UNIVERSITÀ
DEGLI STUDI DI TRIESTE

Intronic triplet repeat expansions cause a transcriptional downregulation in Friedreich ataxia

Group 5:

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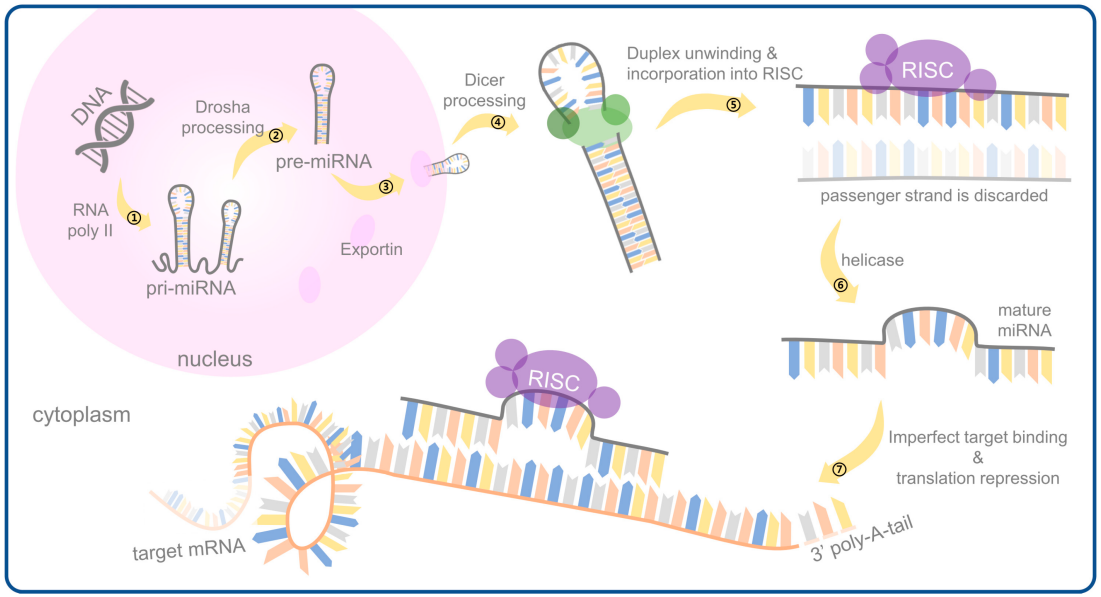
Intronic triplet repeat expansions cause a transcriptional downregulation in Friedreich ataxia

Introduction:

- DNA Repeats
- Friedreich ataxia
- Triplet repeat in Arabidopsis thaliana
- Triplet repeats in FRDA

Paper analysis:

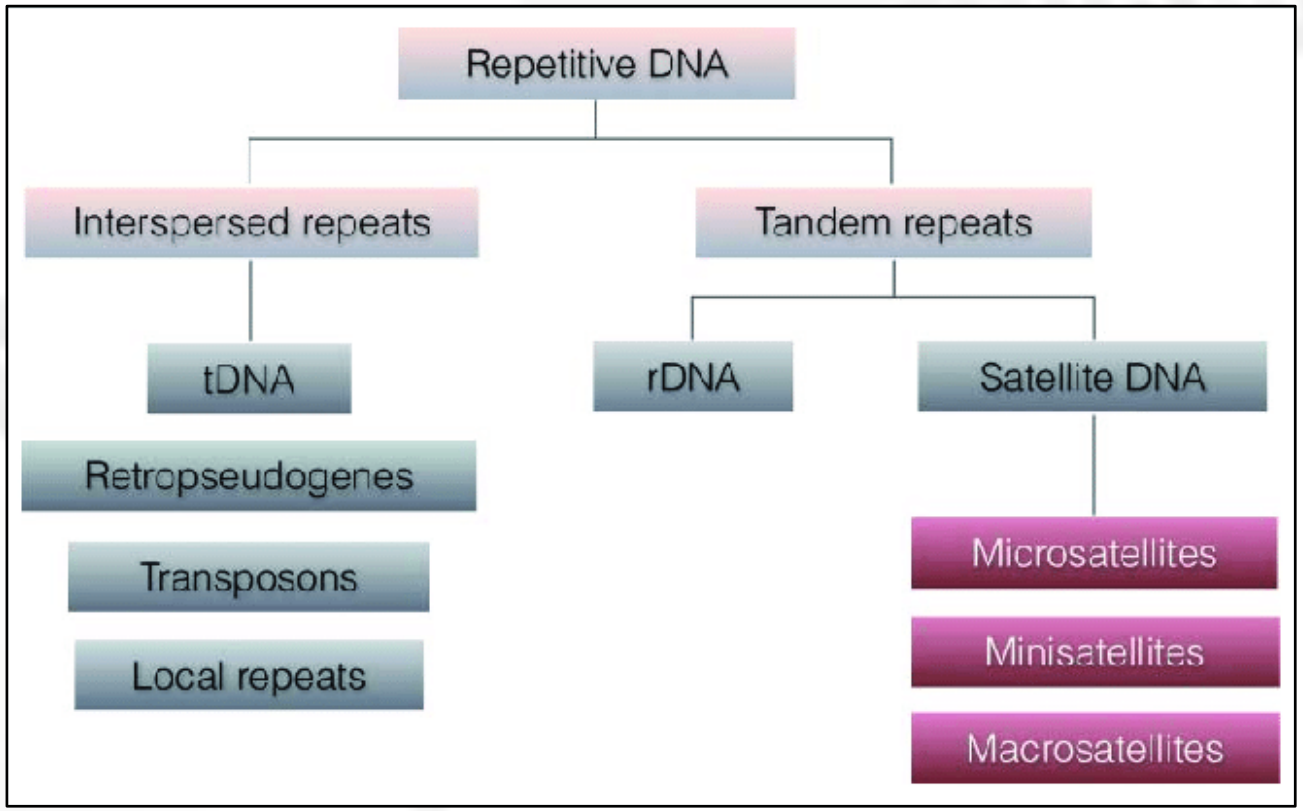
- Downregulation of IIL1
- Increased accumulation of small RNAs
- 24nt miRNA produced by DCL3
- Downregulation of Pol IV and RDR-2
- HEN1, AGO4 and Pol V for transcriptional downregulation
- Perturbation of DNA methylation
- Histone methyltransferases for transcriptional downregulation



DNA Repeats

Distribution of different types of repetitive sequences is represented diagrammatically on a plant chromosome with different colors.

- Red: centromeric tandem repeats
- Blue: telomeric repeats
- Yellow: sub-telomeric tandem repeats
- Green: intercalary tandem repeats
- Brown: dispersed repeats
  White: genes and low-copy sequences.

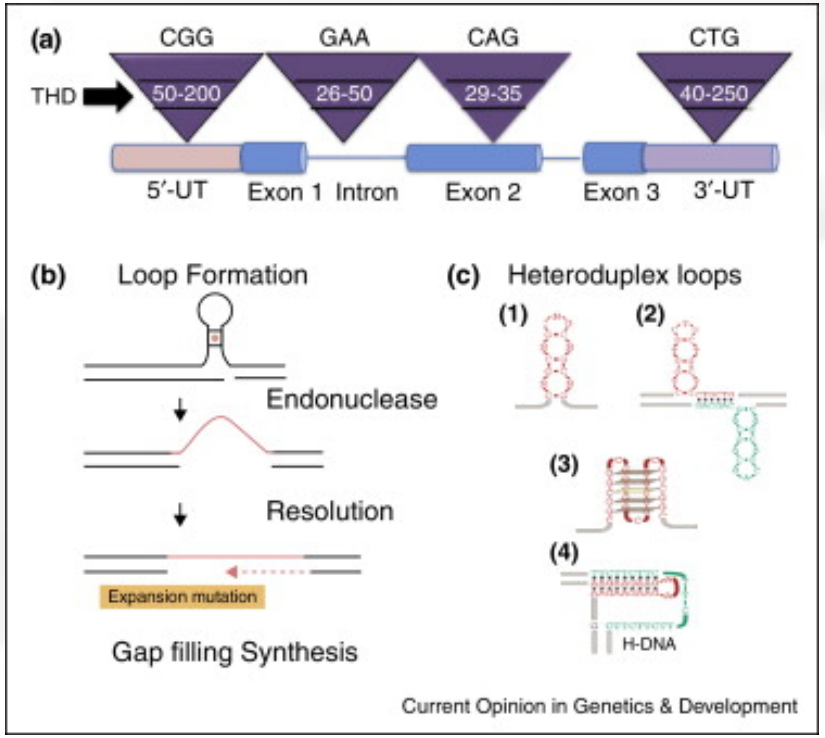


DNA triplet expansion

- Some genetic conditions are caused by an unusual genetic change – an expansion of a segment of DNA that contains a repeat of 3 nucleotides (triplet repeat), such as CAGCAGCAG . . . CAG.

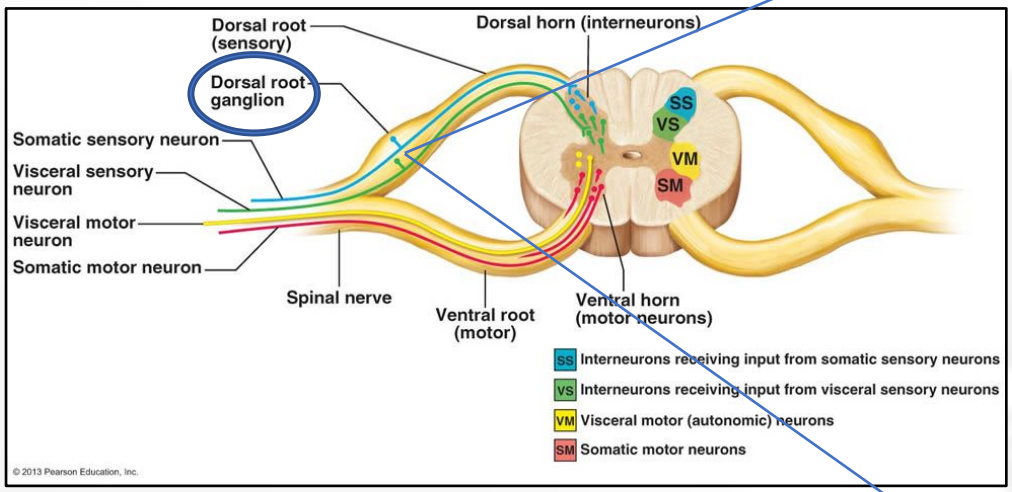
- In these disorders, healthy individuals have a variable number of triplet repeats, but there is a threshold beyond which a high number of repeats causes disease. This threshold varies in different disorders.

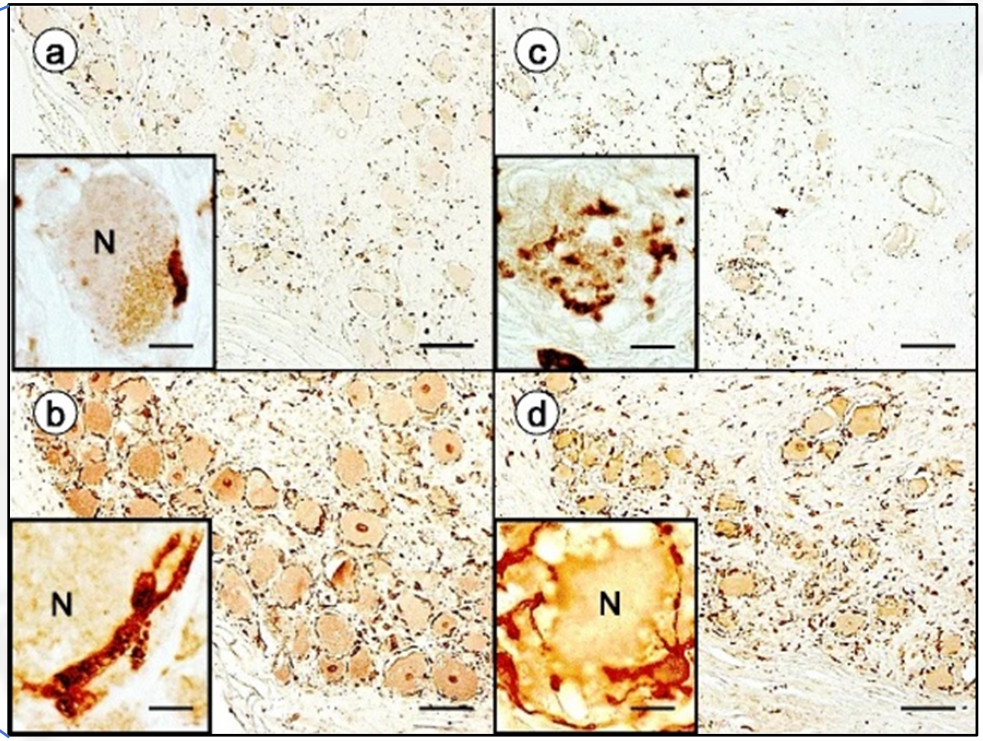
- The triplet repeat expansion is sometimes called a dynamic or unstable mutation.



