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A comprehensive machine-readable view of the mammalian cholesterol biosynthesis pathway

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ABSTRACT

Cholesterol biosynthesis serves as a central metabolic hub for numerous biological processes in health and disease. A detailed, integrative single-view description of how the cholesterol pathway is structured and how it interacts with other pathway systems is lacking in the existing literature. Here we provide a systematic review of the existing literature and present a detailed pathway diagram that describes the cholesterol biosynthesis pathway (the mevalonate, the Kandutch-Russell and the Bloch pathway) and shunt pathway that leads to 24(S),25-epoxycholesterol synthesis. The diagram has been produced using the Systems Biology Graphical Notation (SBGN) and is available in the SBGN-ML format, a human readable and machine semantically parsable open community file format.

1. Introduction

Cholesterol is an intensively studied, multi-functional lipid that is key to many aspects of immunological, neuronal, viral and hepatocyte biology. It is an essential component of cellular membranes and is a precursor to steroids, bile acids and oxysterols whilst its own precursors contribute to prenylation and dolichylation and the formation of vitamin D_3. One of the oxysterols known to be involved in linking sterol metabolism to innate immunity [1,2] is 25-hydroxycholesterol. However its place in the sterol metabolism has not yet been well established.

Despite the importance of the cholesterol synthesis pathway to cellular function and its value in pharmaceutical therapies, an integrative picture of how the pathway is structured has not been well described in the literature, impeding the development of a more rigorous understanding of the role of the cholesterol metabolism in cellular processes. Publications typically focus on segments of the cholesterol biosynthesis pathway showing variable level of details. Kovacs and co-authors focus on the mevalonate section of the pathway and on the subcellular location of the enzymes involved [3]. Wang and co-authors concentrate on the steps leading to 24(S),25-epoxycholesterol synthesis and their similarity to steps in the cholesterol biosynthesis pathway [4]. Previous work studying the role of the cholesterol biosynthesis pathway has shown a modest level of detail on the sterol arms of the pathway [5–7] in innate immunity. The LIPID MAPS consortium offers the most detailed descriptions of the Bloch and Kandutsch-Russell branches of cholesterol biosynthesis, but these lack cell compartment information and lack integration with the 24(S),25-epoxycholesterol shunt arm and other branching pathways [8].

Here we present a comprehensive literature review of the cholesterol synthesis pathway and we implement this as a detailed pathway that captures enzymatic activity and compartmental localization and summarizes all intermediate metabolic forms. Our review also clearly indicates what information is missing and where additional research is required.

2. Materials and methods

The model of the cholesterol biosynthesis pathway presented in this work has been assembled using a variety of publicly available resources including the research findings of the LipidMaps

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consortium [8] and results obtained from thorough searches of the published literature that have been manually curated and validated by domain experts.

In cases where there were conflicting reports, preference was given to the more recent papers and to the works in which more reliable and advanced methods were used. The suggested order of events is supported by a number of independently obtained research results. The principles of the Evidence Ontology (ECO) [9], the Gene Ontology Evidence Codes [10] and the Evidence Code Decision Tree [11] were considered during the pathway reconstruction.

A brief summary is provided for each enzyme and the corresponding metabolic reactions involved in the pathway. For each enzyme we endeavored to capture the following information where available: corresponding gene name approved by HUGO Gene Nomenclature Committee [12], alternative names, enzymological activities according to the Enzyme Nomenclature Committee of the IUBMB [13], enzyme function description, subunit structure, subcellular location and related disorders.

We have included a list of UniProt IDs for the proteins captured in the model (Table 1) and a list of metabolite names (common and systematic) as used in the LipidMaps database [8] (Table 2). Common names are used on the map where available.

The pathway that we present here is described using the Systems Biology Graphical Notation (SBGN) [14], a community driven consensus graphical schema for capturing the molecular details of pathway systems. In particular, we use the SBGN Process Description language [15]. A machine-readable model is available as part of the supplementary material in SBGN-ML format [16] and we present it graphically in Figs. 1–3, in an enhanced form. The SBGN-ML format files can be read using a variety of software packages.

