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Review

Structure and function of the nucleus: anatomy and physiology of chromatin

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Abstract. A review of the literature accumulated recently on nuclear structure and function reveals that: (1) The nucleus is the interphase form of chromosomes (chromatin organizes and compartmentalizes the nucleus). (2) These organizational programs are morphogenetic in nature and are regulated by both DNA content and by epigenetic interactions. (3) In mammals with a diploid complement, it is very likely that chromosomes construct interphase domains based on their structural milieu (including any imprinted areas). These are the same structured areas that correspond to G- and R-bands with their varying DNA content and early versus late replication. (4) Changes in a position of a segment of DNA from one chromatin environment to another changes its availability to early replication factors and transcription factors as well as its nuclear positioning and chromatin architecture. This process was first described as positional effect variegation in *Drosophila* but is now found to be more general and explains many cases of direct clinical relevance. Examples in mammals include spreading of X inactivation, imprinting and changes in chromatin associated with chromosome translocation. (5) Chromosomal autoconstruction and reconstruction into a functional nucleus are altered during cell cycle and during differentiation (much more work needed on this area).

Key words. Nuclear structure; chromatin; epigenetic modifications; chromosome rearrangements; imprinting; interphase chromosomes.

Introduction

nucleus (Brown 1831)—a membrane-enclosed cell organelle which represents one of the two main compartments of the eucell and contains the bulk of its genetic information (nuclear DNA) in the form of chromatin. (From: Rieger et al., *Glossary of Genetics*, 5th ed., 1991, Springer-Verlag, Berlin).

Research on nuclear structure is as old as the suggestion over 110 years ago that chromosomes occupy specific domains in the interphase nucleus [1, 2]. However, the nucleus remains far more complicated and misunderstood than any other cellular organelle, and only in the past decade have advances in technology allowed us to make significant progress in understanding the structure and function of chromatin in the interphase nucleus. This is not intended as a review of chromosome structure and function; excellent summaries of these areas abound (Wolffe [3] as an example). Rather, I will provide a selective review that supports new models based on the ideas that chromosomes and chromatin orchestrate an interphase nuclear environment that regulates gene activity not only during the cell cycle but also in diverse processes from differentiation to malignancy. In other words, I review how chromatin changes its environment to produce the functioning structure we call an interphase nucleus.

A problem of terminology

Much of the literature in this field uses terminology that keeps us thinking in terms of distinct objects (chromosomes, subnuclear compartments, nuclear matrix etc.) affecting or regulating each other. Thus, we use such statements as 'the nucleus contains chromosomes' or 'chromosomes attached to the nuclear envelope.' Chromosomes are the building blocks as well as the builders for the nucleus (they form structures and function). Nuclear formation following metaphase is essentially a morphogenetic process, and chromatids can be thought of as prenuclei [4–6]. We should then realign our thoughts to think of the nuclear structures as byproducts of chromosomes (i.e. the nucleus is just an interphase form of chromatin). After all, chromosomes do acquire many new proteins for each stage of the cell cycle (including interphase and metaphase). This remodeled chromatin at interphase *is* the nucleus with its various compartments. I would prefer that we think in terms of chromosomal assembly and remodeling into functional nuclear or chromatin structure (i.e. nuclear assembly) or alterations of chromosomal states to create various functioning entities. These include chromosomal compartments (CCs), subchromosomal domains (SCDs), and interchromosomal compartments (ICCs). We are then able to address the dilemma articulated by Singer and Green [7] of which came first 'in the nucleus': the concentration of transcription and splicing factors or the gene activity (recruiting such factors into compartments). Recent work on one transcription regulator (yeast GCN5p) suggests that it functions in collaboration with other molecules as a histone acetyltransferase and thus alters nucleosomal (chromatin) conformation [8]. Histone acetylation plays a central role in chromatin accessibility and transcriptional activity [9]. Since all such transcription factors bind specific segments of DNA, we can think of DNA as the ultimate builder of its own functioning environment (the chromatin state). Of course, there are certain situations where DNA states can be modified by external factors (e.g. imprinting by methylation of CpG residue), but then these modified DNA sequences will build the correct structural chromatin milieu. We review several lines of evidence that demonstrate the ordered complexity of chromatin and factors that influence chromatin function in its metaphase and interphase states.

