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Review

Spatial organization of the fission yeast nucleus

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ABSTRACT

One important challenge for cell biology in the post-genomic era is to understand how genomes are organized within the nuclear space and how this organization contributes to genome stability, gene expression and DNA metabolism. In yeasts, chromosomes are anchored to the nuclear envelope via specific DNA loci such as centromeres, telomeres and TFIIIC binding sites. This remarkable topological organization requires the presence of distinct DNA sequences and chromatin domains and relies on a large number of proteins that mediate chromatin interactions with the nuclear envelope. Therefore, the nuclear envelope serves as a dynamic scaffold to anchor and organize distinct chromosomal domains, thus generating a higher-order nuclear architecture. This review summarizes the current knowledge on the spatial organization of the fission yeast genome and its implications for nuclear function.

KEYWORDS: fission yeast, pombe, nucleus, nuclear architecture, nuclear envelope, chromatin boundaries, TFIIIC

1. INTRODUCTION

In recent years, significant progress has been made in understanding the importance of nuclear architecture for the regulation of nuclear processes. Examples of such regulation include control of transcriptional activity and replication timing in mammalian cells by DNA locus repositioning to the nuclear periphery [1, 2], the direct role of nuclear pores in transcriptional control in budding yeast [3], as well as the control of meiotic recombination by cytoplasmically generated cytoskeletal forces transmitted to nuclear DNA in fission yeast [4]. In mammalian cells, chromosomes occupy specific territories within the intranuclear space that change in response to developmental gene regulation [5]. Most of the key nuclear processes such as transcription, replication and DNA repair, take place in specialised structures, termed foci, that recruit protein complexes involved in the different steps of these processes [6, 7].

Fission yeast nuclear organisation shares many features with that of more complex nuclei of higher eukaryotes. These features include large repeat-rich centromeres and peripherally localised heterochromatin relying on RNAi-dependent pathway for assembly and maintenance [8-10]. This, in combination with a small 13.8 Mb genome containing 5123 protein coding genes, just three chromosomes [11] and a relatively large nucleus (2.5-3 μm in diameter) [12] suitable for microscopic observation, makes fission yeast a convenient tool for understanding conserved principles underlying eukaryotic nuclear organisation and function.

Both fission yeast and metazoans possess evolutionarily conserved physical links between the DNA and the nuclear envelope that contribute to creating and maintaining nuclear architecture [13]. Such links are prominent at centromeric and telomeric heterochromatin but are also found at a large number of other sites within the fission yeast genome [14-16]. These NE connections are critical for nuclear architecture during vegetative growth and meiotic progression [17, 18].

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The present review focuses on characteristic features of fission yeast chromatin organization within the interphase nucleus, describes the role of heterochromatin, boundary elements and their associated proteins in the spatial organization of the genome and discusses recent progress in understanding how the nuclear envelope creates a dynamic scaffold organising complex nuclear architecture.

2. An overview of the fission yeast nucleus

The fission yeast nucleus occupies approximately 8% of the cell volume and is positioned in the middle of the cylindrical fission yeast cell. Nuclear size increases during cell growth in proportion to the cytoplasmic volume and is largely independent of the DNA content [12]. The double membrane of the nuclear envelope (NE) spanned by the nuclear pore complexes (NPCs) provides the barrier and allows for selective transport between the nucleus and the cytoplasm. In addition, NPCs are able to recruit specific DNA sequences and regulate transcription [13]. While outer nuclear membrane (ONM) is topologically linked to the endoplasmic reticulum, the inner nuclear membrane (INM) is separated from the other cell membranes, creating the need for a specialized molecular pathway that delivers newly synthesized transmembrane INM proteins to their destination [19].

These INM proteins and their nucleoplasmic partners fulfill some of the roles that in mammalian cells are performed by the nuclear lamina that is not present in the fission yeast. These roles include providing anchoring platforms for heterochromatin and other DNA loci at the NE surface, contributing to mechanical integrity and maintaining the shape of the nucleus [20-25]. A key example of such anchoring platform is provided by the nucleoplasmic face of the spindle pole body (SPB; centrosome equivalent) that during the interphase is attached to all three fission yeast centromeres [26]. Since the cytoplasmic surface of the interphase SPB is connected to interphase microtubule bundles, SPB provides a molecular bridge able to transmit microtubule generated forces to the nuclear DNA [27]. Similar to clustering of centromeres at the SPB, telomeres are also clustered in two to three spots at the nuclear surface away from the SPB and close to the nucleolus [8] that occupies around one third of the nuclear volume.

