Unpacking chromatin remodeling in germ cells: implications for development and evolution

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Abstract: Germ cells reflect the evolutionary history and the future potential of a species. Understanding how the genome is organised in gametocytes is fundamental to understanding fertility and its impact on genetic diversity and evolution of species. Here, we explore principles of chromatin remodeling during the formation of germ cells and how these are affected by genome reshuffling.

Key words: HiC; TADs; compartments; spermatocytes; oocytes; chromosomal fusions

The spatial conformation of genomes arises from a close interplay between gene function and chromatin organisation. It is now well-stablished that the hierarchical organisation of the genome is widely conserved across mammalian species comprising as it does, **chromosome territories** (Glossary) in which chromatin is organised into **compartments** (open/closed), **topologically associated domains** (TADs) and DNA loops [1]. However, it was not until recently that the basis of chromatin remodeling during the formation of germ cells and early development began to be understood [2–4]. Here we provide an overview of the structural and functional plasticity characterising higher-order genomic organisation and how this is transmitted to offspring, with a focus on evolution.

Principles of genome organisation in mammalian germ cells

Highly differentiated haploid gametes (sperm in males and oocytes in females) are generated during **gametogenesis**; a regulated cellular process in which the proliferation and differentiation of gonium reproductive cells is followed by meiosis, which consists of two rounds of cell divisions (meiosis I and II).

Male and female gametogenesis show fundamental differences in timing, cell morphology and cell cycle regulation. During spermatogenesis, chromatin undergoes dramatic structural changes, mainly correlated with transcriptional activity and **cohesin** occupancy (Figure 1). Moreover, meiotic progression is accompanied by chromatin-transcriptional relationships in DNA promoters, where promoters with high CG content present active transcription marks (H3K4me3, H3K9ac and H3K27ac) and promoters with low CG content appear to be methylated [5] (Figure 1). Interestingly, between 20-45% of DNA methylated promoters also harbor active transcription marks and 5hmC

48 modifications (the so-called atypical promoters), that allow for wave-specific
49 transcription despite methylation, giving to poised transcriptional regulators during
50 gametogenesis [5].
51
52 As for the dynamics of chromatin remodeling during spermatogenesis, spermatogonia
53 present a somatic-like genome organisation, which harbor clear A/B compartments and
54 TADs but exhibit changes in chromosome occupancy, DNA methylation, histone
55 modifications and transcription, highlighting their commitment to enter meiosis (Figure
56 1) [4,5]. It is during prophase I when major chromatin remodeling takes place in
57 primary spermatocytes; homologous chromosomes are organised into DNA loop
58 anchored to the chromosomal axes formed by the axial element of **synaptonemal**
59 **complex** and meiotic cohesins (i.e., REC8 or RAD21L). Evidence suggests a weak
60 compartmentalization [2,4] that would serve to accommodate the major events that take
61 place during prophase I, such as chromosomal movements, chromatin condensation and
62 the formation and repair of DNA **double-strand breaks** (DSBs). Moreover, meiotic
63 chromosomes accommodate transcriptional activity associated with cohesin occupancy
64 [4] that could result from transient chromatin contacts and be locally regulated. The
65 molecular mechanisms behind cohesin based organisation of active transcription in
66 primary spermatocytes remain to be elucidated.
67
68 After male meiosis, haploid cells (round spermatids and sperm) adopt a distinctive
69 higher-order chromatin structure to accommodate histone-to-protamine transition and
70 cellular differentiation. Although A/B compartments are re-established in round
71 spermatids, TADs are not as defined as in other cell types, appearing more transient
72 (Figure 1). This phenomenon could represent an organisational preamble for the

spermiogenic chromatin remodeling, as flexibility and accessibility are needed for an efficient protamine transition. Importantly, round spermatids harbor active transcription, with cohesins present at the vicinity of promoters of genes relevant for fertilization and embryogenesis [4]. Thus, the activation of transcription, which accompanies chromatin remodeling during meiosis and spermiogenesis, might play a role in the development of the future embryo by providing transcripts needed upon zygote genome activation.