Chromosomes organize distinct domains in the interphase nucleus

The seminal observations of Rabl and Boveri [1, 2] clearly suggested a nonrandom arrangement of chromosomes when the nucleus is reconstructed following division. An ordered chromosomal structure is visible in microscopic examinations of the ultrastructure of the nucleus [10]. With the advent of fluorescence in situ hybridization (FISH) it became possible to examine the positions of specific chromosomes in interphase, further confirming an ordered nuclear structure [11–14]. Even in somatic cell hybrids, it appears that genomes and chromosomes are allotted certain domains [15, 16]. Mathematical models based on data from chromosome exchanges following irradiation also support a confinement of chromosomes to domains in the interphase nucleus [17]. The segregation of chromosomal territories in the interphase nucleus is even detected in living cells [18, 19]. In three-dimensional (3D) reconstruction of G0 rat lymphocyte nuclei, bodies of chromatin are most condensed at the nuclear envelope, and there were 22 such domains approximating the haploid chromosome number [20]. This suggested that chromosomes are paired somehow in these interphase nuclei. The possibility that heterologs (chromosomes that are not homologous) form clusters is not excluded.

Chromosome painting and 3D reconstruction provide some additional data to support a domain organization for each chromosome. However, this is not to suggest a fixed chromosome orientation in the interphase nucleus. A repositioning of the centromeres from the periphery towards the nuclear center occurs with cell cycle events in human [21] and mouse [22] lymphocytes. In mouse lymphocytes, 65% of the centromeres are found in the outer 50% of nuclear volume at G1, but this percentage drops significantly as centromeres reposition to the interior of the nucleus from S to G2 [22]. Human lymphocytes show 85–100% of centromeres on the nuclear periphery, and these reorganize to the interior earlier in the cell cycle from G1 to S phase [21]. In *Drosophila*, chromosomes maintain a Rabl orientation (polar orientation of telomeres and centromeres) for less than 2 h following mitosis and then reorient while maintaining heterochromatic areas at the nuclear periphery [23, 24]. Work with pulse labeling of living cells demonstrated that subchromosomal foci in interphase (of about 400–800 nm) exhibit slight changes in both position and size [19]. Changes in chromatin distribution have also been documented during other development and differentiation events such as during chick embryo chondrogenesis [25] and during myogenesis in rat L6E9 cells [26]. Nuclear and chromosomal changes accompany and could induce differentiation [27]. As an example, reverse transformation of a malignant fibrosarcoma cell line results in clustering of the acrocentric chromosomes in association with the nucleolar periphery [28]. Different cell types in the same organism may carry different chromosome positioning in interphase suggesting a possible role in differentiation [29–34]. These data and calculations based on electric

charge differences and viscosity values [35] suggest that chromosome territories are important functional and structural components of interphase chromatin.

Functional subchromosomal domains in interphase related to those of metaphase

There are several reported subnuclear organizational centers which have (or are presumed to have) defined specific functional and structural correlates:

1) *Nucleolus*. The nucleolus represents a distinct subnuclear structure which may provide (at least partially) a model for other nuclear compartments. In humans, the nucleolus as a compartment is organized by a subset of the chromatin localized at the stalks of acrocentric chromosomes (areas named nucleolar organizer regions, NORs). The position of the short arms containing NORs of the acrocentric chromosomes appears to be highly organized forming the nucleolus [36]. NORs contain the ribosomal RNA coding genes. In the interphase nucleolus, these genes form a fibrillar center with the regulatory and nucleoprotein complexes including RNA polymerase I, class I transcription factors and topoisomerase [37–41]. This center is surrounded by a dense component that includes nascent transcripts and associated processing machinery and this in turn is surrounded by a granular area which includes mature 28S and 18S ribosomal RNA (rRNA) as well as various stages of ribosome assembly (reviewed in [41]). Electron microscopic studies suggest that the heterochromatic regions are located on the periphery of the nucleolar mass [42, 43]. FISH studies confirm a peripheral location of the centromeric repeats at the outside of the nucleus with stalk elements inside the nucleolus and rDNA sequences located at the periphery [44] (fig. 1A).

2) *Coiled bodies*. While first described in 1903 [45] as nucleolar accessory bodies, coiled bodies are now known to include small nuclear ribonucleoproteins, nucleolar DNA and proteins, and a peculiar protein called coilin [46, 47].

