Architecture of the chromatin in the interphase Nucleus

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Introduction

Since the XIX\textsuperscript{th} century and the work of Rabl, several hypotheses had been emitted as of the existence or not of an organized and controlled architecture of the chromatin during the interphase, hypotheses which had evolved in function of the available techniques to be checked with. Thus, for Rabl, a nucleus compartmentalization should existed, each chromosome occupant a defined territory. This hypothesis was essentially based on the theoretical concepts since the techniques of optical microscopy. If they permitted to describe some structures like the nucleoli, they did not have the necessary resolution in order to distinguish the chromatin fibers from each others. The idea of nucleus in interphase has been called into question by the studies in electronic microscopy, carried out in the years of 60 and 70. The results were disappointing despite the excellent resolution obtained; not particular architecture of DNA molecules could be described. The prevailing idea was that the nucleus was a simple envelope containing DNA molecules, discondensed and mixed in a random way. Since a score of years, the emergence and the rapid development of Fluorescent In Situ Hybridization (FISH) techniques lead to reexamine the topic. Thanks to new tools of imageries which finally permitted to specifically visualize each molecule of DNA within the nucleus.

I. 3D organization of the chromatin in the interphase nucleus
   1. 1. Relationship of the chromatin with the nucleus structures
The chromatin principal component of the nucleus, is in relationship with two principal nuclear structures identified in microscopy: the nuclear membrane and the nucleoli.

a. The Nucleoli were identified since a while as the place for (rRNA) synthesis. These intra-nuclear structures are not limited by envelope and they assemble in variable number (in general 2 per nucleus) at the time of transcription in G1, after the mitosis. They are composed of several domains visible in electronic microscopy:

- One or several fibrillary centers, corresponding to the transcription zone starting from DNA molecules.
- A dense fibrillary component which surround the fibrillary center(s), containing the primary transcripts.
- A granular component less dense in periphery, corresponding to assemblage zone of pre-ribosome.

Thus, it is a functional structure linked to the rRNA synthesis and not pre-established indispensable to this synthesis. This synthesis is carried out starting from repeated genes, few hundred of times, in the genome and all are located at the short arms of acrocentric chromosomes (chromosomes 13, 14, 15, 21 and 22). These regions are called Nuclear Organizing Regions (NOR). Because bringing together these NORs involves the nucleolus installation (by concentration) within necessary components for the transcription and the maturation of rRNA. The physical proximity of short arms of acrocentric chromosomes could be continued some times until the mitosis, where a juxtaposition of several acrocentric chromosomes NORs can be observed.

b. The nucleus is delimited by envelope constituted of a double membrane (internal and external nuclear membranes, separated by the peri-nuclear space). At the internal face of the internal nuclear membrane, a felting of intermediary filaments was found, the lamina, composed of three fundamental proteins: the lamina A, B and C. The lamina has many interactions with the proteins of the internal nuclear membrane and with the chromatin, in relationship with its functions in the nuclear envelope organization and probably in the regulation of gene expression.

Indeed, a number of transcriptional regulators interact with the laminas, just as the HP1 protein fix specifically to the heterochromatin. By the lamina means, the nuclear envelope could participate at the heterochromatin organization in the nucleus and more largely at the regional control of by the gene relocalization on the contact of the heterochromatin to inactivate them.

2. 2. Notion of chromosomal territories

In the beginning of the eighties, thanks to laser irradiation experiences of Hamster cells, the first elements of observation come to support the existence
of chromosomal territories. But it is the in situ hybridization with painting chromosomal probes, which permitting to directly visualize the specific genetic material at each chromosome, showed that each chromosome occupies a well delimited territory without mixing or covering with neighbor territories, confirming therefore the Rabl theory. These results are shown for all the chromosomes pairs and constitute the first proof of the chromatin organization during the interphase. Within these territories, the chromatin fiber preserves an organization which permits to identify sub-domains corresponding to arms and chromosomal bands. The surface occupied by these territories is proportional coarsely to the chromosomal size, but other parameters could modulate, for example, the chromosome global level expression. These chromatin territories do not constitute nuclear compartments as well as the nucleoli, because they are permissible; as demonstrated by the passive diffusion of with a size that could reaches 500kDa in the entire nucleus.

3. Topography of the territories
Is there an accurate and regulated arrangement of chromosomes when compared to each others during the interphase? It is a difficult question, which is not resolved in definitive way, because divergent or even contradictory results were reported en favor or against this hypothesis. It should be underlined that the first investigations were carried out in different cell types, even in different species, which suggests the existence of functional and/or tissue variability.

