# QnAs with Mitchell A. Lazar

**Sandeep Ravindran,** *Science Writer*

Mitchell A. Lazar has spent his career studying the transcriptional regulation of metabolism, particularly the role of nuclear receptors. He has discovered several nuclear receptors and elucidated the mechanisms by which they interact with the genome and epigenome. He has made key findings related to the nuclear receptor PPARγ, including the discovery of one of its targets, the previously unidentified hormone resistin. Lazar is currently a professor at the University of Pennsylvania and was elected to the National Academy of Sciences in 2017. In his Inaugural Article (1), Lazar and colleagues investigated the necessity of the nuclear receptor REV-ERBα/β, which links circadian rhythms and metabolism, for the effects of a putative ligand, SR9009. He recently spoke to PNAS about his findings.

**PNAS:** How did you first become interested in the REV-ERBα/β nuclear receptors?

**Lazar:** When I was a postdoctoral fellow in William Chin's laboratory at Brigham and Women's Hospital, I cloned REV-ERBα. I moved to the University of Pennsylvania in 1989 to start my own laboratory, and one of my goals was to understand what REV-ERB did. We discovered the unique DNA-binding motif for REV-ERB, and we discovered that it represses transcription, but we still didn't know its physiological function. In 1998, Ueli Schibler made an amazing discovery. He discovered that if you synchronized cells in culture, about 10% of the transcriptome became circadian, and when he looked to see which genes had the highest circadian amplitude, REV-ERB was at the top of the list (2). Later, Ueli discovered a subtle but clear circadian phenotype in REV-ERBα knockout mice. I was interested in metabolism, and there was a body of work that showed that metabolism is circadian. So that's when I thought there could be a link with REV-ERB, and I had all of the tools in my laboratory to study REV-ERB.

**PNAS:** How did you decide to explore the role of REV-ERB on the effects of SR9009?

**Lazar:** In 2007 my laboratory and other groups found that molecular heme could bind to REV-ERB and stabilize its repressive conformation. That implied that maybe you could find a drug that works through this receptor. In 2012, SR9009 was described as a molecule that could affect the transcriptional activity of REV-ERB but not other nuclear receptors (3). Its activity was described as specific to REV-ERB, and the initial paper showed that it had major effects on locomotion, among other things. Since then, the compound has been used as a REV-ERB agonist, and a lot of laboratories noticed beneficial effects on obesity, diabetes, cardiovascular disease, rheumatoid arthritis, inflammation, and cancer. Over the years, people were using the compound but instead of saying "the compound does so-and-so" or "the compound, which is a REV-ERB agonist, does so-and-so," the papers were saying "REV-ERB does so and so," which really is not what they showed. Because of all of these really positive effects, including increased exercise endurance and weight loss and so on, commercial entities that sell nutraceuticals started selling the compound online. Then papers began appearing in the scientific literature describing methods for testing for illicit use of the compound by athletes. So it seemed to us that it was very important to determine whether the compound has its effects exclusively through REV-ERB or not.

**Mitchell A. Lazar. Image courtesy of David DeBalko (photographer).**

**PNAS:** What did you need to do to test this?

**Lazar:** We needed to make a REV-ERBα/β knockout. There was a previously available conditional knockout of REV-ERBα and -β, but we discovered that although the knockout strategy had knocked out the REV-ERBα DNA-binding domain, it did not result in a knockout. The protein not only is made, but we published a paper showing that it binds to the genome at sites to which it is tethered rather than binding directly (4). That paper sort of turned lemons into lemonade because we went from being disappointed that we



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didn't have a knockout to making a discovery about another mode through which REV-ERB can regulate gene expression. That was great, but we were still stuck because nobody had a conditional knockout, and we set out to make one. After a lot of work, we finally got a conditional REV-ERBα/β double knockout and validated it. That's shown in the Inaugural Article (1). We now have an exciting tool that my laboratory is planning on using to compare phenotypes in tissue-specific knockouts. But we also thought this was a good chance to actually test whether the compounds that are out there, such as SR9009, are specific for REV-ERB.

**PNAS:** What did you learn about SR9009 using the REV-ERBα/β double knockout?

**Lazar:** We generated embryonic stem cells as well as liver cells that lacked both forms of REV-ERB, and we treated these and wild-type cells with SR9009. In the case of rapidly dividing embryonic stem cells, we showed that the compound blocks their proliferation, but it blocked the proliferation of double-knockout cells too. In liver cells we discovered that the majority of genes regulated by the compound were also regulated to the same degree in the double knockout. In fact, there were only a small number in which the compound regulated genes in the opposite direction from the double knockout, which is what you'd expect if it really was an activator of the repression function of REV-ERBs.

**PNAS:** What is your main takeaway from the Inaugural Article (1)?

**Lazar:** The first is that some of the biological activities of SR9009 are occurring in a REV-ERB–independent manner, and thus it is incorrect to attribute any particular effect of SR9009 to REV-ERB without careful experimental controls. The second is that whenever you use a compound, you're studying the effects of that compound, not necessarily its putative target. Every compound is going to have some off-target effects, some more than others. Whenever a new compound is introduced as a specific modulator, it's critical to show that it doesn't function in the absence of its putative target before ascribing its effects as such. This is a general principle that extends far beyond the interrogation of REV-ERB biology.


**1** P. Dierickx *et al.*, SR9009 has REV-ERB–independent effects on cell proliferation and metabolism. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 12147–12152 (2019).
**2** A. Balsalobre *et al.*, A serum shock induces circadian gene expression in mammalian tissue culture cells. *Cell* **12**, 929–937 (1998).
**3** L. A. Solt *et al.*, Regulation of circadian behaviour and metabolism by synthetic REV-ERB agonists. *Nature* **485**, 62–68 (2012).
**4** Y. Zhang *et al.*, GENE REGULATION. Discrete functions of nuclear receptor Rev-erbα couple metabolism to the clock. *Science* **348**, 1488–1492 (2015).