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Mini Review

Chromatin Organization and Transcriptional Regulation at TADs and Loops Depleted Pachytene Stage during Spermatogenesis

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ABSTRACT

In mammals, high-order chromatin structures are reorganized during spermatogenesis. Topologically Associating Domains (TADs) and chromatin loops are almost lost at the pachytene stage while CTCF and cohesion are still bound on pachytene chromatin, and the promoters and enhancers on pachytene chromatin remain open. The fact that pachytene chromatin is actively transcribed when TADs and chromatin loops are lost indicates that gene transcriptional regulation at pachytene stage can be independent of TADs and chromatin loops. TADs and chromatin loops loss is also found in CTCF and cohesion acute degraded cells in vitro. In this review, we discuss the reorganized chromatin organization during spermatogenesis, especially at pachytene stage. We also discuss recent progress in our understanding about fine-scale chromatin structures beyond TADs and chromatin loops and their relationship with transcriptional regulation in normal and CTCF or cohesion depleted cells. Finally, we show that recent findings about chromatin structures beyond TADs and chromatin loops may explain the gene transcriptional regulation at pachytene stage.

Keywords: Chromatin reorganization; Pachytene; Spermatogenesis; Fine-scale chromatin structures; Transcriptional regulation

INTRODUCTION

In mammals, interphase chromatin is organized into A/B compartments, Topologically Associating Domains (TADs), and chromatin loops in the nucleus, revealed by Hi-C and its related methods [1]. The A/B compartments divide the chromosomes into two parts, where A compartments associate with opened, actively transcribed chromatin regions while B compartments overlap with closed, transcription silenced chromatin regions. TAD is often insulated by CCCTC-binding factor (CTCF) and cohesion complex, in which chromatin interacts more frequently with each other than with linearly nearby chromatin outside the TAD. Structural loops often form between two boundary regions of TADs while regulatory loops often occur within TADs. TADs and loops are believed crucial for gene transcriptional regulation, and dynamic TADs and loops are associated with many biological processes [2]. However, acute disruption of TADs and loops by CTCF or cohesion protein depletion only have a relatively modest effects on gene expression [3,4].

The mammalian spermatogenesis is a highly specialized developmental process which involves spermatogonia renewal and proliferation, meiosis, and spermatogenesis [5]. Recent studies based on Hi-C have examined the dynamic 3D genome organization during spermatogenesis in mice and the rhesus monkey with isolated spermatogenic cells at different stages of spermatogenesis, containing spermatogonia (primitive type A Spermatogonia (priSG-A) and type A Spermatogonia (SG-A)), meiosis (pachytene), and spermiogenesis (round Spermatid (rST) and Spermatozoa (SZ)) [6-8]. During spermatogenesis, A/B compartments on autosomes are largely unaltered while the strength of A/B compartments was weakened at pachytene stage

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during meiosis [6,8]. In mice, Hi-C analysis revealed that TADs and chromatin loops are existed in priSG-A, weakened in SG-A, almost disappeared in pachytene and rST, and re-established in SZ [8]. In the rhesus monkey, TADs also strongly depleted at pachytene stage [6]. Other studies focusing on pachytene stage also confirmed this finding [9-11]. Previous studies have shown that CTCF and cohesion loss can result in acute disruption of TADs and chromatin loops [3,4], but both immunofluorescence (IF) and ChIP-seq results showed that CTCF and cohesion are still bound on pachytene chromatin [7,8], which indicates that the loss of TADs and chromatin loops on pachytene chromatin are largely independent of CTCF and cohesion. Although TADs and chromatin loops are largely lost in Pachytene stage, pachytene chromatin is actively transcribed [8], indicating that gene transcriptional regulation can be independent of TADs and chromatin loops at specific developmental stages. Although the promoters and enhancers are still open on pachytene chromatin [8,12], how enhancers recognize their target promoter(s) and regulate gene transcription at pachytene stage is still unclear. The regulated gene transcription at the pachytene stage is crucial for post-meiotic process and the production of mature sperm. Mutations of meiotic genes or abnormal gene expression often resulting in infertility [13]. Thus, unveiling the mechanisms of gene transcriptional regulation at pachytene stage during spermatogenesis will also promote the understanding and treatment of infertility.

In this mini review, we will discuss recent findings about chromatin structures in TADs and chromatin loops depleted cells and their relationship with gene transcriptional regulation based on the high resolution chromatin interaction analysis and super-resolution imaging methods. Results from these studies also provide new insights on chromatin structures and gene transcriptional regulation at pachytene stage during spermatogenesis.

