Phase II - Conjugation of compounds

D'Eustachio, P., Jassal, B.

European Bioinformatics Institute, New York University Langone Medical Center, Ontario Institute for Cancer Research, Oregon Health and Science University.

The contents of this document may be freely copied and distributed in any media, provided the authors, plus the institutions, are credited, as stated under the terms of Creative Commons Attribution 4.0 International (CC BY 4.0) License. For more information see our license.

This is just an excerpt of a full-length report for this pathway. To access the complete report, please download it at the Reactome Textbook.

16/11/2022
Introduction

Reactome is open-source, open access, manually curated and peer-reviewed pathway database. Pathway annotations are authored by expert biologists, in collaboration with Reactome editorial staff and cross-referenced to many bioinformatics databases. A system of evidence tracking ensures that all assertions are backed up by the primary literature. Reactome is used by clinicians, geneticists, genomics researchers, and molecular biologists to interpret the results of high-throughput experimental studies, by bioinformaticians seeking to develop novel algorithms for mining knowledge from genomic studies, and by systems biologists building predictive models of normal and disease variant pathways.

The development of Reactome is supported by grants from the US National Institutes of Health (P41 HG003751), University of Toronto (CFREF Medicine by Design), European Union (EU STRP, EMI-CD), and the European Molecular Biology Laboratory (EBI Industry program).

Literature references


Reactome database release: 82

This document contains 7 pathways (see Table of Contents)
Phase II - Conjugation of compounds

Stable identifier: R-HSA-156580

Phase II of biotransformation is concerned with conjugation, that is using groups from cofactors to react with functional groups present or introduced from phase I on the compound. The enzymes involved are a set of transferases which perform the transfer of the cofactor group to the substrate. The resultant conjugation results in greatly increasing the excretory potential of compounds. Although most conjugations result in pharmacological inactivation or detoxification, some can result in bioactivation. Most of the phase II enzymes are located in the cytosol except UDP-glucuronosyltransferases (UGT), which are microsomal. Phase II reactions are typically much faster than phase I reactions therefore the rate-limiting step for biotransformation of a compound is usually the phase I reaction.

Phase II metabolism can deal with all the products of phase I metabolism, be they reactive (Type I substrate) or unreactive/poorly active (Type II substrate) compounds. With the exception of glutathione, the conjugating species needs to be made chemically reactive after synthesis. The availability of the cofactor in the synthesis may be a rate-limiting factor in some phase II pathways as it may prevent the formation of enough conjugating species to deal with the substrate or it's metabolite. As many substrates and/or their metabolites are chemically reactive, their continued presence may lead to toxicity.

Literature references


Editions

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004-11-29</td>
<td>Authored</td>
<td>Jassal, B.</td>
</tr>
<tr>
<td>2008-05-19</td>
<td>Edited</td>
<td>Jassal, B.</td>
</tr>
</tbody>
</table>
Glucuronidation

Location: Phase II - Conjugation of compounds

Stable identifier: R-HSA-156588

Glucuronidation conjugation utilizes UDP-glucuronosyltransferases (UGTs; EC 2.4.1.17) to catalyze a wide range of diverse endogenous and xenobiotic compounds. Glucuronidation is the major pathway in phase II metabolism and accounts for approximately 35% of drug conjugation. UGTs are microsomal membrane-bound and catalyze the transfer of a glucuronate group of uridine diphosphoglucuronate (UDPGA, a co-substrate) to the functional group of specific substrates. UDPGA is synthesized from glucose-1-phosphate (G1P). G1P is required for glycolysis and is present in high concentrations in the cell, making it unlikely to be a limiting factor in UDPGA synthesis. UDP is added to G1P to form UDP-glucose which is then dehydrogenated to form UDPGA. The basic reaction is

$$\text{UDP-Glucuronate} + \text{acceptor} \rightarrow \text{UDP} + \text{acceptor-\beta-D-glucuronide}$$

The effect of this conjugation is to confer polarity to the substrate which can then be easily excreted in urine or bile. Functional groups acted on include hydroxyl, carboxylate, amino and sulfate groups. There are 2 families of UGTs, UGT1 and UGT2 which are further sub-divided into 3 subfamilies, UGT1A, UGT2A and UGT2B. There are more than 26 different isozymes in humans, of which 18 are functional proteins. They are composed of 527-530 residues and have a molecular weight of 50-57KDa.

The UGT1 family comprises of 9 proteins (UGT1A1, 1A3-1A10) but only 5 have been isolated in humans. Example substrates which are glucuronidated are acetaminophen by UGT1A6 and bilirubin by UGT1A1. Members of the UGT2 subfamily are each encoded by their own genes, in contrast to UGT1As which are encoded at the UGT1 locus. Example substrates are morphine conjugation by UGT2B7 and androgenic steroid conjugation by UGT2B17.

