Cell. Mol. Life Sci. 66 (2009) 1198 – 1208
1420-682X/09/071198-11
DOI 10.1007/s00018-008-8584-2

Cellular and Molecular Life Sciences

Review

Chromatin structure contribution to the synaptonemal complex formation

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Received 18 September 2008; received after revision 10 October 2008; accepted 24 October 2008
Online First 16 December 2008

Abstract. Meiosis is a key cellular and molecular process for sexual reproduction contributing to the genetic variability of organisms. This process takes place after DNA replication and consists in a double cellular division, giving rise to four haploid daughter cells or gametes. Meiotic recombination between homologous chromosomes, in the meiotic prophase I, is mediated by a tripartite structure named Synaptonemal Complex (SC). The SC is a peptidic scaffold in which the chromatin of homologous chromosomes is organized during the pachytene stage, holding chromosomes together until the meiotic recombination and genetic exchange have taken place. The role of chromatin structure in formation of the SC and the meiotic recombination at meiotic prophase I remain largely unknown. In this review we address the epigenome contribution to the SC formation at meiotic prophase I, with particular attention on the chromatin structure modifications occurring during the sub-stages of meiotic prophase I.

Keywords. Synaptonemal complex, chromatin structure, epigenetic modification, meiotic prophase I, lateral element associated repeat sequences (LEARS).

Introduction

Molecular changes during the meiotic prophase I and chromatin structure.

Chromosomal morphogenesis during the meiotic prophase I involves three main components: a) establishment of a physical inter-relation between sister chromatids along with formation of axial chromosomal structures, b) interaction between chromosomes involved in homologous recognition, DNA recombination and SC formation, and c) temporally programmed variations in molecular composition and compaction of bulk chromatin and axis-associated components [1]. It is worth mentioning that all these processes occur in a chromatin template structured in multiple levels of compaction. Briefly, chromatin consists of a complex and dynamic array of nucleosomes, and non-histone proteins. Nucleosomes are composed by 146 base pairs of nuclear DNA wrapped around the octamer of histones [2]. The linker histone H1, which interacts with inter-nucleosomal DNA, contributes to form the next higher order level of chromatin organization known as the solenoid or 30-nm fiber. Chromatin dynamics is achieved through the action of numerous binding factors and/or modifying

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enzymes inducing histone covalent modifications, as well as complexes mediating ATP-dependent chromatin remodeling [3]. Furthermore, histone modifications (acetylation, phosphorylation, methylation, ubiquitination) constitute highly specific regulatory signals affecting the epigenetic control of gene expression through chromatin structure [3]. Therefore, we predict that specific epigenetic profiles established by dynamic chromatin structure during meiotic prophase I are related to chromosome morphogenesis.

Pre-meiotic S phase: homologous chromosomes recognition

During pre-meiotic S phase, the chromosomes begin a search for their homologous counterpart for subsequent pairing and exchange of genetic material in the pachytene stage [1]. One model postulates that this homologous recognition occurring in a large scale is mediated through DNA transcription [4]. Homology recognition mediated by transcription has been demonstrated recently in female stem cells as a prerequisite for X-chromosome inactivation, which allows gene dosage compensation [5]. Electron microscopy observations demonstrated the presence of axial elements (AEs) surrounded by loops formed by chromatin associated with ribo nucleo particles (RNPs) (lampbrush structures) at pre-leptotene stage. These lampbrush structures progressively associate between them [4] (Fig. 1). During this pre-meiotic S-phase, the chromatin is diffuse with reduced sectors of heterochromatin, indicating a relaxed chromatin configuration at this stage [1]. This correlates with early studies on DNase I accessibility in which specific DNA sequences showed increased DNase I sensibility in pre-meiotic S-phase, as compared with pre-meiotic stages. Furthermore, nuclease sensibility seems to be associated with transcriptionally active DNA; however, in pachytene cells nuclease action is observed in transcribed and non-transcribed genomic sequences [6], pointing to a more relaxed general chromatin configuration in cells entering meiotic prophase I. By contrast, the chromatin of sexual chromosomes in leptotene, zygotene, and pachytene stages presents a gradual decrease in nuclease digestion sensibility. In the pachytene stage the heterochromatin of the sexual chromosomes (XY body) is clearly seen under light and electron microscopy [6].

