JCB Mini-Review

Retinosomes: new insights into intracellular managing of hydrophobic substances in lipid bodies

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Lipid bodies form autonomous intracellular structures in many model cells and in some cells of specific tissue origin. They contain hydrophobic substances, a set of structural proteins such as perilipin or adipose differentiation-related protein, enzymes implicated in lipid metabolism, and proteins that participate in signaling and membrane trafficking. Retinosomes, particles reminiscent of lipid bodies, have been identified in retinal pigment epithelium as distinct structures compartmentalizing a metabolic intermediate involved in regeneration of the visual chromophore. These observations suggest that lipid bodies, including retinosomes, carry out specific functions that go beyond those of mere lipid storage organelles.

Lipid bodies are autonomous intracellular structures with metabolic functions

The properties of hydrophobic substances, such as self-aggregation, interference with cellular membrane structure and stability, and binding to hydrophobic proteins, impede normal cellular processes and need to be counteracted effectively. Therefore, cells across phyla simply store several of these substances in storage depots termed lipid droplets, storage droplets, lipid bodies, lipid particles, or adiposomes. In the retinal pigment epithelium (RPE) of the eye these retinyl ester storage particles are termed retinosomes.

Typically, lipid bodies contain triacylglycerides, cholesteryl esters, and/or retinyl esters in their hydrophobic core, which is most likely surrounded by a hemi-membrane of unique fatty acid composition (Tauchi-Sato et al., 2002). Although they are clearly identified as morphologically distinct and autonomous entities found in close proximity to the plasma membrane or intracellular organelle membranes (Franke et al., 1987; Steiner et al., 1996; Imanishi et al., 2004), their formation is not fully understood. Most likely, the lipid bodies are born at or in the ER, which is where acyltransferases are catalyzing the last steps in the synthesis of triacylglycerides and where cholesteryl ester and all-trans-retinyl ester syntheses take place. An attractive model proposes that the triacylglycerides and sterol esters accumulate between the two leaflets of the ER bilayer and, after reaching a certain critical concentration, bud off into the cytoplasm. Thereby the highly hydrophobic esters would form the core of a particle surrounded by a monolayer of ER membrane lipids oriented with their acyl chains toward the particle core and with their polar headgroups toward the cytoplasm. Because the limiting ER monolayer corresponds to the cytoplasmic leaflet of the ER membrane, proteins residing in this membrane or those peripherally bound to it can be found on the surface of the lipid bodies (for review see Londos et al., 1999; Brown, 2001; van Meer, 2001).

Lipid droplets have traditionally been regarded as inert inclusions used as storage vessels for hydrophobic intermediates such as triacylglycerides and all-trans-retinyl esters. However, the recent identification of proteins involved in lipid metabolism, signaling, and membrane trafficking in fractions of purified lipid droplets is suggestive of a more active role (Fujimoto et al., 2004; Liu et al., 2004). Among others, key enzymes of sterol metabolism, some of which are controlled in their expression by the sterol regulatory element-binding protein, are found in lipid droplet fractions. Likewise, lipid droplet fractions of yeast cells contain enzymes involved in sterol and triacylglyceride metabolism. Important metabolic enzymes found in lipid particles of the yeast *Saccharomyces cerevisiae* are Erg1p, Erg6p, and Erg7p (ergosterol biosynthesis) and Faa1p, Faa4p, and Fat1p (fatty acid metabolism; Athenstaedt et al., 1999). Thus, anabolic as well as catabolic steps in lipid metabolism might occur in or at lipid bodies. In most cases the exact location of metabolic enzymes in the lipid bodies remains to be determined, along with how and when they gain access to their substrates.

Several small GTPases of the Rab family, which are known to participate in membrane trafficking events, have also been identified in lipid droplet fractions (Liu et al.,

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Key words: retinoid cycle; retina; lipid body; retinal pigment epithelium; adipose differentiation-related protein

Abbreviations used in this paper: ADRP, adipose differentiation-related protein; LRAT, lecithin retinol acyltransferase; RPE, retinal pigment epithelium.

2004). These GTPases include Rab1 and Rab2, which have been shown to regulate ER-Golgi transport, and Rab5, Rab7, and Rab11, which regulate different steps in early and late endocytosis. Another protein possibly involved in signaling and trafficking steps, which is targeted to lipid bodies under certain conditions, is the cholesterol-binding protein caveolin (Ostermeyer et al., 2001, 2004; Pol et al., 2001). Most caveolin isoforms are found in the cytosolic leaflets of cellular membranes, e.g., ER, Golgi membranes, and the plasma membrane (caveolae). However, caveolin-1 was recently also identified in lipid droplets (Robenek et al., 2004). In this study, immunolabeling of freeze-fracture replicas revealed the presence of caveolin-1 in the exoplasmic leaflets of ER membranes and in lipid droplet cores. This localization is not easily compatible with previous findings identifying caveolin at the surface of lipid droplets (for review see Brown, 2001). Thus, it remains to be seen whether and how caveolin-1 transits the lipid bilayer at some point, how it gains access to the hydrophobic core of lipid droplets, and how hydrophilic regions of the protein are organized in the hydrophobic interior of the droplet. Regardless of these controversial issues, it is evident that several caveolin isoforms can associate with lipid bodies. Ectopic expression studies reveal that an NH_2-terminally truncated caveolin-2 isoform, caveolin-2β, is also present in lipid bodies (Fujimoto et al., 2001). Moreover, NH_2-terminal truncation mutants of other caveolins as well as full-length caveolins tagged with an ER retention signal are directed to lipid bodies (Ostermeyer et al., 2001, 2004; Pol et al., 2001). Interestingly, one of the mutants accumulating on lipid bodies also blocks microtubule-dependent lipid droplet motility and the redistribution and/or catabolism of lipids out of the lipid bodies (Pol et al., 2004). Together with the finding that endogenous caveolins can redistribute to lipid bodies in lipid-loaded cells (Pol et al., 2004), these data suggest a direct or indirect role for caveolin in the transport of lipid from lipid bodies to other destinations within the cell. This process could include the transport of cholesterol, although the major biosynthetic and endocytic transport routes of cholesterol, requiring either vesicular carriers or carrier protein complexes (for review see Maxfield and Wustner, 2002), appear not to include lipid bodies.

