# The lipodystrophy protein seipin is found at endoplasmic reticulum lipid droplet junctions and is important for droplet morphology

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**Lipodystrophy is a disorder characterized by a loss of adipose tissue often accompanied by severe hypertriglyceridemia, insulin resistance, diabetes, and fatty liver. It can be inherited or acquired. The most severe inherited form is Berardinelli-Seip Congenital Lipodystrophy Type 2, associated with mutations in the *BSCL2* gene. *BSCL2* encodes seipin, the function of which has been entirely unknown. We now report the identification of yeast *BSCL2*/seipin through a screen to detect genes important for lipid droplet morphology. The absence of yeast seipin results in irregular lipid droplets often clustered alongside proliferated endoplasmic reticulum (ER); giant lipid droplets are also seen. Many small irregular lipid droplets are also apparent in fibroblasts from a BSCL2 patient. Human seipin can functionally replace yeast seipin, but a missense mutation in human seipin that causes lipodystrophy, or corresponding mutations in the yeast gene, render them unable to complement. Yeast seipin is localized in the ER, where it forms puncta. Almost all lipid droplets appear to be on the ER, and seipin is found at these junctions. Therefore, we hypothesize that seipin is important for droplet maintenance and perhaps assembly. In addition to detecting seipin, the screen identified 58 other genes whose deletions cause aberrant lipid droplets, including 2 genes encoding proteins known to activate lipin, a lipodystrophy locus in mice, and 16 other genes that are involved in endosomal–lysosomal trafficking. The genes identified in our screen should be of value in understanding the pathway of lipid droplet biogenesis and maintenance and the cause of some lipodystrophies.**

BSCL2 | lipid bodies

Lipodystrophies are disorders in the development or maintenance of adipose tissue, the storage site for triglycerides and sterol esters (1–3). These conditions can be acquired or inherited, partial or generalized. A notable example of acquired partial lipodystrophy occurs in HIV-infected patients on highly active retroviral therapy (HAART) who lose subcutaneous adipose tissue from the face and limbs but gain it elsewhere, such as the upper back and neck (4). The most severe lipodystrophies are the Berardinelli-Seip congenital generalized forms. Patients affected are born with little or no adipose tissue. As children, they have ravenous appetites and grow more rapidly than normal. In general, patients with lipodystrophy have high circulating levels of triglycerides, develop deposits of fat in their muscles and liver, and often acquire insulin resistance and diabetes, similar to obese patients.

Two Berardinelli-Seip Congenital Lipodystrophy (BSCL) genes have thus far been identified. *BSCL1* encodes an acylglycerol phosphate acyltransferase, which catalyzes a critical step in the biosynthesis of triglycerides (5). The more severe form of the disease, in which adipose tissue is virtually absent, is caused by mutations in *BSCL2*. *BSCL2* encodes a protein, seipin, the function of which is entirely unknown (6, 7); a role in the differentiation of mesenchymal cells into preadipocytes has been postulated to explain the lack of adipose tissue (6, 8). Human seipin is predicted from its primary sequence to span a membrane twice, with both termini facing the cytoplasm, and a glycosylation site in the luminal segment (9); seipin-GFP partially localizes to the endoplasmic reticulum (ER) (10). Interestingly, mutations in the glycosylation site cause Silver syndrome and motor neuropathy (10), a result of a severe ER stress response (11). Whereas *BSCL2* mutations that cause lipodystrophy are recessive, Silver syndrome is the result of a dominant inheritance.

We reasoned that defects in the assembly of lipid droplets (lipid bodies; adiposomes) might cause lipodystrophy, because they comprise the fat storage site within adipose tissue. Although several lines of evidence suggest that lipid droplets are derived from ER (12), there is no agreement on mechanism. Most models offer a budding pathway, whereas an alternative idea is that they are ''nursed'' alongside the ER (13). No ER assembly factors have been identified. To this end, we screened a yeast deletion library for aberrant lipid droplets. We report the results of the screen, including the identification of yeast seipin as a factor in lipid droplet assembly or maintenance.

