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Article

Lipid Droplets Control the Maternal Histone Supply of *Drosophila* Embryos

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Summary

Background: Histones are essential for chromatin packing, yet free histones not incorporated into chromatin are toxic. While in most cells multiple regulatory mechanisms prevent accumulation of excess histones, early *Drosophila* embryos contain massive extranuclear histone stores, thought to be essential for development. Excess histones H2A, H2B, and H2Av are bound to lipid droplets, ubiquitous fat storage organelles especially abundant in embryos. It has been proposed that sequestration on lipid droplets allows safe transient storage of supernumerary histones.
Results: Here, we critically test this sequestration hypothesis. We find that histones are anchored to lipid droplets via the previously uncharacterized protein Jabba: Jabba localizes to droplets, coimmunoprecipitates with histones, and is necessary to recruit histones to droplets. *Jabba* mutants lack the maternal H2A, H2B, and H2Av deposits altogether; presumably, these deposits are eliminated unless sequestered on droplets. *Jabba* mutant embryos compensate for this histone deficit by translating maternal histone mRNAs. However, when histone expression is mildly compromised, the maternal histone protein deposits are essential for proper early mitoses and for viability.
Conclusions: A growing number of proteins from other cellular compartments have been found to transiently associate with lipid droplets. Our studies provide the first insight into mechanism and functional relevance of this sequestration. We conclude that sequestration on lipid droplets allows embryos to build up extranuclear histone stores and provides histones for chromatin assembly during times of high demand. This work reveals a novel aspect of histone metabolism and establishes lipid droplets as functional storage sites for unstable or detrimental proteins.

Introduction

In eukaryotes, histone expression levels need to be carefully balanced. Histones are essential for assembling genomic DNA into chromatin, protecting genome integrity, and regulating transcription [1]. Already mild histone reduction disturbs transcription and increases sensitivity to DNA damage [2]; it may even underlie the increased genomic instability of aging cells [3, 4]. Overabundance of histones is also detrimental; it disrupts gene expression, causes chromosome loss, increases DNA damage sensitivity, and may promote cancer [5–9].

Multiple regulatory mechanisms cooperate to balance the cellular histone supply [7, 10]. Biosynthesis of new histones and their assembly into chromatin are typically tightly coupled. Transcriptional and posttranscriptional regulation limit histone biosynthesis to times of need, and excess histones are proteolytically degraded [7, 11]. Thus, many cells have only a few extranuclear histones, e.g., just ~0.1% of the entire histone content in human somatic cells [12, 13].

Sometimes, however, significant amounts of extranuclear histones accumulate on lipid droplets, the cellular organelles dedicated to fat storage. Proteomic analyses detected histones in lipid-droplet preparations from yeast to insects to humans [14]. For early *Drosophila* embryos, the presence of histones on lipid droplets has been confirmed in vivo [15]; here, histones equivalent to thousands of diploid nuclei are bound to lipid droplets. We proposed that lipid-droplet binding prevents these supernumerary histones from interfering with cellular processes and protects them from surveillance mechanisms that usually eliminate excess histones [14, 15]. A direct test of these proposals was previously not possible.

The biological role of droplet-bound histones remains unclear. One possibility is that they have novel, droplet-specific functions, e.g., a moonlighting role in regulating lipid metabolism. Alternatively, droplet binding may allow storage of histones for later use in chromatin assembly; droplet-bound histones can indeed be transferred to nuclei [15].

Proteins from diverse cellular compartments have been reported to accumulate on lipid droplets under specific conditions [14, 16]. For example, the cytosolic Hsp70 and inosine monophosphate dehydrogenase relocate to lipid droplets after heat stress or insulin signaling, respectively [17, 18]. Lipid droplets may thus have a general role in sequestering proteins. Droplet binding has been proposed to inactivate harmful proteins, promote intracellular protein delivery, facilitate assembly of protein complexes, or aid protein degradation [14, 15, 19]. For several viral proteins, transient droplet association is indeed crucial for virus assembly [20, 21]. But it remains unknown if any endogenous proteins are indeed sequestered on lipid droplets to subsequently function elsewhere.

In this manuscript, we investigate the mechanism and significance of histone binding to lipid droplets in *Drosophila* embryos. We find that the novel protein Jabba physically interacts with histones and recruits them to lipid droplets. *Jabba* mutant embryos lack the extranuclear histone deposits. When mild disruption of histone gene expression is combined with *Jabba* mutants, embryos die during stages of highest histone demand. Our findings demonstrate that lipid droplets store histones for use in early embryogenesis.

Results

Histones on Lipid Droplets

Lipid droplets have a hydrophobic core of neutral lipids surrounded by amphipathic lipids and proteins. Their low buoyant