Adipose differentiation-related protein (ADRP) and related proteins are present on lipid bodies

In addition to the enzymes and putative transport proteins mentioned in the previous section, at least one of a set of four related proteins (ADRP, perilipin, S3-12, and TIP47) has been found to associate with all lipid bodies analyzed thus far. The role of these proteins is not fully understood, but they may stabilize the droplet structure, control the lipolysis of core lipids, and/or provide an anchor for specific subcellular locations within the cell. Structurally, these proteins share a 100-residue-long region of limited homology at the NH_2-terminal part called the PAT (perilipin, ADRP or adipophilin, and TIP47) domain.

Perilipin is the predominant PAT protein in mature adipocytes. It coats the hydrophobic particles, limiting the access of lipases to their substrates in the particle core and thereby restraining lipolysis in resting cells (Miura et al., 2002). Upon β-adrenergic receptor stimulation and subsequent PKA activation, perilipin becomes phosphorylated, allowing lipases to gain access to the lipid bodies (for review see Birnbaum, 2003). Consistently, perilipin null mice have a reduced fat cell mass and a resistance to obesity combined with an elevation in basal lipolysis (Martinez-Botas et al., 2000; Tansey et al., 2001).

The amino acid sequence of ADRP (~50 kD) is not suggestive of transmembrane domains, but the protein appears to be acylated (Heid et al., 1996). Fluorescent protein-tagged ADRP is directed to the surface of lipid bodies in human hepatocyte HuH7 and Chinese hamster ovary K2 cells (Fujimoto et al., 2004; Liu et al., 2004). FRAP experiments using GFP-tagged ADRP protein demonstrate that photobleached lipid bodies do not recover fluorescence, suggesting that ADRP is not recruited rapidly to the surface of lipid bodies (Targett-Adams et al., 2003). ADRP and perilipin are important in serving as a nucleation center for the assembly of lipids to form nascent lipid bodies (Wang et al., 2003), and overexpression experiments in COS-7 cells suggest that ADRP can enhance the uptake of long-chain fatty acids by increasing their influx velocity (Gao and Serrero, 1999). PAT proteins are not found in yeast (*S. cerevisiae*) lipid bodies. This finding raises the question of possible substitution or different mechanisms of lipid droplet generation and/or physiology in yeast as compared with animal cells.

Retinosomes are lipid bodies allowing retinoid compartmentalization during the production of visual pigment chromophore in the eye

Studies on lipid bodies were advanced recently when a novel method of imaging these structures was introduced (Imanishi et al., 2004). This method took advantage of the intrinsic fluorescent properties of vitamin A combined with noninvasive infrared two-photon microscopy (Denk et al., 1990; Piston, 1999; Piston and Knobel, 1999), which allowed for deep tissue penetration in live animals to monitor regeneration of rhodopsin without introduction of artificial fluorophores. Using this approach, we were able to identify in RPE cells previously uncharacterized structures, which were termed retinosomes (or REST particles, for retinyl ester-storage particles). Retinosomes have been shown to participate in 11-cis-retinal recycling in vivo, a process that is necessary for production of rhodopsin and maintenance of the light sensitivity of the photoreceptors (Imanishi et al., 2004). The retinosomes were characterized as specific sites of all-trans-retinyl ester accumulation because of genetic evidence (Imanishi et al., 2004; see the following section) and because spectrally sensitive detectors revealed that the λ max of retinosome fluorescence (488–499 nm) corresponds to that of model all-trans-retinyl esters (Fig. 1, A and B). They appear to exist as stable organelles because the number of retinosomes does not change after all-trans-retinol is mobilized from photoreceptor cells, whereas their all-trans-retinyl ester content significantly increases (Imanishi et al., 2004). However, they appear to participate actively in 11-cis-retinoid production because the amount of all-trans-retinyl esters in retinosomes increases after bleaching of visual pigments