## Results

To identify genes in this pathway, we screened 4,936 yeast deletion clones for lipid droplet abnormalities by growing the strains overnight and treating them with BODIPY 493/503, a dye commonly used to stain lipid droplets (14). Most strains showed a wild-type phenotype, an average of 5–6 brightly stained lipid droplets of fairly uniform size per cell (Fig. 1). Although there was significant variability in lipid droplets among cells of any particular strain, inspection of several fields of cells revealed 59 deletion strains with clearly different phenotypes (Table 1). Traits were scored for differences in droplet number, size, dispersion throughout the cytoplasm (droplets in some strains were aggregated or centralized around the nuclear envelope), staining intensity, resolution of droplets from the cytoplasm or from each other (clear or indistinct), or a combination [supporting information (SI) Table 2]. To determine the dependence of phenotype on growth conditions, each strain was observed both in mid-log and stationary phase. Interestingly, 14 strains showed a wild-type phenotype in log phase but developed a high number of lipid droplets in stationary culture (SI Table 3).

Author contributions: R.G.W.A. and J.M.G. designed research; K.M.S., D.B., R.B., and W.-P.L. performed research; A.K.A. and A.G. contributed new reagents/analytic tools; K.M.S., D.B., R.B., N.V.G., R.G.W.A., and J.M.G. analyzed data; and J.M.G. wrote the paper.

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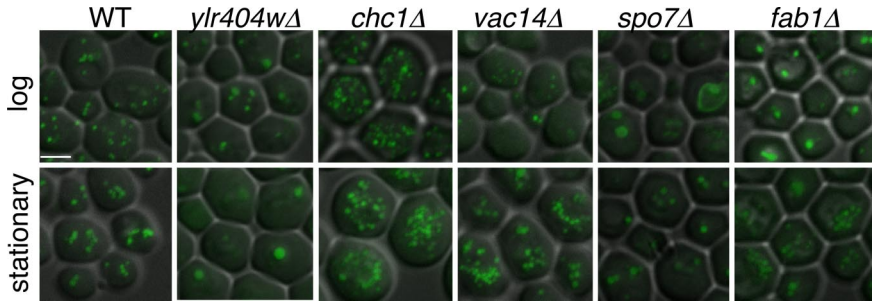


**Fig. 1.** Examples of strains with aberrant lipid body morphology. Cells were harvested in log or stationary phase, and lipid droplets were stained with BODIPY. (Scale bar, 2 $\mu$m.)

The largest functional group of proteins represented in Table 1 are those in endosomal/vacuolar (lysosomal) trafficking (16 genes), of which five encode subunits of the vacuolar ATPase, indicating the direct or indirect importance of this pump to lipid droplet assembly and/or maintenance. Defects in retrograde transport from Golgi to ER (in the *erd1*Δ and *sec22*Δ strains) suggest that factors are lost from the ER that are important for droplet morphology. Several genes encoding mitochondrial and endoplasmic reticular proteins also gave aberrant phenotypes; these two organelles apparently make tight contacts with lipid droplets (15). Also in the gene collection were *SPO7* and *NEM1*, which form a membrane complex that activates *PAH1* (16), the ortholog of lipin, a phosphatidic acid hydrolase (17) that is defective in a form of mouse lipodystrophy (18). Indeed, a *pah1*Δ strain obtained from Carman (17) showed a similar phenotype-neutral lipid frequently localized to the ER (data not shown).

We focused on determining the identity of *YLR404w*, a gene of unknown function. Combined analysis of primary sequence and predicted structure of multiple sequences by using PROMALS (19) strongly suggested that *YLR404w* encodes the *Saccharomyces cerevisiae* seipin (Fig. 2*A*). Seipins share a conserved structure in animals, plants, and fungi. As is true for human seipin, all members of the family share a core structure, ≈210 aa residues in length, flanked by two predicted transmembrane helices, one at each end (red cylinders), with several additional patches of hydrophobic residues (yellow). The amino and carboxyl termini of the yeast protein have been predicted to face the cytosol with the large central domain in a luminal compartment, similar to human seipin (9, 20). The core sequence illustrated in Fig. 2*A* comprises most of yeast seipin, but other species have extensions at either or both ends, which display low sequence similarity.

