

## Chapter 2

# Interphase Chromosome Behavior in Normal and Diseased Cells

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**Abstract** Interphase chromosomes are nonrandomly positioned in the nuclei of normal cells. They occupy specific locations with respect to a radial distribution from the nuclear edge to the nuclear interior. Furthermore, there is some evidence that interphase chromosomes reproducibly have the same neighbors that can be involved in creating translocations which lead to cancer. Not only are chromosomes nonrandomly positioned but they are anchored to certain regions of the cell nucleus by cellular structures such as the nuclear lamina and the nucleolus. Global screening of the genome has identified both lamina-associated domains and nucleolar-associated domains. Increasingly, researchers are finding that interphase chromosomes are mislocalized in disease situations. The consequences of chromosome mislocalization are not yet that clear, but gene expression can be affected with interphase chromosomes being located in another compartment of the nucleus, changing

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their interactions with nuclear structures. This chapter outlines how chromosomes behave in interphase nuclei and with what they interact. We discuss many examples of when chromosomes, and the genes housed upon them, change their location and behavior in disease situations such as cancer and the premature aging syndrome called Hutchinson–Gilford progeria syndrome. We also describe new findings whereby genes in the host are relocated and expressed after a parasitic infection.

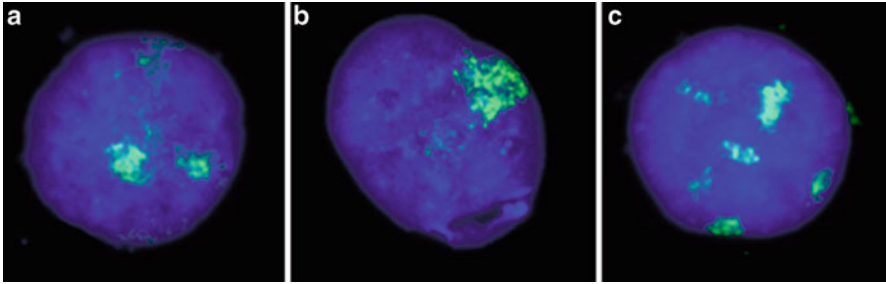
## Introduction

The eukaryotic cell nucleus is a highly complex organelle that contains the cell genome in the form of interphase chromosome territories. Interphase chromosomes are described thus because as they decondense, after cell division within the new daughter nuclei, they remain in and interact with a particular area of the nucleus, a so-called territory of the nucleus. Although there is some intermingling and ‘coming together’ of genes from different chromosomes, most of the body of one chromosome is maintained together, and thus to all intents and purposes chromosomes in interphase are separate entities. The interphase chromosomes are nonrandomly positioned in nuclei, revealing that there must be a high level of genome reorganization post mitosis to obtain individual chromosome territories in the right compartments of the nucleus (Fig. 2.1).

The structures within nuclei are intimately involved in organizing and positioning interphase chromosomes to allow the coordination of a wide range of functions centred around the genome, such as gene expression and silencing, splicing and processing, and DNA replication and DNA repair. These nuclear structures are all linked and are part of a functionally responsive cellular network (Starr 2009). Such architecture comprises the nuclear envelope with all its components: integral membrane proteins (IMPs) and the nuclear lamina, nucleoli, the nucleoskeleton, and a range of nuclear bodies (Foster and Bridger 2005). All these structures interact with and anchor interphase chromosomes. Misorganization or disruption of this nuclear architecture can lead to problems in regulating normal chromosome behavior, producing compromised cells with the possibility that diseases such as cancer or degenerative syndromes may arise.

## Nuclear Structures

The most prominent subcompartment of the nucleus is the nucleolus. The nucleoli are where ribosomal RNAs are synthesized and processed, thereby providing a site for efficient assembly of ribosomal subunits. In humans the acrocentric chromosomes containing the ribosomal repeat genes are embedded in the nucleoli, providing a functional anchorage site for these genes and their chromosomes (Bridger et al. 1998). tRNA genes are also clustered at the nucleoli (Boisvert et al. 2007; Nemeth et al. 2010). Furthermore, other chromosomes that do not contain ribosomal DNA or tRNA genes are also associated with nucleoli (Bridger et al. 1998).