3) *Gems* (*Gemini of coiled bodies*). A novel class of nuclear structures occurring near coiled bodies, contain the 'survival of motor neuron' proteins but are otherwise of unknown function [48].

4) *PML oncogenic domain (POD)*. This is a dense fibrillar ring occurring in normal cells but is fragmented in promyelocytic leukemia (PML) [49, 50]. Structural disintegration of the POD accompanies the classic translocation 15;17 that produces a fusion protein containing PML and retinoic acid receptor α [51–53].

5) *Perinucleolar compartment (PNC)*. This structure contains several RNAs transcribed by RNA polymerase III including RNAase P, multi-drug resistant protein RNAs and multiple Y RNAs as well as a polypyrimidine tract-binding protein [54, 55].

Besides these five specific subnuclear structures, the chromatin is organized in specific fashion into SCDs. The first identification of an order in chromosomal DNA was the recognition that *Drosophila* chromosomes have bands and chromatin loops that correspond to actively transcribed and more repressed domains [56–58]. Similarly, mammalian chromosomes are divided into distinct areas with correlated transcriptional and structural differences: G-bands, R-bands and T-bands [59–62]. R-bands (G-band negative regions) are GC rich, replicate early in S phase, have a higher concentration of CpG islands, high gene concentration and high transcriptional and recombinational activities [62–65]. In contrast, G-bands (R-negative) are AT rich, replicate late in S phase, contain more repetitive DNA and have low gene concentration (mostly tissue specific genes). It is likely that waves of replication correspond to the structural components of the chromosome, that is R- and G-bands [66]. The presence of expanded repeats in the genome can delay replication [67]. In general, spatial variations in chromatin structure as seen in metaphase is related to replication and transcription [63].

The patterns of G- and R-bands along the length of the metaphase chromosome, when decondensed in interphase, are maintained and are also clearly related to both replication and transcription. Transcribed genes occur in early replicating regions of the genome, areas of chromatin that are generally more decondensed in interphase [68, 69]. While each chromosome occupies a discrete domain [70, 12], the G-chromatin of that particular chromosome is more condensed and localizes on the nuclear periphery and the perinucleolar area, while the R-chromatin areas are more diffuse and localize to the interior and at the periphery of chromosomal domains (fig. 1A). Evidence for these structural and functional relationships come from recent FISH studies. Active genes localize in the periphery of the respective chromosomal domains [35, 71, 72]. These transcribed areas will also recruit a high concentration of splicing factors to their locations between chromosomal domains [73]. A subset of the R-band positive areas is the so called T-bands for telomeric bands. These are usually areas that are over 100–300 kb proximal to the telomere and they are GC-rich, suggesting that they have a high gene concentration [64]. Figure 1A summarizes results of many experiments and is a simplification of this nuclear substructure. It is based on the now well-documented correlation between structural elements of the metaphase chromosome and structural and functional elements of the interphase form of the chromosome. Let us explore these concepts further.

Even at metaphase, chromosomes begin to attract lamin proteins, which would form the mesh of proteins at the inner surface of the nuclear membrane [4]. All SCDs including telomeres are attached to the nuclear matrix