We assume that the relaxed chromatin status in pre-meiotic S phase is attributed to specific epigenetic events. In spermatogonia and pre-leptotene cells the whole genome displays histone H4 acetylation (euchromatic mark). However, this mark is absent in the following stages, until it reappears in elongated spermatids with a loose chromatin structure, which allows the protamines-histones exchange [7, 8]. Histone H3 lysine 9 acetylation (acH3K9), considered an open chromatin mark, is detectable from type-B spermatogonia up to the zygotene stage [8]. On the other hand, the reduced constitutive heterochromatin found at the beginning of the meiotic prophase in type-B spermatogonia corresponds to centromeric and telomeric regions, where H4K20me3 and H3K9me3, which are modifications incorporated by the histone methyltransferase Suv39h, are enriched [9, 10]. Both histone marks are present throughout the pre-meiotic (spermatogonia B-type), as well as leptotene and zygotene stages [9]. At pachytene stage, when the homologous chromosomes are paired, H4K20me3 is present in centromeres, telomeres and nearby chromatin. These repressive chromatin marks are present until late pachytene (Table 1). Probably such histone marks are needed for regulated incorporation and preservation of centromeric and telomeric chromatin in the synaptonemal complex. Both marks are no longer present in elongated spermatids where the histones-protamines replacement takes place [9]. The relaxed status of chromatin and the presence of euchromatin epigenetic marks during pre-meiotic S phase correlate with high rates of DNA transcription, including genes in anti-sense orientation [4, 11], as well as replacement of H3 by the histone variant H3.3 [11] (Table 1). It is interesting to note that in this stage splicing factors are scarce [12], while unspliced pre-mRNAs are highly abundant, as shown by immunolocalization of heterogeneous nuclear ribonucleoproteins (hnRNPs) [4]. All these facts indicate that during meiotic S phase DNA is intensively transcribed and that nascent pre-mRNAs are processed only to a low extent, supporting the idea of the requirement of transcription for homologous chromosomes recognition. Nevertheless, this hypothesis remains poorly explored.

Pre-leptotene stage: Chromatin commitment for chromosomal pairing and recombination.

Differential epigenetic profiles contribute to the configuration of pre-leptotene chromatin. Phosphorylated serine 10 of histone 3 (H3S10P) begins to be discernible at this stage. The mark decreases in leptotene, and by the zygotene stage, H3S10P is diffusely detected [13] (Table 1). H3S10P is also associated with chromatin relaxation and compaction during the meiotic prophase I [13]. These observations indicate that chromatin dynamics, potentially involving H3S10P, is needed for prophase progression. Furthermore, other global chromatin rearrangements