**Fig. 2.1** Chromosome territories in interphase nuclei. Individual territories for human chromosome 10 (*green*) have been delineated using two-dimensional (2D) fluorescence in situ hybridization (FISH). (a) Normal immortalized human dermal fibroblast interphase nucleus. (b) Immortalized Hutchinson–Gilford progeria syndrome fibroblast nucleus. (c) Nucleus from a transformed cell derived from a breast tumor. Note that the nuclei in (a) and (b) contain only two territories whereas (c) displays many territories of chromosome 10 that are derived from ploidy and translocations

In genome-wide screens, two studies have revealed many sites throughout the genome that are anchored at the nucleoli, including chromosome 17 (van Koningsbruggen et al. 2010) and chromosome 19 (Nemeth et al. 2010). Both these chromosomes have been found to be interiorly located and associated with nucleoli in extracted nuclei (J. Bridger, unpublished data). These studies demonstrate that the nucleolus is a major player in anchoring and organizing chromosome territories in interphase nuclei.

The interior of a nucleus is thought to be more conducive to transcription of active genes, whereas a correlation has been shown between gene repression and positioning at the periphery (Zink et al. 2004). In yeast the nuclear periphery has been shown to consist of two distinct compartments: a region permissive to transcription near the nuclear pore complexes, and a repressive region that contains foci of silencing factors (Andrulis et al. 1998; Taddei et al. 2006). Components of the nuclear periphery, such as nucleoporins and lamin proteins, are thought to interact with repressors of transcription. For example, emerlin interacts with the transcriptional repressors germ cell-less (GCL) and barrier to autointegration factor (BAF) (Holaska et al. 2003), and the nuclear envelope protein LAP2 $\beta$  interacts with HDAC3 to cause histone H4 deacetylation and gene repression (Somech et al. 2005). Genes can become anchored at the periphery of the cell, which affects their local chromatin environment. For example, genes that become tethered to the nucleoporin Nup2p are blocked from becoming heterochromatic and therefore remain active, whereas tethering of telomeres to other nucleoporins results in gene silencing (Ishii et al. 2002; Feuerbach et al. 2002).

The nuclear envelope is made up of the inner and outer nuclear membranes, which house nuclear pore complexes, and the nuclear lamina. The inner nuclear membrane, facing the nuclear interior, contains a large number of IMPs (Trinkle-Mulcahy and Lamond 2007; Gomez-Cavazos and Hetzer 2012). By proteomic analysis, at least 67 IMPs have been identified. The better known IMPs are lamin B

receptor, lamin-associated polypeptides 1 and 2, emerin, MAN1, and nesprins (Schirmer et al. 2003). Many of these IMPs have chromatin/DNA-binding capabilities and are believed to be involved in anchoring interphase chromosomes to the nuclear periphery (Zuleger et al. 2011). Furthermore, the nuclear envelope components can be very different in different tissue types (Korfali et al. 2012), which could explain why different areas of the genome become localized to the nuclear envelope in different cell types (Peric-Hupkes et al. 2010).

The nuclear lamina, found subjacent to the nuclear membrane, consists of type V intermediate filament proteins nuclear lamins A, B, and C and is known as a structural scaffold under the nuclear envelope, which provides mechanical strength. There are three mammalian lamin genes: *LMNA*, *LMNB1*, and *LMNB2*, encoding at least six proteins. *LMNA* encodes four alternatively spliced mRNAs for lamin A, A $\Delta$ 10, and C1 and C2 proteins, which are called A-type lamins. *LMNB1* encodes lamin B1, and the lamin B2 mRNA can be spliced to yield B2 or B3 proteins. The presence of lamins A and C is limited to differentiated cells; however, lamins B1 and B2 are expressed in all cell types both in adults and in embryos. Furthermore, expression of certain lamin proteins such as C2 is restricted to the testis and during meiosis, whereas lamin B3 exists only in oocytes and spermatozoa (Rodríguez and Eriksson 2010). Lamin proteins have DNA/chromatin-binding abilities but also bind to a number of the IMPs of the nuclear membrane. Thus, there are a plethora of sites at the nuclear periphery for interphase chromosomes to bind and be anchored. A large study, in which the human genome was probed for lamin B-binding sites, revealed 1,300 lamin-associated domains (LADs); many of these LADs were found to be in gene-poor regions of the genome (Guelen et al. 2008). Interactions with the nuclear lamina are associated with gene silencing and repression markers such as H3K4 dimethylation (Ferrai et al. 2010), increasing evidence for the idea that the periphery of the nucleus is associated with gene repression. Moreover, the dysregulation of expression of both types of nuclear lamin has been correlated with cancer and degenerative disease (Butin-Israeli et al. 2012), including neurological degeneration (Coffinier et al. 2011).