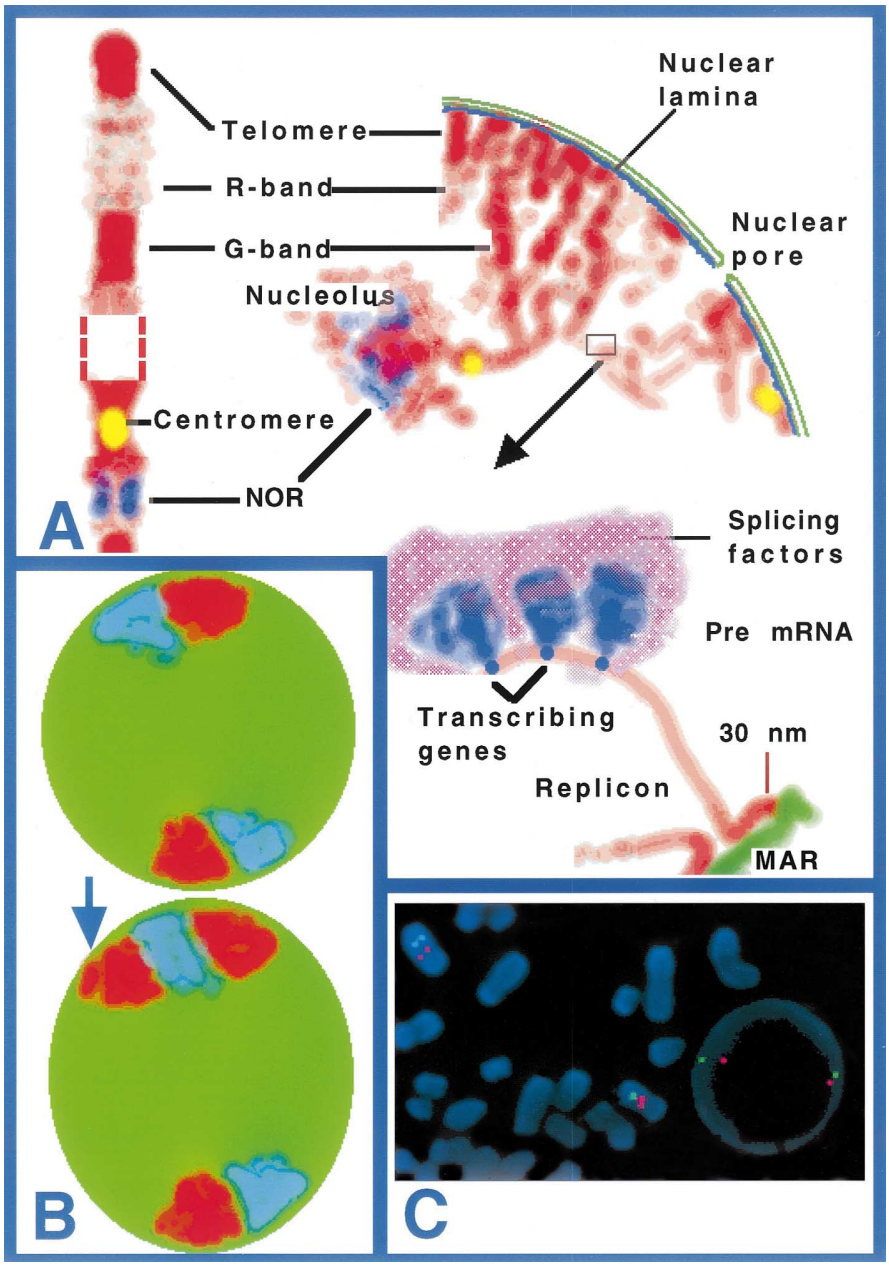

Figure 1. (*A*) Consensus model of nuclear structure and function relationship. Top left shows a partial representation of an acrocentric chromosome (short arm pointing down) at prometaphase. Chromosomes reconstitute the nucleus at telophase by using specific subchromosomal domains (SCDs, e.g. telomeres, and heterochromatic regions) to attract lamins, nuclear matrix and other nuclear components. Some SCD (e.g. R-bands) are important in constructing the inter- and intrachromosomal compartments (ICCs) which are a network of areas in the nucleus for transcription and mRNA processing (insert) and are linked to the nuclear pores via channels. The functioning genes in those areas are in extended DNA loops of 100–200 kb likely representing replicons. The transcribed genes are thought to occur in the decondensed fibers (11 nm), whereas the untranscribed repressed chromatin is in more condensed fibers (30 nm to 100–200 nm). This explains the difference microscopically between areas of condensed chromatin (e.g. at the nuclear periphery and just outside the nucleolus) and more open areas (at the chromosome domain periphery). Modified from various sources [41, 43, 98, 171–174]. (*B*) A potential model for the impact of an extra chromosome or chromosome segment (such as in a translocation) on nearby chromosomal and subchromosomal domains. Extra material or repositioned chromatin in translocations shown by an arrow in the nucleus at bottom could cause changes analogous to those seen with the *Drosophila trans* position effects of $Brown^{Dominant}$ mutation. See text for detail. (*C*) FISH using two probes on the long arm of chromosome 7 (green and red) at metaphase and interphase. Note parallel orientation at interphase of both homologues.