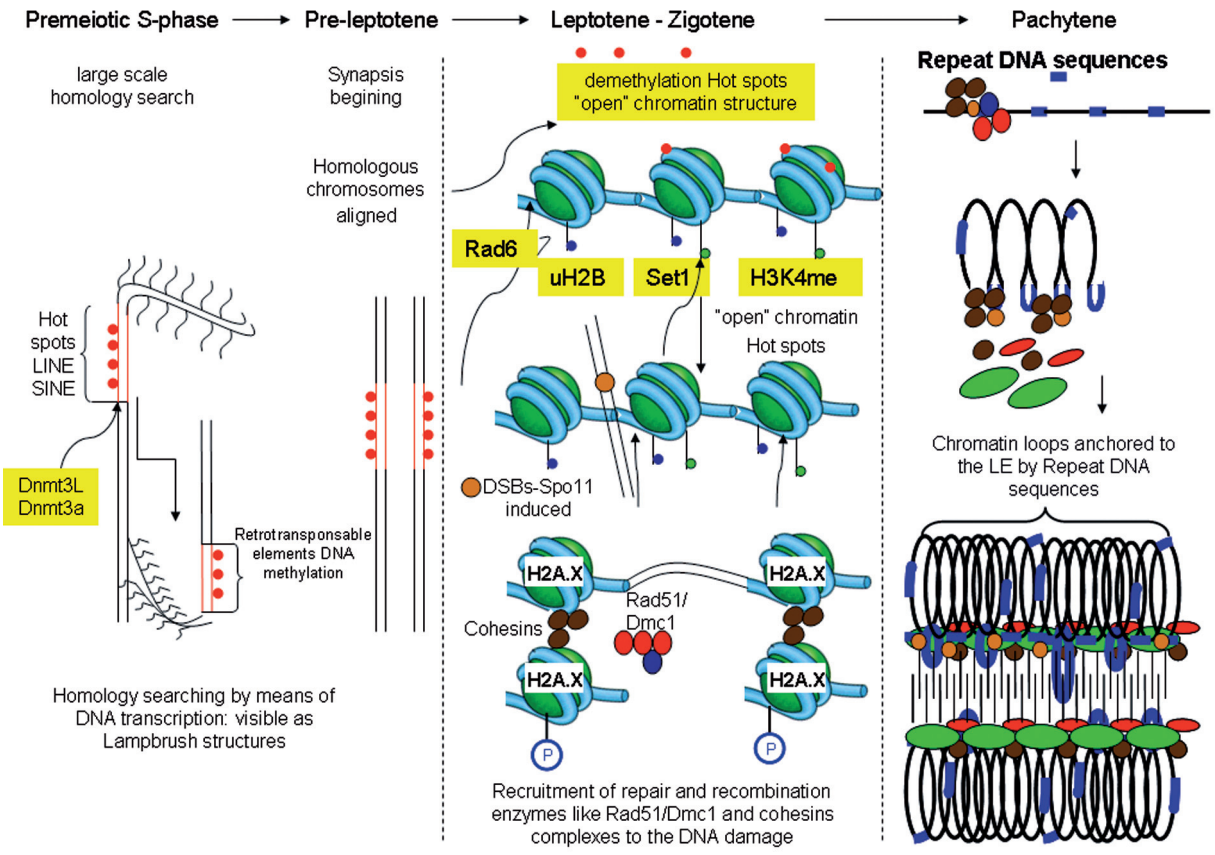
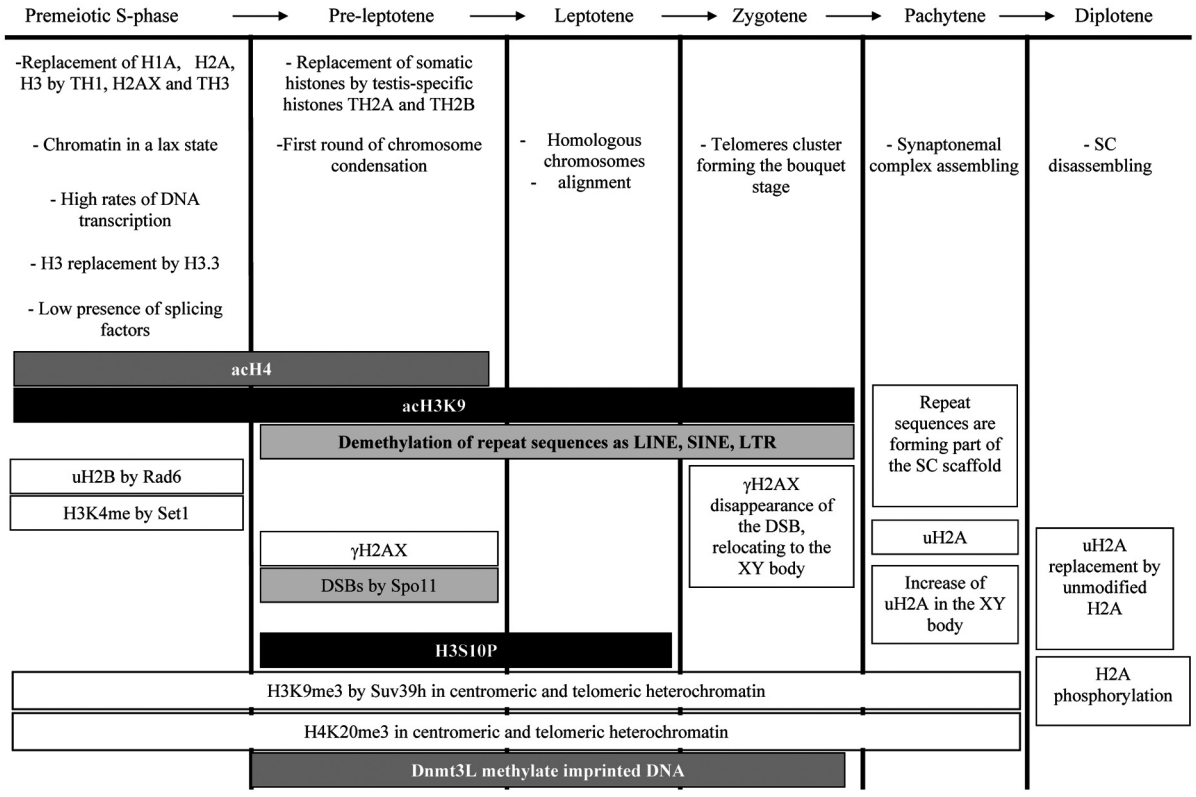


Figure 1. Scheme exemplifying the chromatin modifications at DNA sequences, which become part of the DNA-protein scaffold of the SC. At pre-meiotic S-phase the chromosome homology search takes place at big scale. One theory points out transcription as a relevant mediator for the homology search. In this stage repeat sequences are DnmtL-methylated, perhaps avoiding their participation in the homology search. At leptotene stage the homologous chromosomes become aligned. At leptotene-zygotene transition, the chromatin of the repeat elements related to hot spots undergoes changes and an active demethylation process allowing the ubiquitination of histone H2B (uH2B) and chromatin relaxation through Set1 dependent H3K4 methylation. Then, DSBs mediated by Spo11 are induced on this repeat sequences after – as a response to DNA damage – H2A becomes phosphorilated (γH2A), a signal that favors the recruitment of other factors to remodel the chromatin structure and permit access to the recombination machinery, as Rad51 and DMC1 proteins, responsible for the early recombination nodule formation. Likewise, cohesin complexes are recruited to DSB, keeping together the chromatids and homologous chromosomes, allowing successful DNA repair. Spo11 is not removed from DSB and appear in the LEs of the SC in pachytene stage. In this way, as the pachytene stage is established, the LE of the SC is formed by LEARS; the LE-specific proteins, SYCP2, SYCP3; the cohesin complexes SMC1, SMC3, Rec8, and by the protein Spo11. In this way the repeat DNA sequences anchor whole chromosomes to the SC scaffold.

occur in this stage. The clumps of compact chromatin contacting the nuclear envelope progressively decrease and the nucleoli migrate towards the nuclear envelope. The homologous chromosomes, anchored to the nuclear envelope, migrate until they locate in proximity. Then, the chromatin fibers anchored to the early AEs act as a highly specific bar code for the recognition of homologous chromosomes, allowing pairing and formation of the rope-ladder structures [4, 14]. In conclusion, not only morphology but also the chromatin dynamics are required in these early steps of meiosis.

