THE COMPLEX ORGANIZATION OF EUKARYOTIC CELL NUCLEUS (II): CHROMOSOME TERRITORIES AND THE INTERCHROMATIN DOMAINS

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The **chromosome territories** (CTs) could be defines as the total amount of space filled by all the chromosomes inside the nucleoplasm of an interphasic cell nucleus. They represent one of the major parts of nuclear architecture.

The term *chromosome territory* was first introduced by Theodor Bovery (1909), following some microscopic studies of the time, which suggest that each mitotic visible chromosome remain distinct during interphase; also, it occupies a specific part in the nuclear space (Cremer and Cremer, 2010).

Although, during the 1970s and the early 1980s, the existence of CTs was seriously contested, especially by a number of electronic microscopic observations (which lead some authors to consider that the chromatin fibers of distinct chromosomes intermingle), other researches tested and successfully demonstrated the validity of Bovery's concept (by experimental methods such laser – UV – microirradiation, in situ hybridization, flow citometry of fluorescently labeled mitotic chromosomes and other) (Cremer and Cremer, 2010). These methods, combined with the 3D and 4D (space plus time) fluorescence microscopy and the image analysis, have shaped the understanding of human nuclear chromatin as a dynamic, high – ordered structure. Moreover, some authors consider that the three main nuclear compartments are: an *"open" higher-order chromatin compartment* (containing active genes), a *"closed" chromatin compartment* (with inactive genes), and an *interchromatin domain* (ICD or IC) (Cremer et al., 2000).

Each chromosome which form a particular CT is bound through its telomeres by proteins of the nuclear lamina in discrete sites. The homologous chromosomes from a pair are not necessarily situated in vicinity one to each other (Robinson et al., 2003).

Even though the CTs exist only in cells with real nucleus (eukaryotes), some lower eukaryotes (such as the yeast *Saccharomyces cerevisiae)* lack chromosome territories. Morphologicaly, the CTs (observed in fluorescence microscopy in vivo) appear as spherical structures of about 2 micrometers in diameter.

If the interchromatin (interchrosomal) compartment could be viewed as a network of channels which separate the individual CTs, the hypothetical CT structure in the CT-IC model can be conceived as a sponge of chromatin permeated by intraterritorial IC channels (Cremer and Cremer, 2010); therefore, the CTs are not compact, solid entities.

The intimate ultrastructural and molecular organisation of the CTs, their involvment in some essential nuclear functions (such as replication and translation) and also their complex relationships with the IC and other nuclear compartments are issues treated in different ways by various authors.

Generally and theoretically, a CT is considered a heterogenous and dynamic structure, containing at least two subdomains: one is the DNA with later interphasic replication, which is locates nearby the nuclear envelope, whereas the earlier – replicating DNA occupies a more

central position inside each CT, toward the center of the nucleus, where it projects (Robinson et al., 2003).

A number of microscopic studies show that chromatin fibers present different folding modalities inside the CTs, according the type of cell they belong.

For instance, in plants, the chromatin fibers have large loops, that are mutually associated at their base; in higher eukaryotes (e. g. mammals) the fibers appears to be folded into many interconnected and distinct megabase – sized domains, which could represent a functional unit each; finally, some observations suggest the existence of a hybrid model of chromatin organization (Meaburn and Misteli, 2007).

One of the most accepted conception about the molecular organization and the functions of CTs chromatin states that each CT have an internal, more condensed, high – orderdered chromatin fibers region (*chromatin domain*) and an outermost, thin (up to 200 nm) layer of loose chromatin, in contact with the IC, named *perichromatin region* (PR) (Fakan and van Driel, 2007). EM evidences, together with other observations, suggest that the chromatin fibrils located in the PR play a major role in supporting the DNA processing and the gene expression: the DNA replication (Jaunin and Fakan, 2002), the DNA repair (hypothetical) (Solimando et al., 2009), the DNA transcription (which generates pre – mRNA trancripts of single genes under RNA Pol II catalysis) and the cotranslational splicing (by means of the splicing factors provided by the splicing speckles located nearby PRs, in the proximal IC (Cremer and Cremer, 2010).

Besides the *CT – IC model*, other models concerning the architecture and functions of nuclear chromatin were elaborated; these conceptions emphasize or disagree more or less the idea of chromosome territories.

