Pumping up the metabolic Rev-ERB

By Michael J. Haas, Senior Writer

Two teams have shown that the nuclear receptors Rev-ERBAα and Rev-ERBAβ play a central role in regulating the circadian clock and metabolism,[1,2] and a third group has found that agonizing both receptors treats obesity in mice.[3] Collectively, the findings suggest Rev-ErbA agonists could help treat a range of metabolic diseases.

Next, agonists with longer half-lives will need to be developed and patient populations that might benefit from the molecules will have to be identified.

The circadian clock regulates sleep/wake cycles, metabolism and other physiological parameters in relation to the 24-hour day. Previous studies have suggested that among the many regulatory components of the circadian clock, two transcription factors—Rev-ERBAα (nuclear receptor subfamily 1 group D member 1; NR1D1) and Rev-ERBAβ (nuclear receptor subfamily 1 group D member 2; NR1D2)—only played minor roles in regulating circadian cycles. Indeed, mice deficient in either Rev-erbaα or Rev-erbaβ had only mild circadian disruptions.[4,5]

To better understand the function of the two proteins, separate teams from the **Salk Institute for Biological Studies** and the **Perelman School of Medicine at the University of Pennsylvania** conducted genomic analyses of normal mouse livers. Both groups found Rev-erbaα and Rev-erbaβ bound many of the same target genes, including genes that regulate the core circadian clock and lipid metabolism.

Additionally, both teams showed that compared with wild-type mice or those lacking only one *Rev-erba* gene, mice lacking *Rev-erbaα* and *Rev-erbaβ* had decreased nocturnal locomotor activity, which is indicative of disruptions in normal circadian rhythms. The double-knockout animals also had higher plasma levels of glucose and triglycerides and greater levels of hepatosteatosis.

Collectively, these results showed Rev-ErbAα and Rev-ErbAβ cooperated to regulate the core clock and metabolism in ways that were not evident in earlier studies that focused only on one of the proteins, the teams wrote in their respective papers.

One team was led by Ronald Evans, professor and director of the Gene Expression Laboratory at Salk and an investigator at the **Howard Hughes Medical Institute** (HHMI), and included researchers from the **University of California, San Diego School of Medicine**, **The University of Sydney**, **Westmead Hospital**, **Westmead Millennium Institute** and the **Swiss Federal Institute of Technology Lausanne**.

The other team was led by Mitchell Lazar, professor of medicine and genetics and chief of the Division of Endocrinology at Perelman and director of Perelman's Institute for Diabetes, Obesity and Metabolism.

Results were reported in *Nature* and *Genes & Development*, respectively.

Revisiting Rev-ERB

Meanwhile, a team from **The Scripps Research Institute** developed two small molecules that were dual Rev-ErbAα and Rev-ErbAβ agonists to evaluate their *in vivo* effects on the circadian clock and metabolism. In mice, the agonists delayed the onset of nocturnal locomotor activity and increased energy expenditure and decreased fat mass compared with vehicle, all without altering food intake or activity levels.

In mouse models of obesity, the agonists decreased fat mass and plasma levels of glucose, triglycerides and cholesterol compared with vehicle.

Thomas Burris, professor of molecular therapeutics at **Scripps Florida**, led the team, which included researchers from HHMI and **The University of Texas Southwestern Medical Center**.

Results were reported in *Nature*.[3]

"We've always considered Rev-Erbs as an auxiliary or backup loop in the core circadian clock, but these papers show that the receptors are probably a critical component of that clock in mammals and humans," said Timothy Willson, director of chemical biology at **GlaxoSmithKline plc**.

Willson noted that a previous Lazar-led team showed Rev-ErbAα plays a role in energy expenditure, glucose homeostasis and other liver functions.[6] "So the idea of Rev-Erb as a metabolic target is not unprecedented, and the effects of double-knockout or dual agonism on metabolic parameters shown in the three new studies is not surprising," he said. "But the effects are more profound than in previous studies using only *Rev-erbaα* knockouts."

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"While there has been a lot of evidence over the past four or five years linking metabolism and the circadian clock, there have not been many papers about targeting clock proteins with small molecules," added Ross Bersot, president of **ReSet Therapeutics Inc.** "It's interesting to see data on the metabolic effects" of the Scripps team's small molecule Rev-ErbA agonists.