Xenobiotics conjugated with glucuronic acid can be substrates for beta-glucuronidase, an enzyme common in gut microflora. This enzyme can release the parent or phase I metabolite which can be reabsorbed. It can then either re-exert it's original effects or be conjugated by glucuronic acid again. This cycle is called enterohepatic circulation and can delay the elimination of the xenobiotic.

Literature references


### Editions

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004-11-29</td>
<td>Authored</td>
<td>Jassal, B.</td>
</tr>
<tr>
<td>2008-05-19</td>
<td>Edited</td>
<td>Jassal, B.</td>
</tr>
</tbody>
</table>
Two groups of sulfotransferase (SULT) enzymes catalyze the transfer of a sulfate group from 3-phosphoadenosine 5-phosphosulfate (PAPS) to a hydroxyl group on an acceptor molecule, yielding a sulfonated acceptor and 3-phosphoadenosine 5-phosphate (PAP). One is localized to the Golgi apparatus and mediates the sulfonation of proteoglycans. The second, annotated here, is cytosolic and mediates the sulfonation of a diverse array of small molecules, increasing their solubilities in water and modifying their physiological functions. There are probably thirteen or more human cytosolic SULT enzymes; eleven of these have been purified and characterized enzymatically, and are annotated here (Blanchard et al. 2004; Gamage et al. 2005). These enzymes appear to be active as dimers. Their substrate specificities are typically broad, and not related in an obvious way to their structures; indeed, apparently orthologous human and rodent SULT enzymes can have different substrate specificities (Glatt 2000), and none has been exhaustively characterized. The substrates listed in the table and annotated here are a sample of the known ones, chosen to indicate the range of activity of these enzymes and to capture some of their known physiologically important targets. Absence of a small molecule - enzyme pair from the table, however, may only mean that it has not yet been studied.

**Literature references**


Acetylation

Location: Phase II - Conjugation of compounds

Stable identifier: R-HSA-156582

N-acetyltransferases (NATs; EC 2.3.1.5) utilize acetyl Co-A in acetylation conjugation reactions. This is the preferred route of conjugating aromatic amines (R-NH2, converted to aromatic amides R-NH-COCH3) and hydrazines (R-NH-NH2, converted to R-NH-NH-COCH3). Aliphatic amines are not substrates for NAT. The basic reaction is

\[ \text{Acetyl-CoA} + \text{an arylamine} \rightarrow \text{CoA} + \text{an N-acetylarylamine} \]

NATs are cytosolic and in humans, 2 isoforms are expressed, NAT1 and NAT2. A third isoform, NATP, is a pseudogene and is not expressed. The NAT2 gene contains mutations that decrease NAT2 activity. This mutations was first seen as slow acetylation compared to the normal, fast acetylation of the antituberculosis drug isoniazid. Incidence of the slow acetylator phenotype is high in Middle Eastern populations (70%), average (50%) in Europeans, Americans and Australians and low in Asians (<25% in Chinese, Japanese and Koreans). N-acetylation and methylation pathways differ from other conjugation pathways in that they mask an amine with a nonionizable group so that the conjugates are less water soluble than the parent compound. However, certain N-acetylations facilitate urinary excretion.

N-acetylation occurs in two sequential steps via a ping-pong Bi-Bi mechanism. In the first step, the acetyl group from acetyl-CoA is transferred to a cysteine residue in NAT, with consequent release of coenzyme-A. In the second step, the acetyl group is released from the acetylated NAT to the substrate, subsequently regenerating the enzyme.

Literature references


Methylation

**Location:** Phase II - Conjugation of compounds

**Stable identifier:** R-HSA-156581

Methylation is a common but minor pathway of Phase II conjugation compared to glucuronidation or sulfonation. The cofactor used in methylation conjugation is S-adenosylmethionine (SAM). SAM is the second most widely used enzyme substrate after ATP and is involved in a wide range of important biological processes. SAM is synthesized from methionine's reaction with ATP, catalyzed by methionine adenosyltransferase (MAT). There are two genes, MAT1A and MAT2A, which encode for two homologous MAT catalytic subunits, 1 and 2.

During conjugation with nucleophilic substrates, the methyl group attached to the sulfonium ion of SAM is transferred to the substrate forming the conjugate. SAM, having lost the methyl moiety, is converted to S-adenosylhomocysteine (SAH). SAH can be hydrolyzed to form adenosine and homocysteine. Homocysteine can either be converted to glutathione or methylated to form methionine, thus forming the starting point for SAM synthesis and completing the cycle.

