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Review

Biology and pathobiology of lipid droplets and their potential role in the protection of the organ of Corti

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ABSTRACT

The current review article seeks to extend our understanding on the role of lipid droplets within the organ of Corti. In addition to presenting an overview of the current information about the origin, structure and function of lipid droplets we draw inferences from the collective body of knowledge about this cellular organelle to build a conceptual framework to better understanding their role in auditory function. This conceptual model considers that lipid droplets play a significant role in the synthesis, storage, and release of lipids and proteins for energetic use and/or modulating cell signaling pathways. We describe the role and mechanism by which LD play a role in human diseases, and we also review emerging data from our laboratory revealing the potential role of lipid droplets from Hensen cells in the auditory organ. We suggest that lipid droplets might help to develop rapidly and efficiently the resolution phase of inflammatory responses in the mammalian cochlea, preventing inflammatory damage of the delicate inner ear structures and, consequently, sensorineural hearing loss.

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1. Introduction

The observation of intracellular lipids is old in the field of cell biology. However, the study of the organelle chiefly involved in its storage, known as lipid droplet (LD), has just recently gained significant attention by basic scientists, clinical investigators, practitioners from many medical disciplines, and pharmaceutical companies. Fundamental studies in the field of cell biology, biophysics, and biochemistry, in particular those performed during the last twenty-five years, have revealed that LDs are not only involved in the storage but also in the release and metabolic processing of lipids and proteins involved in a number of intra-cellular and multi-cellular mechanisms. This field of research has been galvanized by extensive biochemical investigations that revealed that LDs are not simple inclusions of fat, but they have a constitutive cohort of resident molecules, including simple and complex

lipids, steroids, and proteins, involved in a variety of critical cellular functions (Brown, 2001; Cermelli et al., 2006; Martin and Parton, 2006). In fact, a large number of laboratories around the world are currently working on extending these fundamental observations. The conceptual framework emerging from these studies is that, at a subcellular level, some components are involved in the regulation of the structure and function of the LD itself while others perform functions integrated at either the cellular or the whole organism level. Another focus of intensive investigation seeks to test the hypothesis that the molecular composition of LDs varies among cells, and even inside a single cell, according to their contributions to the structure and function of the resident tissue. This line of investigation is providing clues to the type and complexity of the contributions of LDs to cell specialization.

The study of LDs is pushing the development of technology ahead. This is evident by the increase in the application of very sophisticated biochemical and biophysical methods as well as genetically engineered model organisms. The most transformational impetus for the advancement of this field, however, can be found in the current need of translating the knowledge from the laboratory to the clinic at a faster pace. For instance, one of the

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most rapidly growing cause for disease in USA, and in many places around the world, are those associated with fat accumulation, storage, metabolism, and its conversion to energy. At the organelle level, the management of fat begins with the function of the LDs in adipocytes. The obesity epidemic has endowed us with bodies that carry and recycle a large amount of fat, which in many cases has direct toxic effects. For instance, the accumulation of fat in LDs underlies the pathogenesis of NASH (Non-Alcoholic SteatoHepatitis), a liver disease which has become one of them most frequent cause of death in modern medical practice (Karagozian et al., 2014). It appears, that unbalanced lipid management by cells, leads to “lipoapoptosis”, with concomitant inflammation, fibrosis, and organ failure, a process further enhanced by various toxins, including some commonly used drugs (Anderson and Borlak, 2008).

A nascent but very promising area of investigation involves studies on the function and dysfunction of LDs in other organs and cell populations, besides the liver and adipocytes. This research is guided by the hypothesis that LDs would play a role in the biology and pathobiology of every organ and cell population just like in liver and adipocytes. It is on this shore of the current research tide that our laboratory is actively investigating the potential contribution of LDs to maintaining the health of the auditory organ. We want to understand how these organelles and their component mediate or antagonize the effects of ototoxic agents, as well as whether and how they participate in the development of human diseases. Therefore, in the current article we review the existing knowledge in this field, highlight the most current research directions, and describe a useful paradigm that can help in better understanding the role of LDs in the biology and pathobiology of auditory diseases.

2. Early functional paradigms for understanding the contribution of LDs to the function of the auditory organ

In most cells other than adipocytes, LDs are too small or too few to be seen in histological sections. Confocal and electron microscopy, however, revealed that LDs exist nearly ubiquitously from bacteria to mammals, and current theories coincide in that all mammalian cells contain LDs but their size and number is cell type- and species-dependent (Ohsaki et al., 2014). For example, LDs are a well-known feature of guinea pig Hensen cells ((Hallpike, 1936; Kimura, 1975; Vinnikov and Titova, 1964) as cited by Merchan et al. (1980)). In contrast, LDs in mouse Hensen cells are usually very small, making them near undetectable by light microscopy and hard to identify in EM images because their content is not electron-dense. LDs function in Hensen cells (or any other auditory cell), or its importance for the organ of Corti as a whole, is still unknown.

Merchan and coworkers proposed in the 1980's the first, to the best of our knowledge, hypothesis about a potential role of Hensen cell LDs in auditory function (Merchan et al., 1980). In their original study and two other reports published in the following years (del Canizo Alvarez and Merchan Cifuentes, 1986; del Canizo-Alvarez et al., 1987), these authors speculated that, since the tectorial membrane (TM) is attached by its lateral end to Hensen cells, changes in Hensen cells' height might influence the size of the sub-tectorial space. In other words, the distance between the reticular lamina (RL) and the TM will be dependent of the height of Hensen cells (Merchan et al., 1980). Thus, Hensen cell LDs might be considered a factor of modulation in the interaction between the hair cells' stereocilia and the TM. In addition, since exposure to high-intensity noise induces release of LDs from guinea pig Hensen cells (del Canizo Alvarez and Merchan Cifuentes, 1986; del Canizo-Alvarez et al., 1987; Merchan et al., 1980), and produces a significant reduction in the height of these cells in a murine model (Wang

et al., 2002), they could have an important and active part in the protection of the organ of Corti against trauma during high-intensity sound exposure.

