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Proteins under new management: lipid droplets deliver

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Lipid droplets are ubiquitous organelles that store neutral lipids and have crucial roles in lipid metabolism. Recent studies have uncovered many examples of lipid droplets recruiting proteins from other cellular compartments, in a cell type-specific and regulated manner. Some droplet-recruited proteins are destined for destruction, whereas others are released and reused when conditions change. Droplets might therefore have a general role in managing the availability of proteins, and they have been proposed to serve as generic sites of protein sequestration. The implications of this emerging role of lipid droplets include regulated inactivation of proteins, prevention of toxic protein aggregates and localized delivery of signaling molecules.

Introduction: from blobs of fat to dynamic organelles

Most organisms cope with unpredictable access to nutrients by stockpiling reserves for times of deprivation. Neutral lipids, such as triacylglycerol and sterol esters, are low-volume, high-energy reserve materials. They also provide precursors for membrane components and signaling molecules. Neutral lipids are not present free in the cytoplasm but are stored in specialized structures called lipid droplets [1–5]. These structures include triacylglycerol inclusions in mycobacteria, lipid bodies of adipose tissues, retinosomes in photoreceptors, oil bodies in plant seeds, lipid particles of yeast, and the lipid globules of many animal eggs.

Although recognized since the early days of cell biology, lipid droplets were long ignored as passive, boring blobs of fat. However, pioneering research over the past decade has established lipid droplets as dynamic organelles and as central, active players in lipid homeostasis. Lipid droplets are, for example, sites for triacylglycerol breakdown [2], for the production of steroid hormone precursors and eicosanoids [6] and for the recycling of retinoids [7]. Not surprisingly, lipid-droplet abnormalities are characteristic of many health problems, including obesity, diabetes, *Chlamydia* infections [8], liver disease and atherosclerosis.

Yet this new view of lipid droplets might already require a major update. Several laboratories have recently found that lipid droplets can become the temporary home to proteins seemingly unrelated to lipid metabolism, including histones, enzymes involved in purine synthesis and viral capsids (Table 1). Recruitment to droplets is frequently massive, regulated and cell-type specific, suggesting that there is more to lipid droplets than managing fat. Here, I review these findings and discuss the hypothesis that lipid droplets generally serve as protein sequestration sites, a notion with wide-ranging implications for cell and developmental biology.

Defining the proteins of lipid droplets

Lipid droplets have a simple structure: a hydrophobic core of neutral lipids is surrounded by a monolayer of polar lipids (Figure 1a). Proteins are present at the surface, bound through hydrophobic or electrostatic interactions (Figure 1b). Whether proteins also reside within the core is controversial; several studies report such localization using electron microscopy [9,10]. In contrast to our detailed knowledge of the protein repertoire of other organelles, the proteins of lipid droplets are not well characterized. For comprehensive summaries, I refer the reader to up-to-date reviews [1–5].

The high lipid content and consequent low buoyant density of lipid droplets enables rapid isolation by floatation. Proteomic analysis of droplets thus purified has identified many new candidate droplet proteins [10–17]. But are they authentic droplet proteins or unavoidable contaminants of the purification procedure? It has been proposed that lipid-droplet purification is especially prone to artifacts (Figure 1c): droplet fragmentation during isolation might expose the hydrophobic droplet core, to which random proteins from the cell lysate could then stick nonspecifically [3]. It is therefore crucial to verify lipid-droplet localization in intact cells.

Because droplets are typically large and perfectly round, *bona fide* droplet proteins – if present all over the droplet surface – display a characteristic ring-like distribution (Figure 2a). Simultaneous detection of neutral lipids confirms such rings as lipid droplets. Thus, if specific probes are available, standard light microscopy can provide strong evidence for *in vivo* droplet association.