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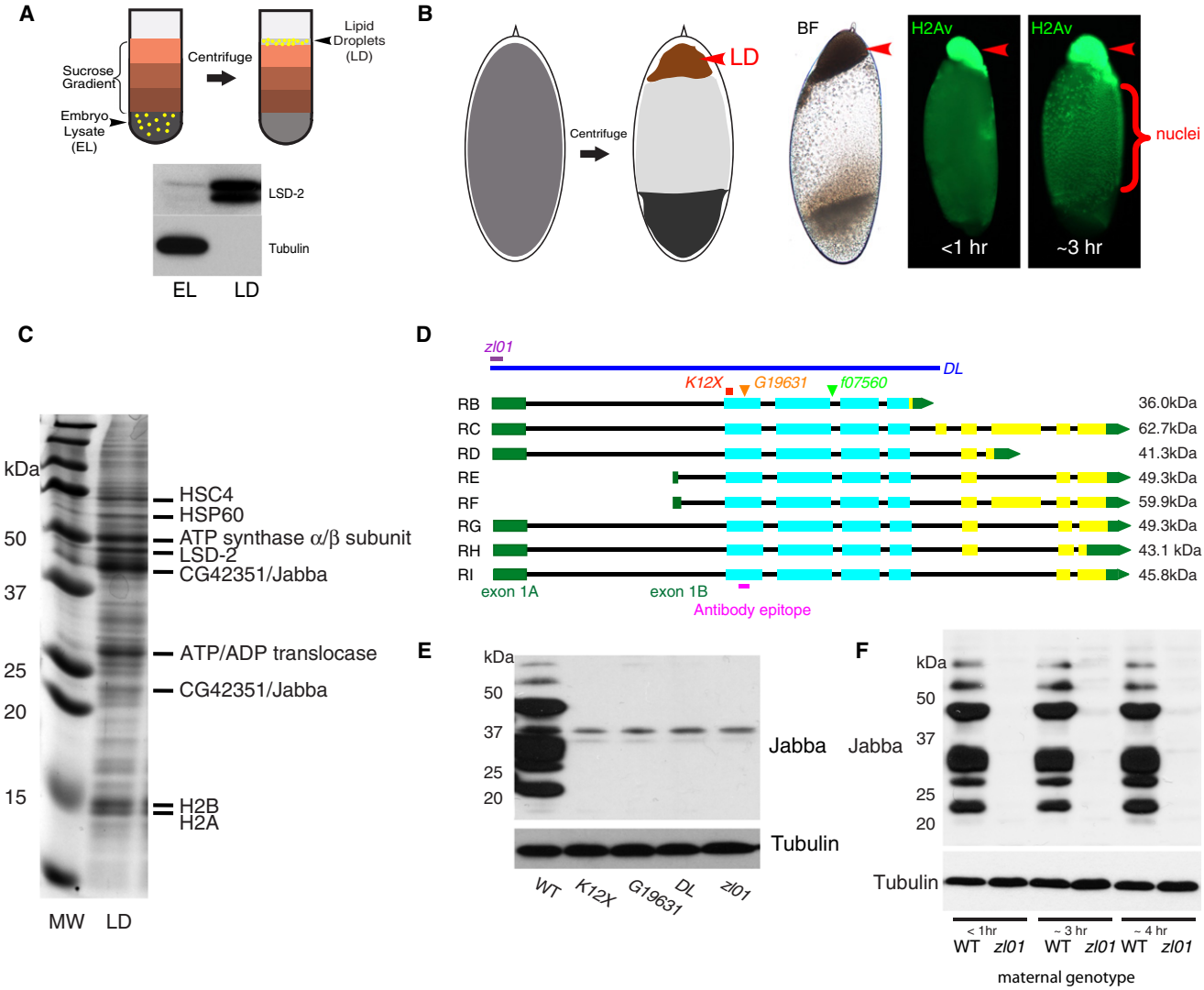


Figure 1. Identification and Characterization of Jabba

(A) Purification of lipid droplets from embryo lysate (EL) by flotation. During centrifugation, lipid droplets float to the top of the sucrose gradient. The lipid-droplet fraction (LD) is enriched for the droplet protein LSD-2 and depleted for the cytoplasmic protein tubulin.

(B) Enrichment of lipid droplets by in vivo centrifugation. The droplets form a distinct lipid-droplet layer (LD; arrowheads), visible by bright-field (BF) microscopy. Anti-H2Av staining reveals H2Av on the droplet layer of embryos of different ages, as well as within nuclei.

(C) Proteins from purified wild-type droplets were separated by SDS-PAGE and stained with Coomassie blue (MW = molecular weight markers, LD = lipid-droplet fraction). Major bands were excised and identified by mass spectrometry.

(D) *Jabba* locus. Exons are shown as boxes: green = UTR, cyan = coding regions shared between all isoforms, yellow = coding regions restricted to some isoforms. Pink bar = peptide used for antibody generation. The extent of deletions in $Jabba^{DL}$ and $Jabba^{zl01}$ is indicated, as well as the location of the nonsense mutation $Jabba^{K12X}$ and of two transposable element insertions.

(E) Lysates from ~3-hr-old embryos analyzed by anti-Jabba immunoblotting.

(F) Embryos from reciprocal crosses between wild-type and $Jabba^{zl01}$ parents analyzed by anti-Jabba immunoblotting. See also Figure S1.

density allows lipid droplets to be separated from other cell components. For example, lipid droplets can be purified from *Drosophila* embryo lysates by sucrose-gradient centrifugation (Figure 1A). In the complementary "in vivo centrifugation" approach [22], intact embryos are centrifuged: the buoyant droplets float up, forming a distinct layer (Figure 1B).

Using both approaches, we previously showed that histones H2A and H2B are massively present on lipid droplets of early embryos [15]. Proteomic analysis had also identified histone variant H2Av as candidate droplet protein. Endogenous H2Av is indeed present on biochemically purified lipid droplets (see below) and highly enriched in the droplet layer of centrifuged embryos (Figure 1B): In very young embryos, H2Av resides almost exclusively in the droplet layer; in older embryos, it is present also in nuclei.

Identifying Jabba as Candidate Histone Docking Protein

Histones are bound to the droplet surface via electrostatic interactions [15]. If they bind via specific proteins, such histone anchors should be present on lipid droplets in comparable amounts and, just like histones, constitute major droplet proteins. We purified droplets by flotation, separated copurifying proteins by SDS-PAGE, and identified the most prominent bands by mass spectrometry (Figure 1C). In addition to

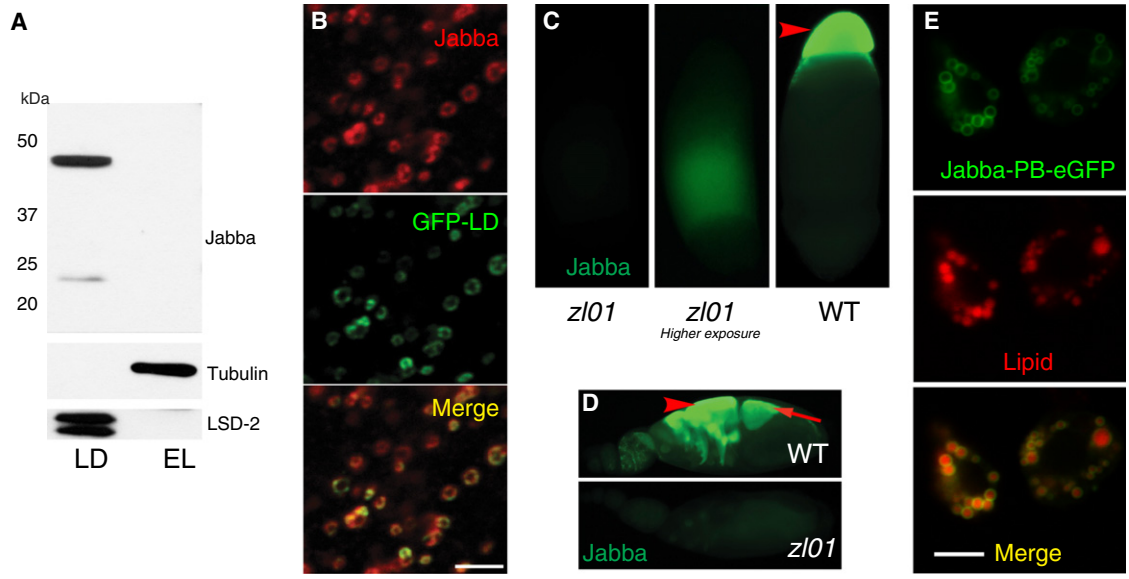


Figure 2. Jabba Is a Lipid-Droplet Protein

(A) Equal amounts of protein from wild-type lipid-droplet samples (LD) and embryo lysate (EL) analyzed by immunoblotting (see Figure 1A). Two Jabba bands are highly enriched in the droplet fraction; under these conditions, no signal is detected in the lysate.