In addition to decreasing the number of lipid droplets, deletion of the yeast seipin homolog drastically alters lipid droplet morphology when cells are grown in glucose medium, as shown by electron microscopy (Fig. 3 *Aa–Af*). Lipid droplets in normal cells are small electron-transparent organelles (Fig. 3*Aa*). In contrast, the deletion strain typically contained clusters of organelles of complex morphology, with both electron-transparent and -opaque areas (Fig. 3*Ab*, arrow; higher resolution in Fig. 3*Ad*). The opaque regions could sometimes be resolved as layers of membrane. Membranes continuous with both cortical and perinuclear ER often penetrated the cluster and were frequently engorged with lipid (Fig. 3*Af*). Organelles within the cluster sometimes appeared as grapes on a stem of ER (Fig. 3*Ae*). A less common phenotype for the strain was the presence of very large lipid droplets (Fig. 3*Ac*). The images suggest that lipid droplet assembly is unregulated in the absence of seipin, yielding immature organelles that bud chaotically from the ER.

The aberrant assembly of lipid droplets in *YLR404w*-deficient cells is further highlighted when cells are grown on oleic acid. Growth of wild-type yeast on fatty acids causes the production of large lipid droplets of fairly uniform size (21) (Fig. 3*Ag*). In contrast, seipin-deficient cells produced a large number of lipid droplets of widely varying sizes and often of irregular shapes (Fig. 3*Ah*), including giant ones (Fig. 3*Ai*), which might represent products of fusion of smaller organelles.

To determine whether protein targeting to lipid droplets is disrupted in the *YLR404w* knockout (KO), we monitored the localization of fluorescently tagged Erg6p, a protein abundant in yeast lipid droplets (22). Erg6p localized to individual lipid droplets in the wild-type strain and to the ER/lipid droplet clusters in the seipin KO strain, indicating that protein targeting,

**Table 1. Genes involved in lipid body morphology**

| Function/localization | Gene |
|---|---|
| Endosome/vacuole | FAB1, PEP5, PEP7, PPA1, TFP1, VAC14, VMA2, VMA4, VMA6, VMA7, VMA22, VPS4, VPS16, VPS21, VPS24, VPS35, VPS51, VPS66 |
| ER | ERD1, OST4, SCP160, SEC22, SPO7, YIL039W |
| Mitochondria | ATP3 MDM20, MRM2, TOM5 |
| Other trafficking pathways | ANP1, CHC1, MOG1 |
| Actin or tubulin related | BEM2, CNM67, SLA2, SRV2 |
| Base and nucleotide metabolism | ADE8, ADE12, ADK1 |
| Transcription/expression | BUR2, DOA1, HPR1, KEM1, MED2, MSN1, PAF1, ROX3, SPT10, STO1 |
| Other nuclear activities | APN1, ECM1, EST3, NEM1 |
| Other | BUD32, DRS2, PLC1, SSD1, TPD3 |
| Unknown | YLR404W, YOR333C |

Genes whose deletions produce aberrant lipid bodies are grouped by localization or function, based on www.yeastgenome.org.