Droplets harbor proteins from other cellular compartments

Among the reported candidate droplet proteins are numerous instances of proteins previously thought to be unique to other cellular compartments. Examples include a nuclear spliceosome activator [18], viral capsid components [19,20] and enzymes from the cytosol [21] and endoplasmic reticulum (ER) [22]. Table 1 lists those examples where droplet association has been verified in

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Table 1. Refugee proteins on lipid droplets

Protein	Typical compartment	Characterized function	When found on lipid droplets	Species or cell type	Remarks	Refs
Histones	Nucleus	Nucleosome components	In oocytes and early embryos	*Drosophila*	Can leave droplets and move to nuclei	[14]
IMPDH	Cytosol	Purine biosynthesis	After insulin signaling or growth in oleate	Mammalian cell lines	Droplet localization depends on PI3-kinase pathway	[21]
Caveolins	Plasma membrane	Component of caveolae; cholesterol homeostasis	Overexpression, certain mutant constructs, block in transport from ER to Golgi, lipid influx	Mammalian cell lines	Caveolin traffic between plasma membrane and lipid droplets might depend on cholesterol levels	[5,57]
Stomatin	Plasma membrane, late endosomes	Lipid-raft protein	Small amounts basally; massively following overexpression	Mammalian cell lines	Redistributes to vesicles when overexpression stops	[16]
Hsp70	Cytosol	Chaperone	During heat stress	Rat adipocytes	Other chaperones were not recruited	[23]
Hepatitis C core protein	Viral capsid	Structural component of capsid	After viral infection	Mammalian cell lines	When core protein is expressed in the liver – in the absence of other viral proteins – it causes droplet accumulation	[19,20,30]
Squalene epoxidase	ER	Sterol biosynthesis	When enzymatically inactive	Yeast	Activatable in the presence of ER	[22]
α-synuclein	Predominantly cytosolic		Overexpression	Mammalian cell lines	Overexpression causes droplet accumulation	[29]
ApoB	Secreted lipoproteins, ER	Primary protein of VLDL[a]	When VLDL assembly is inefficient or when proteolytic turnover is blocked	Human hepatocytes	Degraded through proteasome and by autophagy	[31]
Nir2	Golgi apparatus	Regulation of membrane transport	Growth in oleate or when carrying a mutation in PI-transfer domain	HeLa cells	Droplet targeting might be the result of threonine phosphorylation	[24]
Prp19p	Nucleus	Spliceosome activator	Constitutive in adipocyte lineage?	Murine adipocytes	Implicated in droplet biogenesis	[18]

[a]Abbreviations: PI, phosphatidylinositol; VLDL, very low density lipoprotein.

intact cells. This list will probably grow as the presence of additional surprising candidates is verified *in vivo*.

Many of these unexpected proteins associate with lipid droplets conditionally. In mammalian cells, insulin signaling causes translocation of inosine monophosphate dehydrogenase (IMPDH), an enzyme involved in purine biosynthesis, to the droplet surface [21]. In adipocytes, the cytosolic chaperone Hsp70 becomes highly enriched on droplets after heat shock [23], and the peripheral membrane protein Nir2, typically present on the Golgi apparatus, partially relocalizes to droplets when cells are exposed to oleic acid [24].

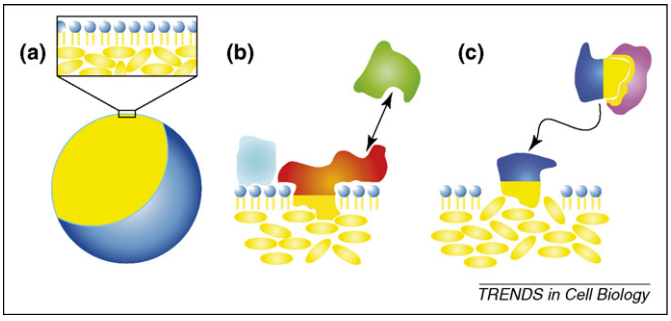

Figure 1. Structure of lipid droplets. **(a)** Lipid droplets have a large hydrophobic core, made up of neutral lipids (yellow ovals), which is surrounded by a monolayer of polar lipids (blue, hydrophilic head groups; yellow, hydrophobic tails). **(b)** The polar shell also contains several types of proteins: some (red) bound through hydrophobic interactions with the core, some bound through electrostatic interactions with polar lipids (blue) or with resident proteins (green). Regulation of such protein–protein interactions might mediate conditional recruitment of proteins to the droplet surface [47,48,58]. **(c)** Damage to the phospholipid layer during droplet isolation has been proposed [3] to partially expose the central core, providing a sticky site for artificial adsorption of proteins with hydrophobic patches (yellow). *In vivo*, these hydrophobic patches are bound up by partner proteins (pink) and droplet association does not occur.

