

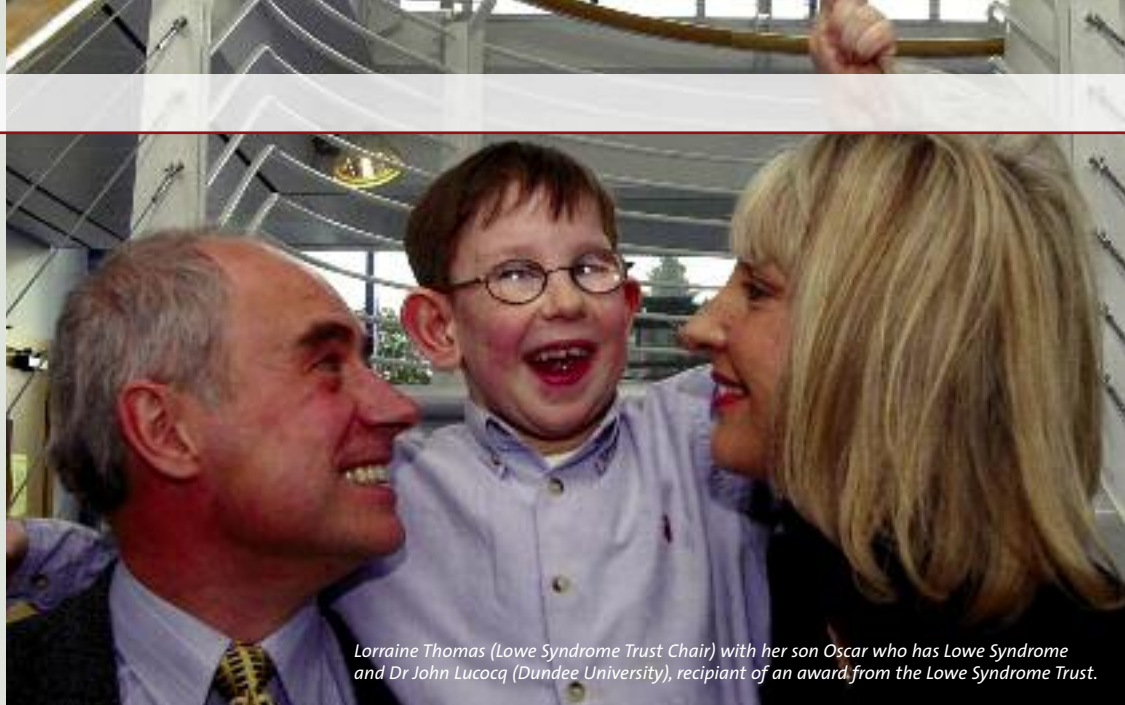


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Lorraine Thomas (Lowe Syndrome Trust Chair) with her son Oscar who has Lowe Syndrome and Dr John Lucocq (Dundee University), recipient of an award from the Lowe Syndrome Trust.

The Cell Biology of Lowe Syndrome and its Effects in the Eye

PIP₂ is a signalling lipid regulated by OCRL1

Lowe Syndrome is a rare genetic disorder that has caught a good deal of attention from cell biologists and clinical scientists, but still resists our full understanding and for which no treatment is available. From the clinical point of view, the syndrome is also known by the acronym OCRL, standing for the oculo-cerebro-renal syndrome of Lowe, which indicates the widely dispersed problems patients, all boys, face. Although symptoms are not absolutely confined to the three organs suggested by the name, the major problems are: (1) Ocular: the first symptoms are congenital cataracts. Later in childhood, half of patients develop glaucoma. Sadly this is a common finding after early surgery for bilateral cataract;¹ (2) Cerebral / CNS: developmental delay, hypotonia, behavioural abnormalities, and sometimes seizures; (3) Renal, this being the most dangerous clinically, with a tubulopathy usually detectable in infancy and progressing to life-threatening renal failure in teenage / early adulthood. This article will examine the progress that has been made in our understanding the cell biological basis of Lowe Syndrome, emphasising aspects that apply to the ocular symptoms.

