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Review

Symmetry applied to nuclear microanatomy: a review of gene function and cell differentiation

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Summary. The purpose of this paper is to review current knowledge and understandings of gene control and cell differentiation, based upon an appreciation of a possible role that nuclear microanatomy and considerations of steric symmetry might play.

Metaphase sister chromatids have identical base codes but show a mirror image symmetry of higher order coiling. Chromosomes in the interphase nucleus have spatially well defined domains and are anatomically distinct and ordered. Chromosomes are known to have interactions i.e. sex chromosome inactivation, PEV etc

An hypothesis of gene activation is made based on steric interactions among chromosomes and between chromosomes and activating and repressor proteins. These interactions may be influenced by the handedness of higher order chromatid coiling, since homologues show mirror-image symmetrical coiling in metaphase, which might be retained to a certain degree in interphase. This may result in a binary switching of genes.

All possible combinations of chromatids in the interphase nucleus, would be enabled by a differential segregation of homologous chromatids at mitosis. To conserve patterns of interchromatid interactions, there must be a programmed segregation of chromatids towards one of the two spindle pole attachments. This orientation might be effected by preferential attachment of microtubules to kinetochore attachment sites, by steric hindrance of the kinetochore by condensed chromatin which initially allows only unidirectional tubule attachment, or possibly by a tethering of interacting chromatids which would migrate en masse.

An attempt to apply this hypothesis to some illustrative pathological conditions is made.

Key words: Cell diferentiation, Chromosomes, Mitotic spindle

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Introduction

The great advances in the understanding of gene function and differentiation were facilitated by the breath-taking technological advances in molecular biology and information technology. The discoveries of mechanisms of gene functioning, of the multitudes of promoters and repressors and their involvement in human disease conditions have overshadowed the newly gained understanding of chromosomal microanatomy and its application to the above knowledge. Until the application of confocal ultramicroscopy and in situ hybridization techniques (i.e. FISH), very few anatomical insights could be gained about chromosomal microstructure. To many biologists and investigators, the nucleus was regarded as little more than a reaction vessel for the interactions of nucleotides and their associated enzymes and cofactors.

The purpose of this review is to correlate newly gained knowledge of chromosomal microanatomy with present knowledge of gene functions and genetics.

Chromosomal spatial domains in the interphase nucleus

With classical light microscopy chromosomes are only discrete, and identifiable during mitosis. During interphase however, light microscopy of the nucleus only reveals nucleoli, and clumps of heterochromatin. Visser et al. (2000) examined BrdU-labeled chromatids by immunoelectronmicroscopy after several generations. They found that the labeled chromatin formed distinct domains, with occasional areas of contact between adjacent chromosomes. Abney et al. (1997) used photobleaching of fluorescently labeled chromatin to demonstrate the relative immobility of chromatin, which implied that the chromosomes are attached to underlying nuclear substructures. By plotting intranuclear positions of FISH signals for nine different q arm markers, Sun et al. (2000) concluded that there was a size dependent intranuclear organization of chromosomes within the interphase nuclei of cultured human fibroblasts, with the

q arm telomeres of large chromosomes more peripherally located than the telomeres of smaller chromosomes. A relationship of gene function with chromatin organization has been claimed by Cremer et al. (2000). This is supported by immunolabelling of nascent RNA and FISH labeling of cultured HeLa cells (Verschure et al., 1999).

Physical and functional attachments are described (Pfaller and Newport, 1995; Ye and Worman, 1996; Vladimirskaya et al., 1999) of chromosomes to the nuclear envelope and easily discerned intranuclear structures such as the nucleoli (Chubb et al., 2002). As well, Barboro et al. (2003) propose the attachment of chromosomes to a nuclear frame consisting of lamins stabilized with RNA and nuclear mitotic apparatus protein (NuMa), which determines functional domains and higher order organization of chromatin (Gotzman and Foisner, 1999). Weipoltshammer et al. (1999) has demonstrated telomeric but not centromeric attachments to the nucleoskeleton. The nuclear matrix is thought to be pivotal in the functional organization of chromatin, allowing for the attachment of transcription factors and enabling chromatin remodeling by histone-DNA and histone-histone interactions (Stein et al., 2000). Associations have been made between the nuclear matrix and gene regulation and their interactions with intranuclear cytoplasmic intermediate filaments (Traub 1995; Tolstonog et al., 2001, 2002).

