Zinc mediates the SREBP-SCD axis to regulate lipid metabolism in Caenorhabditis elegans

Jing-Jing Zhang,† Jun-Jun Hao,§ Yu-Ru Zhang,** Yan-Li Wang,*, Ming-Yi Li,† Hui-Lai Miao,† Xiao-Ju Zou,†,‡‡ and Bin Liang*,†

Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences and Yunnan Province* and State Key Laboratory of Genetic Resources and Evolutionary and Functional Genomics,† Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, China; Department of Hepatobiliary Surgery,† Affiliated Hospital of Guangdong Medical University, Zhanjiang 524001, China; College of Fisheries,** Henan Normal University, Xinxiang, Henan 453007, China; and Department of Life Science and Biotechnology,† Key Laboratory of Special Biological Resource Development and Utilization of University in Yunnan Province, Kunming University, Kunming 650214, China

Abstract Maintenance of lipid homeostasis is crucial for cells in response to lipid requirements or surplus. The SREBP transcription factors play essential roles in regulating lipid metabolism and are associated with many metabolic diseases. However, SREBP regulation of lipid metabolism is still not completely understood. Here, we showed that reduction of SBP-1, the only homolog of SREBPs in Caenorhabditis elegans, surprisingly led to a high level of zinc. On the contrary, zinc reduction by mutation of sur-7, encoding a member of the cation diffusion facilitator (CDF) family, restored the fat accumulation and fatty acid profile of the sbp-1(ep79) mutant. Zinc reduction resulted in iron overload, which thereby directly activated the conversion activity of stearoyl-CoA desaturase (SCD), a main target of SREBP, to promote lipid biosynthesis and accumulation. However, zinc reduction reversedly repressed SBP-1 nuclear translocation and further downregulated the transcription expression of SCD for compensation. Collectively, we revealed zinc-mediated regulation of the SREBP-SCD axis in lipid metabolism, distinct from the negative regulation of SREBP-1 or SREBP-2 by phosphatidylcholine or cholesterol, respectively, thereby providing novel insights into the regulation of lipid homeostasis.—Zhang, J-J., J-J. Hao, Y-R. Zhang, Y-L. Wang, M-Y. Li, H-L. Miao, X-J. Zou, and B. Liang. Zinc mediates the SREBP-SCD axis to regulate lipid metabolism in Caenorhabditis elegans. J. Lipid Res. 2017, 58: 1845–1854.

Supplementary key words sterol regulatory element-binding protein • stearoyl-CoA desaturase • lipid homeostasis

Lipids, including fatty acids, triglycerides, and cholesterol, are important membrane components, signaling molecules, and energy reservoirs. To grow, proliferate, and survive, the cell must evolve certain mechanisms to quickly respond to lipid requirements or surplus. The transcription factors, SREBPs, play essential roles to regulate the biosynthesis of fatty acids, triglycerides, and cholesterol to meet the needs of the cell (1–5). In mammals, SREBPs are encoded by two genes, SREBF-1 and SREBF-2. SREBF-1 is transcribed by alternative promoter usage into two isoforms, SREBF-1a and SREBF-1c, of which SREBF-1c mainly regulates fatty acid metabolism (2). SREBF-2 regulates the transcriptional expression of genes mostly participating in cholesterol metabolism (1, 2, 6). Reciprocally, SREBF-1 is also negatively regulated by phosphatidylcholine (PC) (7) and cholesterol negatively regulates the processing of SREBF-2 (6).

The expression and activity of SREBPs have been implicated in metabolic syndromes (8). SREBF-1c has been implicated in type 2 diabetes mellitus, insulin resistance in skeletal muscle, and the pathogenesis of β-cell dysfunction (9–11). Hepatic SREBF-1c levels are increased in animal models of insulin resistance (12, 13). Sequence variations at the SREBF1 locus were linked to type 2 diabetes mellitus (14–16) and increased expression levels of SREBPs and genetic polymorphisms have shown associations with CVDs (17, 18). Meanwhile, many rodent models generated by either overexpression or knockout of SREBPs clearly indicate that dysregulation of SREBPs results in dyslipidemia: aberrant lipid metabolism in the liver, adipose

Abbreviations: BP, 2,2′-dipyridyl; CDF, cation diffusion facilitator; PC, phosphatidylcholine; QPCR, quantitative real-time PCR; RNAi, RNA interference; SCD, stearoyl-CoA desaturase; TAG, triacylglycerol; TPN, N,N,N′,N′-tetrakis (2-pyridylmethyl) ethylenediamine; WGS, whole genome sequencing.