Disease	Mutant gene	Expanding repeat	Normal number of repeats	Pathogenic number of repeats
FRAXA (fragile X syndrome)	*FMR1*, on the X-chromosome	**CGG**	6–53	230+
FXTAS (fragile X-associated tremor/ataxia syndrome)	*FMR1*, on the X-chromosome	**CGG**	6–53	55–200
DM (myotonic dystrophy)	DMPK	**CTG**	5–37	50+
FRDA (Friedreich's ataxia)	FXN or X25, (frataxin–reduced expression)	**GAA**	7–34	100+
FRAXE (Fragile XE mental retardation)	AFF2 or FMR2, on the X-chromosome	**CCG**	6–35	200+

Friedreich Ataxia (FRDA)

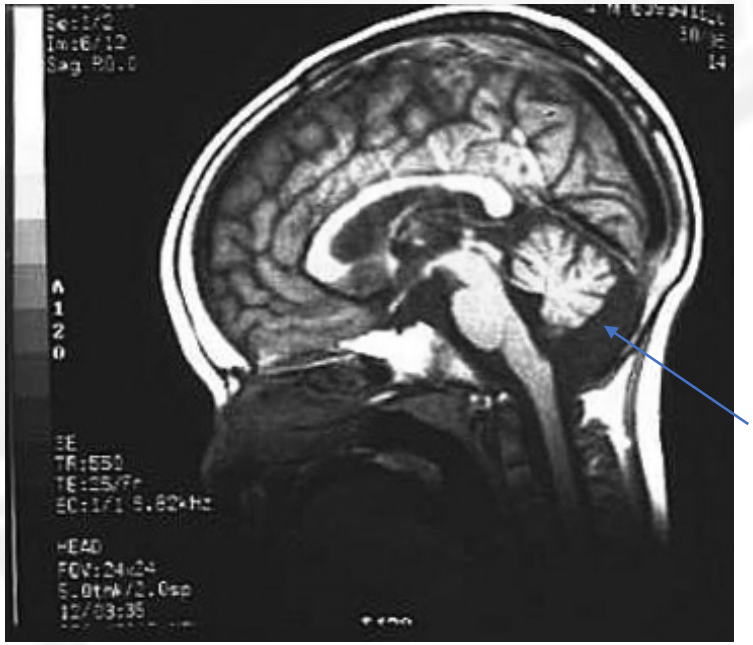




Dorsal root ganglia in Friedreich ataxia: satellite cell proliferation and inflammation

Friedreich Ataxia (FRDA)

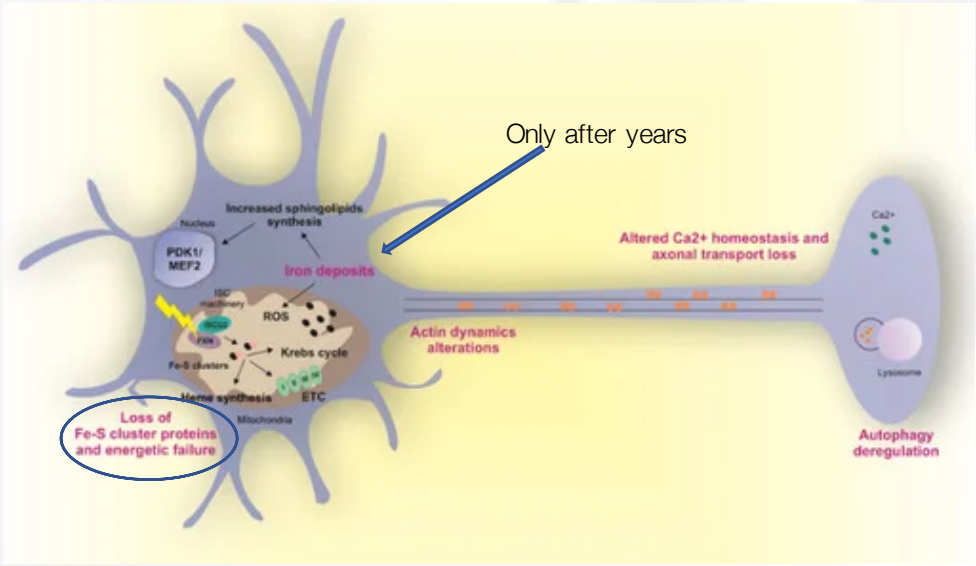
Man with FRDA showing postural ataxia

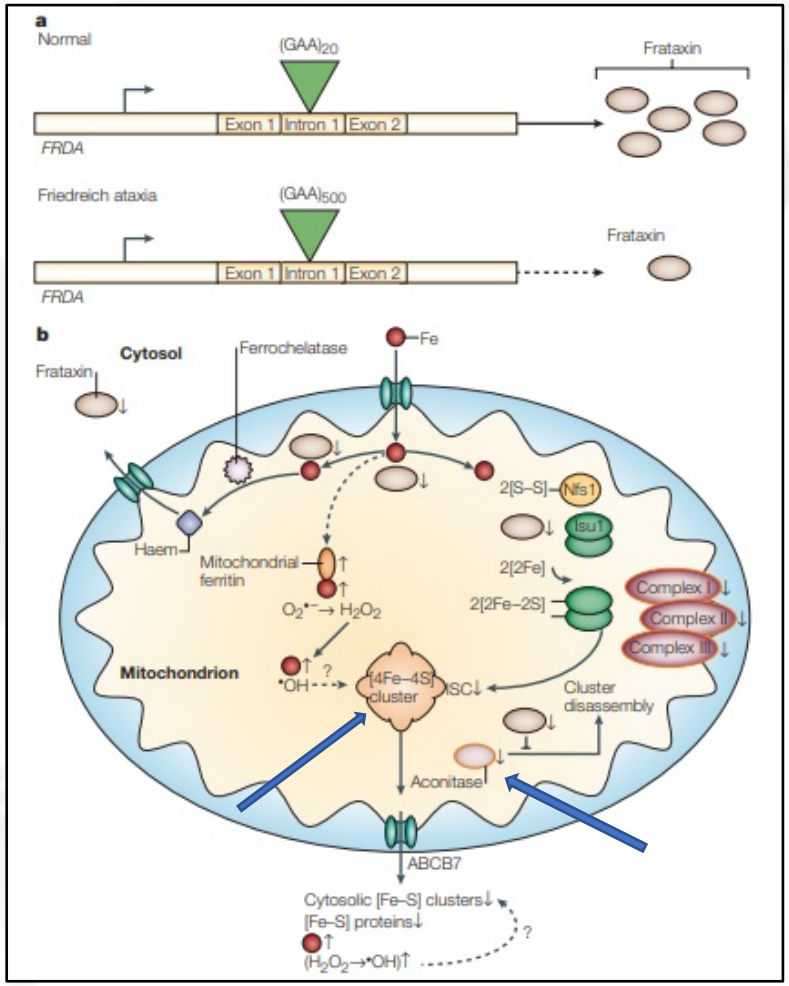


MRI of a man affected by FRDA

Friedreich Ataxia (FRDA)

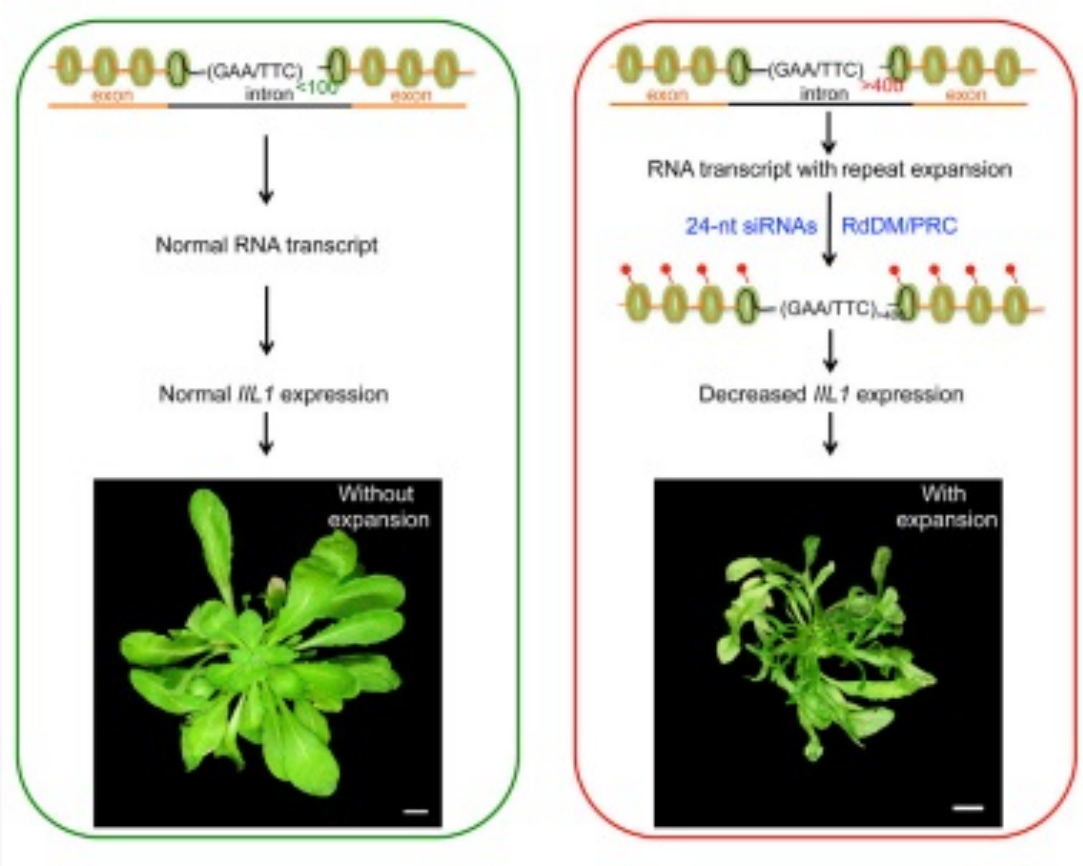
A recent study has identified two novel tissue specific transcript variants, encoding two isoforms of frataxin that lack the mitochondrial targeting sequence and are therefore different from the canonical transcript.



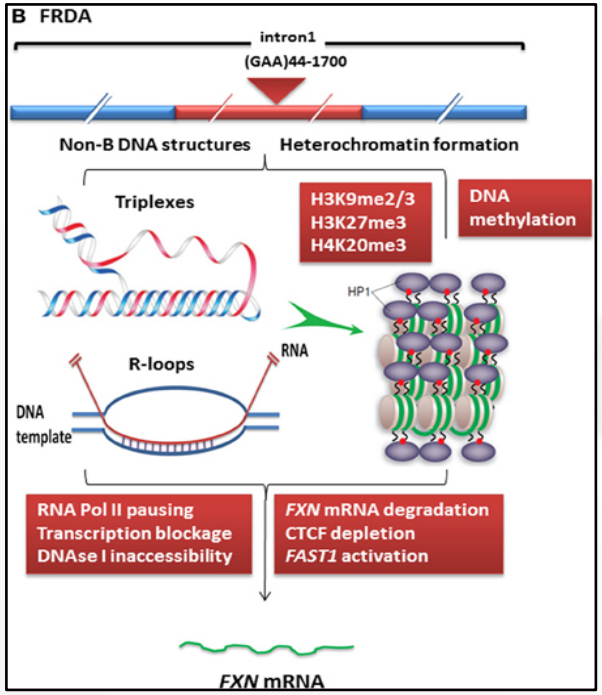


What can we learn about triplet repeats from other species?

