REVIEW

OPEN

A new SPRING in lipid metabolism

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Purpose of review
The SREBP transcription factors are master regulators of lipid homeostasis owing to their role in controlling cholesterol and fatty acid metabolism. The core machinery required to promote their trafficking and proteolytic activation has been established close to 20 years ago. In this review, we summarize the current understanding of a newly identified regulator of SREBP signaling, SPRING (formerly C12ORF49), its proposed mechanism of action, and its role in lipid metabolism.

Recent findings
Using whole-genome functional genetic screens we, and others, have recently identified SPRING as a novel regulator of SREBP signaling. SPRING is a Golgi-resident single-pass transmembrane protein that is required for proteolytic activation of SREBPs in this compartment. Mechanistic studies identified regulation of S1P, the protease that cleaves SREBPs, and control of retrograde trafficking of the SREBP chaperone SCAP from the Golgi to the ER as processes requiring SPRING. Emerging studies suggest an important role for SPRING in regulating circulating and hepatic lipid levels in mice and potentially in humans.

Summary
Current studies support the notion that SPRING is a novel component of the core SREBP-activating machinery. Additional studies are warranted to elucidate its role in cellular and systemic lipid metabolism.

Keywords
C12ORF49, cholesterol metabolism, MBTPS1, posttranscriptional regulation, S1P, SPRING, sterol-responsive element binding protein

INTRODUCTION

Lipids are essential components of mammalian cells. They serve a multitude of functions, most notably as integral constituents of membranes and for storage and generation of energy. Owing to their pivotal role, lipids are implicated in a range of physiological conditions, and dysregulated lipid metabolism is implicated in the development of numerous diseases. It follows that tight regulation of lipid metabolism is essential.

The sterol-responsive element-binding proteins (SREBPs) are the master regulators of lipid metabolism. The SREBP family of transcription factors consist of three isoforms: SREBP1a, SREBP1c, and SREBP2 [1–3]. The two SREBP1 isoforms are transcribed from the same gene through differential promotor use that results in them having a different first exon [4], while SREBP2 is derived from a different gene. SREBP1a is mainly expressed in highly proliferative cells and can activate fatty acid synthesis as well as cholesterol synthesis. SREBP1c is predominantly active in the liver where it drives the nutrient-dependent transcription of genes involved in fatty acid synthesis, and SREBP2 specifically controls the expression of genes involved in cholesterol synthesis and uptake [5].

SREBPs are produced as precursor endoplasmic reticulum (ER)-resident membrane proteins. To attain their transcriptionally active forms, SREBPs undergo regulated trafficking and proteolytic steps (Fig. 1) [6]. Briefly, in the ER, SREBPs form a complex with SREBP cleavage-activating protein (SCAP) [7]. This complex is retained in the ER when the cholesterol content of the ER membrane exceeds 5% by

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Curr Opin Lipidol 2023, 34:000–000

DOI:10.1097/MOL.0000000000000894

KEY POINTS

- SPRING is a recently discovered component of the core SREBP-activating machinery.
- Loss of SPRING markedly attenuates SREBP signaling in cells.
- SPRING promotes the proteolytic maturation of S1P, the protease, which cleaves SREBP in the Golgi, and is also required for retrograde transport of SCAP from the Golgi to the ER.
- Mice lacking hepatic *Spring* have markedly reduced lipid levels in the liver and circulation.

virtue of SCAP binding to insulin-induced gene (INSIG) proteins. A drop in the cholesterol content of the ER membrane below 5% leads to a conformational change in SCAP that releases INSIG and exposes a MELADL motif that interacts with coat protein complex II (COPII) [8,9]. This promotes transport of the SCAP–SREBP complex to the Golgi, where SREBP is sequentially processed by two proteases. First, a luminal loop of SREBPs is cleaved by Site-1 protease (S1P), and in a second step, intramembrane cleavage by Site-2 protease (S2P) releases the N-terminal helix–loop–helix–leucin zipper domain of SREBP that translocates to the nucleus [10]. There, SREBPs bind to sterol regulatory element DNA sequences located in promotors of their target genes (Fig. 1). Non SCAP-dependent activation of

