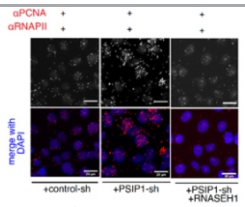
कोशिकाओं में जीनोमिक स्थिरता

6

जीनोम अखंडता को बनाए रखने में एपिजेनेटिक कारकों की भूमिका का अध्ययन

एस . जयकुमार*
मुक्त मूलक जैविकी अनुभाग, विकिरण जीवविज्ञान और स्वास्थ्य विज्ञान प्रभाग, भाभा परमाणु अनुसंधान केंद्र, ट्रांबे-400085, भारत



नियंत्रण और PSIP1-KD RWPE-1 कोशिकाओं से प्राप्त α-PCNA और α -RNAPII एंटीबॉडी के बीच प्रतिनिधि पी. एल. ए. छवि

सारांश

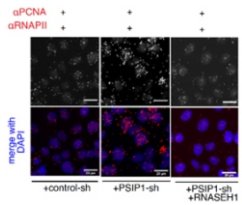
रोग के विकास और उसके उपचार का अध्ययन करने के लिए कोशिकाओं में जीनोमिक अखंडता को बनाए रखने में शामिल आण्विक तंत्रों का अध्ययन महत्वपूर्ण है। इस अध्ययन में, ट्रांसक्रिप्शन के दौरान अनिर्धारित आर-लूप को हल करने के लिए एक एपिजेनेटिक कारक, PSIP1 की भूमिका का अध्ययन किया गया है। PSIP1 की कमी से सक्रिय रूप से ट्रांस्क्रिप्ट करने वाले जीन निकायों के आर-लूप में उल्लेखनीय वृद्धि होती है, जिससे ट्रांसक्रिप्शन-रेप्लिकेशन द्वंद्व में वृद्धि के कारण डीएनए की क्षति में वृद्धि होती है। ये परिणाम कोशिकाओं में जीनोमिक स्थिरता बनाए रखने में PSIP1 की भूमिका के बारे में एक नई अंतर्दृष्टि प्रदान करते हैं।

Genomic Stability in Cells

6

Understanding the Role of Epigenetic Factors in Maintenance of Genome Integrity

S. Jayakumar*
Free Radical Biology Section, Radiation Biology & Health Sciences Division, Bhabha Atomic Research Centre, Trombay-400085, INDIA



Representative PLA image between α-PCNA and α-RNAPII antibodies obtained from control and PSIP1-KD RWPE-1 cells

ABSTRACT

Studying the molecular mechanisms involved in maintaining genomic integrity in cells is important for understanding disease development and their treatment. In this study, the role of an epigenetic factor, PSIP1 has been understood in resolving unscheduled R-loops during transcription. Depletion of PSIP1 lead to significant increase in R-loops in the actively transcribing gene bodies leading to increase in DNA damage by increased transcription-replication conflicts. These results show a novel insight into the role of PSIP1 in maintaining genomic stability in the cells.

KEYWORDS: *Genome Integrity, R-loops, PSIP1, Epigenetics, DNA damage*

**Author for Correspondence: S. Jayakumar*
E-mail: kumarsj@barc.gov.in

Introduction

Deoxyribonucleic acid (DNA) is the central molecule in the functioning of the eukaryotic cells. They code all the necessary information for the cells to thrive, organise themselves and propagate. Faithfully maintaining the integrity of the genome is vital to this cycle. However, numerous internal cellular processes and external factors, both physical and chemical, pose threats to DNA stability. To counteract potential damage, cells employ antioxidant mechanisms and various repair pathways, ensuring the restoration of DNA integrity. Any deviations or dysregulations in this process can lead to deleterious consequences to the cells. The fundamental reasons for many of the diseases like cancer is traced back to the failure of the cells to maintain the integrity of their genome. Hence, comprehending the pathways and mechanisms responsible for maintaining genomic integrity is imperative for disease understanding and therapeutic development. Compounding the challenge of genomic maintenance is the inherent length of DNA, requiring significant compaction within the confines of the cellular environment. Furthermore, they have to be decompacted during replication and transcription (the process of decoding the information from DNA). This compaction and packaging of the DNA in the form of chromatin is achieved by the cells by deploying special class of basic (alkaline) proteins called histone proteins. These histone proteins undergo multitude of post-translational modifications (PTMs) like acetylation, methylation, phosphorylation, ubiquitination etc., in their amino acid residues present especially in their N-terminal regions. These modifications further acts as signals and anchoring points for recruitment of other regulatory proteins. In this way, the histone proteins not only provide the structural fabric for the genome but also play core role in functional regulation of the genome. The histone PTMs themselves are regulated by complex network of reader, writer and eraser proteins and functional regulation of genome through these histone PTMs and its associated factors is called as epigenetic mechanism of gene regulation. Beyond regulating gene expression, the role of epigenetics in DNA damage and repair is emerging as a important area of research [1]. PC4 and SF2 interacting protein (PSIP), is a multifunctional chromatin protein, that binds to methylated