The movement of chromatin in the nuclei appears to be largely constrained and thus reflects the physical attachment of chromatin to nuclear compartments, such as the nucleolus, nuclear periphery, and nucleoskeleton. Individual chromosomes occupy discrete compartments, and therefore distinct genomic regions localize to specific subnuclear positions. From several studies, it is becoming evident that nuclear position may have a crucial role for gene regulation. Moreover, it has been shown that there is a strong correlation between transcriptionally silent, late-replicating chromatin and a nuclear peripheral localization in several model systems (Boyle et al. 2001; Andrulis et al. 1998). Fluorescence recovery after photobleaching (FRAP) studies on mammalian cell nuclei indicate that in time periods of more than 1 h, chromatin becomes immobile over distances greater than 0.4  $\mu$ m. Chubb et al. demonstrated that nucleoli and the nuclear envelope constrain the motion of interphase chromosomes that are located at these nuclear structures. In addition they demonstrated that the mobility of chromatin not associated with nucleoli or the nuclear periphery was much less constrained (Chubb et al. 2002).

## Interphase Chromosome Positioning

We have been discussing how gene-poor regions of the genome are associated with the nuclear periphery. These data come from sophisticated global screening experiments. This distribution of more inactive areas of the genome at the nuclear periphery fits with earlier studies whereby whole gene-poor chromosomes were found to be located at the nuclear periphery. The first of these studies was performed by Bickmore and colleagues and demonstrated the differential distribution in interphase nuclei of the similarly sized chromosomes 18 and 19 (Croft et al. 1999). Chromosome 18, a gene-poor chromosome, was located at the nuclear periphery whereas chromosome 19, a gene-rich chromosome, was located in the nuclear interior. This gene density-correlated chromosome positioning in interphase nuclei was confirmed for all chromosomes in proliferating lymphoblastoid cells (Boyle et al. 2001). For human fibroblasts this gene density distribution is found in proliferating cells and not in nonproliferating cells (Bridger et al. 2000; Meaburn et al. 2007a, 2008; Mehta et al. 2007, 2010). Nonproliferating cells display a size-correlated distribution with large chromosomes toward the nuclear periphery and smaller chromosomes in the nuclear interior. Thus, when doing chromosome positioning studies it is critical to know whether the cells are proliferating. This point is especially important when comparing transformed and immortalized cancer cells with primary control cells, which will have a greater proportion of nonproliferating cells in the culture or tissue section. We use immune detection of the proliferation marker Ki-67, commonly used in neoplastic diagnostics (Kill 1996). The nuclei with very bright staining are in the proliferative cell cycle, and negative nuclei or nuclei with very dull staining are nonproliferating and are either quiescent or senescent. It is important that the cells with very dull staining are not counted as positive because this will lead to misinformation about proliferative status. In a primary culture of fibroblasts the maximum number of proliferating cells is usually never more than 65 %, and this is for the youngest of cultures. Therefore, pKi67 is a very important marker to use in chromosome positioning assays, but it must be analyzed correctly. During the past decade there have been a number of studies that have compared chromosome territory position between cancer cells and suitable control cells. However, very few of these have taken into account proliferative status.

We have found that individual chromosome territories change location in the cell nucleus when primary fibroblasts change proliferative status (Meaburn et al. 2007a; Mehta et al. 2007, 2010), meaning that some specific chromosomes are relocated whereas some stay where they are. Indeed, when cells are induced to become quiescent by serum starvation, interphase chromosomes either remain where they are, such as chromosome X, or move from a peripheral location to a more interior location, such as chromosomes 13 and 18, or move toward the nuclear periphery, as, for example, chromosome 10. We have shown that energy is required for the movement of these chromosomes, and nuclear motor proteins actin and nuclear myosin I $\beta$  are involved. Others have also found that nuclear motor proteins are involved in chromatin relocation in the nucleus (Chuang et al. 2006; Dundr et al. 2007; Ondrej et al.