TRANSCRIPTION ASSOCIATED FINE-SCALE CHROMATIN STRUCTURES

The findings that gene transcriptional regulation can precede normally in TADs and loops depleted cells [3,4,7,8], suggest that there may be other chromatin structures more tightly relate to gene transcriptional regulation, which are hard to be revealed by Hi-C due to its resolution limitation. The resolution of chromatin contacts maps generated by Hi-C depends on the use of restriction enzymes and the sequencing depth. Smaller chromatin fragments after restriction enzymes digestion and deeper sequencing of the Hi-C libraries give higher resolution of chromatin contacts maps. However, even when sequencing by billions of reads, local chromatin contacts like Enhancer-Promoter (E-P) interactions are still inefficiently detected by Hi-C, due to the relatively long distance between restriction sites. To overcome this issue, micrococcal nuclease is used for chromatin digestion in Micro-C, replacing the restriction enzymes used in Hi-C to obtain high resolution chromatin contacts maps [14]. Recent work in mammalian cells based on nucleosomes-resolution Micro-C reveals that there are fine-scale chromatin structures beyond TADs and chromatin loops [15]. Micro-C data reveal that previously obscured chromatin structures within TADs contain sharper "loop-like" and "stripe-like" structures formed between promoter-promoter and enhancer-promoter sites (P-P/E-P dots and P-P/E-P stripes), and local self-interacting E-P domains [15]. P-P/E-P stripes can link multiple genes or genes and enhancers together which are greatly reduced after acute transcription inhibition, yet acute transcription inhibition have little effect on chromatin organization at the level of TADs and chromatin loops [15]. In addition, recent work of NicE-C, replacing the restriction enzymes used in Hi-C with nicking enzyme digestion, also reveals the fine-scale P-P/E-P interactions at high resolution [16]. Unlike HI-C or Micro-C measures genome-wide chromatin contacts (all versus all), NicE-C measures the chromatin contacts formed between open chromatin regions (many versus many) [16]. As functional promoter and enhancer regions are usually open chromatins, NicE-C can detect P-P/E-P interactions similar to Micro-C with less sequencing depth. In summary, after breaking the resolution limit of Hi-C, recent studies uncover fine-scale transcription-associated chromatin structures, such as P-P/E-P interactions.

CHROMATIN STRUCTURES BEYOND TADS AND CHROMATIN LOOPS IN CTCF AND COHESION DEPLETED CELLS

As only a subset of fine-scale chromatin structures appear to be CTCF and cohesion-specific [15], how acute depletion of CTCF and cohesion can affect P-P/E-P interactions remains unclear. A recent preprint work addressed this question by performing high-resolution Micro-C with CTCF and cohesion, acute depletion cells [17]. After CTCF and cohesion depletion, Micro-C data reveals similar chromatin organization changes at the levels of TADs and chromatin loops compared to previous studies based on Hi-C [3,4,17]. However, the Micro-C data showed that the fine-scale P-P/E-P interactions and gene transcription are largely insensitive to acute depletion of CTCF and cohesion [17]. Thus, acute depletion of CTCF and cohesion can abolish TADs and chromatin loops, but have little effect on P-P/E-P interactions. This might be the reason for the modest changes of gene expression under these conditions. In addition to the resolution breakthrough by Micro-C which reveals fine-scale unaffected P-P/E-P interactions in CTCF and cohesion depleted cells, super-resolution chromatin tracing studies also reveal the chromatin organization features in individual cells compared to that from cell population averaging revealed by Hi-C [18]. In super-resolution chromatin tracing, targeted genomic region is partitioned into numerous segments and these individual segments were imaged by sequential rounds of fluorescence in situ hybridization (FISH). Pairwise interactions between chromatin segments can then be measured from the super-resolution chromatin images generated from each cell [18]. The imaging results reveal an abundance of TAD-like structures in single cells. The boundaries of TAD-like structures in single cells vary from cell to cell while with a preference at CTCF and cohesion binding sites, which is consistent with the finding that TAD boundaries are often bound by CTCF and cohesion in bulk cells revealed by Hi-C. The super-resolution chromatin

tracing results in cohesion depleted cells show that TADs are abolished at the population average level while the TAD-like structures in single cells are still exist [18]. The reason for this discrepancy might be the loss of preferential positioning of TAD boundaries at CTCF and cohesion binding sites in the absence of cohesion. Thus, although at the population level TADs are lost, the TAD-like structures in individual cells may still function in E-P contacts and gene transcriptional regulation.

CONCLUSION

During spermatogenesis, TADs and chromatin loops are lost at pachytene stage, which is similar to that in CTCF and cohesion depleted cells. In addition, although TADs and chromatin loops are lost, chromatin accessibility of promoters and enhancers and the gene transcriptional regulation are still normal at pachytene stage, which are also similar to that in CTCF and cohesion depleted cells. However, CTCF and cohesion are still bound on pachytene chromatin, suggesting that the mechanism of disappeared TADs and chromatin loops at pachytene stage is different from that in CTCF and cohesion depleted cells. The exist of P-P/E-P interactions and TAD-like structures at single cell level in CTCF and cohesion depleted cells might be the reason why TADs and chromatin loops loss have modest effects on gene transcription. Thus, high resolution chromatin structures such as P-P/E-P interactions at fine-scale and TAD-like structures at single cell level may also explain the normal gene transcription regulation at TADs and chromatin loops depleted pachytene stage during spermatogenesis. Future studies with high resolution Micro-C or NicE-C at population level and super-resolution chromatin imaging at single cell level will help us to determine whether there are such fine-scale chromatin structures at Pachytene stage.

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CONFLICT OF INTEREST

None

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