In sperm, chromatin adopts a unique folding organisation in which highly condensed DNA spatially constraints genome architecture. Chromosomes appear to be arranged into **chromosome territories**, with increased pericentromeric interactions attributable to centromere clustering into the chromocenter [4,6]. Moreover, sperm presents clear A/B compartments and defined TADs with low variance scores defining their borders, possibly linked to the sub-Mb scale toroidal organisation that characterises sperm chromatin [7].

In the case of female gametogenesis (oogenesis), late-stage oocytes present specific chromatin configurations known as Polycomb-Associating Domains (PADs) (Figure 1). PADs play a pivotal role during early embryogenesis, in which epigenetic reprogramming of histone modifications and DNA methylation are essential for the formation of the embryo [8] (see S1 in the supplemental information online). They are compartment-like and cohesin-independent structures, which are marked by distinct H3K27me3 profiles. PADs arise as A/B compartments that weaken in late-stage oocytes, an organisational transition that might facilitate chromosomal segregation [8,9]. Even though PADs have not been detected in MII oocytes, they are clearly

97 defined after fertilization at the 2-cell stage, in which maternal H3K27me3 might enable
98 the formation of PADs even before the zygote genome activation upon fertilization [10].
99

100 ## Disruption of genome topology in germ cells by genome reshuffling

101 Exploring the implications of genome reshuffling on 3D genome folding in the germ
102 line can provide fertile grounds for investigating the evolutionary dynamics of genome
103 function and, ultimately, speciation. Large-scale **chromosomal reorganisations** can
104 potentially be fixed in a population eventually contributing to the formation of new
105 allelic variants on which natural selection can work (see S2 in the supplemental
106 information online). However, they can also trigger the development of inherited
107 diseases, genome instability and cancer by altering gene expression of the reorganised
108 genomic regions.
109

110 While it has been reported that disturbances of domain architecture due to inversions,
111 fusions or indels can lead to oncogene activation and novel gene functions [11], the role
112 of balanced chromosomal changes, such as **Robertsonian (Rb) fusions**, are just
113 beginning to be elucidated [12]. Rb fusions provide an example of genome plasticity,
114 representing the most common large-scale chromosomal structural change in nature. In
115 fact, there is evidence of Rb fusions having an impact on fertility as they are linked to
116 recurrent miscarriages and aneuploid offspring in humans [13]. However, recent data
117 suggest that the situation could be more complex and may also affect genome topology
118 in germ cells with associated functional and evolutionary implications [12].
119

120 In fact, Rb fusions can drastically alter the 3D chromatin conformation in spermatocytes
121 and round spermatids [12] (Figure 2). This spatial chromosome reorganisation can

prompt novel interactions between domains, exposing them to new regulatory environments that can potentially affect gene expression and/or regulation, as initially proposed by the **Integrative Breakage Model** of genome architecture [14]. This can have implications for both fertility and evolution that need further exploration. First, the presence of new chromosomal interactions may rewire or attenuate gene networks, providing new grounds for evolutionary novelty. Secondly, Rb fusions have a direct impact on meiotic **recombination**, resulting in a detectable genomic footprint that has implications for genetic diversity [12] (see S2 in the supplemental information online). This represents the empirical demonstration of the **interchromosomal effect** [15], showing that the presence of chromosomal fusions in the germ line can alter segregation patterns.

Concluding remarks

The spatial folding of chromosomes, and their organisation in the nucleus, has a significant regulatory impact on gene expression and, as a result, potentially important evolutionary consequences. Understanding the structural and functional implications of genome reshuffling in the germ line will be fundamental for elucidating the evolutionary forces that drive genome plasticity. Here we have summarized how large scale chromosome reorganisations (such as Rb fusions) can alter the 3D topology in germ cells. Further functional studies on the effect of CTCF/cohesins mutations in disrupting genome topology in germ cells will certainly provide new insights into our understanding of the mechanism responsible of the heritability of 3D genome folding. In this context, the advent of multidisciplinary approaches that combine computational and experimental methods, underpinned by high-throughput technologies, will provide impetus for the broader exploration of the functional and structural basis of genomes

147 reinforcing the link between the 3D genome architecture, developmental biology,
148 fertility and evolution.
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159 ## Declaration of interests
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161
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Figure Legends