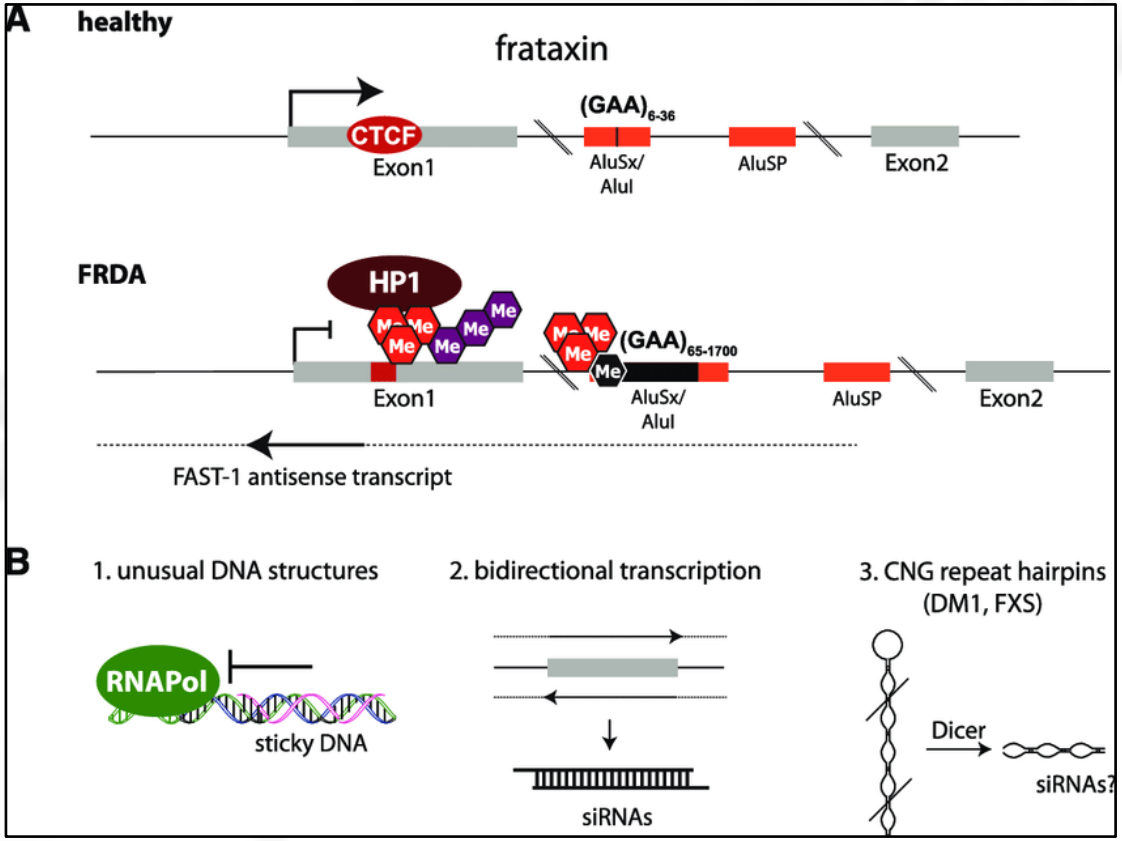
Triplet repeats in Arabidopsis thaliana



Triplet repeats in FRDA



Repression of frataxin correlates with upregulation of the FAST-1 antisense transcript.



Paper presentation

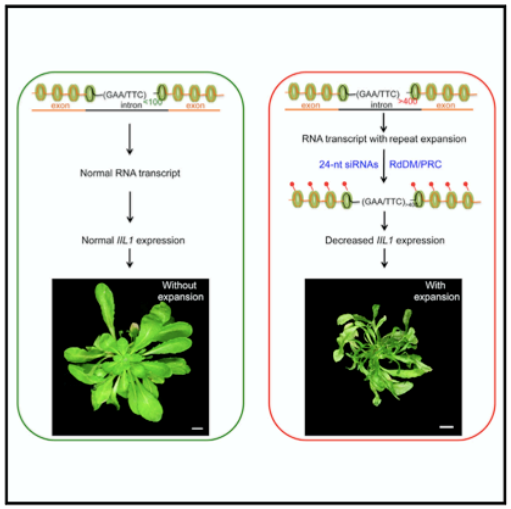
- Triplet repeat expansions can trigger local production of 24-nt small RNAs
- Expansion-induced siRNAs correlate with the downregulation of gene expression
- Intronic repeat expansions cause gene silencing through the RdDM pathway

Article

Cell

RNA-Dependent Epigenetic Silencing Directs Transcriptional Downregulation Caused by Intronic Repeat Expansions

Graphical Abstract



Highlights

- Triplet repeat expansions can trigger local production of 24-nt small RNAs
- Expansion-induced siRNAs correlate with the downregulation of gene expression
- Intronic repeat expansions cause gene silencing through the RdDM pathway

Authors

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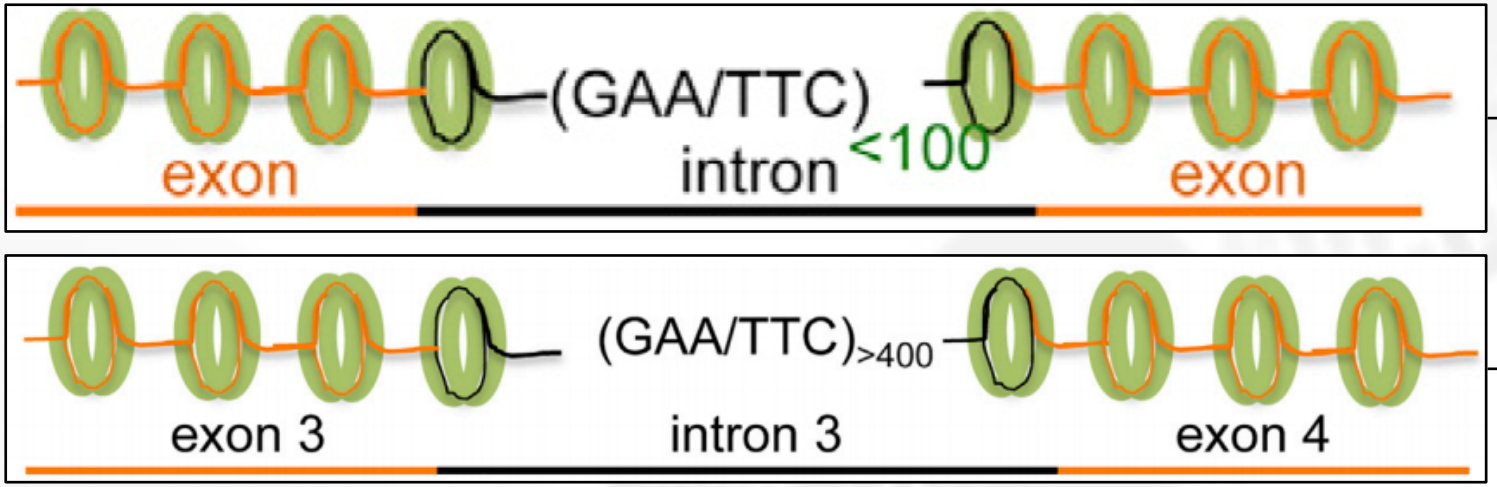
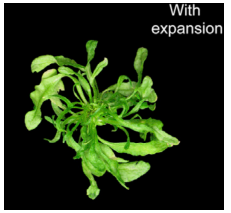
In Brief

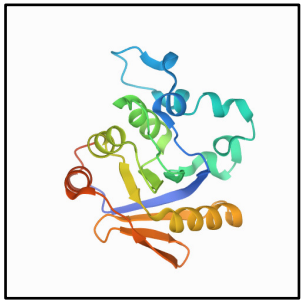
Triplet expansions within an *Arabidopsis* gene leads to local siRNA biogenesis and consequent transcriptional downregulation, suggesting how intronic repeat expansions may affect gene expression in other systems.

IIL1 locus





IIL1 Locus transcribes for the small subunit 1 (ISOPROPYLMALATE ISOMERASE SMALL SUBUNIT 1) of isopropyl malate isomerase (IPMI).
This enzyme is involved in the formation of leucine in Arabidopsis thaliana.
Inside the *IIL1* locus, under normal conditions, there are less than 100 repeats inside 3, if the repeats are more than 400, the phenotype of the plant has changed, resulted in a severe phenotype (*iil*) with stunted growth, narrow pale leaf blades with green vasculature.



Samples analyzed in paper:

Bur-0

Bur-0: has many ripetitions, more than 400 in IIL1 locus, with a iil phenotype.

NS15

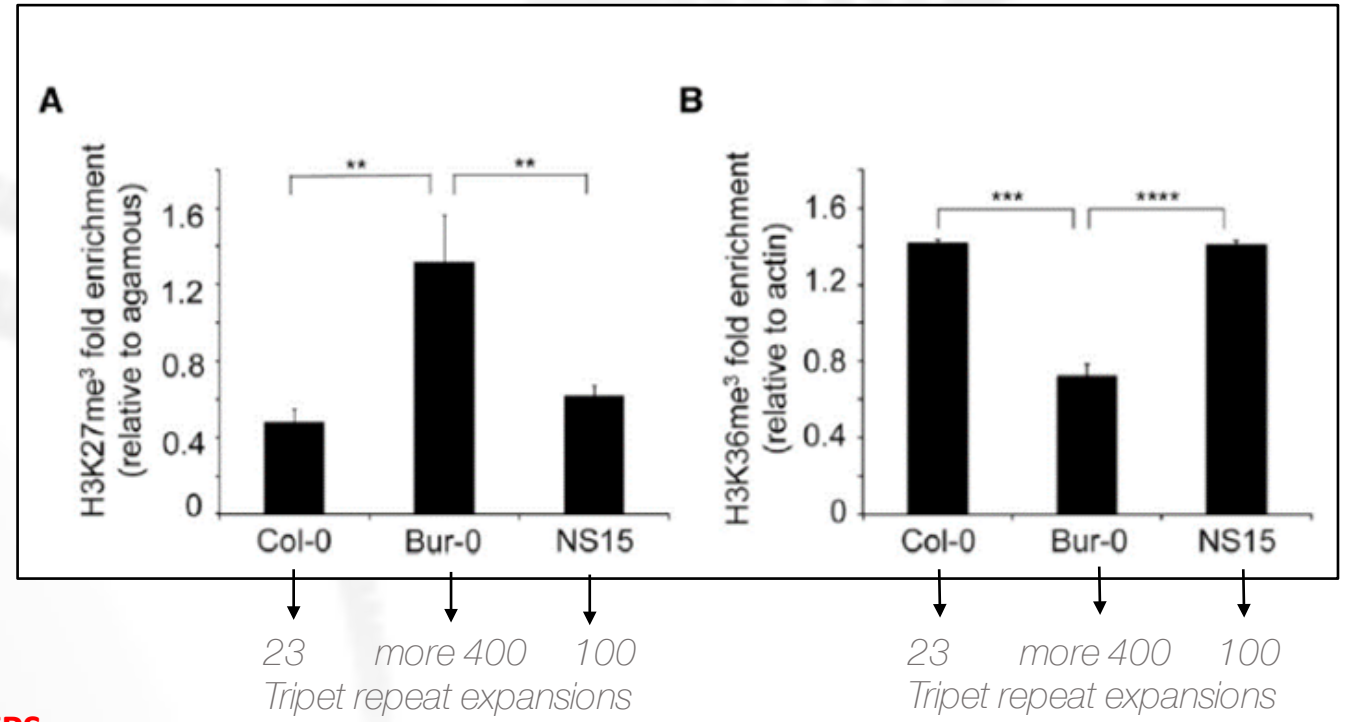
NS15: spontaneuos phenotype revertant, lost partially the repeat expansion in IIL locus.

Col-0

Col-0: contains only 23 copies of GAA repeats in IIL1 locus.

Triplet Expansion-Induced Transcriptional Downregulation of *IIL1* is associated with Epigenetic Changes

Relative enrichment of H2K27me3, a mark for repressive chromatin in Bur-0 compared to NS15 and Col-0 that lack the repeat expansion and depletion of H3K36me3, associate with an active chromatin, in Bur-0 compared to NS15 and Col-0.



Conclusion:

The repeat-expansion-associated transcriptional downregulation of *IIL1* in Bur-0 can be mediated through epigenetic changes.