(B) Embryos expressing the droplet marker GFP-LD stained for GFP (green) and Jabba (red). Jabba is present in rings that colocalize with GFP-LD. Scale bar represents 2.5 μm.

(C) Anti-Jabba staining of centrifuged wild-type and *Jabba*^{zl01} embryos. Jabba is highly enriched in the droplet layer (arrowhead) in the wild-type. The middle panel shows a ~10-fold longer exposure of the mutant.

(D) Anti-Jabba staining of centrifuged egg chambers. Lipid-droplet layers in the oocyte (arrow) and in nurse cells (arrowhead) are indicated.

(E) Kc167 cells transiently expressing Jabba-PB-eGFP (green) stained with LipidTOX (red) to reveal lipid droplets. Scale bar represents 5 μm. See also Figure S2.

histones H2A and H2B, we found the cytosolic chaperone HSC70-4 and the mitochondrial proteins HSP60, ATP synthase, and ATP/ADP translocase. Because these proteins are abundant in nonnuclear and nondroplet compartments, they seemed poor candidates for factors that recruit histones specifically to lipid droplets.

We therefore focused on the remaining two candidates, the perilipin LSD-2 [23] and the novel protein CG42351. LSD-2 is not the histone anchor: histones remain bound to purified droplets even if LSD-2 is dislodged with detergents [15], and, in embryos lacking LSD-2, histones are still present on the droplet layer after centrifugation (see Figure S4A available online). The prominent band at 42 kDa was identified as CG42351, in the following called Jabba. A minor band at 23 kDa also contained Jabba peptides and likely represents a breakdown product or alternative isoform.

Embryos Express Multiple Forms of Jabba

Jabba is a novel protein, evolutionarily conserved among insects, but not obviously present in other species. Based on FlyBase annotations and our own cloning of *Jabba* cDNAs, the *Jabba* locus is predicted to encode eight transcripts (Figure 1D). These transcripts arise from two different promoters, have coding exons 2 through 5 in common, and then diverge. All peptides recovered by mass spectrometry map to the common region and do not distinguish between isoforms.

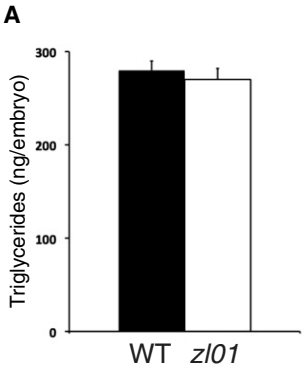
Using multiple strategies, we identified eight mutant *Jabba* alleles, including deletions, nonsense mutations, and transposable-element insertions (Figure 1D and Supplemental Experimental Procedures). We also generated an antibody against an epitope in the shared exon 2. Immunoblotting (Figures 1E, S1A, and S1B) detected several prominent bands in wild-type embryos. All these proteins are encoded by the *Jabba* locus, as they are absent in several *Jabba* mutant alleles. It is unknown which *Jabba* splice variants give rise to the Jabba proteins present in embryos. Several Jabba bands detected by immunoblotting have a lower molecular weight than the smallest predicted isoform, suggesting posttranslational processing.

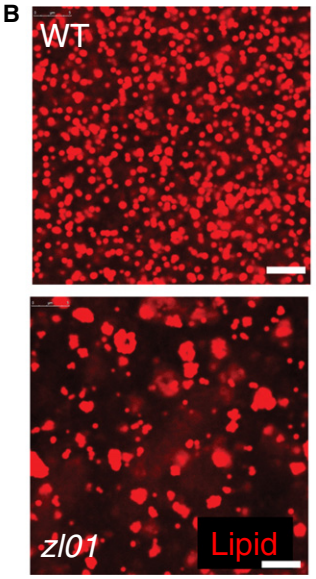
To test whether the Jabba protein of early embryos is maternally provided or due to new transcription in the embryo, we performed reciprocal crosses between wild-type and *Jabba* mutant parents (Figures 1F and S1C). Because the presence of Jabba protein depended strictly on the genotype of the mother, Jabba is maternally provided—like histones and lipid droplets.

Jabba Is Lipid Droplet Associated

We had identified Jabba as 42 and 23 kDa proteins prominent in purified lipid droplets. By immunoblotting, Jabba bands of these sizes were indeed highly enriched on purified droplets (Figures 2A and S2B). Other Jabba protein forms were not detected; either these forms localize elsewhere or are lost from droplets during purification.

Anti-Jabba immunostaining revealed round cytoplasmic structures in the size range of embryonic lipid droplets [24]; these structures showed the ring pattern typical for proteins present on the surface of lipid droplets (Figure 2B). No such structures were evident in *Jabba*^{zl01} embryos. Jabba signal indeed colocalized with GFP-LD (Figure 2B), a fusion protein present on most embryonic droplets [25]. Jabba signal was also highly enriched in the droplet layer of centrifuged embryos (Figure 2C). Finally, lipid droplets in early embryos





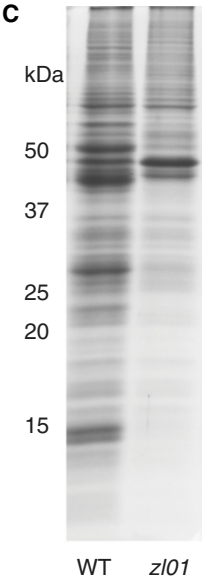


Figure 3. *Jabba* Mutants Have Abundant Lipid Droplets with Altered Protein Content

(A) Triglyceride levels in 2-hr-old wild-type and *Jabba*^{zl01} embryos are similar. Error bars represent SDs.

(B) Lipid droplets in 2-hr-old wild-type and *Jabba*^{zl01} embryos, revealed by Nile Red staining. *Jabba*^{zl01} droplets are unevenly distributed and irregularly shaped, a phenotype that inspired the name Jabba. Scale bar represents 5 μm.

