Reply to “How Free Retinol Behaves Differently from RBP-Bound Retinol in RBP Receptor-Mediated Vitamin A Uptake”

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I acknowledge the letter of Zhong et al. (1). Our observations in references 2 to 5 demonstrated that, in addition to functioning as a retinol transporter, STRA6 is a cytokine signaling receptor. We showed that, upon activation by holo-RBP, STRA6 triggers a JAK/STAT cascade leading to induction of STAT target genes. As one such gene is the SOCS3 gene, a negative regulator of the insulin receptor, activation of STRA6 leads to insulin resistance. We showed further that the contribution of STRA6 to retinol uptake by mouse tissues *in vivo* is modest, except in the eye, and that the receptor is not necessary for maintaining vitamin A homeostasis either during embryonic development or in adults (6).

Zhong et al.'s statement that these observations contradict “many studies” misrepresents the literature. Their cited reference 3 reported on experiments in zebrafish, their cited references 4 to 8 studied only STRA6-mediated transport, their cited reference 10 only characterized the effects of ablation of STRA6 on the eye, and their cited references 2 and 14 did not study STRA6 at all. Curiously, Dr. Sun is an author of a recent publication (7) that, while failing to cite our previously published observations (6), corroborates our findings that ablation of STRA6 does not generally impair retinoid status in mice.

To label holo-RBP with [³H]retinol, we typically mix [³H]retinol with holo-RBP (~0.9 retinol/RBP mole ratio) to obtain [³H]retinol-RBP at ~5,000 cpm/nmol. Due to the high specific radioactivity of commercially available [³H]retinol (~50 Ci/mmol), the addition does not significantly change the total concentration of retinol. The mixture is incubated for 2 h, comprising 20 half-lives ($t_{1/2}$ s) for the dissociation of retinol from RBP ($t_{1/2}$ = 6.17 min [8]). The procedure thus allows complete equilibration of [³H]retinol onto holo-RBP. The basis for Zhong et al.'s criticism of this protocol is not clear.

Zhong et al. note that work from the Blaner laboratory demonstrated that RBP is critical for embryonic development under vitamin A-deficient conditions, attesting to the importance of RBP in supplying vitamin A to extrahepatic tissues (9). The observations that STRA6-null mice develop normally even under vitamin A deficiency (6) demonstrate that, unlike RBP, STRA6 is not critical for maintaining vitamin A homeostasis during development.

Retinol can enter cells from extracellular holo-RBP either by free diffusion through the plasma membrane (reviewed in reference 10) or by STRA6-mediated transport. Zhong et al.'s observations that retinol can be readily taken up from the complex of holo-RBP with its binding partner in blood, TTR, simply reflect our findings that TTR does not interfere with uptake by diffusion (2, 11). In contrast, TTR competes with STRA6 for binding holo-RBP and thus blocks both retinol transport and cell signaling by the receptor (2). Zhong et al.'s statement that the affinity of STRA6 for RBP is higher than that of TTR does not faithfully represent findings in the literature. In fact, available information

indicates that the dissociation constants (K_d s) for association of RBP with STRA6 and with TTR are essentially identical (K_d of RBP/STRA6 = 60 nM [12]; K_d of RBP/TTR = 70 nM [11]). Consequently, STRA6 is functional only when its local concentration is exceptionally high, e.g., in the eye (13), or when holo-RBP level exceeds that of TTR, e.g., in obese mice (2). Other physiological circumstances under which STRA6 can function remain to be identified.

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