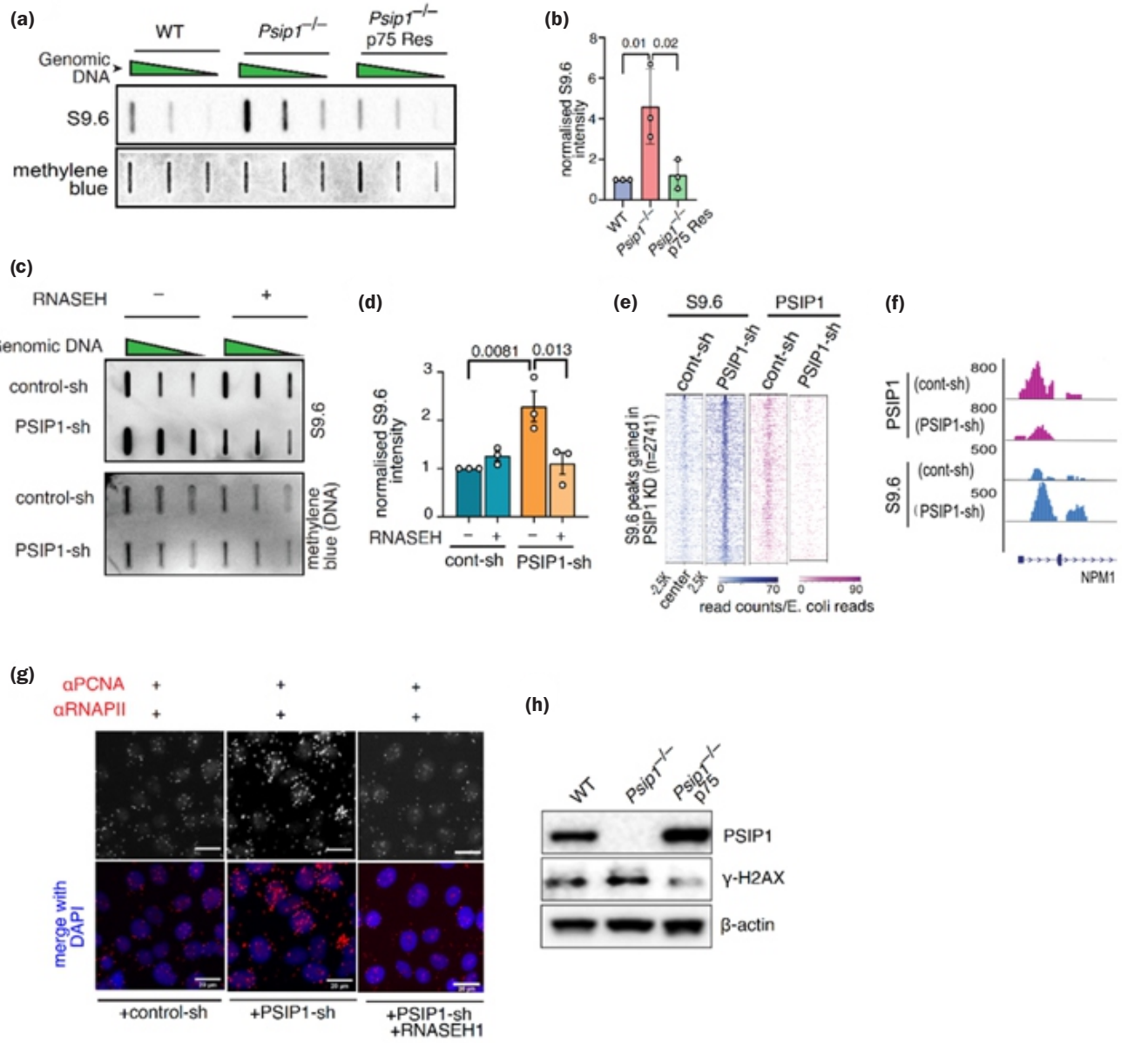


Fig.1: (a). R-loops levels seen by slot blot using S9.6 antibody in wild-type, Psip1$^{-/-}$ and Psip1$^{-/-}$ p75R MEFs. The band intensity was normalized to loading control (methylene blue) and plotted (b). (c). Similar to (a) but in RWPE-1 cells and the normalized band intensity has been plotted (d). (e). Heatmap showing R-loop and PSIP1 levels in PSIP1-KD and control RWPE-1 cells. (f). Genome browser track showing the CUT&Tag signal (read counts) for PSIP1 and R-loop in control and PSIP1-KD RWPE-1 cells. (g). Representative PLA image between α-PCNA and α-RNAPII antibodies obtained from control and PSIP1-KD RWPE-1 cells. (h). Immunoblot images of PSIP1, γ-H2AX and β-actin for lysate from wild-type, Psip1$^{-/-}$ and Psip1$^{-/-}$ p75R MEFs. (adopted from [5]).

histone H3 lysine 36 (H3K36me) via the PWWP domain, and PSIP1 binding is enriched at the sites of RNAPII transcription and helps in transcription initiation and elongation. Previous study has shown that the absence of PSIP1 in the cells leads to increased DNA damage [2]. Additionally, studies employing immunoprecipitation of PSIP1 revealed interactions with various proteins involved in transcription, RNA processing, and DNA repair. Notably, these interacting proteins overlap with those identified in R-loop complexes [2,3].