In pre-leptotene stage the chromatin structure undergoes changes that allow chromosome morphogenesis, such as formation of the recombination intermediates and SC formation. Furthermore, DNA replication in the pre-leptotene stage in mouse and rat spermatocytes is accompanied by a wave of *de novo* synthesis of histones, besides replacement of somatic histones by testis-specific histone variants such as TH2A and TH2B [15]. Nevertheless, partial replacement of somatic histones H1A, H2A, H3 by TH1, H2AX and TH3 initiates during spermatogonial differentiation [15]. This histone replacement is necessary for further chromatin modification (see below). At this stage specific epigenetic events controlling the SC formation become more evident than in previous stages. Ubiquitination of the histone H2B (uH2B) by Rad6 allows H3K4 methylation by Set1, contributing to chromatin remodeling and generation of double strand breaks (DSBs) induction by Spo11 [7, 16] (Fig. 2). Thus, the first recombination intermediates,

Table 1.



namely DSBs, are governed by epigenetic modifications inducing changes in the chromatin structure potentially influencing SC formation.

Leptotene stage: Chromosomal dynamics and establishment of the SC nucleation spots

At leptotene stage the chromosomes are discernible as thin and filamentous structures [1]. At this point, sister chromatids organize into structures called AEs and most of the compact chromatin clumps are dissociated [17, 18]. At the early-mid leptotene the DSBs, produced by enzyme Spo11, recruit proteins related to the meiotic mismatch repair machinery (MMRM) and stabilize the association between the homologous chromosomes. Such interactions can be seen in association with the axial elements of the homologous chromosomes to be aligned [19]. These numerous interactions are described as inter-axial bridges of around 400 nm in width [18]. As leptotene progresses, a fraction of these bridges mature into axial associations (AAs), which hold together the paired Lateral Elements (LEs). Moreover, the AAs nucleate the formation of SC between the associated AEs [20]. Despite the fact that chromosomal homologous sequences are aligned at this stage, the DSB are necessary for accurate SC formation. In the absence of functional Spo11 protein, synapses can be restored if DSBs are induced by other means, as has been shown for *spo11* mutant mice. In such mice, the DSB produced by the administration of the chemotherapeuthic agent cisplatin rescues meiotic arrest and formation of SC, which are compromised otherwise [21]. During this stage, the DSBs are generated and the histone variant H2A.X is phosphorylated (γH2A.X). This covalent histone modification is present from leptotene up to zygotene. Later, in early pachytene, this modification is not observed in autosomes, whereas its presence is evident in the XY body; however this epigenetic modification seems not to be implicated in the formation of DSB on the XY body. Mammalian histone variant H2A.X is similar to H2A; however, it contains a unique SQ motif on its C-terminus, which is evolutionarily conserved. Upon DNA damage or DSBs, the H2A.X SQ motif becomes phosphorylated (γH2A.X), which tags DNA for recruitment of recombination enzymes [15, 22] (Table 1). After double strand cleavage, the ends of the broken molecule must be modified by the 5'-to-3' exonucleolytic activity of MRE1 and RAD50 enzymes, resulting in single stranded DNA (ssDNA) which, through the action of Rad51 and Dmc1, invade an unbroken DNA duplex in either sister chromatin,