Figure 1: Genome organisation in the mammalian germline and early stages of embryonic development. (**A**) Representation of chromatin organisation during spermatogenesis. Spermatogonia present a somatic-like organisation with the genome folded into compartments (A and B) and subsequent TADs. Active transcription (RNA-seq) correlates with A (open) compartments. Subsequently, there is an attenuation of compartments and TADs in primary spermatocytes (here exemplified as leptonema, zygonema, pachynema and diplonema stages). In pachynema and diplonema active transcription correlates to meiotic cohesin occupancy in promoters of transcriptionally active genes. Post-meiotic cells (round spermatids and spermatozoa) recover a somatic-like configuration, although with particularities such as that TAD borders are not clearly defined, but active transcription correlates with A compartments. Adapted from [4]. (**B**) Representation of chromatin organisation in the female germline. Late oocyte stages (growing oocytes (GO) I and II) show blurrier TAD organization than in somatic cells, as transitioning to specific local chromatin-interacting regions known as Polycomb-Associating Domains (PADs), which are associated to H3K27me3 marks. Chromatin organisation dramatically changes in MII oocytes, where neither PADs nor other folding configurations are detected. Adapted from [8]. (**C**) After fertilization, the PAD organisation detected in late oocytes re-appears, being the most evident at the two-cell stage of the embryo. Adapted from [8]. (**D**) Schematic representation of promoter activity during gametogenesis (from spermatogonia to round spermatids). Active promoters (green arrows) can be either the typical high CG-content promoters with active transcription marks, or atypical promoters that present both methylation (black lollipops) and 5hmC modifications (green lollipops) accompanied by active

226 transcription marks. Low-CG-containing promoters present DNA methylation and are

227 transcriptionally silent (red arrow). Adapted from [5].