Fifteen years ago a great step forward was taken with the ground-breaking work by the lab of Prof Robert Nussbaum identifying a gene on the X chromosome called OCRL1 that is not expressed in all cases Lowe Syndrome.² This codes for an enzyme that normally eliminates the highly active lipid molecule phosphatidylinositol (4,5) bisphosphate (hereafter referred to as PIP₂, Figure 1). When PIP₂ was discovered in the 1950s, it was the first lipid known to have a role in intracellular signalling. PIP₂ is found in the cytoplasmic leaflet of cellular membranes, and it is based on a more simple precursor that can be phosphorylated at three biochemically distinct positions (carbons 3, 4 and 5 of a sugar ring). Different combinations of this phosphate code produce PIP₂ and six other PIPs, each of which is now known to have unique functions (Figure 1). OCRL1 is a 5-

phosphatase enzyme, removing the phosphate at position 5. Even after 50 years, the function of PIP₂ is still the subject of intense research, and its importance is undisputed for many cellular processes. One rule that applies widely is that when a protein binds PIP₂, the protein is activated, and conversely unbinding from PIP₂ leads to inactivation. Since Lowe Syndrome was the first human genetic disorder known to affect PIP₂ directly, research into OCRL1 not only offers the chance to discover cures for patients with Lowe Syndrome, but also is an important avenue to understand the whole PIP₂ pathway.³



Oscar with Jonathan Ross, a Trustee of the Lowe Syndrome Trust, visit: www.lowetrust.com

Basic ideas about the effects of OCRL1 deficiency

The eventual goal of research into Lowe Syndrome is to gain a practical understanding of its pathogenesis and to devise cures. As with any similar medical question, the problem is knowing where to start. This has been driven by basic biochemical and cell biological research.

General – excess PIP₂: OCRL normally acts to remove PIP₂, so Lowe Syndrome is a manifestation of increased PIP₂. But this is not simply a uniform increase of PIP₂ in all parts of all cells. This is because human

cells have nine other 5-phosphatases.⁴ OCRL1 is clearly likely to be responsible for the normal destruction of just a small proportion of the total amount of PIP₂. Therefore, an important task for cell biologists in studying Lowe Syndrome is to determine precisely what happens to PIP₂ in cells lacking OCRL1, in particular asking two different questions: (a) How much extra PIP₂ is there? and (b) Where is the extra PIP₂? While the first question is in the domain of biochemistry, the second question belongs very much to cell biology, which addresses questions of sub-cellular anatomy. And it is this second question that is particularly relevant, because it has been shown that OCRL1 and PIP₂ are normally in different parts of the cell. PIP₂ is mainly on the inner face of the plasma membrane (Figure 2). However, OCRL1 is most prominently

found on internal membranes, in particular the Golgi apparatus, and the closely related *trans*-Golgi network (Figure 2),⁵ where there is very little PIP₂.⁶ Thus, this enzyme cannot be acting on the bulk pool of the lipid.

The physical separation of PIP₂ and OCRL1 leads naturally to the most simple model (the null hypothesis) proposing that OCRL1 prevents PIP₂ accumulating at the Golgi. Although experiments on cells lacking OCRL1 have all (so far) failed to find the build-up of an internal pool of PIP₂, I believe that this is because of a repeated, systematic experimental mistake. In searching for internal pools of PIP₂, we have used a protein module that binds PIP₂, for example the pleckstrin homology domain of phospholipase C. Such domains bind PIP₂ reasonably well in the plasma membrane, and are useful for comparisons of PIP₂ in different parts of the plasma membrane. However, these domains have evolved over hundreds of millions of years to bind PIP₂ only at the plasma membrane, and so they have evolved secondary, weak interactions with the plasma membrane. By the same token, these domains do not bind well to PIP₂ on internal membranes. Once we accept how biased these PIP₂-detecting tools are, we can begin to adapt them to reveal internal pools of PIP₂, for example by hooking them directly to other protein domains that weakly target internal membranes. This unnatural combination of domains may be the best way to detect the presence of the unnatural pool of PIP₂ that we suspect exists in cells lacking OCRL1.

Membrane traffic: PIP₂ regulates a large number of specific processes inside cells. Out of this long list, so far just two processes have been implicated as likely suspects in the pathogenesis of Lowe Syndrome. The strongest candidate is membrane traffic (Figure 2). The normal flow of proteins inside cells includes exchange between Golgi membranes and endocytic compartment. Lack of OCRL1 has been shown to alter this interchange.⁷ To support its role in these events, OCRL1 has been shown to bind directly both to components involved in this traffic step, and to proteins involved in endocytosis at the plasma membrane.^{7,9} In addition, a minority of the OCRL1 protein can be detected at key sites early in the endocytic pathway (Figure 2). In sum total, we now know that many aspects of intracellular traffic could be affected by loss of OCRL1. The way in which this relates to the symptoms of Lowe Syndrome is discussed below.