Functional groups attacked are phenols, catechols, aliphatic and aromatic amines and sulfhydryl-containing groups. The enzymes that catalyze the transfer of the methyl group to these functional groups are the methyltransferases (MT). MTs are small, cytosolic, monomeric enzymes that utilize SAM as a methyl donor. There are many MTs but the best studied ones are named on the basis of their prototypical substrates: **COMT** (catechol O-methyltransferase), **TPMT** (thiopurine methyltransferase), **TMT** (thiol methyltransferase), **HNMT** (histamine N-methyltransferase) and **NNMT** (nicotinamide N-methyltransferase). An example of each enzyme mentioned is given. In each case, a typical substrate for the enzyme is shown.

**Literature references**


https://reactome.org

**Editions**

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005-02-03</td>
<td>Authored</td>
<td>Jassal, B.</td>
</tr>
<tr>
<td>2008-05-19</td>
<td>Edited</td>
<td>Jassal, B.</td>
</tr>
</tbody>
</table>
Glutathione S-Transferases (GSTs; EC 2.5.1.18) are another major set of phase II conjugation enzymes. They can be found in the cytosol as well as being microsomal membrane-bound. Cytosolic GSTs are encoded by at least 5 gene families (alpha, mu, pi, theta and zeta GST) whereas membrane-bound enzymes are encoded by single genes. Soluble GSTs are homo- or hetero-dimeric enzymes (approximately 25KDa subunits) which can act on a wide range of endogenous and exogenous electrophiles. GSTs mediate conjugation using glutathione (GSH), a tripeptide synthesized from its precursor amino acids gamma-glutamate, cysteine and glycine. A generalized reaction is

\[ RX + GSH \rightarrow HX + GSR \]

Glutathione conjugates are excreted in bile and converted to cysteine and mercapturic acid conjugates in the intestine and kidneys. GSH is the major, low molecular weight, non-protein thiol synthesized \textit{de novo} in mammalian cells. As well as taking part in conjugation reactions, GSH also has antioxidant ability and can metabolize endogenous and exogenous compounds. The nucleophilic GSH attacks the electrophilic substrate forming a thioether bond between the cysteine residue of GSH and the electrophile. The result is generally a less reactive and more water-soluble conjugate that can be easily excreted. In some cases, GSTs can activate compounds to reactive species such as certain haloalkanes and haloalkenes. Substrates for GSTs include epoxides, alkenes and compounds with electrophilic carbon, sulfur or nitrogen centres. There are two types of conjugation reaction with glutathione: \textit{displacement reactions} where glutathione displaces an electron-withdrawing group and \textit{addition reactions} where glutathione is added to activated double bond structures or strained ring systems.

**Literature references**

Amino Acid conjugation

Location: Phase II - Conjugation of compounds

Stable identifier: R-HSA-156587

Xenobiotics that contain either a carboxylic group or an aromatic hydroxylamine group are possible substrates for amino acid conjugation. Xenobiotics with a carboxylic group conjugate with an amino group of amino acids such as glycine, taurine and glutamine. The hydroxylamine group conjugates with the carboxylic group of amino acids such as proline and serine. The amino acid is first activated by an aminoacyl-tRNA-synthetase which then reacts with the hydroxylamine group to form a reactive N-ester. N-esters can degrade to form electrophilic nitrenium (R-N^+ -R') and carbonium (R-C^+ H_2) ions. The pyrolysis product of tryptophan, an N-hydroxy intermediate, can potentially form these reactive electrophilic ions.

Literature references


Editions

<table>
<thead>
<tr>
<th>Date</th>
<th>Action</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005-03-10</td>
<td>Authored</td>
<td>Jassal, B.</td>
</tr>
<tr>
<td>2008-05-19</td>
<td>Edited</td>
<td>Jassal, B.</td>
</tr>
</tbody>
</table>
## Table of Contents

- **Introduction**  
- **Phase II - Conjugation of compounds**  
  - Glucuronidation  
  - Cytosolic sulfonation of small molecules  
  - Acetylation  
  - Methylation  
  - Glutathione conjugation  
  - Amino Acid conjugation

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Phase II - Conjugation of compounds</td>
<td>2</td>
</tr>
<tr>
<td>Glucuronidation</td>
<td>3</td>
</tr>
<tr>
<td>Cytosolic sulfonation of small molecules</td>
<td>5</td>
</tr>
<tr>
<td>Acetylation</td>
<td>6</td>
</tr>
<tr>
<td>Methylation</td>
<td>8</td>
</tr>
<tr>
<td>Glutathione conjugation</td>
<td>10</td>
</tr>
<tr>
<td>Amino Acid conjugation</td>
<td>11</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>12</td>
</tr>
</tbody>
</table>