†To whom correspondence should be addressed.
e-mail: xiaojuzou@163.com (X-J.Z.); liangb@mail.kiz.ac.cn (B.L.)
The online version of this article (available at http://www.jlr.org) contains a supplement.
tissue, pancreas, and other organs and tissues (19–26). Therefore, the biological functions of SREBPs must be tightly regulated to maintain lipid homeostasis. In spite of the above established regulatory circuits of SREBP-2 by cholesterol (6), SREBP-1 by PC (7), and Drosophila (d)SREBP by phosphatidylethanolamine (27), it is still arguable that other unknown factors may participate in the SREBPs’ regulation of lipid metabolism. The activities and functions of SREBPs in lipid metabolism are highly evolutionarily conserved across metazoans. The model organism, Caenorhabditis elegans, contains only one SREBP family member encoded by shp-1. Similar to its mammalian homologs, SREBPs also regulate fatty acid (28–31), PC (7), and cholesterol metabolism (32, 33). Depletion of shp-1 by RNA interference (RNAi) knockdown (29, 32, 34) or deletion (28) leads to altered fatty acid profiles and lowered fat stores. Through a forward genetic screen of suppressors of the shp-1(ep79) mutant, we identified that mutations in sur-7, encoding a member of the cation diffusion facilitator (CDF) family, could restore the altered lipid profiles of the shp-1(ep79) mutant. Furthermore, we revealed a distinct zinc-mediated SREBP-stearoyl-CoA desaturase (SCD) regulatory circuit to maintain lipid homeostasis.

MATERIALS AND METHODS

Strains and culture conditions

C. elegans strains were maintained on NGM plates with Esche-nichia coli OP50 under standard culture conditions, unless otherwise specified. The WT strain was N2. CB4856 is a WT isolated from Hawaii used for SNP mapping. The organisms and strains used in this study are shown in supplemental Table S1.

RNAi

RNAi was performed by feeding bacterial strains from the Ahringer C. elegans RNAi library, as we described previously (35). Young adult animals were harvested for further analysis.

Genome screening and isolation of shp-1 suppressors

Genome screening of shp-1(ep79) suppressors was carried out following a previously described method (36, 37). Briefly, L4 shp-1(ep79) worms were treated with ethyl methanesulfonate. Individual F2 worms that displayed morphologies of growth, body size, and color obviously close to the WT N2 were singled out and continuously cultured to the F3 generation. Then, F3 worms were harvested for analysis of fat accumulation by Nile red staining of fixed cells and analysis of fatty acid composition by GC, as well as quantification of the growth rate, to confirm the real suppressors of the shp-1(ep79) mutant.

Mutation mapping by SNP markers and whole genome sequencing

Mutation mapping of shp-1(ep79) suppressors (kun82 and kun84) was carried out using SNP-SNP, as previously described (38, 39). Whole genome sequencing (WGS) of kun82;shp-1(ep79) and kun84;shp-1(ep79) mutants was performed using the method of Sarin et al. (40). Briefly, the genomic DNA of kun82;shp-1(ep79) and kun84;shp-1(ep79) worms was isolated and subjected to WGS on the Illumina HiSeq2000 platform. The sequence data were mapped to the sequence of the WT N2 reference genome using ELAND via the BWA and SAMtools.

Nile red staining of fixed worms and quantification of lipid droplet size

Nile red staining of fixed worms was performed as previously described (28, 41). The images were captured using identical settings. At least 20 worms were visualized. The quantification of lipid droplet size was as previously described (42). Approximately six to eight worms were measured for each worm strain or treatment.

Analysis of fatty acid composition and triacylglycerol

C. elegans lipid extraction, the separation of triacylglycerol (TAG) and phospholipids by TLC, and the determination of fatty acids by GC were performed as previously described (42). Fatty acid compositions were analyzed with an Agilent 7890A gas chromatograph equipped with a 15 m × 0.25 mm × 0.25 μm DB-WAX column.

Analysis of quantitative real-time PCR and semi-quantitative PCR

Total RNA and cDNA preparation, quantitative real-time PCR (QPCR), and semi-QPCR were performed as previously described (43). For semi-quantitative PCR, we used 25 PCR cycles for shp-1. For QPCR, the relative abundance was determined using the ΔΔCt method and the reference gene, act-5, was used as a control for template levels.

