

Srebp2: A master regulator of sterol and fatty acid synthesis¹

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Sterol regulatory element-binding proteins (SREBPs, including SREBP1a, SREBP1c, and SREBP2) are basic-helix-loop-helix leucine zipper (bHLH-Zip) transcription factors that regulate the synthesis and cellular uptake of two major building blocks of cell membranes: cholesterol and fatty acids. For cholesterol biosynthesis, SREBPs activate expression of genes such as HMG-CoA reductase (*HMGCR*), HMG-CoA synthase (*HMGCS*), and mevalonate kinase (*MVK*). For cholesterol uptake, SREBPs activate expression of the LDL receptor (*LDLR*). For fatty acid synthesis, SREBPs activate expression of genes such as fatty acid synthase (*FASN*) and acetyl-CoA carboxylase (*ACC*). Because of the essential role of cholesterol and triglycerides for all cells, it is perhaps no surprise that SREBPs are tightly regulated, exhibiting extensive crosstalk and some redundancy. It has long been viewed that SREBP2 is primarily responsible for activation of genes involved in cholesterol synthesis, as opposed to fatty acid synthesis. In contrast, SREBP1c functions primarily in the liver to drive fatty acid synthesis, while SREBP1a can drive both pathways in all tissues. However, understanding the tissue-specific and differential genetic requirements for each SREBP has proven elusive. In this issue of the *Journal of Lipid Research*, Vergnes et al. (1) from the laboratory of Karen Reue confirm the role of SREBP2 in regulating cholesterol biosynthesis genes, but discover that SREBP2 has an unanticipated function in survival and limb patterning during development and that it also possesses an important role for fatty acid synthesis via compensatory upregulation of SREBP1 genes (Fig. 1).

Vergnes et al. use a gene trap allele of murine *Srebf2* to examine deficiency of Srebp2 in embryos and adult animals at various dosages through an ingenious conditional allele. This conditional allele is a genetic modification of *Srebf2* through a gene trap, which is an insertional mutation, usually in an intron, that contains a strong splice acceptor and polyadenylation signal that disrupts splicing, resulting in a severely truncated protein product. The novelty of this gene trap, within the first intron of *Srebf2*, is that it enables the conversion of a null allele to a hypomorphic (reduced function) allele through Cre-Lox recombination. This is due to the nature of this specific gene trap, in

which the splice acceptor is flanked by loxP sites, followed by the β -GEO fusion protein/reporter. *Srebf2* splicing with this gene trap produces β -GEO instead of Srebp2 protein. Removal of the splice acceptor with Cre recombinase largely inactivates the function of the gene trap, after which the remaining β -GEO sequence *partially* disrupts expression of *Srebf2*, likely through modest effects on splicing or transcriptional activation. Because Srebp2 autoregulates its own expression in a positive feedback loop (2), an effective gene trap is vital to generating Srebp2 depleted animals by combining one hypomorphic (hyp) Cre-recombined allele and one null “fully-trapped” allele. Vergnes et al. use this allele to generate Srebp2 deficient (*Srebf2*^{-/-}) and Srebp2 depleted (*Srebf2*^{-hyp}) mice. This gene trap is also likely advantageous in that no region of the *Srebf2* locus has been deleted to generate the null (or hypomorphic) alleles, and thus regulatory sequences *and* other important genes are less likely to be affected unintentionally. This is especially important because engineered mutations could affect the *Srebf2*-embedded microRNA gene, miR-33, which represses Srebp1 and cellular cholesterol efflux 33 (3).

Using the fully trapped null allele, Vergnes et al. document the lethal embryonic phenotype caused by loss of Srebp2, importantly, with no effect on miR-33 expression caused by a lack of Srebp2. The critical importance of *Srebf2* is perhaps not too surprising given the embryonic lethal phenotype of engineered *Srebf2*^{-/-} mouse knockouts that was noted, although not described, more than 18 years ago (4). This early lethality is likely due to the widespread expression of Srebp2 in the developing embryo described by Vergnes et al. and its known role in cholesterol synthesis. However, the cause of embryonic lethality remains unknown along with a gender dimorphism where ~50% female *Srebf2*^{-hyp} mice died between 8 and 12 weeks of age.