In embryos of the fruit fly *Drosophila*, lipid droplets are associated with 50% or more of the total embryonic pool of certain histones [14] (Figure 2b). This recruitment is both cell-type and histone specific: droplet association occurs in ovaries and early embryos, yet not in older embryos or cultured cells; it involves H2A, H2B and H2Av but not other histones. Recent proteomic studies detected histones also in lipid-droplet preparations from the yeast *Saccharomyces cerevisiae* [25], the fat body of *Drosophila* larvae [15] and human leukocytes [10]. In *Drosophila* embryos, H2Av was initially described as 'cytoplasmic' [26]; thus, 'cytoplasmic' histone stockpiles reported for many animal eggs (e.g. by Pauls *et al*. [27]) might also reflect droplet localization and should be carefully re-examined. In the germ line of the nematode *Caenorhabditis elegans*, at least, yet another histone (H1.1) accumulates in cytoplasmic 'granules' of unknown nature [28]; it will be exciting to test if these histone dots represent lipid droplets.

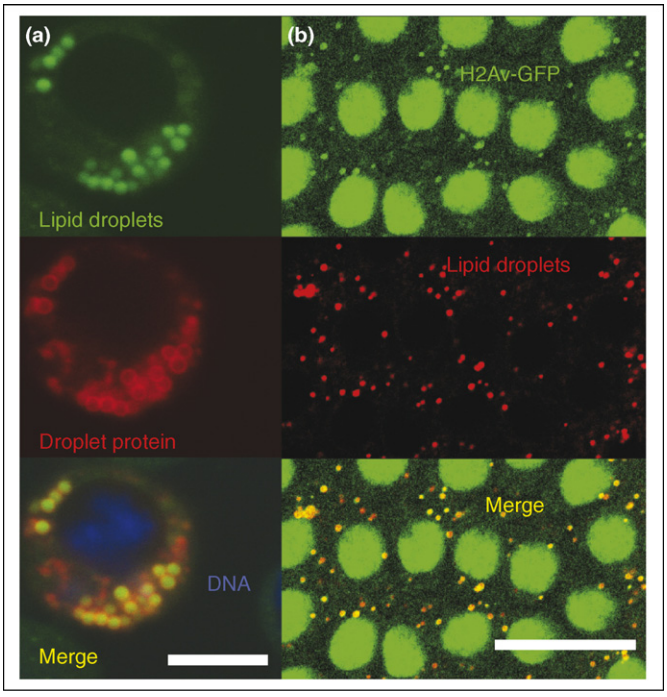

Figure 2. Proteins localized to lipid droplets. **(a)** *Drosophila* cultured cells expressing a red fluorescent protein fusion protein (red) targeted to lipid droplets and stained for neutral lipids (green) and DNA (blue). The droplet protein is present in characteristic rings surrounding the neutral lipid core (the scale bar represents 8 μm). Modified, with permission, from Ref. [59]. **(b)** H2Av–GFP (green) is found not only in nuclei (large disks), but also in abundant cytoplasmic dots that look like lipid droplets, move like lipid droplets [14] and stain with a lipid droplet-specific marker (red) (the scale bar represents 20 μm). Modified, with permission, from Ref. [14].

A hypothesis: sequestration of refugee proteins

It is conceivable that these proteins have a moonlighting function in lipid-droplet biology, in addition to their characterized role in their 'normal' compartment. However, in several cases, droplet localization occurs in response to elevated protein levels – for histones when large maternal stockpiles are present [14]; for stomatin [16] and the Parkinson's disease protein α-synuclein [29] when artificially overexpressed; for viral capsid proteins following infection [19,20,30]; for caveolin when levels in the ER rise owing to overproduction or aberrant vesicle trafficking [5]; and for apolipoprotein B (ApoB) when proteolytic turnover is inhibited [31]. It has been proposed [14] that droplet binding removes excess proteins from other cellular compartments and stores them for later use. It has also been suggested that droplets keep aggregation-prone proteins on hold until degradation [3,31]. A further hypothesis is that unfolded proteins reside on lipid droplets until they are refolded by droplet-associated chaperones [14,23].