Spatial order of chromosomes in the interphase nucleus and in mitosis

In addition to chromosomes occupying discrete intranuclear loci i.e. “domains”, evidence has accumulated that they also evidence preferred spatial relationships to each other. In plants, yeasts, and diptera, interphase chromosomes have the Rabl configuration i.e.telomeres at one nuclear pole and centromeres at the opposite pole (Marshall et al., 1996, 1997; Leitch, 2000). In human and murine cells, specific genes occupy constant positions relative to each other, irregardless of transcriptional activity or cell differentiation (Nagele et al., 1999; Parada and Misteli, 2002; Parada et al., 2002). Some investigations have found that genes occupy constant radial positions between the nuclear center and the nuclear membrane (Parreira et a1., 1997). Ferguson and Ward (1992) and Kozubek et al. (2002) have described a centromeric orientation at the nuclear membrane of human cells. Both studies failed to show evidence of homologue pairing. The studies of Skalnikova et al. (2000) and Nogami et al. (2000) also found peripheral centromeric orientation in human blood cells, with genes and early replicating domains situated centrally in the nucleus. Kirsch-Volders et al. (1980) measured association tendencies of chromosomes and found preferential associations for pairs of autosomes at both centromeres and telomeres. Tanabe et al. (2002) have found that the chromosomes of both human and chicken cells show similar radial positions with large chromosomes peripherally located and smaller ones more central. Lukasova et al. (2002) have shown that human lymphocyte chromosomes have a nonrandom three dimensional structure, with highly expressed genes found close to the nuclear center, on the inner sides of the chromosomal territories. The findings of Cremer et al. (2001), are consistent with the above, but find some differences in the positioning of chromosomes (number 18 and Y) between the ellipsoid nuclei of human amnion and fibroblast nuclei and the spherical nuclei of human lymphocytes. Sadoni et al. (1999) have demonstrated in human cultured fibroblasts, a higher order compartmentalization of polar chromosomes with gene replication times and hence gene function corresponding to mitotic chromosomal banding patterns. From their pulse labeling experiments, they concluded that sister chromatids are mitotically segregated in an apparent random manner. Alcobia et al. (2000) used centromeric chromosomal probes and confocal microscopy to study centromere associations in human blood cells and fibroblasts. While they demonstrated specific associations of centromeres especially the acrocentrics with nucleoli, they also found tissue specific association patterns. Manders et al. (1999) pulse labeled living cultured Indian Muntjac cells and followed specific labeled foci through the cell cycle. They concluded that ”the gross structure of an interphase chromosome territory is directly related to that of the prophase chromosome”. Furthermore, Nagele et al. (1999), found that “some chromosome homologues of diploid cells exhibited a preferred intranuclear position that may correspond to the spatial order of chromosomes in rosettes of mitotic cells”. A study of chromosome positioning of post-mitotic daughter cells suggested that chromosome positioning was dependent upon their configuration at the preceding mitosis (Sun and Yokata, 1999). This conclusion was supported by the findings of Gerlich et al. (2003), who also suggested that the specific timing of sister chromatid separations transmits chromosomal positions from one cell generation to the next.

Many studies reveal that chromosomal order is also maintained during mitosis. Coll et al. (1980), using Giemsa-banding chromosomal identification techniques, found that the chromatids show symmetrical orientation on the mitotic plate which in their text figures, appear not to show "mirror-image" symmetry, but form identical arrays of haploid sets which show “head to tail” orientation on the mitotic plate. Recently, Klein et al. (1998) have shown that chromosomes maintain a nonrandom and symmetrical order during mitosis. In contrast, Allison and Nestor (1999) have found that human chromosomes show a random but symmetrical distribution on the mitotic ring and describe symmetrical chromatid position in daughter cells. Nagele et al. (1995), describe an "antiparallel disposition" of the two haploid sets on the mitotic rosette, which is consistent with Coll et al’s findings (1980).