[74]. However, only a small subset of these SCDs organize and are attached to the nuclear lamina (nuclear envelope). Telomeres have a unique peripheral organization in interphase [75–77]. Since they must recombine with each other to maintain homogeneity of telomere sequences, it is possible that clusters of nonhomologous telomeres occur in compartments at the nuclear periphery [78]. Constitutive heterochromatic regions also form SCDs that are preferentially organized, locating to (and perhaps essential for constituting) the nuclear periphery and the perinucleolar domains [79–81]. SCDs with active transcription (R-bands and T-bands) organize internal nuclear compartments for gene transcription [82]. As an example, the gene ERBB-2 is localized on the surface of the chromosome 17 domain in interphase nuclei in a DNase-hypersensitive domain facing the nuclear periphery [83]. The data support the concept that chromosomes and SCDs build unique spatial relationships with other SCDs when constructing the nucleus at telophase (see fig. 1A). In a separate section I will discuss how such organization could directly impact function (gene expression) and how it may be altered in certain situations (chromosome abnormalities, differentiation etc.).

The published data collectively support a close structure-function relationship of both metaphase and interphase chromatin at the visible microscopic level. The structures are also related at the submicroscopic level. In examined eukaryotic nuclei, loops likely representing a single replicating unit (replicons) are attached to the nuclear matrix at areas called matrix attachment regions (MARs) or scaffold attached regions (SARs). These MARs are found both in mitosis and in interphase. They contain topoisomerase II, DNA polymerase α and primase as well as other structural and functional proteins and function in replication as well as in transcription [84–92]. The latter studies taken together support a model of chromatin structure whereby chromatin fibers are attached to MARs which regulate both replication and transcription of genes in units representing the replicon (fig. 1A, insert).

RNA processing along compartmentalized inter- and intrachromosomal domains in the interphase nucleus

The nature and function of the 'spaces' between chromosomes and those between subchromosomal domains is debated. There are specific nuclear compartments for transcribed DNA and for RNA synthesis and processing [93–96]. It is also becoming clear that areas of active nuclear transcription release their RNA into distinct interchromatin granule clusters (IGCs) [97] or interchromosomal compartments (ICCs) [98]. These areas can also form 'channels' along which RNA is transported out of the nucleus [35, 98–100] ICCs also extend to the interior of chromosome domains [87]. There is a correlation between the positions of certain genes, their RNA products and splicing factors (as measured by anti SC-35 antibodies) [101]. Basically, active genes are at the edge of a compartment of splicing factors of which pre-messenger RNA (mRNA) forms only a smaller portion (in some genes only coincident with the transcribing DNA). By contrast, inactive genes are not related in their position to the common SC-35 domains and were shown by numerous works to be more interior in the chromosome domain [35, 98]. Since there are about 50 SC-35 domains [102], clearly each domain must encompass multiple active genes. A summary of these findings is illustrated in the insert in figure 1. From an evolutionary standpoint, one can assume a selective advantage for this model based on the relative conservation of diffusible factors controlling gene expression and splicing factors as well as in shielding inactive genes from potential reactivation.

Dynamic genetic and epigenetic changes in chromatin state and gene activity

Although much more work is needed, numerous studies have documented the effect of chromatin state on gene transcription both in cis and in trans. The best examples of these effects are in position effect variegation (PEV) in *Drosophila*. Basically, PEV occurs when genes normally expressed in euchromatic areas are translocated to areas with heterochromatin or vice versa, resulting in variegated (mosaic) expression patterns. While these genes are intact, their expression suffers significantly if they are not in their correct 'chromatin environment', and such altered chromatin environment changes gene expression [103–105]. Most earlier work on PEV dealt with the effects of the translocation on gene expression in the vicinity and on the same chromosome (i.e. in cis) [106–111]. More fascinating are the newer data on position effect variegation in trans (on other genes not colinear with the disrupted gene). The best example of this is the *Brown* (*bw*) eye mutation called 'dominant.' The mutated allele results from an insertion of a large block of heterochromatin into *bw*. The curious result is that the expression of the normal homologous allele is effected in a variegated pattern. Detailed cytological investigations showed that this is explained by heterochromatic associations of the bw^D with centromeric heterochromatin and bringing in the wild-type allele to associate with this repressive heterochromatic complex at the nuclear periphery [23, 24, 112, 113].