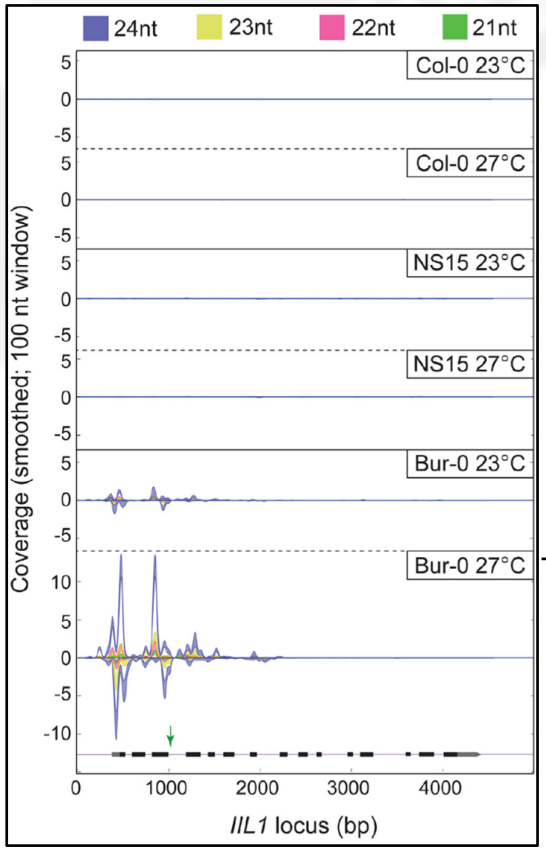
CONLUSIONS ALWAYS IN BOLD LETTERS

Triplet Repeat Expansion is associated with increased accumulation of small RNAs

Performed genome-wide deep sequencing of small RNAs in Bur-0, NS15, and Col-0 plants grown at 23°C and 27°C figure.
This figure shows the 24, 23, 22, 21 -nt small RNAs in the locus *IIL1*.
Quantification of the sense and antisense small RNAs is shown in the positive and negative dimensions, respectively, along the y axis

Conclusion:

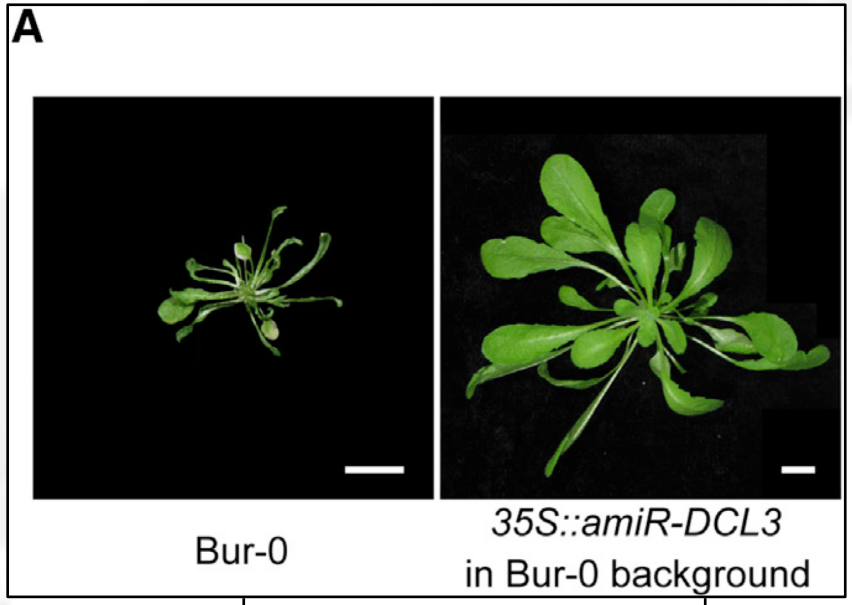
There is a temperature-dependent and locus-specific increase in the abundance of *IIL1* small RNAs in Bur-0, correlating with the length of the triplet repeat expansion and its associated phenotype.



Other title

24 nt-siRNA and Dicer Like 3

In plants, different molecules of DICER produce different species of small RNA, which can be distinguished by their size. These different small RNA were analyzed on the *IIL1* locus and it was seen that the dominant one is 24-nt Small RNA.
These small 24-nt RNA are produced by DICER LIKE 3. To verify if siRNA-24nt have a role on the *iil* phenotype, eliminated DICER LIKE 3 in Bur-0 through artificial microRNA.
The *iil* phenotype was suppressed within the microRNA treated plant against DICER LIKE 3.



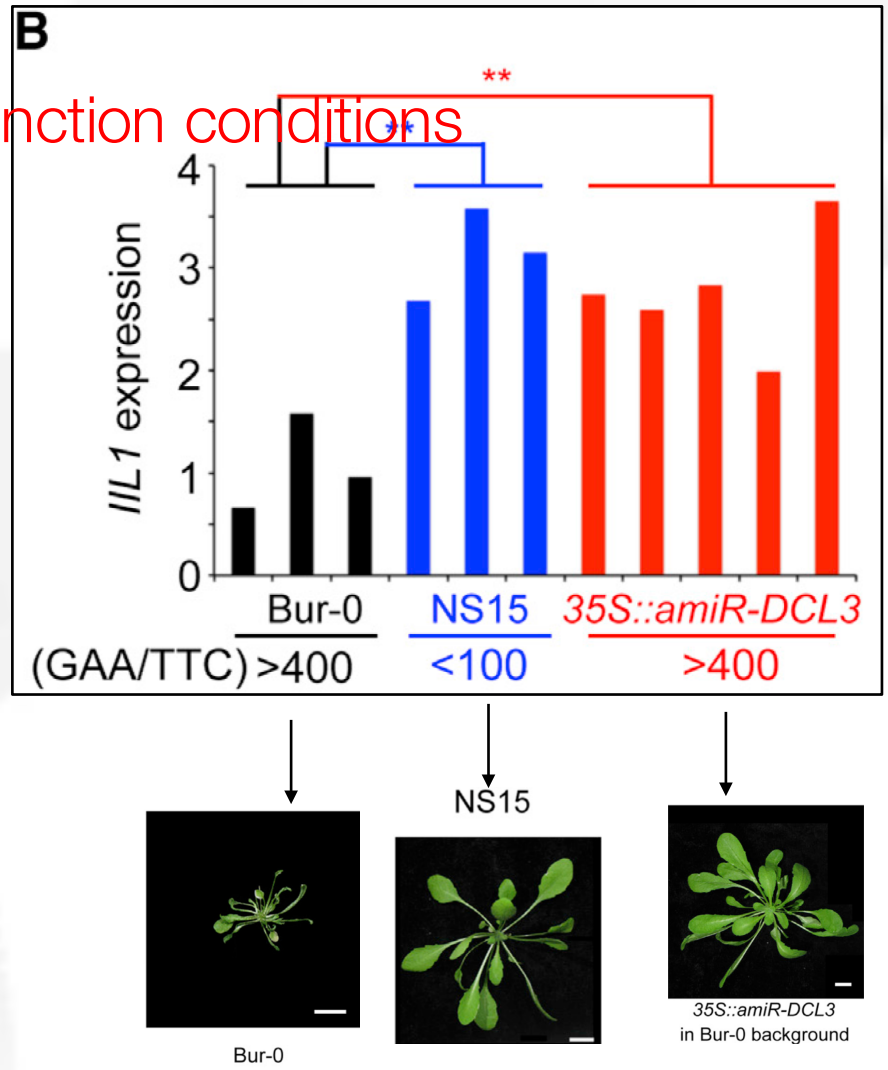
More 400 GAA/ iil phenotype

More 400 GAA, but suppressed iil phenotype

CONLUSION?

Expression levels of *IIL1* in different genotypes.in DCL3 loss of function conditions

The suppression of the *iil* phenotype, as can be seen from this image, was coupled by an increase in the expression of *IIL1* in miRNA-treated plants.
This suggests that DCL3 and consequently the 24nt-siRNA map to *IIL1*, are fundamental for the downregulation observed in the presence of a triplet repeat expansion at the *IIL1* locus of Bur-0.

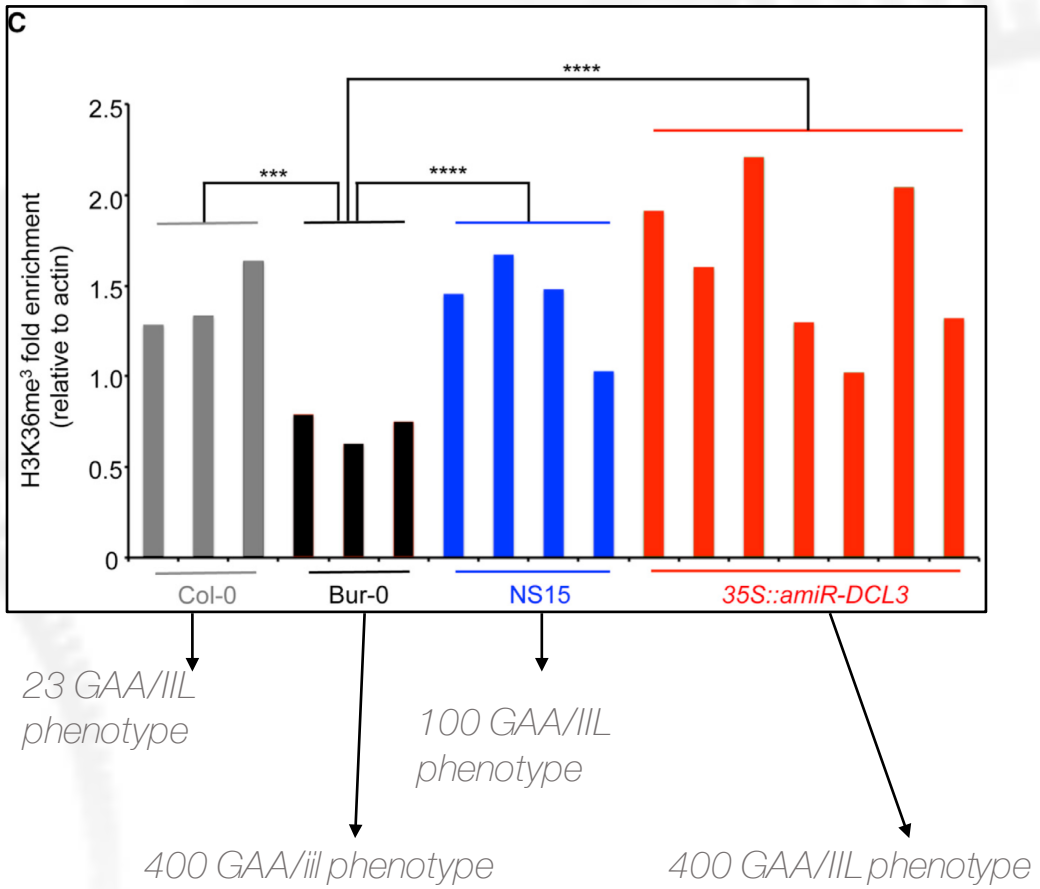


CONLUSION?

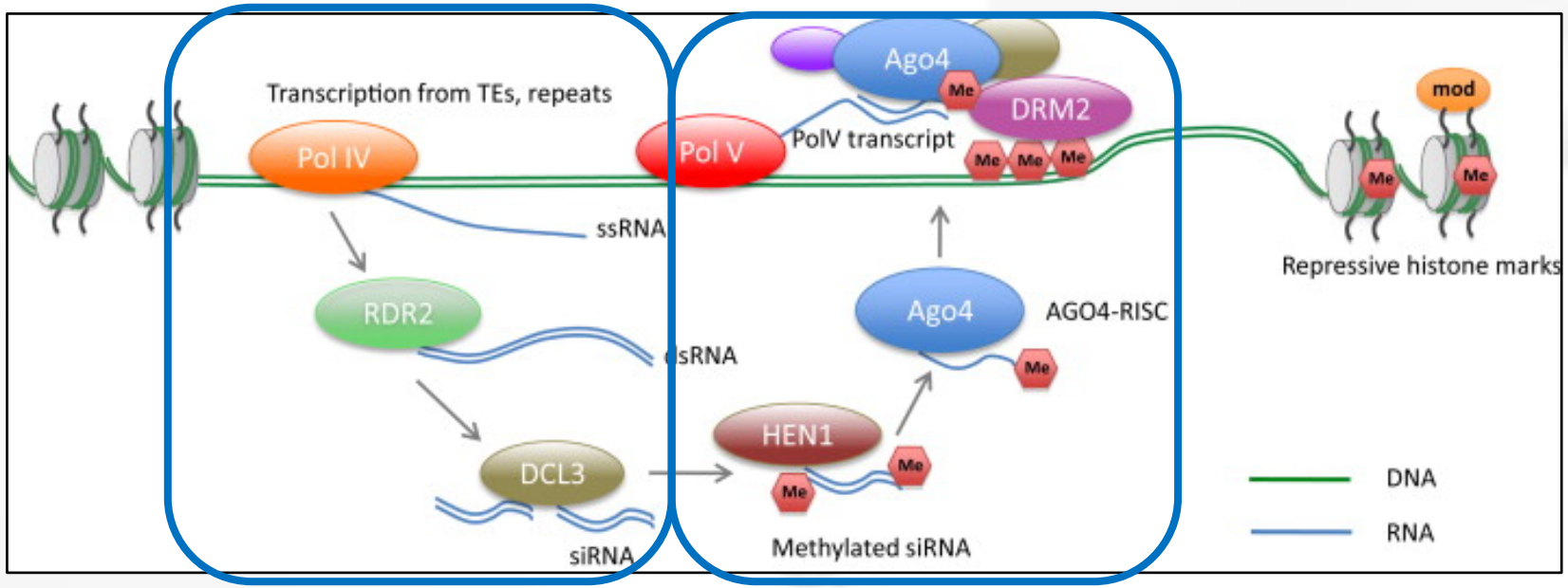
Relative enrichment of H3K36me3 across various genotypes.