However, the most recent results seem to confirm the existence of a chromosomal territories arrangement, not in random way, during the interphase. This arrangement is function of the chromosomes size and/or their genes containing. Thus, there is a preferential localization of small chromosomes towards the inner of the nucleus while the tall ones are frequently observed towards the periphery. Nevertheless, the gene containing intervenes as it had been demonstrated for the case of two chromosomes: 18 and 19. Though of comparable sizes, the chromosome 19, rich in genes, is located more towards the nucleus center than the 18 one, poor in genes, which is observed towards the periphery. This relationship, between the increased gene density and the more central position in the interphase nucleus, had been reported since for other pairs.

Therefore, if we admitted the existence of none random organization of territories within the nucleus, there is another question to resolve: is this organization associated to a particular arrangement of homologue chromosomes or of certain pairs together? Once again, the obtained results so far are discordant and do not permit yet to answer in a definitive way. However, some recent observations relative to respective positions of implicated chromosomes in some reciprocal translocations support the regular positioning of chromosomes when compared to each other.

4. Interface between neighbor territories (Figure 1)
Two models are proposed to describe the relationships which exist between the neighbor chromosomal territories.

a. In the first model, Interchromatin Domain (ICD model), should exist spaces devoid of chromatin called interchromatin
spaces. These spaces constitute a three dimension channels network which starts at the nuclear membrane pores level and spread between the chromosomal territories with invagination. These gaps have variable sizes with few micrometers of diameter, while the finest regions could have only some nanometer of wide. The interchromatin spaces would be the place where concentrated of all the non chromatin material (nuclear corps, pre-mRNA, transcription factors) and would constitute as such a simple place for macromolecules stocking or a place of enzymatic reactions. Anyway, it is established in return that these spaces serve as circulation tracks within the nucleus per simple passive diffusion, permitting the distribution of proteins necessary for the transcription and exporting in return the (RNAm) products.

b. The second model, Interchromosomal network (ICN model), suppose, on the contrary, the existence of recovering zones between neighbor territories, in which the chromatin fibers of the two chromosomes are closely associated. This model is based on observations done upon ultra fine cuts which permit to better preserve the chromatin architecture than the standard preparations of 3D FISH. Nearly 40% of each territory would be mixed, in periphery, with the neighbor territories. The importance of the boundary zone would be function in particular to the chromosomal territory compaction (more the chromatin is compact less is the interpenetration possibilities), where it self proportional on the chromosome richness in genes and on its transcriptional activity. In this model, the proteins necessary to the transcription, replication, DNA reparation and the RNAs would diffuse freely between the chromatin loops within each territory and are not confined within specialized spaces. The principal interest of this model is to conciliate the chromosomal territory with the observed frequency of reciprocal translocations. Indeed, for each chromosome, there is a very good correlation between the mixed territory proportion, with the neighbor territories, and the reciprocal translocation frequency implicated the considered pair.
5. Organization model of the chromatin in the interphase nucleus
So far, a functional organization model of the interphase nucleus, carried out of different observations, is constituted of chromosomal territories containing genetic information under the form of chromatin. Within the nucleus, the proteins and the RNA products can diffuse freely to reach their fixation site or to be exported towards the cytoplasm, or via a network of channel spaces connected to nuclear pores separating the territories (ICD model), or directly through the chromatin loops of different territories (ICN model). In both models, transcription activities, carried out with the chromatin contact, can be regulated with modifying the accessibility to genes. Active genes are accessible to transcription complexes because they are close of interchromatin space in the ICD model or because they are located upon DNA large loop in the ICN model. The observation that gene activation can be associated to its relocalization in a neighbor territory permits to consider new modes of co-regulations of participating genes - located eventually on distinguish chromosomes - in common metabolic pathways. What ever the model is which turns out the closer to the reality, this chromatin organization permits to inactivate easily the gene groups by modifying their position within the territories and by returning them inaccessible to the transcriptional machinery. Among the number of points to be elucidated is the question whether the organization in the chromosomal territories is a preliminary, permitting to organize and to regulate the transcription or, on contrary, it would not be the
consequence of the cell transcription activity, by managing compact chromatin regions since inactive and others more “spaced”, in contact of rich zones with proteins and mRNA, result of the transcription.

II. Relative evolution of the territories when compared to each other during the succession of the cell cycles

Are the chromosomes positioned in random way within the nucleus or on contrary they are presenting a specific arrangement with respect to each other? The question is not yet solved but it opens interesting perspectives as far as such arrangement would constitute important epigenetic information within the context of cellular differentiation and would explain (or be explained by) a differential expression of some genes in different cell lines. If this hypothesis is checked, the transmission at the identical of this positional information takes an essential importance to preserve the peculiar features of each cell type. Two arguments at the present time are in favor of a coordinated organization of territories with respect of each other.

