Genetic Engineering

Luciferase

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1. Preface

When we first started researching the topic genetic engineering, we came across a genetically modified species of zebra fish that would glow in the dark. We looked into that and soon found out that they aren't only used for entertainment, but also for scientific purposes. We were fascinated by that technique of making parts of a cell or an entire animal glow and decided to focus on that topic.
At first we wanted to focus on the green fluorescent protein (GFP), but after contacting Novartis about that topic we were suggested to look into luciferase, a similar but still fundamentally different scientific area. We decided to write about luciferase because we came to the conclusion that we believe it to be a more interesting research topic with other scientific applications. What makes luciferase so interesting is the fact that it makes cellular activity visible and it lets you follow cellular growth and transportation of molecules.
Our goal was to find out how you can modify DNA so that it will synthesize proteins that will glow in the dark. Furthermore, we wanted to find out how and for what purpose this method was developed and to which scientific findings it led or will lead in the future.

2. Introduction

Luciferase is an enzyme responsible for a form of bioluminescence that has been known for more than two thousand years. It was first discovered in fireflies because they glow in the dark. People have been fascinated by those glowing animals ever since, but it wasn't until the 17th century that scientists started researching this topic. In 1667, Robert Boyle found out that air is required for luminescence. Years later, Raphaël Dubois introduced the terms luciferin and luciferase. Another scientist who documented observations of luciferase was Charles Darwin; he saw glowing waves. Today we know that what he saw were dinoflagellates in the ocean. (1)

The first scientist to discover that multiple forms of luciferase and luciferin exist was E. Newton Harvey in the 20th century. He also found out that luciferins and luciferases are species-specific and not interchangeable. Harvey took this as an evidence for evolution. (2)

Nowadays, luciferase is a widely researched topic and is mainly used to visualize cellular activity over time, cell growth and transport systems.

At the moment, one of the main goals of scientists is to find a luciferase with a smaller gene sequence and a smaller corresponding luciferin molecule that sets free more photons in the reaction, thus producing more light and making it possible to visualize even smaller activities. In 2008, a US-American team of scientists was able to visualize fewer than ten mouse T cells with an enhanced firefly luciferase in immunocompetent mouse models of cancer. The sensitivity was increases 200-400 times, making it possible to monitor gene expression even from just three T cells. (3)

The main research areas are monitoring toxins and also to study pathways of cell that are over or under activated in diseases. Using this knowledge, scientists are hoping to find cures for cancer and other diseases (interview; question 5).

An alternative treatment is for example the green fluorescent protein (GFP) but there are fundamental differences. The main difference between those two methods is that GFP is fluorescent and luciferase is luminescent, meaning that unlike luciferase, GFP is not able to produce light on its own. Also, GFP is not an enzyme and therefore can't amplify the reaction.

3. Description of engineering technique

The first step is the location and then the isolation of the genes of interest using polymerase chain reaction (PCR). After the isolation, the gene sequence is inserted into a vector and then the vector is transferred into the organism to be modified. Then, the cells of the organism integrate the gene sequence into their own DNA. At last, the successfully modified organisms have to be separated from the unsuccessfully modified organisms.

In our chosen topic, luciferase, the gene sequence can be found in different organisms, for example in fireflies or in dinoflagellates. After successfully evolving the modified cells, luciferase enzymes are being produced. For the light emitting reaction, luciferin and ATP are required. ATP is already in the cells, however in many research applications extra ATP is added to the reaction to increase the amount of light generated. Luciferin, on the other hand, can't be produced by the cell itself and therefore has to be synthesized in the lab. It is then added into the organism and will diffuse into the cells.

In the reaction (called reporter gene assay), luciferin reacts with oxygen and ATP and turns into oxyluciferin. The reactions sets free energy in form of visible light. Other products are CO_2, AMP and PP1. Luciferase and Magnesium ions (Mg^{2+}) are required as catalysts to run the reaction. This is only the case if beetle luciferin and the corresponding luciferase (firefly luciferase) are used. Other luciferases require different luciferins and thus may also have different byproducts.

In the lab, the emitted light can't be seen by eye but the signal intensity (photons per time) can be measured by extremely sensitive devices. Because luciferases generate light on their own, background radiation is very low. Due to that, measurements are very precise and barely biased.