Cytoskeleton: Another cellular function that is affected in Lowe Syndrome is the actin cytoskeleton. Many actin binding proteins interact with PIP₂.¹⁰ Another link with actin is that OCRL1, in addition to its 5-phosphatase domain, contains a domain that binds Rac, which is a master regulator of actin dynamics, and which recruits some OCRL1 to parts of the cell where actin is being actively polymerised, such as the leading edge of migrating cells.¹¹

Linking these ideas to eye disease

Given these models, what are the likely events that lead to cataract? The defect must be quite severe, as cataracts are usually bilateral from birth. Another sign of the strong effect of loss of OCRL1 on the lens is that carriers (typically the mothers and sisters of patients) who have one null and one fully active OCRL1 gene have small, sub-clinical, punctate cataracts, indicating that Barr body inactivation and X-chromosomal mosaicism create islands of defective cells within the forming lens.

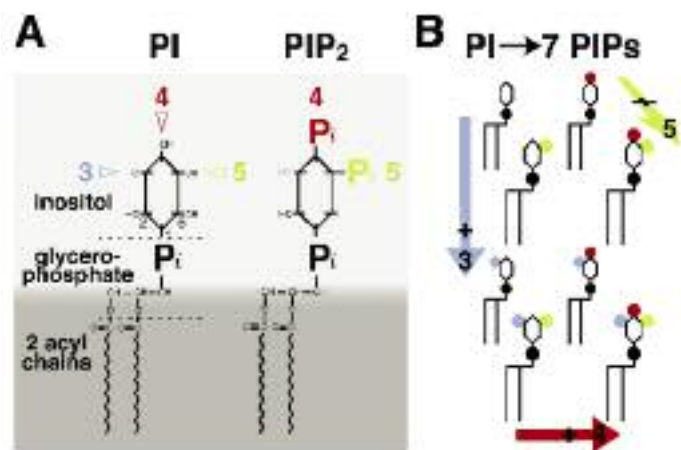


Figure 1: PIP₂ is a signalling lipid.

A. Phosphatidylinositol (PI) is a common lipid that contains two acyl chains buried in the membrane, glycerophosphate lying at the membrane / cytoplasm interface, and inositol, a 6-carbon sugar ring in the cytoplasm. Positions 3, 4 and 5 of this ring (colour coded blue, red and yellow respectively) can all be modified by reversible addition of phosphate groups (Pi). PIP₂ is the common name for PI(4,5)P₂, which has phosphates at positions 4 and 5.

B. Phosphorylation of PI at positions 3, 4 and 5 of its inositol ring produces seven PIP species. These are: top row: PI, PI(4)P; second row: PI(5)P, PI(4,5)P₂; third row: PI(3)P, PI(3,4)P₂; bottom row: PI(3,5)P₂ and PI(3,4,5)P₃. Arrows indicate three separate sets of enzymes that add phosphates (circles) at the three positions. Arrows and circles are coloured as in A. Phosphatases remove these phosphates, and OCRL1 is one of 10 proteins in humans that remove phosphates from position 5 (reversing the yellow arrow), turning PI(4,5)P₂ into PI(4)P.

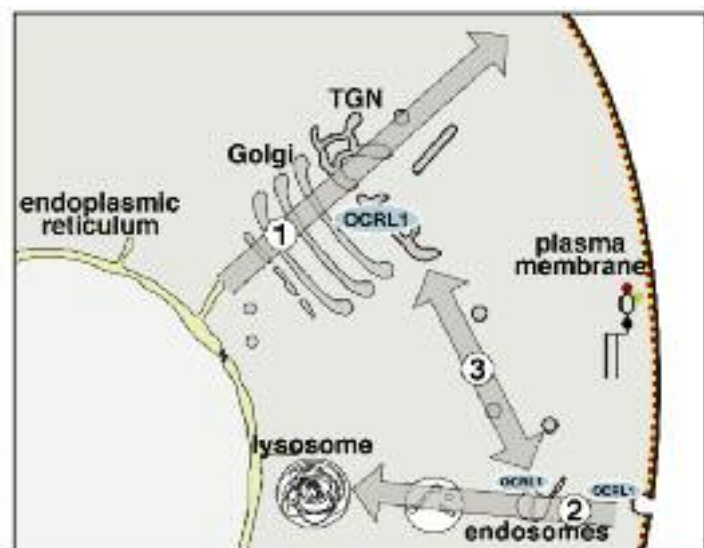


Figure 2: OCRL1 is mostly separated from the bulk of PIP₂.