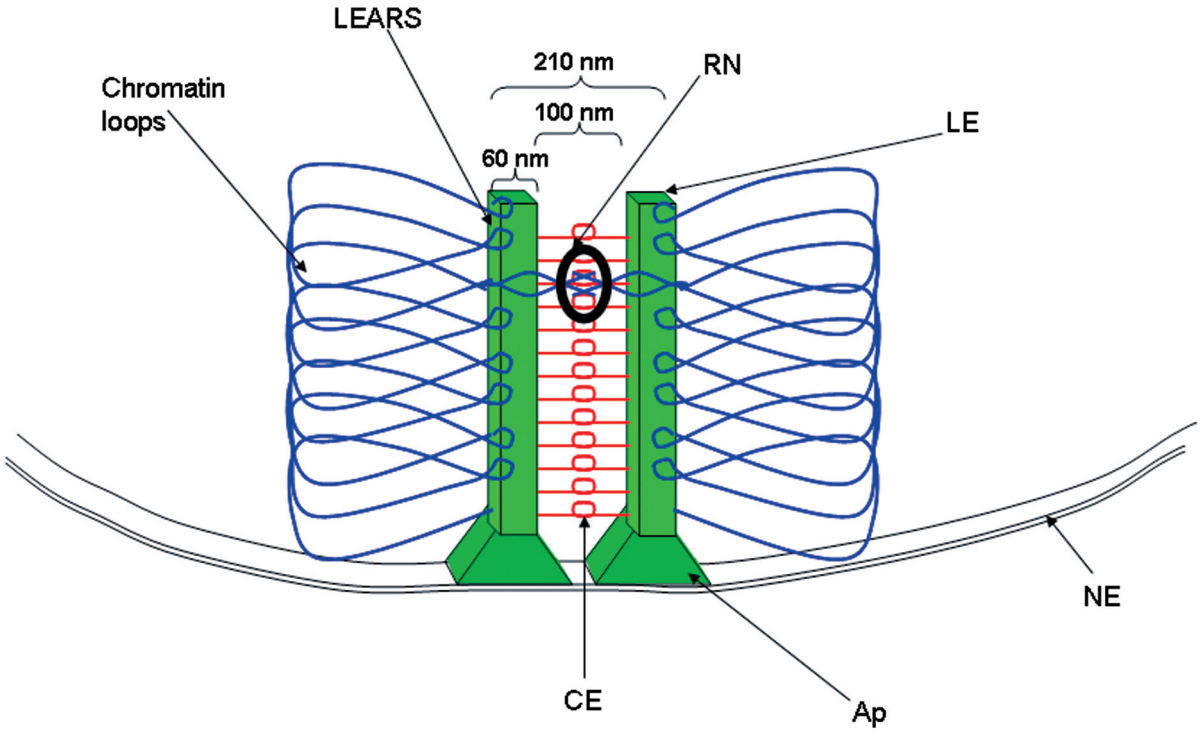


Figure 2. Synaptonemal complex (SC) is a tripartite composed of two lateral elements (LE) and a central element (CE). The thickness of the complex is 210 nm with two LEs of approximately 60 nm in width, and a central space of around 100 nm thick. The homologous chromosomes anchor to the LE by Lateral Element-Associated Repeat Sequences (LEARS). The ends of the LEs are attached to nuclear envelope (NE) by the adhesion plate (ap).

or the homologous chromosomes, for further use as a template for DSB repair. These interactions, occurring between homologous chromosomes, are critical for proper chromosome pairing and synapse [23]. During branch migration, which takes place in the mid-phase of the homologous recombination-repair process, nucleosomes are displaced causing dramatic chromatin structure changes in the region where formation of joint molecules occurs, and dsDNA is unwound through the activity of RAD54, which is a sub-unit of the SWI2/SNF2 ATP-dependent chromatin remodeling complex [15]. Thus, epigenetic modifications induced as response to DNA damage are relevant for the proper chromatin rearrangements associated with the SC formation.

Upon DBS in *S. cerevisiae* DNA damage response kinases Tel1, Mec1 and Rad53 (ATM, ATR and Chk2 in mammals, respectively), phosphorylate H2AX and the repair protein Mre11 is recruited. Furthermore, it has been demonstrated that cohesin complexes are recruited to DSBs by Mre11, suggesting that cohesion contributes to keeping chromatids and homologous chromosomes together, facilitating DNA repair, and avoiding homologous chromosome dissociation due to DSB. Moreover, in human cells cohesions recruitment to DSBs requires the Smc5/6 complex, in addition to Mre11, Smc5 and Nse2 subunits [24]. The picture emerging from all these data includes a complex network where DSBs produced by Spo11 at the meiotic hot spots, many of them associated with repeat rich sequences, becomes the recruitment signal for DNA damage-dependent kinases, which in turn phosphorylate H2AX. Such a process is required for damage-sensing and recruitment of DSB repair proteins to the break sites, with subsequent loading of cohesin complexes (Fig. 1). As the meiotic prophase I continues, most of these hot spots – linked to repeated, rich sequences which are coupled with proteins as Spo11, cohesins and LE specific-proteins – build up the LE of the SC in the pachytene stage [18, 24, 25] (Fig. 1). From these data it is clear that chromatin structure, which is deeply affected by its modifications, is involved in the anchorage of chromosomes to the SC scaffold. However, studies attempting to elucidate the complex network of DNA-proteins that compel to the SC formation are required.

First evidences of the SC formation

Ultrastructural evidence from our laboratories supports the beginning of SC formation near the nuclear

envelope (NE) [4]. The attachment of the SC scaffold to the NE is observed as an electron-dense structure named the adhesion plate (Fig. 2), which is also associated with telomeric DNA repeats [26]. Knockout mice for the SYCP3 gene present dissociation of telomeric repeats from the adhesion plate along with perturbed SC [26]. Therefore, the connection between SYCP3 and telomeric repeats seems to be required for the initial steps of the SC formation. Telomeric and centromeric sequences represent the main source of constitutive heterochromatin and are highly enriched in epigenetic marks associated with gene silencing [27] (Table 1). Such features prompted us to propose that heterochromatin in telomeric sequences facilitate the beginning of the SC formation. This is supported by the fact that knockdown of Suv39 h and G9a histone methyltrasferases causes synapsis establishment failure between homologous chromosomes [9, 28].

