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medical & biological sciences

Pyrimidine Biosynthesis

PYRIMIDINE BIOSYNTHESIS

• Here, we will draw the process of pyrimidine biosynthesis.

OVERVIEW

Pyrmidine biosynthesis occurs via two key pathways:

De novo synthesis

• Involves ring synthesis followed by PRPP (activated ribose (phosphorylated ribose) attachment in the formation of the nucleotide.

- Ring synthesis involves bicarbonate, ammonia, and 2 ATP.

- These components undergo a reaction that is catalyzed by CPS 2 (cabamoyl phosphate synthetase 2).

- The next set of necessary components are aspartate and NAD+.

Salvage pathway

- Means the base is reincorporated into the nucleotide.

Nucleoside vs Nucleotide

• A nucleoside is a BASE + a SUGAR.

• A nucleotide is a BASE + a SUGAR + PHOSPHATE

5-phosphoribosyl-1-pyrophsophate (PRPP) formation

Also, let's review the formation of 5-phosphoribosyl-1-pyrophsophate (aka 5'-phosphoribosyl-1'-pyrophosphate), commonly abbreviated: PRPP or activated ribose (meaning it's activated to accept nucleic bases).

• Indicate that Ribose 5-Phosphate (R5P) converts to PRPP via PRPP synthetase, which catalyzes the addition of 2 phosphate (a pyrophosphate) from ATP, which then converts to AMP.

The Liver & Pyrimidine Ring Production

For reference, we now show the liver produce a standard pyrimidine ring, so we can refer to it throughout the production of the ring we will build during this tutorial.

• The liver is the primary organ that produces nucleic acids – we can remember this because it manages ammonia, and as we'll see ammonia is a key component of purine biosynthesis.

- Although "pyrimidine" is a longer name, it is the smaller of the two bases whereas purine (the shorter name) is the larger of the two bases:

- This is easy to misremember if you don't pay attention!

PYRIMIDINE BIOSYNTHESIS: 4 PARTS

We divide pyrimidine biosynthesis into 4 parts:

• Part 1: Formation of carbamoyl phosphate

• Part 2: Carbamoyl phosphate conversion to ororotate

• Part 3: Ororotate combination with PRPP to form orotidylate (OMP)

• Part 4: Formation of the uracil base of UMP, the thymine base of TMP, and the cytosine base of CTP.

PART 1: FORMATION OF CARBAMOYL PHOSPHATE

de novo synthesis of a pyrimidine

Requires

• 2 ATP

• Bicarbonate (HCO_3^-)

• Ammonia (NH_3) [which is derived from glutamine hydrolysis].

- Again, this is why nucleotide production occurs in the liver, because it is the organ that can best handle nitrogen (ammonia) waste – so it makes sense that if nucleotide synthesis relies on ammonia formation, it ought to occur in a body organ that can best manage ammonia!

Forms

Carbamoyl phosphate

• Show these molecules combine together with a phosphate as carbamoyl phosphate.

• Indicate that 2 ADP and a phosphate are released in the reaction (remember that glutamine hydrolysis, itself, converts

glutamine to glutamate).

• And show that carbamoyl phosphate synthetase II catalyzes the reaction (in the cytosol).

- (In comparison to CPS I, which acts within mitochondria)

PART 2: CARBAMOYL PHOSPHATE CONVERSION TO ORROTATE

• Show that aspartate is added to form carbamoylaspartate.

• A phosphate is released.

• Then, indicate that carbamoylaspartate cyclizes to dihydroorotate.

• And dihydroorotate oxidizes to orotate.

THE PYRIMIDINE RING

So, now we can trace the molecular origins of the pyrimidine ring:

• Show that N1, C6, C5, C4 – all derive from aspartate.

• Show that C2 and N3 derive from carbamoyl phosphate.

• Now, we show the completed orotate molecule, for reference.

Clinical Correlation*: **hereditary orotic aciduria**

- There are elevated levels of orotic acid in the urine from a failure of orotate conversion to uracil, which we will learn about next.

PART 3

Ororotate combination with PRPP to form orotidylate (OMP)

• Redraw orotate

• Then, add it to PRPP: 5-phosphoribosyl-1-pyrophsophate (aka 5'-phosphoribosyl-1'-pyrophosphate).

• First, draw the ribose, draw a pentagon with an oxygen atom inserted at the top.