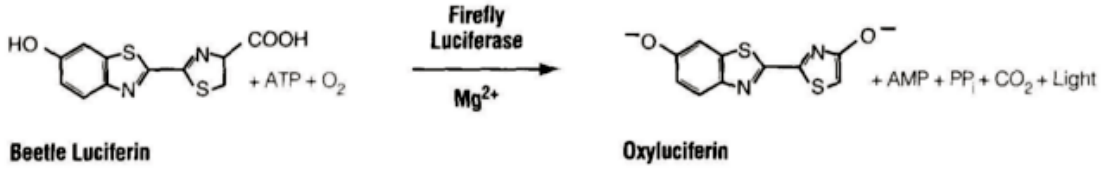


Figure 1 light emitting reaction of luciferin with luciferase

4. Documentation

Interview with Dr. Christian Parker from Novartis:

1. *How do you insert luciferin and ATP into a living cell without destroying it?*
   ATP is already in the cell as the "universal" energy currency and luciferin is actually able to diffuse into cells (in a similar way to how drugs get into cells).

2. *What are the advantages and disadvantages of luciferases that require ATP compared to those which don't need ATP?*
   I think all luciferase enzymes used for research purposes require ATP. I think there are some bacterial luciferases that use a special flavin mononucleotide. You cannot use them as a reporter gene in mammalian cells as the flavin mononucleotide is too large to diffuse into the cell like Luciferin can.

3. *Is there an advantage of having different luciferases with different properties?*
   Yes, the most common use is to have one luciferase be linked to the pathway of interest and another linked to cell growth or number so that you can normalize the signal from the pathway for effects on cell growth - or death if there is toxicity. There are also luciferases with different colors that allow you to monitor two different pathways at once.

4. *In comparison to GFP, what are the advantages and disadvantages of luciferase?*
   The advantages of luciferases are the reaction is catalytic and the detectors for luminescence are also more sensitive (with a larger dynamic range for detection) than for fluorescence.
   GFP though can be used for things like "lineage tracing" where you express the GFP in a certain cell type and then it is switched off as the cells grow and change and you can track which cells have grown from those original cells.

5. *How can you use the knowledge about luciferase to improve daily life?*
   Reporter gene assays using luciferase readouts have been used to generate assays to monitor toxins such as heavy metals or pesticides in water.
   But we use them to look at pathways in cells that are over or under activated in a

disease and then look for ways to regulate these pathways back to what they should be. This has applications in all sorts of diseases from different types of cancer to lung diseases, types of kidney diseases and even immune diseases.

6. *What adaptations do you expect in the future?*
   It's almost impossible to imagine. If you had asked me this just last year I could not have told you about the new "nanoluciferases" that have been found and are less than 20kDa (i.e. very small enzymes) and which are even brighter. There are now examples of "split" luciferases where the enzyme only becomes active when the two halves are held close together on a macromolecule complex, so you could "watch" complexes such as the ribosome coming together and working one day.
   The biggest advance will be if the instrument makers can combine FACS sorting with single cell Luminescence (LACS). That would be great.

7. *Is there hope to find a cure for any kind of human diseases with the help of luciferase?*
   I mentioned that earlier and to boast a bit my group ran screens looking for inhibitors of the pathway that drives fibrosis in lung diseases. We also helped the project team find a compound that is starting clinical trials

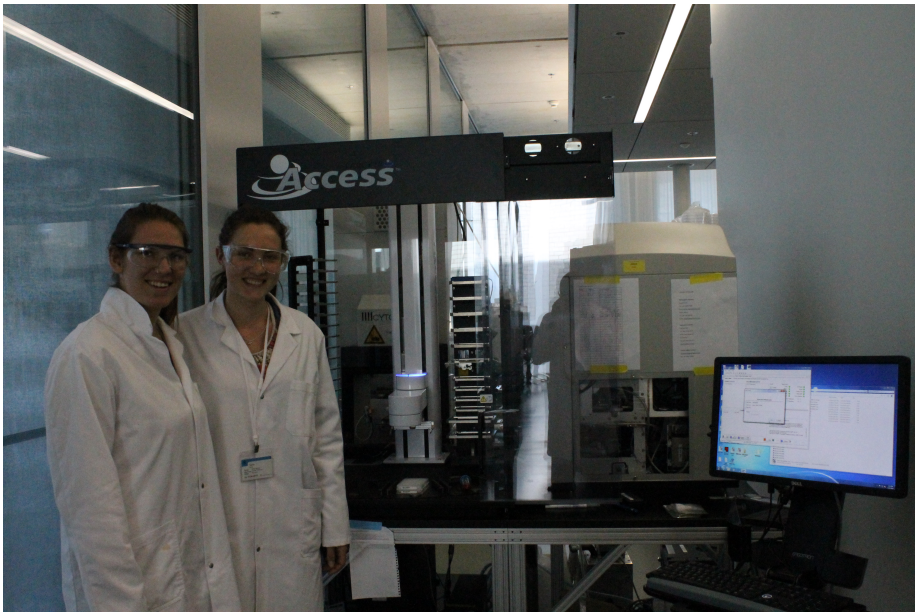
8. *Is it possible to genetically manipulate organisms so that they will synthesize luciferin on their own?*
   It might be possible (probably is) but as Luciferin is small and easy to synthesize, so we let the chemists do that.