During cell division, fission yeast nucleus undergoes closed mitosis where intranuclear spindle segregates chromosomes while the nuclear envelope remains assembled [28, 29]. At the beginning of mitosis, chromosomes undergo condensation and many elements of the nuclear architecture disassemble, to re-assemble later in the daughter cells after division.

3. Fission yeast chromatin organization

Similarly to other eukaryotes, fission yeast contain large blocks of heterochromatin that show characteristic patterns of histone and DNA modifications such as acetylation and methylation of histone N-terminal tails and methylation of DNA [9, 30-32]. These modifications result in differential binding of regulatory proteins to heterochromatin nucleosomes that, in turn, lead to stabilisation and spreading of heterochromatin domains and create the highly condensed nucleosome arrangement. This prevents access of RNA polymerase II, resulting in the transcriptional repression of these genomic areas.

The heterochromatin domains located at fission yeast centromeres and telomeres contribute to three-dimensional genomic organisation by enabling clustering and NE attachment of these DNA regions [21, 33]. In addition, centromeric heterochromatin is key for kinetochore assembly and establishment of sister chromatid cohesion, ensuring faithful DNA segregation [34, 35]. At the sequence level, these regions contain DNA repeats and a small number of protein coding genes. Transcription of these repeats has been shown to recruit the RNAi machinery and is required for heterochromatin establishment and maintenance [9, 31, 32, 34, 36-38].

S. pombe centromeres are functionally and structurally similar to those of higher eukaryotes [10, 39]. They range from 35 to 110 kb in length and are composed of a central core (*cnt*) packed into nucleosomes containing the histone H3 variant Cnp1 (CENPA) which regulates kinetochore assembly (Figure 1). The central core is flanked by two different regions: the inner repeats (*imr*), specific to each chromosome, and the outer repeats (*otr*). This domain structure is conserved from fission yeast to humans [10, 40, 41].

Centromeric heterochromatin is disrupted during a brief period in S-phase allowing for RNAPII dependent transcription of inverted repeat sequences at heterochromatin nucleation sites which are processed into siRNA [9]. These siRNAs guide the RNA-induced initiation of transcriptional gene silencing (RITS) complex to these regions, which in turn recruits the Clr4-containing complex (CLRC) to promote H3K9 methylation [31, 36, 38, 42, 43]. H3K9me constitutes a binding site for chromatin organization modifier Swi6, homolog of human HP1 (Heterochromatin Protein 1), and Chp2. These two proteins self-associate and interact with other proteins leading to the spreading of heterochromatin from nucleation sites to adjacent regions and maintaining the heterochromatin environment [30, 31, 44]. In addition to RNAi-dependent heterochromatinization, other mechanisms initiate and maintain heterochromatin, including several histone deacetylases (Sir2, Clr6 and the SHREC complex) [44-46]. Once established, centromeric heterochromatin spreads *in cis* until it reaches a boundary element (see below).

At the mating type locus, heterochromatin formation is essential for the sexual dimorphism in the fission yeast and for the mating type switching. The mating type region is located on chromosome II, and contains three mating type genes as well as two identical inverted DNA repeats (IR-L and IR-R, Figure 1). These repeats, similar to those at the centromeres, are transcribed, triggering the RNAi pathway of heterochromatin assembly [31, 32, 47]. In addition, a second redundant pathway operates at the *mat* locus. It involves ATF/CREB transcription factors that bind to the *mat* region and recruit heterochromatin assembly regulators, such as the SHREC complex (containing Clr3 deacetylase as well as Clr1 and Clr2) and Clr6 that, in turn, target Clr4 methyltransferase and Swi6 to the repeat loci [48]. In the absence of the RNAi machinery, the ATF/CREB pathway becomes essential for H3K9 methylation and heterochromatin assembly at the *mat* locus [31, 43, 44, 49, 50].