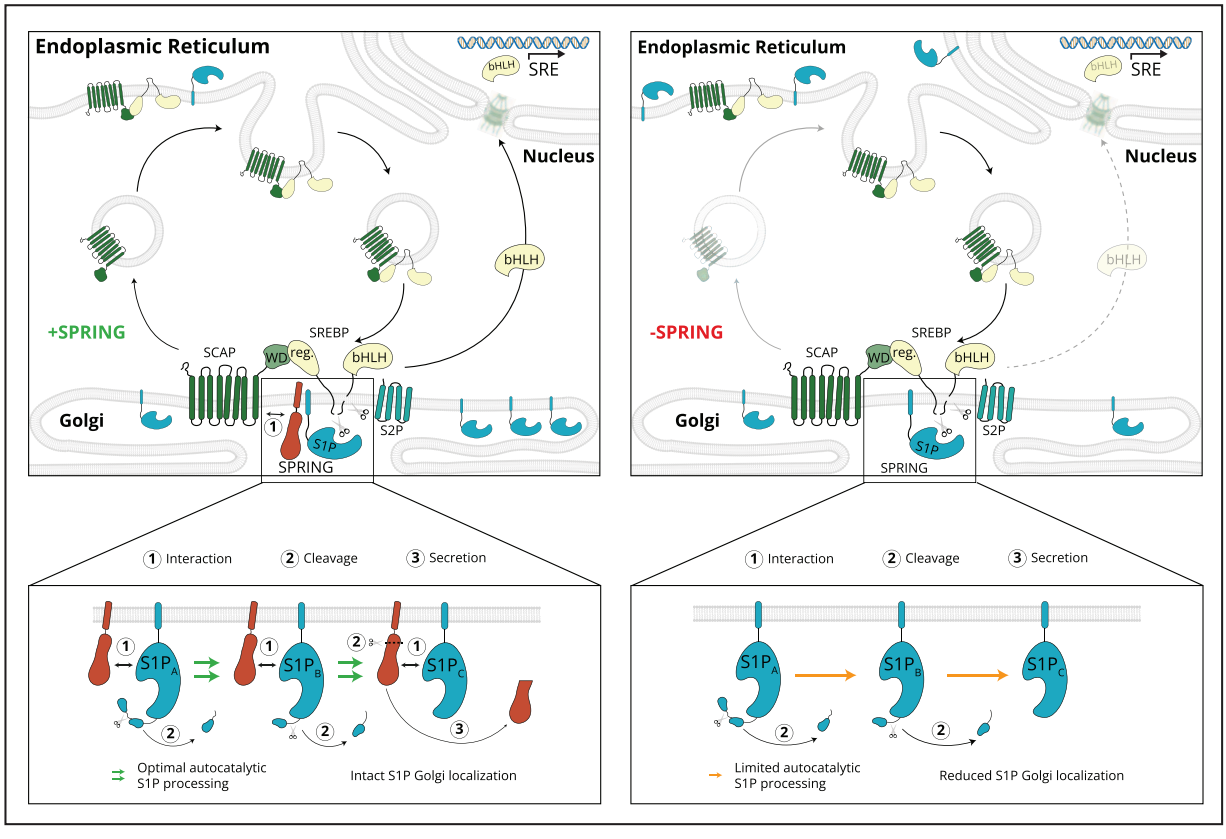

FIGURE 1. Simplified scheme of proteolytic activation of sterol-responsive element-binding proteins and the proposed role of SPRING. (Left) The sterol-responsive element-binding protein (SREBP) (yellow) and SREBP cleavage-activating protein (SCAP) (green) complex is retained in the endoplasmic reticulum (ER) when sufficient sterols are available. A drop in sterol levels results in trafficking of the complex to the Golgi where SREBPs are proteolytically cleaved by the sequential activity of S1P (blue) and S2P. This releases the transcriptionally active SREBP domain that enters the nucleus and activates expression of SRE-containing targets genes. SCAP undergoes retrograde trafficking to the ER following cleavage of SREBPs by S1P to enter a new round of cycling. SPRING has been proposed to: interact with both S1P and SCAP, and to be required for arrival of S1P to the Golgi and retrograde trafficking of SCAP, promote the autocatalytic proteolysis of S1P from the precursor $S1P_A$ to the mature $S1P_C$ form in the Golgi, and undergo S1P-mediated cleavage resulting in secretion of its ectodomain. (Right) In the absence of SPRING, delivery of S1P to the Golgi is reduced, as is the retrograde transport of SCAP to the ER. Furthermore, proteolytic maturation of S1P to its active form is markedly attenuated. Collectively, this results in decreased SREBP signaling in cells lacking *SPRING*.

SREBPs has also been recently proposed and attributed to caspase-2-mediated cleavage of ER-resident SREBPs in inflammatory states [11]. Whether this is a general or context-specific activation mechanism is unclear.