What these proposals have in common is that the normal function of these unexpected proteins is elsewhere in the cell and that droplet localization provides them with temporary shelter. To capture this notion, I propose the term 'refugee proteins'. The broader hypothesis underlying these proposals, namely that lipid droplets are general sites for regulated sequestration and that they manage the availability of refugee proteins, has far-ranging implications for general cell biology, for developmental mechanisms and for disease processes. Four scenarios are discussed below concerning how protein sequestration might be biologically advantageous (Figure 3). As of yet, there is no conclusive proof for these possibilities but the literature offers intriguing hints.

Droplets as sequestration sites: keeping proteins inactive

Because even low levels of free histones can interfere with growth and development [32,33], histone synthesis and turnover are usually elaborately controlled, such that little free histone is present. Yet, newly laid *Drosophila* eggs contain histones in thousandfold excess relative to DNA [14]. Why do these histones not interfere with other cellular processes? Because excess histones H2A, H2B and H2Av are largely present on lipid droplets, it was proposed that droplet sequestration inactivates them [14].

Could recruitment to lipid droplets be a general regulatory mechanism to inactivate proteins? Squalene

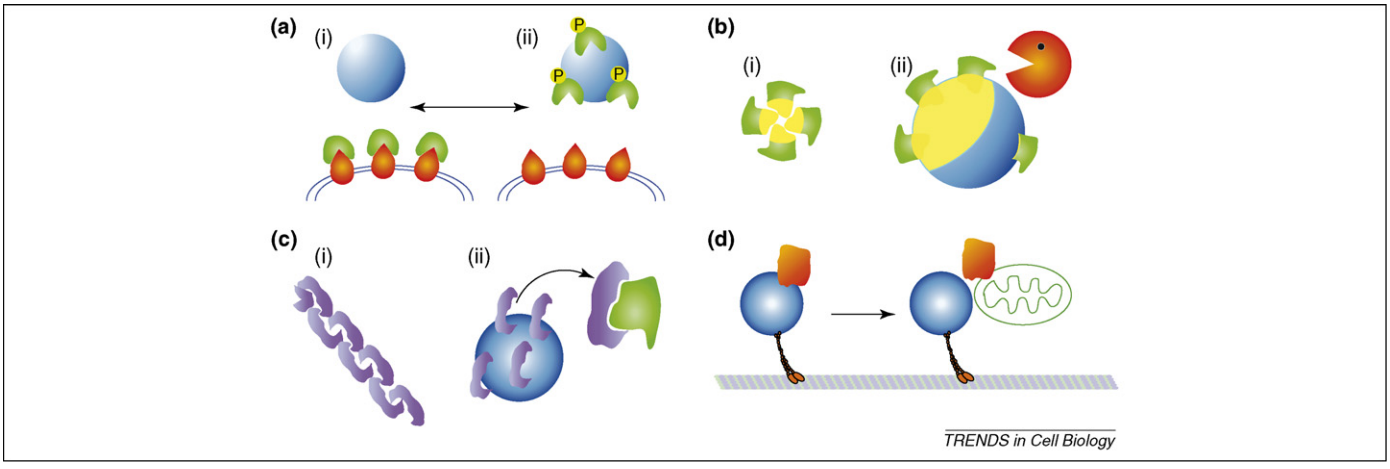

Figure 3. Droplets as managers of protein availability: proposed roles. **(a)** Sequestration site. Functional inactivation of a protein (green) by physically separating it from a binding partner (red) which resides in a different compartment. (i) Active state; (ii) sequestered, inactive state. Mechanisms of sequestration are unknown but might involve post-translational modifications, such as the addition of phosphate groups (P). **(b)** Garbage dump. Damaged proteins (green) with exposed hydrophobic patches (yellow) form potentially toxic aggregates (i). Recruitment to lipid droplets through these hydrophobic patches prevents aggregation, enabling ordered degradation (ii). **(c)** Chaperone. Proteins lacking proper binding partners will aggregate irreversibly (i). Droplet association keeps them in an assembly-competent state until their partners become available (ii). **(d)** Vehicle. As lipid droplets move along microtubules through the cytoplasm, passenger proteins (red) come along for the ride and are delivered when droplets reach the target compartment (green).

epoxidase is indeed enzymatically inactive when present on lipid droplets [22], and ApoB accumulation on droplets might prevent the formation of toxic aggregates [31]. Sequestration might inactivate proteins directly (e.g. by blocking the catalytic site of an enzyme) or indirectly (e.g. by physically separating the protein from binding partners or substrates that remain at their original location) (Figure 3a). Sequestration in an innocuous location might prove superior to alternative control mechanisms (e.g. post-translational modifications, allosteric regulators and chaperones) if protein activity cannot easily be turned off through conformational changes.

