COMMENTARY

Evolutionary paradox: Conserved zinc finger proteins target rapidly evolving pericentric sequences

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Received: July 23, 2024; Accepted: October 22, 2024; Published Online: October 30, 2024; https://doi.org/10.59717/j.xinn-life.2024.100100

Citation: Ma R., Wang H. T., and Zhu B. (2024). Evolutionary paradox: Conserved zinc finger proteins target rapidly evolving pericentric sequences. The Innovation Life **2(4)**: 100100.

Pericentric heterochromatin represents a classic example of constitutive heterochromatin. It is essential for various biological functions, including ensuring precise chromosomal segregation, maintaining genome stability, and preventing inappropriate recombination.[1] These domains form a repressive environment with conserved characteristics across species from fission yeast to mammals, such as H3K9 methylation, HP1 proteins, and histone hypoacetylation. In mammals, H3K9me3 is produced by SUV39H1 and SUV39H2 (SUV39H homologues), which contain a SET domain with histone methyltransferase activity and a chromodomain that binds H3K9me3 specifically. H3K9me3 also serves as a docking site for HP1 to attach to pericentric heterochromatin. HP1 has a chromodomain responsible for H3K9me3 binding and a chromoshadow domain for HP1 dimerization and interaction with other proteins. HP1's binding to H3K9me3 and its interaction with SUV39H homologues enable the spreading of H3K9me3 to neighboring nucleosomes, thus providing a scaffold for additional HP1 molecules (Figure 1).[1] However, H3K9me3 is also present outside pericentric heterochromatin, indicating that the interaction among SUV39H homologues, H3K9me3, and HP1 homologues does not solely explain the recruitment of SUV39H homologues to these regions.

Despite the consistent repressive epigenetic marks, the DNA sequences within pericentric heterochromatin vary significantly among different species. Major satellite DNA in mice comprises pericentric heterochromatin regions and several megabase pairs of 234 bp units. These A/T-rich sequences make up ~5% of the mouse genome. Unlike the simpler chromosomal distribution of mouse major satellite DNA, human pericentric heterochromatin includes various repetitive DNA classes such as satellites I, II, and III, which differ in nucleotide compositions and length and are differentially distributed across chromosomes.

What triggers the targeting of SUV39H homologues to pericentric heterochromatin? How does the conserved machinery target variable pericentric sequences in vertebrates? In this commentary, we describe findings from a recent study by Ma et al. that reveals the conserved mechanism of pericentric heterochromatin initiation by ZNF512 and ZNF512B in vertebrates.[2]

ZNF512 AND ZNF512B RECRUIT SUV39H HOMOLOGUES TO PERICENTRIC HETEROCHROMATIN

The hidden DNA-binding proteins recognizing pericentric repeats and directly interacting with SUV39H homologues might have been missed in previous genetic studies due to redundancy. To identify these candidates, Ma et al. employed the highly specific targeting capability of the CRISPR/Cas9 system alongside the spatial precision of the APEX2-based proximity labeling system. This allowed them to characterize the protein composition of pericentric heterochromatin. They found that the zinc finger proteins ZNF512 and ZNF512B specifically localize to pericentric regions throughout the cell cycle. Furthermore, these two zinc finger proteins are constitutively expressed in most mouse cell types, fitting the definition of constitutive heterochromatin.[2]

To investigate the physiological function of ZNF512 and ZNF512B, they introduced ZNF512 or ZNF512B into a reporter cell line with 256 copies of LacO repeats. Remarkably, both ZNF512 and ZNF512B could initiate heterochromatin formation at this ectopic repetitive region. They also demonstrated that ZNF512 and ZNF512B interact directly with SUV39H homologues. In cells with a double knockout of *Znf512* and *Znf512b*, H3K9me3 foci remained at pericentric heterochromatin. These results indicate that ZNF512 and ZNF512B are sufficient to initiate heterochromatin formation by directly recruiting SUV39H homologues but are not necessary for maintaining heterochromatin.[2]

