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Minireview

Chromatin organization in relation to the nuclear periphery

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Abstract In the limited space of the nucleus, chromatin is organized in a dynamic and non-random manner. Three ways of chromatin organization are compaction, formation of loops and localization within the nucleus. To study chromatin localization it is most convenient to use the nuclear envelope as a fixed viewpoint. Peripheral chromatin has both been described as silent chromatin, interacting with the nuclear lamina, and active chromatin, interacting with nuclear pore proteins. Current data indicate that the nuclear envelope is a reader as well as a writer of chromatin state, and that its influence is not limited to the nuclear periphery.

Keywords: Chromatin; Nuclear lamina; Lamin; Nuclear pore complex; Nucleoporin; Nup

1. Chromatin organization by compaction

To fit into the limited space of the nucleus and still carry out its function, human genomic DNA is extensively folded, making it about 10 000-fold more compact. Several levels of compaction have been described: the nucleosome, the 30 nm fiber and higher order chromatin structure.

The lowest level of chromatin compaction is the nucleosome. A 5–10-fold compaction is achieved when 146–165 base pairs of DNA are wound around an octamer of histone proteins, which is referred to as the nucleosome core particle. Besides providing a structural basis for the first compaction level, histones can also affect chromatin organization by being chemically modified at their tail or by being replaced by variants of the core histones. These modifications have a major impact on chromatin structure and gene expression by influencing the binding of proteins to the nucleosome, the affinity of DNA for the histone octamer and the stability of higher order structures [1]. Thus, at this low level of organization the nucleosome offers a powerful mechanism for controlling chromatin structure in a local, non-random manner.

Findings on the second level of compaction are more ambiguous. In vitro, oligonucleosomes are able to organize themselves into a compact fiber with a diameter of 30 nm in absence of nuclear proteins but in the presence of divalent cations. In vivo, estimated nuclear cation concentrations are even higher than the concentration used in experiments, aiding the compaction [2]. This compaction could be further modulated by the involvement of numerous nuclear proteins in vivo. For example, histone tails and histone H1 further stabilize this structure by binding to linker DNA.

All condensation levels above the 30 nm fiber are indicated as higher order chromatin structure. This poorly defined structure may consist of several levels of condensation and is very dynamic and thus hard to study. The question has even been raised whether there is a uniform higher order structure at all, or whether chromatin is too dynamic to form stable structures at a higher order level [3].

All levels of compaction are not equal throughout the cell, leading to more accessible and less accessible regions. Dynamic chromatin-binding proteins and histone modifications play key roles in dynamically compacting the chromatin or opening it up, giving the cell the possibility to rapidly alter chromatin compaction at multiple regions when necessary. Chromatin compaction can control processes like transcription, duplication and repair by limiting the accessibility of chromatin by proteins. Knowing this, it is not surprising that disturbance of chromatin structure has been linked to several types of disease, including cancer [4].

2. Chromatin organization by insulator activity

To prevent spreading of condensed, silent chromatin to more open and active regions, insulators can form a barrier between these distinct chromatin domains. Insulators have been defined as genomic elements and their interacting proteins can block distal enhancer activity or protect chromatin against effects from a neighboring chromatin region when positioned adjacent to it [5]. The first insulator discovered was the *gypsy* transposable element, which blocked enhancers from activating the yellow gene when inserted upstream of the *yellow* gene promoter [6]. A complex of proteins binding to the *gypsy* insulator has been identified, consisting of Su(Hw), Mod(mdg4), CP190 and dTopors [7–9]. In yeast, insulators have been found to form boundaries that block spreading of silenced chromatin at telomeres and from the mating-type loci HML and HMR. In vertebrates, examples of insulators are those in the chicken β-globin genes and the human T cell receptor-α/δ locus [5]. The boundary function of some insulators has been shown to be dynamic, as the insulator function can be modified or abrogated by modifying factors and DNA methylation [5]. Although still several models exist for the mechanism of insulator function, much data points in the direction of a loop-domain model. For instance, inserting two copies of the Su(Hw) insulator

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element instead of one copy inhibited the insulator function, suggesting that the insulator activity can be overcome by a loop formed by the insulator elements interacting together [10,11]. When an enhancer was flanked by two Su(Hw) insulator sites, blocking of activity was more severe, suggesting that by forming a loop around the enhancer, enhancer–promoter interactions are being blocked [10]. Thus, insulators are suggested to establish a higher order chromatin structure by the formation of loops or possibly more complicated structures.