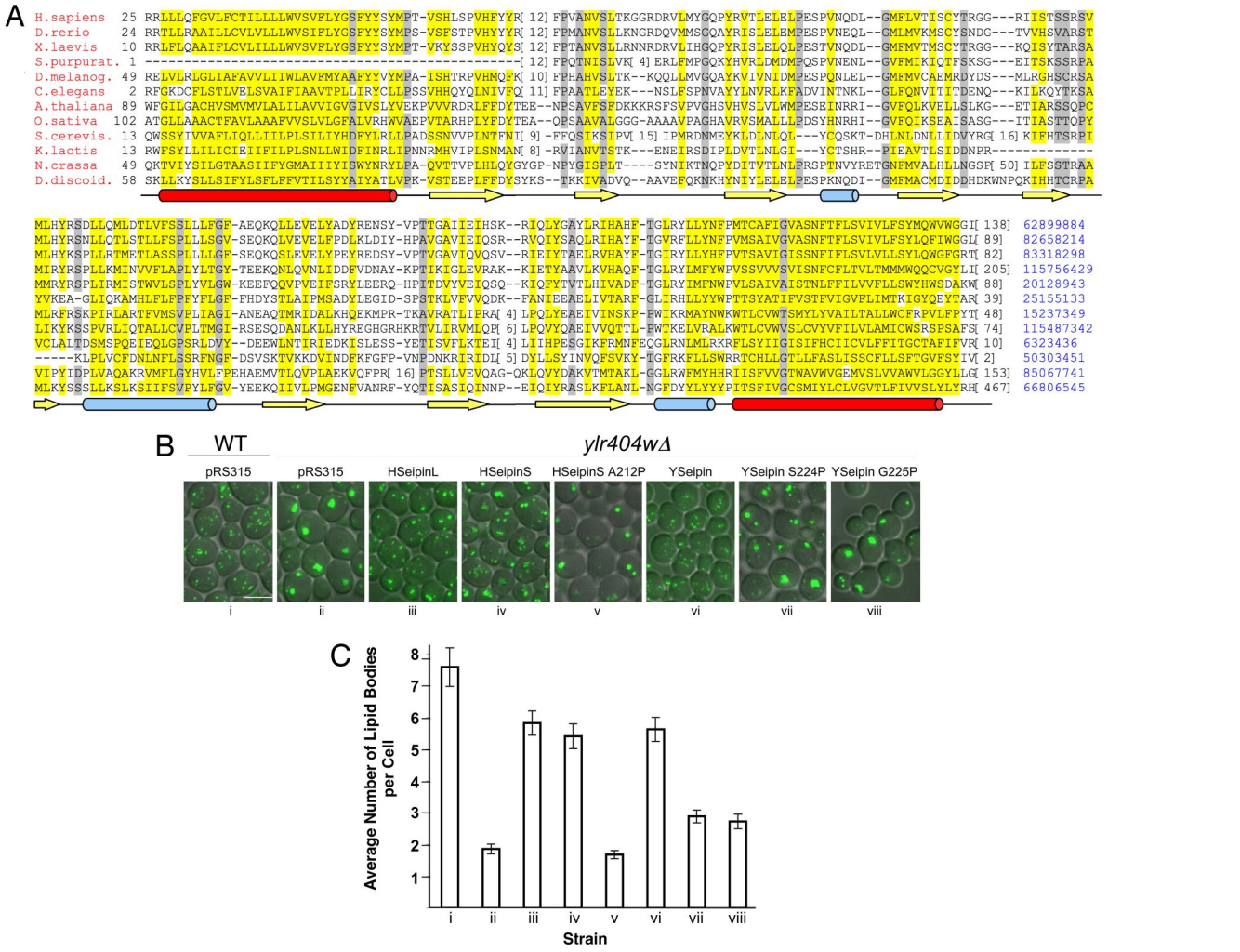


**Fig. 2.** *YLR404w* is yeast *BSCL2*/seipin. (*A*) Alignment of seipins by using PROMALS (19). NCBI gene identification numbers (gi numbers) are shown after the sequences. Regions are highlighted yellow to indicate hydrophobic patches and conserved small amino acids are highlighted gray. Predicted secondary structure (PSIPRED) (29) is shown below the sequences. Predicted transmembrane $\alpha$-helices are colored red. (*B* and *C*), Complementation of lipid droplet phenotype in *ylr404w*Δ by human and yeast seipins. Wild-type or the seipin-deleted strain was transformed with empty plasmid (pRS315) or plasmid-containing sequences encoding the long or short form of human seipin (HSeipinL or HSeipinS), A212P human seipin, yeast seipin (YSeipin), or yeast seipin with the mutations S224P or G225P. BODIPY-stained images are shown in *B* (scale bar, 5 $\mu$m); the histogram in *C* shows the number of lipid droplets per cell.

at least to the vicinity of lipid droplets, does not require seipin (Fig. 3*C*). To confirm the identity of ER membranes in the cluster, we expressed GFP fused to a secretion signal sequence and the four amino acid ER retention signal HDEL. Interestingly, >90% of lipid droplets in wild-type cells appeared in close proximity to the ER, suggesting that they may remain attached. In the mutant, a bright patch of labeled ER corresponded to the lipid droplet clusters (arrow), suggesting a proliferation of ER within this structure (Fig. 3*D*).

