How are metabolic pathways and reaction chains discovered in living organisms?

3 Answers

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Answered 3 years ago · Upvoted by Roger Morton, PhD. In Plant Molecular Biology, ANU 1994 · Author has **555** answers and **392.4K** answer views

How are metabolic pathways and reaction chains discovered in living organisms?

The pathways that have been elucidated were a combination of radio label tracer studies with tissue homogenates and tissue samples. I read many of the original papers when I was in grad school, but just remember how clever the scientists were in these studies. The analytical methods they used were so simple but yielded elegant results.

Another approach that has been used was done in bacteria and they figured out the pathways using point mutations. I did a rotation in a lab in grad school where we had many different mutants. We had a lot of fun figuring out the steps and the structures of the intermediates. We used many techniques and many years after I left the lab they figured out the entire pathway and all of the intermediates. That three week rotation through that lab got me interested in analytical chemistry.

All these types of studies require multidisciplinary teams.

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What is your favorite metabolic pathway?

How do metabolic pathways work?

What are the experimental approaches for studying a metabolic pathway?

What are the big research areas in metabolic pathways?

Harsh Oza, Integrated PhD Biotechnology, Regional Centre For Biotechnology (2026)
Answered 2 years ago · Upvoted by Rashi Kumar, BSc Biotechnology & Biochemistry, St. Xavier's College, Ahmedabad (2019) · Author has **855** answers and **2M** answer views

What are the experimental approaches for studying a metabolic pathway?

For elucidating the metabolic pathways we need to discover the sequence of the pathway, the reaction mechanism, and the regulatory mechanism of that pathway.

Let us discuss some of the ways we can study and elucidate the molecular pathways with some historical examples.

→ Identify the organ or organelle.

The very important step in elucidating the molecular pathway is to know the location where this series of reaction is occurring. The organs or organelles which are carrying out the reactions need to be identified before going for any further studies.

In 1880s, death due to diabetes was very common. However, very less information related to cause of diabetes was known. The researchers of that era only knew that diabetes was associated with eating too much. In 1921, Banting and Best discovered insulin and the organ associated with diabetes. They did the experiment on dogs. The

the former ones did. After that, they isolated and prepared a homogenous mixture from extracts of the pancreas which was injected into a human suffering from diabetes [1].

Organelle separation was difficult until the 1960s when Christian de Duve discovered organelles like peroxisomes, lysosomes, etc. [2].

→ **Perfusion technique.**

It is one of the old techniques used by many many researchers to study the metabolic reactions. Earlier **scientist used pigeon chest muscles for studying the metabolic reactions**. Microbes such as *Mycoplasm gallisepticum* were also used to study the glucose metabolism using NMR and continuous perfusion technique [3]. Otto Warburg's manometer is often included in such experiments.

→ **Labelling the biomolecules with Radioactive and Heavy isotopes.**

Heavy isotopes and radioisotopes such as P-32, S-35, C-14, I-131, N-15, H-3, H-2, C-13, Ca-46, O-18, Cobalt, etc. have been used very frequently to distinguish the molecules of interest from the non-labeled molecules.

Meselson and Stahl used **N-14 and N-15 isotopes to deduce the semi-conservative replication** of the DNA [4].

Georg Franz Knoop (who discovered the beta-oxidation of fatty acid) used **chemical labels to study the metabolic pathway**. In his experiment, he used the phenyl group to label the ω-ends of the fatty acid. He fed two types of fatty acids to the dogs- ω-phenylvaleric acid and ω-phenylbutyric acid. The urine of the dogs was analyzed which gave hippuric acid when dogs were fed with odd-chain fatty acid and phenaceturic acid with even fatty acid. This concluded that fatty acids are broken into two carbons at a time during metabolism [5][6].

Arsenate which is the structural analog of phosphate was also used for the studying the thermodynamic aspects of glycolysis [7] and other metabolic pathways associated with the inorganic phosphate. In the sixth step of glycolysis, i.e. conversion glyceraldehyde 3-phosphate to 1,3 bisphosphoglycerate, inorganic phosphate is required. In absence of Pi, the reaction does not happen; however, when arsenate is added, there is the formation of 1,3 bisphosphoglycerate indicating the role of inorganic phosphate in that step.

Paul D. Boyer used **O-18 oxygen for studying the ETC** (electron transport chain), synthesis of ATP by ATP synthase [8].

Radioisotopes was promoted by Rosalyn Yalow when she developed the radioimmunoassay technique[1].

Dorothy Hodgkin used cobalt isotope for finding the vitamin B12[2].

→ **Fluorescence tagging.**

This is one of the most widely used technique nowadays. Tagging the enzyme or the substrate with the GFP or any other fluorescent tag allows the researchers to trace the substrate, its conversion, location where it is metabolized, etc.

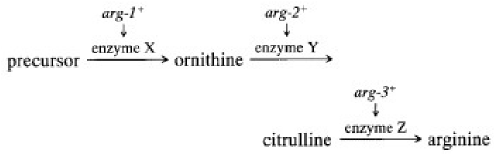
FACS (Fluorescence activated Cell Sorters) has made the isolation of selected cells from the cell culture.