Twenty years later, Bell and Fletcher proposed a different role for the Hensen cell LDs (Bell and Fletcher, 2004). These authors developed a model for cochlear fine-tuning based on Symmetric Lloyd–Redwood (SLR) waves, known in ultrasonics as “squirting” waves, and pointed out that their distinctive properties make them well suited to generate standing-wave resonance between the rows of OHCs. Briefly, OHCs excited by the traveling wave will become electromotile, deforming the RL and the TM and launching a secondary wave from each cell. These secondary waves will interact with other OHCs, causing them to respond with further waves, and so on. Since there are three parallel rows of OHCs, a positive feedback at a resonance frequency related to the OHC spacing would occur, with a “radial” wave in a direction normal to the rows generating a standing-wave resonance. There is, however, a major problem with this model: the propagation of SLR waves in the narrow sub-tectorial space would be strongly dampened by viscous forces, particularly at low frequencies. However, since it is known that the effects of viscosity in narrow channels can be greatly diminished when hydrophobic surfaces are involved, the authors proposed that LDs secreted by Hensen cells into the sub-tectorial space would coat both TM and RL surfaces with oil, reducing their viscous drag and making the standing-wave resonance possible (Bell and Fletcher, 2004). Interestingly, other models considered radial fluid motion in the RL-TM gap, but rejected it because of viscosity considerations (e.g., (Cai et al., 2004; Chadwick et al., 1996)). Assigning a role to Hensen cell LDs in overcoming limitations imposed by viscosity could also provide support to these other models, opening new avenues for understanding cochlear fine-tuning mechanism.

In 2009, a completely different role for Hensen cell LDs was proposed by the laboratory of one of the authors of this Review (Kalinec et al., 2009). Experimental evidence indicated that Hensen cell LDs contain significant amounts of the protein annexin A1 (ANXA1), and that glucocorticoids activate a cellular mechanism that results in the release of ANXA1 from the LDs first, and then from the Hensen cells to the extracellular milieu. Since ANXA1 is known to inhibit leukocyte migration and extravasation by receptor-mediated signaling, as well as to regulate cell death signaling and the phagocytic clearance of apoptotic cells (D'Acquisto et al., 2008), it was proposed that Hensen cell LDs would play a major role in preventing leukocytes from invading the organ of Corti and facilitating the resolution of inflammatory responses in the mammalian inner ear (Kalinec et al., 2009). The potential immunological and anti-inflammatory roles of LDs in the cochlea are discussed in more detail in Section 8.

Thus, since we now know that LDs have many important structural, energetic, and paracrine functions in practically every cell from every organ, a better knowledge of the cell biology, biochemistry and biophysics of LDs is essential to elucidate their actual function/s in Hensen cells and other cell populations of the cochlea, and their importance for the auditory function.

3. Lipid droplets: from static fatty inclusions to dynamic organelles

LDs are ubiquitous dynamic organelles that synthesize, store, and supply lipids in cells from diverse organisms, including bacteria, yeast, plants, insects and animals. While the earliest descriptions of this organelle date back to the 19th century (Altmann, 1890; Wilson, 1896), they were ignored for decades by cell biologists thereafter because of their perceived role as inert fat particles (Farese and Walther, 2009). In the early 1900s, LDs achieved

recognition as organelles present in most cells, receiving the name of liposomes. Curiously, in the late 1960s, artificial liposomes engineered for the delivery of molecules to cells and animals usurped this name, and consequently scientists working in these field begun to call them by various names, including lipid bodies, fat bodies, fat droplets, and adiposomes. In the field of plant biology, researchers adopted more frequently the term of oil bodies. The term “lipid droplets” became popular in the 1990s, and it is now the most widely accepted (Farese and Walther, 2009).

LDs consist of a phospholipid monolayer surrounding a core of mostly neutral lipids (Ohsaki et al., 2009). The stored lipids support vital cellular functions such as energy metabolism, membrane synthesis, and production of essential lipid-derived molecules such as lipoproteins, bile salts, or hormones (Farese and Walther, 2009). Recent cell biology, proteomics, lipidomics and metabolomics analyses have solidly demonstrated that LDs also contain structural and membrane proteins, proteins involved in lipid synthesis, histones, and aliphatic and aromatic lipids in free and esterified forms (Fig. 1) (Athenstaedt and Daum, 1997; Bartz et al., 2007; Blaner et al., 2009; Bostrom et al., 2007; Brasaemle et al., 2004; Cermelli et al., 2006; Fujimoto et al., 2004; Katavic et al., 2006; Larsson et al., 2012; Liu et al., 2004; Tauchi-Sato et al., 2002; Turro et al., 2006; Wan et al., 2007; Zehmer et al., 2009; Zhang et al., 2011). It has also been demonstrated that cell autonomous mechanisms and extracellular signals can also regulate LD formation and function (Hapala et al., 2011), indicating that these organelles may play a dynamic role in the regulation of cell homeostasis and processes not directly related to lipid metabolism, such as protein degradation and immunity (Pol et al., 2014). This idea is further supported by the observation that accumulation of LDs also occurs during progression of pathologies not obviously related to lipid biology, such as cardiomyopathies, neuropathies, or during viral hepatitis caused by, among others, the human immunodeficiency virus (Vallet-Pichard et al., 2012).

Importantly, although structurally similar, LDs should not be confused with plasma lipoproteins. Plasma lipoproteins are another kind of particles, also wrapped in a phospholipid monolayer and containing lipid esters, which transport lipids through the aqueous circulation to different regions of the body (Ohsaki et al., 2009). In

addition to having LDs, hepatocytes and intestinal epithelial cells make very low-density lipoprotein (VLDL) and chylomicrons, respectively, which are secreted by exocytosis (Murphy, 2001). Thus, in some cells two different lipid particles of a similar structure exist in opposite compartments, lipoproteins and LDs (Ohsaki et al., 2009). In addition, LDs should not be confused either with exosomes or with microvesicles, which are lipid bilayer-bound structures. Exosomes play a very important role in paracrine cell-to-cell communication (Bobrie et al., 2011; Record et al., 2014a, 2011, 2014b), whereas microvesicles participate in the transport of phospholipids from specialized areas of the cells (Muller et al., 2009). While diameter ranges from 30 to 90 nm for VLDL, from 70 to 1000 nm for chylomicrons, and from 100 nm to 1 μ m for microvesicles, LDs range from 15 nm to more than 100 μ m in diameter, which can either increase or decrease in response to cellular signals.

4. Biogenesis of lipid droplets

Biogenesis of LDs is a poorly understood process involving the formation of a monolayer-bound organelle from a bilayer membrane. Additionally, large LDs can form either by growth of existing LDs or by the combination of smaller LDs through several distinct mechanisms. The most accepted theory of the origin of LDs postulates that they are most likely generated at the ER membrane (Jacquier et al., 2013, 2011). LDs are often found in close proximity to the ER and, in some instances, they are connected to the ER through ER–LD membrane bridges (Soni et al., 2009). Detection of ER membrane proteins, ER chaperones, and caveolins in LDs, also support this theory (Bozza et al., 1997; Dvorak et al., 1992; Ghosal et al., 1994; Robenek et al., 2004). These facts, however, do not exclude the possibility that other cellular organelles contribute to the LD-forming process (Ohsaki et al., 2009).