We studied a BSCL2 fibroblast line [taken from a patient with a nonsense mutation (23)] to see whether human cells also showed aberrant droplets. Compared with normal human fibroblasts, the seipin-deficient cells had many smaller lipid droplets, often not resolved from each other when stained either with Oil Red O or an antibody against the lipid droplet membrane protein ADRP (24) (Fig. 3*B*). Oleate caused accumulation of lipid droplets in normal fibroblasts and a more intense staining of the small lipid droplets in the mutant. Electron microscopy confirmed that the cytoplasm of the mutant cells was filled with many tiny clear organelles, which we assume to be lipid droplets, instead of the few distinct ones seen in normal cells (Fig. 3*E*).

To test the functional relationship between human and yeast seipin, human seipin was expressed in the *ylr404w*Δ deletion strain. This protein can be generated from two putative alternative translational start sites from mRNA of different lengths, yielding proteins of 398 and 462 aa (6, 9). Both forms complemented the yeast deletion strain and reversed the phenotype to the same extent as did the yeast protein with respect to morphology (Fig. 2*B*) and number of lipid droplets (Fig. 2*C*). In contrast, the missense mutation A212P in human seipin that causes lipodystrophy (7) failed to complement. Two analogous mutations in the yeast protein (S224P and G225P, each predicted by different algorithms to correspond to human A212P) only weakly complemented the strain. Thus, we conclude that *YLR404w* encodes yeast seipin and that the human and yeast proteins have similar functions in lipid droplet assembly or maintenance.

To gain information on the role of seipin in lipid droplet function, we determined its localization in yeast. When tagged at either the amino or carboxyl terminus with mCherry and expressed from the strong PGK promoter, the protein was clearly localized to the ER based on colocalization with GFP-HDEL (Fig. 4*A*). Human seipin also resides in the ER (10). However, the pattern of seipin in the ER was not uniform; patches of seipin