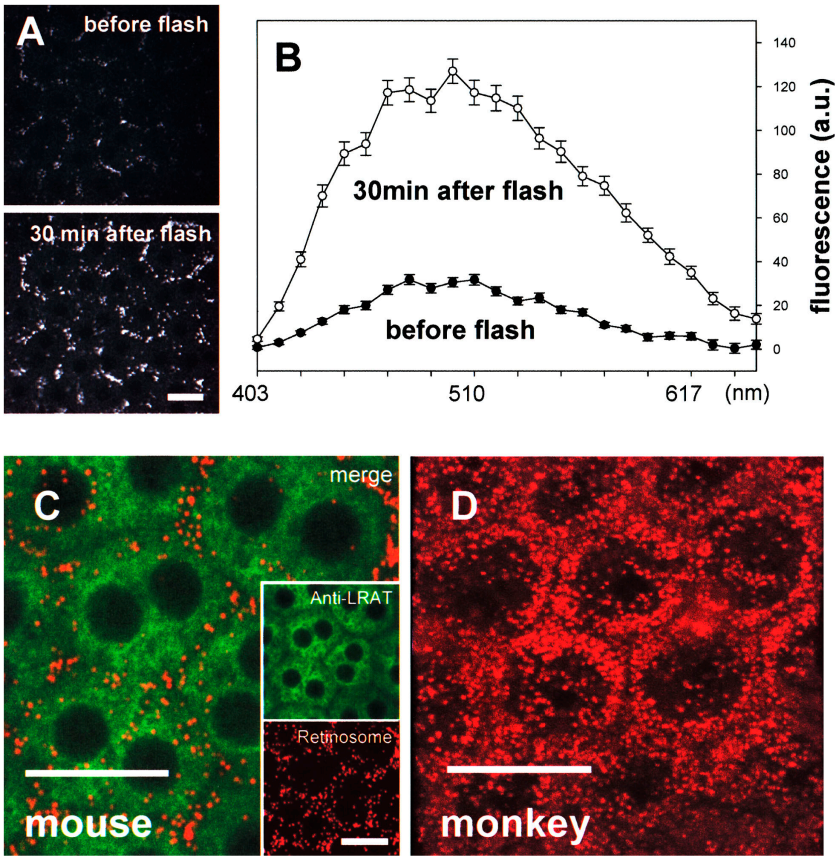

Figure 1. **Immunocytochemical and spectral identification of retinosomes as the storage particles of all-trans-retinyl esters.** (A) Light-dependent change in the fluorescent level of retinosomes in RPE cells. Images were collected before and after flash stimulation (Imanishi et al., 2004). (B) Emission spectra of retinosomes were measured by the LSM 510 META detector before and after flash stimulation (n = 40, error bars indicate SEM; Imanishi et al., 2004). (C) Covisualization of retinosomes (red) and LRAT (green) identified by immunofluorescence. LRAT is localized to the smooth ER, whereas retinosomes are located close to the plasma membranes. (D) Retinosome-like structures of monkey RPE cells. Cells were imaged as described previously (Imanishi et al., 2004) by penetrating laser light through the apical side of the macular RPE cells. Bars, 20 μm.

and subsequently decreases during the process of rhodopsin regeneration. Interestingly, the density of retinosomes seems to vary between species. Mouse RPE appears to have fewer retinosomes (Fig. 1 C) as compared with monkey RPE within the macular region (Fig. 1 D). These differences loosely correlate with the much faster regeneration of visual pigments, particularly cones, in primates as compared with the slow regeneration process in rodents (McBee et al., 2001).

In mice, retinosomes are uniquely elongated structures of lipid particles oriented perpendicularly to the cell layer with a length of 6.9 ± 1.1 μm, a diameter of 0.8 ± 0.2 μm, and a density of 36.2 ± 2.2 per double-nuclei cell (Fig. 2 A; Imanishi et al., 2004). They are clearly distinct from Golgi membranes, mitochondria, the majority of lysosomes, the plasma membrane, and the endoplasmic reticulum. Retinosomes participate in 11-cis-retinal recycling, as demonstrated by in vivo experiments using wild-type and certain knockout mice (Imanishi et al., 2004).

Formation and utilization of retinosomes in transgenic mice

Genetically engineered mice lacking key components of the retinoid cycle have been generated to define the role, formation, and utilization of retinoid intermediates in the live RPE. In the retinal photoreceptor cells, photoisomerization of the chromophore 11-cis-retinal that is coupled to the visual pigment leads to the production and release of all-trans-

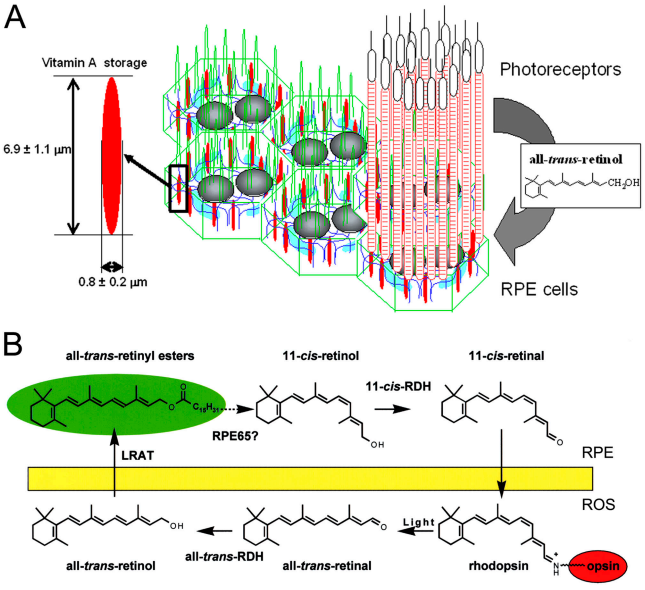

Figure 2. **Cell biology and chemistry of the retinoid cycle reactions in the vertebrate retina.** (A) Cell biology of the retinoid cycle. The RPE cells (hexagons) inter-digitate with several rod outer segments of the photoreceptors. Large curved arrows symbolize the flow of retinoids from photoreceptors to the RPE. Retinosomes (~6.9 μm in length) are depicted as elongated red ovals. (B) Simplified chemistry of the retinoid cycle (modified version from Jang et al., 2000). The retinoid cycle reactions were reviewed previously (McBee et al., 2001).