The prevailing hypothesis of LDs biogenesis postulates that they originate by pinching off from the ER lipid bilayer. This process would occur when a nucleus of lipid esters accumulate between the two leaflets of the ER membrane, gradually grow into a globular shape, and are finally pinched off from the ER to become independent LDs (Murphy and Vance, 1999; Wanner et al., 1981). The

Fig. 1. Structure of a LD. A phospholipid monolayer encloses a core containing mostly triacylglycerols (TAGs) and sterol esters (SEs). Proteins are associated to the LDs surface and also found inside the core, which also contains histones, RNA, lipid micelles, and ribosomes. Free cholesterol is in the monolayer, but probably in the LD's core too.

nascent LDs would be very small. A mathematical model predicted that, in *Saccharomyces cerevisiae*, LD bud-off diameter would be of about 12 nm (Zanghellini et al., 2010); millisecond-scale coarse grained molecular dynamics simulations, on the other hand, suggest that they could be around 17 nm in diameter (Khandelia et al., 2010). One potential controversy in this model is that the phospholipid and fatty acid composition of the ER differs from that of the LDs, with the LDs having a greater amount of unsaturated fatty acids (Ivanova et al., 2009). However, it has been suggested that such changes in LD phospholipids could be due to lipid remodeling (Tan et al., 2014). Another potential problem with the scheme described above is that lipid esters are supposed to bulge as a globule within a relatively small area of the ER membrane, but it is not clear whether such a globule formation occurs based on the lipid properties alone. It is probable that some protein-based mechanism restricts thin lateral spreading of the lipid ester between the two membrane leaflets. In fact, a number of studies have revealed that LDs contain proteins that contribute to the LD formation process, membrane integrity, biosynthetic process, cytoskeletal association, transport, and secretion. We discuss this repertoire of proteins in Section 5.

There is a clear relationship between LDs biogenesis and inflammatory responses, with specific and well-regulated signaling pathways associated with LDs biogenesis in cells involved in inflammatory and/or neoplastic reactions (Bozza and Viola, 2010). For instance, saturated fatty acids do not trigger LD formation while cis-unsaturated fatty acids are potent inducers of LDs (Bozza et al., 1996; Moreira et al., 2009; Weller et al., 1991), suggesting that their formation involves more than simple incorporation of exogenous lipids. Moreover, stimulation with cytokines/chemokines and hormones induces receptor-mediated LD biogenesis not only *in vivo* but even *in vitro* in the absence of exogenous lipids (Bandeira-Melo et al., 2001; Bozza et al., 1998; Maya-Monteiro et al., 2008; Pacheco et al., 2007; Vieira-de-Abreu et al., 2005).

Interestingly, it has been demonstrated that, at least in *Drosophila* but potentially also in mammals, LDs could be part of a histones-based antibacterial defense system (Anand et al., 2012). Histones are sequestered on LDs under normal conditions but, in the presence of bacterial lipopolysaccharide (LPS) or lipoteichoic acid (LTA), they are released and kill bacteria efficiently *in vitro*. LDs-bound histones also function *in vivo*: when injected into *Drosophila* embryos lacking LDs-bound histones, bacteria grow rapidly; in contrast, bacteria injected into embryos with LDs-bound histones die (Anand et al., 2012). Middle ear infections, one of the most common pediatric diseases, may be of bacterial origin and induce inner ear inflammation (Paparella et al., 1980), which may lead to sensorineural hearing loss (Paparella et al., 1984). Thus, the intimate association of LDs with inflammation and with this potential antibacterial defense mechanism, suggest that they could have an important role in the protection of the auditory system against infections and inflammatory responses.

5. Lipidomics of lipid droplets

All LDs functions are rooted in their unique architecture. In contrast to other organelles that have aqueous content within a phospholipid bilayer membrane, the LDs basic structure is a phospholipid monolayer (or hemi-membrane) surrounding a core of neutral lipids (Fig. 1). In mammalian LDs the phospholipid monolayer contains phosphatidylcholine (PC) and phosphatidyl ethanolamine (PE) as in other membranes, but it is peculiar in that also contains lyso-PC and lyso-PE with abundant unsaturated fatty acids and the ether-linked form of PC and PE (Bartz et al., 2007; Tauchi-Sato et al., 2002). The overall composition of phospholipids appears to be similar irrespective of the cell type (Bartz et al.,

2007), but its heterogeneity among LDs in a single cell has not been addressed. The amount of free cholesterol is also significant at the surface monolayer of LDs from many cell types, which is not surprising since LDs function as a cholesterol sink for the whole body (Prattes et al., 2000; Zweytick et al., 2000). Whether free cholesterol exists only in the surface or both in the surface and the core is not known yet (Ohsaki et al., 2014). The core, in turn, consists mostly of triacylglycerols (TAG) and sterol esters (SE), but depending on cell type, may also include retinyl esters, waxes, and ether lipids (Ohsaki et al., 2009; Wilfling et al., 2014). TAG and SE can colocalize in the same LDs (Cheng et al., 2009; Kellner-Weibel et al., 2001), but TAG-dominant and SE-dominant LDs may form separately (Hsieh et al., 2012). Acyl chain composition in TAGs can also vary among LDs (Horn et al., 2011; Rinia et al., 2008).

In addition to storing lipids for use in a variety of metabolic processes, by compartmentalizing them LDs are able to buffer cells from the toxic effects of excessive amounts of lipids (Farese and Walther, 2009). For example, lipid detoxification and sequestration from the cytoplasm is a phenomenon clearly observed in LDs from macrophages, which can incorporate large amounts of cholesterol that would otherwise induce cell death via endoplasmic reticulum stress (Maxfield and Tabas, 2005).

It should be noted that LDs are found both in the cytoplasm (*aka* cLDs) and the cell's nucleus (nLDs), and they have different lipid composition and, possibly, origin (Layerenza et al., 2013). For example, nLDs isolated from rat-liver nuclei were contained 19% TAG, 39% SE, 27% cholesterol, and 15% polar lipids, whereas cLDs from the same cells contained 92% TAG, 5% SE, 2% cholesterol, and 1% polar lipids (Layerenza et al., 2013). Since very little is known about nLDs, in this review we used LDs as synonymous of cLDs.