For example, based on modern imaging techniques, (especially on electron spectroscopic imaging (ESI) a *„lattice" model* for the organization of chromatin in the interphase mammalian cells was proposed (Dehghani et al., 2005). The authors of this model afirm that the chomatin fibers of 10 and 30 nm in diameter form an extensive network that holds a distributed interchromosomial space (IC), which lack chromatin.

According to the *interchromatin network model* (ICN) (Branco and Pombo, 2006), the CTs are not so clearly physically separated one to another; the chromatine fibers (or loops) from one CT, or from neighboring CTs, intermingle and can mutally contact each other (intrachromosomal and interchromosomal contacts). The degree of intermingling between specific chromosome pairs could influence the frequency of chromosome translocations in a particular cell type (e.g. the human lymphocytes). The interchrosomal associations via the interchromatin network, conditioned by transcriptional events, is important for chromosomes organization in mammalian cells.

Another model (based on existence of CTs) affirms that genes present on chromatin loops formed at the surface of neighboring CTs, which deeply penetrate inside the separating IC, are colocalised at this level in order to ensure their expression or coregulation (Fraser and Bickmore, 2007). These sites are rich in transcription factories (Pol II), speckles containg splicing factors and also other regulatory elements which interact with genes *in cis* or/and *trans* positions.

Besides this models, other models consider that interphase chromatin could form giant loops which expands throughout the nuclear space; some giant chromatin loops could transport genes to repressive nuclear compartments situated at great distance from their origin (Alberts et al., 2008).

None of the structural and functional chromatin organisation models in eukaryotic cells could be considered as ideal, because the lack of strong experimental evidences supporting one of them particularly. Clarifications in this respect could be made by using different experimental methods, such as: the chromatin polymers, which can reveal important aspects of active nuclear architecture and the combination of focused ion beams with high resolution SEM for 3D analysis of chromosome architecture (Schroeder - Reiter et al., 2009 cited by Cremer and Cremer, 2010).

As mentioned above, the interphase nuclear chromatin has a high - ordered three - dimensional structure, essential in supporting the DNA functions (DNA replication, RNA transcription and processing).

In higher eukaryots, especially (mammals), a key role in setting and regulation of chromatin organization was assigned to *boundary* or *insulator elements* (Labrador and Corces, 2002). According to this concept, highly condensed chromatin regions alternate with open active chromatin domains. Because of the presence of boundary/ insulator elements, the chromatin of interphasic nuclei adopts a rosette-like (or flower) spatial structure; within it, the chromatin loops (open nucleosomal strands) occupie an external, functional position, whereas the highly condensed chromatin regions (heterochromatin domains, which also could contain inactive insulators) are located in a central position; the active insulators are located at the limit of these regions, separating them. During development, or in a particular tissue, a portion of central chromatin could transform into an open loop, by activation of the appropriate boundary element, while an active external domain becomes heterochromatic, by inactivation of its corresponding insulator.

The **interchromatin (interchrosomal) compartment** (IC) represents the totality of channel – like spaces situated between the heterochromatin blocks in interphasic nuclei (according to the CT – IC model). It could be considered a dynamic and very active nuclear compartment, strongly related, both structurally and functionally, with the cromosomes territories.

The IC contain a large diversity of nucleoplasmatic components (subnuclear bodies, protein complexes, heterogenous RNA molecules, newly formed DNA molecules).

Due to its molecular composition and based on the present knowledge, the IC could be conceived as a compartment where the essential functions of a nucleus and, in particular, the genetic information are expressed (DNA replication, RNA synthesis and processing). For example, as was previously shown, the RNA molecules are transcribed at different sites of perichromatin fibrils, in IC, and these transcripts (pre - mRNA) are further modified by the splicing factors present in the interchromatin speckles.

The IC play also an important role in the assembly of various molecules in functional units, for instance the rRNAs which form ribonucleoproteins (RNP) particles with different proteins (ribosome subunits) (Kee Brouwer A., 2010).

Because their direct connection with the nuclear envelope pores, many of the products processed and packaged within the IC could be transported through this space to the nuclear pores and exported in cytosol. This transport, together with the active movement of some components in order to fiind their targets (e.g. histones, DNA repair enzymes, hormone receptors) highlights the role of the IC in trafficking molecules with high efficiency (Robinson et al., 2003). All these functions make the IC to be a very crowded and active space.

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