Intriguingly, several of the examples from Table 1 involve proteins that spend at least part of their life cycle in the ER, such as squalene epoxidase when enzymatically active or ApoB during assembly into lipoprotein particles. The ER is a versatile organelle with broad and divergent cellular functions, from the control of lipid homeostasis to the synthesis, assembly and quality control of secretory proteins, to vesicular traffic. On the one hand, this diversity might require particularly nuanced mechanisms of regulation; on the other hand, because lipid droplets are thought to arise from the ER [1–5], it might be especially easy to reroute proteins from the ER to lipid droplets and thus modulate ER function.

Droplets as garbage dumps: last stop before destruction

What happens to proteins once sequestered on lipid droplets? In hepatocytes, droplet-bound ApoB is polyubiquitinated, and droplets are enriched for proteasome subunits. When protein turnover is compromised pharmacologically, the amount of ApoB on droplets increases [31] – as do levels of other ubiquitinated proteins, suggesting that they accumulate on droplets to be handed off to the cellular degradation machinery [3]. The reported droplet accumulation of a mutant of the lysosomal regulator Arl8b has been attributed to misfolding [34] and might similarly represent an intermediate before degradation. In liver cells of mice impaired in the autophagy pathway of protein turnover, ubiquitin-positive aggregates accumulate near lipid droplets [35].

Why would cells bother to relocate such proteins to lipid droplets? Partially unfolded proteins expose hydrophobic residues normally buried within the interior, and, as a result, tend to aggregate (Figure 3b). Such cytoplasmic aggregates can be extremely toxic and are implicated as the cause of a broad range of human diseases [36]. The droplet core might provide an innocuous interaction surface for the exposed hydrophobic patches and might thus prevent aggregate initiation and growth.

This proposal of lipid droplets as selective garbage dumps leads to an interesting speculation [14]. Lipid-droplet numbers increase in many disease states, from osteoarthritis to liver degeneration, to cartilage overproliferation (discussed in Ref. [14]), and also in response to the overexpression of α-synuclein [29,37]. Although these conditions might promote droplet accumulation because they interfere with lipid metabolism, increased droplet number might also be a protective response that generates additional droplet surface to sequester misfolded proteins.

Droplets as chaperones: storing proteins for later use

However, not all droplet-associated proteins are degraded (Table 1). Droplet-bound stomatin redistributes to vesicles when overexpression stops [16], and squalene epoxidase on droplets becomes enzymatically active when mixed *in vitro* with ER [22]. When *Drosophila* droplets carrying green fluorescent protein (GFP)-tagged H2Av are transplanted into recipient embryos, the droplet-bound histone is released and travels to nuclei [14].

If these proteins remain functional, why recruit them to lipid droplets in the first place? As discussed earlier, droplet association might silence inappropriately active proteins (Figure 3a). Alternatively, these proteins might be unstable without their normal binding partners, and would aggregate or be degraded (Figure 3c). Histones face both risks: *in vitro*, free histones are prone to aggregation [38], and *in vivo* they are rapidly degraded by active mechanisms [32]. Sequestration on lipid droplets might enable embryos to stockpile large amount of histones for later development. Histones H2A and H2B not present in chromatin are usually kept competent for assembly by binding to histone chaperones (such as nucleoplasmin or nucleosome assembly protein-1) [39]. Yet, proteomic analysis of the *Drosophila* lipid droplets so rich in H2A and H2B did not identify such histone chaperones [14]. If the absence of chaperones can be confirmed independently, it suggests that sequestration to droplets provides an analogous chaperone function.

In adipocytes, a third of the cellular Hsp70 relocates to lipid droplets following heat stress [23]. One explanation proposed [23] is that Hsp70, a molecular chaperone, refolds denatured proteins that have accumulated on the droplet surface – if so, droplet association would have kept them in a refolding-competent state. Such a role of droplets in the recovery of unfolded protein might also explain the abundance of lipid droplets in many diseases.

