of solute transport within the cell. This recycling process is crucial for the survival of cells, particularly neurons, which are no longer capable of cell division. Defects in this process cause many hereditary neurodegenerative diseases. But because of their rarity, pharmaceutical companies are not particularly interested in finding cures. However, the diagnosis of neurodegenerative disease is devastating for the family of any afflicted child. One group of neurodegenerative diseases is termed Batten disease. While symptoms vary, all are caused by lysosomal dysfunction, resulting in the steady build-up of waste material in the cell. Disease progression is typically characterised by the development of blindness, followed by the onset of seizures and early death, typically in the twenties. Understanding the disease would not only help its sufferers but also provide valuable information on a crucial cell function, i.e. how cells recycle their waste. We will describe how biophysical measurements are helping to provide some insights into the molecular physiology of lysosome function and the pathology of Batten disease.

Keywords: lysosome, Batten disease, membrane dipole potential, solid-supported membrane, electrophysiology

INTRODUCTION

The key to sustainable living is recycling. In the long term our planet and our species cannot survive if we continue to rely on non-renewable supplies of resources and energy. This is becoming increasingly obvious, realised by the general public, and even by the majority of politicians. But this realisation has only come in the last few years, accelerated by the increasing frequency of extreme bushfires and floods. In comparison, Nature has known this to be true and has been practising recycling for millions of years. Almost every eukaryotic cell, including human cells, contains cell organelles known as lysosomes. These are microscopic recycling depots, where biological macromolecules, such as proteins, nucleic acids, polysaccharides and lipids which have reached their 'use-by', date are broken down into smaller building blocks, so that these can be recycled for the synthesis of new macromolecules.

LYSOSOME FUNCTION

The recycling function of lysosomes is a vital part of any eukaryotic organism's survival. However, how the lysosome carries out its recycling activity is still incompletely understood. This is due to the multitude of processes occurring and their complexity. Nevertheless, here the basic principles are presented.

Lysosomes can be considered as the cell equivalent of a stomach, i.e. the site of digestion of old or defective macromolecules. These are sequestered from the rest of the cell cytoplasm within autophagosomes by a process known as autophagy. The autophagosomes subsequently fuse with a lysosome to allow the digestion of its contents. In the stomach the digestion of protein occurs via the enzyme pepsin, which is only active under acidic conditions. The low pH of the stomach is created by a proton pump or H^+,K^+-ATPase, embedded in the plasma membrane of parietal cells lining the stomach wall. A similar situation occurs in the lysosome. H^+ ions are pumped into the lysosome by a V-type H^+-ATPase. This creates an acidic environment within the lysosome with a pH of 4.5–5. Also similar to the stomach, the proteases which degrade proteins within the lysosome are only active at this pH. This is a crucial point. If the proteases were active at neutral pH, they would degrade proteins within the cell cytoplasm before they reached the lysosome, leading to major cell damage and probably cell death. The low pH of the lysosome ensures that degradation only occurs where it is required, inside the lysosome.

Once macromolecules within the lysosome have been degraded, the resulting building blocks, such as amino acids, simple sugars, nucleosides and the breakdown products

of lipids, must be transported back into the cytoplasm, so that they can be used in various biosynthetic pathways for the synthesis of new macromolecules. However, these small building block molecules are impermeable to the surrounding lipid membrane of the lysosome. Therefore, the lysosome membrane is packed full of a multitude of different integral membrane proteins which facilitate the transfer of these molecules across the membrane and into the cytoplasm.

BATTEN DISEASE

The importance of lysosome function becomes even clearer when one considers what happens when it malfunctions. Batten disease is the common name of a group of disorders known medically as neuronal ceroid lipofuscinoses (NCL). It is a hereditary neurodegenerative disease which can be caused by mutations in one of at least 13 different proteins. Some of these proteins are enzymes within the lysosome which are responsible for degradation processes. Others are integral membrane proteins responsible for the transport of the molecular building blocks out of the lysosome. Characteristic for all forms of the disease is the build-up of the pigment lipofuscin with the lysosome. Although all cells are in principle affected, neurons are particularly affected, because they no longer undergo cell division and cannot be replaced. Therefore, as waste material continues to accumulate within a neuron its function becomes increasingly impaired.

The most common form of Batten disease is due to a mutation in a lysosomal integral membrane protein called Battenin or CLN3, which in humans belongs to the family of atypical solute carriers. The function of this protein was only discovered last year (Laqtom et al. 2022). It is responsible for the transport of glycerophosphodiesters across the lysosome membrane. These are the building blocks of the phospholipids that make up cell and cell organelle membranes. Without glycerophosphodiesters a cell would not be able to repair any damage occurring within its membranes. Disease progression involves first vision loss, followed by the onset of seizures, intellectual impairment, and early death (usually 20–30 years). Although it is the most common form of the disease, CLN3 is still a rare disease with an occurrence of only about 1 per 140,000 births. Because of this, pharmaceutical companies are not particularly interested in carrying out research or developing a treatment. There simply isn't enough money to be made. In such circumstances the best hope for developing a drug treatment is probably to re-purpose a drug that is already on the market for the treatment of another condition. The treatment of any disease, however, is significantly enhanced by a molecular understanding of its cause. The discovery of the function of the CLN3 protein this year was, therefore, a major step forward (Laqtom et al. 2022).