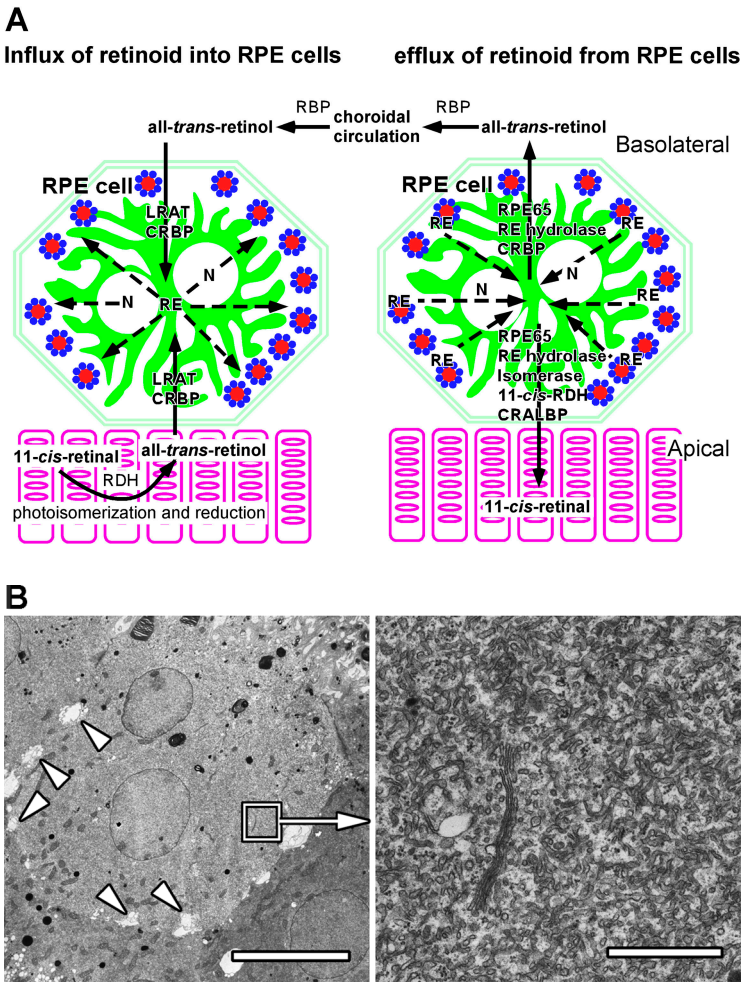

Figure 3. **Putative role of retinosomes in the retinoid cycle and ultrastructure of mouse RPE.** (A) Retinoid cycle in the eye and exchange of all-trans-retinol between RPE cells and choroidal circulation. In the left RPE cell, influx of retinoid is emphasized. All-trans-retinol enters from choroidal circulation or interphotoreceptor space into the RPE, where it is esterified. This process is perturbed in *Lrat−/−* mouse. Retinyl esters (RE) are transported to retinosomes by yet unknown mechanisms. In the right RPE cell, efflux of retinoid is highlighted. All-trans-retinyl esters in retinosomes are used to produce 11-cis-retinal, a chromophore of rhodopsin, or hydrolyzed to all-trans-retinol and exit to choroidal circulation. These processes are prohibited in *Rpe65−/−* mouse. Red, retinosomes; blue, ADRP; green, endoplasmic reticulum; purple, rod outer segments. (B) Ultrastructure of a mouse RPE cell. (left) RPE cells are filled with internal membranes (low magnification view). Bar, 10 μm. (right) The higher magnification image reveals that the internal membranes mostly correspond to smooth endoplasmic reticulum. Bar, 1 μm. Many retinoid processing enzymes are thought to localize to the ER. Retinosomes tend to localize proximally to the lateral plasma membrane (some retinosome structures are indicated by arrowheads). This unique architecture of RPE cells may facilitate the localized processing of retinoids and formation of 11-cis-retinal.