Symmetry of chromatids

Evidence for mirror image symmetry can also be found in the topology of sister chromatids. Boy de la Tour and Laemmli (1988) observed mirror symmetry of sister chromatids in metaphase chromosomes. Using electron tomography of metaphase nucleolar organizer regions, Heliot et al. (1997) described the same phenomenon. Using FISH probes, Baumgartner et al. (1991), described mirror symmetry of fluorescent spots on sister chromatids. Ushiki et al. (2002), using atomic force microscopy, demonstrated the symmetrical coiling of sister chromatids, as well as the existence of bridging fibers between Q-bands of sister chromatids. Stack and Anderson (2001) have dealt with the theoretical implication of the above findings in some depth. Goradia and Davis' investigation (1978), entitled "Asymmetry in sister chromatids of human chromosomes" deals with nonsymmetrical patterns of G-banding of chromatids, rather than with the "handedness" of chromatin coiling. The chromosome scaffold is thought to provide the template for the spiral structure of mitotic chromosomes (Hirano, 2000; Maeshima and Laemmli, 2003), but no explanation for the mirror-image symmetrical coiling could be found.

Changes in chromatin structure related to gene activity and cell differentiation

Epigenetic mechanisms (Hendrich and Willard, 1995) play a central role in gene silencing and involve methylation of DNA and histone deacytelation (Lee and Chan, 2001; FASEB Meeting Report, 2001; Maison et al., 2002; Richards and Elgin, 2002). As well, various protein factors such as heterchromatin protein 1 (Kellum, 2003) bind to DNA, alter the chromatin coiling structures, as well as gene expression. Transcription factors may alter chromatin coiling and affect gene activity (Lundgren et al., 2003). A link between alterations in chromatin structure, the binding of transcription factors to the nuclear matrix with RNA polymerase II, and gene activity is discussed by Stein et al. (1999). There is current interest in the BAF (SW1/SNF) family of chromatin remodeling proteins which are thought to allow access of transcription factors to DNA (Kassabov et al., 2003; Katsani et al., 2003; Wang, 2003). Olave et al. (2002) discussed the role of actin intermediate filaments in the chromatin remodeling complexes. Histone octamers and DNA topoisomerase I and II form the chromosomal scaffold (Sumner,1996), contribute to DNA supercoiling and accessibility, and to the spatial accessibility of interaction sites (Caserta and diMauro, 1996). In addition to epigenetic mechanisms, gene expression is affected by mutations and is protected from silencing by "insulators" or boundary elements (Bell and Felsenfeld, 1999; Burgess-Beusse et al., 2002).

The actions of the above-mentioned chromatin-modifying factors have been related to cell differentiation and embryonic development (Renard, 1998; Cunliffe, 2003). Spatial position of a gene relative to other chromosomal elements has also been linked to gene activity (Bridger et al., 2000). In human differentiated cells, both homologous and some nonhomologous chromosomes show pairing and close spatial associations (Chandley et al., 1996) which show tissue specific patterns (Alcobia et al., 2000). Gene inactivation has been linked to its distance from centromeric heterochromatin (Bartova et al., 2002) and has been presented as an example of epigenetic silencing. TIF1, a transcriptional intermediary factor involved in cell differentiation concentrates in the pericentromeric heterochromatin of embryonic cells induced to differentiation by retinoic acid (Cammas et al., 2002). The retinoic acid receptor itself is silenced by DNA methylation and histone deacetylation (Sirchia et al., 2002). Beil et al. (2002) describe "a global remodeling of interphase chromatin territories" in leukemic cells which have undergone retinoic acid-induced differentiation.