Fission yeast telomeres, similar to telomeres of higher organisms, contain repeated sequences ensuring chromosomal end maintenance during replication. Telomeres are composed of three parts: a 3'-single stranded overhang, a 300 bp long repeat area and a subtelomeric region (Figure 1). Telomeric ends are regulated by Taz1, the homolog of mammalian TRF1/TRF2 [51, 52]. Little is known about heterochromatin formation at telomeres, although the presence of DNA repetitive sequences, siRNA hot spots and heterochromatin factors near the telomeres of the three chromosomes suggests that heterochromatin formation at telomeres might share similar mechanisms with other genomic domains [37, 43, 53]. Telomeric heterochromatin is regulated by the RNAi-RITS pathway and also by a second mechanism based on Taz1 protein. Both pathways bring about binding of Swi6 to telomeric regions that, in turn, recruits SHREC complex, thus promoting heterochromatin assembly [37, 48, 54].

Another part of the fission yeast genome that contains heterochromatin is rDNA, localized in the nucleolus and consisting of a tandem array of 150 rDNA repeats [55, 56]. Transcription of these repeats is essential to maintain nucleolar integrity [55, 57]. Organization of rDNA chromatin in *S. pombe* is poorly understood. The RNAi pathway is required for rDNA heterochromatin silencing and for rDNA repeat stability, as deletion of RNAi components or *clr4* results in derepression of reporter genes inserted at silenced rDNA repeats and leads to an increase in mitotic recombination that affects the integrity of rDNA [9, 58]. In *S. cerevisiae* and mammalian cells, the transcriptional state of rDNA has been shown to be heterogeneous, with active and inactive repeats interspersed. While the transcriptionally active repeats contain the typical features of active chromatin such as hypomethylated promoters and highly acetylated histones, inactive rDNA promoters harbor methylated histone H3K9 and are associated with HP1 [9, 59-62]. In *S. pombe*, Clr3 is the only histone deacetylase that localizes to the nucleolus and is required for rDNA silencing [63].

4. Chromatin boundaries

One of the interesting features of the *S. pombe* genome that it shares with higher metazoans is the presence of chromatin boundaries, or insulators. While in multicellular organisms insulators have the ability to block interactions between enhancers and promoters [7, 64], functionally similar sequences in *S. pombe* mainly act to prevent heterochromatin from spreading outside of the repressed areas such as centromeres and the mating type locus.

