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CELL SCIENCE AT A GLANCE

Membrane trafficking in the retinal pigment epithelium at a glance

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ABSTRACT

The retinal pigment epithelium (RPE) is a highly specialised pigmented monolayer sandwiched between the choroid and the photoreceptors in the retina. Key functions of the RPE include transport of nutrients to the neural retina, removal of waste products and water from the retina to the blood, recycling of retinal chromophores, absorption of scattered light and phagocytosis of the tips of the photoreceptor outer segments. These functions place a considerable membrane trafficking burden on the RPE. In this Cell Science at a Glance article and the accompanying poster, we focus on RPE-specific adaptations of trafficking pathways. We outline mechanisms underlying the polarised expression of membrane proteins, melanosome biogenesis and movement, and endocytic trafficking, as well as photoreceptor outer segment phagocytosis and degradation. We also briefly discuss theories of how dysfunction in trafficking pathways contributes to retinal disease.

KEY WORDS: Melanosome, Membrane trafficking, Phagocytosis, Polarity, Retinal pigment epithelium

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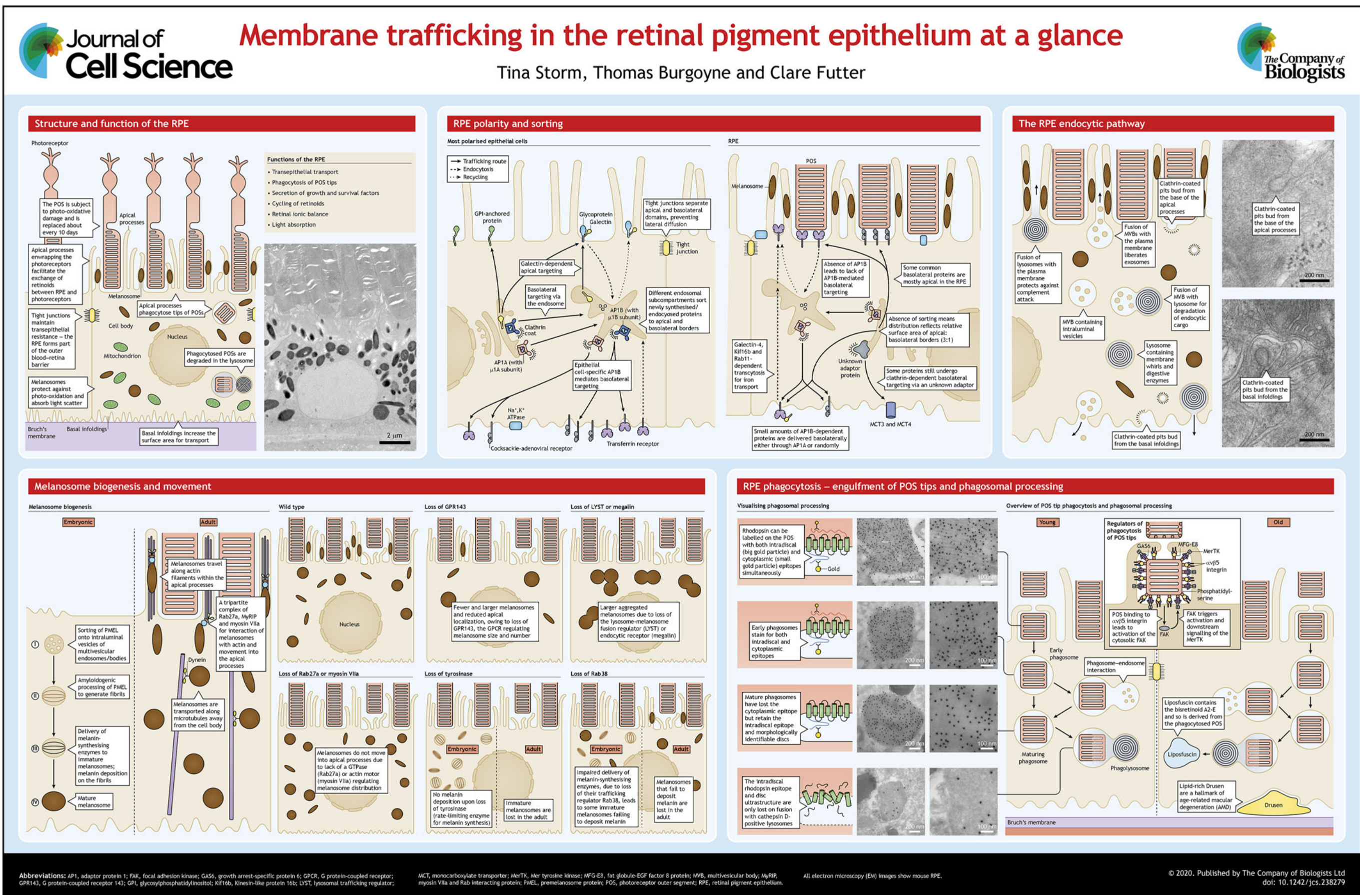
Introduction