R-loops are RNA-DNA hybrid structures formed during transcription when newly produced RNA molecules bind back to the DNA template strand. Unscheduled R-loops, if not resolved, can impede transcription and trigger DNA damage due to transcription-replication conflicts. The complete pathway and the proteins involved in R-loops resolution in cells remain unclear. This work proposes a novel role for PSIP1 in resolving R-loops, thereby safeguarding genome integrity during transcription.

Materials and Methods

For the study, human prostate epithelial cells (RWPE-1) and mouse embryonic fibroblast cells (MEFs) were used. For estimating the R-loops, cells were lysed in lysis buffer with proteinase K and incubated at 55^{0}C overnight. Isopropanol was added to the lysate and the DNA was pelleted using centrifugation. The DNA was quantified and different quantities of DNA were blotted onto the N+ nitrocellulose membrane using a slot blot apparatus. Then the membrane was baked at 80^{0}C for 2 hours, incubated in blocking buffer (5% skimmed milk in PBS) for 1 hour, followed by incubation with S9.6 antibody overnight at 4^{0}C in a nutator. Blots were washed and then incubated with secondary antibody for 2 hours at room temperature, washed and developed. The same blots were stained with methylene blue to quantify total DNA. Cleavage under targets & Tagmentation (CUT&Tag) was performed and the sequence reads were processed according to protocol [4]. Proximity Ligation Assay (PLA) was performed using Duolink In Situ Red Starter kit (Mouse/Rabbit) according to the manufacturer protocol (Merck, DUO92101).

Results and Discussion

To understand the role of PSIP1 in R-loop homeostasis, the levels of R-loops were studied in MEF cells with PSIP1 knock out, using S9.6 antibody through blotting experiment. Knockout of PSIP1 lead to stark increase in the R-loops accumulation that could be reversed by restoring the expression of PSIP1 in those cells (Fig. 1a and b), implicating the role of PSIP1 in R-loop resolution. Similarly, in RWPE-1 cells, when PSIP1 was depleted it lead to increase in R-loops and that increase could be reversed by overexpression of RNASEH1-an enzyme that can act specifically on RNA-DNA hybrid and degrades them. (Fig. 1c and d). Further to understand the genome wide accumulation of R-loops and its link with PSIP1 depletion, CUT&Tag-seq, a modified form of chromatin immunoprecipitation was used. In this assay, the cells were permeabilized, the targets were bound with antibody and the antibody bond region was cleaved using transposase. These cleaved DNA fragments are isolated, amplified, sequenced, aligned to the reference genome and the enriched regions are found out. From the CUT&Tag analysis, it was found that upon PSIP1 depletion 2741 peaks were showing accumulation of R-loops and these regions are occupied by the PSIP1 in the control cells, again indicating the role of PSIP1 in R-loop resolution (Fig. 1e and f). These peaks are mostly seen in the gene bodies. The consequence of this R-loop accumulation in terms of transcription-replication conflict (TRC) was then probed by performing PLA between PCNA (part of replication) and RNAPII (part of transcription). Results indicated the increased TRCs linked to PSIP1 depletion and that can be reversed by overexpressing RNASH1 (Fig. 1g). Further this increase in TRC lead to DNA damage as seen by the increased γ-H2AX levels, and that damage could be reversed by re-expression of PSIP1 (Fig. 1h).

Conclusion

These results demonstrated a novel role of PSIP1 in resolving unscheduled R-loops that arise during transcription and thereby preventing TRC and DNA damage in the transcriptionally active part of the genome [5].

Acknowledgements

The author thank the European Research Commission for providing with Marie-Curie Individual Fellowship and Dr. Pradeepa Madapura and other Lab members of Madapura Lab, Queen Mary University of London, London, UK, for help in carrying out the project. Author also thank Dr. Santosh K. Sandur, Head, RB&HSD, BARC for the help and encouragement.

References

[1] Millán-Zambrano, G., Burton, A., Bannister, A.J. et al., Histone post-translational modifications — cause and consequence of genome function, Nat Rev Genet., 2022, 23, 563–580.

[2] Jayakumar, S., Taylor, G. C. A., von Kriegsheim, A. & Pradeepa, M. M., H3K36me3 and PSIP1/LEDGF associate with several DNA repair proteins, suggesting their role in efficient DNA repair at actively transcribing loci., Wellcome Open Res., 2021, 2, 83.

[3] Pradeepa, M. M., Grimes, G. R., Taylor, G. C. a, Sutherland, H. G. & Bickmore, W. a. Psip1/Ledgf p75 restrains Hox gene expression by recruiting both trithorax and polycomb group proteins, Nucleic Acids Res., 2014, 42, 9021–32.

[4] Kaya-Okur, H. S. et al. CUT & Tag for efficient epigenomic profiling of small samples and single cells, Nat. Commun., 2019, 101(10), 1–10.

[5] Jayakumar, S., Patel, M., Boulet, F. et al., PSIP1/LEDGF reduces R-loops at transcription sites to maintain genome integrity, Nat Commun., 2024, 15, 361.