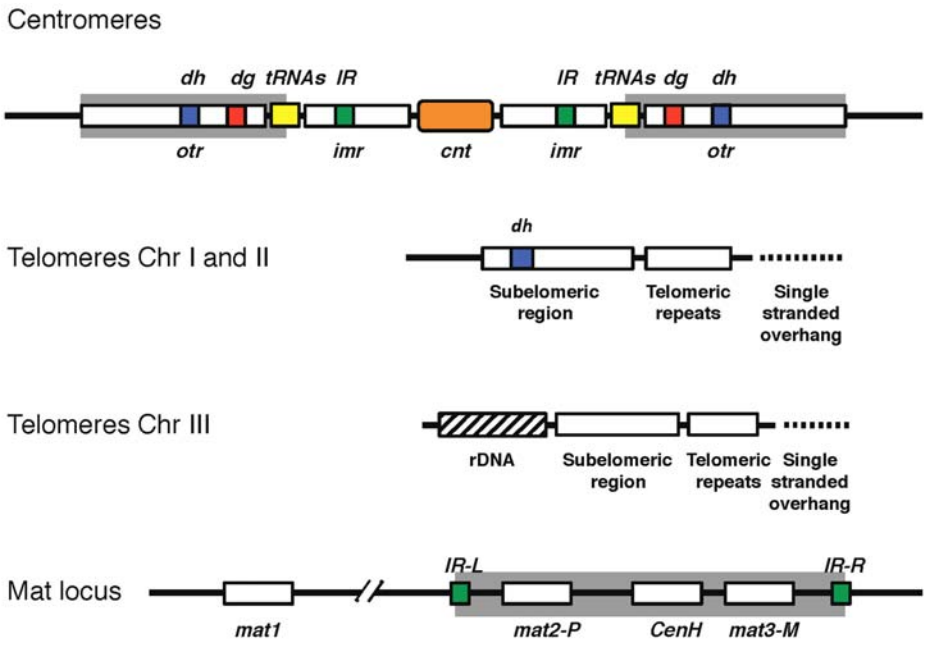

Figure 1

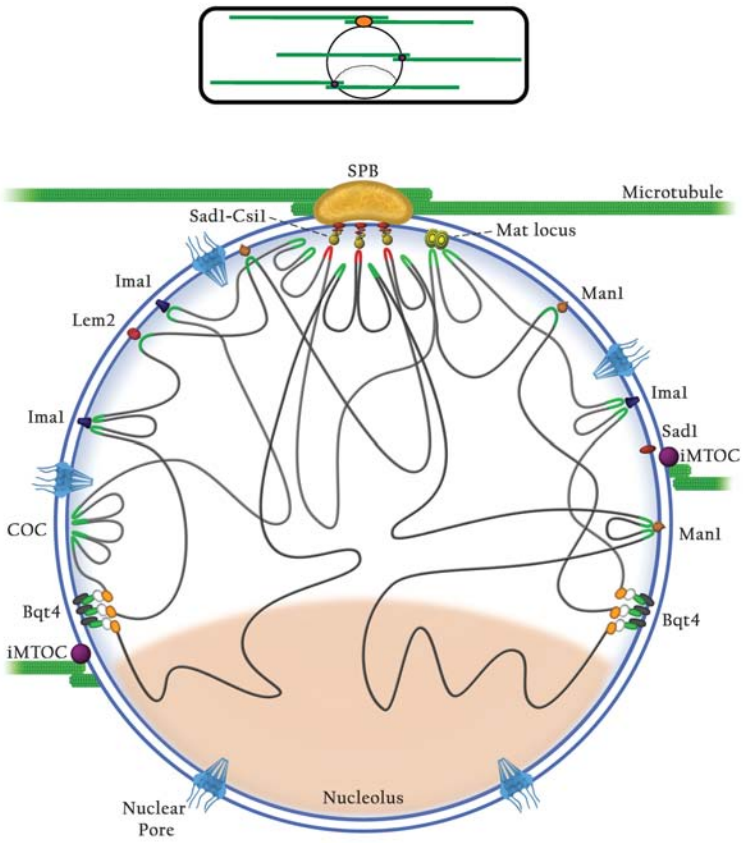

Figure 2

Specific examples of *S. pombe* boundary elements include inverted repeats that flank the *mat* locus as well as centromeric boundaries such as the tRNA genes at the edge of the inner centromeric repeats. It has been proposed that transcription of these tRNA genes by RNA polymerase III could be responsible for the boundary activity of this element [36, 65-67]. In addition to tRNA genes, two inverted repeat elements located at both sides of centromeres 1 and 3 (*IRC1*, *cen1* and *IRC3*, *cen3*) are acting as alternative barrier structures. IRC1 contains the typical histone modifications of active chromatin and RNAPII transcripts from these regions are converted into siRNAs by RNAi machinery [9, 68].

Both tRNA genes and IR boundaries contain sequence motifs known as B-boxes. B-Boxes are high affinity binding sites for TFIIIC that recruits RNA polymerase III transcriptional machinery, contributing to promoter activity of tRNA and 5S rRNA genes. Consistently, TFIIIC and RNAPIII are enriched at tRNA and 5S rRNA genes [66]. tRNA genes and IRs may use a similar mechanism based on B-boxes to prevent the spreading of heterochromatin into neighbouring euchromatin regions. Thus, deletion of B-boxes positioned inside the 500 bp fragment within IR-R and IR-L results in abnormal spreading of heterochromatin [66].

Boundaries of telomeric heterochromatin in *S. pombe* do not contain TFIIIC binding sites, and are characterized by a gradual transition between heterochromatin and euchromatin, which is due to a balance of histone modification activities [9]. Both subtelomeric regions and centromeric boundaries bind the SNF2 family chromatin remodeling factor Fft3 that maintains heterochromatin by preventing euchromatin assembly via H3K9 acetylation [69].