The chromodomain of SUV39H homologues and HP1 homologues recognize H3K9me3, and their interactions reinforce heterochromatin maintenance (Figure 1). This self-sustaining loop mechanism for heterochromatin maintenance, independent of initial recruitment, is well established in fission

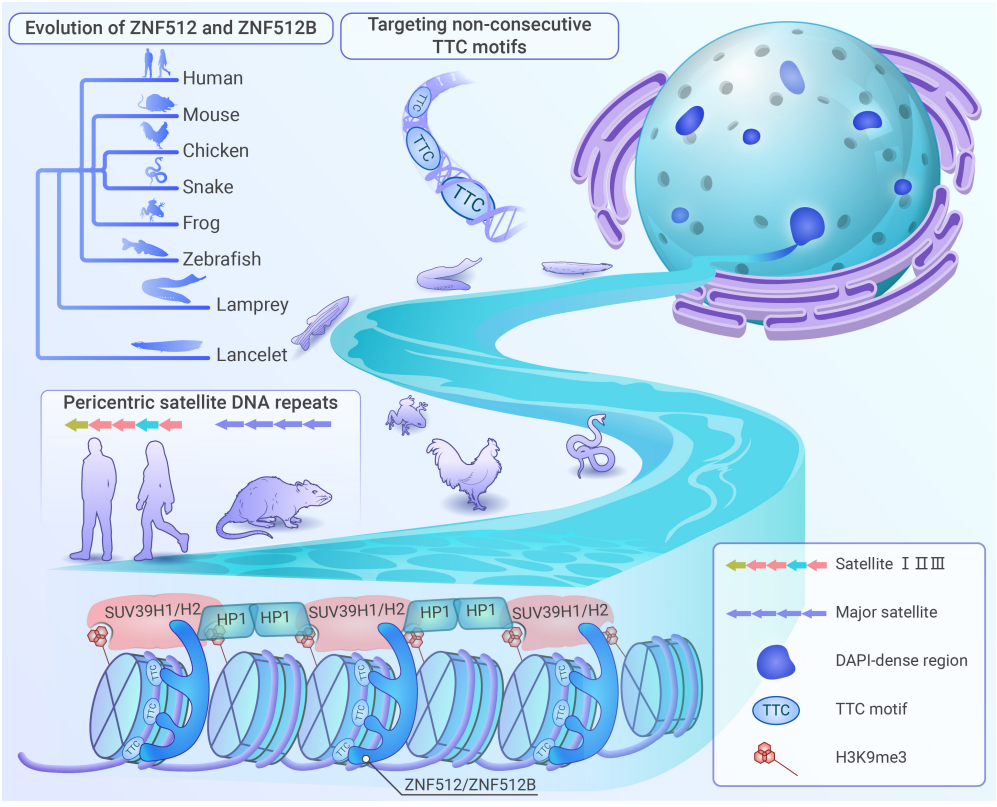

Figure 1. ZNF512 and ZNF512B, conserved among vertebrates, recognize non-consecutive TTC sequences in pericentric heterochromatin and recruit histone H3K9 methyltransferase SUV39H1/SUV39H2, leading to the establishment of constitutive heterochromatin. Despite the divergence of pericentric satellite DNA repeats, the non-consecutive TTC motifs remain conserved.

yeast.[3] This paradox between initiation factor and maintenance factor also occurs in X chromosome inactivation, another classic epigenetic phenomenon. During X chromosome inactivation in murine epiblasts, the XIST lncRNA is required to silence one X chromosome. However, removing XIST lncRNA at later development stages does not affect X chromosome sciencing.[4] To distinguish initiation and maintenance of pericentric heterochromatin, Ma et al. created quadruple-knockout cells by knocking out *Suv39h1* and *Suv39h2* in cells deficient in the presumed initiation factors ZNF512 and ZNF512B. Reintroducing ZNF512 or ZNF512B allowed the relocalization of catalytically inactive SUV39H homologues. These results suggest that ZNF512 and ZNF512B are initiation factors responsible for pericentric recruitment of SUV39H homologues.[2] In summary, exogeneously expressed ZNF512 and ZNF512B can recruit SUV39H homologues to exogenous non-heterochromatin sequences or pericentric regions in cells lacking initiation factors and maintenance factors.