In particular, the supplementary files provide a description of the pathway that can be edited and modified in accordance with the SBGN standard in order to be of future use to the research community. The SBGN-ML file format encodes the biological meaning associated with each component of the model. This allows the model to be parsed by software (i) to ensure that modification is biologically valid and (ii) to facilitate automatic generation of mathematical descriptions of the pathway biology. It should be possible to open these files in any software designed to comply with the SBGN-ML standard, including but not limited to VANTED and Cytoscape [17,18]. For the purpose of this review, we compiled and tested the files using the VANTED software tool [17]. Here we shall outline how the files can be opened and accessed using the VANTED and CYTOSCAPE [18] software tools.

2.1. Accessing supplementary SBGN-ML files using the VANTED software tool

2.1.1 Download the files with '.sbgn' file extension from the supplementary material.
2.1.2 Download and install VANTED from http://vanted.ipk-gatersleben.de/.
2.1.3 Open VANTED and you will be greeted by a screen divided into two regions: an empty area on the left for diagrams and a column on the right containing settings under various tabs.
2.1.4 Using the automated installer to obtain the SBGN-ML add-on.
2.1.4.1 In the right hand column select the 'Help' tab and then the 'Settings' tab beneath and click on the 'Install/Configure Add-ons' button. This will open the Add-on Manager.
2.1.4.2 Click on the 'Find Add-ons/Updates' button on the bottom of the Add-on Manager window. This, in turn, opens the 'Direct Add-on Download' window.
2.1.4.3 At the top right of the Add-ons window, left and right arrows allow the user to move through a list of the available Add-ons. Find the Add-on entitled, 'SBGN-ED' and click the corresponding 'Install Add-on' button. VANTED will now automatically download the SBGN extension.
2.1.4.4 Click 'OK' and you will return to the 'Add-on Manager' where 'SBGN-ED' will now be listed as an Add-on. Ensure that the Active button is ticked beside the SBGN-ED entry to the list.
2.1.4.5 Click 'OK' on the 'Add-on Manager'. The software is now installed.
2.1.4.6 From the menus at on the top of the VANTED window, select File>Open and choose your downloaded file with the '.sbgn' file extension, in the usual way.
2.1.5 Manual installing the SBGN-ML add-on
2.1.5.1 *From* http://vanted.ipk-gatersleben.de/ Select Add-ons and then 'SBGN-ED – Editing, Translating and Validating of SBGN Maps'.
2.1.5.2 Select 'Download & Installation' and then 'SBGN-ED' under downloads. A file called sbgn-ed.jar should start to download.
2.1.5.3 Return to Vanted and in the right hand column select the 'Help' tab and then the 'Settings' tab beneath. Click on the 'Install/Configure Add-ons' button. This will open the Add-on Manager.
2.1.5.4 Click the 'Install Add-on' button and select the sbgn-ed.jar file downloaded previously. Click the 'Install' button. This will return you to the 'Add-on Manager'.
2.1.5.5 A message will appear the top of the Add-on Manager window stating that 'Add-on ''sbgn-ed.jar'' will be updated when application is restarted'. Select OK and quit the program, before relaunching it.
2.1.5.6 From the menus at on the top of the VANTED window, select File>Open and choose your downloaded file with the '.sbgn' file extension, in the usual way.

2.2. Accessing supplementary SBGN-ML files using the Cytoscape software tool

2.2.1 Download and install Cytoscape from http://www.cytoscape.org/.
2.2.2 Open Cytoscape and select the Plugins menu followed by 'Manage Plugins'.
2.2.3 In the search bar, type sbgn and hit return. Folders will appear in the window and under 'Available for install' will appear a Utility folder.
2.2.4 Open the utility folder and select the latest version of CySBGN before hitting the install button. The CySBGN plugin will then be downloaded and installed. Once it is installed, close the 'Manage Plugins' window.
2.2.5 From the File menu select import followed by 'Network (Multiple File Types)'. In the window that opens, make sure that the 'Local' option is chosen and high the 'Select' button to bring up a file selector. Choose the downloaded file with the 'sbgn' file extension in the usual way.

3. Results and discussion

3.1. Pathway maps

Fig. 1 shows the mevalonate arm of the cholesterol biosynthesis pathway and includes enzymatic activity in the mitochondria, peroxisome, cytoplasm and endoplasmic reticulum. The arm starts with the consumption of acetyl-CoA, which occurs in parallel in three cell compartments (the mitochondria, cytoplasm and peroxisome) and terminates with the production of squalene in