

METHODS IN MOLECULAR BIOLOGY™

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Retinoids

Methods and Protocols

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Preface

Technical advancement is a major driving force in the experimental sciences, and retinoid research is no exception. Ancient Egyptians recognized that fresh liver can cure night blindness. More than 3000 years later, vitamin A was identified as the essential ingredient in liver. Since then, the pace of discovery has accelerated due to the advent of new techniques, especially during the recent decades. The molecular mechanism for vitamin A's physiological function was first elucidated in vision. Today, the biological functions of vitamin A have been found in almost all vertebrate organs, and its multi-tasking ability has continued to surprise researchers. In addition to vision, known biological functions of vitamin A include its roles in embryonic growth and development, immune competence, reproduction, maintenance of epithelial surfaces, and proper functioning of the adult brain. At the biochemical level, vitamin A derivatives serve distinct functions as photoreceptor chromophores, as transcriptional regulators through the control of nuclear hormone receptors, and as translational regulators, a function discovered recently. Since vitamin A derivatives have potent biological activities, especially in their effects on cellular growth and differentiation, imbalances in vitamin A homeostasis are associated with a wide range of pathological conditions, such as visual disorders, cancer, infectious diseases, diabetes, teratogenicity, and skin diseases. New biological functions are still being discovered for vitamin A derivatives. For example, it was recently discovered that retinol inhibits adipogenesis. Retinol, but not retinoic acid, has the ability to maintain the pluripotency of embryonic stem cells. Retinoic acid plays surprising roles in regulating protein translation in neurons. Certain tissues have the ability to accumulate surprisingly high concentrations of retinoids under physiological conditions. For example, when channelrhodopsin, which uses all-*trans*-retinal as its chromophore, and rhodopsin, which uses 11-*cis*-retinal as its chromophore, are expressed in different regions of the mouse brain in the optogenetic technique to study neural circuits, they become light sensitive without the addition of exogenous retinoid. The physiological functions of retinoids in the adult brain are beginning to emerge, including their roles in sleep, learning, and memory.

The purpose of this book is to summarize recent technical tools to help researchers in diverse fields to uncover more surprises in the future. The target audience of this book includes both beginning researchers and experienced researchers who would like to learn new techniques. All chapters were written by experts on the subjects. Topics cover diverse techniques for both *in vitro* and *in vivo* studies. A special chapter provides advice on the practical use of diets in both animal and human research on vitamin A. Biochemical techniques include the detection and quantitation of retinoids using HPLC, mass spectrometry, and fluorescence and techniques to study visual pigments, retinoid isomerase, a membrane transporter for retinoid, A2E, retinoic acid catabolism, and cellular vitamin A uptake. Biophysical techniques include fluorescence anisotropy of retinol binding protein, electrophysiology to study retinoid cycle in vision, visualization of retinoid in native tissues, two-photon microscopy to study retinoid transport, and epifluorescence to study

retinol in photoreceptor cells. Cell biological techniques include cell culture models for studying retinoid transport and the role of retinol in embryonic stem cell culture.

Hui Sun
Gabriel H. Travis

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