DNA contains all the genetic information necessary to build and maintain an organism. Inside the cell nucleus, DNA is wrapped around histone proteins to form chromatin fibers. Depending on the degree of compaction, the chromatin is classified as euchromatin (less condensed, gene-rich, and more accessible to transcription machinery) or heterochromatin (highly condensed, gene-poor, and transcriptionally silent; Huisinga et al., 2006; Figure 1A).

Heterochromatin had long been considered as genetically inert. However, it turned out to be crucial to the biology of cells and contributes, for example, to chromosome segregation during cell division. It is also important for silencing mobile genetic elements – segments of DNA that can move within the genome or jump into the genome of other cells. Mobile genetic elements can pose a real threat to the integrity of the genome, and consequently, several defence mechanisms have evolved to identify them and prevent them from multiplying.

Some heterochromatic loci, formed of remnants of mobile genetic elements, are at the center of these defence mechanisms. These parts of the genome, also known as piRNA clusters, are often compared to a ‘memory system’. Despite being heterochromatic and usually silent, they are transcribed and processed into small non-coding RNAs. These RNAs are called PIWI-interacting RNAs, or piRNAs for short, because they are loaded by proteins of the PIWI family (Brennecke et al., 2007).

In many eukaryotes, the molecular feature defining heterochromatin is the enrichment of a specific histone mark called H3K9me3. This mark is recognized by proteins belonging to the HP1 (Heterochromatin Protein 1) family, which help to pack DNA into its condense structure (Bannister et al., 2001; Lachner et al., 2001). A member of this family, known as Rhino, facilitates the transcription of piRNA clusters in the germline of fruit flies. Although Rhino displays specific affinity for H3K9me3, it only binds to a subset of heterochromatin regions that contain this histone mark. So far, it was unclear how Rhino is guided to these specific parts of the genome (Mohn et al., 2014).

Now, in eLife, Julius Brennecke and colleagues – including Lisa Baumgartner as first author – report the results of experiments that clarify this process (Baumgartner et al., 2022).

The team (who are based at the Vienna BioCenter and the Institute of Molecular Biotechnology of the Austrian Academy of Sciences) used a combination of genetic, genomic and imaging approaches to study Rhino in the
germline of female fruit flies. The experiments revealed that Rhino interacts with a DNA-binding protein, which Baumgartner et al. named Kipferl. Indeed, a depletion of this protein in the ovaries of fruit flies leads to a broad redistribution and concentration of Rhino at the nuclear periphery in a form that evokes the shape of a Kipferl, an Austrian croissant.

They found that both Kipferl and Rhino are bound at many piRNA source loci, suggesting that Kipferl may act to identify the heterochromatin regions to which Rhino must bind. In the absence of Kipferl, Rhino is sequestered to another part of the genome and its binding to some piRNA clusters is lost, despite the presence of H3K9me3 marks. As a result, piRNA production in ovaries lacking Kipferl is reduced, several mobile elements are reactivated, and the females are less fertile than flies expressing Kipferl.

Kipferl is a DNA-binding protein that specifically binds to DNA sequences that are rich in the guanine nucleotide. This study suggests for the first time that such DNA sequences could help to attract Kipferl, which then recruits Rhino to participate in defining piRNA clusters. However, while Kipferl guides and stabilizes Rhino to some chromatin domains enriched in H3K9me3 to convert
them into piRNA clusters, some Rhino-dependent piRNA clusters do not need Kipferl (Figure 1B).

The study of Baumgartner et al. also suggests that additional factors help guide Rhino to piRNA clusters during early oogenesis, as Kipferl is not expressed during this developmental stage. Furthermore, piRNAs provided by the mothers may also help to recruit Rhino to specific heterochromatin regions in the embryo (Akkouche et al., 2017; Le Thomas et al., 2014). However, the relative contribution of maternally deposited piRNAs and Kipferl in recruiting Rhino to specify piRNA clusters during embryogenesis requires future investigations.

The study of Baumgartner et al. shows that the relationship between Rhino, Kipferl and DNA is complex, and their elegant dissection of the role of Kipferl provides substantial new insight into how piRNA clusters are defined in the genome.

Akdou Akkouche iGReD, Université Clermont Auvergne, CNRS, INSERM, Faculté de Médecine, Clermont-Ferrand, France
http://orcid.org/0000-0002-4781-2861

Emilie Brasset iGReD, Université Clermont Auvergne, CNRS, INSERM, Faculté de Médecine, Clermont-Ferrand, France
emilie.brasset@uca.fr
http://orcid.org/0000-0003-2479-7969

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