The retinal pigment epithelium (RPE) is a pigmented monolayer adjacent to the light-sensing photoreceptors (rods and cones) and is essential for photoreceptor survival and, hence, vision. Unlike most epithelia, which are exposed apically to fluid, the RPE is sandwiched within the multiple cell layers of the back of the eye (see poster). Its apical processes enwrap the photoreceptor outer segment (POS), whereas the highly infolded RPE basal membrane is attached to the Bruch's membrane, which separates the RPE from a layer of fenestrated capillaries (the choriocapillaris). The morphological specialisations of the RPE facilitate its multiple functions (Lakkaraju et al., 2020; Strauss, 2005). They include the transport of nutrients from the blood to the neural retina and of waste products and water from the retina to the blood. Ion channels and



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transporters within the RPE regulate water transport and the ionic composition of the subretinal space to maintain the excitability of the photoreceptors. The pigment-containing melanosomes, which give the RPE its name, protect retinal cells from photo-oxidation and absorb light scatter. Approximately 10% of POSs are replaced each day as part of a renewal process. Phagocytosis and degradation of the damaged POS tips by the RPE is essential for retinal survival.

In this Cell Science at a Glance article and accompanying poster, we focus on the unique demands that the functions of the RPE place on membrane traffic. Reflecting these functions, some RPE plasma membrane proteins display reverse polarity compared to most epithelia, and we will thus describe RPE-specific mechanisms of polarised sorting. We will review how melanosome biogenesis and fate in RPE cells compares with that in the better-characterised melanocyte. We will then focus on recent advances in our understanding of the endocytic pathway in RPE cells, which is of particular interest because of its role in transport across the RPE. Additionally, this pathway provides a potential route of exocytosis through exosome secretion and lysosome exocytosis. Finally, the daily phagocytosis of POSs gives the lysosomes of the RPE an unparalleled degradative burden, and accumulation of the aging pigment lipofuscin, due to incomplete processing of POS, may lead to lysosome dysfunction and be detrimental in age and disease. Therefore, we will summarise current knowledge of POS-specific phagocytic uptake mechanisms and review what is known about phagosome processing in the RPE compared with other better-characterised professional phagocytes.

RPE polarity and sorting

More than 25 years ago, RPE cells were discovered to exhibit reverse polarity of key proteins, like the Na,K-ATPase, which is basolateral in most epithelia but is apical in the RPE (Gundersen et al., 1991). However, the mechanisms underlying that reverse polarity are yet to be fully resolved. In all epithelia, multiple polarised sorting mechanisms are utilised by different sets of plasma membrane proteins (see poster). Newly synthesised cell surface proteins can be sorted in the trans-Golgi network (TGN) into carriers that are targeted to apical or basolateral plasma membranes either directly or via the endocytic pathway. Additionally, polarised sorting mechanisms exist in endosomes, not only to sort biosynthetic proteins trafficked from the TGN, but also those endocytosed from the plasma membrane. Subdivisions of the endocytic pathway that play different roles in polarised sorting have been extensively reviewed (Lehmann et al., 2014) and will not be described here. Finally, polarised sorting mechanisms can operate on the basolateral plasma membrane to selectively transcytose membrane proteins to the apical border. This was shown in RPE cells transfected with vesicular stomatitis viral G protein (VSV-G) and influenza haemaglutinin (HA), examples of basolateral and apical proteins, respectively. Newly synthesised VSV-G and HA were both delivered to the basal surface prior to transport of HA to the apical surface (Bonilha et al., 1997).