DISCOVERY OF SPRING (C12ORF49)

The core SREBP-activating machinery and process has been established in a series of elegant studies from the Brown and Goldstein lab in the early 2000s [6,7]. In recent years, genome-wide genetic screens have emerged as a powerful strategy to interrogate metabolic processes in a robust and unbiased manner. To interrogate SREBP signaling, we used an insertional mutagenesis strategy in the near haploid cell line Hap1 and conducted two independent screens [12▪▪]. Using Hap1 cells in which endogenous Squalene Epoxidase (SQLE) was tagged with the green fluorescent protein mNeon we identified regulators of SQLE, an SREBP2 target and a rate limiting-enzyme in de novo cholesterol biosynthesis. In parallel, we conducted a synthetic lethality screen in Hap1 cells deficient in Fatty Acid Synthase (*FASN*), an SREBP1-regulated gene and a key enzyme in de novo fatty acid synthesis. These screens identified the genes encoding the core SREBP activation machinery (*SCAP*, *MBTPS1*, *MBTPS2*, *SREBF2*). The only other shared denominator of these two screens was a hitherto uncharacterized positive regulator of SREBP signaling, *C12ORF49*, which we named SREBF Pathway Regulator In Golgi (*SPRING*) [12▪▪]. Soon thereafter, Aregger *et al.* [13▪▪] performed a genome-wide CRISPR/Cas9 screen in *FASN*-deficient Hap1 cells, similar to the ones used in our study, with the goal of identifying genetic interactions in the framework of defective de novo fatty acid synthesis. By scoring all combinations of double knockouts, a strong genetic interaction between *FASN* and *SPRING* was also identified. In parallel, using a computational approach, Bayraktar *et al.* generated a co-essentiality network using datasets from the DepMap project [14,15▪▪]. Their hypothesis was that perturbations of genes that are associated with the same metabolic pathway should present similar phenotypes across various cancer cell lines. This approach also uncovered a correlation between SPRING and genes regulated by the SREBP pathway [15▪▪]. Collectively, these distinct genetic approaches identified the previously uncharacterized gene *SPRING* and provided compelling evidence for *SPRING* being a novel regulator of SREBP function. Moving forward from the discovery of SPRING, in the next section we will take a closer look on what is currently known about its structure and mechanism of function.

CHARACTERIZATION OF SPRING

SPRING is evolutionary conserved in metazoa and is ubiquitously expressed, with expression being highest in the thyroid and lung [12▪▪,15▪▪]. In humans, *SPRING* is encoded by five exons, and four transcript variants are annotated (NM_024738.4; NM_001353623.2; NM_001353624.2: NM_001353625.2, respectively). Exons 1 and 5 are shared by all variants, whereas exons 2, 3 and 4 are subject to alternative splicing. Variant 2 (NM_001353623.2) contains a unique 5′ sequence, as its transcription is initiated at a different transcriptional start site. Only transcript variant 1 encodes full-length SPRING containing all five exons and biological relevance, if any, of the other variants remains is unknown. Three *SPRING* pseudogenes are also annotated. These are located on chromosome 10 (*SPRING1P1*), chromosome X (*SPRING1P2*) and chromosome 16 (*SPRING1P3*). Like the majority of pseudogenes, they likely originate from retro-transposition of processed mRNA. Whether pseudogenes in general are transcriptionally active and whether their biological relevance is understudied [16]. A study investigating the fate of pseudogenes predicts that *SPRING1P* and *SPRING1P2* are not transcribed and contained no information regarding *SPRING1P3* [17]. Intriguingly, a SNP present in the 3′ UTR of *SPRING1P3* (rs9937036) was reported to be associated with fasting serum alanine aminotransferase (ALT) levels in a Hispanic cohort of obese children [18]. The full-length transcript of *SPRING* encodes for a protein comprised of 205 amino acids with a calculated size of 23.6 kDa (Fig. 2). SPRING contains a signal peptide, followed by a single transmembrane domain and an uncharacterized conserved domain (DFU2054) that contains a cysteine-rich region with 14 highly conserved cysteine residues [12▪▪,13▪▪,14,15▪▪]. Furthermore, SPRING carries a single N-linked glycosylation (Asn67), and in cells is predominantly localized to the Golgi with its C-termini facing the lumen [12▪▪,13▪▪,14,15▪▪]. The structure of SPRING and the unique DFU2054 domain has not been reported to date.