- Label carbons 1' through 4' going clockwise from the oxygen atom,

- Add carbon 5' as an attachment to carbon 4'.

- Add hydroxyl groups to carbons 2', and 3'.

• Next, add a phosphate to the 5' carbon: hence, Ribose (the sugar), 5-Phosphate).

- We leave the 5' and 3' in different colors, so our attention is brought to the 5' to 3' orientation of the sugar/phosphate backbone.

• At the 1' hydroxyl add a pyrophosphate.

• Now, show that in the combination, the pyrophosphate is released and orotidylate (OMP) is formed:

- We draw it as the PRPP but with the orotate attached at the 1' carbon where the pyrophosphate had previously been.

PART 4

Formation of the uracil base of UMP, the thymine base of TMP, and the cytosine base of CTP

It's important that we skip to the end, now, and show where we want to end up in order to understand the biochemistry of the next steps in pyrimidine biosynthesis.

Uracil

• First, draw uracil, which is the base of UMP

- The decarboxylated monophosphate of OMP.

• Show that it forms the standard pyrimidine ring.

• Then, include the oxygens that were found in orotate at carbons 2 and 4.

• And, then, leave off, for reasons we'll see, the carboxyl, at carbon 6 (it's decarboxylated).

Thymine

• Now, draw thymine, the thymine base of the monophosphate TMP.

- Show that it forms the standard pyrimidine ring.

• Then, include the oxygens that were found in orotate at carbons 2 and 4.

• Again, leave off, for reasons we'll see, the carboxyl, at carbon 6: it's decarboxylated.

- *If this looks exactly the same as uracil, you've drawn the structure correctly.*

- BUT draw a methyl at carbon 5 on the thymine and indicate that thymine is a DNA base whereas uracil is an RNA base and is unmethylated at carbon 5.

Cytosine

• Now, let's draw cytosine, which is the base of the triphosphate, CTP.

• Again, draw the pyrimidine ring.

• Include the oxygen that was found in orotate at carbon 2 but at carbon 4, add an amino group, instead.

- Leave carbon 5 unmethylated.

- Leave carbon 6 decarboxylated.

Orotidylate (OMP) to Uridylate (UMP)

Now, we're ready to see how we get from orotidylate to these three base structures.

• First, show that OMP is then decarboxylated via orotidylate decarboxylase to UMP (uridylate), which is the monophosphate of uracil.

- Consider that without the enzyme orotidylate decarboxylase, it would take ~ 78 million years (a million life-times!) for the decarboxylation to occur.

UTP (Uracil) to CTP (Cytosine)

• Show that uridylate (uridine monophosphate) is phosphorylated to UDP and UTP then that via glutamine hydrolysis ammonia is added to convert UTP to CTP (via CTP synthetase).

UMP to dUMP

• Requires **Ribonucleotide reductase**

- Let's address the catalytic action of ribonucleotide reductase, which is the enzyme that catalyzes the replacement of the 2' hydroxyl group on the ribose moiety with a hydrogen: in this instance, we need it to convert the uridylate (uridine monophosphate) to deoxyuridylate (deoxyuridine monophosphate).

• Indicate that ribonucleotide reductase catalyzes the following reductions where the precursors are:

- ADP, GDP, CDP, UDP

- *and the products are:*

- dADP, dGDP, dCDP, dUDP

• Thus, in our reaction, let's omit the phosphorylation of UMP to UDP and subsequent dephosphorylation that will occur and simply show the role of ribonucleotide reductase in the conversion of uridylate to deoxyuridylate (dUMP), because that will lead us to the key knowledge point that we are converting uracil an RNA base to thymine a DNA (a deoxyribonucleic acid base).

dUMP to dTMP

• Now, show that N5, N10-Methylene-tetrahydrofolate adds a methyl group to deoxyuridylate.

- Note that you'll see thymine indicated either as dTMP (to emphasize that the 2' hydroxyl is deoxygenated or, simply, as TMP.

- Dihydrofolate is released.

Dihydrofolate reductase & Tetrahydrofolate reformation

• For completion, show that dihydrofolate reductase uses NADPH as the reductant to reform tetrahydrofolate.

FULL-LENGTH TEXT

• Here, we will draw the process of pyrimidine biosynthesis.

• To begin, start a table.

