Retinoid metabolism: a balancing act

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The recent characterization of the aldehyde dehydrogenase Aldh1a2 and the cytochrome P450 Cyp26, two enzymes involved in retinoid metabolism, has helped to explain how bioactive retinoids are made and catabolized. By the elegant definition of an *Aldh1a2* null mutation as a dominant suppressor of a *Cyp26* null mutation, it is now unequivocally demonstrated that the main function of Cyp26 is to degrade endogenous all-*trans* retinoic acid rather than to synthesize bioactive hydroxylated retinoids.

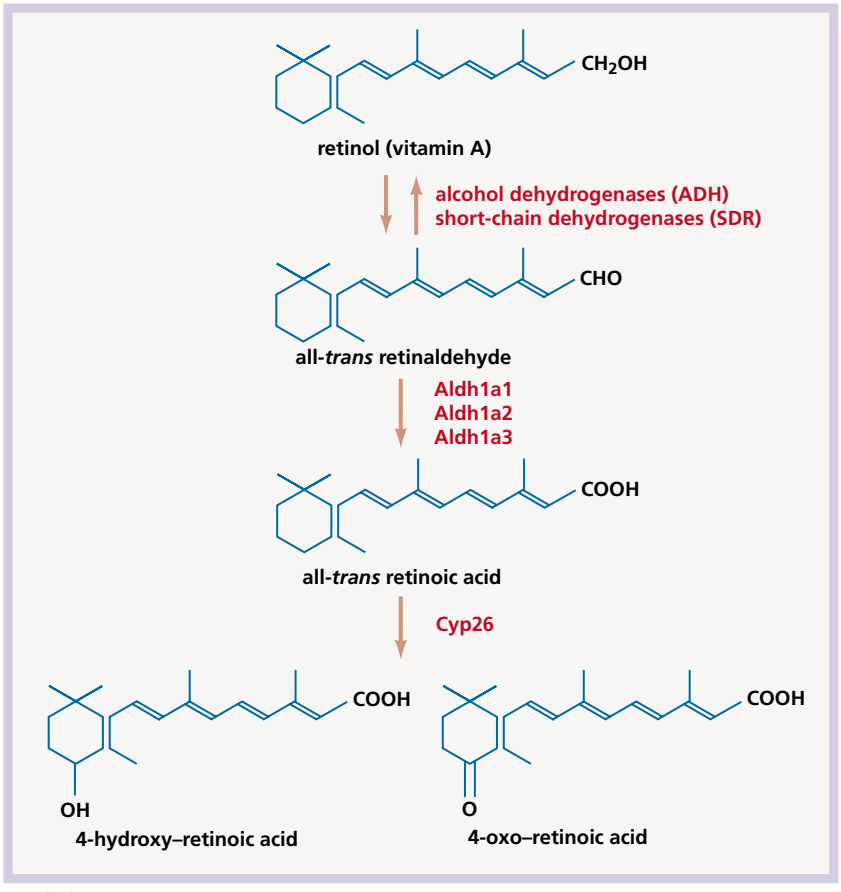
Using mice as flies is a mouse geneticist's dream. With an ever-increasing number of mouse knockout strains available, the dream is fast becoming a reality. We can now carry out experiments to uncover genetic interactions that until recently were almost exclusively identified in invertebrates such as *Drosophila melanogaster* and *Caenorhabditis elegans*. An example of such an experiment is presented in the accompanying paper[1] by Karen Niederreither and colleagues. These authors have identified genetic interactions between two enzymes involved in retinoid metabolism by crossing mouse knockout strains carrying targeted mutations in each. Their results deepen our understanding of retinoid biology.

Retinoid metabolism

Retinoids are derived from dietary vitamin A. Their main function is in cell signaling, in which they bind two classes of retinoid receptors, RARs and RXRs, that belong to the family of nuclear hormone receptors. These receptors are ligand-regulated transcription factors with essential roles in embryonic development and adult physiology. The biological importance of retinoids has long been known, as major developmental abnormalities follow retinoid deprivation or exposure to excess retinoids. Some of these abnormalities resemble congenital birth defects that are relatively common in humans, including spina bifida and cleft palate.

How are retinoids synthesized and catabolized? Vitamin A (retinol) is converted to retinaldehyde and then to all-*trans* retinoic acid by way of two oxidation steps (see figure). All-*trans* retinoic acid is present in embryonic and adult tissues at high levels, binds efficiently to RARs, and is a major biologically active retinoid *in vivo*[2]. Some of the remaining uncertainties stem from the existence of additional retinoid metabolites. For example, 4-hydroxy-retinoic acid and 4-oxo-retinoic acid (see figure) have been suggested to be inactive breakdown products of all-*trans* retinoic acid. Other results, however, have raised the possibility that these retinoids have important biological functions[3,4].

Although the picture remains incomplete, many of the critical enzymes involved in retinoid metabolism have been identified. Oxidation of retinol to retinaldehyde requires the activities of several alcohol dehydrogenases (see figure)[5]. The second step—oxidation of retinaldehyde to all-*trans* retinoic acid—requires the action of three related retinaldehyde dehydrogenases and is generally believed to be the rate-limiting step in the biosynthesis of all-*trans* retinoic acid. This conclusion is supported by several observations. For example, there is a striking correlation between embryonic expression of the aldehyde dehydrogenase



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Retinoid roundup. The biosynthetic pathway for generating all-*trans* retinoic acid involves two oxidation steps, the first leading to the generation of all-*trans* retinaldehyde. The rate-limiting step in production of all-*trans* retinoic acid in the embryo is mediated by three related aldehyde dehydrogenases, Aldh1a1, Aldha2 and Aldha3. All-*trans* retinoic acid is converted to hydroxylated metabolites by Cyp26 (refs 7,8). These hydroxylated derivatives are metabolized to other polar retinoid metabolites by additional enzymatic pathways. Niederreither *et al.*[1] show that the impaired retinoid balance resulting from a *Cyp26* null mutation is rescued on an *Aldh1a2* heterozygous background. This finding indicates that the main, if not only, function of Cyp26 is to protect tissues from excess all-*trans* retinoic acid.

