IRGM-deficient settings drives gut inflammation in models of colitis.

The study from Mehto et al. (2019) raises several interesting lines of future investigation. The central role of NLRP3 in propagation of inflammation makes it an attractive drug target, with several NLRP3 inhibitors identified and tested preclinically (Mangan et al., 2018). Despite this, the molecular targets and biochemical interactions mediating NLRP3 inhibition remain unclear. Further structural and biochemical investigation of the IRGM/ASC/NLRP3 interaction may provide insight into important motifs mediating NLRP3 oligomerization and could aid in the development of small molecules specifically targeting critical NLRP3 sites.

Additionally, studies investigating mechanisms regulating IRGM levels are warranted. While murine homologs of IRGM are interferon inducible, the IRGM gene was inactivated early in primate evolution due to a retrotransposon event that disrupted the open reading frame. The gene was reestablished in a common ancestor of humans and great apes due to an ERV9 retroviral insertion at the 5′ end of the *IRGM* gene (Bekpen et al., 2009). Considering the link between IRGM and NLRP3, understanding mechanisms regulating IRGM levels may provide insight into the inflammatory process. Finally, as NLRP3 dysregulation has been implicated in the pathogenesis of several chronic inflammatory diseases, therapeutic strategies targeting the IRGM-NLRP3 interaction could be useful for a variety of diseases with inflammatory etiologies.

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Pach-ing It in: The Peculiar Organization of Mammalian Pachytene Chromosomes

Yoon Hee Jung[1] and Victor G. Corces[1,*]
[1]Department of Biology, Emory University, 1510 Clifton Road NE, Atlanta, GA 30322, USA
*Correspondence: vgcorces@gmail.com
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In this issue of *Molecular Cell*, Wang et al. (2019) use Hi-C to visualize at high resolution the complex reprogramming of chromatin architecture during spermatogenesis in rhesus monkeys and mice. They find that pachytene spermatocytes have a unique chromosome organization that may result from the presence of the synaptonemal complex and transcription-associated proteins.

Mammalian spermatogenesis can be divided into three crucial phases: mitosis (self-renewal of spermatocytes), meiosis (formation of haploid spermatids), and the post-meiotic phase known as spermiogenesis (morphological and biochemical changes). The critical events of homologous recombination, programmed DNA double-strand breaks, and chromosome synapsis occur during meiosis. To facilitate these events, the prophase of meiosis I is prolonged and can be further subdivided into four stages named leptotene, zygotene, pachytene and diplotene (Clermont, 1972).

Changes in the epigenomic landscape during spermatogenesis have been characterized in terms of chromatin accessibility, histone modifications, transcription, and DNA methylation (Guo et al., 2017; Hammoud et al., 2014; Lesch et al., 2016; Maezawa et al., 2018). However, little is known about the three-dimensional (3D) chromatin organization in spermatogenesis intermediates. In this issue of *Molecular Cell*, Wang et al. (2019) describe the dynamic reprogramming of chromatin architecture during spermatogenesis in rhesus monkeys and mice by comparing four different stages—spermatogonia,