Basolateral targeting

Clathrin-based basolateral targeting mechanisms operate at both the TGN and endosomes (Deborde et al., 2008; Folsch et al., 1999). Clathrin is recruited to the membrane by clathrin adaptor complexes (APs) that recognise short basolateral targeting signals in the cytoplasmic domains of plasma membrane proteins. These signals often resemble endocytosis motifs that are recognised by AP2 at the plasma membrane but it is AP1 that recognises basolateral targeting motifs. The tetrameric AP1 adaptor contains either the ubiquitously expressed µ1A subunit (in AP1A) or the epithelial specific µ1B subunit (in AP1B). Whereas both AP1A and AP1B contribute to basolateral sorting, a subset of basolateral targeting motifs in proteins, such as the transferrin receptor and Cocksackie-adenoviral receptor (CAR, also known as CXADR), are recognised by the µ1B, but not the µ1A, subunit (Carvajal-Gonzalez et al., 2012; Folsch et al., 1999; Gravotta et al., 2012; Ohno et al., 1999). Intriguingly, RPE cells lack the µ1B subunit, and hence AP1B (Diaz et al., 2009). For some proteins, like CAR, this means that there is no active sorting and its polarised distribution (75% apical) simply reflects the relative surface areas of the apical and basolateral borders, with the apical border being ∼3 times the surface area of the basolateral border (Marmorstein et al., 1998; Okami et al., 1990). A clathrin-based basolateral sorting mechanism nevertheless exists in RPE cells, because the monocarboxylate transporters (MCTs) MCT3 and MCT4 are efficiently basolaterally targeted in RPE cells (and other epithelia) by a mechanism depending on clathrin and an unknown adaptor (Castorino et al., 2011). The MCT1 transporter is targeted basolaterally in most epithelia but is apical in RPE cells because MCT1 lacks a basolateral targeting signal and relies on the AP1B-dependent recognition of the basolateral targeting motif within its dimerization partner, CD147 (also known as BSG and EMMPRIN), to be targeted to the basolateral border (Deora et al., 2005).

Apical targeting

Targeting signals in apical membrane proteins are more diverse than those in their basolateral counterparts and include cytoplasmic domain signals of variable length, glycosylphosphatidylinositols (GPI) anchors and glycans on the luminal domain. Intriguingly, clathrin and AP1, which operate in basolateral targeting, have been implicated in the apical targeting of the endocytic receptor megalin (also known as LRP2) (Gravotta et al., 2019) and GPI-anchored proteins (Caceres et al., 2019). At least for megalin, this apical sorting does not depend on the AP1B subunit (Gravotta et al., 2019) and so potentially operates in RPE cells. N-glycans on the luminal domain of apical proteins can promote their apical targeting in kidney and gut epithelia through interaction with galectins (Delacour et al., 2006, 2005). In the RPE, galectin-4 promotes Rab11- and KIF16b-dependent transcytosis of the transferrin receptor to the apical border (Perez Bay et al., 2013; Perez Bay et al., 2014), contributing to the predominantly apical localisation of this receptor in RPE cells. How galectins, which lack a signal sequence, gain access to the extracellular (i.e. luminal) domains of transmembrane proteins and then dictate apical sorting is not fully established.

Our knowledge of the mechanisms underlying polarised sorting of transmembrane proteins in the RPE is incomplete. Even less well understood are the mechanisms underlying the polarity of secretion of soluble growth factors and cytokines by the RPE, which is essential to maintain the health of the neighbouring photoreceptors and endothelial cells of the choriocapillaris.

Melanosome biogenesis and movement

Melanosomes are membrane-bound organelles in which melanin is synthesised and stored to protect retinal cells from photo-oxidation. As model lysosome-related organelles, their biogenesis has been extensively studied in skin melanocytes, in which their molecular regulation is well understood (Sitaram and Marks, 2012). Here, we focus on RPE-specific features of melanosome biogenesis and function.