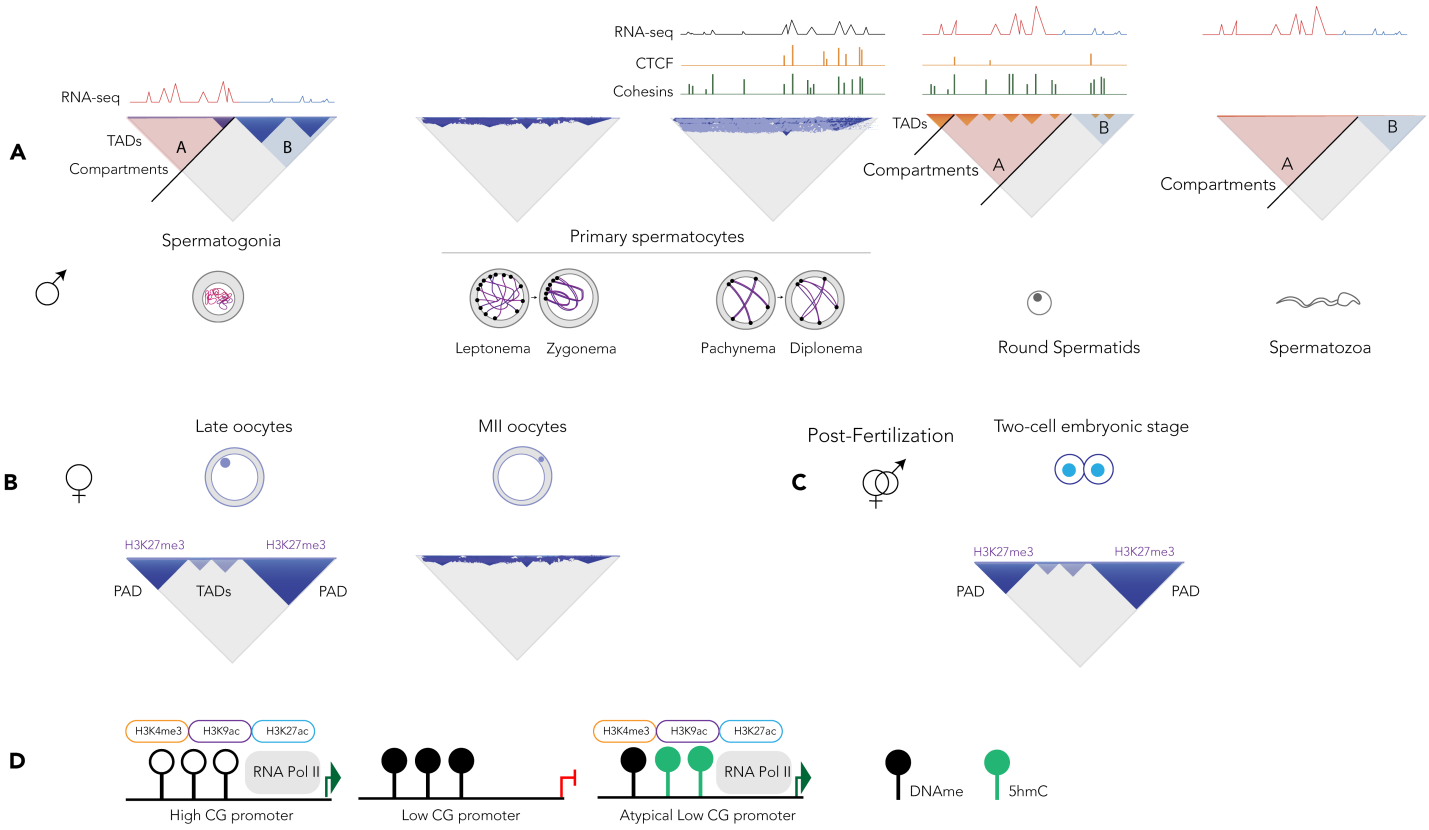

Figure 2: Impact of Rb fusions in genome folding. Schematic representation of chromatin organisation described in standard (no chromosomal fusions) and Rb mice (Robertsonian fusions) depicting chromosomes non-involved in Rb fusions (chromosomes 1 and 2) and chromosomes involved in Rb fusions (chromosomes 3 and 8) as examples in (**A**) somatic interphase (fibroblasts), (**B**) primary spermatocytes (meiotic cells) and (**C**) round spermatids (post-meiotic cells). Adapted from [12]. Centromeres are presented as black circles whereas telomeres are shown as red circles at one tip of the chromosomes. Two types of interactions are represented for each cell type: genome-wide interactions and heterologous chromosomal interactions. Grey represents no interactions, while the scale color towards red represents increasing interactions, where orange represents detectable interactions and red highlights an increase of interactions. (**A**) In somatic interphase, genome-wide interactions HiC maps depict *cis* (diagonal for each chromosome) and *trans* interactions (shadowed squared outside the diagonal). In the case of Rb mice, genome-wide HiC maps represent high interactions between fused chromosomes (3 and 8). Representation of heterologous interactions between pairs of chromosomes (1 vs. 2 and 3 vs. 8) shows high interaction at the centromeric regions as the result of Rb fusions. (**B**) Genome-wide HiC maps in primary spermatocytes present attenuated compartments in both standard and Rb mice and centromeric interactions in fused chromosomes. Heterologous interactions maps show how the presence of Rb fusions disrupts interaction patterns in both fused (3 vs. 8) and non-fused (1 vs. 2) chromosomes. (**C**) Post-meiotic cells present a somatic-like chromatin configuration genome-wide. The presence of the chromocenters (centromeric associations present in mouse round spermatids) results in a reduction of heterologous contacts genome-wide. As such, the presence of Rb fusions restricts interactions between non-fused chromosomes in round spermatids.