3. Chromatin organization at the nuclear periphery

Are differently compacted or structured chromatin regions distributed in a random way inside the nucleus, or do some regions prefer certain sites? To address this question, a fixed nuclear viewpoint is required. For this reason extensive research has been performed into localization of chromatin in relation to the nuclear envelope and the putative role of the nuclear envelope in chromatin organization. The first hint that differently compacted and structured chromatin regions are distributed non-equally in the nucleus dates from about a century ago. Classical cytological characterization of the nucleus discerned two types of chromatin: the relatively dark staining heterochromatin that stays condensed throughout interphase and lighter staining euchromatin (''real'' chromatin) which decondenses in interphase and is traditionally associated with transcriptional activity [12]. Interestingly, in many cell types, classically defined heterochromatin has a different subnuclear distribution than euchromatin, with heterochromatin enriched at the nuclear periphery and around nucleoli [13]. It has been suggested for a long time that this non-random distribution of heterochromatin and euchromatin has a function and that attachment of chromatin to the nuclear envelope is important to obtain the three dimensional organization of the chromatin fibers [14,15]. These suggestions are based both on the rationale that the nuclear envelope is the only stable structure in the nucleus at which chromatin can be organized structurally and on experimental data showing that chromatin fibers are attached to the nuclear envelope [16,17]. In 1968, Comings concluded on the basis of electron microscopic images of labelled nuclei that there is a certain degree of order in interphase chromatin and suggested that the order might be maintained by attachment of chromatin to the nuclear envelope. Blobel extended this view by suggesting a 'gene-gating' hypothesis: compact chromatin associates with the nuclear lamina, while expanded transcribable genes associate with the nuclear pore complex, aiding in nuclear export of RNA. He proposed that the non-random distribution of nuclear pore complexes in the nuclear envelope reflects the non-random organization of chromatin. However, whereas the models of Comings and Blobel were logically and intuitively sound, at their time not many data were present to confirm their ideas. Now the situation is different, as many new techniques in both microscopy and the use of microarrays have boosted research in the chromatin field.

4. Chromatin at the nuclear periphery: from stainings to genes

The first genetic elements that were found to be localized to the nuclear periphery were telomeres, the ends of chromosomes. Already in 1885, observations about the positioning of chromatin in cells were made by Carl Rabl, who observed in salamander nuclei that centromeres clustered at one pole and telomeres at the opposite pole [18]. Peripheral telomeres have also been observed in *Drosophila* [19,20], Trypanosoma, plant cells, vegetatively growing fission yeast, but not in mammalian cells [21,22].

The first studies that systematically mapped genomic loci in relation to the nuclear periphery were performed in *Drosophila* polytene-chromosome containing cells. It was found that specific chromosomal loci associated with the nuclear envelope with a high frequency [19,23–25]. Interestingly, those loci often corresponded to ''intercalary heterochromatin'', linking the concept of inactivity of peripheral heterochromatin to genomic maps. Two decades later, high resolution molecular mapping in *Drosophila* cells confirmed this link and revealed that genes that associate with the nuclear lamina are transcriptionally silent (further described below) [26]. In human cells, the first study that went beyond localization of bulk staining was the localization of the inactive X chromosome at the nuclear periphery [27]. Chromosome-specific fluorescent in situ hybridization revealed that autosomes too have their preferred position [28], correlating with gene density: gene-poor chromosomes tend to localize to the nuclear periphery (e.g. human chromosome 18), while gene-rich chromosomes tend to localize at intranuclear positions [29].

5. Silencing at the nuclear periphery: cause or consequence?

Does localization at the periphery cause chromatin silencing or is the peripheral localization a consequence of inactivation? In yeast, presence at the nuclear periphery has been correlated with inactivity of genes by a study in which a RNA polII transcribed gene was inserted adjacent to telomere sequence and thereby was repressed, a process called telomere position effect (TPE) [30]. Yeast telomeres cluster at the nuclear periphery as do proteins that are essential for TPE. However, it was shown that localization to the nuclear periphery is not necessary nor sufficient for TPE [31] and there is no correlation between TPE levels and extent of localization [32].

Telomere-independent silencing of genes at the nuclear periphery was tested in yeast by tethering genes artificially to the nuclear envelope by fusing integral membrane proteins to the Gal4 DNA-binding domain [33]. Several of these membrane proteins caused silencing. The mechanism by which the silencing occurs has been suggested to be the higher concentration of SIR proteins at the periphery, as overexpression of SIR3 and SIR4 improved silencing in strains with defective silencers. Telomere clusters colocalize with Sir3p, Sir4p and Rap1 [34]. Seventy percent of these foci is at the nuclear periphery and does not directly associate with nuclear pore complexes, nor does provocation of nuclear pore clustering at one side of the nucleus affect the position of the telomere foci. In this study, resolution was too low to determine whether subnuclear position was altered in absence of Sir3p or Sir4p. Recently, it has been found that the Sad1-UNC-84 (SUN) domain protein Mps3 is required for anchoring of telomeres to the nuclear periphery by binding Sir4 [35].

Observations in higher eukaryotes also indicate a repressive role of the nuclear envelope. IgH loci move away from the nu-