Unlike skin melanocytes, where melanosome biogenesis and transfer to keratinocytes occurs throughout life, RPE cells retain

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their melanosomes and so the majority of melanosome biogenesis is embryonic. Melanosome biogenesis in the RPE follows the same well-characterised stages as in melanocytes (see poster) (Lopes et al., 2007b; Raposo et al., 2001). PMEL is a protein that is expressed in melanogenic cells, where it is targeted to multivesicular endosomes. Subsequent amyloidogenic processing of PMEL leads to the formation of striations within the endosomal lumen and formation of immature melanosomes. After delivery of melanin-synthesising enzymes to the immature melanosome, melanin is deposited on the striations to form mature melanosomes. If melanin deposition fails, then the striated immature melanosomes that can be readily identified in embryonic RPE are lost in the adult through, as yet, unclear mechanisms (Lopes et al., 2007b).

Another distinctive feature of RPE melanosomes is that they access the apical processes of the RPE. In amphibians and fish, this light-dependent movement is extensive, but in mammals it is limited and involves the transfer of melanosomes from microtubules of the cell body to the actin filaments of the apical processes (Futter et al., 2004). A tripartite complex of Rab27a on melanosomes, linked to myosin VIIa on actin via the linker protein MyRIP, is required for this transfer (El-Amraoui et al., 2002; Futter et al., 2004; Gibbs et al., 2004; Lopes et al., 2007a) and bears a striking parallel with the Rab27a–melanophilin–myosin V complex required for the interaction of melanosomes with the actin cytoskeleton in melanocytes (Hume et al., 2002, 2001).

Clues about melanosome biogenesis and function in the RPE have come from studies of pigmentary disorders (Box 1). Albinism can have multiple genetic causes that result in varying loss of pigmentation, but all forms of albinism share visual symptoms that can include light sensitivity, involuntary eye movements (nystagmus), impaired eye alignment (strabismus) and underdeveloped fovea (the cone-rich part of the retina responsible for high visual clarity) (McKay, 2019). These symptoms result from developmental defects that occur when the RPE is the only pigmented cell in the body. Paradoxically, however, the main clinical manifestations are in neurons that do not express the genes involved in albinism. The most common cause of albinism is the loss of function of tyrosinase, which catalyses the conversion of tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) and then assists in melanin generation. Exogenous expression of tyrosine carboxylase in the RPE allows tyrosinase-deficient RPE to synthesise L-DOPA but not melanin (Lavado et al., 2006). This is sufficient to rescue ocular developmental defects, suggesting that L-DOPA, rather than melanin, is critical for ocular development. L-DOPA is the ligand for GPR143 (also known as OA1) (Lopez et al., 2008). Signalling from this G-protein-coupled receptor regulates melanosome number, size (Schiaffino and Tacchetti, 2005) and distribution (Palmisano et al., 2008), but additional functions of this receptor, for example in regulating secretion of growth and survival factors, may be critical regulators of neuronal behaviour during ocular development.

Loss of function of lysosomal trafficking regulator (LYST; also known as CHS1) (Collier et al., 1985; Robison et al., 1975) or loss of function of megalin (Storm et al., 2019) causes abnormalities of melanosome size and shape. The presence of acid hydrolases within RPE melanosomes raises the possibility that melanosomes could have a degradative function in addition to their established roles (Lopes et al., 2007b). In the RPE, a major substrate for degradation is phagocytosed POSs (see below). The identification of gold particles within melanosomes after delivery of gold-labelled POSs *in vivo* (Schraermeyer et al., 1999) and the accumulation of lipofuscin within melanosomes (melanolipofuscin) with age (Feeney-Burns et al., 1984) would be consistent with a role for melanosomes in phagosome degradation. The bisretinoid-containing lipofuscin is generally considered to be at least partly derived from undegraded POSs, but proteomic analysis suggests that lipofuscin associated with melanin has a different composition (Warburton et al., 2007), making the trafficking pathways leading to melanolipofuscin formation unclear.