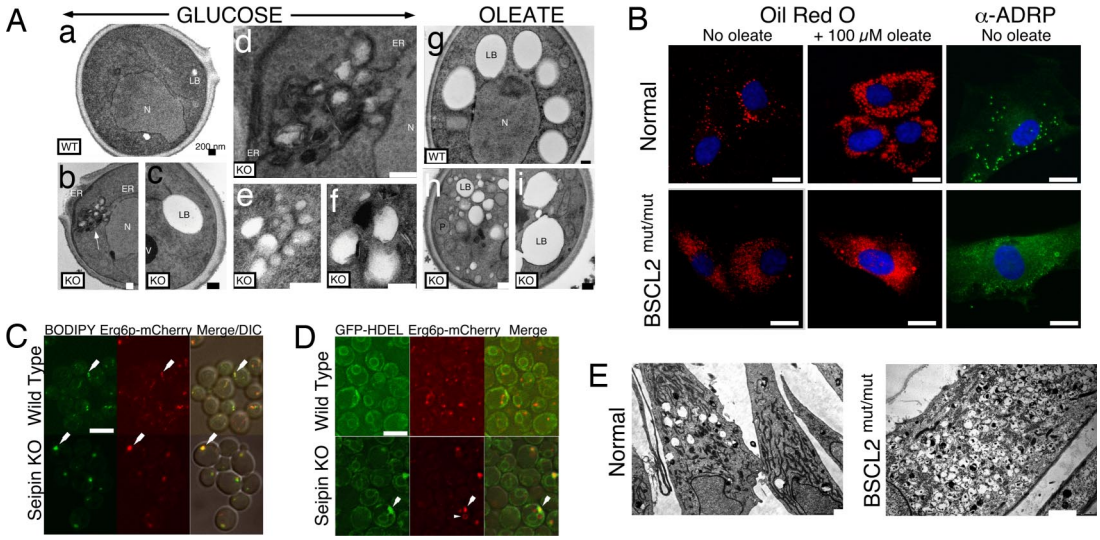


**Fig. 3.** Abnormal lipid droplets in seipin-deficient cells. (*A*) ultrastructure of wild-type or the seipin KO yeast strains. Cells were cultured in glucose or oleate as indicated. Lipid droplets are in clusters in the KO (arrow in *b*, and in higher resolution in *d–f*). In oleate medium, lipid droplets in wild type are of uniform size (*g*) but are irregular in size, shape, and number in the KO strain (*h* and *i*). (All scale bars, 200 nm.) ER, endoplasmic reticulum; LB, lipid droplet; N, nucleus; V, vacuole. (*B*) Lipid droplets are aberrant in seipin-deficient human fibroblasts. Cells were stained with Hoechst and either Oil Red O (with or without culturing with oleate) or antibodies against the lipid droplet marker protein ADRP (without oleate). (Scale bars, 10 $\mu$m.) (*C*) Erg6p is targeted to the aberrant clusters (arrows), suggesting normal protein targeting. The merge image also incorporates brightfield to outline the cells. (Scale bar, 5 $\mu$m.) (*D*) The ER is concentrated in the aberrant clusters (arrows). Arrowhead indicates a giant lipid droplet. Note that lipid droplets appear to be attached to ER in the wild-type strain. (Scale bar = 5 $\mu$m.) (*E*) Proliferation of small lipid droplets in seipin-deficient fibroblasts, grown without oleate. Note the presence of many small clear organelles in the mutant cells. (Scale bars, 2 $\mu$m.)

were observed with overexpressed protein. We looked in more detail at the colocalization of droplets and ER (Fig. 4*B*). When a series of *z*-sections through whole cells were examined, 92–97% of lipid droplets (values obtained from two observers) could not be resolved from the ER. These results suggest that lipid droplets remain tethered to the ER. When droplets, ER and seipin were simultaneously imaged by using BODIPY, CFP-HDEL, and chromosomally expressed seipin-mCherry (which was active and punctate), most lipid droplets colocalized or overlapped with seipin staining in the ER (a *z*-section is shown for each row in Fig.