retinal. The photoisomerized chromophore must then be reverted back to 11-cis-retinal to maintain the light sensitivity of the photoreceptors (Fig. 2 B). Specific metabolic transformations of retinoids (i.e., the retinoid cycle) are responsible for this conversion (McBee et al., 2001). In the photoreceptor cells, the majority of all-trans-retinal is released from the photoactivated pigment and accessible to a set of short-chain alcohol dehydrogenases on the surface of the disk membrane. However, a fraction of all-trans-retinal escapes into the intracellular disk space and needs to be transported across the disk membrane. This process requires the action of the ATP-binding cassette transporter (ABCR), which can transport all-trans-retinal or flip all-trans-retinylidene-phosphatidylethanolamine across the membrane, thereby allowing efficient reduction of the aldehyde to all-trans-retinol (Fig. 2 B). The significance of transport across the disk membrane is underscored by the finding that mutations in the ABCR gene in humans (Allikmets et al., 1997) or ABCR gene ablation in mice cause a buildup of bis-retinoids in photoreceptor cells (Mata et al., 2000). Two nucleotide-binding domains of ABCR have interdependent functions (Ahn et al., 2003). One domain appears to play a noncatalytic, regulatory role, whereas the second binds and hydrolyzes ATP in a process that produces energy needed for the transport of hydrophobic substrates across photoreceptor disc membranes. The newly formed all-trans-retinol then diffuses to RPE, where the exclusive enzymatic activity of lecithin retinol acyltransferase (LRAT) catalyses the esterification of all-trans-retinol (vitamin A) with fatty acids. Coenzyme A-retinol transferase cannot substitute for LRAT in this reaction (Batten et al., 2004; Imanishi et al., 2004). Importantly, LRAT is present in the ER, indicating that this organelle is the site of esterification and genesis of retinosomes (Fig. 1 C and Fig. 3 A).

In the next step of the cycle, all-trans-retinyl esters, which have the propensity to self-aggregate, bud off from the ER as independent structures, forming the retinosomes. This process is followed by trans-cis isomerization of the retinoid 11–12 double bond. In the dissected eye, this isomerization does not occur, and the retinoid cycle stops at either all-trans-retinol at lower temperatures or all-trans-retinyl esters at body temperatures. It is possible that exhaustion of ATP and GTP blocks isomerization under those conditions. However, such an explanation is not compatible with the conventional view of isomerization by an isomerohydrolase, where hydrolysis of the all-trans-retinyl esters is coupled to the isomerization reaction (Rando, 1996). Such reactions would not require energy input because high-energy esters are formed in the dissected eye. Thus, small G-proteins or other energy-using enzymes could be required, not for the actual isomerization reaction but to achieve a specific cellular distribution of all-trans-retinyl esters.

One possible site of isomerization could be the ER. However, in this case all-trans-retinyl ester would need to be transported from retinosomes back to the ER. RPE65 (an RPE-specific 65-kD protein), which binds retinyl esters (Gollapalli et al., 2003; Mata et al., 2004), could mobilize them and function as a retinosome-ER shuttle protein. This shuttle would be unidirectional, as retinosomes probably form by budding off from the ER and the lack of RPE65 in knockout mice does not affect the formation of retinosomes. However, *Rpe65*−/− mice accumulate all-trans-retinyl esters in overgrown structures. Why are these overgrown structures formed? In wild-type mice, all-trans-retinol enters the RPE efficiently from the bloodstream, but also exchanges rapidly with a pool of all-trans-retinol present in the blood (Qtaishat et al., 2003). RPE65 could be a key protein that works together with a hydrolase (REH) to produce all-trans-retinol, an alternative substrate for a putative isomerase (for review see McBee et al., 2001). The absence of RPE65 prevents all-trans-retinol's exchange with the blood circulation. Thus, once all-trans-retinol enters the eye and is esterified, it remains in the retinosomes and cannot be liberated to diffuse to blood circulation (Fig. 3 A). Two types of RPE65 were characterized, a membrane-associated and a soluble form (Xue et al., 2004). The molecular switch between the two forms was proposed to involve triple palmitoylation of specific Cys residues catalyzed by LRAT. However, two out of three Cys residues are not conserved in vertebrate RPE65s, raising the questions of whether or not triple palmitoylation is a general feature of the isomerization process and whether the proposed mechanism is relevant.

The retinoid isomerization reaction could take place in retinosomes. Consistent with this concept is the phenotype of CRALBP (soluble 11-cis-retinol/11-cis-retinal binding protein) knockout mice. CRALBP appears to be crucial for an efficient and stereospecific isomerization reaction (McBee et al., 2000), and CRALBP-deficient mice accumulate all-trans-retinyl esters, most likely in the retinosomes (Saari et al., 2002). Because the isomerization to 11-cis-retinol is drastically attenuated in these mice, retinosomes are a likely place for the actual isomerization reaction. However, it must be noted that sluggish isomerization does occur in the absence of CRALBP due to the presence of other retinoid-binding proteins, eventually restoring the visual pigment.

Once produced, 11-cis-retinol is oxidized to 11-cis-retinal and exported to the photoreceptors (Fig. 2 B), where it recombines with opsins to form 11-cis-retinylidene-opsins (and rhodopsin and cone pigments; for review see Filipek et al., 2003). Thus, retinosomes appear to be essential structures for retaining esterified vitamin A in the eye to support its utilization in forming the chromophore for visual pigments.