Box 1. Examples of monogenic diseases affecting melanosome biogenesis and trafficking in the RPE

Mutation of a functionally diverse set of genes results in aberrant RPE melanosome formation and/or trafficking in both the RPE and melanocytes. Here, we focus on examples where analysis of mouse models has revealed RPE-specific aspects of melanosome formation, fate or trafficking.

Rab38 (Bultema and Di Pietro, 2013): mutation of the *Rab38* gene in mice (‘Chocolate’/*Cht* mouse) (Lopes et al., 2007b) results in a dramatic reduction in the number of RPE melanosomes in adult RPE. Rab38 and the closely related Rab32 regulate the delivery and retrieval of melanin-synthesising enzymes to immature melanosomes.

TYR (Lambert et al., 2019): oculocutaneous albinism type 1 (OMIM #606933) is caused by mutation of the *TYR* gene (Lewis, 1993) and leads to hypopigmentation of the skin, hair, and eyes. The encoded tyrosinase catalyses melanin synthesis and, although immature striated melanosomes are formed in embryonic RPE, there is a total absence of RPE melanosomes later in life, indicating instability of immature melanosomes.

MYO7A (Williams, 2008): Usher syndrome 1B (OMIM #276900) is characterised by progressive retinal degeneration, profound deafness and vestibular defects and is caused by a mutation in *MYO7A* (Petit, 2001). The encoded myosin VIIa regulates melanosome movement into the apical processes via a tripartite complex with Rab27a and MyRIP in the RPE and has been shown to have a role in phagocytosis and phagosome maturation in the RPE (Gibbs et al., 2003).

RAB27A (Van Gele et al., 2009): Griscelli syndrome type 2 (OMIM #607624) is a rare autosomal recessive disorder caused by mutation of the *RAB27A* gene. Rab27a belongs to the family of small GTPases and is part of the tripartite Rab27a–myosin VIIa–MyRIP complex regulating melanosome motility in the RPE. As observed with absence of myosin VIIa, defects of Rab27a lead to an absence of melanosomes in RPE apical processes.

GPR143 (Schiaffino, 2010): mutation of the *GPR143* gene leads to ocular albinism type 1 (OA1) (OMIM #300500), which impedes the stop signal for melanosome growth and results in a reduced number of enlarged but otherwise normally shaped RPE melanosomes (Incerti et al., 2000; Young et al., 2008).

LRP2 (Christensen and Birn, 2002): mutation of the *LRP2* gene (encoding megalin) causes the very rare Donnai–Barrow syndrome (OMIM #222448) (Kantarci et al., 2007; Pober et al., 2009). Patients with this disease suffer from a diverse range of developmental and functional abnormalities including high-grade myopia, large protruding eyes, retinal dystrophy, retinal detachment and formation of abnormally shaped macromelanosomes (Khalifa et al., 2015; Pober et al., 2009; Storm et al., 2019). Absence of megalin greatly affects the endocytic machinery and leads to aggregation and fusion of melanosomes in the RPE.

LYST (Kaplan et al., 2008): Chediak–Higashi syndrome (OMIM #214500) (Kaplan et al., 2008) is a rare lethal disorder caused by mutations of the *LYST* gene, which generally result in systemically abnormal and enlarged lysosomes and lysosome-related organelles including the RPE melanosomes (Collier et al., 1985; Robison et al., 1975; Valenzuela and Morningstar, 1981).

Roles of the RPE endolysosomal system

Endocytosis

Phagocytosis, pinocytosis, clathrin-dependent and clathrin-independent endocytosis all enable material from the immediate environment to be taken into the cell, but each pathway has distinct

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functions. Here, we will discuss the significance of these endocytic mechanisms for RPE function (see poster), although RPE phagocytosis is discussed separately below.

Caveolae mediate one type of clathrin-independent endocytosis and are especially prevalent in endothelial cells. Although present in cultured RPE, they are rarely observed in RPE cells *in vivo* (Mora et al., 2006), suggesting that this route might not be a significant uptake route in the RPE (Gu et al., 2017). Clathrin-dependent endocytosis is believed to play an essential role in the uptake and transfer of essential nutrients across the RPE (Alberts et al., 2007), which constitutes the outer blood–retina RPE barrier. This allows for a tightly regulated movement of nutrients and solutes to and from the retina, thus creating a controlled retinal environment.