6. Proteomics of lipid droplets

LDs contain numerous proteins at their surfaces, where they control lipid synthesis, initiate lipid droplet fusion and promote lipid hydrolysis. Interestingly, some studies showed presence of proteins, including soluble ones, in the LD core (Bozza et al., 1997; Dvorak et al., 1992; Robenek et al., 2005, 2006) (Fig. 1), where they play still unknown functional roles. It is not plausible that hydrophilic proteins exist alone in the core, but amphiphilic proteins may complex with phospholipids to make structures compatible with the hydrophobic environment (Fujimoto and Parton, 2011). Importantly, LDs proteome varies between LDs of different sizes (Krahmer et al., 2011; Wilfling et al., 2013; Wolins et al., 2006) or different lipid composition (Hsieh et al., 2012) within the same cell.

Depending on the species examined, LDs may contain from 40 to 300 different proteins (Beller et al., 2006; Bouchoux et al., 2011; Ding et al., 2012; Ivashov et al., 2013; Krahmer et al., 2013b). Since the cytoskeleton plays a role in controlling their distribution, LDs proteome is rich in cytoskeletal proteins. Several different families of membrane trafficking proteins and vesicular traffic proteins have been identified in LDs from yeast, *Drosophila* and mammals, supporting the view that they could be specialized for bi-directional, intermembrane molecular transport (Tan et al., 2014; Zehmer et al., 2009). The presence of dynein and kinesin-1, for instance, is consistent with the reported long-range directional movement of LDs along microtubules (Welte, 2009). Short-distance Brownian movement, on the other hand, suggests that the LD distribution is also affected by factors like intermediate filaments (Franke et al., 1987) and other organelles (Wolinski et al., 2011). In addition, LDs might have a role in signaling due to the presence on their surface of several protein kinases that are involved in signal transduction such as phosphatidylinositol 3-kinase, mitogen-activated protein (MAP) kinases and protein kinase C (Yu et al., 2000, 1998).

The more characteristic LDs proteins, however, are the perilipins, the first proteins identified in LDs (Greenberg et al., 1991; Londos et al., 1995, 1996). Originally, they were termed “PAT family of proteins”, with PAT deriving from the names of three major proteins of this family, Perilipin, ADRP (adipocyte differentiation-related protein), and TIP47, with each having highly related N-terminal sequences and common affinity for LDs (Lu et al., 2001). In 2010, the name “perilipins or PLINs” was adopted as a unifying nomenclature for these proteins, with PLIN1 corresponding to the original perilipin (aka perilipin 1), PLIN2 to ADRP and PLIN3 to TIP47, while the gene symbols *Plin* and *PLIN* were adopted for murine and human genomes, respectively (Kimmel et al., 2010). Currently, five different perilipins, PLIN1 to PLIN5, have been characterized (Bickel et al., 2009; Brasaemle, 2007). PLIN1 and PLIN5, in adipocytes and oxidative cells, respectively, function both as a lipolytic barrier and as regulators of TAG hydrolyzing enzymes. Ubiquitously expressed PLIN2 and PLIN3 also seem to act protectively against lipolysis, implying that perilipins are crucial for mammalian LDs (Ohsaki et al., 2014). PLIN2 coats the LDs surface of all mammalian cells except mature white adipocytes, where it is replaced by PLIN1 (Bickel et al., 2009; Londos et al., 1999). PLIN2 prevents lipase association with the surface of LDs and slows TAG turnover (Listenberger et al., 2007). Consequently, the levels of PLIN2 expression are closely linked to LD accumulation (Imamura et al., 2002; Larigauderie et al., 2004; Magnusson et al., 2006). In contrast to PLINs 3, 4, and 5, which can exist as soluble cytosolic proteins, in the absence of LDs PLIN2 is degraded by the proteasome (Tan et al., 2014). Interestingly, the size of LDs in adipocytes can determine the type of PLIN proteins that associate with the LD surface. This is exemplified by the finding that inducing LD formation in pre-adipocytes resulted in the localization of PLIN4 and PLIN5 on smaller LDs while PLIN1 and PLIN2 are found on larger LDs (Wolins et al., 2005).

Results from cell culture, mouse models, and human studies indicate that the primary function of the collective PLIN protein family is to sequester lipids into LDs by protection to neutral lipase action. Exquisite regulation of energy and substrate release also suppresses lipocytotoxicity. Intriguingly, although all five PLIN proteins share an ability to inhibit the action of the same set of LD lipases (Lass et al., 2011), their actions seem to involve very different mechanisms. The tissue-specific distributions of the different PLIN variants may reflect the relative degree toward either lipid storage or utilization (Sztalryd and Kimmel, 2014).

7. Biophysics of lipid droplets

Although LDs emerging from the ER would be very small (Khandelia et al., 2010; Zanghellini et al., 2010), many cells possess large LDs. Moreover, LDs size varies not only between different cells but also inside a single cell and at different time-points, growing and shrinking in response to cellular signals. Although, from a biophysical point of view, LDs may be considered an emulsion (Thiam et al., 2013), the precise control of LDs size makes them different from a simple two-phase system. The determinants of LD size are incompletely understood, but may be affected by rates of TAG and phospholipid synthesis and turnover as well as cell's energy requirements (Egan et al., 1992; Tan et al., 2014), and clearly depend on one or more types of proteins associated to the LDs' surface. To give just an example, myocytes produce many small LDs with high turnover rates to fulfill the needs of these cells to produce energy from fatty acids (Egan et al., 1992; Tan et al., 2014); adipocytes, in contrast, usually have a single, huge LD that works essentially as a fat reservoir.