Supplementation or sequestration of zinc

ZnSO4 supplementation and zinc reduction by N,N,N,N-tetraakis (2-pyridylmethyl) ethylenediamine (TPEN) were analyzed as described previously (44, 45). In brief, ZnSO4 and TPEN were added to NGM plates to a final concentration of 50 and 5 μM, respectively. Later, synchronized L1 worms were placed and cultivated on NGM plates supplied with either ZnSO4 or TPEN. L4 worms or young adults were harvested for analysis.

Zinpyr-1 staining and visualization

Zinpyr-1 staining was performed as described previously (46). The fluorescence of Zinpyr-1 was visualized under an OLYMPUS BX53 fluorescence microscope (Olympus, Japan). Images were captured using identical settings and exposure times, unless specifically noted. The fluorescence intensity was quantified using Photoshop software.

Visualization of GFP fluorescence

At least 20 GFP worms were picked and mounted on an agarose pad and anesthetized using 10 mM sodium azide. GFP fluorescence was visualized under an OLYMPUS BX53 fluorescence microscope (Olympus, Japan). GFP reporter expression was quantified using Photoshop software. At least six worms were used for each biological sample.

Statistical analysis

The data were presented as the mean ± SEM, except when specifically indicated. Statistical analyses included the t-test or ANOVA. All figures were generated using GraphPad Prism 6 (GraphPad Software, La Jolla, CA).

RESULTS

Whole genome screen of genetic suppressors of shp-1(ep79) mutant

Previously, we reported a reduction-of-function allele, ep79, of shp-1 encoding a homolog of SREBPs, which was created by the excision of a Tc1 element (supplemental
The snb-1(ep79) mutant displayed decreased fat accumulation with reduced lipid droplet size compared to WT N2, as quantified by TLC/GC and indicated by our established Nile Red staining of fixed worms (supplemental Fig. S2A–C) (28, 41). There was a different fatty acid profile of the snb-1(ep79) mutant, especially the levels of C18:0 and C18:1(n-9) (supplemental Fig. S2D), which led to the reduced conversion activity of SCD as indicated by the desaturation index C18:1(n-9)/C18:0 (supplemental Fig. S2E) (47). Moreover, the growth rate of snb-1(ep79) was delayed (supplemental Fig. S2F). To identify genes or signaling pathways that might bypass SBP-1 to regulate lipid metabolism, we performed a forward genetic screen for suppressors of the snb-1(ep79) mutant. We treated the metabolism, we performed a forward genetic screen for pathways that might bypass SBP-1 to regulate lipid delayed (supplemental Fig. S2F). To identify genes or signal-

Zinc regulates lipid homeostasis via SREBP-SCD
ZnSO₄ treatment apparently reduced the fat accumulation and lipid droplet size in strains with a sur-7 mutant background, including sur-7(tm6523), sur-7(tm6523); sbp-1(ep79), sur-7(kun84), and sur-7(kun84); sbp-1(ep79) mutants (Fig. 3F, G). On the contrary, reduction of zinc by TPEN treatment remarkably exacerbated the fat accumulation and increased lipid droplet size in all tested strains (Fig. 3F, G). In brief, these data explicitly supported that zinc negatively affected fat accumulation, and the restored fat accumulation of the sbp-1(ep79) mutant was due to the reduction in zinc levels.

Zinc negatively regulates the activity of SCD, a main target of SREBP

SCD introduces the first double bond and converts saturated fatty acids (C16:0 and C18:0) to MUFAs [C16:1(n-7) and C18:1(n-9)], which are the primary substrates for the biosynthesis of TAGs, phospholipids, and cholesterol esters (53). SCD is a main target of SREBP, which transcriptionally regulates SCD expression from C. elegans to mammals (28, 53, 54). Although the expression of sbp-1 was not affected in sur-7(tm6523); sbp-1(ep79) and sur-7(kun84); sbp-1(ep79) mutants, as in the sbp-1(ep79) mutant alone...
Zinc regulates lipid homeostasis via SREBP-SCD, the level of C18:1(n-9), which is the de novo synthesized fatty acid by SCD in *C. elegans*, and the conversion activity of SCD were significantly elevated in *sur-7; sbp-1* double mutants compared with the *sbp-1* mutant (Fig. 2G), implying a mechanism independent of SBP-1 transcription to regulate SCD conversion activity. In fact, dietary ZnSO₄ slightly, but significantly, decreased both the level of C18:1(n-9) and the conversion activity of SCD (Fig. 4A, B) in the *sur-7* mutant background. In contrast, zinc reduction by TPEN treatment dramatically increased the C18:1(n-9) level and the conversion activity of SCD in all tested strains except the *fat-6(tm331); fat-7(wa37)* mutants (Fig. 4A, B), which lack SCD enzymes for the conversion of C18:0 to C18:1(n-9) in *C. elegans*. Moreover, TPEN treatment obviously reduced the GFP expression of GFP::SBP-1 (*KQ377*) in the nucleus compared with the control (Fig. 4F), whereas it did not affect the mRNA expression of *sbp-1* (Fig. 4G). Therefore, zinc reduction