The data on PEV make sense in light of our understanding of the relationship between nuclear positioning, chromatin assembly and disassembly, and

transcription. Transcription requires nucleosome displacement [114] and thus a loose chromatin environment. It is also known that replication-coupled chromatin assembly can inhibit basal transcription [115]. Chromatin decondensation is needed during differentiation to accommodate transcription and vice versa [116–120]. Experimental evidence in *Saccharomyces* shows that perinuclear localization of chromatin facilitates transcriptional silencing [121]. There are genes that regulate chromatin condensation and PEV in *Drosophila* [122, 123] and those that regulate chromatin condensation in mammals [124]. It is becoming apparent that PEV and other forms of chromatin changes can be considered epigenetic modification but are also directly and intimately effected by mutations and chromosome rearrangements both in cis and trans. Even earlier recognition of the impact of epigenetic chromatin modification came from studies of X chromosome 'Lyonization' (inactivation) in the XX female. The inactive X chromosome replicates later than the active X chromosome, and many genes are inactivated on the inactive and condensed X chromosome and are thus functionally hemizygous in the female. Late replication appears to be a prerequisite step for X inactivation but is then followed by CpG island methylation and histone H4 deacetylation, which stabilizes the inactivated region [68, 69, 125–128]. The inactivation process involves expression of Xist initially solely from the paternal X chromosome and suppression of the maternal Xist gene (maternal X active). Then, at the morula stage, the parental imprints are erased, and a mechanism counting X chromosomes is initiated that results in random X inactivation [129]. The organization of the inactive X in the interphase nucleus is unique and is formed by telomere association to form the Barr body [130]. While roughly equivalent in nuclear total volume, the active X is more elongated with a larger surface area and shows much less condensation and less methylation [35, 131]. In X-autosome translocations, the chromatin environment of inactivation can spread from the X segments to several G-bands of the translocated autosome [132].

Other epigenetic changes in chromatin structure were reported. Carcinogens can alter chromatin states by epigenetic mechanisms involving DNA methylation without affecting the sequence [133]. Methylation induces acetylation and is a very strong modifier of chromatin structure [134–136]. As any cytogeneticist and pathologist knows, cancer cells have altered chromatin shape in both metaphase and anaphase. Cells undergoing senescence can also have significant chromatin reorganization [137]. It is interesting to note that telomere lengths can increase in cancer cells and are decreased in senescent cells, suggesting a possible role for telomeres in these chromatin changes.

Genetic factors that lead to chromatin changes include GC content and other factors that regulate formation of R-, G- and T-bands at metaphase as discussed above [63]. There are many other mutational changes that can lead to different chromatin configurations. An example of interest is that the expansion of the CTG triplet repeats in myotonic dystrophy is believed to increase assembly of nucleosomes and thus to a repressed chromatin configuration [138]. This is similar to the observation in *Drosophila* that expansion of transgene repeats leads to heterochromatin formation and gene silencing [103]. Other studies in humans and mice show that certain sequences (e.g. CD2 locus control region) operate by altering the chromatin environment, thus insuring an open reading frame [139]. Experimental approaches to reconstituting active and inactive chromatin states in vitro are at their infancy, but it is clear that coding sequences recruit proteins that can alter the nearby chromatin environment [81]. These factors likely explain why some rearrangements cause human disease even when the breakpoints are far (few to hundreds of kb) from the target gene [140]. While the chromatin environment is correlated to transcriptional states in many (perhaps most) cases, there are some conflicting data (perhaps exceptions to the rule). Experimental evidence, such as DNase sensitivity, suggests that differential chromatin states can be maintained through both meiosis [141] and mitosis (see data discussed above) despite the suppression of transcriptional activities. Further, the male X chromosome becomes inactivated (facultatively heterochromatized) during meiosis but reactivates at the blastocyst stage in all tissues except trophoectoderm in mammals [142].

Genomic imprinting provides another cogent example of regulation of gene expression by chromatin environment and epigenetic modifications. There are many mammalian autosomal genes that are now known to have normal expression from only one homolog (paternal or maternal) but not both. Mutation or abnormality of these genes show different phenotypic effects depending on the chromosome involved (paternal or maternal). A review of imprinting is beyond the scope of this paper, but there is some evidence that the silencing of some imprinted genes is likely explained by models involving a protein factor(s) that regulates transcription by affecting chromatin structure [143–145].