Large droplets can arise from two general mechanisms: growth of a LD or by processes in which LDs combine to form a single, larger

LD. LD growth would occur by local synthesis of TAGs at the surface of LDs. Thus, droplet growth would require a cellular trafficking pathway to deliver the necessary enzymes for TAG synthesis, probably from the ER although other trafficking routes cannot be precluded, to the LDs (Ohsaki et al., 2014). The generation of a large LD from combination of smaller LDs can occur either by direct fusion or by ripening, a diffusion-mediated transfer of core lipids (Thiam et al., 2013). Direct fusion of LDs in cells is rare under normal circumstances, but it can be induced by modulating the LD surface by limiting available phosphatidylcholine (Fei et al., 2011; Krahmer et al., 2011), or by the addition of surfactants or other fusogenic agents (Ariotti et al., 2012; Murphy et al., 2010). In adipocytes, large LDs form by what appears to be a ripening process called permeation. Specifically, the fat-specific protein of 27 kDa (FSP27), a member of the cell death-inducing DFF45-like effector (CIDE) family mainly expressed in adipocytes, is involved in transferring lipids between two adjacent LDs (Gong et al., 2011). Experimental observations are consistent with TAG molecules diffusing from the smaller to the larger LD at a contact site, driven by differences in Laplace pressures of the two LDs. Ripening-mediated transfer of TAG by FSP27 is likely regulated by binding of FSP27 to perilipin 1, which increases transfer by increasing pore size (Sun et al., 2013).

From a physical point of view, LDs are different from vesicular organelles in that they are not membrane-bound structures, their internal content is highly hydrophobic and their shape is invariably spherical. Vesicular organelles, with their aqueous lumen, may change shape in a flexible manner, whereas LDs need to be round to minimize the interface between hydrophobic lipid esters and the cytosol. Because of this requirement, the amount of LD surface molecules must be controlled in accordance with the volume of lipid esters to prevent formation of packing defects in the phospholipid monolayer (Bigay and Antonny, 2012; Ohsaki et al., 2009). Due to its unique characteristics, quantitative discordance between the surface and the volume may occur in the growth and/or involution processes of the LDs. These processes may give rise to the structural and functional diversities of LDs (Ohsaki et al., 2009). For instance, and in contrast to common vesicles with true membranes that after fusion change their volume but preserve their surface area, after fusion LDs preserve the total volume generating an excess of about 15–20% surface area (Fujimoto et al., 2008; Murphy et al., 2010; Ohsaki et al., 2009). This surface area surplus can be either released from the cell as vesicles or internalized by the LDs as micelles with or without associated proteins (Fujimoto et al., 2008; Ohsaki et al., 2009) (Fig. 1). Conversely, LD division result in loss of surface (Fujimoto et al., 2008; Ohsaki et al., 2009), which facilitate the interaction of the lipids in the core with external lipases in a process regulated by perilipins (Sztalryd and Kimmel, 2014).

The membranous structures and/or various proteins observed in the core of LDs in several cell models could be a natural consequence of the fusion process. For example, the protein annexin A1 was found inside the LDs of guinea pig Hensen cells (Kalinec et al., 2009), membranes and ribosomes were observed in LDs of leukocytes (Wan et al., 2007), and RNA was reported in the mast cell LD (Dvorak et al., 2003). Moreover, detection of ER membrane proteins, ER chaperones, and caveolins by immunoelectron microscopy (Bozza et al., 1997; Dvorak et al., 1992; Ghosal et al., 1994; Robenek et al., 2004) imply that LDs may have an internal structure other than a simple mass of lipid esters (Fig. 1). In addition, heterogeneous labeling patterns of LD associated proteins in the cross-fractured core by immunoelectron microscopy (Robenek et al., 2005, 2009) suggest that LDs with different internal structures may coexist in a cell (Fujimoto and Parton, 2011; Ohsaki et al., 2014, 2009).

8. Lipid droplets and human diseases

The worldwide pandemic of obesity has serious health consequences, and constitutes serious challenges to both biomedical research and treatment (Popkin et al., 2012). It is widely accepted that we become overweight and even obese because our intake of energy (food) exceeds our caloric expenditure. Our bodies first adapt to this chronic state of increased energy supply by accessing the high capability of white adipose tissue to store surplus energy as neutral lipids in large, unilocular LDs. With time, and for reasons not yet fully determined, LDs in adipose tissue become limited in its lipid storage capacity. Lipids “spill over” to non-adipose tissue, such as skeletal and heart muscles, liver, and pancreas, which increase its own LDs number and size to entrap these excess lipids. Chronic excess lipid flux into non-adipose tissues, commonly termed “ectopic fat”, can either cause or potentiate insulin resistance, lipotoxicity, and eventually tissue dysfunction (Boden, 2011). Therefore, a distinction that has both mechanistic and biomedical importance is that of normal excessive fat accumulation in adipocyte’s LDs versus ectopic fat in LDs of non-adipose tissue.

The pathophysiological consequences of ill-distributed adipose lipid storage and the importance of adipose LDs function to maintain systemic glucose and lipid homeostasis are best illustrated in lipodystrophies, which are often associated with problems in adipose LDs’ growth and function (Vigouroux et al., 2011). While the presence of ectopic fat is highly correlated with insulin resistance, dyslipidemia, diabetes type 2, reproductive abnormalities, and cardiovascular diseases, the relationships among ectopic fat and insulin resistance in skeletal and cardiac muscles and liver are complex, and indeed puzzling (Coen and Goodpaster, 2012; Muoio, 2010; Sun and Lazar, 2013). Skeletal muscle from exercised-trained subjects can display high insulin sensitivity, despite intramuscular TAG levels that exceed those of obese and diabetic individuals, a phenomenon described as the “athlete paradox” (Coen and Goodpaster, 2012; Muoio, 2010). This dissociation between insulin resistance and accumulated LDs is not restricted to muscle tissue, but is also described for liver (Sun and Lazar, 2013). In these organs, high lipid intake combined with the endocrinological and metabolic abnormalities, stimulate gene expression that directly affect the biology of LD. In fact, the mechanism underlying this important phenomenon is an area of active investigation, which is leading to the development of drugs that can reverse these alterations. For instance, LDs function is dependent upon gene expression induced by several nuclear transcription factors of the PPAR (Peroxisome Proliferator-Activated Receptors) family (Bindesboll et al., 2013; Dalen et al., 2004; Kershaw et al., 2007; Schädinger et al., 2005). In turn, PPARs are activated by ligands produced through the catabolic functions of the LDs (Haemmerle et al., 2011; Mottillo et al., 2012; Ong et al., 2011; Sapiro et al., 2009), a feed-forward loop. In addition, PPARs drive expression of fatty acid oxidative genes. Thus, by regulating PPAR nuclear activities that promote an increased fatty acid flux thru the mitochondrial oxidative pathway, LDs may play a significant protective role against lipid-induced cytotoxicity (Georgiadi et al., 2012). Similarly, a large number of drugs that can manipulate these pathways and impact LD formation are being actively used in the clinical setting. For example, several studies showed that during steatogenesis the expression pattern of several LD associated perilipins changes in a PPAR γ -dependent manner (Inoue et al., 2005; Matsusue et al., 2008; Schädinger et al., 2005). Notably, PLIN1, which under normal conditions is only expressed in adipose tissue, is found in human hepatocytes of fatty liver (Fujii et al., 2009; Straub et al., 2008). PLIN2 levels are also up-regulated in steatosis in humans and in mice (Motomura et al., 2006), whereas PLIN2-deficient mice

are resistant to diet-induced fatty liver, implicating PLIN2 in hepatic lipid accumulation (Chang et al., 2006).