Another important discovery was made in 2018 by Schulz et al., who found that the sterols carbenoxolone, 7-ketocholesterol, enoxolone and prednisolone reverse the symptoms of Batten's disease on brain cells isolated from CLN3-deficient mice (Schulz et al. 2018). The origin of this effect is still unclear, but all of these molecules have the common property that they bind to cell membranes. There appears, therefore, to be a link to the function of the CLN3 protein which is now known (Laqtom et al. 2022) to be necessary for the supply of the building blocks for synthesising the phospholipids of cell membranes.

MEMBRANE STRUCTURE AND FUNCTION

Chemistry is the science of the electron and interactions between molecules are based primarily on electrostatic forces, caused by a non-uniform distribution of electrons. A prime example is the water molecule, which is often considered as a molecular dipole which exerts an electrostatic force on neighbouring water molecules. However, liquid water is an isotropic solvent, meaning that the orientations of water molecules relative to one another are random. As such, the electric fields caused by individual water molecules cancel each other out and there is no macroscopic electric field. However, this is not the case for a biological membrane, which can be considered as an anisotropic solvent. Due to the hydrophobic effect and van der Waals forces, the lipid molecules organise themselves into a lipid bilayer, which in its headgroup region incorporates oriented water molecules. Under these circumstances of non-random distribution, the electric fields of individual molecules or functional groups do not cancel. Within the headgroup region of the membrane there is a large electric potential difference, positive in the membrane interior, called the membrane dipole potential because it arises from the preferential orientation of molecular dipoles in the headgroup region. The dipole potential produces an electric field strength of $\sim 10^9$ V m^{-1}. This is more than enough to influence the conformation and hence activity of integral membrane proteins, such as ion channels, pumps and transporters.

Previous experiments using a membrane-bound fluorescent probe sensitive to the local electric field strength have shown (Starke-Peterkovic et al. 2006) that 7-ketocholesterol, a compound which Schulz et al. (2018) found alleviated the symptoms of Batten disease in mice, significantly decreases the dipole potential in mice. This suggests that modification of the dipole potential may be a possible strategy for the treatment of Batten disease. To test this a robust method for reliably quantifying the dipole potential is required.

SURFACE ELECTROGENIC EVENT READER (SURFE²R)

It is not possible to measure the magnitude of the dipole potential directly, because this would require the insertion of electrodes at different depths within the membrane, probably not more than a nanometre apart. This is not technically feasible. Therefore, one must calculate the dipole potential indirectly from its effect on another observable quantity. One possibility is to measure the difference in the strength of membrane binding of two hydrophobic ions: tetraphenylborate and tetraphenylphosphonium. These two ions have very similar structures, except that tetraphenylborate has a negative charge and tetraphenylphosphonium has a positive charge. Therefore, any difference in their binding strengths should be caused by the dipole potential.

To follow the binding process we are planning to use an instrument from the German company Nanion GmbH, which is based in Munich. The technology on which the instrument is based was initially invented in the 1980s at the University of Constance within the group of Professor Peter Läuger, the author's host for his initial Humboldt fellowship in Germany, but the initial method used black lipid membranes, which are prone to rupture. In the late 1990s, Professor Klaus Fendler, at the Max Planck Institute of Biophysics in Frankfurt on the Main, developed the method into a much more robust instrument by combining it with solid-supported membrane technology (Tadini-Buoninsegni & Fendler 2015; Bazzone et al. 2017).

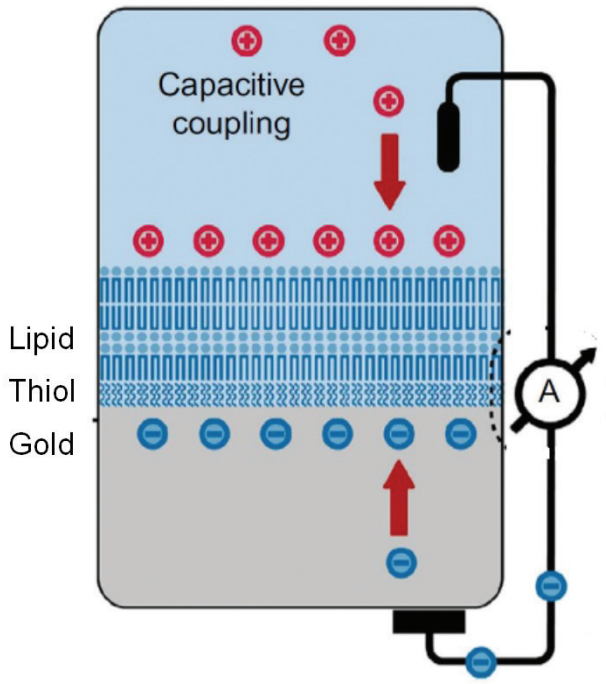

Figure 1: Principle of capacitive coupling on which the surface electrogenic event recorder (SURFE²R) from Nanion Technologies GmbH is based.

This allowed the instrument to subsequently undergo commercialisation. The technique involves tethering a lipid bilayer membrane to a gold electrode surface via an alkanethiol linkage. The gold-supported membrane structure can be considered as an electrical capacitor, so that when an ion binds to the membrane surface, this produces a compensating capacitive current within the gold substrate (Figure 1). Thus, the binding of ions causes a current pulse, and the strength of ion binding to a membrane can be determined by measuring the concentration dependence of the magnitude of the current pulses.

The instrument being installed in the author's laboratory in Sydney is the first of its type in Australia. It represents an excellent example of technology transfer between Germany and Australia and demonstrates the great benefit for Australia through collaboration both with German research institutions and German companies.

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Conflict of interest

The author declares no conflict of interest.

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