(supplemental Fig. S6), the level of C18:1(n-9) (Fig. 2F), which is the de novo synthesized fatty acid by SCD in *C. elegans*, and the conversion activity of SCD were significantly elevated in *sur-7; sbp-1* double mutants compared with the *sbp-1* mutant (Fig. 2G), implying a mechanism independent of SBP-1 transcription to regulate SCD conversion activity. In fact, dietary ZnSO₄ slightly, but significantly, decreased both the level of C18:1(n-9) and the conversion activity of SCD (Fig. 4A, B) in the *sur-7* mutant background. In contrast, zinc reduction by TPEN treatment dramatically increased the C18:1(n-9) level and the conversion activity of SCD in all tested strains except the *fat-6(tm331); fat-7(wa37)* mutants (Fig. 4A, B), which lack SCD enzymes for the conversion of C18:0 to C18:1(n-9) in *C. elegans*. Moreover, TPEN treatment obviously reduced the GFP expression of GFP::SBP-1 (*KQ377*) in the nucleus compared with the control (Fig. 4F), whereas it did not affect the mRNA expression of *sbp-1* (Fig. 4G). Therefore, zinc reduction

To examine whether the increased level of C18:1(n-9) and SCD conversion activity by TPEN treatment was due to upregulated expression of *scds*, we investigated the mRNA and GFP expression of *fat-5, fat-6*, and *fat-7*, encoding three separate SCDs, in *C. elegans*. Interestingly, the GFP expression of all three SCDs and the mRNA expression of *sbp-1* were significantly reduced in TPEN-treated worms compared with the control worms, while ZnSO₄ treatment slightly, but significantly, increased the fluorescence intensity of FAT-6::GFP and FAT-7::GFP (Fig. 4C–E). Moreover, TPEN treatment obviously reduced the GFP expression of GFP::SBP-1 (*KQ377*) in the nucleus compared with the control (Fig. 4F), whereas it did not affect the mRNA expression of *sbp-1* (Fig. 4G). Therefore, zinc reduction
might repress the nuclear translocation of SBP-1 and then downregulate the transcriptional and translational expression of scls. However, these results were contradicted by the increased level of C18:1(n-9) and the conversion activity of SCD (Fig. 4A, B).

In spite of that, RNAi knockdown of fat-6, which had a stronger RNAi effect than fat-7 RNAi to some degree, apparently decreased fat accumulation with decreased lipid droplet size in all test strains (Fig. 4H, J), even under TPEN treatment (Fig. 4I, K). In addition, although the fat-6(tm331);fat-7(wa37) double mutants were similar to the sbp-1(ep79) mutant in terms of fat accumulation, they were partially resistant to TPEN-induced fat accumulation (Fig. 4L, M). Altogether, these results suggest that zinc reduction-induced fat accumulation probably depends on the SCD function, in which its conversion activity was elevated, but its transcriptional and translational expression was repressed inversely.

Zinc antagonizes iron to determine SCD conversion activity and fat accumulation

To explore genes responding to zinc reduction, we performed RNA-seq analysis in worms treated with 2.5 and 5 μM TPEN. Transcriptome profiles revealed that the expression of 78 genes was responsive to TPEN in a concentration-dependent manner (Fig. 5A, B; supplemental Table S2). Consistent with our previous QPCR results (Fig. 4E), the expression of fat-5 and fat-7 was also downregulated in the RNA-seq data (Fig. 5B). Surprisingly, we found that the expression of ftm-1 and smf-3, encoding ferritin and divalent metal-ion transporter 1 (DMT1), respectively, was reversely responsive to zinc concentration. Previous reports, including ours, show that the expression of the ftm-1 gene and protein are induced; in contrast, the expression of smf-3 is suppressed to reduce iron uptake under iron overload (56–58). QPCR confirmed that the mRNA expression of ftm-1 and smf-3 indeed displayed an opposite pattern in
Zinc regulates lipid homeostasis via SREBP-SCD response to zinc concentrations (Fig. 5C, D). Consistently, TPEN treatment dramatically increased the fluorescent expression of FTN-1::GFP, while dietary ZnSO₄ had a negative effect (Fig. 5E). Thus, zinc had an antagonizing role to affect iron homeostasis.