(C) Equal amounts of proteins of droplets purified from wild-type and *Jabba*^{zl01} embryos were separated by SDS-PAGE and stained with Coomassie blue.

accumulate around the central yolk just before cellularization; this transport requires the Halo protein [26]. Jabba rings also accumulated around the yolk, in a Halo-dependent manner (Figure S2A). We conclude that Jabba is associated with lipid droplets.

During oogenesis, lipid droplets originate in nurse cells and are transferred through cytoplasmic bridges to the growing oocyte. We employed in vivo centrifugation to separate the constituents of nurse cells and oocytes by density [15, 22]. Anti-Jabba signal was present exclusively in the droplet layer of nurse cells and oocytes (Figure 2D). Jabba apparently already localizes to lipid droplets in nurse cells and is deposited with them into the oocyte.

Jabba localizes to lipid droplets also in *Drosophila* Kc167 cultured cells. Transient expression of GFP-tagged Jabba isoforms yielded the characteristic ring pattern. These rings represent lipid droplets, as shown by colabeling with a droplet-specific dye or coexpression of Tdt-tagged droplet proteins (Figures 2E and S2C–S2G). All three isoforms tested displayed this localization, suggesting that droplet targeting is inherent in the common region.

Jabba Is Required for Histone Recruitment to Lipid Droplets

Embryos lacking Jabba have normal overall triglyceride content and display abundant lipid droplets (Figures 3A, 3B, and S3C). In the mutant embryos, lipid droplets were less evenly distributed than in the wild-type and often clustered. The mutant droplets also frequently appeared larger, though currently we cannot resolve whether this is due to fusion of clustered droplets upon fixation.

Droplets purified from *Jabba*^{zl01} and wild-type embryos differed dramatically in their protein composition (Figure 3C). Several major bands were missing from the mutant sample, including H2A and H2B. Many other bands were still present. In particular, LSD-2 was abundant on the mutant droplets, a fact confirmed by immunoblotting (Figure S3A). We also found the known droplet proteins kinesin-1 and Klar on *Jabba*^{zl01} droplets (Figures 4A and S3B). Thus, absence of Jabba leads to loss of specific droplet proteins.

We confirmed the absence of histones from *Jabba* droplets with antibodies. By immunoblotting, we found that H2A and H2B are abundantly present on wild-type, but not *Jabba* mutant droplets (Figure 4A). By immunostaining, we found that histone signal was strong in the droplet layer and nuclei of centrifuged wild-type embryos, but absent from the droplet layer of *Jabba* mutants: Similar results were observed for unfertilized eggs and for embryos of various ages (Figures S4C, 4B, and S4D) as well as for several independently derived *Jabba* alleles (Figures 4B and S4B).

Histone variant H2Av displayed the same pattern. In *Jabba* mutants, H2Av was absent from purified droplets and from the droplet layer of centrifuged embryos (Figures 4A, 4B, S4C, and S4D). We also employed H2Av-GFP transgenes and live imaging of embryos. For centrifuged embryos, presence of H2Av-GFP in the droplet layer requires Jabba (Figure 4C). In uncentrifuged embryos that express Jabba, H2Av-GFP was present in nuclei and in cytoplasmic rings, previously shown to be lipid droplets [15]. In *Jabba* mutants, nuclear signal was prominent, but no cytoplasmic rings were detectable (Figure 4D). We conclude that Jabba is required for H2A, H2B, and H2Av to localize on lipid droplets.

Droplet-Bound Jabba and Histones Form Protein Complexes

Jabba might act as the histone anchor, physically connecting histones to lipid droplets, or it might act indirectly, e.g., by recruiting or modifying the actual histone anchor. If Jabba recruits histones directly, the amount of Jabba should influence how much histone is present on lipid droplets. In embryos from mothers with a single copy of the *Jabba* gene (1× *Jabba*), Jabba levels are roughly halved compared to the wild-type (2× *Jabba*) (Figure 5A). When centrifuged 3-hr-old embryos were stained for H2B, nuclear signal was comparable between embryos from 1× and 2× *Jabba* mothers, but the droplet-layer signal was greatly reduced for 1× *Jabba* (Figure 5B). We observed a similar dependence of histone levels on *Jabba* dosage, using H2Av-GFP (Figure 5C).

To directly test if Jabba physically recruits histones to droplets, we purified lipid droplets from H2Av-GFP-expressing embryos and precipitated H2Av-GFP with anti-GFP antibodies. The 42 kDa form of Jabba, but not LSD-2, coprecipitated under these conditions (Figure 5D). Apparently, Jabba is specifically brought into the pellet, not simply via precipitation of entire droplets. We propose that Jabba acts as

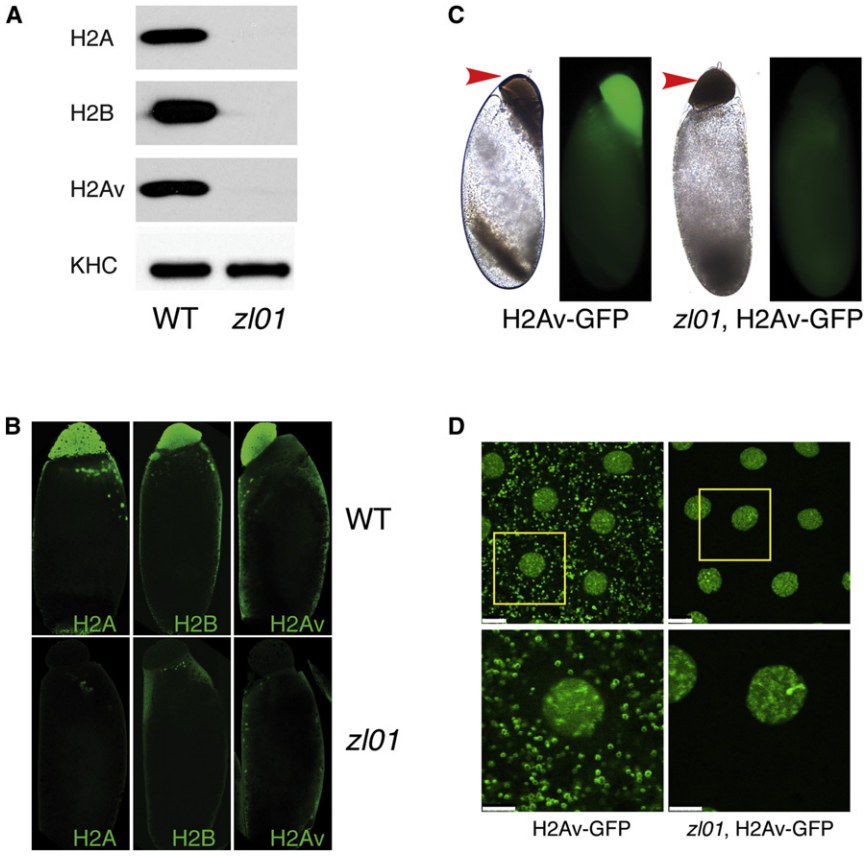


Figure 4. Jabba Is Necessary for Histone Recruitment to Lipid Droplets

(A) Immunoblotting of purified droplets from wild-type and *Jabba*^{zl01} embryos. Equal amounts of total protein were loaded. Kinesin heavy chain (KHC) levels are similar. H2A, H2B, and H2Av levels are dramatically reduced in the mutant.