2007, 2008a, b). The idea that chromatin and chromosomes are translocated around the nucleus by nuclear motor proteins is a relatively new area of study for nuclear biologists, and as yet very little is known about the distribution and mode of action of the nuclear motor proteins themselves. Our studies have shown that NM1 $\beta$  is found throughout the nucleoplasm, with a concentration around the nuclear envelope and nucleoli in proliferating cells (Bridger and Mehta 2011; Mehta et al. 2010). It is extremely likely, given the importance and reproducibility of chromosome and gene positioning, that motor proteins involved in repositioning chromosomes and chromosomal subregions could be altered in disease states and cause issues for gene regulation. Indeed, we have observed in nonproliferating cells that NM1 $\beta$  distribution is very different, with large aggregates of the protein deep within the nucleoplasm. A similar distribution is apparent in cells derived from patients with the premature aging disease called Hutchinson–Gilford progeria syndrome (HGPS) (Mehta et al. 2011). Little or no research has been performed assessing nuclear myosins in cancer; however, one study correlated the presence of nuclear myosin VI with prognosis in renal cancer (Ronkainen et al. 2010) and another with nuclear myosin 18b in ovarian cancer (Yanaihara et al. 2004).

## The Link Between Interphase Chromosome Location and Gene Expression

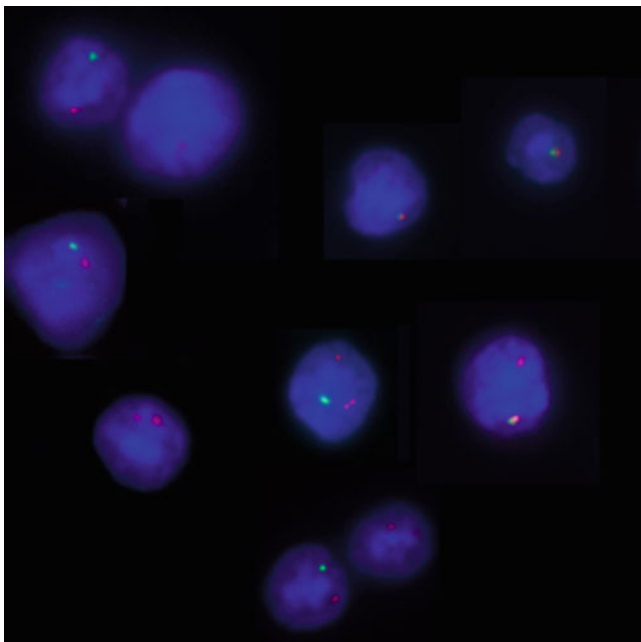
There is now evidence supporting the hypothesis that nuclear location of a chromosome and/or gene could play a role in regulating specific gene expression. For example, when resting human lymphocytes are activated by phytohemagglutinin, changes result in the intraorganization of chromosome territories, both in the degree of intermingling between territories and in their volume. More importantly, however, the radial positioning of the chromosome territories is changed. This alteration has been postulated to be a response to an altered transcriptional program (Branco et al. 2008). Furthermore, during *ex vivo* stem cell differentiation into adipocytes, the radial position of important genes involved in adipogenesis altered dramatically, with genes that become switched on when moving from the nuclear periphery toward the nuclear interior and back again when switched off. Control genes in this system that were either on or off did not respond to the adipogenic growth factors and did not change location (Szczerbal et al. 2009). In this differentiation system, there was little whole chromosome movement, but genes were looped out from chromosomes into the nuclear interior to associate with the nuclear structure SC35 speckles (Szczerbal and Bridger 2010).

Other studies have gone further, to identify where in the nucleus and to what nuclear structures the genes are targeted. Genes have been found to relocate to structures associated with active transcription and processing of RNA. Indeed, the activation of gene loci can involve a repositioning of genes toward areas of the nucleus where RNA polymerase II molecules aggregate into superstructures called transcription factories (Osborne et al. 2007). Other studies have shown genes