SCDs are iron-containing enzymes that require iron for their activity (53, 59). Because zinc reduction increased FTN-1 expression and SCD conversion activity, we hypothesized that the elevated conversion activity of SCD by zinc reduction was probably due to iron overload. The iron chelator, 2,2'-dipyridyl (BP), was used to reduce the iron level (60). Indeed, the level of C18:0 was increased and the conversion activity of SCD was decreased in a BP concentration-dependent manner (Fig. 5F, G). However, TPEN treatment significantly increased the level of C18:1(n-9) and the conversion activity of SCD, even under BP treatment (Fig. 5F, G). Although either TPEN or BP treatment apparently reduced the growth rate of worms, TPEN treatment obviously improved the slow growth rate of worms treated with BP (Fig. 5H). In addition, compared with the control, TPEN treatment led to high fat accumulation with increased lipid droplet size, while BP treatment had the opposite effect in worms (Fig. 5I, J). Moreover, TPEN treatment restored the low level of fat accumulation in...
BP-treated worms (Fig. 5L, J). Altogether, these lines of evidence consistently suggest that iron competes with zinc to determine the SCD conversion activity and fat accumulation, and zinc reduction-induced fat accumulation depends on the iron-activated SCD conversion activity.

**DISCUSSION**

The evolutionarily conserved transcription factors, SREBPs, play central roles to transcriptionally regulate genes involved in fatty acid, triglyceride, and cholesterol metabolism, from *C. elegans* to mammals. To our great surprise, we found that the *sbp-1(ep79)* mutant displayed an elevated zinc level, as indicated by Zinpyr-1 fluorescence and *mtl-1* expression (Fig. 3A–D). In contrast, zinc reduction repressed the nuclear translocation of GFP::SBP-1 (Fig. 4F), consequently downregulating the transcriptional expression of the *scd* genes, *fat-5*, *fat-6*, and *fat-7* (Fig. 4C–E). Furthermore, zinc was sufficient to directly regulate SCD function and lipid homeostasis, independent of SREBP function (Fig. 6). Thus, we uncovered a distinct zinc-mediated regulation of SREBP-SCD in lipid metabolism, in which SREBP repressed the zinc level to upregulate SCD conversion activity, while, reciprocally, zinc affected the transactivation of SREBP on *scd* genes (Fig. 6), although the details need to be further characterized.

SCD1 catalysis is dependent on its di-iron center (53, 61). Interestingly, the recent crystal structure of mouse SCD1 showed that zinc, instead of iron, was the predominant ion in the structure (62). We found that zinc reduction led to iron overload, as well as increased SCD conversion activity and fat accumulation, consistent with a recent report that zinc deficiency promotes insulin resistance by exacerbating iron overload in the liver and induces hepatic steatosis in patients (63). On the other hand,
the reduction of iron by BP indeed decreased the SCD conversion activity and fat accumulation, whereas it was absolutely reversed by zinc reduction (Fig. 5F–J). Thus, our results, together with those of others, convincingly revealed an evolutionarily antagonistic role between zinc and iron to determine SCD conversion activity and fat accumulation from *C. elegans* to humans.

Zinc has been reported to play an important role in lipid metabolism. The zinc content was significantly lower in patients with alcoholic liver disease (64). In contrast, zinc supplementation significantly reduced total cholesterol, LDL cholesterol, and triglycerides in a meta-analysis of 24 studies on humans (65) and reversed alcoholic steatosis in mice (66). Here, we showed that zinc reduction promoted SCD conversion activity, but increased levels of zinc conversely impaired SCD conversion activity to positively or negatively affected fat accumulation in *C. elegans* (Fig. 6). Altogether, these results revealed an evolutionarily conserved role of zinc that negatively regulated fat accumulation, from *C. elegans* to humans. Reduction in iron or targeting SCD may hold potential promise for the treatment of zinc- or iron-related metabolic diseases.

Some strains were provided by the CGC, which is funded by National Institutes of Health Office of Research Infrastructure Program.

### REFERENCES

1. Goldstein, J. L., R. A. DeBose-Boyd, and M. S. Brown. 2006. Protein sensors for membrane sterols. *Cell*. **124**:35–46.