The diagram shows three major pathways of membrane traffic: 1. secretion (exocytosis), 2. endocytosis, and 3. exchange between the trans Golgi network (TGN) and endosomes (see numbered arrows). OCRL1 is largely found in the TGN (and to a lesser extent early in the endocytic pathway), while PIP₂ is mainly found on the cytoplasmic side of the plasma membrane (red and yellow dashed line). Loss of OCRL1 affects interchange between TGN and endosomes (step 3). Despite circumstantial evidence that OCRL1 is important in endocytosis (step 2),⁷ such an effect has yet to be reproduced in the laboratory.

Despite the strength of lens phenotype, there is no obvious mechanism by which Lowe Syndrome causes cataracts. One way to identify how loss of OCRL1 causes cataracts is to look for parallels in other mutations that induce cataracts. Do any of these share the same pathogenesis?

In many cases of hereditary cataracts, the underlying mutation has been mapped, and the underlying cellular pathway involved can be determined.¹² The most commonly affected pathways have specific roles in lens cell function. Many mutations are in crystallins, cytoplas-

mic chaperones that prevent other proteins unfolding, keeping them from aggregating, when they lose transparency. Chaperones are particularly important in the lens because once laid down, lens tissue must function without being replaced for an entire lifetime. However, there is no known mechanism by which dysregulation of PIP2 might affect crystallin function. Another pathway where mutation commonly leads to cataract is the transcriptional regulation of lens development, but again there is known link between this and PIP2.

There are three pathways implicated in hereditary cataract that are also known to be linked to PIP2. One revolves around aquaporin-0, a major integral protein of plasma membrane only found in lens fibres, which pumps water in and out of the cells. Water circulation into the deepest part of the lens requires this pumping. Incorrect intracellular trafficking of aquaporin-0 in the developing lens fibre, such as might occur with loss of OCRL1, might fail to deliver it to the correct portions of the plasma membrane. This parallels the renal defect in Lowe Syndrome, where current research proposes that the fault is in endocytosis and recycling of megalin and cubulin,⁹ plasma membrane receptors for components that need to be recovered from the glomerular filtrate. A second interesting cataract mutation is in the beaded filament structural protein 2, a lens-specific filament-forming protein that forms part of the cytoskeleton only in the lens. Little is known about these filaments, but they are likely to be related to membrane traffic and so to PIP2. There are also clear links between disordered actin and lens cell malfunction. For example, congenital cataracts occur as part of a hereditary multi-organ syndrome called Nance-Horan Syndrome where a putative actin binding protein is mutated.¹³

The third and final category of mutation are in connexins, the main component of intercellular bridges between lens cells. These so-called gap junctions form channels that link the cytoplasm of neighbouring cells, allowing small molecules such as electrolytes to circulate through the pulp of the lens. Similar to loss of aquaporin-0, loss of lens connexins causes congenital cataracts. Significantly, new research shows that connexin function is regulated by PIP2.¹⁴ Another link between cataract and intercellular junctions is that the Nance-Horan syndrome protein NHS-A targets these sites. These links led our lab to investigate whether OCRL1 functions at intercellular junctions. Promisingly, preliminary results show a direct interaction between OCRL1 and the junctional protein ZO-1 that binds connexins. We also find that some OCRL1 is localised to junctional areas under certain conditions, indicating that the interaction is functionally relevant. The dysregulation of intercellular junctions is therefore a candidate mechanism by which loss of OCRL1 affects lens cell function, although it may not relate to the problems of the kidney or CNS, where disordered membrane traffic may be the main problem.⁹

To progress, scientists must go beyond reductionism, which dissects individual interactions down to the minutest detail. This approach must be supplemented by models that more accurately reflect the disease state in human lens, renal tubule and CNS

Conclusion

Loss of OCRL1, one of ten enzymes that inactivate PIP2, causes Lowe Syndrome by means we cannot currently explain, despite our knowledge of the protein's complex interactions and intracellular localisation. This means that clinicians and patients may have to wait for a thorough understanding of the syndrome, despite some valiant attempts to develop treatments.¹⁵ To progress, scientists must go beyond reductionism, which dissects individual interactions down to the minutest detail. This approach must be supplemented by models that more accurately reflect the disease state in human lens, renal tubule and CNS. Primary cells from these sites are very difficult to work with: they can only be obtained in low numbers, and they rapidly change in tissue culture. Another approach is to develop animal models. Even though the simple experiment of deleting OCRL1 in mice was disappointing, because the mice show no signs of disease,¹⁶ the group of Prof Nussbaum (San Francisco) is attempting to 'humanise' the 5-phosphatase pathway in mice to such a degree that loss of OCRL1 accurately reproduces symptoms. Here in the UK, the group of Martin Lowe (University of Manchester) have already found that loss of OCRL1 in zebrafish (a species with genome and organs very close to humans in evolutionary terms) affects the CNS in that species, indicating that this animal model

may be informative. This makes the announcement of the new project in Manchester funded by the Lowe Syndrome Trust so exciting and timely. **EN**