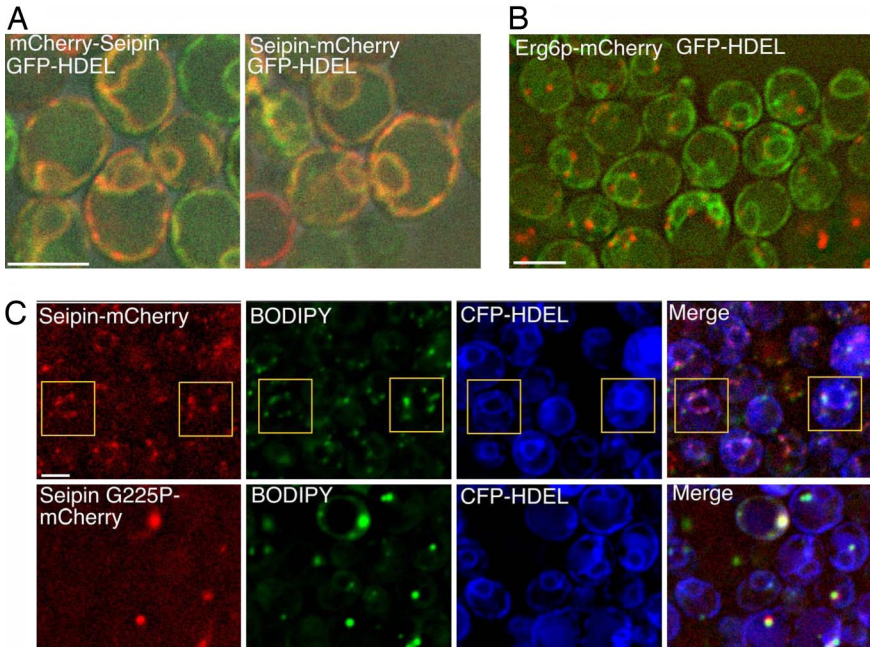


**Fig. 4.** Yeast seipin localizes in the ER and marks sites of contact with lipid droplets. (*A*) Seipin, tagged at either terminus with mCherry, localizes to the ER. Both seipin and GFP-HDEL were expressed on plasmids and driven by the *PGK* promoter. (*B*) Most lipid droplets appear bound to the ER. Droplets that were chromosomally tagged with Erg6p-mCherry also expressed the GFP-HDEL ER marker. (*C*) Seipin marks docking sites of lipid bodies. Seipin-mCherry, or inactive seipin-G225P-mCherry was chromosomally expressed at the seipin locus. CFP-HDEL, driven by the PGK promoter, was plasmid expressed. Lipid droplets were stained with BODIPY. Yellow boxes frame two cells showing clear seipin droplet proximity. Droplet clusters in cells expressing the mutant protein (null background) also colocalize seipin. (Scale bars, 5 $\mu$m.)

4*C*). Colocalization was even clearer with inactive seipin G225P, where lipid droplet clusters were intensely stained with seipin. These results strongly suggest that lipid droplets normally reside on the ER and that seipin is found at the junctions.

## Discussion

We report the identification of 59 genes that are important for normal lipid droplet morphology in yeast, which should help to elucidate pathways of droplet biogenesis and maintenance. Many disparate classes of protein and organellar functions apparently collaborate to maintain droplets. Some of these, for example transcription factors, would alter droplets simply by modifying basal metabolic rates leading to changes in droplet volume. The appearance of several genes that control vesicle transport through the central vacuolar system in the gene set, however, suggests a major role for these pathways. Although much work is required to understand the action of these genes in droplet function, the appearance of three lipodystrophy genes among the 59 validates the screen as a means to approach the study of these fascinating organelles.

The data presented here indicate that lipid droplets are aberrant in the absence of seipin, implicating this protein in droplet assembly and/or maintenance. Our finding that >90% of droplets are in close proximity to the ER and that seipin is in these apparent junctions suggest that seipin is involved in communication between these two organelles. For example, the ER may serve as a transient reservoir for excess phospholipids or droplet proteins during periods of rapid lipolysis during which the volume of the droplet core, and therefore the surface of the organelle, decreases. During periods of rapid fatty acid esterification, ER droplet junctions may provide a direct path for enlarging the droplet without a requirement for *de novo* biogenesis of more organelles. Although this concept appears not to be consistent with the proliferation of droplets in yeast and human fibroblasts in the absence of seipin, this phenotype could reflect a cellular response to a deficiency in droplet function rather than a direct effect of the absence of seipin itself. Determining the kinetics of the appearance of the phenotype vs. the disappearance of seipin expressed on a regulated promoter should address this issue. Although the precise role of seipin is not clear, the seipin-deficient phenotype in yeast, where the ER is often found wrapped around droplets, suggests that the protein is not required for binding of the two organelles to each other.

Our data strongly suggest that failure to form normal lipid droplets in adipocytes or adipocyte precursors is the primary cause of BSCL2 disease. Patients deficient in *BSCL2*/seipin lack essentially all adipose tissue even though *BSCL2* mutant fibroblasts can still produce lipid droplets, albeit aberrant ones. In contrast, patients develop fatty liver and muscles, which our data suggest is caused at least in part to a lack of seipin in these cells rather than high circulating triglyceride levels. Why is there no apparent adipose tissue in BSCL2 patients? It is possible that adipocytes are more dependent on seipin compared with other cells for generation of lipid droplets and that identifiable adipocytes fail to appear in the absence of seipin. Alternatively, formation of structurally and functionally normal lipid droplets may be required for adipocyte differentiation and, in the absence of seipin, preadipocytes either revert or are shunted to an apoptotic pathway. Such a mechanism would clarify the previous idea that seipin plays some role in differentiation of mesenchymal cells into adipocytes (6, 8). An answer will require a detailed study of adipocyte differentiation in *BSCL2*-deficient animals.