THE FUNCTION OF SPRING IN THE STEROL-RESPONSIVE ELEMENT-BINDING PROTEIN PATHWAY

The identification of *SPRING* as a novel regulator of the SREBP pathway was rapidly followed by functional studies directed at elucidating the underlying molecular mechanism(s). These studies revealed that SPRING is critically required for SREBP signaling in cells [12▪▪,13▪▪,14,15▪▪,19▪▪]. Accordingly, loss of *SPRING* was associated with attenuated proteolytic processing of SREBPs into their mature

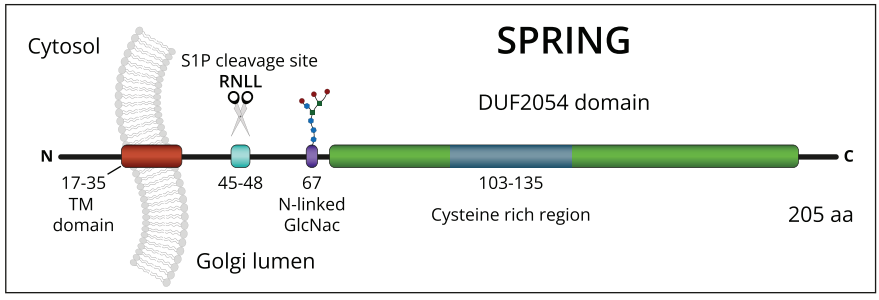

FIGURE 2. Schematic domain structure of SPRING. The domain structure of SPRING is schematically presented with the transmembrane and DUF2054 domains highlighted. Additionally, the single glycosylation site and the identified S1P cleavage site are marked.

form, decreased SREBP reporter signal, and lower basal-stimulated and sterol-stimulated expression of SREBP-regulated target genes. Functionally, these changes resulted in reduced LDLR-dependent LDL uptake, and attenuated cholesterol and fatty acid synthesis. Collectively, these studies position SPRING as an essential determinant of SREBP signaling in cells.

Two mechanisms have been suggested to explain how SPRING governs SREBP signaling (outlined in Fig. 1). First, using co-immunoprecipitation, we demonstrated that SPRING interacts with SCAP, and that in Hap1 cells devoid of SPRING, retrograde trafficking of SCAP from the ER is impaired [12▪▪]. As a consequence, these cells display functional 'SCAP deficiency' and limited SREBP activation. Consistent with this model, overexpression of SCAP rescued SREBP signaling in Hap1 cells lacking SPRING [12▪▪]. Yet this model cannot fully explain why SREBP processing is not rescued by collapsing the Golgi into the ER in Brefeldin A-treated SPRING-deficient cells [15▪▪,19▪▪]. In this setting, S1P would not be spatially separated from SREBPs, and cleavage would not require SCAP-dependent anterograde trafficking to the Golgi.

The second mechanism arose through the analysis of interaction proteomics and TurboID-mediated proximity labelling, which revealed that SPRING also interacts with S1P, the protease responsible for the first cleavage step of SREBPs in the Golgi [13▪▪,15▪▪,19▪▪]. S1P is a member of the proprotein convertase family and is made in the ER as an inactive precursor protein ($S1P_A$). In two sequential posttranslational processing steps, S1P autocatalytically cleaves itself to reach its mature and active form in the Golgi ($S1P_C$; Fig. 1) [20–22]. Mechanistic studies revealed that the interaction between SPRING and S1P stimulates the autocatalytic maturation of S1P [19▪▪,23▪]. Accordingly, loss of SPRING decreases maturation of S1P, whereas overexpression of SPRING results in more efficient processing of S1P to its mature form. Decreased trafficking of S1P to the Golgi has also been reported in the absence of SPRING [19▪▪,23▪]. As proper spatiotemporal activity of S1P is essential for the proteolytic activation of SREBPs, these findings provide a potential basis for how the absence of SPRING leads to dysfunctional SREBP activation and impaired cellular lipid metabolism. Intriguingly, while the two proposed mechanisms put forward to explain SPRING-dependent regulation of SREBP signaling, that is, retrograde SCAP trafficking and regulation of S1P function are seemingly unrelated, the two processes are not independent of each other. Genetic or pharmacologic inhibition of S1P function impairs retrograde trafficking of SCAP and results in SCAP degradation and functional deficiency [24]. This linkage may provide a unifying scenario to explain the reported roles of SPRING in SREBP signaling.