Relative enrichment of H3K36me3 in chromatin immunoprecipitation (ChIP) experiments across WHICH GENE – IN PLANTS WITH DIFFENT REPET NUMBER
various genotypes measured through qPCR.
As can be seen from the image, we note that levels of H3k36me3, known to be associated with an active chromatin state, were significantly increased in transgenic plants compared to the Bur-0 control.
Conclusion:

DCL3-dependent siRNAs are essential for the observed epigenetic changes caused by the expanded repeat in *IIL1* of Bur-0



RdDm PATHWAY



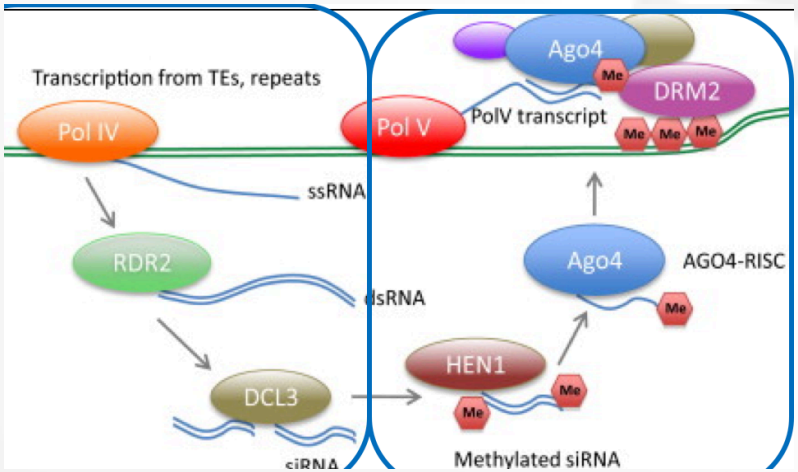
RNA-directed DNA methylation (RdDM) is a biological process in which non-coding RNA molecules direct the addition of DNA methylation to specific DNA sequences.
The first part of the RdDM pathway revolves around the biogenesis of sRNAs. In this process interact togheter Pol IV , RDR2 and DCL3. At the end of this first part of the pathway we have 24-nt sRNAs.
In the second part we have different actors that processed siRNA and methylated the DNA complementary loci. These actors are: HEN1, AGO4, Pol V and DRM2.

Downregulation of Pol IV and RDR2 pathway in Bur-0 suppresses Triplet Expansion-Associated growth defects

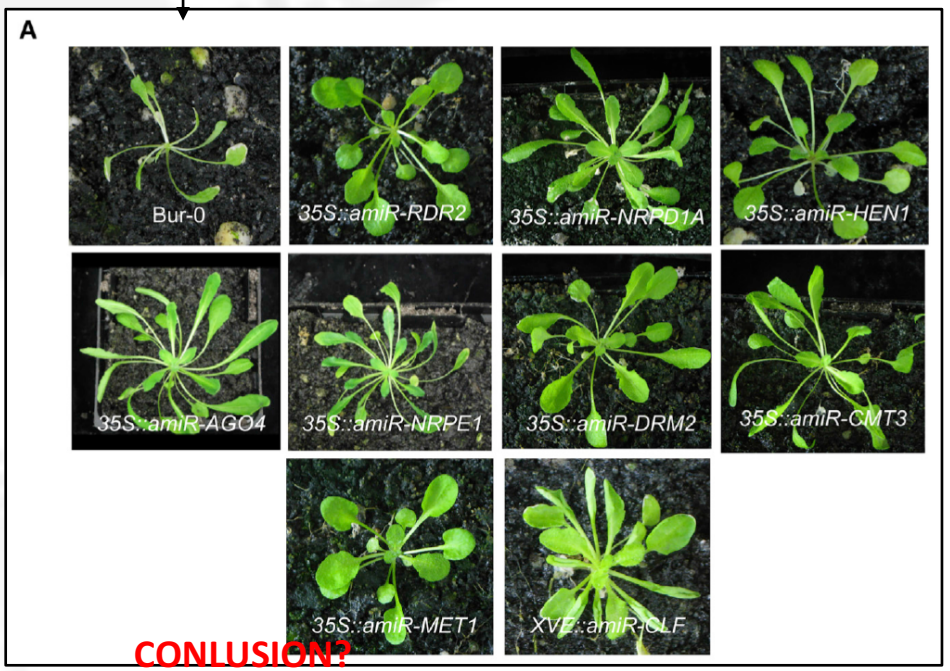
The researchers evaluated the involvement of RdDm pathway.

To do this, they shot down RDR2 and other actors using artificial microRNA and analyzed the impact of this knockdown on the *iil* phenotype.

It was also seen in this case that through the treatment with miRNA, there was a phenotypic suppression of *iil*.



Bur-0 is the only one with iil phenotype



CONLUSION?

Indicate which genes were eliminated in experiment

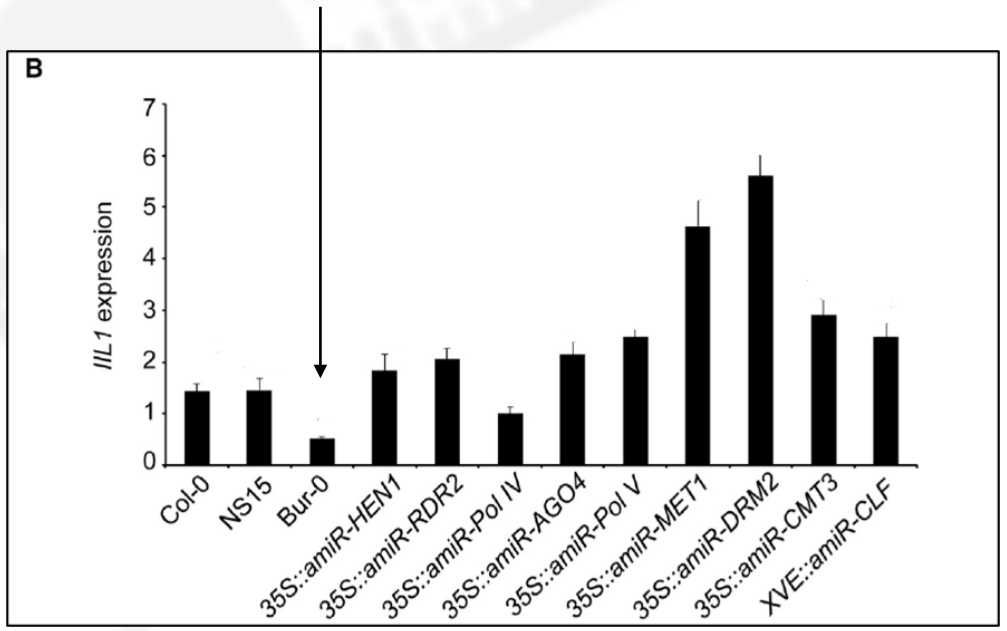
The expression levels of *IIL1* in Col-0, NS15, Bur-0, and across various primary transgenic lines.

The expression levels of *IIL1* in Col-0, NS15, Bur-0, and across various primary transgenic lines harboring artificial microRNAs against the individual components of the RdDM pathway in Arabidopsis.

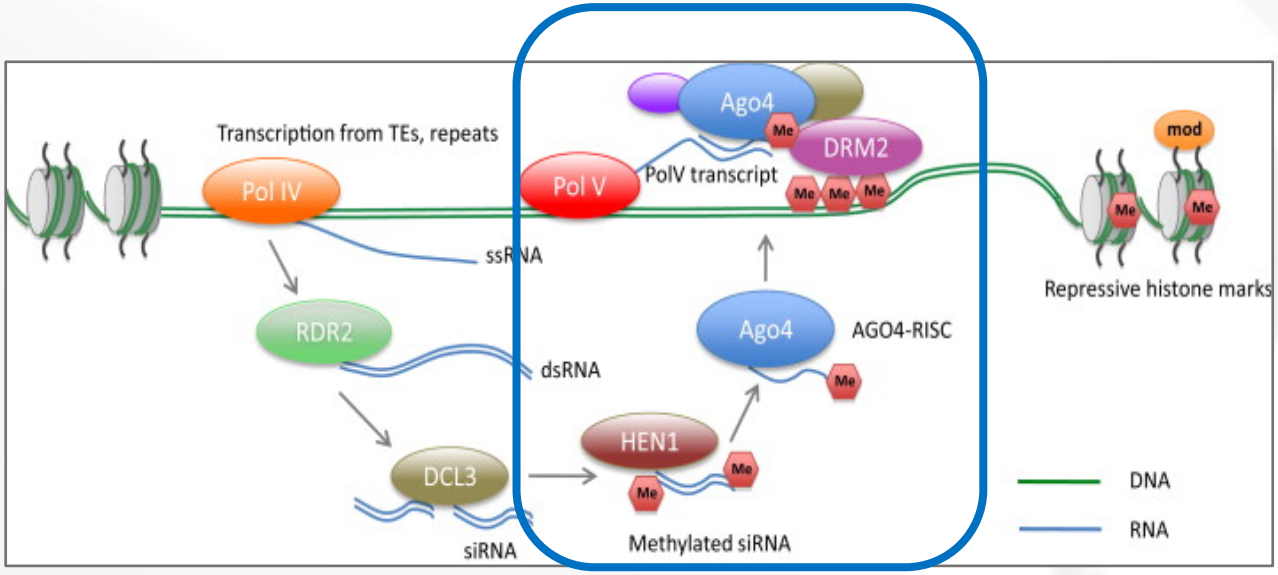
Conclusion:

Pol IV/RDR2 are among the components involved in the transcriptional downregulation of *IIL1* caused by the triplet repeat expansion.

Bur-0 is the only one with iil phenotype



HEN1, AGO4, and Pol V are required for the Triplet Expansion-Associated Transcriptional Downregulation of *IIL1*



Multiple loci play a role in mediating siRNA-dependent epigenetic silencing:

HEN1 mediates one of the initial steps in the biogenesis of siRNA.

AGO4 interact with 24-nt siRNAs.

Polymerase V (Pol V) interact with AGO4 for transcriptional downregulation.

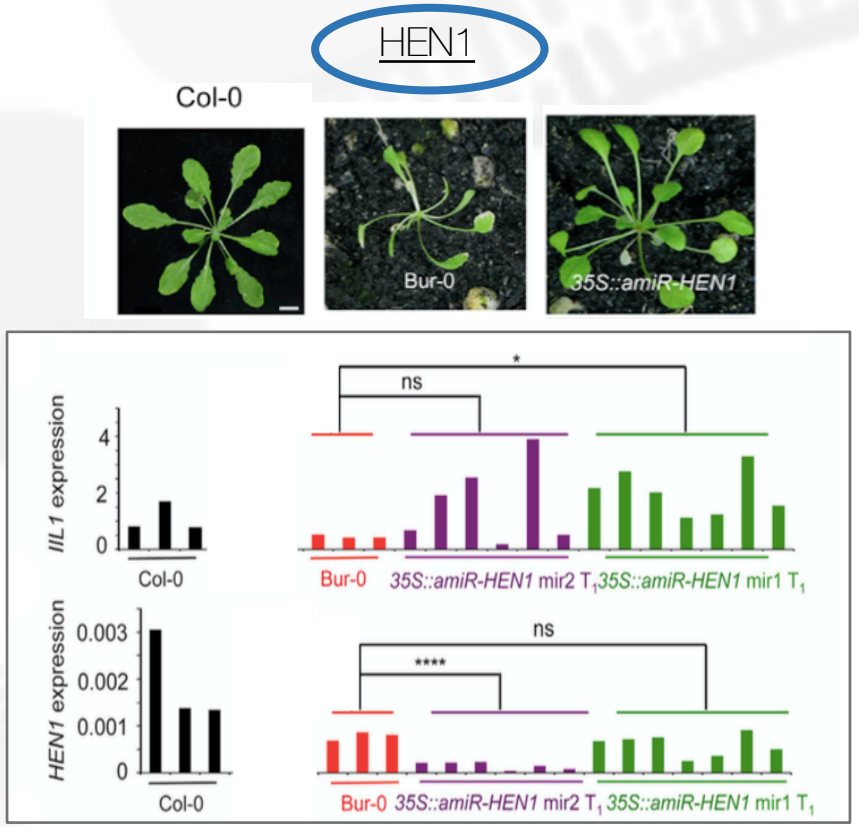
HEN1, AGO4, and Pol V are required for the Triplet Expansion-Associated Transcriptional Downregulation of *IIL1*

Transgenic lines which have artificial microRNAs against individual component.