Droplets as vehicles: delivering passenger proteins

Lipid droplets have 'extracellular cousins', the lipoproteins, also characterized by a core of neutral lipids surrounded by polar lipids and dedicated proteins. Lipoproteins ferry lipids between tissues and – at least in *Drosophila* – signaling proteins, such as the morphogens Wingless and Hedgehog [40,41].

Lipid droplets might serve the same function intracellularly. Depending on the cell type, lipid droplets display intimate physical association with other organelles, including the ER, mitochondria and peroxisomes. Such contacts are thought to enable the exchange of lipids but might also promote the delivery of proteins. In many cells, lipid droplets move vigorously along microtubules (summarized in Ref. [42]), a property that could bring them into transient contact with many compartments. Sequestered proteins might ride as passengers on these droplets and be released when droplets bump into their target compartment (Figure 3d).

No such protein delivery has yet been demonstrated but there are intriguing hints. For example, lipid droplets undergo transient 'kiss-and-run' interactions with phagosomes [43]; and it has been hypothesized that such contacts transfer both arachidonic acid and Rab proteins

[43] and thus promote phagosome maturation. Lipid droplet-recruited stomatin can redistribute into endosomal/lysosomal vesicles, an exchange probably enhanced by microtubule-based motion of both vesicles and droplets [16]. In *Drosophila* embryos, it is possible to misdirect droplet motion to generate droplet-depleted cells. In such cells, zygotic production of histones starts prematurely [14], as if to compensate for failed delivery of maternal histones.

Because lipid droplets can display polarized transport along microtubules [44], cells might even use droplets to control the local concentration of cytoplasmic determinants. Thus, droplets have the potential to modulate establishment of cell polarity and spatial differentiation of embryos. Yet, *Drosophila* embryos with abnormal droplet distributions are viable and grossly normal [42,44,45]. Because development frequently utilizes redundant pathways, and embryos often compensate for early problems, defects might only become apparent in sensitized genetic backgrounds or following detailed examination [14].

Several proteins involved in lipid metabolism are recruited to droplets through members of the droplet-specific perilipin, adipophilin, TIP47 (PAT) family [46–48]. PAT proteins are also important for correct motion of lipid droplets [42,49]. Both the recruitment and transport functions are thought to be regulated by PAT protein phosphorylation [42,48,50]. Whether this dual role of PAT proteins enables cells to coordinate droplet motion and release of passengers is an exciting but unexplored possibility.

Multiple modes of sequestration?

Although the four scenarios discussed earlier share certain features, they represent distinct models: for example, inactivation by sequestration does not necessarily mean that the protein is stored (it could be degraded instead), and storage might not keep the protein inactive (it might rather prevent premature degradation while its binding partner is missing). However, these scenarios are also not mutually exclusive; for example, proteins riding piggyback on droplets might be kept inactive during transit. As more examples of droplet sequestration are examined, it will be important to test these concepts separately to determine which – if any – represent the real biological relevance of sequestration.

Sequestration might not only have diverse biological functions, but might also involve multiple, distinct mechanisms: ApoB is thought to bind through hydrophobic interactions [31], whereas for histones and Hsp70, electrostatic interactions predominate [14,23]. It has also been proposed that some proteins are sequestered in an unfolded state [14], so that hydrophobic residues normally buried in the protein interior can interact with the droplet core (similar to the situation in Figure 1c) – although there is no evidence yet for this possibility. Because PAT family members recruit several proteins involved in lipid metabolism, they might also mediate sequestration events. However, at least for histones in *Drosophila* embryos, PAT proteins cannot be the full story because LSD2 (lipid storage droplet 2), the only PAT protein detected in significant amounts, could be washed off the droplets under conditions where histones remained attached [14].

Molecular chaperones have been hypothesized to have important roles in sequestration: they might unfold proteins to make them sequestration competent or refold them to promote release [14]; they might repair denatured proteins gathered at the droplet surface during stress [23] or they might present droplet-bound proteins to the degradation machinery. Chaperones are indeed abundant in many droplet proteomes [10,13–16,25], although whether or not they perform any – or all – of these functions remains to be tested. One particular interesting question will be if – as implied in the hypotheses – their activity can be regulated to promote unfolding under some circumstances and refolding under others.