Aldh1a2 and the activity of transgenic retinoid-responsive reporter genes, and Aldh1a2-deficient mouse embryos have abnormalities that are also evident under retinoid deprivation[6].

A cytochrome P450 enzyme, Cyp26, was recently identified and shown to be essential for the generation of 4-hydoxy-retinoic acid and 4-oxo-retinoic acid, as well as other hydroxylated retinoid derivatives[7,8]. Studies of Cyp26-deficient mice have largely supported the conclusion that 4-hydroxy-retinoic acid and 4-oxo-retinoic acid are inactive degradation products of the all-*trans* isomer. Accordingly, embryos from these mice have abnormalities that mimic all-*trans* retinoic acid-induced teratogenicity[9,10]. It is possible, however, that some of the abnormalities observed in *Cyp26*$^{-/-}$ mice result from a deficiency in Cyp26-generated metabolites that have active biological roles *in vivo*. Moreover, all-*trans* retinoic acid could have an inhibitory influence on activities promoted by hydroxylated retinoic-acid derivatives, thus explaining why abnormalities due to Cyp26 deficiency and exposure to excess all-*trans* retinoic acid would resemble each other. Niederreither *et al.*[1] have now settled this somewhat controversial issue through genetic experiments using knockout strains harboring mutations in *Aldh1a2* and *Cyp26*.

Retinoids in the balance

As the amount of all-*trans* retinoic acid is diminished in mice heterozygous for *Aldh1a2*, Niederreither *et al.*[1] hypothesized that if Cyp26-generated bioactive metabolites are essential, the phenotypes of *Cyp26*$^{-/-}$ mice should be exaggerated on an *Aldh1a2*$^{+/-}$ background. Conversely, if Cyp26 metabolites are merely degradation products, and Cyp26 simply functions to protect the embryo from excess all-*trans* retinoic acid, the phenotype should be attenuated in the *Aldh1a2*$^{+/-}$ background.

The results are dramatic and quite revealing. The early lethal phenotype of Cyp26-deficient mice was phenotypically rescued by heterozygous disruption of *Aldh1a2* (ref. 1). Complete rescue of the spina bifida and urogenital tract abnormalities was observed, whereas other abnormalities were partially rescued, notably abnormal hindbrain development and vertebral transformations. Strikingly, most of the *Cyp26*$^{-/-}$*Aldh1a2*$^{+/-}$ embryos were viable, survived into adulthood and were fertile. The remaining abnormalities in these animals are milder versions of phenotypes that occur in *Cyp26*$^{-/-}$ embryos. Thus, the results provide compelling evidence that the main, if not sole, function of Cyp26 is to protect tissues from inappropriate exposure to all-*trans* retinoic acid.

The model morphogen?

All-*trans* retinoic acid used to be considered the model 'morphogen'—a signaling substance that is locally synthesized and forms a concentration gradient by diffusion[11]. Graded responses to the morphogen can thus allow patterning of otherwise undifferentiated embryonic tissues such as the limb bud and early neural tube. The morphogen hypothesis seemed consistent with a requirement for a balanced exposure to retinoids evident from the severe abnormalities resulting from either its deprivation or excess.

How has characterization of Aldh1a2 and Cyp26 influenced the morphogen hypothesis? Although the analyses of Aldh1a2- and Cyp26-deficient mice have also emphasized the requirement for finely tuned retinoid signaling, it seems probable that, in most cases, a given tissue is either uniformly exposed or protected from retinoids in an on/off mode of signaling. Such a conclusion is consistent with the uniform *in vivo* expression patterns of *Aldh1a2* and *Cyp26*, which occur in sharply defined, mostly non-overlapping domains. Moreover, *Aldh1a2*$^{-/-}$ embryos can be partially rescued by feeding pregnant mothers with all-*trans* retinoic acid[6]. Such treatments would be expected to result in uniform retinoid exposure in zones devoid of retinoid-degrading enzymes and are not obviously compatible with a requirement for graded distribution of retinoic acid.

Clearly, the identification and characterization of retinoid-metabolizing enzymes has dramatically expanded our perspective on retinoid biology, and the work by Niederreither *et al.*[1] provides compelling evidence that 4-oxo-retinoic acid is mainly an inactive breakdown product of all-*trans* retinoic acid. Questions about other retinoid metabolites remain, however, including the didehydroretinoids found in chick embryos, and retro-retinoids, a class of hydroxylated retinoids whose biological activities are apparently not mediated by nuclear receptors[12–14]. Perhaps most enigmatic is the role of 9-*cis* retinoic acid, a high-affinity ligand for RXR, whose existence *in vivo* has been questioned because of difficulties in extracting it from tissues[2,15]. As exemplified by the work of Niederreither *et al.*[1], continuing efforts focusing on retinoid metabolism will provide important answers. Their study also provides a powerful example of the potential of genetic approaches in mice to uncover intricate relationships in signaling and other essential processes. □

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