The number of markers that have been used to follow the endocytic pathway in RPE cells is somewhat limited. Both clathrin and non-clathrin dependent endocytosis of the interphotoreceptor matrix protein interstitial retinol binding protein (IRBP; also known as RBP3) by the RPE, followed by delivery to multivesicular bodies (MVBs) and lysosomes, has been observed in culture and in retinal explants (Cunningham et al., 1999; Hollyfield et al., 1985). IRBP has been implicated in transport of the visual chromophore 11-*cis* retinal and all-*trans* retinol between the photoreceptors and RPE. Endocytosis of IRBP by the RPE could contribute to the uptake of all-*trans* retinol by the RPE for enzymatic reformation of 11-*cis* retinal as part of the normal visual cycle or it could be required for clearance of damaged IRBP.

Several endocytic receptors have been identified in the RPE, including the two low-density lipoprotein receptor-related proteins, LRP1 (Hollborn et al., 2004) and megalin (Storm et al., 2014), as well as the epidermal growth factor receptor (Yan et al., 2007). Immunocytochemical detection of megalin supports the idea that basal and apical receptor-mediated endocytosis is active, since the receptor was identified in vesicles throughout the RPE (Storm et al., 2014). Both LRP1 and megalin have large numbers of structurally and functionally distinct ligands, including vitamin-binding proteins, enzymes, lipoproteins, hormones and signalling proteins (Christensen et al., 2009; Strickland et al., 2002). Thus, likely roles for both receptors in the RPE are the tightly controlled delivery of essential nutrients across the outer blood–retina barrier as well as the modulation of signalling pathways.

Heparan sulfate proteoglycans (HSPGs) (Sarrazin et al., 2011) represent another class of surface proteins present on the RPE (Clark et al., 2011). They may act as endocytic receptors for a number of ligands including chemokines and tyrosine kinase-type growth factor receptors. In addition, HSPG has been identified as the primary cell surface receptor for adeno-associated virus type 2 (AAV2) virions (Summerford and Samulski, 1998) and suggested to interact with fibroblast growth factor receptor 1 (Qing et al., 1999) and αvβ5 integrin (Summerford et al., 1999) as co-receptors in receptor-mediated endocytosis of AAV2. This is currently of particular interest as AAV2 is being used in gene therapy clinical trials for the delivery of *RPE65* (Bainbridge et al., 2008) and *REP1* (MacLaren et al., 2014) genes to treat Leber congenital amaurosis and choroideremia patients, respectively.

Another interesting role for endocytosis in the RPE with potential clinical importance is protection from complement-mediated membrane attack. The complement cascade is a critical component of the innate immune system, and comprises complement proteins that are capable of osmotic lysis of invading pathogens through formation of a transmembrane pore also known as the membrane attack complex (MAC) (Morgan et al., 2017). Aberrant activation of the complement cascade may be central in the pathogenesis of age-related macular degeneration (AMD) (Clark and Bishop, 2018), a multifactorial disease discussed in more detail below. We have shown that RPE cells are able to mitigate the effects of complement membrane attack by endocytosis and delivery of the C5b-9 membrane attack complex (MAC) to the lysosome (Georgiannakis et al., 2015).

Lysosome exocytosis

Recent studies have shown that raised levels of intracellular Ca^{2+} can trigger the fusion of lysosomes with the plasma membrane (Jaiswal et al., 2002; Xu et al., 2012). Lysosome exocytosis is important for plasma membrane repair and the expulsion of cellular debris as well as hindering pathogen infection. In RPE cells, lysosome exocytosis may occur as a membrane-protective response to Ca^{2+} influx through sublytic membrane attack complex pores caused by the activation of the complement cascade (Tan et al., 2016). Additionally, exocytosis of incompletely digested phagolysosomes has been observed in the RPE in a number of reports. In *Rana ridibunda* frog retina (Rungger-Brandle et al., 1987) and chloroquine-treated Long-Evans rats (Peters et al., 2006), basolateral exocytosis of incompletely digested material in the lateral and basal intercellular space with deposition in Bruch's membrane was observed.