In contrast to genetic inherited forms of lipodystrophies, as those described above, acquired forms due to autoimmune diseases or drug treatment are much more common (Krahmer et al., 2013a). Protease inhibitors used to treat HIV infection are one of the most frequent causes of acquired lipodystrophy. HIV protease inhibitors might change the expression and localization of transcription factors, such as PPAR γ , that mediate adipocyte differentiation (Caron et al., 2001). Moreover, they increase basal and stimulated lipolysis in adipocytes by decreasing PLIN levels on the LDs by increasing lysosomal degradation of PLINs (Adler-Wailes et al., 2005). Combined, these effects alter the structure and composition of LD and thereby their function.

Regarding lipodystrophies with immune association, it was observed that accumulation of LDs within leukocytes, epithelial cells, hepatocytes and other non-adipocytic cells is a frequent phenotype in infectious, neoplastic and other inflammatory conditions (Bozza and Viola, 2010). Importantly, presence of eicosanoids within LDs is not restricted to leukocytes or to inflammatory conditions, the enzymes involved in eicosanoid synthesis localize at LDs, and LDs are the sites for eicosanoid generation in most cells in normal conditions as well as during inflammation and cancer. Cells that produce high quantities of eicosanoids under physiological conditions, for example those involved in normal ovulation and pregnancy, exhibit high numbers of LDs containing eicosanoid-synthesizing enzymes (Arend et al., 2004; Meadows et al., 2003, 2005; Seachord et al., 2005). Moreover, endothelial and epithelial cells involved in pathological conditions such as in cancer, hypoxia and during infections were shown to contain increased numbers of eicosanoid-synthesizing LDs (Accioly et al., 2008; Dvorak et al., 1994, 1993; Scarfo et al., 2001; Weller and Dvorak, 1994). Thus, inflammation leads to lipodystrophies, and because in lipodystrophies there is accumulation of lipids that are also mediators of inflammation, these disease states appear to be maintained by a positive-feedback loop.

The hypothesis of LD inhibition as target for anti-inflammatory therapy has been tested in different model systems. Although no specific LDs inhibitors have been described so far, different classes of drugs as well as gene knockdown of PLIN proteins can inhibit the formation of this organelle. Aspirin and selected other non-steroidal anti-inflammatory drugs (NSAIDs), for example, inhibit LD formation *in vivo* and *in vitro* (Bozza et al., 2002, 1996; Vieira-de-Abreu et al., 2005). An important point, however, is that studies targeted to the development of selective LD inhibitors should guarantee the safety of LD inhibition, since lipid accumulation within LDs is part of a protective mechanisms of lipid homeostasis against cellular lipotoxicity. Nevertheless, it has become clear that there is a tight link between inflammatory mediators and LDs biology, which extends to the effect of anti-inflammatory drugs on these organelles.

As it relates to malignant diseases, the up-regulated lipogenesis is a common phenotype to numerous human carcinomas and has been associated with poor prognosis in breast, prostate and colon cancer (Kuhajda, 2006; Swinnen et al., 2006). Moreover, a role for LDs as a potential target to generate new drugs for cancer treatment has also been suggested (Accioly et al., 2008; Tirinato et al., 2015). Altered lipid metabolism in cancer cells involves modulation of numerous lipogenic enzymes (Kuhajda, 2006; Swinnen et al., 2006), and culminates in the accumulation of newly formed lipids in cytoplasmic LDs. Indeed, enhanced LD numbers have been described in several neoplastic processes, including adenocarcinoma of the colon (Accioly et al., 2008), invasive squamous cervical carcinoma (Than et al., 2003), human brain tumor (Opstad et al., 2008) and hepatocarcinoma (Dvorak et al., 1993). The observation

that neoplastic cells and tissues exhibited highly increased numbers of LDs and increased expression of PLIN2, has highlighted the possibility of using the detection of LDs and/or PLIN2 as biomarkers in cancer (Bozza and Viola, 2010). Importantly, a very recent paper investigating Cancer Stem Cells (CSC), which have been associated with cancer recurrence after therapy, showed that high levels of LDs are a distinctive mark of CSCs in colorectal (CR) cancer (Tirinato et al., 2015). This is a relevant conceptual advance, since it demonstrated that a cellular organelle, the LD, is a signature of CSCs (Tirinato et al., 2015). A further functional characterization of LDs could lead soon to design new target therapies against CR-CSCs.

In summary, the identification of key pathways, molecules and functions of LDs and their relationship to pathological processes is enabling the development of therapeutic targets for future intervention in common human diseases including atherosclerosis, hepatic steatosis, cancer and inflammation.

The central role of LDs in human diseases ranging from obesity and diabetes to cancer raises intriguing questions: Which could be the role of LDs in the organ of Corti? How pathological processes and pharmacological manipulations modify LDs structure and function?

9. LDs in the cochlea: part of an anti-inflammatory mechanism?

The cochlea was originally considered an immunologically privileged organ because it is separated from the systemic circulation by a blood-labyrinth barrier physiologically similar to the blood–brain barrier of the central nervous system. However, this postulate has been challenged by the observation that inflammatory responses in the cochlea occur in the presence of bacterial or viral pathogens, antigens that cause labyrinthitis, as well as noise- or drug-induced damage (Fujioka et al., 2014a; Tan et al., 2013). Although the main purpose of the inflammatory reaction is to protect the auditory organ, the inflammatory response can also cause significant bystander injury to the delicate structures of the cochlea. For example, the migration of leukocytes and macrophages to the sites of injury or infection is a defining step of inflammatory responses. In the ear, however, migration of these cells into the cochlear scala media must be prevented because it has the likelihood of abolishing the endocochlear potential through the disruption of the tight-junction barrier at the organ of Corti reticular lamina, leading to apoptosis of outer hair cells and irreversible, profound deafness. Thus, there is a need to prevent the potential deleterious effect of inflammatory responses by maintaining the process at controllable levels and/or accelerating the return of the system to the normal conditions. It has been suggested that interleukin-10 (IL-10) could be involved in controlling the level of the inflammatory response. In particular, autoimmune hearing loss is intensified in IL-10 deficient mice and this response is reversed by IL-gene transfer (Zhou et al., 2012), and the early phase of cochlear inflammation in a murine model, before the recruitment of leukocytes and macrophages, is significantly exacerbated by IL-10 deficiency (Woo et al., 2015). A second way to protect the auditory organ would be to enhance the resolution phase of inflammatory responses.