(B) Immunostaining of centrifuged embryos (<1 hr). *Jabba*^{zl01} embryos lack H2A, H2B, and H2Av signal in the droplet layer (top).

(C) Centrifuged embryos from mothers expressing H2Av-GFP, by bright-field and fluorescence microscopy. GFP signal on the droplet layer (arrowhead) is dramatically reduced in the mutant.

(D) In uncentrifuged embryos, H2Av-GFP is present in nuclei for both genotypes, but is absent from cytoplasmic puncta (which represent lipid droplets [15]) in *Jabba*^{zl01}. The exposure for the wild-type is double that of the mutant sample; for unknown reasons, H2Av-GFP signal in the nuclei is stronger in *Jabba*^{zl01} embryos. Scale bars represent 7.5 μm (top), 5 μm (bottom). See also Figure S4.

histone-docking protein and recruits histones to droplets by physical interactions, either directly or via bridging proteins. Since monomeric histones are unstable, it seems likely that the droplet-bound histones are H2A/H2B and H2Av/H2B heterodimers.

Jabba Mutants Lack Maternal H2A/H2B/H2Av Deposits

If histones are unable to bind to lipid droplets, they might be present elsewhere in the embryo or they might be eliminated, like excess histones in yeast [7, 11]. Unfertilized *Jabba*^{zl01} mutant embryos had massively reduced levels of H2A and H2B, and reproducibly less H2Av (Figure 6A). Levels of H3, a histone not bound to lipid droplets, were unchanged (Figure 6B). Embryos from 1× *Jabba* mothers had intermediate maternal histone deposits (Figure 6C).

In addition to the maternally supplied histone proteins, wild-type embryos also contain abundant histone messages [10]: maternally deposited histone mRNAs [27, 28] are later supplemented by zygotic transcription [29, 30]. Translation of these messages contributes to the histone protein supply, as overall histone protein levels increase several-fold during the first 3 hr of embryogenesis [15] (Figure 6E). This source of new histones is apparently unaffected by the absence of Jabba: H2A and H2B levels in *Jabba*^{zl01} embryos go up as embryos age (Figure 6D), such that by stage 5 they have reached close to wild-type levels (Figure 6E). We also detect similar levels of maternally provided H2A mRNAs in both wild-type and *Jabba*^{zl01} embryos (Figure 6F). Thus, *Jabba* mutations do not interfere with histone biosynthesis per se. We propose that *Jabba* mutant embryos lack the maternal histone deposit because extranuclear histones are degraded.

What are the consequences for the embryo? During wild-type development, nuclei initially divide in the center of the embryo (cleavage stages), and most subsequently migrate to the embryo cortex (syncytial blastoderm). Cellularization occurs after four additional cortical mitoses [31]. Severe reduction in maternal histone expression causes aberrant nuclear shapes and distributions, nuclei arrested in mitosis, and, ultimately, embryonic death before cellularization [28]. Yet DNA staining of *Jabba* mutant embryos revealed the wild-type pattern of nuclear distributions; in particular, in blastoderm embryos, nuclei at the cortex were evenly spaced and uniform in size (Figure S5A). *Jabba* mutant embryos also hatched at wild-type rates (Figure 7A) and gave rise to fertile adults.

Limited histone supply can cause defects during mitosis [28, 32]. In blastoderm embryos, the products of aberrant mitoses are eliminated by nuclear fallout [33]: Defective nuclei dissociate from their centrosomes and move into the central yolk. Free centrosomes at the cortex are therefore a hallmark of DNA damage and/or defective mitoses. Yet in both wild-type and *Jabba* mutant embryos, we only rarely detected centrosomes unattached to nuclei (Figure S5A). Apparently, the lack of the maternal histone protein deposit is neither essential for embryo viability nor does it cause widespread mitotic defects.

Jabba Mutations and Reduced Histone Expression Are Synthetically Lethal

Jabba mutants are surprisingly normal, even though a sufficient histone supply is essential for early embryogenesis [28]. We reasoned that biosynthesis of new histones in the zygote (Figures 6D and 6E) might generate just enough histones for *Jabba* embryos to survive. Cultured mammalian cells and yeast, for example, maintain viability even when overall histone incorporation into chromatin is substantially reduced [2].

To test whether *Jabba* embryos are particularly dependent on new histone synthesis, we employed mutants in the