Exosome secretion

Exosomes are small secreted extracellular vesicles that are released into the surrounding environment through fusion of MVBs with the plasma membrane (Hessvik and Llorente, 2018). The differential protein and lipid composition of basolateral and apical exosomes suggest that controlled polarised exosomal secretion may occur in epithelia (Hessvik and Llorente, 2018). Exosomal cargo may include nucleic acids, proteins and lipids, and is not only cell and tissue specific, but also influenced by the metabolic state of the cell. Thus, exosomes have been implicated in diverse physiological and pathological functions, including waste excretion, intercellular communication, immune response modulation, extracellular matrix turnover, stem cell division and differentiation and neovascularization (Hessvik and Llorente, 2018; Klingeborn et al., 2018). Unsurprisingly, exosomes have been proposed to harbour important roles in RPE–retina communication. A recent *in vitro* study suggests that RPE exosomes may be involved in increased vascular endothelium growth factor secretion and, consequently, neovascularization in response to oxidative stress (Atienzar-Aroca et al., 2016). RPE exosomes have furthermore been suggested to play a central role in AMD pathology through modulating complement activation in the RPE-surrounding milieu (Wang et al., 2009). Interestingly, the G-protein coupled receptor GPR143 was recently shown to inhibit exosomal release in the RPE in response to binding of its endogenous ligand L-DOPA (Locke et al., 2014) suggesting that RPE exosomal release may be tightly controlled.

RPE phagocytosis – POS engulfment and phagosome processing

Phagosome engulfment

Externalisation of phosphatidylserine in POS membranes is the main 'eat me' signal for RPE cells to engulf the tips of the POS. Engulfment of POS discs is under circadian control and is dependent on the light–dark cycle. A burst in the number of RPE phagosomes soon after light onset was first identified in albino rats (LaVail, 1976), and circadian control of POS engulfment has subsequently been observed numerous times in different species. Secreted milk fat globule-EGF factor 8 protein (MFG-E8) in the sub-retinal space bridges the phosphatidylserine residues on the

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POS to αvβ5 integrin on the RPE, thereby promoting POS engulfment (Finnemann et al., 1997; Nandrot et al., 2007, 2004; Ruggiero et al., 2012). Annexin A5 was recently shown to regulate the number of αvβ5 receptors at the apical RPE membrane (Yu et al., 2019), whereas annexin A2 has been implicated in POS engulfment and F-actin recruitment to the newly formed phagosome (Law et al., 2009). The scavenger receptor CD36 is also required for efficient phagosome engulfment but its exact role remains elusive (Finnemann and Silverstein, 2001; Ryeom et al., 1996). POS binding to αvβ5 integrin increases complex formation with and activation of the cytosolic focal adhesion kinase (FAK; also known as PTK2), triggering activation and downstream signalling of the Mer tyrosine kinase (MerTK) and thus promoting POS disc engulfment (Finnemann, 2003; Finnemann and Nandrot, 2006). MerTK also requires binding of its ligand Gas6 (or protein S), which links the MerTK on the RPE to phosphatidylserine on the POS (Burstyn-Cohen et al., 2012; Hall et al., 2005). Defects in the efficient engulfment of POSs, as occurs in the Royal College of Surgeons rat (Bok and Hall, 1971; Dowling and Sidman, 1962; Herron et al., 1969), which lacks functional Mer tyrosine kinase (D'Cruz et al., 2000), lead to rapid retinal degeneration.

Phagosome processing

In professional phagocytes, phagosome maturation subsequent to internalisation of pathogens and other foreign particles involves sequential interactions between the maturing phagosome, endosomes and lysosomes (Bosch et al., 1993; Vieira et al., 2002). This is also likely to be the case for phagosome maturation in the RPE (see poster), since prelysosomal partial digestion of the visual protein rhodopsin after POS engulfment, which coincides with the interaction of the phagosomes with the endosome, is followed by full rhodopsin degradation after lysosomal delivery (Wavre-Shapton et al., 2014). Delivery of the phagocytosed POS to the lysosome involves movement of the phagosome from the apical to the basal region of the cell, which requires myosin VIIa- and KLC1 (Esteve-Rudd et al., 2018; Gibbs et al., 2003).