- The probability for an alteration to occur between two chromosomes when exposed to irradiation depends notably on the distance existing between them during the interphase. From one cell type to another, variable frequencies were observed of different possible associations, suggesting therefore that the relative position of chromosomes, when compared to each other, varies in function of considered cell line.

- The relative position of chromosomal territories, when compared to each other, is transmitted at the identical at each cell division to daughter cells. Fluorescence observations realized on alive cells show that all territories located in the same half of the mother cell nucleus is associated to a half nucleus of each cell daughter. The retained mechanism, at the present time, to explain the preservation position after the metaphase, where all chromosomes are assembled on the same plan, consists of an asynchronous chromatide separation at the anaphase: the chromosomes that are going to position in the most peripheral half of the nucleus (in daughter cells), compared to the metaphase division plan, will separate before those that are destined to occupy a more central position. (See figure 2).

A key element for the chromosome asynchronous separation control could be the quantity of centromeric heterochromatin, which is indispensable for the chromatide cohesion at the level of centomere. Indeed, if this heterochromatin is altered (by incorporation of Hoechst which prevents a correct condensation, for example), a random repartition of chromosomes is observed, this time, in the daughter cells.

If the relative position of different chromosomal territories constitute epigenetic information (which is to be demonstrated in a definitive way), it is already probable that the transmission of this information is not absolute and that variations occur after a number more or less important of permitting a functional adaptation of cells.
III. The nucleus: a dynamic structure of 4 dimensions

The chromosomal territories have a stable position in the nucleus during the cell cycle, expressing the global immobility of the chromatin in the nucleus. Fluorescence analysis techniques on alive cells show indeed a high stability of used tracers during the G1, S and G2 phases. This immobility is probably in relation with the little available space inside the nucleus, but it can arise in part of the chromatin anchorage in some nuclear compartments, nucleoli and principally envelope.

Meanwhile, if the analysis scale is changed, we will see that the chromatin is not a coagulate in the nucleus and two kinds of movement will be observed.

a. On the one hand, fluorescence marking of tiny chromosomal regions (about 10 kb) underlined the existence of a small scale movement, on a distance less than 0.5 µm. These movements are, brownian type, turned in all directions but because of their weak amplitude, the considered locus remains delimited to a reduced region of the nucleus (about 1/1000th of the total volume). Therefore, these movements are consistent with the chromosomal territory notion, clearly individualized and relatively immobile. All the investigated loci do not show the same possibilities of movements; some seem less mobile as the case of telomeres, the centromeres or some domains along the chromosomes, corresponding may be to chromatin anchoring sites of nuclear structures.

b. On the other hand, large scale movements are susceptible to be in relation with modification of cell transcriptional activity, for example, when a cell does the re-entry in the cycle in the quiescent phase, or during the cell differentiation, as it had been observed on Lymphocytes B. These movements could have an important functional role during the cell differentiation process by modulating the expression of some genes with a repositioning in regions favoring or, on contrary, inhibiting the transcription. Furthermore, the chromatin movements permit to explain the co-localization within gene transcription complexes located on distinguish chromosomes, as much as a correlation is found between the cell transcriptional activity and the importance of chromosomal territory recovering.

Conclusion
Since the XIXth century, Rabl had the right intuition: the nucleus is not an organelle serving to separate only the genome of the cytoplasm but it plays an essential role probably in the chromatin organization and the gene expression control. Within this organelle, the genome is arranged on a none random way; each chromosome is occupying a well defined territory and it is globally maintained in place by contacts with diverse sub nuclear structures. This arrangement, eventually particular to certain cells or tissues, is transmitted to daughter cells during the cell divisions. Finally, movements are possible, after all, at some loci levels which can be relocalized in other regions of the nucleus notably during cellular differentiation phenomena or transcription renewal. The position modifications and the existence of none random chromosomal territories arrangement suggest that may be exist, in the nucleus, zones more or less in favor of the transcription and therefore of the gene expression. According to its location, such or such gene group will be entirely activated without assuring an individual control of each. Furthermore, the existence of exclusion zones would facilitate the protein action assuming the transcription (enhancers, polymerases, etc…) by reducing the number of potential targets and by increasing the apparent concentration of transcription factors towards the active genes. Therefore, it would be existed a genetic plasticity correlated with the chromatin mobility which would participate to the cell adaptation to a moving environment.

The importance of this organization and the structures which maintain it (notably the nuclear envelope) in the functional regulation of genome is attested by the laminopathies associated troubles. The accurate investigation of the physiopathology of these diseases will contribute probably of new clarifications on the mechanisms of meta regulation of gene expression at the regional level.

Among those, DNA epigenetic modifications (cytosine’s methylation, histone’s methylation/ acetylation) constitute an axe of privileged research because of argument number existing in favor of a relationship between the epigenesis (chromatin structure, genome organization) and gene expression.

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