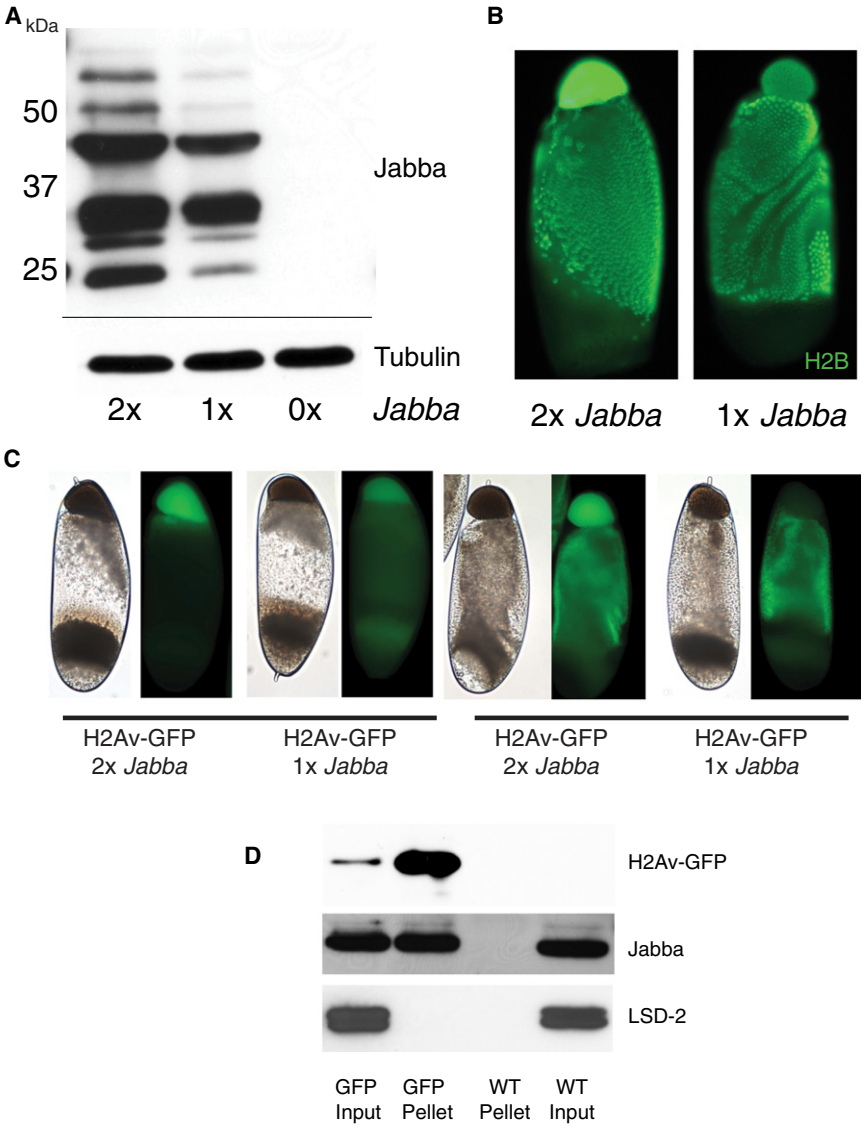


Figure 5. Jabba Is the Histone-Docking Protein on Droplets

(A) Anti-Jabba immunoblotting in equal numbers of embryos from mothers carrying two, one, or zero copies of a wild-type *Jabba* gene. Jabba protein levels scale with *Jabba* gene dosage (2× *Jabba* = wild-type; 1× *Jabba* = *Df(2R)Exel7158*/+; 0× *Jabba* = $Jabba^{zl01}$).

(B) H2B signal on the lipid-droplet layers of centrifuged 2× *Jabba* embryos (~3-hr-old) is much stronger than that of 1× *Jabba*, while nuclear signal is similar.

(C) Centrifuged H2Av-GFP/+ and $Jabba^{zl01}$/+, H2Av-GFP/+ embryos. Embryo ages: < 1 hr (left panel), ~3 hr (right panel). Reduced droplet signal with age presumably represents transfer of histones from lipid droplets to nuclei [15].

(D) H2Av-GFP coimmunoprecipitates with Jabba. Lipid droplets purified from H2Av-GFP and wild-type embryos were exposed to anti-GFP antibodies for immunoprecipitation.

Drosophila stem-loop-binding protein (dSLBP). dSLBP binds the messages of core histones and promotes their correct processing, stability, and translation [28, 34]. Simple reduction in *dSLBP* dosage caused no overt defects: Embryos from 1× *dSLBP* mothers were morphologically normal, cellularized, and hatched at near wild-type rates (Figure 7A). In contrast, embryos from $Jabba^{zl01}$ *1×dSLBP* mothers typically reached syncytial stages but then became morphologically abnormal (Figure 7B). Cellularization, if initiated at all, frequently proceeded abnormally (Figure S5B). Most embryos failed to hatch (Figure 7A), and about half ultimately rounded up inside the egg shell (Figure S5C), reminiscent of the "cannonball" phenotypes observed when precellularization embryos are massively irradiated [35, 36]. The hypomorphic allele $dSLBP^{10}$ caused similar synthetic lethality (Figure S5E). Finally, we already observed some reduction in embryo hatching even when we reduced histone expression only zygotically (Figure S5F).

Two additional strategies to impair histone expression yielded similar results: First, we reduced the dosage of H2Av. Embryos from 1× *H2Av* mothers developed grossly normally and hatched at wild-type rates. Embryos from $Jabba^{zl01}$ 1× *H2Av* mothers displayed severe hatching defects (Figure S5E); ~10% died as cannonballs. Second, we halved the dosage of core histone genes. Embryo hatching was normal when mothers had one or two copies of *Jabba*, but was variably reduced when *Jabba* was absent (data not shown).

DNA staining of embryos from $Jabba^{zl01}$ *1×dSLBP* mothers revealed multiple defects. Frequently, nuclei had different sizes and were misshapen and unevenly spaced (Figure 7D). We also observed trailing chromosomes during anaphase and telophase (Figure 7E), indicative of defective chromosome segregation. Finally, mutant embryos apparently experienced massive nuclear fallout because they displayed nuclei in between the cortex and the central yolk and abundant free centrosomes at the cortex (Figure 7F). Nuclear fallout was also evident by bright-field microscopy (Figure S5D; Movie S1). We conclude that under sensitized conditions the droplet-bound histones are essential for early embryogenesis.

Discussion

Lipid-Droplet Binding Allows Accumulation of Excess Histones

The maternal histone protein deposit is dramatically reduced in *Jabba* embryos. In principle, lack of these histones could be due to defective histone biosynthesis during oogenesis, lack of transport of histones from nurse cells to oocytes, or degradation of histones if they are not droplet-bound. A defect in histone protein production seems unlikely (Figure 6): *Jabba* embryos contain normal levels of maternal histones messages; these messages are abundantly translated in the zygote, and the histone deposit scales with *Jabba* dosage.

During oogenesis, lipid droplets originate in nurse cells and are then transferred to oocytes. Histones might be absent

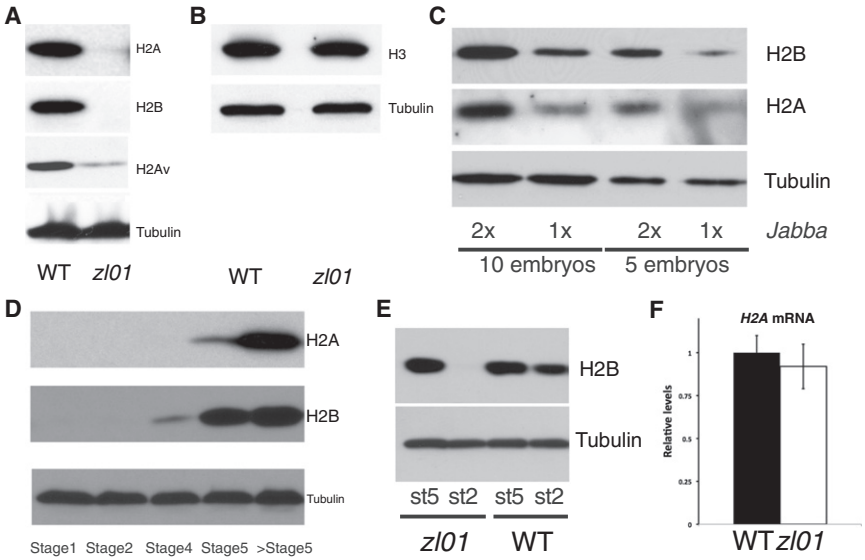


Figure 6. *Jabba* Mutant Embryos Lack Maternal Histone Deposits, but Can Synthesize Histones Zygotically

(A and B) Compared to wild-type, unfertilized *Jabba*^{zl01} embryos have barely detectable H2A and H2B, less H2Av, but similar amounts of H3.

