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What helps plants get just the right amount of iron?

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Abstract

Some things are crucial for life in small doses, but too much of it can be harmful or even fatal. Take salt, for example. We cannot survive without it, but it can poison or even kill us if we eat too much of it. Iron works similarly for many organisms, including plants. Plants cannot grow or function without it, but too much iron can do a lot of damage. Therefore, they need to carefully regulate how much of it they take in. How exactly do plants do this? We looked at how the normal version and a *mutant form* of Thale cress regulate their iron uptake by comparing their gene products, root growth, and the amount and location of chemical gene tags. We found that epigenetic factors are involved in controlling iron uptake. Read on to find out exactly what that means!

Introduction

Plants face two dilemmas when it comes to iron. First, it is essential for their growth and survival; however, plants only need tiny amounts of iron. That's why it's called a **micronutrient** – and too much of it can do a lot of damage. For that reason, plants need to be careful not to take up either too little or too much iron from the soil.

Secondly, even though iron is very common in the ground, it's mostly in a form that cannot be used by plants directly. So plants have to perform a series of chemical reactions to make the iron in the ground accessible.

How do plants deal with these dilemmas? That is, how do they regulate their iron uptake to make sure they get enough but not too much of it? We used genetic analysis of Thale cress, a **model organism**, and one of its **mutant forms** to figure this out.

Like other researchers before us, we knew that **gene regulation** (the process of turning **genes** on or off) controls the uptake of iron and the needed chemical reactions. We already knew the genes that encode proteins, which carry out the chemical reactions involved in iron uptake, and the sequence of DNA base pairs (genetic code) of these genes.

But we also wanted to know if **epigenetic** factors such as **chemical tags** attached to the DNA or the proteins it's wrapped around (which together make up the **chromatin** structure of genetic material) could be involved in turning genes on and off.

Figure 1:
Thale cress (*Arabidopsis thaliana*) is a model organism whose genome has been extensively studied. It has allowed us to find out more about how plants regulate their iron uptake.
Photo:
Bruna, CC BY-SA 3.0

Ever heard of epigenetics? It's a really hot topic in molecular biology and something we still have tons to learn about. "Epi" is Greek for "above" and refers to things other than the regular DNA sequence that determine which genes are turned on or off. These can be chemical tags that attach to DNA or the proteins the DNA is wrapped around, which change how DNA is packaged to fit into the nucleus, a very small compartment within the cell where DNA is kept. This could, for instance, turn genes off by making them inaccessible for the enzymes that read the DNA and make the relevant **gene products**. So while epigenetic factors do not change genes, they change how genes are read and then expressed. And here comes the crazy part: environmental factors or behavior can change these epigenetic markers, and these changes can sometimes even be passed on to the next generation!

Methods

We selected the Thale cress plant (*Arabidopsis thaliana*, Fig. 1) as our study subject because its **genome** has been extensively studied, and we have a good idea what's going on in its body at a **molecular** level.

To get a better understanding of the gene regulation process, we designed the following experiment:

- We took normal Thale cress plants (also called "the wild type") as well as a mutant form (*clf-29*) with a slightly different genome and grew them both from seeds in our lab. We picked that particular mutant form because it lacks the ability to effectively add the epigenetic chemical tag (H3K27me3) that we were interested in studying. That meant we could compare the two types to see what effect H3K27me3 has.
- We then subjected all the plants to either low or high iron levels in a **growth medium** for three days.
- Next we compared the type and amount of gene products in the root cells of all the plants.
- We also used a special process called chromatin immunoprecipitation (ChIP) to see if our epigenetic marker was in fact affecting the genes involved in the response to iron in the growth medium.
- Finally, we measured and compared the roots of both plants, the wild type and the mutant form, to see which grew better under low and high iron conditions.

Results

- When there were high levels of iron in the growth medium, we found similarly low amounts of gene products (indicating equally low **gene expression**) for both the wild type and the mutant form cress plants (Fig. 2).
- Among the plants that we grew in growth medium with low levels of iron, however, the mutant form had much higher amounts of gene products (and thus higher gene expression) than the wild type of Thale cress.
- Our ChIP test found the chemical tag H3K27me3 in higher amounts on the genes that deal with iron uptake in the wild type cress as compared with those of the mutant form.
- Finally, when comparing root length between the wild type and mutant form cress, we found longer roots in the mutant plants when iron levels were low (Fig. 3).

Please see Figure 3 on Page 3

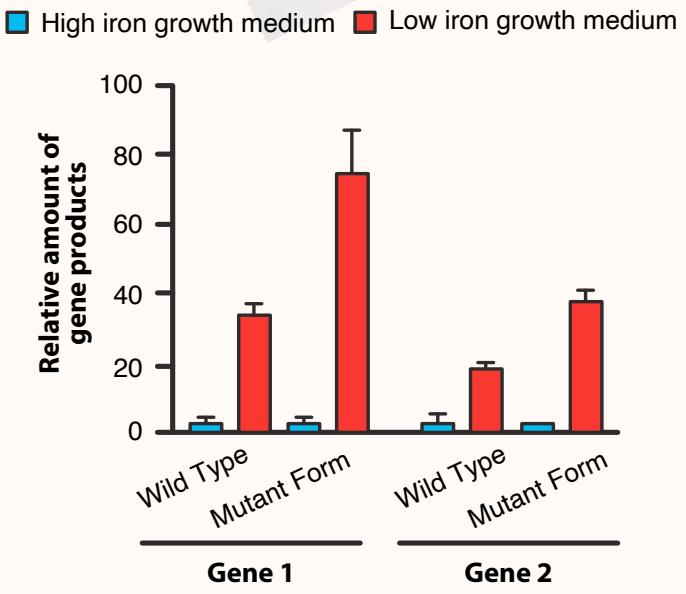


Figure 2:
Comparison of the amount of gene products (which show us gene expression) between our wild type and mutant form cress plants grown in different iron conditions (high/low)