Droplets and RNA

There are early indications that droplets might also recruit ribonucleoprotein particles and polysomes. Several droplet proteomes report RNA-binding proteins, ribosomal subunits and translation factors [10,12,14,15,25]; and electron microscopy reveals the presence of ribosomes on lipid droplets of leukocytes [10] and of RNAs on droplets of mast cells [51,52]. Because IMPDH can bind to nucleic acids [53], droplet-localized RNAs might recruit IMPDH to droplets – or vice versa. It is not yet known how widespread RNA localization to droplets is and whether specific RNAs are recruited. In an extension of the sequestration hypothesis, recruited RNAs might be kept translationally silent or might be passengers to be delivered locally.

Are droplets unique as sequestration sites?

There do not seem to have been any systematic attempts to identify refugee proteins – proteins that find temporary shelter in a particular intracellular location but usually function elsewhere. Therefore, it is not known if such sequestration happens exclusively on lipid droplets. Many examples in Table 1 were identified because the characteristic 'rings' formed by proteins on the droplet surface (Figure 2a) are striking and hard to ignore. Sequestration on less obvious structures might have escaped attention or been misidentified as general cytoplasmic distribution. Alternative sequestration sites might, for example, explain why in *Drosophila* embryos some histones, although in excess, are not found on lipid droplets [14].

Alternatively, droplets might be particularly suited as storage sites because of features unique to these organelles. First, the extensive hydrophobic core of lipid droplets might provide a perfect site to store proteins with exposed hydrophobic patches. Second, the sizes and numbers of lipid droplets vary tremendously, from single huge droplets in white adipocytes to many dozens of tiny droplets per cell in *Drosophila* embryos [44]. Thus, cells might be able to adjust total droplet surface area and therefore total sequestration capacity over wide ranges. Third, the protein repertoire of droplets can vary greatly (e.g. in response to changes in physiology [13,25]), a property which might enable different droplets to sequester distinct proteins. There are even several examples where – within a single cell – a given protein localizes to only a subset of droplets [2,15,21,54,55], implying the presence of droplets with distinct surface properties. Such droplet variation might enable cells to sequester incompatible proteins in

distinct locations or to ship passenger proteins to diverse destinations. How cells control size, numbers and surface properties of lipid droplets in such a sophisticated manner is a fascinating but completely open question.

Conclusions and perspectives

Although the sequestration hypothesis is certainly far from proven, the persistent association of refugee proteins with droplets cannot be ignored. To test this hypothesis critically and determine the importance of droplets as managers of protein availability, it will be necessary to address how widespread sequestration is, what the underlying mechanisms are, how sequestration is regulated and what biological roles it serves.

The published droplet proteomes list many candidates whose droplet association has yet to be verified. One lesson of the examples in Table 1 is that 'educated guesses' about the best candidates might miss some of the most interesting cases. An unbiased, systematic analysis will be necessary to identify the true spectrum of droplet-bound proteins. Situations where the proposed sequestration functions of droplets are probably most active (e.g. in early embryos full of maternally provided proteins [14] or in cells under stress [23]) will probably become the richest sources of new examples of sequestered proteins.

A first step in understanding the mechanism of sequestration will be to determine the physical state of sequestered proteins [56]: are they folded or unfolded? Are they present as monomers or in complexes? What are their binding partners on droplets? Is regulated droplet targeting achieved by controlling the availability of some 'receptor' on droplets or by marking the protein with unique post-translational modifications?

Once sequestration mechanisms are understood, it should be possible to design strategies to prevent sequestration – or prevent the release of sequestered proteins – and examine the biological consequences. For example, if IMPDH cannot bind to droplets in response to insulin, does that alter the outcome of insulin signaling? Or if, in *Drosophila* embryos, histones are not sequestered, are those free histones degraded or do they accumulate and interfere with development? Given the wide range of refugee proteins already discovered on lipid droplets, it seems likely that systematic analyses will uncover biological roles for sequestration beyond the scenarios imagined here. Because lipid droplets are no longer the stepchildren of cell biology, many new insights can be expected into the biochemical functions and biological roles of lipid droplets in the coming years – and many new refugee proteins are likely to be waiting for us on the droplet surface.

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