(C) By immunoblotting, the amount of H2A and H2B in unfertilized 2× *Jabba* embryos is roughly double that of 1× *Jabba* embryos.

(D) *Jabba*^{zl01} embryos can generate H2A and H2B. Levels of both histones go up dramatically as development proceeds (stage 1, ~30 min; stage 2, ~60 min; stage 4, ~2 hr; stage 5, ~3 hr).

(E) By ~3 hr, *Jabba*^{zl01} embryos have reached H2B levels similar to wild-type embryos.

(F) Unfertilized wild-type and *Jabba*^{zl01} embryos have similar levels of H2A mRNA (measured by qRT-PCR, normalized to the wild-type value). Error bars represent SDs.

from oocytes because without droplet-attachment they are left behind in the nurse cells. We disfavor this possibility: first, most nurse-cell contents are transferred to the oocyte later in oogenesis anyway; second, oocytes contain abundant histone mRNAs that could contribute to the intraoocyte histone pool regardless of transport problems; third, we have failed to detect obvious accumulation of H2B or H2Av-GFP in the nurse-cell cytoplasm of *Jabba* mutants (not shown).

In yeast, excess histones are destroyed by the proteasome [5, 11]. Indirect evidence suggests that similar pathways are active in animal cells [2]. We propose that in *Jabba* mutants the maternal histone protein deposit is reduced because histones not bound to lipid droplets are degraded.

Droplet-Associated Histones Support Early Development

To ensure proper chromatin packaging, the cellular histone supply has to match the available DNA. In early *Drosophila* embryos, the demand for histones is particularly high: by blastoderm stages, new DNA equivalent to thousands of diploid nuclei needs to be packaged, during S phases that last less than 5 min [31]. It had long been thought that this demand was met by the maternal deposits of histone mRNA and protein [10]. Our data now show that embryos in which the maternal histone protein deposit is selectively impaired are viable.

Severe impairment of the embryonic histone supply has been achieved by two distinct strategies: 10-fold reduction of histone mRNA levels via *dSLBP* mutations [28] or by combining mild disruption of histone expression with a lack of the histone protein deposit via *Jabba* mutants (this manuscript). In either case, the overall histone supply apparently falls below a critical threshold: many embryos become abnormal by or before blastoderm stages. Under these sensitized conditions, *Jabba* mutants display massive nuclear fallout as well as trailing chromosomes in mitosis, consistent with the aberrant mitoses and increased DNA damage sensitivity known to result from reduced histone levels. We conclude that when histone biosynthesis in the embryo is compromised, *Jabba* mutants die because they cannot compensate for the lack of the maternal histone deposit.

This analysis strongly argues that the droplet-bound histones are one of the sources of histones that sustain early development. Indeed, histones can be transferred from droplets to nuclei (as previously shown by droplet transplantation [15]), and levels of droplet-bound histones decrease as embryogenesis proceeds [15] (Figure 4C). In embryos with impaired histone biosynthesis (1× *dSLBP*), the histone pool on droplets is markedly reduced by cellularization, compared to wild-type (Figures S5G and S5H), consistent with the notion that these embryos are particularly dependent on the maternally provided histone store.

Microarray and RNA-seq data available on FlyBase [37] suggest that Jabba expression is not restricted to early embryos. What role Jabba may play in other cell types is an exciting challenge for future research. As in embryos, it may modulate histone availability, though presumably the need for excess histones is reduced later in development. Our analysis (Figure 3) raises the intriguing possibility that Jabba may also buffer other proteins or may control morphology and spatial distribution of droplets.

Lipid Droplets as General Protein Storage Sites

Droplet-associated histones may be widespread: By immunolocalization, histones have been detected on lipid droplets in housefly embryos [15] and mouse oocytes [38], as well as on the lipid-droplet-related microvesicles of mammalian sebocytes [39]. In addition, many droplet proteomes report the presence of specific histones (see, e.g., [14]). In these cases, histones may also be transiently stored on lipid droplets for later use in the nucleus, just as in *Drosophila* embryos. Storage capacity is likely enhanced by additional mechanisms, since both classical histone chaperones and certain importins can buffer limited amounts of histones in the cytoplasm [5, 40]. We speculate that the abundant surface of lipid droplets provides high-capacity storage and also keeps the histones from inappropriately entering the nuclei. The droplet-bound histones might buffer histone availability if histone synthesis and DNA replication transiently get out of sync, provide a source of histones for DNA repair, or even protect against bacterial pathogens [41].

The specificity of histone-droplet interactions is remarkable: H2A, H2B, and H2Av are droplet localized, and their stability in

