Chapter 4

The evolution of the retinol metabolism

Abstract

Introduction

The retinol metabolism comprises a series of enzymatic reactions that convert dietary vitamin A (retinol) into various bioactive compounds, primarily retinal for vision (REF) and retinoic acid for gene regulation (REF), ensuring the proper functioning of visual processes and other physiological roles in the body (REFS).

Retinol (Vitamin A1) is an essential micronutrient derived primarily from diet. It can be obtained directly from animal sources as retinyl esters or indirectly from plant sources as pro-vitamin A carotenoids, which are then converted into retinol in the body (REFS). Once in the cell, retinol is esterified to retinyl ester by the enzyme lecithin retinol acyltransferase (LRAT) (REF). When needed, retinyl ester is hydrolysed back to retinol (REF). Retinol is oxidized to retinal by retinol dehydrogenases (RDHs). Several other enzymes are involved in various steps of the retinol metabolism pathway as schematically shown in Figure 4.1 that summarizes what is known about the pathway according to the Kegg Pathway Database (REF). In addition, Table 4.1 provides a comprehensive list of these enzymes ranked by the number of pathways they participate in according to Kegg. Involvement in one or few pathways serves as an indicator of enzyme specificity to the retinol metabolism, as opposed to broad spectrum enzymes.

Retinal, particularly 11-cis-retinal, plays a crucial role in vision (REFS). 11-cis-retinal binds to the protein opsin in photoreceptor cells forming rhodopsin. Upon absorbing a photon, 11-cis-retinal is isomerized to all-trans-retinal, leading to a conformational change in opsin, and initiating a cascade of events called phototransduction (REFS) (see Chapter 3). After light exposure, all-trans-retinal is reduced to all-trans-retinol and then converted back to 11-cis-retinal through a series of enzymatic reactions. This part of the visual cycle is essential as it ensures the retina’s responsiveness to light (REFS). The regulation of the metabolic steps ensures sufficient 11-cis-retinal availability and prevents toxic build-up of intermediates. Additionally, retinal can be further oxidized to retinoic acid by retinaldehyde dehydrogenases (RALDHs). Retinoic acid serves as a signalling molecule that regulates gene expression and is critical for numerous developmental processes (REFS).

Given the importance of the retinol metabolism, it is compelling to delve into its evolutionary history, especially when considering the broader evolution of vision. Hence, the work presented in this chapter aimed to unravel this intricate history. The initial step was to identify the genetic components involved and determine their evolutionary relationships to answer questions such as: Do the gene families belong to overarching orthogroups? How closely related are they? The subsequent objective was to uncover the distribution of these components across the animal kingdom and, more broadly, within eukaryotes, to pinpoint the specific point in time when all the components came into place. The final endeavour was to delineate the main evolutionary events characterizing each orthogroup, to discern, for instance, if certain gene families have undergone a greater number of evolutionary events and contextualizing them within the evolutionary tree of life.

Results and Discussion

Enzymes involved in retinol metabolism belong to 12 major orthogroups.

To understand the evolution of the retinol metabolism, I decided to reconstruct the evolution of all the enzymes involved in the pathway, as described by Kegg (REF) (Figure 4.1 and Table 4.1). To do this, I explored the genes encoding these enzymes in 101 species spanning all of Eukarya (Table 4.2 and its supp version with more info).

The first step to study the evolution of these genes was to first determine to which gene families or orthogroups they belonged to. In fact, while the number of enzymes participating in the pathway is relatively high, some of them might belong to a broader gene family, or to put it more precisely, orthogroup, i.e., a group of orthologs and paralogs deriving from the same original gene duplication.

Therefore, the first part of the analyses aimed to identify the orthogroups that the enzymes belong to. For this, a preliminary blastp was performed using kegg orthologs as queries versus our database of 101 eukaryotes. The results of this blast were used as input for orthogroup identification pipeline. The details can be found in the Methods section, but briefly, two alternative softwares (Broccoli (REF) and Orthofinder (REF)) were used to independently assess orthogroups, then the results were compared, and consensus groups were defined.

The results of the orthogroup identifications and the comparison between the two methods is shown in Figure 4.2. First of all, we can see how the two methods are largely consistent, with many cases of one-to-one correspondence of orthogroups. However, it is also immediately noticeable that Orthofinder tended to provide fewer and larger orthogroups, while Broccoli provided more and in some cases smaller orthogroups. As a consequence, some gene families appeared fragmented into multiple smaller orthogroups according to Broccoli only. Table 4.3 summarizes the final 12 orthogroups identified and shows the comparison of Orthofinder and Broccoli with each other and the original Kegg groups.

Overall we identified some interesting and unexpected findings: such as that DGAT and DGAT2L4 are not to be considered the same gene familiy/orthogroup (according to both Orthofinder and Broccoli) and that the SDR and RDH families are intermingled, possibly indicating that they belong to a broader orthogroup. For the latter, to discriminate more rigorously the relationship between SDR and RDH, all orthougroups were collected as one big orthogroup for phylogenetic analysis.

Possvm and Generax describe substructure of orthogroups.

RETSAT

PNPLA4

ALDH1

BCMO1/RPE65

LRAT

RDH/SDR

Even when this was one of the largest orthogroups (~5000 sequences) with by far the most complex substructure, it may very well be that the mega orthogroup could be expanded even more to include other enzymes. The possible incompleteness may stem from the fact that our original seeds for the blast came only from enzymes used by the retinol metabolism (focus of this chapter) however other enzymes are involved in other pathways. Future research that aims to understand the details of the evolution of RDHs and SDRs should broaden even more the scope of investigation, even if this will be extremely challenging due to the difficulties in performing phylogenetic analysis with such large datasets.

DGAT

DGAT2LA4

CYP

AOX

ADH

UGT

Conclusions

Evolution:

Origins: The visual pigments, opsins, and the associated visual cycle have ancient origins. It's believed that the basic phototransduction machinery was present in the common ancestor of most extant animals.

Opsin Diversification: Over evolutionary time, different opsin classes evolved, allowing animals to detect light across different wavelengths. This diversification is tightly linked with changes in retinoid usage and the visual cycle.

Vertebrate Adaptations: The transition from aquatic to terrestrial environments necessitated changes in the visual system, including modifications in the retinoid pathway.

Retinoid Binding Proteins: Evolutionary changes in retinoid-binding proteins and enzymes have fine-tuned retinoid transport, storage, and metabolism, allowing diverse organisms to adapt to their specific visual environments.

Methods

Identification of orthogroups for retinol metabolism enzymes.

**Species list (and species tree)**

**Data mining**

- Collection of kegg “orthologs” for each components as queries

- preliminary loose blastp

**Orthogroup definition**

- with orthofinder

- with broccoli

- comparison of the two, visualisation with Cytoscape

- final definition of consensus orthogroups

Reconstruction of evolutionary history of orthogroups.

**Phylogenetic trees**

- alignments, trimmings

- trees

- ETE (only for generax = necessary for some of the subsequent steps)

**Reconstruction substructure/orthologs with Possvm**

**Reconstruction of evolutionary events with Generax**

Data Availability

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References