On average, between 20–40 rod outer segments project toward one RPE cell (for review see McBee et al., 2001; Fig. 2 A). For efficient transfer of retinoids between the RPE and the photoreceptor cells, the retinoid processing enzymes should be widely distributed throughout the cell. This prediction has been confirmed by revealing the ER distribution of LRAT poised to trap all-trans-retinol to form all-trans-retinyl esters (Fig. 1 C). Self-associating complexes of all-trans-retinyl esters (Li et al., 1996) would facilitate retinosome formation, and the resulting clustering of all-trans-retinyl esters in retinosomes may prevent diffusion of retinoids throughout the retina. However, the symmetric nature of these structures and their intracellular distribution suggest that additional proteins are involved in forming or maintaining the particles. ADRP, which is present on retinosomes as well as other lipid bodies, could play such a role, either alone or in conjunction with other yet to be identified proteins. Interestingly, neither retinosomes nor ADRP are found in mouse or bovine retinal Müller cells, which are implicated in the cone-dominant retina (e.g., chicken) as cells functioning alternatively to RPE in 11-cis-retinal production in the course of cone photoreceptor regeneration (Mata et al., 2002).

Increased accumulation or lack of lipid bodies causes diseases

Several conditions or genetic alterations can result in an aberrant intracellular accumulation of lipids, either in the endosomal system when internalized lipids are not properly degraded or transported or in overgrown lipid bodies. Altered lipid storage is clearly evident in several cells, e.g., in adipocytes and hepatocytes after excess food intake. Modified retinosomes are also observed in the retina, where they are the result of blinding disorders affecting the enzymes of the retinoid cycle (for review see Rattner et al., 1999; Dryja, 2000; Baehr et al., 2003). As discussed, retinosomes accumulate in mice incapable of carrying out the enzymatic isomerization process (RPE65 knockout) and are absent in mice deficient in vitamin A in the eye (LRAT knockout; Fig. 3 A), which are animal models of inherited early onset dystrophies that are a subset of Leber congenital amaurosis. Thus, although many retinoid processing enzymes are thought to localize to the ER and cytoplasm, retinosomes that are found close to the plasma membrane of RPE cells (Fig. 1 and Fig. 3 B) could not only be a storage place for retinoids, but could also play a direct, metabolic role in the regeneration of visual pigment chromophores (Imanishi et al., 2004). Therefore, retinosomes are novel components of the retinoid cycle critical to the formation of 11-cis-retinal, and their structure is aberrant in certain disease stages.

Conclusions: the eye as an experimental model system to study lipid bodies

Spectrally sensitive noninvasive two-photon microscopy in conjunction with genetically engineered mice lacking key components of the retinoid cycle can be used to follow the production of the visual chromophore 11-cis-retinal in intact eyes. Two-photon microscopy permits deep tissue penetration of infrared excitation light in anesthetized mice and allows monitoring of the regeneration processes of rhodopsin without introducing external fluorophores. This novel approach has revealed the existence of novel compartments, retinosomes, which are critical to the formation of 11-cis-retinal. It also provides an opportunity to study the formation of the lipid bodies in vivo with spatiotemporal resolution, taking advantage of the accessibility of the visual system to physiological testing. Thus, the eye is one of the most accessible systems for studying the formation, utilization, and disease-causing abnormalities of lipid bodies.

We would like to thank Dr. David Piston for his help in recordings of fluorescence emission, Daniel Possin for his technical support with the EM analysis, and Gerrit van Meer (University of Utrecht), Kevin Ridge (University of Texas), Paul Hargrave (University of Florida), and members of the laboratory for helpful comments on the manuscript.

This research was supported by National Institutes of Health grants EY08061 and EY13385, a grant from Research to Prevent Blindness, Inc. (RPB) to the Department of Ophthalmology at the University of Washington, the Stargardt and Retinal Eye Disease Fund, and a grant from the E.K. Bishop Foundation. K. Palczewski is the RPB Senior Investigator. V. Gerke acknowledges support from the VolkswagenStiftung for a sabbatical stay at the Department of Ophthalmology, University of Washington.

Submitted: 19 May 2004
Accepted: 1 July 2004

References

Ahn, J., S. Beharry, L.L. Molday, and R.S. Molday. 2003. Functional interaction between the two halves of the photoreceptor-specific ATP binding cassette protein ABCR (ABCA4). Evidence for a non-exchangeable ADP in the first nucleotide binding domain. *J. Biol. Chem.* 278:39600–39608.

Allikmets, R., N. Singh, H. Sun, N.F. Shroyer, A. Hutchinson, A. Chidambaram, B. Gerrard, L. Baird, D. Stauffer, A. Peiffer, et al. 1997. A photoreceptor cell-specific ATP-binding transporter gene (ABCR) is mutated in recessive Stargardt macular dystrophy. *Nat. Genet.* 15:236–246.

Athenstaedt, K., D. Zweytick, A. Jandrositz, S.D. Kohlwein, and G. Daum. 1999. Identification and characterization of major lipid particle proteins of the yeast *Saccharomyces cerevisiae*. *J. Bacteriol.* 181:6441–6448.

Baehr, W., S.M. Wu, A.C. Bird, and K. Palczewski. 2003. The retinoid cycle and retina disease. *Vision Res.* 43:2957–2958.

Batten, M.L., Y. Imanishi, T. Maeda, D.C. Tu, A.R. Moise, D. Bronson, D. Possin, R.N. Van Gelder, W. Baehr, and K. Palczewski. 2004. Lecithin-retinol acyltransferase is essential for accumulation of all-trans-retinyl esters in the eye and in the liver. *J. Biol. Chem.* 279:10422–10432.