Data results:
The expression of *IIL1* was increased along with the suppression of the *iil* phenotype.

In conclusion:

HEN1 is required for the observed transcriptional downregulation.



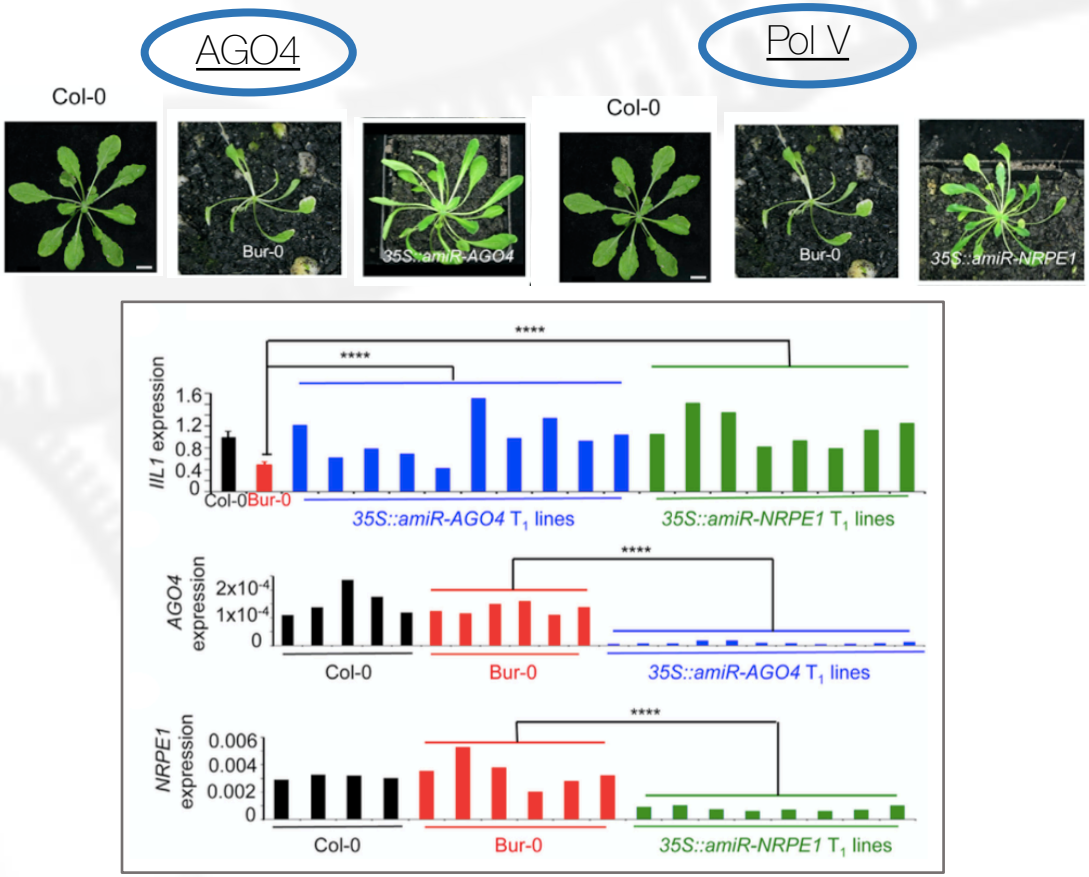
HEN1, AGO4, and Pol V are required for the Triplet Expansion-Associated Transcriptional Downregulation of *IIL1*

Transgenic lines which have artificial microRNAs against individual component.

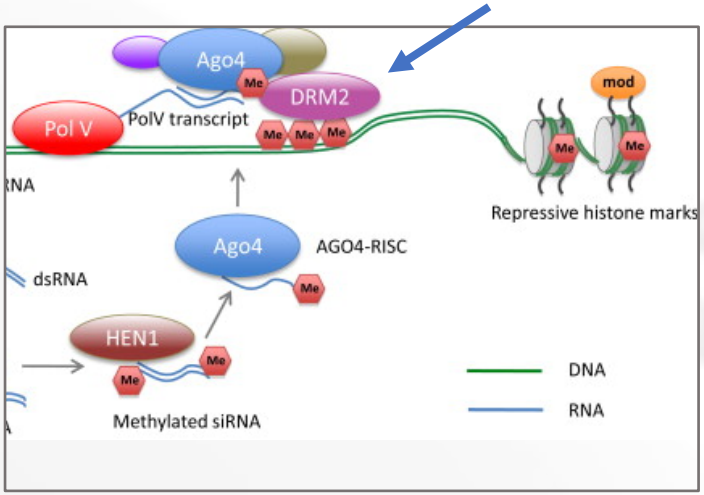
Data results:
The expression of *IIL1* was increased along with the suppression of the *iil* phenotype.

In conclusion:

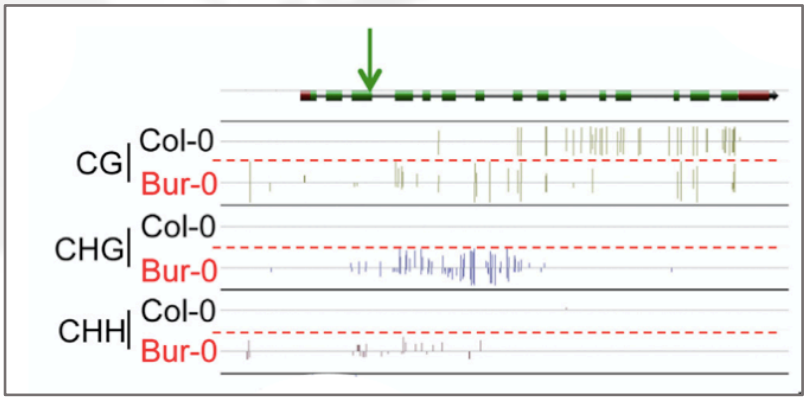
AGO4 and Pol V are essential for the observed transcriptional downregulation.



Perturbation of DNA Methylation suppresses Triplet Expansion-Associated Transcriptional Downregulation



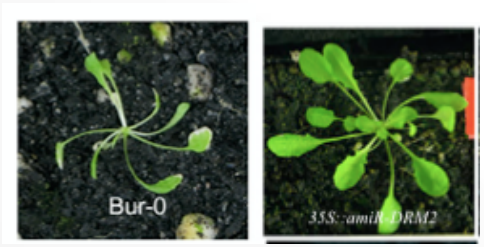
DOMAINS REARRANGED METHYL TRANSFERASE 2 (DRM2) has an important role in DNA methylation for transcriptional silencing.
DRM2 carries out de novo methylation of the DNA.



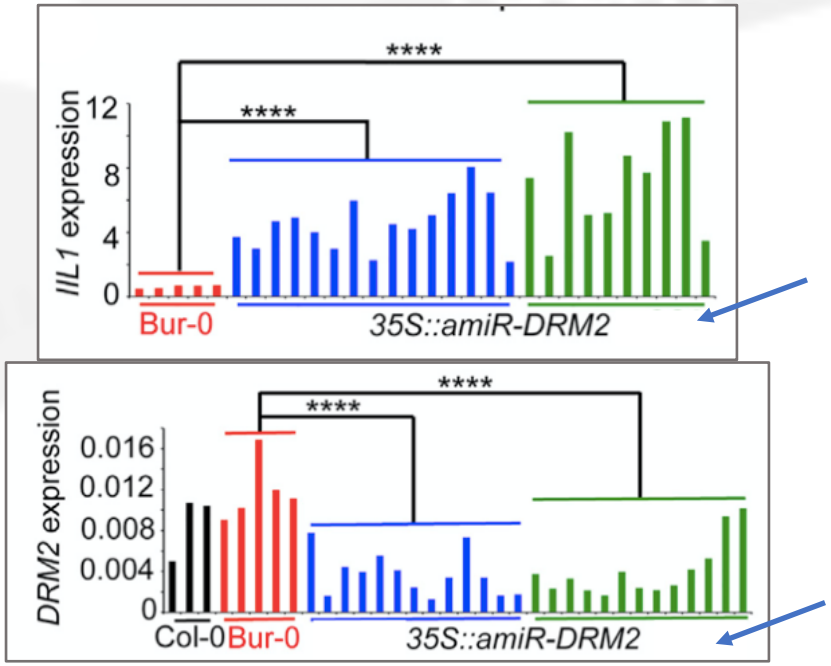
IIL1-Bur-0 appears to be hypermethylated in the 5' region, particularly in CHG and CHH sequence.

Perturbation of DNA Methylation suppresses Triplet Expansion-Associated Transcriptional Downregulation

They knocked down the component with artificial microRNAs



Data results:
Suppression of the *iil* phenotype coupled with an increase in the *IIL1* expression.

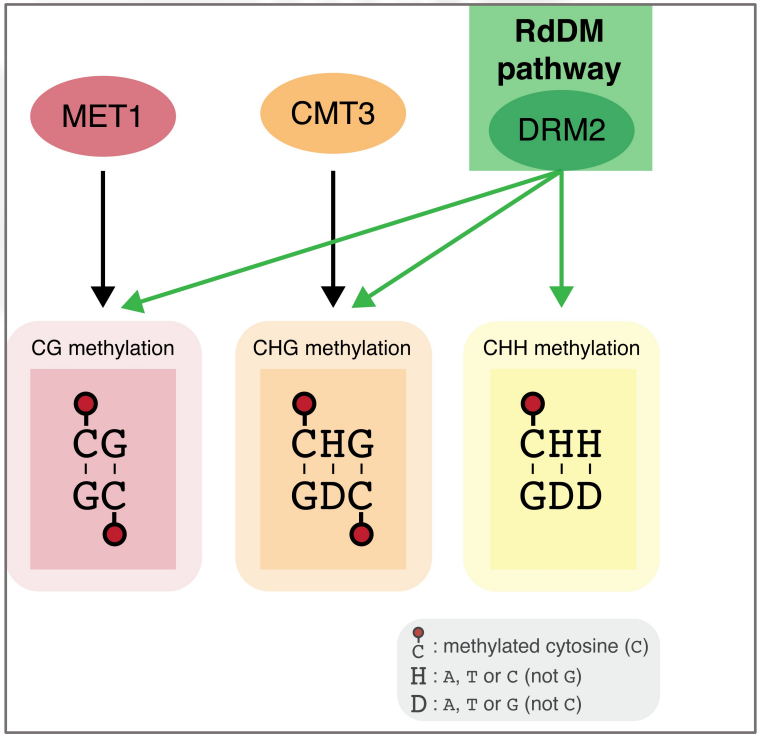


CONLUSION?

Perturbation of DNA Methylation suppresses Triplet Expansion-Associated Transcriptional Downregulation

Maintenance of methylation at CHG and CG require respectively these methyltransferases:

CHROMOMETHYLASE 3 (CMT3)
DNA METHYLTRANSFERASE 1 (MET1)



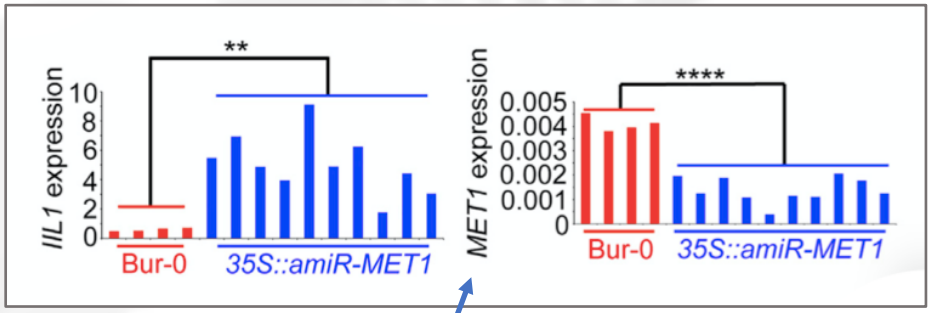
Perturbation of DNA Methylation suppresses Triplet Expansion-Associated Transcriptional Downregulation

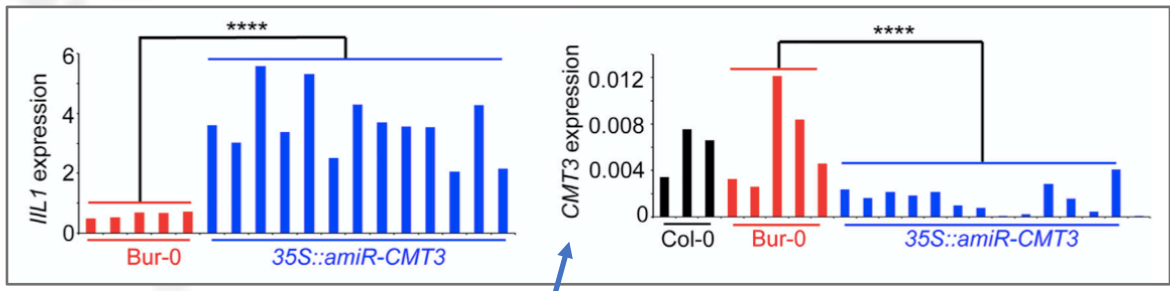
They knocked down the component with artificial microRNAs

Data results:
Suppression of the *iil* phenotype coupled with an increase in the *IIL1* expression.