ReSet's lead compound is SHP-1, a small molecule that targets an undisclosed circadian clock protein. The compound is in preclinical development to treat diabetes. The company also has agonists of orexin 1 receptor (HCRTR1; OX1R) and HCRTR2 (OX2R) in lead optimization to treat narcolepsy and other sleep disorders.

ReSet was cofounded by Salk team leader Evans and Joseph Takahashi, professor and chair of neuroscience at UT Southwestern and investigator at HHMI. Takahashi was a member of the Scripps team, although ReSet was not involved in any of the Rev-Erb papers.

Metabolic shift work

Before any dual Rev-ErbAα and Rev-ErbAβ agonists are developed to treat human metabolic disorders, Willson and Bersot said animal studies

are needed to determine the receptors' roles in specific tissue types and metabolic diseases.

"There is a lot of epidemiological data showing that shift workers have a higher incidence and risk of diabetes and obesity," and those data support the hypothesis that restoring normal clock function with dual Rev-ErbA agonists could treat metabolic disease, Willson said. "But a key question is whether the circadian clock, and especially Rev-ErbAs, are deregulated in all patients with metabolic disease or just a subset" of diseases or patient populations.

He added: "This means studying the clock in metabolic disease patients you might want to treat and ascertaining what, if any, sleep disruptions or other clock perturbations they have."

Wilson said it will be hard to identify associations between circadian disruptions and metabolic disorders in a broad population. "Even in shift workers, it takes 15–20 years before there is a measurable increase in the risk of metabolic disease," whereas short-term disruptions to the circadian rhythms—such as those caused by jet lag or switching between standard time and daylight saving time—have no known effect on people's health, he said.

Bersot noted that circadian clock proteins also have differing tissue-specific metabolic functions in liver, adipose tissue, skeletal muscle and pancreas. "Thus, there are different pathways within the clock mechanism that could help treat different metabolic diseases. You'd have to compare the benefits and liabilities of targeting Rev-ErbAs with other clock components to treat a given disease" and decide which target is most feasible, he said.

He also wanted to know whether the dual agonists are effective in mouse models of diabetes and other metabolic diseases over a longer period than the Scripps team's 12-day study.

Willson said the molecules are not suitable for long-term studies. He noted that the team had to use very high doses of the dual agonists—100 mg/kg, twice daily—to see a therapeutic effect in the obesity models, because of the compounds' short *in vivo* half-lives. He said GSK has observed similarly poor pharmacokinetic properties for its own Rev-ErbAα ligands, GSK4112 and GSK5072, in normal animals.

"These types of compounds—ours and the Scripps team's dual agonists—are not useful for long-term studies," and better compounds are needed before the long-term safety and efficacy of Rev-ErbA agonists can be assessed, he said.

GSK identified GSK4112 and GSK5072 in a screen for ligands of orphan receptors and, in separate collaborations with Lazar's group at Perelman and a group at **The University of Manchester**, used the compounds to investigate Rev-ErbAα's role in metabolism[7] and inflammation,[8] respectively. After determining the compounds were not suitable drug candidates, GSK discontinued their development in 2008 and made GSK4112 available to other researchers as a tool compound. The company's ongoing work includes identifying additional Rev-ErbAα ligands to treat rheumatoid arthritis (RA), asthma and other inflammatory diseases.

Other Rev-ERB-erations

Burris disagreed that the half-lives of the dual agonists made them unsuitable for long-term studies, noting that the compounds had therapeutically relevant plasma levels in mice up to eight hours after dosing. "For the preclinical proof-of-principle studies, we dosed twice daily because we wanted to hit the targets for a long duration," he said. "I don't think this would interfere with safety studies, but these compounds would require further optimization before IND-enabling studies anyway."

Indeed, in ongoing work the team has optimized the agonists to improve their potency about 10-fold and continues to optimize their pharmacokinetic and pharmacodynamic properties, he said.

Burris said the Scripps team is investigating a range of potential metabolic indications for the dual agonists and determining the mechanism by which the agonists increased energy expenditure in the mouse models of obesity.

"We plan to take the agonists into mouse models of atherosclerosis" because the compounds suppressed cholesterol synthesis in mice, he said.

The team's follow-on studies in mice have shown that the agonists could treat sleep disorders, and those results will be reported in a forthcoming publication, he added.

The Scripps Research Institute has filed for a patent covering the dual agonists and related scaffolds and is evaluating opportunities to license the IP or spin it out into a company. "We are having discussions with specific companies, but nothing is settled yet," Burris said.

Team leaders Evans and Lazar could not be reached for comment.

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