Srebp2: A master regulator of sterol and fatty acid synthesis

Blair B. Madison

Division of Gastroenterology, Washington University School of Medicine, Saint Louis, MO 63110

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 (HMGC S), and mevalonate kinase (MVK). For cholesterol uptake, SREBPs activate expression of the LDL receptor (LDL R). 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 and fatty acid synthase, while SREBP1a can drive both pathways in all tissues. However, understanding the tissue-specific and differential genetic requirements for each SREBP has proven elusive.

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 Srebf2 autoregulates its own expression in a positive feedback loop (2), an effective gene trap is vital to generating Srebp2 depleting 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. documented 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−/− mice 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,
transcript levels of Srebp1a and Srebp1c (from the Srebpf1 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 palmitylation (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.
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 Srebf2−/− limb buds, although bizarrely, the sole surviving Srebf2−/− 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 cholesterolylation in Srebf2−/− 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.

REFERENCES