In conclusion:

DNA methylation is an essential component of the triplet expansion induced transcriptional downregulation.

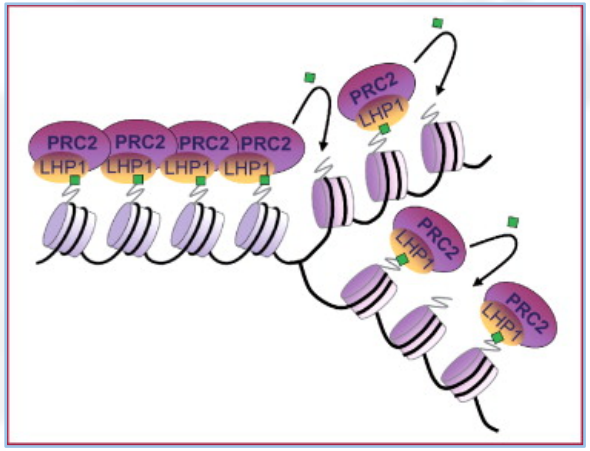


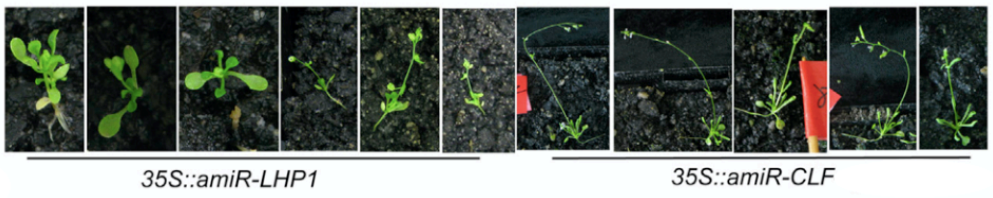


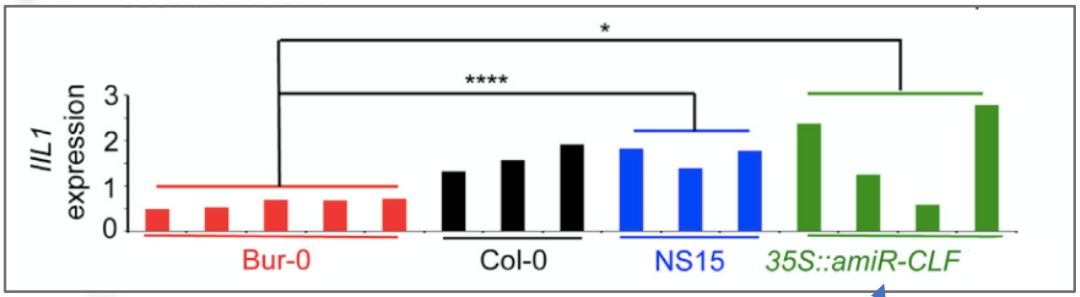
Histone Methyltransferases are essential for the Transcriptional Downregulation caused by Triplet Repeat Expansions

Protein of polycomb group (PRC):

Like heterochromatin protein - LHP1
Histone N-lysine methyltransferase Curly leaf - CLF







CONLUSION?

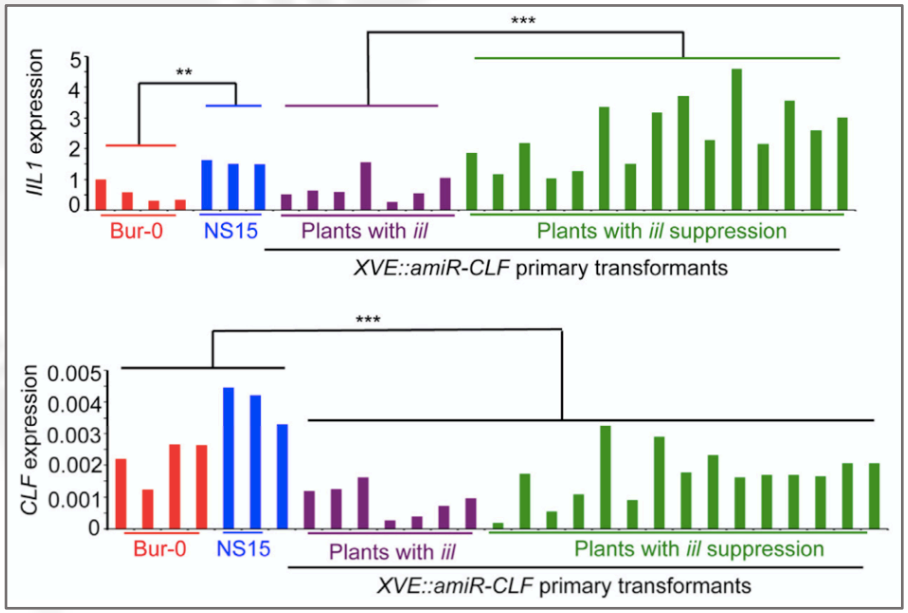
Histone Methyltransferases Are essential for the Transcriptional Downregulation caused by Triplet Repeat Expansions

They knocked down the component with artificial microRNAs

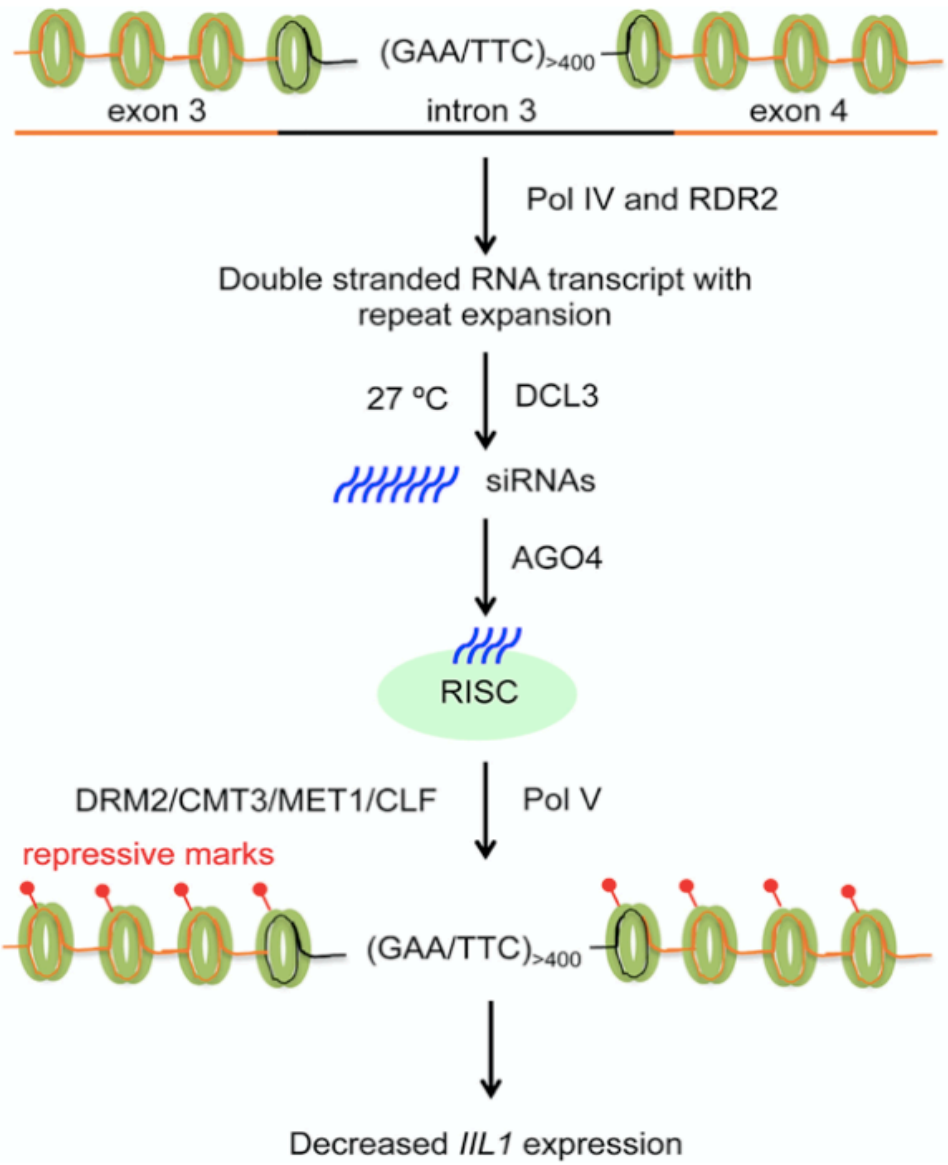
Data results:
The expression of *IIL1* is directly related to the activation of *CLF*.

In conclusion:

the siRNAs appear to modulate epigenetic silencing of the loci harboring the repeat expansion through the RdDM pathway, eventually resulting in repressive epigenetic marks being deposited on chromatin by the PRC