2. Horton, J. D., J. L. Goldstein, and M. S. Brown. 2002. SREBP-1c activation of SCD is associated with type 2 diabetes in French obese and diabetic cohorts. *Diabetes*. **51**:1575–1584.

3. Bi, Y., W. Wu, J. Shi, H. Liang, W. Yin, Y. Chen, S. Tang, S. Cao, M. Cai, S. Shen, et al. 2014. Role for sterol regulatory element binding protein-1c activation in mediating skeletal muscle insulin resistance via repression of rat insulin receptor substrate-1 transcription. *Diabetologia*. **57**:592–602.

4. Wang, H., P. Maechler, P. A. Antinozzi, L. Herrero, K. A. Hagenfeldt-Johansson, A. Bjorkholm, and C. B. Wollheim. 2003. The transcription factor SREBP-1c is instrumental in the development of beta-cell dysfunction. *J. Biol. Chem.* **278**:16622–16629.

5. Vögös, B. 2005. New insight into the pathophysiology of lipid abnormalities in type 2 diabetes. *Diabetes Metab.* **31**:429–439.

6. Shimomura, I., Y. Bashmakov, and J. D. Horton. 1999. Increased levels of nuclear SREBP-1c-associated fatty livres in two mouse models of diabetes mellitus. *J. Biol. Chem.* **274**:30928–30932.

7. Kakuma, T. Y., Lee, M. Higa, Z. Wang, W. Pan, I. Shimomura, and R. H. Unger. 2000. Leptin, troglitazone, and the expression of sterol regulatory element binding protein proteins in liver and pancreatic islets. *Proc. Natl. Acad. Sci. U.S.A.* **97**:8530–8534.

8. Laudes, M., I. Barroso, J. Luan, M. A. Soos, G. Yeo, A. Meirhaeghe, L. Logie, A. Vidal-Puig, A. F. Schaefer, N. J. Wareham, et al. 2004. Genetic variants in human sterol regulatory element binding protein-1c in syndromes of severe insulin resistance and type 2 diabetes. *Diabetes*. **53**:842–846.

9. Eberle, D., K. Clément, D. Meyre, M. Sahbatou, M. Vaxillaire, A. Le Gall, P. Ferré, A. Basdevant, P. Frooguel, and F. Foufelle. 2004. SREBP-1 gene polymorphisms are associated with obesity and type 2 diabetes in French obese and diabetic cohorts. *Diabetes*. **53**:2153–2157.

10. Felder, T. K., H. Oberkoffler, R. Weitgasser, V. Mackevics, F. Krempler, B. Paulweber, and W. Patsch. 2007. The SREBP-1 locus is associated with type 2 diabetes and plasma adiponectin levels in a middle-aged Austrian population. *Int. J. Obes. (Lond)* **31**:1099–1103.

11. Laaksonen, R., K. M. Thelen, H. Paiva, J. Matinheikki, R. Vesalainen, T. Janatuinen, J. Knutti, R. Rontu, K. von Bergmann, D. Luijohann, et al. 2006. Genetic variant of the SREBF-1 gene is significantly related to cholesterol synthesis in man. *Atherosclerosis*. **185**:206–209.

12. Salek, L., S. Latucuta, C. M. Ballantine, A. M. Gotto, Jr., and A. J. Marián. 2002. Effects of SREBP-1a and SREBP-2 polymorphisms on plasma levels of lipids, severity, progression and regression of coronary atherosclerosis and response to therapy with fluvastatin. *J. Mol. Med.* **80**:737–744.

13. Shimanò, H., J. D. Horton, R. E. Hammer, I. Shimomura, M. S. Brown, and J. L. Goldstein. 1996. Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a. *J. Clin. Invest.* **98**:1575–1584.

14. Shimano, H., J. D. Horton, I. Shimomura, R. E. Hammer, M. S. Brown, and J. L. Goldstein. 1997. Isoform 1c of sterol regulatory element binding protein-1c is less active than isofor 1a in livers of transgenic mice and in cultured cells. *J. Clin. Invest.* **99**:846–854.

15. Shimano, H., I. Shimomura, R. E. Hammer, J. Herz, J. L. Goldstein, M. S. Brown, and J. D. Horton. 1997. Elevated levels of SREBP-2 and cholesterol synthesis in livers of mice homozygous for a targeted disruption of the SREBP-1 gene. *J. Clin. Invest.* **100**:2115–2124.