As described above, SPRING promotes the proteolytic maturation of S1P. Reciprocally, we recently found that SPRING itself is an S1P-substrate owing to the presence of an S1P-cleavage motif ($R_{45}NNL_{48}$) (Fig. 2) [23▪]. As a consequence, S1P promotes the proteolytic cleavage of SPRING, resulting in secretion of the SPRING ectodomain (Fig. 1). Pharmacological inhibition of S1P or mutation of the S1P cleavage motif prevents secretion of SPRING, providing strong evidence that this event is S1P dependent [23▪]. Intriguingly, the cleaved SPRING fragment is sufficient for restoring SREBP signaling in cells lacking *SPRING*. It is, therefore, tempting to speculate that SPRING cleavage by S1P acts as a licensing event for S1P-mediated cleavage of SREBPs, potentially by facilitating the interaction between S1P and the incoming SCAP–SREBP complex in the Golgi. However, we point out that forced expression of cleavage-resistant SPRING mutants in SPRING-deficient cells also restores SREBP signaling, indicating that at least in overexpression experiments, cleavage is not a prerequisite. Experiments employing genome-edited cells in which the SPRING cleavage site is altered may be needed to address the functional relevance of SPRING cleavage. Nevertheless,

unbiased proteomics studies performed on human fractionated plasma have identified the cleaved SPRING form in the circulation, supporting the functional significance of this event [25–27].

The substrate specificity of S1P extends beyond SREBPs and includes, amongst others, the activating transcription factor 6 (ATF6; ER stress) [28], the cAMP response element-binding protein (CREB) 3 family member CREB3L3 (fasting response) [29], and N-acetylglucosamine-1-phosphotransferase (GNPTAB; lysosomal biogenesis) [30]. Experiments, mostly conducted in overexpression models support a role for SPRING in their cleavage [15▪▪,19▪▪,23▪]. Results showing that cleavage of S1P targets are affected in the absence of SPRING are more limited. Absence of *SPRING* in HeLa cells results in enlarged lysosomes consistent with impaired GNPTAB processing by S1P [19▪▪], and in Hap1 cells, the tunicamycin-induced ATF6 response is mildly attenuated when *SPRING* is absent [12▪▪]. These findings support the notion that SPRING can affect S1P functionality beyond the scope of SREBP signaling. Yet we emphasize that the screens identifying SPRING prominently pointed towards the SREBP pathway and did not report a global S1P-impairment signature. Moreover, loss of hepatic *Spring* in mice is not associated with lysosomal alterations or overt S1P impairment beyond SREBP signaling in the liver (unpublished), though admittedly these studies were not specifically developed to interrogate other S1P functions. Therefore, whether SPRING-dependent regulation of S1P targets beyond SREBPs is physiologically relevant remains to be established.

THE IN-VIVO FUNCTION OF SPRING

To this point, we described the cellular function of SPRING in regulating SREBP signaling. Studies looking into the in-vivo role(s) of SPRING are more limited. We reported that global ablation of *Spring* in mice results in embryonic lethality [12▪▪]. This outcome is similar to that observed upon deletion of other key genes in the SREBP core machinery like *Mbtps1* [31], *Srebp2* [32] and *Scap* [33]. Given that sustaining embryonic development places a high demand for lipids, it is not surprising that compromised SREBP signaling culminates in in-utero lethality. Germline deletion of *Spring* in zebrafish results in viable offspring and is associated with impaired intestinal lipid uptake. This phenotype, despite have a variable penetrance in *Spring*-ablated fish, is reminiscent of *Mbtps1*-deficient zebrafish [15▪▪]. *Mbtps1*-deficient zebrafish also display cranioskeletal malformations and impaired somite and vertebra development [34,35]. Similarly, individuals harboring rare loss-of-function genetic variants in the *MBTPS1* gene, which encodes S1P, also suffer from skeletal dysplasia [36–38]. Skeletal and developmental defects were not reported in zebrafish that lack *Spring* [15▪▪], suggesting that the functions of S1P and SPRING may not fully overlap *in vivo*.

More recently, we developed mice with conditional *Spring* deletion. Mice lacking hepatic *Spring* are viable and display no overt phenotype. However, ablation of hepatic *Spring* is associated with a strong lipid-centered phenotype (unpublished). Amongst others, circulating and hepatic lipid levels are markedly reduced in these mice because of impaired SREBP-driven de novo lipogenesis. This closely phenocopies mice with hepatic-specific ablation of *Scap* [39] or *Mbtps1* [31], and cements the role of SPRING as a core component of the SREBP-activating machinery. Whether SPRING is also a determinant of lipid and lipoprotein metabolism in humans requires further investigation. Another interesting venue for further explorations will be the potential role of SPRING in cancer, in view of the high demand malignant cells have for lipids to support proliferation. *SPRING* is an essential gene in a broad range of cancer cell lines [12▪▪,14,15▪▪], and sporadic reports suggest SPRING's involvement in tumorigenesis [40–42].