ZNF512 AND ZNF512B RECOGNIZE NON-CONSECUTIVE MOTIFS IN PERICENTRIC HETEROCHROMATIN

The localization of SUV39H homologues in pericentric heterochromatin depends on ZNF512 and ZNF512B, raising the question of what determines the localization of ZNF512 and ZNF512B. By analyzing amino acid sequences and predicting protein structures, they found that ZNF512 and ZNF512B have 3 and 4 zinc finger domains, respectively. Interestingly, unlike classical zinc finger proteins, ZNF512 and ZNF512B have identical fingerprint sequences in their zinc fingers, suggesting each zinc finger can bind the same DNA sequence. The long linker sequences between the zinc fingers imply they may bind non-consecutive DNA sequences. To test these hypotheses, the authors mutated the fingerprint sequences of the zinc fingers and shortened the longer linker sequences, respectively. The pericentric localization of ZNF512 and ZNF512B was abolished by zinc fingerprints mutations or linkers swapping. These experiments showed that the localization of ZNF512 and ZNF512B on pericentric heterochromatin depends on their zinc fingerprints and long linkers. Additionally, ZNF512 and ZNF512B were shown to bind pericentric heterochromatin-derived DNA probes, and ZNF512 with zinc fingerprints mutations or swapped linkers lost this ability.[2]

Despite the lack of highly conserved DNA sequences (Figure 1), pericentric heterochromatin in different species has the same histone H3K9 methylation. To address this paradox, the authors compared the amino acid sequences of ZNF512 and ZNF512B across species. The results showed identical zinc fingerprints and similar linker lengths conserved from lamprey to humans (Figure 1). Additionally, expressing zebrafish or human ZNF512 and ZNF512B genes in a mouse cell line, and mouse genes in a human cell line, demonstrated that ZNF512 and ZNF512B localize to pericentric heterochromatin across species.[2]

SUMMARY AND FUTURE RESEARCH DIRECTIONS

In conclusion, Ma et al. demonstrate that ZNF512 and ZNF512B recognize non-consecutive sequences in pericentric heterochromatin through their zinc fingerprints and long linkers, recruiting histone H3K9 methyltransferase SUV39H homologues to establish constitutive heterochromatin. This mechanism is conserved among vertebrates (Figure 1).[2]

This research raises several interesting questions for future study. One question is the structure of ZNF512 and ZNF512B with their binding DNA sequences. However, obtaining a stable protein-DNA complex may be challenging because the interaction between ZNF512 or ZNF512B and pericentric heterochromatin-derived DNA probes appears relatively weak.[2] Another area of interest is the structure of ZNF512 and ZNF512B with SUV39H homologues. A proposed two-stage activation mechanism for SUV39H1 suggests it is inactive in its free form, and H3K9me3 recognition and chromatin anchoring allosterically promote its methylation activity.[5] Could the interaction between ZNF512 and ZNF512B with SUV39H1 bypass this autoinhibition? Structural study on ZNF512 and ZNF512B with SUV39H homologues might shed light on this issue. Additionally, the recognition of non-consecutive DNA sequences by split zinc fingers reveals a new pattern of DNA recognition, potentially leading to the identification of more such examples. Bioinformaticians could develop new algorithms to identify non-consecutive DNA binding motifs in the future.[2] Finally, long-read sequencing technology now allows for obtaining complete telomere-to-telomere sequence information of pericentric heterochromatin, studying pericentric heterochromatin more precisely, and facilitating functional analysis using genome editing technology.

DECLARATION OF AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the preparation of this work, the authors used ChatGPT to polish the language. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

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FUNDING AND ACKNOWLEDGMENTS

This work was supported by the New Cornerstone Science Laboratory (to B.Z.). The funder had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

AUTHOR CONTRIBUTIONS

R.M. and B.Z. wrote the manuscript, and H.-T.W. drew the figure. All the authors read and commented on the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.