Chromosome abnormalities and interphase chromatin states

Chromosome rearrangements are clearly deleterious in many situations. Rearrangements in a heterozygous state, even when balanced, can cause a direct phenotypic effect on the individual and can also negatively

affect reproduction (a phenomenon known as negative heterosis). The direct phenotypic effects of rearrangements are numerous and well illustrated. Somatically acquired chromosome rearrangements could cause cancer, reproductive problems and development of mosaic conditions with an abnormal phenotype. Constitutional chromosome rearrangements could cause arrested development, fetal loss, growth retardation and/or congenital anomalies [146]. It is not surprising thus that evolution favored development of numerous mechanisms that reduce the rate of chromosome aberrations including [147] (i) increased efficiency of DNA repair, (ii) nuclear architecture including chromosome domains, (iii) increased nuclear size in the gametocytes, (iv) chromatin organization and (v) asynchrony of DNA replication.

Despite these mechanisms, a high incidence of chromosome abnormalities clearly remains, at least in humans [148, 149]. Rearrangements cause reduction in fertility in some situations but not others [150]. This is a rather complicated area of study, but clearly negative heterosis is affected by the type of rearrangement, the species involved and possible other factors [151]. Negative heterosis must be overcome if a rearrangement is to be fixed to a homozygous condition in a population. This can and does happen when a rearrangement has a selective advantage that outweighs its distinct harmful effects in meiosis and reproduction.

The mechanisms by which chromosome rearrangements exert an effect on the phenotype are varied. Clearly, balanced translocations in cancer lead to fusion products or gene regulation changes (e.g. overexpression of certain genes) that have a direct impact on cellular proliferation. In the case of deletions, duplications, trisomies and monosomies, a gene dosage effect can also be involved. However, these two mechanisms (gene regulation at the translocation breakpoint or dosage effects) probably do not explain all cases. Other involved mechanisms include gene interactions, imprinting and/or position effects [16].

As discussed above, each chromosome constructs and occupies a specific compartment including its attendant inter- and intrachromosomal (sub)compartments. The effect of translocations on chromatin configuration and thus gene expression is now well established. Position effect variegation was discussed earlier, but it is not the only example of translocations or chromosome abnormalities leading to chromatin changes and to gene dosage effects. Expression of a growing list of human genes causing disease was found to be affected at a distance by chromosome translocations [152, 140]. In fact, an electron microscopic examination of translocated human chromosomes does reveal structural aberrations detected at metaphase [153]. More dramatic data on such translocation-induced genetic changes at a distance are provided by X-autosome translocations, whereby X inactivation spreads into the translocated autosomal segment [154]. Such an open or repressed chromatin state can be maintained through cell division and differentiation [155, 158]. Other chromatin changes such as the somatic pairing of homologs of chromosome 4 in *Drosophila melanogaster* causes gene suppression [156]. Chromosome position also effects the expression of foreign genes in transgenic animals [157]. As discussed earlier, the effects of rearrangements can cause gene silencing both in cis and trans arrangements.

In diploid organisms each autosome has a homologs and the organization of the homologs can change in cells with aneuploidy (e.g. disomy or monosomy) or structural abnormalities. Recent work using FISH suggests that in normal undifferentiated diploid cells, homologs can be arranged symmetrically on either side of the interphase nucleus [158, 159]. We observed similar patterns in lymphocytes following in situ hybridization (fig. 1C as an example). We had asked the question of the impact of aneuploidy or structural chromosome aberrations on this arrangement. For aneuploidy, our preliminary data, both in lymphocytes with trisomy 18 [16] and polymorphonuclear cells in trisomy 13 [160], showed destabilization of the symmetric arrangement. In the case of trisomy 13, we believe that the extra chromosome is responsible in a structural sense for producing the so-called nuclear projections in the segmented mature neutrophils. For structural abnormalities, some cases of both balanced and unbalanced translocations seem to destabilize nuclear architecture and result in formation of micronuclei [16]. Trisomy 21 patients show loss of the extra 21 with aging [161]. We recently studied a case of maternally inherited balanced translocation between chromosome 7 and 8 in a child with multiple congenital anomalies. The mother (who is phenotypically normal) had a different nuclear organization for these chromosomes than child. This finding, albeit in only one family, supports the mechanism I proposed earlier [16] for the manner in which a balanced rearrangement could produce a phenotypic effect.