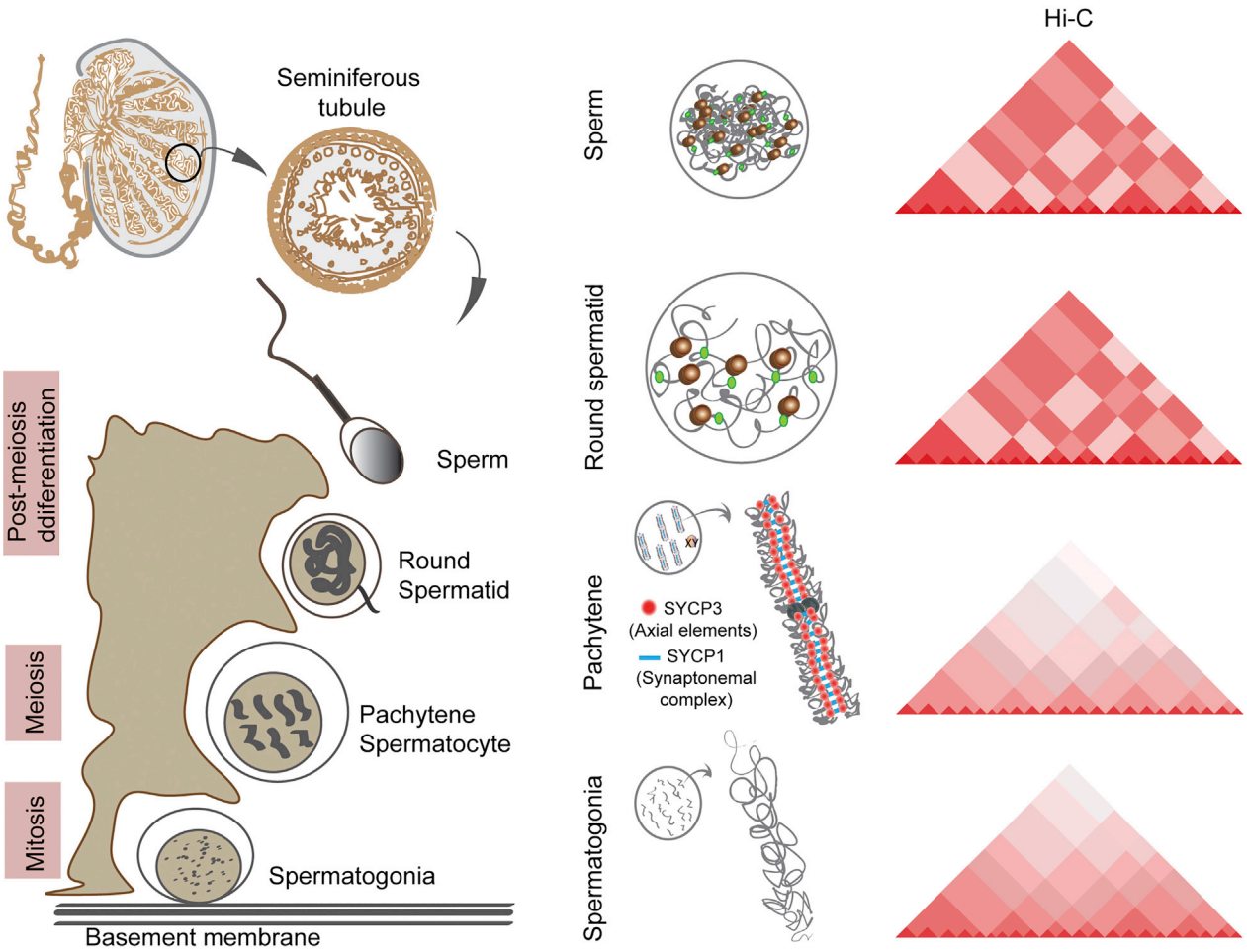

Figure 1. Transitions during Spermatogenesis at the Cellular, Nuclear, and Chromosome Organization Levels
Left side shows a section through a mouse testis and seminiferous tubule indicating the location of male germline cells at different stages of differentiation, from spermatogonia to mature sperm. Center describes the organization of nuclei and chromosomes in the same stages of spermatogenesis. Right side displays Hi-C heatmaps covering the length of a single chromosome and showing intra-chromosome interactions in the nuclei of the corresponding cell types.

pachytene spermatocytes, round spermatids, and mature spermatozoa—using a low-input Hi-C method.

The 3D organization of chromosomes changes throughout the cell cycle. During interphase, results from chromatin conformation capture (3C)-based techniques such as Hi-C suggest that chromosomes are organized into two types of compartmental domains, called A and B, containing transcribed genes with active histone modifications and inactive genes with repressive histone modifications, respectively. High-resolution Hi-C data allows the identification of CTCF loops, which may or may not exactly overlap with compartmental domains. Domains defined based on the directionality of interactions and composed of random combinations of CTCF loops and compartmental domains have been called topologically associating domains (TADs) (Rowley and Corces, 2018). Both compartments and TADs disappear during mitosis and are replaced by small loops located at random positions and extruding from a central axis (Gibcus et al., 2018).