Birnbaum, M.J. 2003. Lipolysis: more than just a lipase. *J. Cell Biol.* 161:1011–1012.

Brown, D.A. 2001. Lipid droplets: proteins floating on a pool of fat. *Curr. Biol.* 11: R446–R449.

Denk, W., J.H. Strickler, and W.W. Webb. 1990. Two-photon laser scanning fluorescence microscopy. *Science.* 248:73–76.

Dryja, T.P. 2000. Molecular genetics of Oguchi disease, fundus albipunctatus, and other forms of stationary night blindness: LVII Edward Jackson Memorial Lecture. *Am. J. Ophthalmol.* 130:547–563.

Filipek, S., R.E. Stenkamp, D.C. Teller, and K. Palczewski. 2003. G protein-coupled receptor rhodopsin: a prospectus. *Annu. Rev. Physiol.* 65:851–879.

Franke, W.W., M. Hergt, and C. Grund. 1987. Rearrangement of the vimentin cytoskeleton during adipose conversion: formation of an intermediate filament cage around lipid globules. *Cell.* 49:131–141.

Fujimoto, T., H. Kogo, K. Ishiguro, K. Tauchi, and R. Nomura. 2001. Caveolin-2 is targeted to lipid droplets, a new "membrane domain" in the cell. *J. Cell Biol.* 152:1079–1085.

Fujimoto, Y., H. Itabe, J. Sakai, M. Makita, J. Noda, M. Mori, Y. Higashi, S. Kojima, and T. Takano. 2004. Identification of major proteins in the lipid droplet-enriched fraction isolated from the human hepatocyte cell line HuH7. *Biochim. Biophys. Acta.* 1644:47–59.

Gao, J., and G. Serrero. 1999. Adipose differentiation related protein (ADRP) expressed in transfected COS-7 cells selectively stimulates long chain fatty acid uptake. *J. Biol. Chem.* 274:16825–16830.

Gollapalli, D.R., P. Maiti, and R.R. Rando. 2003. RPE65 operates in the vertebrate visual cycle by stereospecifically binding all-trans-retinyl esters. *Biochemistry.* 42:11824–11830.

Heid, H.W., M. Schnolzer, and T.W. Keenan. 1996. Adipocyte differentiation-related protein is secreted into milk as a constituent of milk lipid globule membrane. *Biochem. J.* 320(Pt 3):1025–1030.

Imanishi, Y., M.L. Batten, D.W. Piston, W. Baehr, and K. Palczewski. 2004. Noninvasive two-photon imaging reveals retinyl ester storage structures in the eye. *J. Cell Biol.* 164:373–383.

Jang, G.F., J.K. McBee, A.M. Alekseev, F. Haeseleer, and K. Palczewski. 2000. Stereoisomeric specificity of the retinoid cycle in the vertebrate retina. *J. Biol. Chem.* 275:28128–28138.

Li, C.Y., C.L. Zimmerman, and T.S. Wiedmann. 1996. Solubilization of retinoids by bile salt/phospholipid aggregates. *Pharm. Res.* 13:907–913.

Liu, P., Y. Ying, Y. Zhao, D.I. Mundy, M. Zhu, and R.G. Anderson. 2004. Chinese hamster ovary K2 cell lipid droplets appear to be metabolic organelles involved in membrane traffic. *J. Biol. Chem.* 279:3787–3792.

Londos, C., D.L. Brasaemle, C.J. Schultz, J.P. Segrest, and A.R. Kimmel. 1999. Perilipins, ADRP, and other proteins that associate with intracellular neutral lipid droplets in animal cells. *Semin. Cell Dev. Biol.* 10:51–58.

Martinez-Botas, J., J.B. Anderson, D. Tessier, A. Lapillonne, B.H. Chang, M.J. Quast, D. Gorenstein, K.H. Chen, and L. Chan. 2000. Absence of perilipin results in leanness and reverses obesity in Lepr(db/db) mice. *Nat. Genet.* 26: 474–479.

Mata, N.L., J. Weng, and G.H. Travis. 2000. Biosynthesis of a major lipofuscin fluorophore in mice and humans with ABCR-mediated retinal and macular degeneration. *Proc. Natl. Acad. Sci. USA.* 97:7154–7159.

Mata, N.L., R.A. Radu, R.C. Clemmons, and G.H. Travis. 2002. Isomerization and oxidation of vitamin a in cone-dominant retinas: a novel pathway for visual-pigment regeneration in daylight. *Neuron.* 36:69–80.

Mata, N.L., W.N. Moghrabi, J.S. Lee, T.V. Bui, R.A. Radu, J. Horwitz, and G.H. Travis. 2004. Rpe65 is a retinyl ester binding protein that presents insoluble substrate to the isomerase in retinal pigment epithelial cells. *J. Biol. Chem.* 279:635–643.

Maxfield, F.R., and D. Wustner. 2002. Intracellular cholesterol transport. *J. Clin. Invest.* 110:891–898.

McBee, J.K., V. Kuksa, R. Alvarez, A.R. de Lera, O. Prezhdo, F. Haeseleer, I. Sokal, and K. Palczewski. 2000. Isomerization of all-trans-retinol to cis-retinols in bovine retinal pigment epithelial cells: dependence on the specificity of retinoid-binding proteins. *Biochemistry.* 39:11370–11380.