16. Ishikawa, M., Y. Iwashita, S. Yato, T. Kato, S. Kumadaki, N. Inoue, T. Yamamoto, T. Matsuzaka, Y. Nakagawa, N. Yahagi, et al. 2008. Cholesterol accumulation and diabetes in pancreatic beta-cell-specific SREBP-2 transgenic mice: a new model for lipotoxicity. *J. Lipid Res.* **49**:2524–2534.

17. Knebel, B., J. Haas, S. Hartwig, S. Jacob, C. Kollmer, U. Nitzgen, D. Muller-Wieland, and J. Kotzka, 2012. Liver-specific expression of
transcriptionally active SREBP-1c is associated with fatty liver and increased visceral fat mass. *PLOS One.* **7**: e31812.

24. Takahashi, A., K. Motomura, T. Kato, T. Yoshikawa, Y. Nakagawa, N. Yahagi, H. Sone, H. Suzuki, H. Tovoshiba, N. Yamaeda, et al. 2005. Transgenic mice overexpressing nuclear SREBP-1c in pancreas are diabetic. *Cell.* **121**: 897–909.

25. Horton, J. D., N. A. Shah, J. A. Warrington, N. N. Anderson, S. W. Park, M. S. Brown, and J. L. Goldstein. 2005. Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. *Proc. Natl. Acad. Sci. USA.* **102**: 12027–12032.

26. Horton, J. D., I. Shimomura, S. Ikemoto, Y. Bashmakov, and R. E. Hammer. 2009. Overexpression of sterol regulatory element-binding protein-1a in mouse adipose tissue produces adipocyte hypertrophy, increased fatty acid secretion, and fatty liver. *J. Biol. Chem.* **284**: 36562–36566.

27. Dobrosotskaya, I. Y., A. C. Seegmiller, M. S. Brown, J. L. Goldstein, and R. B. Rawson. 2002. Regulation of SREBP processing and membrane lipid production by phospholipids in *Drosophila.* *Science.* **296**: 879–883.

28. Liang, B., K. Ferguson, L. Kadyk, and J. L. Watts. 2010. The role of nuclear receptor NRH-64 in fat storage regulation in *Caenorhabditis elegans.* *PLOS One.* **5**: e9869.

29. McKay, R. M., P. McKay, L. Avery, and J. M. Graff. 2003. *C. elegans* a parsimonious model for exploring the genetics of adipose growth. *Dev. Cell.* **4**: 131–142.

30. Kniazeva, M., T. Euler, and M. Han. 2008. A branched-chain fatty acid is involved in post-embryonic growth control in parallel to the insulin receptor pathway and its biosynthesis is feedback-regulated in *C. elegans.* *Genes Dev.* **22**: 2102–2110.

31. Nomura, T., M. Horikawa, S. Shimamura, T. Hashimoto, and K. Sakamoto. 2010. Fat accumulation in *Caenorhabditis elegans* is mediated by SREBP homolog SBP-1. *Genes Nutr.* **5**: 58, 2017.

32. Yang, F., B. W. Vought, J. S. Satterlee, A. K. Walker, Z. Y. Jim Sun, O. Boss, M. L. Hirsch, S. Ribich, J. J. Smith, et al. 2010. Conserved role of SIRT1 orthologs in fasting-dependent inhibition of the lipid/cholesterol regulator SREBP. *Genes Dev.* **24**: 1403–1417.

33. Kniazeva, M., Q. T. Crawford, M. Seiber, C. Y. Wang, and M. Han. 2004. Monomethyl branched-chain fatty acids play an essential role in *Caenorhabditis elegans* development. *PLOS Biol.* **2**: E257.

34. Kniazeva, M., T. Euler, and M. Han. 2008. SMG-2 is a phosphorylated protein required for mRNA surveillance in *Caenorhabditis elegans.* *J. Biol. Chem.* **283**: 111–119.

35. Wicks, S. R., R. T. Yeh, W. R. Gish, R. H. Waterston, and R. H. Waterston. 2003. Overexpression of sterol regulatory element-binding protein-1a in mouse adipose tissue produces adipocyte hypertrophy, increased fatty acid secretion, and fatty liver. *J. Biol. Chem.* **288**: 266–274.

36. Jorgensen, E. M., and S. E. Mango. 2002. The art and design of genetic screens: *Caenorhabditis elegans.* *Nat. Methods.* **2**: 2102–2110.

37. Parkinson, M. S. Brown, and J. L. Goldstein. 2003. Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. *Proc. Natl. Acad. Sci. USA.* **102**: 12027–12032.