Thus, there are several types of boundaries in fission yeast: tRNA genes that recruit RNAPIII and TFIIIC [68], sites that recruit TFIIIC in the absence of RNAPIII [9, 66, 70], inverted repeat elements flanking the mating type region and *cen1* that are transcribed by RNAPII, and, finally, gradual barriers in subtelomeric regions [69, 71].

Importantly, recent work in fission yeast has shown that these boundary elements have the ability to tether DNA loci to the NE where they cluster, leading to the formation of chromatin loops, thus contributing to the 3D organization of the fission yeast genome [31, 32, 70, 72, 73].

5. Spatial organization of the genome

One of the important molecular mechanisms that is responsible for the spatial arrangement of nuclear DNA in *S. pombe* is clustering of specific DNA loci such as centromeres at the surface of the nuclear envelope (Figure 2). This results in Rabl chromosome configuration where all

Legend to Figure 1. Schematic representation of fission yeast centromeres, telomeres and mating type locus. Centromeres include central core (*cnt*) decorated by nucleosomes containing the histone H3 variant Cnp1 (CENPA) that provides a platform for kinetochore assembly. The central core is flanked by the centromere-specific inner repeats (*imr*) and by the outer repeats (*otr*), composed of a variable number of *dg* and *dh* elements present all three centromeres. Two kinds of DNA sequence elements contribute to centromere anchoring at the NE: tRNA genes and inverted repeats (*IRs*). tRNA genes delimit the heterochromatin boundary between *imr* and *otr*, and are present in the three centromeres. The boundary on the right side of cen1 lacks tRNA genes and depends on the *IRs* within the *imr* region, while cen3 boundaries contain both *IRs* and tRNA genes. Telomeres are composed of three parts: a 3'-single stranded overhang, a 300 bp long repeats and the subtelomeric region. Some of the subtelomeric regions contain sequences similar to centromeric *dh* repeats and could be involved in heterochromatin formation at the subtelomeric areas. rDNA is present near the subtelomeric regions of chromosome III, and is repressed via RNAi-dependent heterochromatin. Mating-type region is located on the right arm of chromosome II and includes three protein-coding genes, *mat1*, *mat2* and *mat3*, as well as cis-acting elements including *IRs* and *cenH* region that are responsible for heterochromatin formation. *CenH* contains repeats similar to *dg* and *dh*, is located between *mat2* and *mat3* and is involved in repressing recombination and transcription via heterochromatin assembly.

Legend to Figure 2. Fission yeast nuclear architecture. Schematic representation of the fission nucleus showing NE components and chromatin elements involved in tethering DNA to the NE. INM proteins Sad1, Ima1, Man1, Bqt4 and Lem2 provide anchors at the NE surface that are connected to chromatin. These connections rely on adaptor proteins specific to individual loci such as Csi1 at centromere-SPB attachment sites and Rap1-Taz1 complex at telomeres. Interphase MT organizing centers (iMTOCs) including the SPB are connected to chromatin.

centromeres are clustered at one site at the SPB while telomeres are attached to the NE part facing away from the SPB [26]. Mating type locus, other heterochromatin sites and TFIIIC binding loci are also attached to the NE at multiple sites that in some cases co-localise with the SPB [66, 70, 74].

Two types of INM protein complexes have been shown to bind heterochromatin. The first type is represented by INM protein Ima1 and the LEM-domain (Lap2/Emerin/Man1) containing proteins Man1 and Lem2, while the second complex involves the SUN domain containing protein Sad1 [14, 22, 75].

Genome-wide studies using Man1 and Ima1 to map chromatin-NE interactions have shown that about one third of the genome is associated with one or both of these proteins [14]. Interestingly, Ima1 and Man1 have overlapping and non-overlapping target regions in the genome. Centromeres are associated with both proteins that preferentially bind central core domains, whereas telomeres are enriched in Man1 at subtelomeric regions where it colocalizes with Swi6. In fact, Man1 and Swi6 show significant co-localization genome-wide that is not shared by Ima1. Ima1 targeted loci, in contrast, contain RNAi machinery and there is a strong correlation between localization of Dcr1 (Dicer), Rdp1 (RNA-dependent RNA polymerase) and Ima1 occupancy [14]. This suggests that the two proteins have functional specialization in anchoring different sets of heterochromatic loci to the NE.