Evidence of chromosome-chromosome interactions

The above discussions are essential in the development of a thesis for a programmed, nonstochastic mechanism for interchromosomal interactions. It appears that chromosomal domains are discrete intranuclear

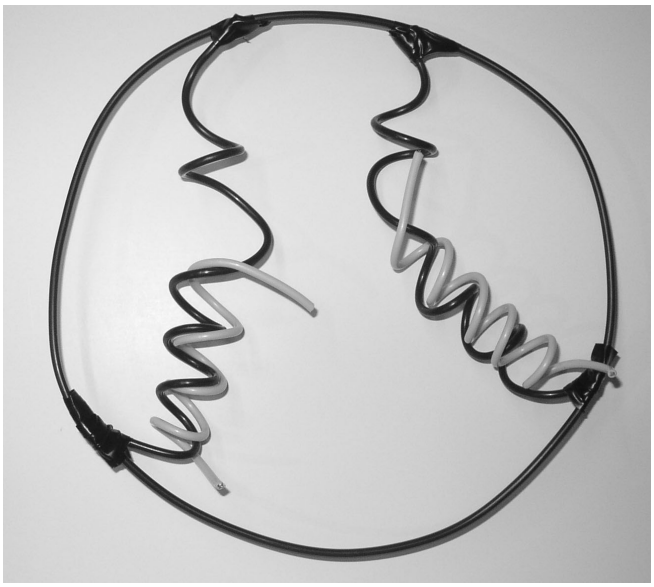

Fig. 1. An interphase nucleus: The black circle represents the nuclear membrane to which a pair of homologue chromosomes are attached at their telomeres and show mirror-image symmetrical coiling. The white coiled structures represent adjacent chromosomes with identical "handedness" of coiling. The pair of black and white chromosomes on the left show identical "handedness" and the white coil is inserted with close apposition into the black one, and suggests that chromosome interactions can occur. The pair of chromosomes on the right show opposite "handedness" in coiling and cannot be apposed.

territories which appear to have preferred intranuclear locations which are perpetuated through cell division into succeeding generations. It is natural to question whether neighbouring chromosomal territories evidence interactions. If this is indeed true, then it is apparent that the degree of chromatin supercoiling and the direction of its symmetry (i.e. left or right-handed coiling) will be important in determining any interactions with neighbouring chromosomes (Fig. 1). The cited findings of chromatid mirror-image symmetry have been described in metaphase chromosomes which evidence "tight" winding and compaction. The visible presence of heterochromatin in interphase nuclei is evidence for the retention of some chromosomal superwinding. Parada et al. (2002), cite the retention of G-banding regions in interphase nuclei.

Some evidence of these interactions in interphase cells have been described at length-Position Effect Variegration (PEV) in Drosophila (Qumsiyeh, 1995; Dernburg et al., 1996; Sabl and Heniloff, 1996; Dorer and Henikoff, 1997; Carvalho et al., 2001; Csink et al., 2002), transvection, wherein phenotypic effects of an allelic mutation are masked by its homologue (Wu and Morris,1999) (Mattick and Gagen,2001), chromosomal silencing from one parent in interspecific hybrids i.e. "nucleolar dominance" (Lewis and Pikaard, 2001) (Sullivan et al., 2001), human X chromosome inactivation i.e. "dosage effects" (Carrel et al., 1999; Bailey et al., 2000; Willard and Carrel, 2001) , transgene imprinting of X inactivation (Monk and Grant, 1990; Migeon et al., 1999; Willard and Carrel, 2001), and translocations involving spatially proximate chromosomes (Kozubek et al., 1999; Neves et al., 1999; Marshall, 2002).

Mitotic chromatid segregation

Previous studies of chromatid separation and segregation have designated this process as a random one. The labeling experiments of Zink et al. (1998) and Neff and Burke (1991) revealed the presence of both labeled and unlabelled chromatids in daughter nuclei. Because sister chromatids are assumed to have identical genetic codes, their random distribution would have no biological significance if their segregation patterns imparted no information. Present theories of inherited differentiation are based on transmission of epigenetic factors such as histone acetylation (Jeppesen, 1997) and methylation as well as associationed RNA and other proteins such as HP1 (Kellum, 2003)

The thesis proposed in the preceding discussion attributes informational significance to the "handedness" of chromosomal coiling, even when there is a degree of unraveling in interphase. Adjacent portions of spiraled chromosomes cannot insert their supercoiled helix and interact with chromosomes showing opposing "handedness". These interactions based on symmetry of adjacent chromosomes can be thought of as binary switches. At least 2^23, (8,388,608) combinations of spiraled chromosomes are possible. In order for specific combinations of interacting chromosomes to be inherited by succeeding cell generations, chromatid segregation must be non-random. The mechanism which effects this programmed segregation is necessary for the existence of multicellular organisms with cellular differentiation, and is probably not present in bacteria or yeasts.