McBee, J.K., K. Palczewski, W. Baehr, and D.R. Pepperberg. 2001. Confronting complexity: the interlink of phototransduction and retinoid metabolism in the vertebrate retina. *Prog. Retin. Eye Res.* 20:469–529.

Miura, S., J.W. Gan, J. Brzostowski, M.J. Parisi, C.J. Schultz, C. Londos, B. Oliver, and A.R. Kimmel. 2002. Functional conservation for lipid storage droplet association among Perilipin, ADRP, and TIP47 (PAT)-related proteins in mammals, *Drosophila*, and *Dictyostelium*. *J. Biol. Chem.* 277:32253–32257.

Ostermeyer, A.G., J.M. Paci, Y. Zeng, D.M. Lublin, S. Munro, and D.A. Brown. 2001. Accumulation of caveolin in the endoplasmic reticulum redirects the protein to lipid storage droplets. *J. Cell Biol.* 152:1071–1078.

Ostermeyer, A.G., L.T. Ramcharan, Y. Zeng, D.M. Lublin, and D.A. Brown. 2004. Role of the hydrophobic domain in targeting caveolin-1 to lipid droplets. *J. Cell Biol.* 164:69–78.

Piston, D.W. 1999. Imaging living cells and tissues by two-photon excitation microscopy. *Trends Cell Biol.* 9:66–69.

Piston, D.W., and S.M. Knobel. 1999. Quantitative imaging of metabolism by two-photon excitation microscopy. *Methods Enzymol.* 307:351–368.

Pol, A., R. Luetterforst, M. Lindsay, S. Heino, E. Ikonen, and R.G. Parton. 2001. A caveolin dominant negative mutant associates with lipid bodies and induces intracellular cholesterol imbalance. *J. Cell Biol.* 152:1057–1070.

Pol, A., S. Martin, M.A. Fernandez, C. Ferguson, A. Carozzi, R. Luetterforst, C. Enrich, and R.G. Parton. 2004. Dynamic and regulated association of caveolin with lipid bodies: modulation of lipid body motility and function by a dominant negative mutant. *Mol. Biol. Cell.* 15:99–110.

Qtaishat, N.M., T.M. Redmond, and D.R. Pepperberg. 2003. Acute radiolabeling of retinoids in eye tissues of normal and rpe65-deficient mice. *Invest. Ophthalmol. Vis. Sci.* 44:1435–1446.

Rando, R.R. 1996. Polyenes and vision. *Chem. Biol.* 3:255–262.

Rattner, A., H. Sun, and J. Nathans. 1999. Molecular genetics of human retinal disease. *Annu. Rev. Genet.* 33:89–131.

Robenek, M.J., N.J. Severs, K. Schlattmann, G. Plenz, K.P. Zimmer, D. Troyer, and H. Robenek. 2004. Lipids partition caveolin-1 from ER membranes into lipid droplets: updating the model of lipid droplet biogenesis. *FASEB J.* 18:866–868

Saari, J.C., M. Nawrot, G.G. Garwin, M.J. Kennedy, J.B. Hurley, N.B. Ghyselinck, and P. Chambon. 2002. Analysis of the visual cycle in cellular retinol-binding protein type I (CRBPI) knockout mice. *Invest. Ophthalmol. Vis. Sci.* 43:1730–1735.

Steiner, S., D. Wahl, B.L. Mangold, R. Robison, J. Raymackers, L. Meheus, N.L. Anderson, and A. Cordier. 1996. Induction of the adipose differentiation-related protein in liver of etomoxir-treated rats. *Biochem. Biophys. Res. Commun.* 218:777–782.

Tansey, J.T., C. Sztalryd, J. Gruia-Gray, D.L. Roush, J.V. Zee, O. Gavrilova, M.L.

Reitman, C.X. Deng, C. Li, A.R. Kimmel, and C. Londos. 2001. Perilipin ablation results in a lean mouse with aberrant adipocyte lipolysis, enhanced leptin production, and resistance to diet-induced obesity. *Proc. Natl. Acad. Sci. USA.* 98:6494–6499.

Targett-Adams, P., D. Chambers, S. Gledhill, R.G. Hope, J.F. Coy, A. Girod, and J. McLauchlan. 2003. Live cell analysis and targeting of the lipid droplet-binding adipocyte differentiation-related protein. *J. Biol. Chem.* 278:15998–16007.

Tauchi-Sato, K., S. Ozeki, T. Houjou, R. Taguchi, and T. Fujimoto. 2002. The surface of lipid droplets is a phospholipid monolayer with a unique Fatty Acid composition. *J. Biol. Chem.* 277:44507–44512.

van Meer, G. 2001. Caveolin, cholesterol, and lipid droplets? *J. Cell Biol.* 152:F29–F34.

Wang, S.M., R.D. Hwang, A.S. Greenberg, and H.L. Yeo. 2003. Temporal and spatial assembly of lipid droplet-associated proteins in 3T3-L1 preadipocytes. *Histochem. Cell Biol.* 120:285–292.

Xue, L., D.R. Gollapalli, P. Maiti, W.J. Jahng, and R.R. Rando. 2004. A palmitoylation switch mechanism in the regulation of the visual cycle. *Cell.* 117:761–771.