The resolution phase of inflammation is a highly controlled and coordinated active process that involves endogenous anti-inflammatory mediators that suppress pro-inflammatory gene expression, phagocyte migration and activation, as well as promote inflammatory-cell clearance by apoptosis and phagocytosis (Serhan et al., 2008, 2007). The resolution phase starts promoting the switch of arachidonic acid–derived prostaglandins and leukotrienes to lipoxins, which, alone or together with the protein ANXA1,

initiate the termination sequence. These events coincide with the biosynthesis, from omega-3 polyunsaturated fatty acids, of resolvins and protectins, which critically shorten the period of neutrophil infiltration by initiating apoptosis. Thus, recruitment of phagocytic cells ceases at this point, and the process of programmed cell death by apoptosis starts. Consequently, apoptotic cells undergo phagocytosis by macrophages, leading to debris clearance and release of anti-inflammatory and reparative cytokines. The anti-inflammatory program ends with the departure of macrophages through lymphatic vessels (Serhan et al., 2008, 2007). Therefore, several well-known molecular components of LDs have the potential to accelerate and enhance the resolution phase of inflammatory responses. As such, the modulation of the synthesis and release of these molecules should be taken into consideration for planning preventive and therapeutic strategies to control the deleterious effects of inflammatory responses to the auditory organ.

Studies performed during the past decade have revealed that the mesenchymal region of the cochlea, including its lateral wall, is a common site of inflammation. Spiral ligament fibrocytes have been suggested to play an important role in cochlear inflammation through the up-regulation of chemokines (Moon et al., 2006, 2007; Oh et al., 2012; Woo et al., 2010, 2015), and resident macrophages, always present in the spiral ligament, are activated in response to various types of insults (Fujioka et al., 2014b). Inflammatory responses usually start at the lateral wall with macrophages migrating from the spiral ligament into the scala vestibuli and scala tympani, but never penetrating into the scala media (see Fig. 2) (Hirose et al., 2005; Tornabene et al., 2006). The mechanism used by the cochlea to prevent migration of leukocytes and macrophages and complete the resolution phase of the inflammatory response is still unknown, except by the clinically exploited fact that it can be stimulated by glucocorticoids (GCs). Indeed, GCs are the most common anti-inflammatory and immunosuppressive drugs used in the treatment of inflammatory diseases in the auditory system.

The classical effects of GCs are mediated by GC receptors (GC-R) and mineralocorticoid receptors (MC-R) (Arriza et al., 1987; Claire et al., 1993; Rupprecht et al., 1993), members of a large family of nuclear hormone receptor transcription factors, (Clark, 2007; Kil and Kalinec, 2013; Lowenberg et al., 2008; Stahn et al., 2007). GC-R and MC-R reside normally in the cytoplasm, but when activated by a ligand they translocate to the nucleus. There, they can interact with other transcription factors, impairing their ability to activate gene expression in a process known as trans-repression (Clark, 2007; Lowenberg et al., 2008; Stahn et al., 2007). In the nucleus the ligand-activated receptors can also bind to positive responsive elements, inducing the synthesis of regulatory proteins that are important for metabolism, thereby exerting many different effects at the cellular, tissue, organ and organism level. This process is termed trans-activation (Clark, 2007; Lowenberg et al., 2008; Stahn et al., 2007). While these well-defined genomic effects take place over a course of hours, rapid responses occurring within minutes or even seconds following the GC application have been described (Boldyreff and Wehling, 2003; Cato et al., 2002; Haller et al., 2008; Losel and Wehling, 2003). These so-called “non-genomic GC responses” are thought to be mediated either by effects on the physicochemical property of cell membranes or through activation of specific cellular targets (Boldyreff and Wehling, 2003; Cato et al., 2002; Haller et al., 2008; Losel and Wehling, 2003). We are focusing our work in characterizing the effects of GCs on the biology of LDs in an effort to understand the mechanism of action of these drugs on the auditory system.

As mentioned in Section 1, the possibility that Hensen cell LDs could have potential immunological and anti-inflammatory roles the cochlea was a natural corollary to initial studies on guinea pig

Fig. 2. Potential mechanism of ANXA1 release from Hensen cells and one of its probable roles in the mammalian cochlea. **A** – Cartoon depicting a possible mechanism of ANXA1 release from Hensen cells. Stimuli-induced LD disintegration would let ANXA1 loose into the cytoplasm; then, in a process mediated by myosin IIc, it would translocate to the cell periphery. Next, ABC transporters would move ANXA1 across the plasma membrane, releasing it into the external milieu (Kalinec et al., 2009). **B** – ANXA1 released by Hensen cells (in green) would prevent migration of phagocytic cells (in red) into the cochlear scala media (SM), protecting the organ of Corti (in yellow).

Hensen cells (Kalinec et al., 2009). Cells lining the scala media express ANXA1, and the majority of ANXA1 in the scala media is stored inside Hensen cell LDs. Moreover, we demonstrated that the glucocorticoid dexamethasone (DEXA) activated a non-genomic mechanism that releases ANXA1 from the LDs and then extrude it to the external milieu as fast as in 3 min. ANXA1 is a lipid and Ca^{2+} binding protein implicated in many aspects of the innate and adaptive immune system (D'Acquisto et al., 2008; Lim and Pervaiz, 2007; Perretti and D'Acquisto, 2009). Specifically, ANXA1 has been shown to inhibit leukocyte and macrophage migration and extravasation, as well as induce cell death and apoptotic cell clearance, during the inflammatory response (D'Acquisto et al., 2008; Gavins et al., 2012). Currently we do not know the entire repertoire of signaling cascades involved in the immunoregulatory functions of ANXA1. For example, it has been shown that the N-terminal region of ANXA1 binds to formyl peptide receptors (FPR), thereby acquiring anti-inflammatory properties; its C-terminal region, in contrast, binds to TANK-binding kinase 1 (TBK1), and thus displays pro-inflammatory properties. Recent studies performed in non-auditory cells demonstrate that ANXA1 links the signaling from Toll-like receptors (TLRs) to enhance type 1 IFN antiviral cytokine response (Bist et al., 2013). TLRs are cell surface receptors that function as key regulators of inflammatory responses in most tissues by playing a pivotal role in the recognition of bacteria and viruses. Members of this family recognize specific structurally conserved molecules derived from pathogens and trigger both MyD88 and TRIF-dependent pathways to stimulate the production of cytokines that participate in both antiviral and antibacterial responses in a plethora of cells. Aberrant activation of these pathways is known to play a critical role in the development of autoimmunity and cancer (Kawasaki and Kawai, 2014). Notably, this pathway has been shown to participate in the progression of experimental otitis media (Li et al., 2013; McCoy et al., 2005). Thus, ANXA1 could be acting through distinct intracellular signaling pathways as a pro-inflammatory factor, an anti-inflammatory mediator and even an antiviral agent (Bist et al., 2013), regulating the inflammatory response, promoting its resolution, and decreasing its deleterious impact on hearing homeostasis.