In addition to the previously discussed symmetry in chromatid structure, Goradia and Davis (1978) described different G banding patterns among human sister chromatids. In contrast, Morris (1977) found no evidence of non-random segregation of sister chromatids. Bekaert et al. (2002) describe different telomere lengths of sister chromatids in human lymphocytes. These findings reinforce the view that sister chromatids are not identical.

Non-random segregation may be effected by physical attachments of interacting chromosomes which retain their associations, even through replication and migration. Avivi and Feldman (1980) cite many investigations describing tethering or connections of chromosomes in plants.

A more likely mechanism would propose differential spindle affinities for sister centromere attachment. As well, there may be qualitative differences in the centrioles which result in preferential kinetochore attachments. Jablonka and Jablonka (1982) theorized that each chromatid contained a directional code which was either masked or unmasked by differences in origin of DNA replication forks. Sullivan et al. (2001), in their review cite evidence supporting an epigenetic role in determining centromere sites. Differing chromatid configurations might conceivably be associated with different centromeric functionality. Van Hooser et al. (2001) suggest that epigenetic factors might assist in establishing the centromeric site and play a role in kinetochore plate assembly. McEwan et al. (1997) in their study of kinetochore fiber attachment describe a sequential attachment of kinetochore fibers to sister chromatids, with one chromatid usually showing twice the number of attached fibers than the leading sister kinetochore. Taylor et al. (2001) describe assymetrical localization of the spindle checkpoint component BubR1 – the kinetochore with the weaker BubR1 signal is usually closer to the nearest spindle pole. At present however, inspite of the above mentioned findings, no strong experimental evidence exists yet for programmed chromatid segregation.

One could suppose that microtubule attachment is opportunistic with attachment of microtubules from one centriole attaching to the most proximal kinetochores of chromatid pairs during anaphase. The consequences of such a segregation would be the formation of daughter cells showing mirror-image symmetry of their respective chromatids. Interchromosomal interactions would still occur within each daughter, but there is no programmed segregation in this model. The segregation of chromatids would be determine by the relation of the spindle axis to the axis of homologue symmetry within the anaphase

nucleus.

Conclusions

Much evidence exists for the existence of ordered and preferred chromosomal territories in interphase nuclei. As well, evidence exists for interactions of interphase chromosomes. It appears that sister chromatids in mitosis show mirror image symmetry and that interphase chromosomes may retain some of the higher order organization seen in mitosis. Very weak evidence exists for programmed separation of chromatids.

The above two concepts, i.e. steric interactions of adjacent chromosomes in the interphase nucleus, and programmed chromatid segregation would explain not only normal differentiation and development but some pathological conditions.

Chromosomal translocations are thought to occur between spatially adjacent chromosomes. In balanced Robertsonian translocations, the translocated chromosomal segments presumably remain in close apposition and interact in the same manner. Unbalanced translocations remove portions of chromosomes from their associated interacting neighbour, thereby inducing lack of repression /activation of genes. Parada et al. (2002) have shown in benign and neoplastic mouse cells that two translocated chromosomes are positioned in close proximity to each other. In cancer-related syndromes like Bloom's syndrome and Fanconi's anemia, the rate of sister chromatid exchanges is markedly increased over normal (Hojo et al., 1995). The insertion of chromosomal segments with chromatin coiling opposite to the normal at that site would cause disruptions of any postulated interactions and steric loss of control for gene expression. Similarly, in aneuploidies of neoplasia, genes on extra chromosomes would not be controlled by steric modulation.

Dedicated to the memory of my brother, Dr. W.P. Bell, *zichrono l'bracha*.

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