Surprisingly, the effect of Srebp2 deficiency on fatty acid synthesis genes was just as severe as the effect on cholesterol biosynthesis genes. In whole *Srebf2*^{-/-} embryos,

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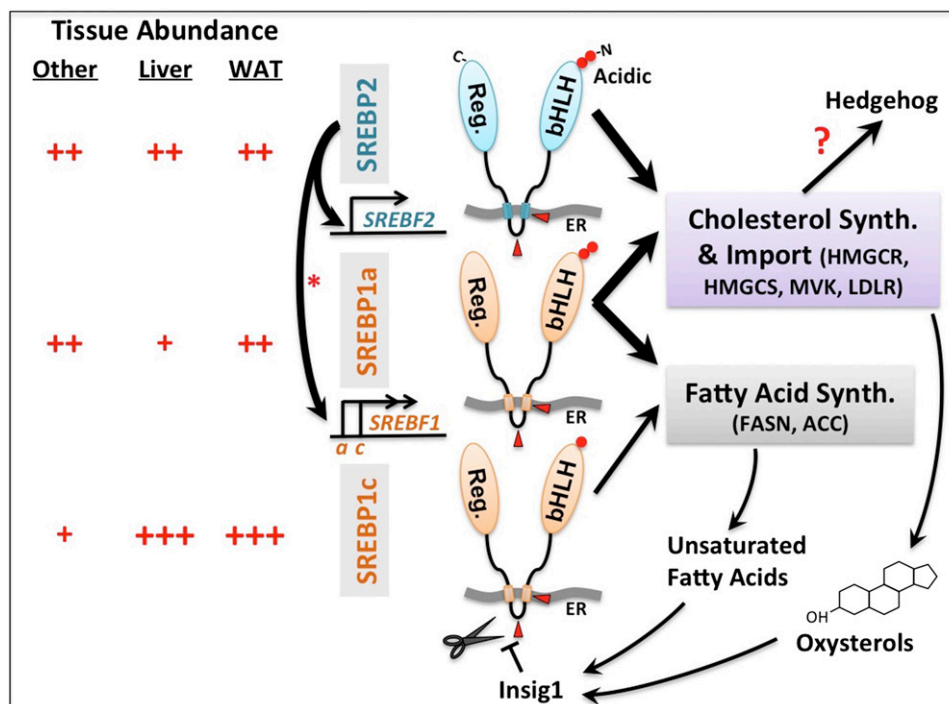



Fig. 1. Regulation of cholesterol and fatty acid bioavailability in cells by SREBPs. SREBPs are conserved bHLH transcription factors localized to the endoplasmic reticulum (ER) via transmembrane domains. Low levels of oxysterols and/or unsaturated fatty acids relieve Insig1-mediated ER-retention, causing SREBP movement to the golgi, proteolytic processing at two cleavage sites (red arrow heads), and nuclear localization. SREBP2 is broadly expressed in embryonic and adult mice and is a potent activator of genes involved in cholesterol synthesis and import, while SREBP1a and SREBP1c play bigger roles in driving expression of genes involved in fatty acid synthesis. Differences in the length and composition of the N-terminal acidic domain (red circles) of each SREBP likely account for differences in their ability to drive transcriptional activation of target genes. Vergnes et al. (1) reveal how loss of *Srebp2* (*Srebf2*) in the mouse greatly compromises expression of both *Srebp1a* and *Srebp1c* in the liver (asterisk), which are cotranscribed from the same locus (*Srebf1*) and differ in their 5' exons, causing defects in both hepatic cholesterol and fatty acid synthesis. Vergnes et al. also describe how defects in cholesterol synthesis caused by *Srebp2* deficiency may negatively impact Hh signaling in developing limb buds of the embryo.