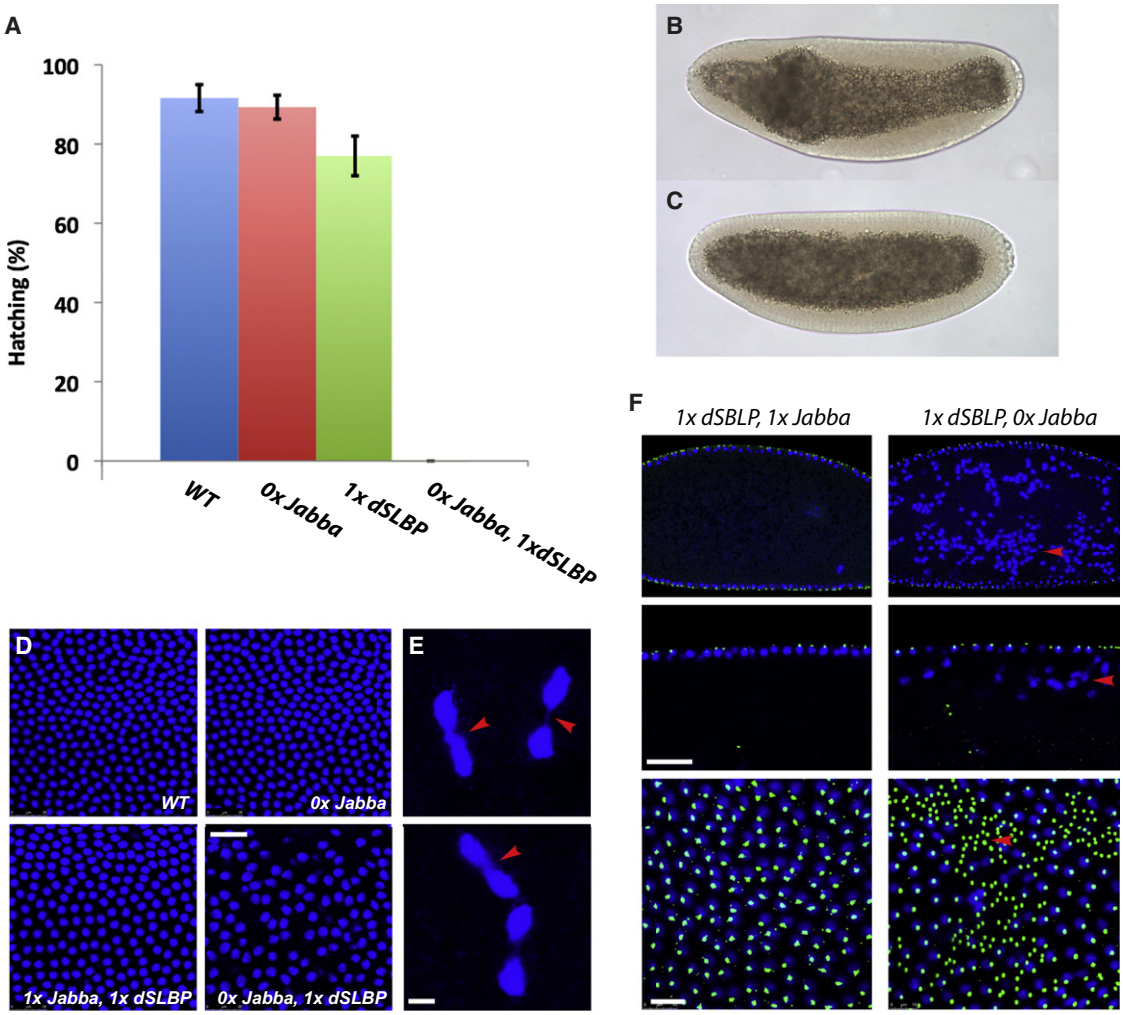


Figure 7. Reduced Histone Expression and *Jabba* Mutants Are Synthetically Lethal

(A) Hatching frequency of embryos from mothers of various genotypes. 0× *Jabba* = *Jabba*^{zl01}; 1× *dSLBP* = heterozygous for *Df(3R)3450*. Error bars represent SDs.

(B and C) Late syncytial blastoderm embryos (heat fixed and inspected by DIC microscopy) from 1× *dSLBP* 0× *Jabba* mothers, with aberrant yolk distribution (B) or grossly normal morphology (C). By cellularization, most embryos displayed some morphological defects.

(D) Surface view of blastoderm embryos is shown; genotype of mothers is indicated (blue = DNA; scale bar represents 25 μm).

(E) Nuclei of an embryo from a 1× *dSLBP* 0× *Jabba* mother showing chromosomes (arrowheads) connecting daughter nuclei late in mitosis. Scale bar represents 5 μm.

(F) Blastoderm embryos stained for DNA (blue) or centrosomes (green): cross-sections at low (top) and high (middle) magnification, plus a surface view (bottom). In the absence of Jabba, nuclei are absent from cortical patches (arrowhead, bottom) and present between the cortex and the central yolk (arrowheads, top and middle). Scale bars represent 10 μm. See also Figure S5 and Movie S1.

the early embryo depends on Jabba. In contrast, H3 is absent from lipid droplets, and its maternal stores are independent of Jabba. It is possible that—as in *Xenopus* oocytes [42]—histones H3/H4 are stored in the cytoplasm associated with histone chaperones. In contrast, droplet-localized histones are apparently not associated with traditional histone chaperones, since no such proteins have been detected on lipid droplets (Figure 1C) [15]. We propose that binding to Jabba stabilizes these histones and that they are later handed off to cytoplasmic chaperones that shuttle them to the nuclei for chromatin assembly.

Like histones, many candidate droplet proteins have known functions or localization in other cellular compartments, such as the cytosolic enzyme inosine monophosphate dehydrogenase, the spliceosome activator Prp19p, and the membrane protein caveolin (reviewed in [14]). We previously proposed that some of these proteins are "refugee proteins," proteins transiently sequestered after or before acting elsewhere in the cell [14, 15]. To our knowledge, our studies on droplet-bound histones provide the strongest evidence yet for droplet sequestration of endogenous proteins and for lipid droplets as a way station for proteins destined elsewhere.

The possibility that lipid droplets modulate protein availability for other cellular compartments suggests that they have novel cellular roles beyond lipid metabolism. If so, altering droplet number or size could affect overall cellular buffering capacity: Too much sequestration surface might reduce available protein below optimal levels, while too little sequestration might allow proteins to build up to toxic levels.

Experimental Procedures

Identification of Droplet Proteins

Lipid droplets were isolated by flotation as described [25], and normalized by protein content for western analysis. For mass spectrometry, isolated droplets were separated by SDS-PAGE, and proteins were digested in gel with trypsin. Tryptic peptides were identified by either MALDI or LC-MS/MS.

Microscopic Analysis

Living embryos and adult females were centrifuged to separate lipid droplets from other cellular components [15, 22]. Lipid droplets were detected in fixed, dechorionated embryos with Nile Red [25]. To detect GFP, Jabba, histones, or centrosomes by immunostaining, dechorionated embryos were heat fixed and devitellinized using standard heptane-methanol procedures. Kc167 cells were cultured in Schneider's medium and induced to accumulate lipid droplets with 400 μM oleic acid. Cells were fixed using 5% paraformaldehyde and counterstained with LipidTOX Deep Red.

Molecular Biology

Jabba isoforms RD, RH, and RI were cloned from an adult female cDNA library. Isoforms were expressed in Kc167 cells as N-terminal GFP fusions. For qPCR analysis, cDNA was synthesized using mRNA from embryos less than 2 hr old. Histone message levels were quantified via a Bio-Rad iQ5 detection system.

Supplemental Information

Supplemental Information includes five figures, one movie, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2012.09.018.

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