Taken together with the data on PEV both in cis and trans, these data suggest that aneuploidy and structural chromosome abnormalities can impact gene expression not only of the affected chromosomes but also of nearby chromosomal regions (fig. 1B). These long-range positional effects, so well documented in the *Drosophila* genome (see discussion above for $Brown^{dominant}$ mutations), are just beginning to be applied to human genetic diseases. Thus, chromosome rearrangements impact nuclear architecture, can destabilize the nucleus predisposing to additional rearrangements and can impact nearby gene expression in cis and trans. Conversely, nuclear architecture can itself predispose to certain rearrangements. A good example of this phenomenon is the

proposed origin of Robertsonian translocations in humans because of the facilitation of proximity at the nucleolar sites [162]. Another example is cited for the repeated establishment of isochromosome 17q in certain cancers [163]. In mammalian evolution, karyotypic orthoselection whereby a lineage can acquire many rearrangements of a particular type [16, 150, 151] may be explained by nuclear position effects. Other examples reported include the predisposition to additional genetic events in individuals with specific abnormalities and the acquisition of 'suites' of particular chromosome rearrangements in cancers following the presumed initial cancer genetic change [16]. Thus, there is an intertwined dynamic relationship between chromosome structure (including rearrangements) and function (including gene regulation and karyotypic evolution).

Outlook and future issues to address

There has been extensive growth of this field of chromatin structural and functional relationships. I had attempted to give a brief entry into this complex area of investigation. It is an area that is bound to see significant growth in the next few years and to have direct impact on both basic genetics and clinical science. While we generally attempt to provide simple models as explanations for the data generated (i.e. parsimony), there are many problematical areas, and the models provided must remain as tentative ideas pending further data. Much more needs to be done to understand chromatin changes affecting transcription, and many questions are raised by the available data.

(1) The nucleus is the interphase form of chromosomes (chromatin organizes and compartmentalizes the nucleus). These organizational programs are morphogenetic in nature and are regulated by both DNA content and by epigenetic interactions as reviewed above. However, the many factors involved in transforming the linear DNA sequence (two-dimensional) to produce the three-dimensional patterns of chromomeres, bands and sub-bands remain to be elucidated [119, 164].

(2) In mammals with a diploid complement, it is very likely that chromosomes construct interphase domains based on their structural milieu (including centromeres, telomeres, bands, heterochromatin and any imprinted areas). Their function and effect on somatic divisions and reconstruction of the interphase nucleus was discussed. Much more remains to be learned about these structures and their impact on cell cycle events and in development. In particular, it would be very important to do more research on the impact of changes in chromatin structure (telomeres, centromeres, bands, translocations etc.) on meiosis [165] and recombination.

(3) We can demonstrate the impact of rearrangements on chromatin environment both in cis and in trans. Changes in a position of a segment of DNA from one chromatin environment to another changes its availability to early replication factors and transcription factors as well as its nuclear positioning and chromatin architecture. This process was first described as positional effect variegation in *Drosophila* but is now found to be more general and explains many cases of direct clinical relevance. Examples in mammals include spreading of X inactivation, imprinting and changes in chromatin associated with chromosome translocation. Much more needs to be learned about how meiotic events (such as crossing over and susceptibility to nondisjunction) are affected by these rearrangements [166–168].

(4) Most current work on chromatin deals with undifferentiated or dedifferentiated cells [14]. More work is needed on a variety of differentiated cells and on how changes in cellular states in general (differentiation, transformation etc.) affect chromatin reorganization and what the significance of such changes is [22, 27, 169].

(5) Chromosomal autoconstruction and reconstruction into a functional nucleus are essentially dynamic morphogenetic processes apparently impacted by stage of the cell cycle, degree of differentiation, and by chromosome abnormalities and epigenetic factors. This area is a fertile field of research that could potentially explain mental retardation and other developmental problems in patients with specific chromosome abnormalities [16].

The study of human anatomy certainly had a long history of development before functional (physiologic) relationships could be established for the various components (organs and tissues). Similarly, recent technological advances such as three-dimensional fluorescence microscopy [170] are very promising in linking structural (anatomical) to functional (physiological) aspects of interphase chromosome states. This understanding will be crucial to many areas of clinical laboratory medicine as well as to basic research in genetics (including gene therapy).

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