→ **Mutants (by gene silencing/knocking).**

various physical and chemical ways.

Beadle and Tatum gave the one gene one protein hypothesis. If any gene for enzyme belonging to a multi-step pathway is inactivated, then the whole metabolic pathway was altered.

> The *arg-1* mutants have a defective enzyme X, so they are unable to convert the precursor into ornithine as the first step in producing arginine. However, they have normal enzymes Y and Z, and so the *arg-1* mutants are able to produce arginine if supplied with either ornithine or citrulline. The *arg-2* mutants lack enzyme Y, and the *arg-3* mutants lack enzyme Z. Thus, a mutation at a particular gene is assumed to interfere with the production of a single enzyme. The defective enzyme, then, creates a block in some biosynthetic pathway. The block can be circumvented by supplying to the cells any compound that normally comes after the block in the pathway[3].



→ **Inhibitor.**

Inhibition of the enzymes is one of the ways to deduce the metabolic pathway and the role of that enzyme. **Idoacetate was used to find the activity of aldolases**. This reagent inactivates the aldolases which caused the accumulation of Frc-1,6-phosphate, Frc 6-phosphate, and Glc 6-phosphate.

Fluorides were used to inactivate the enolase enzyme which causes the accumulation of 3-phosphoglycerate and 2-phosphoglycerate.

→ **Reporter gene.**

The reporter genes[4] are used by researchers for knowing the expression of any gene whose regulatory sequence has this gene. PEP carboxykinase is one of the essential enzymes in gluconeogenesis. The reporter gene used for studying the conditions required for the gene to express was a growth hormone gene. It was found that when glucose levels in the cells fall and protein levels are more, gluconeogenesis in the cell promoted which was concluded by the rise in GH in the blood of a model organism used.

There are many other ways which are used or yet to be discovered for studying and elucidating the metabolic pathways. Bioinformatics has also a role to ease the study and research work in the field of biochemistry.

References

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Footnotes

[1] Rosalyn Sussman Yalow - Wikipedia

[2] Dorothy Hodgkin - Wikipedia

[3] How genes work - An Introduction to Genetic Analysis - NCBI Bookshelf

[4] Reporter gene - Wikipedia

Niharika Dixith, I think, therefore I am.
Updated 1 year ago

What makes the study of metabolic pathways interesting?

I have been asking myself the same question for quite some time now.

My initial impressions of the recommended biochemistry textbook were far from positive. Enzymes, equations, cofactors and pathways. It all seemed so dry. Why would I need to learn all of this anyway?

I did grasp the 'big picture' importance of it. I did realise that the interplay of chemicals is the reason life even exists. I just didn't find the details particularly interesting.

I wondered why I was bored. This quote came to my rescue -

> *the root of boredom is an absence of meaning.*

I wasn't really understanding what I read. I was mostly just memorising. I was too preoccupied with the specifics to Cori-late things (pun intended). And that's why I found the study of metabolic pathways boring.

But now that I've learnt the basics, I can see past the clutter and examine the grand design.

And it is quite intriguing.

It's nice to know that there's an order to the things that happen inside us at the micro-level. We are not just "bags of random chemicals".

There are patterns.

Elegant steps that nature has evolved to keep the engine of life running.

Simple (once familiarised, of course) pathways in which if a single step is hindered, the effects can manifest in dangerous ways. Knowledge of these has been extremely useful in medical science! The mechanisms of a great number of treatments and medications have their principles rooted in biochemistry.

I cannot, even jokingly, say that my understanding of biochemistry is up to the mark. But atleast I no longer sigh at that page in my textbook with a flowchart of the notorious Krebs' cycle. I am amazed instead - how on earth did this even evolve? This is the end result of time, lots and lots of time, and chance. And the hard work and dedication of many researchers, let's not forget.

tl;dr *The study of metabolic pathways is interesting because* ***if you give it enough time and attention****, you will be rewarded with a series of very satisfying Eureka moments. And there is no doubt that knowledge of these is relevant, with a lot of scope for application, and a good measure of unanswered questions to trigger curiosity. The only reason one might find it boring is if they harbour some sort of vague, slightly irrational distaste towards it (that's okay, it happens), or maybe…something to do with that pesky conditional above, in bold?*

Related Questions

How were metabolic pathways discovered?

What is your favorite metabolic pathway?

How do metabolic pathways work?

What are the experimental approaches for studying a metabolic pathway?

What are the big research areas in metabolic pathways?

What are the three pathways of breakdown in living organisms?

What are some of the most common examples of metabolic pathways?

What makes the study of metabolic pathways interesting?

What are the best model systems for studying metabolic pathways?

What is the best topic of research in metabolic pathways?

How do I learn all the metabolic pathways?

How many metabolic pathways are there?

What are the roles of metabolism in living organisms?

What are the most promising untapped metabolic pathways?

What are the 7 participants in metabolic reactions and the 2 different types of metabolic pathways?