TADs and compartments are visible in spermatogonia, but the interactions that give rise to these domains are weaker than in normal somatic cells. These domains become almost undetectable in pachytene spermatocytes, but they are visible again in round spermatids and mature sperm, where they are undistinguishable from those observed in fibroblasts (Figure 1). During the pachytene stage of meiotic prophase, the X and Y chromosomes become transcriptionally inactive. These two chromosomes form the XY body, which displays a 3D organization distinct from the transcriptionally active autosomes. Wang et al. (2019) then analyze how the frequency of contacts between sequences in a chromosome change with the distance between the interacting loci. Based on this analysis, they show that the folding of pachytene chromatin is very similar to that of mitotic metaphase chromatin up to distances of 6 Mb. At longer distances, the decay in contact probability resembles that of fibroblasts, something that is not observed in metaphase chromosomes (Figure 1). Intriguingly, pachytene autosomes display a compartmental organization different from that observed in somatic cells. Instead of

the typical compartments, pachytene chromosomes contain smaller domains detected by local principal component analysis. Wang et al. (2019) refer to these structures as "refined compartments" and find that they correlate very closely with the transcriptional state of the corresponding sequences. When somatic cells are depleted of the cohesin subunit Rad21, interactions within compartments become stronger. Interestingly, the segregation of chromatin into refined compartments correlated with transcription is even stronger than that observed in cohesin-depleted somatic cells.

The correlation between transcriptional state and the location of refined compartments prompted Wang et al. (2019) to further explore the possible connection between chromatin organization and transcription in pachytene spermatocytes by using α-amanitin to inhibit gene expression. This treatment had no effect on the refined A/B compartments. It is possible that, once established, these domains do not require continued transcription for their maintenance. Alternatively, residual RNAPII or other associated transcription factors may be sufficient to maintain compartmental organization. It is also possible that, although the location of refined compartments correlates with transcription, the synaptonemal complex is responsible for promoting the formation of refined compartments while restricting TADs in pachytene chromatin. To test this possibility, Wang et al. (2019) depleted pachytene spermatocytes of Sycp2, a core component of the synaptonemal complex, and Top6bl, a topoisomerase that regulates double-strand break formation during spermatogenesis. Mice deficient in either of these two proteins have attenuation of refined A/B compartments and reappearance of TADs, suggesting an important role for these proteins in the chromosome reorganization taking place at the pachytene stage. This conclusion is moderated by the fact that depletion of either protein leads to arrest in the zygotene or leptotene stages.

Pachytene spermatocytes are arrested in prophase I of meiosis (Figure 1). However, the organization of pachytene chromosomes differs from that of similar stages in mitosis. An interesting difference is that mitotic chromosomes contain loops formed by condensin I and II and extruding at random positions from a central axis (Gibcus et al., 2018). Instead, chromosomes in the pachytene stage appear to contain loops arising at fixed positions from a central axis. The difference in interaction decay rate with respect to distance between pachytene and mitotic chromosomes suggests a less compact chromatin state in the former. This difference in organization may allow transcription of pachytene chromatin and facilitate correct synapsis between homologous chromosomes.

It is possible that chromatin re-organization accompanied by the disappearance of TADs and typical compartmental domains is due to the loss of architectural proteins such as CTCF. However, these changes take place in pachytene spermatocytes despite the maintenance of chromatin-accessible regions during this stage. ATAC-seq analysis of pachytene spermatocytes by Maezawa et al. (2018) shows the persistence of accessible chromatin in intergenic and intron regions, suggesting that DNA-binding proteins—including architectural factors such as CTCF and cohesin—remain bound to DNA regardless of chromosome organization during meiosis. The persistence of architectural proteins is also supported by the observation that depletion of Sycp2 and Top6bl reverts the chromosomes to their normal organization. It is possible that covalent modification of architectural proteins such as CTCF interferes with their normal function in chromosome organization, explaining their preservation in pachytene chromosomes.

Results described by Wang et al. (2019) suggest that the synaptonemal complex and the transcription apparatus play an active role in pachytene chromosome formation. Future experiments should focus on dissecting the relative contributions of specific components of these complexes in the establishment of the unique organization of chromosomes in pachytene spermatocytes.

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