## Materials and Methods

**Yeast Strains and Screen.** For the initial screen, strains of the BY4742-based gene deletion library (Open Biosystems) were cultured overnight in yeast extract/peptone/dextrose (YPD) in 96-well plates, stained for 10 min with 1.25 $\mu$g/ml BODIPY493/503 (Invitrogen), and observed by fluorescence microscopy. At least five fields of cells from each strain were inspected. Strains with aberrant lipid droplets were recultured and checked at least two more times by using two lots of the library. Sixty-three strains were identified with altered lipid droplet morphology (by K.M.S.). For assignment into phenotypic groups, log phase and stationary phase cells were obtained by culturing four dilutions of each strain overnight in YPD. Log phase ($OD_{600}$ of 0.9–2.0) and stationary phase cells ($OD_{600}$ 8–12) of each culture were stained with BODIPY and imaged by obtaining projections of ≈20 *z*-sections through the cell (3 $\mu$m spacing between planes). Two fields, each with ≈30 cells, were obtained for each sample by the microscopy methods described in ref. 15. Cells were fixed with 1% glutaraldehyde and imaged immediately or kept at 4°C for up to several hours. The micrographs were coded, and three of us (K.M.S., D.B., and J.M.G.) collaborated to group strains with similar morphology, considering images from log and stationary cells independently. Four of the original 63 strains were indistinguishable from wild type in this secondary analysis and were not considered further. The *pah1*Δ strain (in the *W303* background) was a kind gift from Gil-Soo Han and George Carman (Rutgers University, New Brunswick, NJ).

**Yeast Complementation Studies.** Cells were transformed with pRS315 (25) that contained yeast seipin or the long or short forms of human seipin (IMAGE clones 3533654 and 3939021; Open Biosystems). Clone 3939021 contains a nine-base insertion in the coding region; its presence or absence did not alter complementation. Expression was driven by the yeast *PGK1* promoter (15), and cells were cultured in synthetic dextrose (2%) medium. Site-directed mutagenesis to generate missense mutations was performed by using *Pfu* polymerase (Strategene).

**Colocalization.** For studies with the lipid droplet protein marker Erg6p, mCherry (26) was fused to the carboxyl terminus in the chromosome. For an ER marker, we expressed a form of GFP-HDEL secreted into the ER lumen from pDN330, a kind gift from David Ng (Temasek Life Sciences), recloned into pRS315-PGK or pRS316-PGK (15) to drive expression from the *PGK* promoter. For some experiments enhanced cyan fluorescent protein (ECFP) (kindly provided by Scott Gibson, University of Texas Southwestern, as modified in ref. 27) was substituted for GFP in pDN330 before insertion of the HDEL-containing fragment into pRS315-PGK. For seipin localization studies, mCherry was fused to either the amino or carboxyl terminus of yeast seipin and expressed in pRS315-PGK.

**Electron Microscopy.** Yeast cells were grown in glucose or oleate medium and processed for electron microscopy as described in ref. 15. Human cells were processed as described in ref. 28.

**Fluorescence Microscopy of Human Fibroblasts.** Primary human fibroblasts were obtained from a patient with a *BSCL2* mutation (n659delGTATC; pF105fsx 111) (23) or from a normal healthy volunteer (written informed consent and approval by the institutional review board were obtained) and cultured in DMEM with 4.5 g/liter glucose and 10% FBS. For immunofluorescence, cells grown on coverslips were fixed with 4% formaldehyde in PBS. Cells were then either stained for neutral lipids with Oil Red O or permeabilized with 0.1% saponin and then stained with a monoclonal antidroplet to ADRP (Research Diagnostics). DNA was stained by using Hoechst 34580. Cells were observed with a Zeiss Axioplan 2E microscope using a Plan-Neofluar 40×/1.3 oil differential interference contrast objective.

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