38. Horton, J. D., N. A. Shah, J. A. Warrington, N. N. Anderson, S. W. Park, M. S. Brown, and J. L. Goldstein. 2005. Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. *Proc. Natl. Acad. Sci. USA.* **102**: 12027–12032.

39. Parks, C. J., K. L. Guan, and M. Han. 2004. Modulation of KSR activity in *Caenorhabditis elegans* by Zn ions, PAR-1 kinase and FPA2 phosphatase. *EMBO J.* **23**: 111–119.

40. Davis, D. E., H. C. Roh, K. Deshmukh, J. J. Brunsma, D. L. Schneider, J. Guthrie, J. D. Robertson, and K. Kornfeld. 2009. The cation diffusion facilitator gene cdfl mediates zinc metabolism in *Caenorhabditis elegans.* *Genetics.* **182**: 1013–1033.

41. Brooks, T. J., J. Browse, and J. L. Watts. 2007. Fatty acid desaturation and the regulation of adiposity in *Caenorhabditis elegans.* *Genetics.* **176**: 865–875.

42. Perez, C. L., and M. R. Van Gilst. 2008. A Zn isotope labeling strategy reveals the influence of zinc signaling on lipogenesis in *C. elegans.* *Cell Metab.* **8**: 260–271.

43. Gourley, B. L., S. B. Parker, B. J. Jones, K. B. Zumbrennen, and E. Leibold. 2003. Cytosolic aconitase and ferritin are regulated by iron in *Caenorhabditis elegans.* *J. Biol. Chem.* **278**: 2927–3234.

44. O’Reilly, A., N. H., J. Cho, O. J. Yoo, and J. Ahn. 2004. Transcriptional regulation and life-span modulation of cytosolic aconitase and ferritin genes in *C. elegans.* *J. Mol. Biol.* **342**: 421–433.

45. Wang, H., X. Jiang, J. Wu, L. Zhang, J. Huang, Y. Zhang, X. Zou, and B. Liang. 2013. Iron overload coordinately promotes ferritin expression and fat accumulation in *Caenorhabditis elegans.* *Genetics.* **203**: 241–253.

46. Kashiwabara, Y., H. Nakagawa, G. Matsuki, and R. Sato. 1975. Effect of metal ions in the culture medium on the stearoyl-coenzyme a 2-oxoacid oxidase activity of *Mycobacterium phlei*. *J. Biochem.* **78**: 805–810.

47. Romney, S. J., C. Thacker, and E. A. Leibold. 2008. An iron enhancer element in the *FTN-1* gene directs iron-dependent expression in *Caenorhabditis elegans* intestines. *J. Biol. Chem.* **283**: 716–725.

48. Behrouzian, B., and P. H. Buist. 2002. Fatty acid desaturation: variations on an oxidative theme. *Curr. Opin. Chem. Biol.* **6**: 577–582.

49. Bai, Y., J. G. McCoy, E. J. Levin, P. Sobrado, K. R. Rajashankar, B. G. Fox, and M. Zhou. 2015. X-ray structure of a mammalian stearoyl-CoA desaturase. *Nature.* **524**: 252–256.

50. Himoto, T., T. Nomura, J. Tani, H. Miyoshi, A. Morishita, H. Yoneyama, R. Haba, H. Masugata, and T. Masaki. 2015. Exacerbation of insulin resistance and hepatic steatosis deriving from zinc deficiency in patients with HCV-related chronic liver disease. *Biol. Trace Elem. Res.* **163**: 81–88.

51. Bode, B. C., P. Hanisch, H. Henning, W. Koenig, F. W. Richter, and C. Bode. 1988. Hepatic zinc content in patients with various stages of alcoholic liver disease and in patients with chronic active and chronic persistent hepatitis. *Hepatology.* **8**: 1605–1609.

52. Ranasinghe, P., W. S. Rathurapatha, M. H. Ishara, R. Jayawardana, P. Galappathth, P. Katulanda, and G. R. Constantine. 2015. Effects of zinc supplementation on serum lipids: a systematic review and meta-analysis. *Nutr. Metab. (Lond).* **12**: 26.

53. Kang, X., W. Zhong, J. Liu, Z. Song, C. J. McClain, Y. J. Kang, and Z. Zhou. 2009. Zinc supplementation reverses alcohol-induced steatosis in mice through reactivating hepatocyte nuclear factor-4alpha and peroxisome proliferator-activated receptor-alpha. *Hepatology.* **50**: 1241–1250.