Heterochromatin is also tethered to the NE via other pathways, some of which are specific to individual heterochromatin regions. For example, centromeres are anchored by Mis6 and Sad1 complexes, while the connection of telomeres to the NE involves the Bqt4 INM proteins that bind to telomere specific Rap1-Taz1 complex [21, 76]. Below, we describe the key elements that connect different DNA regions to the NE.

5.1. Centromere tethering to NE-SPB

The three centromeres of *S. pombe* are localised at the nuclear periphery in close contact with the SPB (Figure 2). Kinetochore proteins and Sad1 complexes at the NE are associated to the centromeric heterochromatin and contribute to centromere clustering. Such clustering has been described in diverse cell types ranging from yeast to plants and animals [8, 77, 78]. In fission yeast, interphase centromere clustering facilitates kinetochore capture by spindle microtubules during mitosis, which is important for correct chromosome segregation [75, 79].

SPBs contain the evolutionary conserved SUN/KASH protein complex that in higher eukaryotes links the centrosomes to the NE [13, 80, 81]. Sad1 is the only member of the SUN family in *S. pombe* [82], whereas the KASH family is represented by Kms1 and Kms2 [83, 84]. In the INM, Sad1 interacts with Csi1, a non-essential coiled-coil protein with a predicted membrane-binding domain that also localizes to the SPB-centromere interface and is essential for binding of centromeres to the interphase SPB [75]. At the beginning of mitosis when the centromeres are released from the SPB, Csi1 dissociates from the centromeres for the duration of mitosis until anaphase, when centromere attachment is reestablished and Csi1 is recruited back to the centromeres [26].

In addition to the SUN protein Sad1 and to Csi1, centromere-SPB connection requires kinetochore proteins Mis6, Ndc80 and Nuf2 [85-87]. Mis6 is an inner kinetochore component required to load the histone variant Cnp1 onto the centromere [87, 88], whereas Nuf2 and Ndc80 are outer kinetochore proteins that form the conserved Ndc80 complex [89]. Mutants in either of the kinetochore proteins Mis6, Ndc80 or Nuf2 lead to centromere dissociation from the SPB. However, centromeres remain connected to the NE in these mutants, suggesting that NE connection may depend on centromeric heterochromatin or on other elements such as boundary sequences [75, 85-87].

5.2. Telomere tethering to NE

Telomeres are clustered and tethered to the nuclear periphery. The clustering depends on the production of siRNAs by RNAi machinery, suggesting a role for heterochromatin in this process [34]. Recently, several proteins have been identified that provide a link between telomeric heterochromatin and the nuclear envelope. Thus, Bqt4 is an INM protein that plays a role in telomere-NE anchoring in vegetative and meiotic cells [21]. Bqt4 interacts with Rap1 at the

telomeres [90] and *bqt4Δ* cells contain telomeres that are not clustered and are dissociated from the NE [21]. Thus, Bqt4 is required to tether telomeres to the NE. However, telomeric silencing is not affected in *bqt4Δ*, suggesting that the heterochromatin environment at the telomeres is not compromised when they are detached from the NE [21, 91]. During mitosis, telomeres temporarily dissociate from the NE due to Cdc2-dependent Rap1 phosphorylation that prevents Rap1 interaction with Bqt4 [76].

5.3. *Mat* locus and *rDNA* tethering to NE

At the mat locus, both heterochromatin and IR repeat boundary elements are required for the correct localization of this locus to the nuclear periphery. It has been demonstrated that in the wildtype cells the *mat* locus is preferentialy associated with the NE in close proximity to the SPB. Deletion of IR repeats does not affect its NE localization but results in the detachment of this locus from the SPB. In contrast, heterochromatin disruption by deletion of *clr4* methyltransferase leads to delocalization of the *mat* region from the NE [74].

Mating type locus boundary elements bind TFIIIC that is not accompanied by any other RNAPIII components. Such TFIIIC binding sites were shown to tether DNA to the NE [66] and could possibly contribute to tethering of the *mat* locus.