9. *Does luciferase influence the metabolism and behavior of animals in any kind of way? If yes, how?*
   Not that I've seen on reporter gene assays (but I have to admit that we do not have the time to investigate "strange" things). Remember though if you are a male firefly or a Click Beetle it will influence your metabolism.

10. *Are there any ethical conflicts about researches in luciferase?*
    I don't think so but people do object to genetic engineering, which you have to use to make reporter gene assays with. Such people often also think the earth is flat, and

that women are the property of their farther or husbands.

We visited Novartis Campus on March 26th and April 11th 2014. Below are our pictures of the lab visitation. On April 11th, we were able to observe a reporter gene assay.



Picture 1 At Novartis, one of the machines required for reporter gene assays

Picture 2 Test results. The machine on the right measures light emission.

5. Discussion

Luciferase is a widely research topic that has gained a lot of attention over the past few years. The knowledge attained from reporter gene assays has enabled scientists to do research on a whole new level. With the help of luciferase assays, essential molecular processes could be understood better and for the first time, researchers were actually able to observe activities in organism over a period of time.
Apart from the application examples given earlier, reporter gene assays with luciferase are also used for many other purposes. Those include the study of the regulation of gene expression in eukaryotes, in blood banks to determine if red blood cells are starting to break down, or even to substitute streetlights by glowing trees.
It is very hard to forecast in which direction future researches will go because there are so many possibilities. One of the most promising projects than scientists are working on right now is the “Glowing Plant Project”. They are developing glowing trees that could at some point replace streetlights, thus saving energy and reducing CO_2 emissions. (4)
From Dr. Parker we learned about another research topic: nanoluciferases. Those luciferases are a lot smaller and a lot brighter than other luciferases.
One of the ethical advantages of luciferase is that it is only used for a scientific purpose and not a commercial one. Like every other genetically engineered organism, there is an ethical conflict when it comes to lab animals but there’s not a specific conflict about luciferase. However, if the Glowing Plant project can be realized and will be commercially used, ethical conflicts are most likely going to come up.

6. Summary

Luciferase is an enzyme that belongs to bioluminescence. With the help of genetic engineering, the enzyme can be inserted into living organisms. When luciferin is added to one of those genetically modified cells, a reaction is going to happen in which luciferin is oxidized and energy in form of visible light is set free. Computers can measure the amount of emitted photons. That enables scientists to study cellular activity, such as growth and transportation.

The knowledge of luciferase has found many applications in today's world. Those include disease research, regulation of gene expression, monitoring toxins and many more.

The topic has gained worldwide interest over the last few years. Scientists assume that with the help of luciferase, many more groundbreaking applications will follow in the future.

7. References

(1) http://guides.library.harvard.edu/content.php?pid=419040&sid=3493478. Accessed 17.04.2014, 16:43.

(2) http://www.nndb.com/people/079/000169569/. Accessed 17.04.2014, 16:58.

(3) http://www.pnas.org/content/105/38/14342.full. Accessed 22.04.2014, 15:37.

(4) http://www.glowingplant.com. Accessed 22.04.2014, 17:21.

General sources:

http://www.encyclopedia.com/topic/Luciferase.aspx. Accessed 22.04.2014, 17:33.

http://pubs.acs.org/doi/abs/10.1021/cb3007264. Accessed 26.04.2014, 16:19.

http://bitesizebio.com/10774/the-luciferase-reporter-assay-how-it-works/. Accessed 26.04.2014, 16:52.

Pictures:

Figure 1 http://bitesizebio.com/10774/the-luciferase-reporter-assay-how-it-works/. Accessed 26.04.2014, 16:52.

Picture 1, Picture 2 own data

We want to thank Mr. Parker for his big help and for inviting us to observe a reporter gene assay at Novartis.