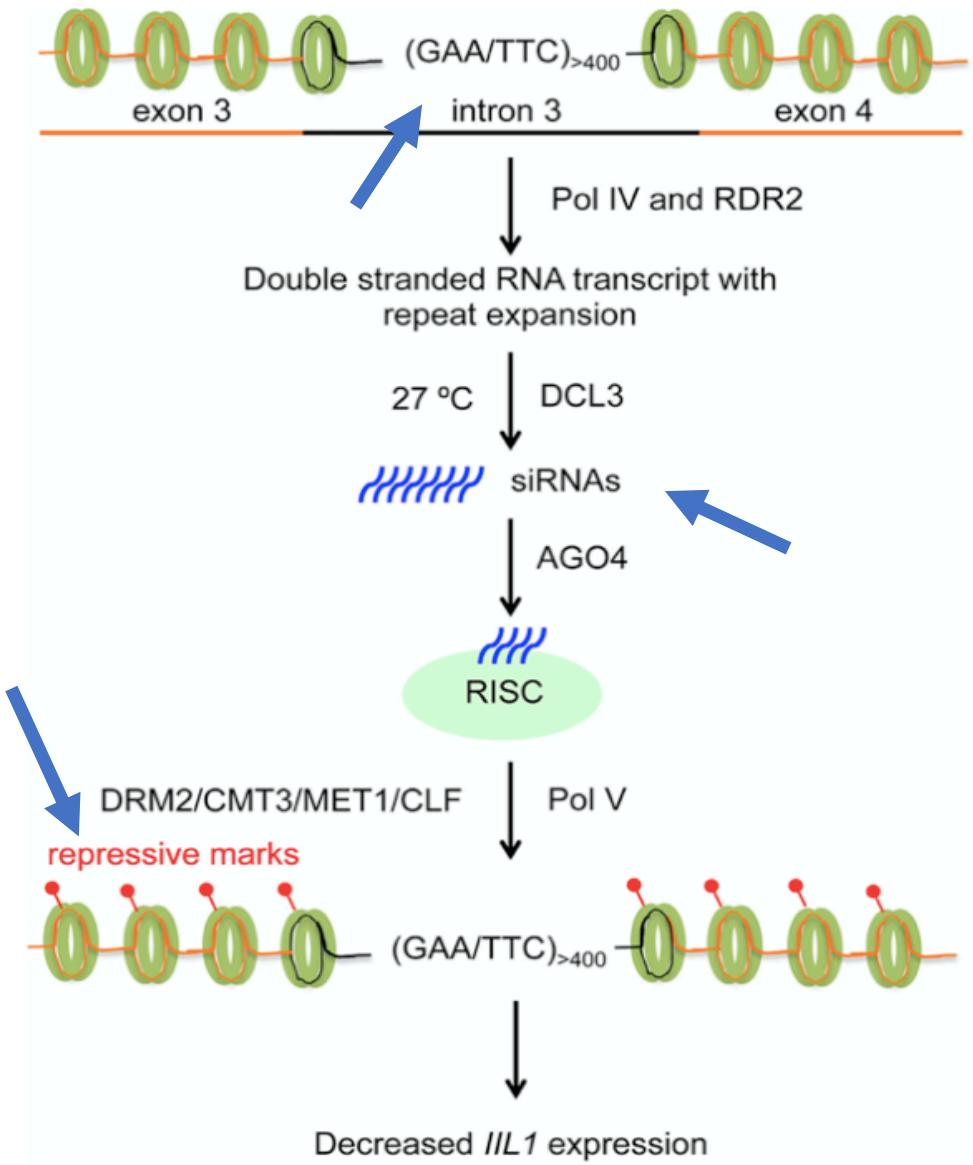


Conclusions



Conclusions

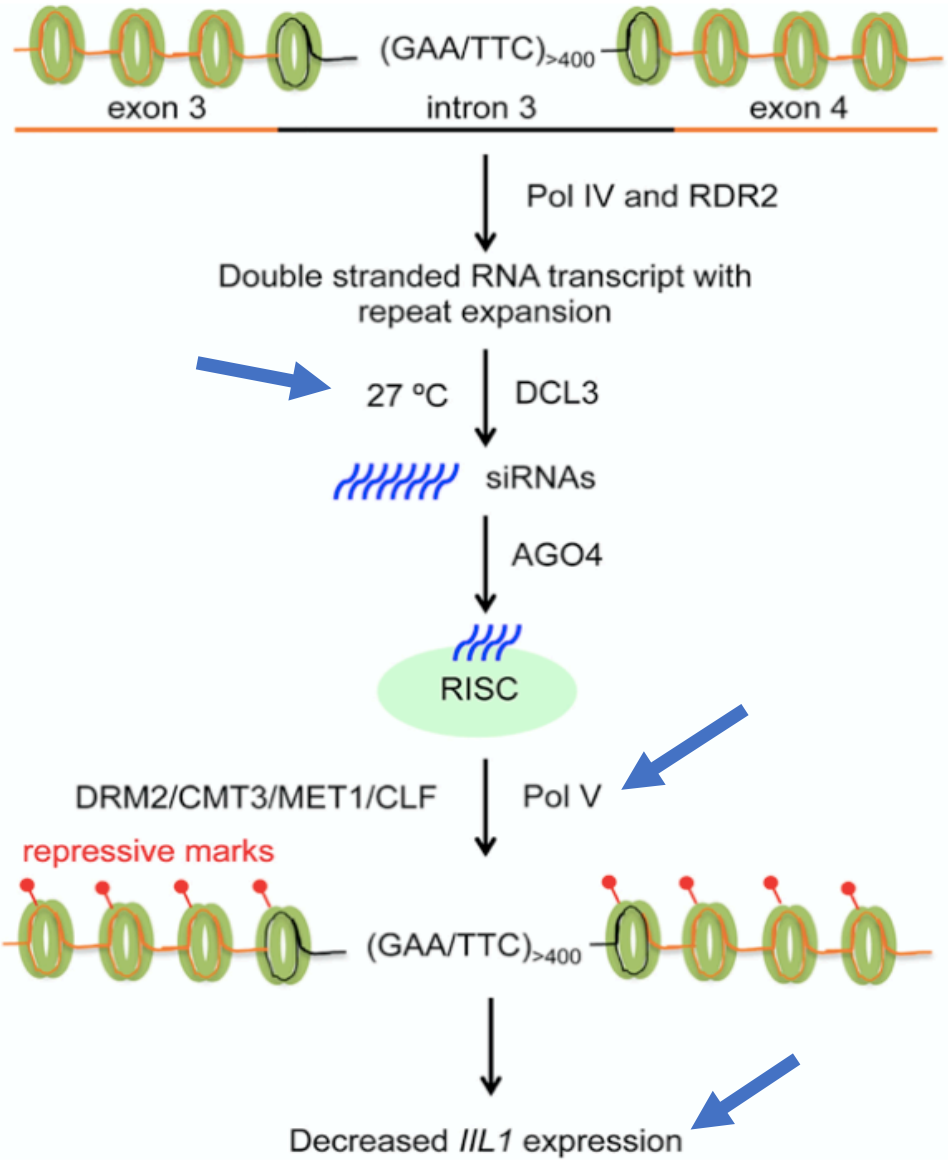
- Triplet Repeat Expansions in transcribed regions can trigger the production of Small RNAs



Conclusions

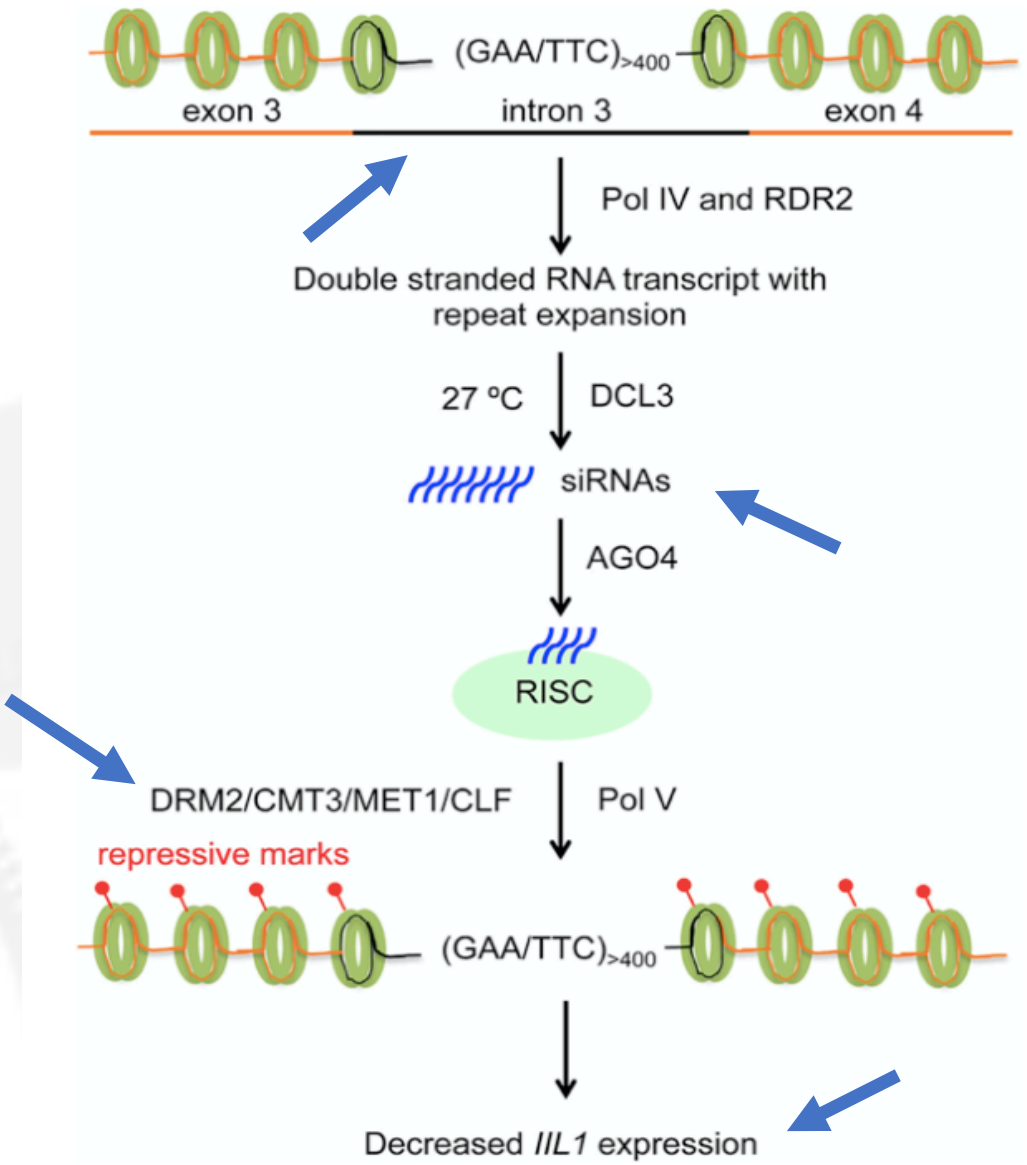
- Triplet Repeat Expansions in transcribed regions can trigger the production of Small RNAs

- Triplet-Derived siRNAs increase at higher temperatures



Conclusions

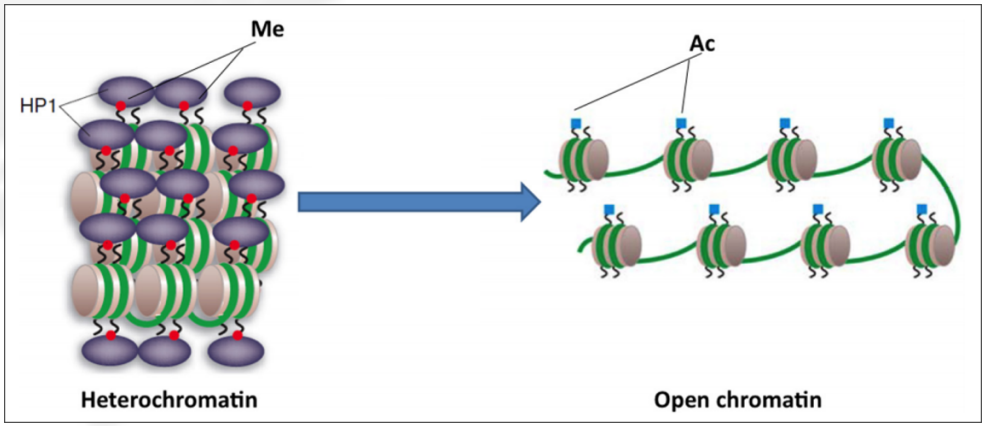
- Triplet Repeat Expansions in transcribed regions can trigger the production of Small RNAs
- Triplet-Derived siRNAs increase at higher temperatures
- siRNA-Mediated epigenetic silencing underlies the Triplet Expansion-Associated Transcriptional Downregulation of the affected gene



Future applications

Trinucleotide repeat expansions have been shown to underlie several neurogenetic diseases, as Friedrich Ataxia. For this reason, in recent years have been discovered posible new therapies:

- DNA Demethylation therapies
- HDAC Inhibitors
- HMTase Inhibitos
- Antigene RNA-base therapies



THANK YOU FOR YOUR ATTENTION

To summarize:

- FRDA is a hereditary form of ataxia given by a triplet repeat expansion on the intron 1 of FXN gene, which leads to mitochondrial dysfunction and neuronal degeneration especially in the DRG.

- It is possible to compare the FXN expansions with the one found on the gene lIL1 of Arabidopsis Thaliana.

- It has been proven that Triplet Repeat Expansions in transcribed regions is able to trigger the production of Small RNAs which lead to the Expansion-Associated Transcriptional Downregulation of the affected gene through epigenetic silencing.

- The level of expression of such siRNAs increases at higher temperatures.

References

- Bologna, N.G., and Voinnet, O. (2014). The diversity, biogenesis, and activities of endogenous silencing small RNAs in Arabidopsis. Annu. Rev. Plant Biol. 65,473–503
- Chan, S.W., Henderson, I.R., and Jacobsen, S.E. (2005). Gardening the genome: DNA methylation in Arabidopsis thaliana. Nat. Rev. Genet. 6, 351–360.
- Chan, S.W., Zhang, X., Bernatavichute, Y.V., and Jacobsen, S.E. (2006). Twostep recruitment of RNA-directed DNA methylation to tandem repeats. PLoS Biol. 4, e363.
- De Biase, I., Rasmussen, A., Monticelli, A., Al-Mahdawi, S., Pook, M., Cocozza, S., and Bidichandani, S.I. (2007). Somatic instability of the expanded GAA triplet-repeat sequence in Friedreich ataxia progresses throughout life. Genomics 90, 1–5.
- Di Prospero, N.A., and Fischbeck, K.H. (2005). Therapeutics development for triplet repeat expansion diseases. Nat. Rev. Genet. 6, 756–765.
- Gatchel, J.R., and Zoghbi, H.Y. (2005). Diseases of unstable repeat expansion: mechanisms and common principles. Nat. Rev. Genet. 6, 743–755.
- Sandi, C., Sandi, M., Anjomani Virmouni, S., Al-Mahdawi, S., and Pook, M.A. (2014). Epigenetic-based therapies for Friedreich ataxia. Front. Genet. 5, 165