References

- Vishwanath M, Cheong-Leen R, Taylor D, Russell-Eggitt I, and Rahi J. Is early surgery for congenital cataract a risk factor for glaucoma? *Br J Ophthalmol* 2004;**88**(7):905-10.
- Attree O, Olivos I M, Okabe I, Bailey L C, Nelson D L, et al. The Lowe's oculocerebrorenal syndrome gene encodes a protein highly homologous to inositol polyphosphate-5-phosphatase. *Nature* 1992;**358**(6383):239-42.
- Pendaries C, Tronchere H, Plantavid M, and Payrastré B. Phosphoinositide signaling disorders in human diseases. *FEBS Lett* 2003;**546**(1):25-31.
- Mitchell C A, Gurung R, Kong A M, Dyson J M, Tan A, et al. Inositol polyphosphate 5-phosphatases: lipid phosphatases with flair. *IUBMB Life* 2002;**53**(1):25-36.
- Lowe M. Structure and function of the Lowe syndrome protein OCRL1. *Traffic* 2005;**6**(9):711-19.
- Watt S A, Kular G, Fleming I N, Downes C P, and Lucocq J M. Subcellular localization of phosphatidylinositol 4,5-bisphosphate using the pleckstrin homology domain of phospholipase C delta1. *Biochem J* 2002;**363**(Pt 3):657-66.
- Choudhury R, Diao A, Zhang F, Eisenberg E, Saint-Pol A, et al. Lowe syndrome protein OCRL1 interacts with clathrin and regulates protein trafficking between endosomes and the trans-Golgi network. *Mol Biol Cell* 2005;**16**(8):3467-79.
- Hyvola N, Diao A, McKenzie E, Skippen A, Cockcroft S, et al. Membrane targeting and activation of the Lowe syndrome protein OCRL1 by rab GTPases. *Embo J* 2006;**25**(16):3750-61.
- Erdmann K S, Mao Y, McCreagh H J, Zoncu R, Lee S, et al. A role of the Lowe syndrome protein OCRL1 in early steps of the endocytic pathway. *Dev Cell* 2007;**13**(3):377-90.
- Suchy S F, and Nussbaum R L. The deficiency of PIP2 5-phosphatase in Lowe syndrome affects actin polymerization. *Am J Hum Genet* 2002;**71**(6):1420-7.
- Faucherre A, Desbos P, Nagano F, Satrie V, Lunardi J, et al. Lowe syndrome protein Ocrh1 is translocated to membrane ruffles upon Rac GTPase activation: a new perspective on Lowe syndrome pathophysiology. *Hum Mol Genet* 2005;**14**(11):1441-8.
- Reddy M A, Francis P J, Berry V, Bhattacharya S S, and Moore A T. Molecular genetic basis of inherited cataract and associated phenotypes. *Surv Ophthalmol* 2004;**49**(3):300-15.
- Sharma S, Ang S L, Shaw M, Mackey D A, Gecz J, et al. Nance-Horan syndrome protein, NHS, associates with epithelial cell junctions. *Hum Mol Genet* 2006;**15**(12):1972-83.
- van Zeijl L, Ponsioen B, Giepmans B N, Ariaens A, Postma F R, et al. Regulation of connexin43 gap junctional communication by phosphatidylinositol 4,5-bisphosphate. *J Cell Biol* 2007;**177**(5):881-91.
- Lowe Syndrome Trust. 2007. Lowe Syndrome Trust research grant to Imperial College, London. <http://www.lowestrust.com/ICLpressreleasemaro7.htm>
- Janne P A, Suchy S F, Bernard D, MacDonald M, Crawley J, et al. Functional overlap between murine Inpp5b and Ocrh1 may explain why deficiency of the murine ortholog for OCRL1 does not cause Lowe syndrome in mice. *J Clin Invest* 1998;**101**(10):2042-53.
- McCreagh H J, Paradise S, Tomasini L, Addis M, Melis M A, et al. All known patient mutations in the ASH-RhoGAP domains of OCRL affect targeting and APPL1 binding. *Biochem Biophys Res Commun* 2008.

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Take home message

- Lowe Syndrome patients have congenital cataracts and glaucoma, as well as renal failure, and no treatments are currently available.
- Lowe Syndrome is a rare genetic disorder in a cellular pathway that controls PIP2, a very important lipid that regulates many signals inside cells.
- Studies of Lowe Syndrome reveal important information about PIP2 in general, and particularly its role in the lens.