Little is known about rDNA localization determinants in *S. pombe*. In the budding yeast, silenced rDNA can be found in association with the NE due to the interaction of the conserved INM proteins HEH1 (ortholog of human Man1) and NUR1 with SIR2 and the cohibin complex [58]. Nuclear envelope binding seems to be required for rDNA repeat stability and integrity of the nucleolus. Artificial tethering of rDNA repeats to the INM suppresses the instability observed in Sir2 mutant cells, suggesting that localization at the NE can promote repeat stability independently of Sir2-silencing activity. Deletion of Sir2, or of cohibin complex proteins Lrs4 or Csm1 also affects silencing at the rDNA independently of HEH1 or NUR1 INM proteins. This suggests that the tethering of the rDNA to the NE also depends on INM protein complexes and on the establishment of silenced heterochromatin and that both mechanisms contribute to rDNA stability by limiting access to the recombination machinery [58].

5.4. 3D organization of other genomic loci

The DamID method based search for DNA loci associated to the INM proteins Man1 and Imal revealed a large number of loci in addition to major heterochromatin domains at centromeres, telomeres and mating type region [14]. Many of these loci contain repressed or low expression genes suggesting that nuclear organization in *S. pombe* is intimately linked to the control of gene expression. Importantly, a new class of DNA elements likely contributing to 3D DNA organization has recently emerged from a genome-wide analysis by Noma *et al.*, who discovered 67 TFIIIC binding sites, dispersed along euchromatin regions of the fission yeast genome [65, 66]. These TFIIIC sites, named Chromosome Organizing Clamps (COC) loci, similar to those present at the *mat* locus, contain little or no RNAPIII. Most of the COC sites are positioned between divergently transcribed genes and ~90% of them are within or relatively close to promoters of RNAPII transcribed genes. Nevertheless, the role of COC sites in gene regulation is unknown [65]. COC sites were shown to be associated to NE in heterochromatin independent and B-box dependent manner. Such chromatin organization could create DNA loops attached to the NE. This topology could influence many aspects of DNA metabolism.

6. Chromatin connection to cytoplasmic MTs

In fission yeast, cytoplasmic microtubules are physically connected to the nucleus. Microtubules are organized into 3-5 antiparallel bundles with plus ends facing the cell tips (Figure 2). Minus end overlap zones are linked to the NE via specialized structures that serve as microtubule nucleation sites, MTOCs. These MTOCs can be connected to chromatin via the SUN-KASH protein complexes [92, 93] and, possibly, via other INM proteins. A well established example of such connection between MTs and chromatin is provided by the SPB that forms a major MTOC at the nuclear surface during the interphase. Microtubule polymerization generates pushing forces against the cell ends that are transmitted to the nucleus via SPB and other MTOCs, and

provide a mechanism for nuclear positioning at the cell center [93, 94]. To what extent such forces are transmitted to chromatin [27] and whether they influence nuclear function is an exciting topic for future research.

7. CONCLUSION

Many elements within the fission yeast genome such as centromeres and telomeres contribute to its 3D organization in the nuclear space. One common feature of these loci is their ability to associate with the NE and to form clusters at the nuclear periphery. One of the most important clusters forms at the nucleoplasmic face of the NE adjacent to the interphase SPB. In addition to the three centromeres, other loci including the *mat* locus, many tRNA genes and 5S rDNA genes are associated with this cluster, creating a unique intranuclear domain rich in heterochromatin and bringing together different parts of the fission yeast genome [70] (Figure 2). This is likely to result in a chromosomal arrangement where DNA loops are formed, defining interphase DNA topology. Such topology is reminiscent of chromosomal loops characteristic of higher metazoans [64].

In addition to the SPB proximal domain of the NE, other sites anchor and position fission yeast genomic loci such as telomeres, transposons and TFIIIC sites. In the future it will be important to find out how these aspects of genome organization contribute to DNA transcription, replication and repair, and also to find out how genome architecture changes in response to environmental conditions such as nutrients and stress [95-97]. Changes in the interphase nuclear architecture could also lead to chromosome segregation defects and genomic instability.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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