Towards the leptotene-zygotene transition, the chromosomes, anchored to the internal nuclear membrane by their telomeres (adhesion plate), move until they are close enough, forming a flower-like arrangement known as the "bouquet stage" [1, 29]. Such stage is transient and occurs simultaneously with SC formation. The bouquet formation has been observed in plants, fungi and animals [1]. In yeast, the leptotene-zygotene stage is associated with formation of the double Holliday junction (dHJ), which represents a stable intermediate stage between the induction of DSBs and DNA exchange. Interestingly, the bouquet structure behaves differently in other organism. In nematodes, including *C. elegans*, only one end of each chromosome anchors to the nuclear envelope and the bouquet is not formed. In contrast, in *D. melanogaster* physical clustering of heterochromatic centromeric regions occurs, visible as a weak polarization of telomeres [1].

In human spermatocytes and oocytes, the telomeric anchorage is mediated by a package of spherical filaments, between 100 and 150 nm, located along the cytoplasmic face of the nuclear envelope. Telomeric anchorage is only visible during zygotene and early pachytene stages, when the bouquet structure is formed. Furthermore, telomeric heterochromatinization is necessary for correct telomere clustering in the bouquet stage at the beginning of meiotic prophase, and for the AE formation, which precedes synaptic pairing [30, 31]. In the newly formed LEs, the optimal attachment of telomeres with the nuclear envelope may need additional structural components such as, for example, actin filaments of the cytoplasmatic matrix and the *trans*-membrane proteins Sun1 and Sun2, which have been directly localized in association with the anchorage of telomeres [32–34]. These facts highlight the involvement of telomeric sequences and associated epigenetic features in the SC formation onset during the transition from leptotene to zygotene stages.

Zygotene stage: assembling the SC

At zygotene stage the chromosomes become shorter, thicker and [1] locate in proximity to the nuclear envelope, at the homologous pairing site, where the SC's classical tripartite structure becomes visible [17]. Chromosomal dynamics initiate during leptotene stage. By early zygotene such movements are most prominent and gradually decrease throughout the zygotene stage, ending at early pachytene. Interestingly, the telomeres and kinetochores are also associated with the nuclear envelope and this interaction remains during the chromosomal movements. In wheat, this nuclear and chromatin mobility can be affected by colcemid, which causes microtubules (MTs) dissociation and destabilizes the nuclear envelope [35]. Actin polymerization seems to be necessary for chromosomal dynamics; however, it is not clear whether it is the nuclear or the cytoplasmic actin which is involved in this process [30]. It has been proposed that chromosomal re-location is needed for attraction of homologous chromosomal segments [1]. This is critical for chromosomal contact initiation and propagation. A novel set of new data supports inter- and intra-chromosomal contacts and chromosomal relocation as relevant for structural and regulatory processes [36, 37]. Such a view probably requires, in zygotenes stage, the fine modulation of chromatin structure, mainly through histone covalent modifications and the distribution of repetitive sequences associated to the SC assembling. Thus, chromosomal movements are needed to attract the already recognized homologous chromosomes and to initiate the SC assembly. Whether telomeric heterochromatin structure is important for these movements is an attractive question to be addressed.

Pachytene stage: synapsis of homologous chromosomes and the SC

In the pachytene stage the homologous chromosome pairs, establish synapses, become shorter, and strengthen their association with the SC. Such association is visible as the characteristic tripartite form [18], in which the four sister chromatids remain aligned along the pachytene stage. At this point, the SC consists of two paired LEs, which are connected by transverse filaments running in a perpendicular orientation (Fig. 2). At electron microscopy level the SCs

present a central element (CE) as a medial linear electron-dense structure. Towards pachytene stage, the homologous chromosomes undergo gradual decondensation until the diplotene stage, in which the SC disassembles and the homologous chromosomes often dissociate, except at the recombination chiasmata [1, 18]. Immunohistochemical studies on mouse cells have shown that the levels of histone H2A ubiquitination (uH2A), which is linked with nucleosomal instability, peak during the pachytene stage [38]. In early pachytene, uH2A is concentrated in the condensed and transcriptionally inactive sex chromosomes XY. By mid-pachytene, uH2A is observed throughout the entire nucleus, but is subsequently restricted to the XY body in late pachytene spermatocytes [38]. Therefore, this uH2A distribution in pachytene chromatin could be related with nucleosome eviction to allow replacement of already modified histones, contributing to regulated meiotic prophase I progression. This is supported by previous studies showing that phosphorylation of H2A during late pachytene is needed for SC disassembling [39]. Furthermore, knockout mice for *hr6b*, which mediates H2A-ubiquitination, present SC structural and length defects [40]. Interestingly, the uH2A distribution pattern in the sex body heterochromatin of such mice does not change [41], supporting the fact that H2A ubiquitination is critical for histone replacement and further modification of newly incorporated histones. Ubiquitinated H2A reappears in elongated spermatids, where it might influence histones-protamines replacement [42, 43].