Interestingly, recent advances provide evidence that POS degradation depends on LC3-associated phagocytosis (Heckmann and Green, 2019), a non-canonical autophagy pathway, and that the intracellular sorting protein melanoregulin links the association of the maturing phagosome with LC3B (Frost et al., 2015). Expression of rubicon (RUBCN) was subsequently suggested to promote LC3-associated phagocytosis in RPE cells during POS degradation (Muniz-Feliciano et al., 2017).

Studies of phagosome processing in the RPE have mainly focussed on processing of the visual pigment, rhodopsin, which is localised on the packed membranous discs of the POS. However, the discs are also rich in lipids, including the highly abundant fatty acid docosahexaenoic acid (22:6) (DHA) and cholesterol, which was recently identified as an additional key factor in RPE phagosome maturation (Ramachandra Rao et al., 2018). Interestingly, accumulating evidence show that up to 80% of the DHA is recycled back to the photoreceptors to be incorporated into newly formed discs (Chen and Anderson, 1993; Chen et al., 1993; Rice et al., 2015), but how the RPE handles this daily challenge is unclear.

One reason for the current interest in phagosome processing in the RPE is the potential role of defects in phagosome processing in the pathology of AMD. Accumulation of intracellular deposits in the form of lipofuscin, and extracellular material in the form of Drusen, are hallmarks of dry AMD. Lipofuscin that accumulates within RPE cells with age, and can be increased in AMD patients, contains the bisretinoid A2E, indicating that it originates at least in part from incompletely digested POS (Kennedy et al., 1995). Drusen are basal extracellular deposits rich in lipids, free and esterified cholesterol and lipoproteins. In mouse models, expression of a dominant-negative form of cathepsin D (Rakoczy et al., 2002), inhibition of lysosome activity with chloroquine (Peters et al., 2006) or loss of function of NPC1 (Claudepierre et al., 2010), a regulator of cholesterol efflux from lysosomes, all lead to accumulation of lipofuscin and/or basal deposits that have some, but not all, of the characteristics of the basal deposits observed in AMD. These data collectively support a role for dysfunctional lysosomal processing of proteins and/or lipids in AMD pathology. The presence of lipids and also exosomal and lysosomal markers within Drusen and Drusen-like extracellular deposits (Wang et al., 2009) supports the idea that lysosome exocytosis could contribute to their generation.

Perspectives

We have highlighted important and unique aspects of membrane trafficking in the RPE. The importance of membrane trafficking in maintaining the specialised functions of the RPE is evident from the impact of partial defects in trafficking pathways on these cells. For example, in X-linked choroideremia (CHM), a disease characterised by the slow degeneration of photoreceptors, RPE and choriocapillaris, loss of Rab escort protein 1 (REP1; also known as CHM), leads to reduced prenylation, and hence function, of Rab GTPases that regulate multiple membrane trafficking pathways in all cells (Seabra et al., 2002). However, CHM is a disease only of the eye. We believe that the trafficking burden of the RPE (and photoreceptors) renders them particularly sensitive to partial defects in membrane trafficking. Progress in understanding aspects of membrane trafficking that are unique to the RPE has been hampered by the limitations of RPE *in vitro* models, which only recapitulate limited RPE features (Lehmann et al., 2014). The development of protocols to differentiate RPE from stem cells, including adult patient-derived cells, is now providing valuable new tools for understanding the molecular regulation of trafficking pathways and how these are compromised in disease (Lehmann et al., 2014). Additionally, new ways to include RPE in organoid cultures will help to reproduce the *in vivo* environment in experimentally tractable systems. A better understanding of membrane trafficking in the RPE is important not only for identifying potential new therapeutic targets for the treatment of eye disease, but also for understanding how drugs and gene therapy vectors are taken up and processed by these cells, which are central to vision.

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