OPEN QUESTIONS REGARDING SPRING

SPRING is rapidly emerging as an important determinant of Signaling. Several venues for future exploration have been mentioned above, and here we highlight, several pertinent questions regarding its mechanism of action.

What is the functional overlap between SPRING and S1P?

While SPRING is required for efficient auto-proteolytic activation of S1P, deletion of *SPRING* seems to preferentially impair SREBP signaling and lipid metabolism. As such, the physiological significance of SPRING for proteolytic processing of other S1P targets requires further investigation. Or is SPRING an S1P co-factor that preferentially couples S1P to SREBP processing?

What is the spatiotemporal sequence of events governing proteolytic activation of S1P by SPRING, and how is this coupled to sterol-responsive element-binding protein processing?

Current studies report on SPRING-stimulated proteolytic maturation of S1P under steady-state

conditions. These studies do not address the spatiotemporal sequence of events underlying this process. Proper proteolytic activation of SREBP requires SPRING activity in the Golgi, yet proteolytic maturation of S1P is initiated in the ER. Hence, does SPRING already interact with S1P in the ER, and if so with which cleavage intermediate(s)? Also, how is association of SPRING with S1P coupled to stimulated S1P maturation and trafficking to the Golgi? In the Golgi, do S1P, SPRING, and SCAP form a tripartite complex, and is this a prerequisite for SREBP cleavage? Addressing some of these questions will require development of reagents to follow endogenous levels of both SPRING and S1P. Our mechanistic understanding of SPRING's function in the SREBP pathway will also be boosted by obtaining a structural framework of SPRING alone and in complex with S1P and SCAP.

Is SPRING expression regulated and what is the significance of SPRING cleavage and secretion?

Expression of *SPRING* is not subject to sterol-dependent regulation [12▪▪], and whether its expression or activity is (metabolically) regulated is not known. As such, the significance of SPRING cleavage by S1P requires further attention. Is this step part of the S1P activation cycle that is required for processing of SREBP by S1P? More broadly, is SPRING secretion a relevant physiological event, and if so, what is the function of secreted SPRING?

Is genetic variation in the SPRING locus associated with lipid traits in humans?

In mice, loss of hepatic *Spring* results in marked effects on circulating lipid levels (unpublished). No studies on human *SPRING* have been reported. In preliminary studies, we have identified both common and rare *SPRING* variants associated with the level of circulating plasma lipids in humans. The significance and the mechanistic underpinning of this observation needs further investigation.

CONCLUSION

The core machinery implicated in proteolytic activation of SREBP signaling has been established two decades ago [6]. Empowered by functional genetic screens, the identification of SPRING as a novel determinant of SREBP signaling represents the latest addition to this system. Elucidating the mechanism of action and physiological roles of SPRING will advance our understanding of the regulation of lipid metabolism.

Acknowledgements

We thank members of the Zelcer lab and Irith Koster for their critical comments and suggestions on this review.

Financial support and sponsorship

N.Z. is an established investigator of the Dutch Heart Foundation (2013T111) and is supported by a Vici grant from the Netherlands Organization for Scientific Research (NWO; 016.176.643).

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

1. Yokoyama C, Wang X, Briggs MR, *et al.* SREBP-1, a basic-helix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene. Cell 1993; 75:187–197.
2. Hua X, Yokoyama C, Wu J, *et al.* SREBP-2, a second basic-helix-loop-helix-leucine zipper protein that stimulates transcription by binding to a sterol regulatory element. Proc Natl Acad Sci U S A 1993; 90:11603– 11607.
3. Shimano H, Horton JD, Shimomura I, *et al.* Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells. J Clin Invest 1997; 99:846–854.
4. Shimomura I, Shimano H, Horton JD, *et al.* Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. J Clin Invest 1997; 99:838–845.
5. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 2002; 109:1125–1131.
6. Goldstein JL, DeBose-Boyd RA, Brown MS. Protein sensors for membrane sterols. Cell 2006; 124:35–46.
7. Brown MS, Radhakrishnan A, Goldstein JL. Retrospective on cholesterol homeostasis: the central role of scap. Annu Rev Biochem 2018; 87:783– 807.
8. Brown AJ, Sun L, Feramisco JD, *et al.* Cholesterol addition to ER membranes alters conformation of SCAP, the SREBP escort protein that regulates cholesterol metabolism. Mol Cell 2002; 10:237–245.
9. Radhakrishnan A, Goldstein JL, McDonald JG, Brown MS. Switch-like control of SREBP-2 transport triggered by small changes in ER cholesterol: a delicate balance. Cell Metab 2008; 8:512–521.
10. Sakai J, Duncan EA, Rawson RB, *et al.* Sterol-regulated release of SREBP-2 from cell membranes requires two sequential cleavages, one within a transmembrane segment. Cell 1996; 85:1037–1046.
11. Kim JY, Garcia-Carbonell R, Yamachika S, *et al.* ER stress drives lipogenesis and steatohepatitis via caspase-2 activation of S1P. Cell 2018; 175:133.e15–145.e15.
12. ▪▪ Loregger A, Raaben M, Nieuwenhuis J, *et al.* Haploid genetic screens identify SPRING/C12ORF49 as a determinant of SREBP signaling and cholesterol metabolism. Nat Commun 2020; 11:1128.