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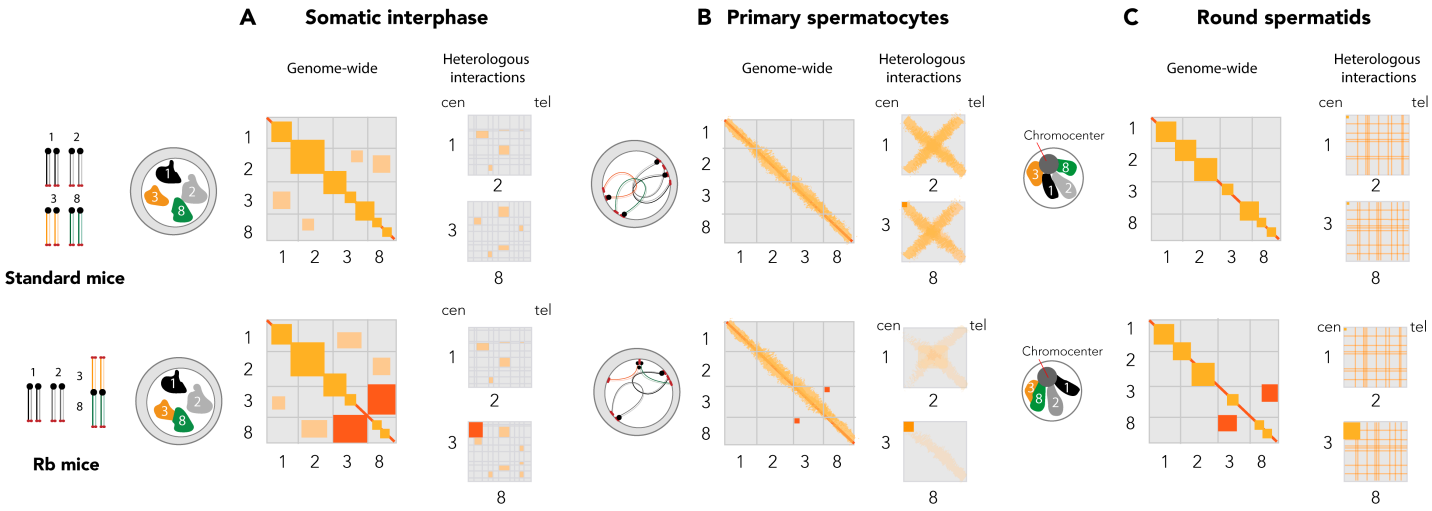
A Somatic interphase
B Primary spermatocytes
C Round spermatids
Genome-wide
Heterologous interactions
cen
tel
Chromocenter
Standard mice
Rb mice
1 2 3 8

Glossary:

Cohesins: Ring-shaped protein complexes that are essential for sister chromatid cohesion. Additionally, they have a determinant role in the assembly of DNA replication factories, chromosome condensation and mitotic spindle assembly, among others.

Chromosome territories: Regions within the nucleus that are preferentially occupied by specific chromosomes. Chromosome territoriality can be influencing by many factors, such as chromosomal size, gene density, and gene expression.

Chromosome reorganisation: The reshuffling of chromosomal regions and can be classified in unbalanced or balanced depending on whether they alter gene dosage. Balanced reorganisations include inversions, reciprocal translocations, fissions, and fusions, while unbalanced reorganisations include duplications and deletions.

Compartments: A hierarchical level of the 3D genome organisation provided by the first principal component of Hi-C interaction matrices and captured by the correspondent eigenvector, which discriminates between interaction frequencies. Chromosomes are organised in A/B compartments that can vary between 1 to 10 Mb in mammals.

DNA Double-Strand Breaks: Programmed DNA breaks catalyzed by the Spo11 endonuclease during early stages of the first meiotic division.

Gametogenesis: The biological process by which haploid gametes are generated. This process is divided into two main stages: (i) proliferation and differentiation of gonium and (ii) meiosis, which consists of two rounds of cell divisions (meiosis I and II).

Integrative Breakage Model: A multidisciplinary hypothesis for the study of genome plasticity that considers that genomic regions involved in evolutionary reshuffling: (i) interact physically inside the nucleus during the formation of the germ line, (ii) present

accessible epigenetic features providing structural and functional accessibility, and (iii) only those reorganisations that do not disturb essential genes and/or gene expression will likely be fixed within populations, thus providing grounds for evolution.

Interchromosomal effect: The mechanism by which chromosomes involved in chromosome reorganisations are proposed to interfere with correct segregation during meiosis of other chromosomes not involved in reorganisations.

Meiosis: Specialized cell division in sexually reproducing organisms that produce haploid gametes.

Recombination: The exchange of genetic material between homologous chromosomes during the first meiotic division.

Robertsonian fusion: A chromosome rearrangement involving centric fusion of two non-homologous acrocentric chromosomes to form a single metacentric chromosome.

Synaptonemal complex: A proteinaceous scaffold, which consists of two lateral elements and a central region that includes the central element and the transverse filaments. The synaptonemal complex maintains the physical connection of homologous chromosomes during the prophase of the first meiotic division and plays a role in meiotic recombination.

Topologically associated domains: Represent genomic loci that preferentially interact with the neighboring *cis* chromatin domains rather than with other regions, conforming functional chromatin domains.