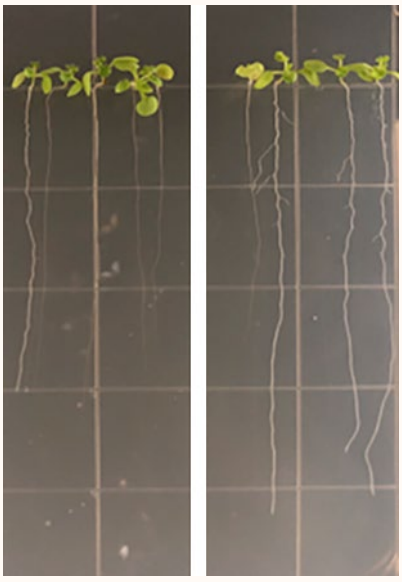
Question:
Which conditions produced different levels of gene expression for wild type and mutant form cress plants?

Discussion

It looks like we were right in our hypothesis that epigenetic factors, such as the chemical tag H3K27me3, are involved in regulating iron uptake in Thale cress plants.

Studying the mutant form we chose made it possible for us to compare the iron uptake mechanisms on Thale cress plants with and mostly without the H3K27me3 chemical tag added to the genes. When Thale cress plants aren't "hungry" for iron, then they keep their iron uptake mechanisms turned down low. That's true for both the wild type and mutant forms. Our root measurements showed that the mutant form plants (without these chemical tags) grew longer roots. That implies the mutant plants were able to take up more iron, which helped them to grow faster in conditions with low iron levels—even though they were risking potential damage from too much iron.

In the wild type, the H3K27me3 tag is added to the relevant genes, which changes the chromatin structure (how DNA is packaged into a compact structure) As a result, it turns off certain genes—in our case those responsible for the uptake of iron—effectively slowing that process down. So, what's the point of the H3K27me3 tags, if the plants need iron? We think the tags help to protect the wild type from an accidental (but harmful!) overdose.



Wild Type | Mutant Form *clf-29*

Question: How did the cress plants grow differently in low levels of iron?

Figure 3: Root growth of wild type and mutant form Thale cress in low iron growth medium

Conclusion

Even though we have known the genetic code of the Thale cress plant for a long time, we still don't fully understand how its genes are turned on or off. Our analysis shows that epigenetic factors are involved in helping the plants take up the iron they so desperately need, without taking in too much of it.

Epigenetic factors are important in your DNA as well, telling your cells which instructions to follow. And here's the surprising part: you can influence your own epigenetics by changing simple things like your diet or lifestyle. And the consequences might be huge, even for future generations!

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Glossary of Key Terms

Chemical tags – in epigenetics, chemicals that attach to the DNA or to the proteins DNA is wrapped around, changing the 3D structure of the DNA.

Chromatin – the material that chromosomes are made of. It consists of DNA wrapped around proteins and packages very long DNA into a dense and compact structure.

Epigenetics – the study of gene expression that is determined not by the sequence of the DNA, but by chemical tags attached to it or the way it wraps around proteins and maintains a compact structure.

Genes – sections of DNA that code for a particular gene product or trait.

Gene expression – the process by which information from a gene is read and leads to making a functional product. If a gene is expressed, it means it is actively being read and the information it contains is translated into a chemical product – usually a protein that plays a specific role in the cell.

Gene products – what is produced when genes are read and translated.

Gene regulation – the process that determines which genes are read (turned on or off) at any given time and in a given location in the body. For instance, different genes are active in your muscle cells than in your nerve cells at any given time.

Genome – all of an organism's genes.

Growth medium – solid or liquid that supports the growth of plants, cells, or microorganisms; often used for studies since its makeup is more controlled.

Model organism – an organism that has been studied a lot and can thus help with answering new questions about a topic. Thale cress is a model organism for gene studies.

Micronutrient – an essential nutrient that is only needed in very small amounts, like iron for plants.

Molecular – having to do with molecules in cells.

Mutant form – an organism (in our case a variety of the Thale cress plant) that has similar but slightly different genes, caused by a mutation in the genome (a change in the sequence of the DNA).

Check your understanding

1. Why is iron a micronutrient?
2. There is a lot of iron in the soil. So it should be easy for plants to take it up, right?
3. What is the difference between genetics and epigenetics?
4. We chose to study Thale cress because it is a model organism. What does that mean?
5. Why did we pick this particular mutant form Thale cress plant to address our question?
6. How did the results show that epigenetic factors are involved in Thale cress iron uptake?