Using a mammalian haploid genetic screen this was the first study to identify SPRING as a regulator of the SREBP pathway. The study reported that loss of SPRING attenuates SREBP proteolytic activation and as a consequence impairs cholesterol synthesis and LDLR-mediated LDL uptake. The work proposed impaired SCAP retrograde trafficking as a potential mechanism to explain how SPRING regulates the SREBP pathway.

13. ▪▪ Aregger M, Lawson KA, Billmann M, *et al.* Systematic mapping of genetic interactions for de novo fatty acid synthesis identifies C12orf49 as a regulator of lipid metabolism. Nat Metab 2020; 2:499–513.

This study identified SPRING/C12ORF49 using a Using a whole-genome CRISPR/Cas9-based synthetic lethality screen in FASN-KO haploid cells. The study further demonstrated that SPRING is essential for SREBP-dependent lipid synthesis.

14. Tsherniak A, Vazquez F, Montgomery PG, *et al.* Defining a cancer dependency map. Cell 2017; 170:564.e16–576.e16.
15. ▪▪ Bayraktar EC, La K, Karpman K, *et al.* Metabolic coessentiality mapping identifies C12orf49 as a regulator of SREBP processing and cholesterol metabolism. Nat Metab 2020; 2:487–498.

By analyzing the Depmap dataset for metabolic co-dependency networks this study identified SPRING/C12ORF49 as an essential component of the SREBP activating process. The study further established the interaction between S1P and SPRING and suggested that this may underlie SPRING's ability to activate SREBP signaling.

16. Cheetham SW, Faulkner GJ, Dinger ME. Overcoming challenges and dogmas to understand the functions of pseudogenes. Nat Rev Genet 2020; 21:191–201.
17. Vinckenbosch N, Dupanloup I, Kaessmann H. Evolutionary fate of retroposed gene copies in the human genome. Proc Natl Acad Sci U S A 2006; 103:3220–3225.
18. Comuzzie AG, Cole SA, Laston SL, *et al.* Novel genetic loci identified for the pathophysiology of childhood obesity in the Hispanic population. PLoS One 2012; 7:e51954.
19. Xiao J, Xiong Y, Yang LT, *et al.* POST1/C12ORF49 regulates the SREBP ■■ pathway by promoting site-1 protease maturation. Protein Cell 2021; 12:279–296.

This study implicated SPRING in the proteolytic activation of precursor S1P to its mature form. Furthermore, the study suggested that SPRING may govern trafficking of S1P from the ER to the Golgi.

20. Seidah NG, Mowla SJ, Hamelin J, *et al.* Mammalian subtilisin/kexin isozyme SKI-1: a widely expressed proprotein convertase with a unique cleavage specificity and cellular localization. Proc Natl Acad Sci U S A 1999; 96:1321–1326.
21. Espenshade PJ, Cheng D, Goldstein JL, Brown MS. Autocatalytic processing of site-1 protease removes propeptide and permits cleavage of sterol regulatory element-binding proteins. J Biol Chem 1999; 274:22795–22804.
22. da Palma JR, Burri DJ, Oppliger J, *et al.* Zymogen activation and subcellular activity of subtilisin kexin isozyme 1/site 1 protease. J Biol Chem 2014; 289:35743–35756.
23. Hendrix S, Tan JME, Ndoj K, *et al.* Intermolecular proteolytic processing of ■ SPRING and Site-1-protease regulate SREBP signaling. bioRxiv 2023; https://doi.org/10.1101/2023.03.28.534447.