transcript levels of *Srebp1a* and *Srebp1c* (from the *Srebf1* locus) were dramatically reduced, which may be responsible for depletion of fatty acid synthesis genes. This effect was also observed in the liver of a sole surviving *Srebf2*^{-/-} adult animal. This trend of reduced expression of *Srebp1a* and *1c* was also encountered in adult *Srebf2*^{hyp} animals, albeit less severe. Unlike effects in the liver caused by *Srebp2* deficiency, in inguinal subcutaneous white adipose tissue (iWAT), *Srebp1a* levels were significantly increased, indicating a tissue-specific compensatory feedback. This may also explain why triglyceride levels are not reduced in white adipose tissue in *Srebf2*^{-/-} or *Srebf2*^{hyp} animals. However, white adipose tissue weight was dramatically reduced in *Srebf2*^{-/-} animals and also moderately reduced in *Srebf2*^{hyp} animals, so the observed compensation may be difficult to parse given the obvious defect in WAT formation. *Srebp2* mediated regulation of *Srebp1c* has been documented, but here Vergnes et al. may have evidence for regulation of both *Srebp1a* and *Srebp1c*, and while they have differing transcriptional start sites, these genes may share sterol-sensitive *cis* regulatory elements. In essence, the liver is highly dependent on *Srebp2*, which provides positive feedback to stimulate expression of *Srebp1a* and *Srebp1c*, whereas other tissues such as WAT may exhibit more plasticity. It may be informative in the future to analyze

Srebp1a and *1c* expression in other tissues in *Srebf2*^{-/-} or *Srebf2*^{hyp} animals in order to understand the range of these compensatory adaptations. Given that whole embryo cholesterol and triglyceride levels appear normal in *Srebf2*^{-/-} mice, it is reasonable to propose that compensation may be provided by maternally-supplied HDL and LDL, via placental transport, and/or by other undefined basal transcriptional activation of cholesterol synthesis genes. Regardless, the findings here are a testament to the robustness of cholesterol and fatty acid synthesis, which can maintain reasonable levels of these essential molecules in the near absence of three master regulators (SREBPs).

Finally, Vergnes et al. found limb patterning defects in *Srebf2*^{-/-} embryos that may reflect altered signaling of the critical “morphogen” Hedgehog (Hh). Hh proteins are secreted signaling proteins that are unusual in that they undergo posttranslational modification in the form of cholesterylation and palmitoylation (5, 6), which have been reported to modulate Hh diffusion throughout tissues during development (forming a so-called morphogen gradient) (7). While whole embryo cholesterol levels were not changed in *Srebf2*^{-/-} embryos, cholesterol-modified Sonic hedgehog protein (Shh) may be locally deficient in *Srebf2*^{-/-} limb buds, suggesting a positive role for this posttranslational modification. Even though the role of

cholesterol-modified Hh proteins remains unclear, Vergnes et al. report that *Srebp2* depletion blocks the expression of key Shh targets (*Ptch1*, *Gli1*, *Bmp4*, *Grem1*) critical to limb bud patterning, with some effects recapitulated by sterol depletion in cultured wild-type fibroblasts. Hh defects may thus be implicated in the pathogenesis of the gross anatomical abnormalities observed in *Srebp2*^{-/-} limb buds, although bizarrely, the sole surviving *Srebp2*^{-/-} mouse possessed normal limbs. Alternatively, other effects of *Srebp2* on limb bud development may occur through cholesterol-independent function of *Srebp2*, perhaps through effects on autophagy genes (8). Further studies are required to explore Hh cholesterylation in *Srebp2*^{-/-} embryos. Taken together, the elegant studies by Vergnes et al. greatly advance our understanding of the pleomorphic effects of SREBP signaling in embryonic lipid homeostasis and development. 

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