We recently found that sub-families of DNA repeat sequences are associated with LEs of the SC in pachytene rat spermatocytes. We called such sequences LEARS, which stands for Lateral Element-Associated Repeat Sequences [25]. This finding opens the possibility that the chromatin structure associated with repeat DNA sequences affects LE structure. Furthermore, the chromatin associated with the DNA repeats is targeted by several epigenetic modifications during meiotic prophase I. Thus, we favor the idea that epigenetic modifications could be linked to the incorporation of LEARS to the SC.

Synaptonemal complex structure

The structure of SC has been well conserved throughout evolution. The basic SC morphology in a longitudinal chromosomal section is the same for plants and animals, and is formed by three parallel peptidic elements. The thickness of the complex is 210 nm with two LEs of approximately 60 nm and a central space of around 100 nm (Fig. 2) [44]. The LEs of the SC are composed of different proteins [44] and loop-shaped DNA filaments crossing at the middle. Several authors have indirectly suggested that the LE-associated DNA corresponds to repeat DNA [45–48]. Recently, our groups confirmed that these DNA filaments correspond exclusively to LEARS [25, 49] and are the chromosome's anchor point to the SC's LE (Figs. 1 and 2). Thus, the whole chromosome is organized around the LE through the SC's protein scaffold (Fig. 2). The SC's CE is built of transverse fibrilar elements of small diameter (1.6–2 nm) composed of protein SYCP1, which is present exclusively during meiosis [18], as well as SYCE1, SYCE2, TEX11 and TEX12, which are associated with the CE [50–53]. The LEs are composed of proteins, such as SYCP2 and SYCP3, and their paralogues in others organisms [18]. Furthermore, LEs also contain cohesin complex components SMC1, SMC3 and STAG3, which associate with REC8 protein forming part of the lateral element in mammals [54].

Chromatin structure during the SC formation

At the beginning of the meiotic prophase I chromatin is mostly relaxed, and the repeat elements, which are normally structured in compact chromatin, suffer configuration changes in order to allow accurate DNA synthesis during the pre-meiotic S phase. Afterwards, during the onset of large-scale homologous chromosome search in the pre-leptotene stage, repeat DNA is methylated, which might avoid interferences with homology recognition mediated by transcribed RNA [7, 8] (Fig. 1). Once the cells have entered leptotene, a large-scale genomic demethylation occurs. Then, Rad6 ubiquitinates histone H2B (uH2B), allowing H3K4 methylation by Set1 and therefore chromatin relaxation at the recombination hot spots, many of them corresponding to repeat sequences [55, 56]. Then, Spo11-dependent DSBs are produced at the hot spots and phosphorylation of histone H2A.X (γH2A.X) occurs, favoring recruitment of meiotic mismatch repair proteins (MMRP) (Fig. 1). During DNA repair in response of DSB damage, Spo11 is not removed from the recombination hot spots, since it is detected in LEs during the SC formation onset, suggesting that Spo11 [21] perhaps plays a role in targeting hot spots to be recruited to the LE. After Spo11 dependent double strand cleavage the ends of the broken molecule must be resected by a 5'-to-3' exonucleolytic activity of the MMRP, Rad50 and Mre11 resulting in a single strand DNA tail (ssDNA). Then, through the action of Rad51 and Dmc1, the ssDNA invades an intact DNA duplex of either the sister chromatin or the homologous chromosome and

uses it as template to repair the DSB as a damage response. At the same time Mer11 recruits cohesin complexes keeping together the chromatids and homologous chromosomes allowing successful DNA repair and avoiding their dissociation due the DSB [7, 8, 15, 22, 24]. In this way, as the pachytene stage is established, the SC's LE is formed of LEARS [25, 47] along with the LE-specific proteins, SYCP2, SYCP3, the cohesin complex proteins SMC1, SMC3, Rec8 [18], and Spo11 [21] (Fig. 1). Hot spots not recruited to the LE participate in the meiotic recombination solved by crossing over, which takes place at the recombination nodule in the SC's CR [55, 56]. Several histone modifications are incorporated to hotspots during the course of the repair process. The idea that such modifications could constitute signals recognized by LE's proteins recruiting LEARS to the SC seem attractive. In this scenario, chromatin modifications in the hot spots could be recognized by cohesin complex components, which have the ability to recognize DNA secondary structures [57, 58] and are loaded to the unpaired AEs in pre-pachytene stages [54, 59], or by LE-specific proteins, which are loaded in the AEs during SC assembly in leptotene and zygotene stages [54]. Furthermore, there is evidence suggesting that certain SC components could be involved in chromatin attachment to the LE. Sequence analysis of major SC proteins predicted the existence of putative DNA binding motifs in SYCP1 and SYCP2 [50, 60–62]. However, whether these proteins can specifically bind repeat DNA sequences is currently unknown. Recent studies indicate that cohesins may also play an important role in chromatin anchoring [63]. Mice lacking cohesin SMC1β, which is present specifically in meiotic cells, present a decrease in LE's associated chromatin as compared with normal mice. In additon, mutants present an altered LE-to-loop ratio with chromatin fiber loops twice as long as those found in wild-type mice [63, 65]. It has been previously shown that cohesins can bind SYCP2, which in turn interacts with SYCP3 [54, 65]. The emerging picture thus points to a complex network of DNA-protein and protein-protein interactions mediating chromosome anchoring to the SC.