• Denote that pyrmidine biosynthesis occurs via two key pathways:

- De novo synthesis, which involves ring synthesis followed by PRPP (activated ribose (phosphorylated ribose) attachment in the formation of the nucleotide.

- Salvage pathway, which means the base is reincorporated into the nucleotide.

• To further our understanding of de novo synthesis, denote that the components necessary for ring synthesis are:

- Bicarbonate,

- Ammonia, and

- 2 ATP

- These components undergo a reaction that is catalyzed by CPS 2 (cabamoyl phosphate synthetase 2). And the next set of necessary components are aspartate and NAD+.

• Let's remind ourselves, now, of some key nucleic acid terminology:

- A nucleoside is a BASE + a SUGAR.

- A nucleotide is a BASE + a SUGAR + PHOSPHATE

Also, let's review the formation of 5-phosphoribosyl-1-pyrophsophate (aka 5'-phosphoribosyl-1'-pyrophosphate), commonly abbreviated: PRPP or activated ribose (meaning it's activated to accept nucleic bases).

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• We divide pyrimidine biosynthesis into 4 parts:

- Part 1: Formation of carbamoyl phosphate

- Part 2: Carbamoyl phosphate conversion to ororotate

- Part 3: Ororotate combination with PRPP to form orotidylate (OMP)

- Part 4: Formation of the uracil base of UMP, the thymine base of TMP, and the cytosine base of CTP.

Part 1:

• Indicate that de novo synthesis of a pyrimidine requires 2ATP + bicarbonate (HCO_3^-) + ammonia (NH_3) which is derived from glutamine hydrolysis.

- Again, this is why nucleotide production occurs in the liver, because it is the organ that can best handle nitrogen (ammonia) waste – so it makes sense that if nucleotide synthesis relies on ammonia formation, it ought to occur in a body organ that can best manage ammonia!

• Then, show these molecules combined together with a phosphate as carbamoyl phosphate.

• Indicate that 2 ADP and a phosphate are released in the reaction (remember that glutamine hydrolysis, itself, converts glutamine to glutamate).

• And show that carbamoyl phosphate synthetase II catalyzes the reaction (in the cytosol).

Part 2:

• Show that aspartate is added to form carbamoylaspartate.

- A phosphate is released.

• Then, indicate that carbamoylaspartate cyclizes to dihydroorotate.

- And dihydroorotate oxidizes to orotate.

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- Show that N1, C6, C5, C4 – all derive from Aspartate.

- Show that C2 and N3 derive from carbamoyl phosphate.

Now, we show the completed orotate molecule, for reference.

• Indicate that in hereditary orotic aciduria, there are elevated levels of orotic acid in the urine from a failure of orotate conversion to uracil, which we will learn about next.

Part 3:

• Redraw orotate.

• Then, add it to PRPP: 5-phosphoribosyl-1-pyrophsophate (aka 5'-phosphoribosyl-1'-pyrophosphate), which allows us to review what we learned in the Nucleic Acids tutorial.

• First, draw the ribose, draw a pentagon with an oxygen atom inserted at the top.

- Label carbons 1' through 4' going clockwise from the oxygen atom,

- Add carbon 5' as an attachment to carbon 4'.

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• At the 1' hydroxyl add a pyrophosphate.

• Now, show that in the combination, the pyrophosphate is released and orotidylate (o-ra-ti-di-late) (OMP) is formed.

- We draw it out below as the PRPP but with the orotate attached at the 1' carbon where the pyrophosphate had previously been.

Part 4:
It's important that we skip to the end, now, and show where we want to end up in order to understand the biochemistry of the next steps in pyrimidine biosynthesis.

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- Show that it forms the standard pyrimidine ring.

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• Show that uridylate (uridine monophosphate) is phosphorylated to UDP and UTP then that via glutamine hydrolysis, ammonia is added to convert UTP to CTP (via CTP synthetase).

Then, in the bottom corner of the page, let's address the catalytic action of ribonucleotide reductase, which is the enzyme that catalyzes the replacement of the 2' hydroxyl group on the ribose moiety with a hydrogen: in this instance, we need it to converts the uridylate (uridine monophosphate) to deoxyuridylate (deoxyuridine monophosphate).

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• For completion, show that dihydrofolate reductase uses NADPH as the reductant to reform tetrahydrofolate.