Consequently, based on our initial studies (Kalinec et al., 2009), we proposed that the majority of ANXA1 in the mammalian cochlea is stored inside Hensen cell LDs. Dexamethasone, in turn, would activate a myosin IIc-mediated mechanism driving ANXA1 from the LDs to the Hensen cells plasma membrane, where it would be released to the external milieu by a process involving ABC transporters (Fig. 2A). ANXA1 released by Hensen cells might act by

preventing phagocytic cells from invading the cochlear scala media, protecting the auditory organ (Fig. 2B). Therefore, GCs would target the LDs, enhancing the release of ANXA1 from Hensen cells and amplifying the pro-resolution and cell-repair mechanisms. Further research, however, indicated that ANXA1 was only one of the factors involved in the process, and other proteins and lipids should be involved in the response stimulated by GCs.

In contrast to other inner ear cell populations, considerable amounts of guinea pig HCs may be isolated quite easily following standard dissection techniques. HCs are located at the external border of the organ of Corti (Fig. 3A), and after dissection remain strongly interconnected by gap junctions, forming large strips resembling glass-beads necklaces because of the LDs located in their cytoplasm (Fig. 3B). Several protocols to isolate near pure fractions of LDs have been developed in different laboratories, with the one developed by Harris and coworkers (Harris et al., 2012) yielding abundant LDs from guinea pig HCs (Fig. 3C).

Looking for an independent confirmation of ANXA1 expression in LDs from guinea pig HCs, we performed nano-LC-ESI-MS/MS studies of LDs isolated from HCs exposed to DEXA for five and 15 min, and complemented these studies investigating the structure and ultrastructure of LDs using confocal and transmission electron microscopy (TEM). In these studies, which were presented at the Inner Ear Biology Kyoto 2014 meeting (Kalinec et al., 2014), we identified more than 300 LDs-associated proteins, with a distinct subset of them being regulated by DEXA. Our TEM studies confirmed that the cytoplasm of guinea pig HCs contain 2 to 3 dozens of small and mid-sized LDs in addition to other typical organelles as Golgi apparatus and relatively small mitochondrias (Kalinec et al., 2009). Importantly, DEXA treatment induced fast fusion of LDs, followed by their disintegration and generation of new LDs (Kalinec et al., 2014, 2015). Confocal studies, on the other hand, characterized the release of protein- and lipid-filled vesicles to the extracellular space (Kalinec et al., 2014). Thus, these studies provide strong evidence that LDs are a cellular target of DEXA, which would induce the release of cargo and constitutive proteins as well as lipids from them in a non-genomic way, in a process initiated by LDs fusion followed by disintegration of the LDs. These results, together with those from ongoing experiments aimed at elucidating the specific changes in LDs lipid content induced by GCs, strongly suggest that HC LDs could have an important role in the inflammatory responses in the cochlea. LDs would help to develop rapidly and efficiently the resolution phase of inflammatory processes, preventing inflammatory damage of the delicate inner ear structures and, consequently, sensorineural hearing loss.

Fig. 3. Isolation of Hensen Cell LDs. **A.** HCs are located at the external border of the organ of Corti (arrowhead). **B.** Long strips of HCs can be easily removed from the cochlear spiral with fine needles. **C.** LDs stained with Nile Red after isolation following the procedure developed by Harris et al. (2012).

10. Concluding remarks

While upon their discovery LDs were thought to be simply an inclusion body, today we know that they are ubiquitous and important organelles present in every cell from many organisms across evolutionary kingdoms. This exquisite conservation suggest that a strong evolutionary pressure must have been exerted on cells to carefully and dynamically partition large concentrations of vital lipids within the cytoplasm. Most current theories infer that this evolutionary pressure relates to the physicochemical and biological properties of lipids, for instance the need to solubilize lipids in a water-rich cytoplasm and store them for later generation and utilization of energy. With the emergence of metazoans, some other properties may have become beneficial during the formation of tissues. This idea is supported by the fact that LD-rich cells can serve as temperature insulators and also contribute to the mechanical homeostasis of tissues and organs. Interestingly, LDs also have the ability to concentrate essential lipid nutrients such as vitamin A. However, other molecules with distinct functions, such as those involved in membrane recycling, paracrine and autocrine signaling, may have arisen to facilitate cell communication. Therefore, all these functions required that cells developed a specialized machinery for maintaining large concentrations lipids separated from the aqueous cytoplasm giving rise to this fascinating organelle.

The revolutionary growth of knowledge in the area of cell and molecular biology, particularly due to the development of high throughput techniques and powerful and varied microscopies, is rapidly providing insights into the particular composition, structure, and dynamics of LDs. In addition, compelling and extensive evidence indicate that LDs are key to maintaining the health of whole organisms, including humans. We have learned that environmental insults (e.g., diet and toxins), metabolic imbalance, and medications can alter the biology of LDs, sometimes contributing to diseases. In fact, these links are so compelling that many clinical societies and government agencies are promoting research in this area with increasing enthusiasm. Unfortunately, there is currently a significant paucity of knowledge regarding the type and function of LDs in the auditory system. To help to fill this gap in knowledge, we

have recently embraced this research by using guinea pig Hensen cells as a model to investigate the structure and composition of LDs in the mammalian cochlea. From this research and, drawing homology to other systems, we believe that LDs must play a significant role in the physiology of hearing. However, much remain to be done to better define both the shared or unique properties of LDs that ultimately contribute to their specific function on the auditory system.

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