SC and DNA methylation

B-type spermatogonia in pre-meiotic S-phase, as well as pre-leptotene and leptotene nuclei which present paternal imprinting, express Dnmt3L. This protein is an isoform of DNA, the methyltransferases Dnmt3a and Dnmt3b, that requires interaction with Dnmt3a to methylate DNA [7]. In B-type spermatogonia Dnmt3L could be involved in methylation of transposable DNA sequences like LINEs, SINEs and LTRs, interfering with their transcription even in relaxed chromatin [8]. During the zygotene stage the genomic non-imprinted regions, including retrotransposable elements, become demethylated [8], what could allow their participation in meiotic recombination [25]. These elements are methylated in following stages probably due to the need to reestablish imprinted patterns and inducing heterochromatin formation during post-fecundation stages, thus avoiding retrotransposition and mutation generation in early embryos [66]. Consistent with this idea, *de novo* DNA methylation of retrotransposable elements by Lsh is important for accurate progression of the meiotic prophase I [67]. In *Dnmt3L* and *Lsh* knockout mice, SCs are not formed, while the LEs appear to be normal [8, 67]. The hypomethylation of repeat sequences at pre-leptotene stages probably affects the homologous chromosomes search, thus impairing SC assembly. Furthermore, DNA hypomethylation prevents eviction of modified histones, such as phosphorylated H2AX, in leptotene-zygotene stages [67]. Therefore, another possibility is that impeding eviction of modified histones from repeat sequences could interfere with their incorporation to LEs, affecting SC formation.

RNA and SC

RNA could be another component that might participate in SC nucleation. Previous ultrastructural analyses revealed the presence of RNA in the SC's LE; moreover, the presence of specific miRNA in the SC has been documented [49, 68]. It is well established that non-coding RNA influences chromatin structure through the RNA interference pathway and in gene dosage compensation through anti-sense transcripts, among others [69, 70]. Recently, the association of RNA to purified chromatin in chicken liver has been reported. Such RNA does not correspond to nascent transcripts, therefore it could be structural RNA [71]. Furthermore, it has been reported recently that repeat DNA sequences correspond to antisense transcription start sites [72]. Thus, the presence of repeat sequences in the SC's LE [25] may favor expression of non-coding transcripts influencing LE nucleation. Hence, the RNA present in SC's LEs could be involved in SC formation. Nevertheless, the exact features and function of LEs-associated RNA remain unknown and are the subject of intense investigation in our laboratories.

Conclusion and prospects

Meiosis is the main source of genetic variability in diploid organisms and implicates homologous recombination, which occurs uniquely in meiotic prophase and is mediated in part by the SC. The LE's anchorage points are constituted by specific DNA sequences called LEARS. These DNA repeat sequences are broadly distributed in the genomes and are subject to many epigenetic modifications, which serve as a means to control their transcriptional status in somatic cells. Epigenetic modifications are dynamic during the meiotic prophase I and are key for SC formation and function.

The chromatin has to undergo structural changes in order to allow progression of the meiotic prophase I, during which meiotic alignment, pairing and recombination of homologous chromosomes occur. The enzymatic machinery controlling chromatin epigenetic modification is critical for SC assembly and therefore for correct meiotic progression. Proper expression of meiosis-specific and mismatch repair genes is also needed. To understand the mechanisms behind SC formation, the complex networks of molecular interactions, as well as the exact succession of protein loading to the SC, have to be addressed. Uncovering the components and functions of the SC has deep implications in human diseases such as male infertility [73], as well as mammalian evolution and genetic variability, highlighting the relevance of understanding the mechanisms of sexual reproduction.

From the chromatin structure perspective a systematic genome-wide analysis is needed to reach deeper insights concerning the structural and functional knowledge all along the meiosis and its associated complexes, such as the SC. We propose detailed molecular studies coupled with cytological experimental approaches to describe in greater detail the components of the chromatin linked to these complexes. In particular, we need a systematic survey of different histone covalent modifications and their correlation with repetitive sequences and other, uncharacterized, genomic components. Of great interest will be to describe the type, abundance, distribution and functional properties of non-coding RNAs in tight relationship with the chromatin associated to the LE. For these purposes, genome-wide protein distribution, histone marks and non-coding transcription of the LEARS in conjunction with their three-dimensional arrangement will be important to better understand meiotic processes and genetic exchange.

Acknowledgements. We would like to thank Paul Delgado-Olguín and members of our research group for stimulating scientific discussions and suggestions. AHH is a fellowship recipient from the Posgrado en Ciencias Biológicas and CONACyT (181375). This work was supported for FRT by grants from the Dirección General de Asuntos del Personal Académico-UNAM (IN209403 and IN214407) and Consejo Nacional de Ciencia y Tecnología-CONACyT (42653-Q and 58767). To GHVN DGAPA-PAPIIT, UNAM grant (IN203308–3).

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