This study investigated the functional interaction between S1P and SPRING, which results in their proteolytic cleavage. The study further demonstrates that SPRING cleavage by S1P results in secretion of the SPRING ectodomain.

24. Shao W, Espenshade PJ. Sterol regulatory element-binding protein (SREBP) cleavage regulates Golgi-to-endoplasmic reticulum recycling of SREBP cleavage-activating protein (SCAP). J Biol Chem 2014; 289:7547–7557.
25. Pernemalm M, Sandberg A, Zhu Y, *et al.* In-depth human plasma proteome analysis captures tissue proteins and transfer of protein variants across the placenta. Elife 2019; 8:e41608.
26. Dey KK, Wang H, Niu M, *et al.* Deep undepleted human serum proteome profiling toward biomarker discovery for Alzheimer's disease. Clin Proteomics 2019; 16:16.
27. Keshishian H, Burgess MW, Gillette MA, *et al.* Multiplexed, quantitative workflow for sensitive biomarker discovery in plasma yields novel candidates for early myocardial injury. Mol Cell Proteomics 2015; 14:2375–2393.
28. Ye J, Rawson RB, Komuro R, *et al.* ER stress induces cleavage of membrane-bound ATF6 by the same proteases that process SREBPs. Mol Cell 2000; 6:1355–1364.
29. Zhang K, Shen X, Wu J, *et al.* Endoplasmic reticulum stress activates cleavage of CREBH to induce a systemic inflammatory response. Cell 2006; 124:587–599.
30. Marschner K, Kollmann K, Schweizer M, *et al.* A key enzyme in the biogenesis of lysosomes is a protease that regulates cholesterol metabolism. Science 2011; 333:87–90.
31. Yang J, Goldstein JL, Hammer RE, *et al.* Decreased lipid synthesis in livers of mice with disrupted Site-1 protease gene. Proc Natl Acad Sci U S A 2001; 98:13607–13612.
32. Vergnes L, Chin RG, de Aguiar Vallim T, *et al.* SREBP-2-deficient and hypomorphic mice reveal roles for SREBP-2 in embryonic development and SREBP-1c expression. J Lipid Res 2016; 57:410–421.
33. Matsuda M, Korn BS, Hammer RE, *et al.* SREBP cleavage-activating protein (SCAP) is required for increased lipid synthesis in liver induced by cholesterol deprivation and insulin elevation. Genes Dev 2001; 15: 1206–1216.
34. Schlombs K, Wagner T, Scheel J. Site-1 protease is required for cartilage development in zebrafish. Proc Natl Acad Sci U S A 2003; 100:14024–14029.
35. Achilleos A, Huffman NT, Marcinkiewicyz E, *et al.* MBTPS1/SKI-1/S1P proprotein convertase is required for ECM signaling and axial elongation during somitogenesis and vertebral development. Hum Mol Genet 2015; 24:2884–2898.
36. Kondo Y, Fu J, Wang H, *et al.* Site-1 protease deficiency causes human skeletal dysplasia due to defective inter-organelle protein trafficking. JCI Insight 2018; 3:121596.
37. Meyer R, Elbracht M, Opladen T, Eggermann T. Patient with an autosomal-recessive MBTPS1-linked phenotype and clinical features of Silver-Russell syndrome. Am J Med Genet A 2020; 182:2727–2730.
38. Carvalho DR, Speck-Martins CE, Brum JM, *et al.* Spondyloepimetaphyseal dysplasia with elevated plasma lysosomal enzymes caused by homozygous variant in MBTPS1. Am J Med Genet A 2020; 182:1796–1800.
39. Moon YA, Liang G, Xie X, *et al.* The Scap/SREBP pathway is essential for developing diabetic fatty liver and carbohydrate-induced hypertriglyceridemia in animals. Cell Metab 2012; 15:240–246.
40. Chang H, Jeung HC, Jung JJ, *et al.* Identification of genes associated with chemosensitivity to SAHA/taxane combination treatment in taxane-resistant breast cancer cells. Breast Cancer Res Treat 2011; 125:55–63.
41. Wang Z, Cheng Y, Abraham JM, *et al.* RNA sequencing of esophageal adenocarcinomas identifies novel fusion transcripts, including NPC1-MELK, arising from a complex chromosomal rearrangement. Cancer 2017; 123:3916–3924.
42. Xie L, Gazin C, Park SM, *et al.* A synthetic interaction screen identifies factors selectively required for proliferation and TERT transcription in p53-deficient human cancer cells. PLoS Genet 2012; 8:e1003151.