becoming associated with other structures. For example, Dundr et al. inserted an artificial U2 snRNA array into the genome of cells and demonstrated that the array moved toward a stably positioned Cajal body for transcription through long-range chromosomal relocation. This movement was inhibited by an actin inhibitor, implying the involvement of actin in interphase chromosome repositioning (Dundr et al. 2007). Other studies have shown genes increasingly associated with SC35 domains upon upregulation (Brown et al. 2008; Szczerbal and Bridger 2010). It has also been shown that repositioning of genes from the periphery to a more interior position can correlate with inappropriate activation of that gene. The formation of chromatin loops for expression from repressed chromatin territories has been suggested as a mechanism of genome regulation, for example, for *Hox* gene activation (Chambeyron and Bickmore 2004). Indeed, most excitingly recent 3C (Ferraiuolo et al. 2010) and 4C conformation capture experiments have shown that actively transcribing *Hox* genes in a cluster are associated with a nuclear compartment for active transcription and that the nontranscribing genes are all located at a region where gene silencing occurs (Noordermeer et al. 2011). When the silenced genes become activated, they then co-compartmentalize with the other active *Hox* genes. This strict co-compartmentalization of genes explains the strict co-linearity rules associated with the *Hox* gene clusters where position in the cluster is correlated with the expression zone down the developing embryo.

The three-dimensional (3D) structure of the chromosomes within the territories also plays a major role in the control of gene expression. Regions of the chromosomes have been shown to interact with other regions of the same chromosome, in *cis*. For example, the locus control region (LCR) of the  $\beta$ -globin gene cluster acts as an enhancer of the  $\beta$ -globin genes, although it is more than 50 kb away. However, the LCR has been shown to be in close physical proximity to an actively transcribed HBB gene, suggesting a direct regulatory interaction (Carter et al. 2002; Tolhuis et al. 2002). This looping in 3D forms an active chromatin hub (ACH) for control of the expression of the  $\beta$ -globin genes (de Laat and Grosveld 2003), which dynamically associate with the LCR (Gribnau et al. 1998). As T-helper cells differentiate from naïve, uncommitted CD4-positive T cells, they show a transcriptional switch. Initially, the cells transcribe low levels of both Th1- and Th2-specific loci and regulators, but as they develop they become committed to either the Th1 or Th2 program (for review, see Murphy and Reiner 2002). Once a lineage has been established, it is retained as a heritable trait. This process of lineage commitment and differentiation involves the physical repositioning of regulators of gene expression. For example, it has been shown that during Th1 differentiation, the *GATA-3* and *c-maf* loci, which encode upstream regulators of Th2 cytokines, were progressively repositioned to centromeric heterochromatin and/or the nuclear periphery and repressed (Hewitt et al. 2004). These findings demonstrate another level of interphase chromosome behavior on gene expression, that is, that the intraorganization of a chromosome territory is also important (Fig. 2.2).

Noncoding RNAs (ncRNA) can control gene expression by establishing local repressive regions. For example, the *Air* ncRNA sets up a local ‘cloud’ of RNA that accumulates at the promoter of the imprinted *Slc22a3* gene and silences it by



**Fig. 2.2** Active genes can coassociate within the interphase nucleus. A montage panel of RNA FISH experiments demonstrates that the *Hbb-b1* gene loci (*green*) and the *Hba* gene loci (*red*) localize together more than 20 % of the time when actively transcribing. This interaction, shown in embryonic day 14.5 mouse erythroblasts, occurs although they are located on different chromosomes and demonstrates the preferential interaction of coregulated genes within the nuclear volume

recruiting G9a, an H3K9 histone methyltransferase (Nagano et al. 2008). The imprinted *Kcnq1* locus is also regulated by a paternally expressed repressive ncRNA, *Kcnq1ot1*, which regulates a domain of up to 750 kb. However, local activation of genes may be able to overcome the regional silencing effects of ncRNAs, as *Kcnq1* transitions from monoallelic to biallelic expression during the development of the heart, and there have recently been shown to be both tissue- and stage-specific chromatin loops between the *Kcnq1* promoter and newly identified DNA regulatory elements (Korostowski et al. 2011). The most notable example of ncRNAs silencing genes is X-chromosome inactivation, where the ncRNA *Xist* silences an entire chromosome. The *Xist* ncRNA covers the chromosome that is going to inactivate and condenses into a smaller, compact structure, which is associated with the periphery (Clemson et al. 1996). As silencing is established, a repressive nuclear compartment forms that excludes RNA polymerase II and transcription factors. Transcriptional repression follows the formation of this compartment, possibly as genes become physically pulled down into the repressive environment, where they are inaccessible to the transcriptional machinery (Chaumeil et al. 2006). The few genes that remain expressed, for example, those in the pseudoautosomal region, loop out of the repressive compartment to be expressed (Splinter et al. 2011).



## ***Interphase Chromosome Behavior in Hutchinson–Gilford Progeria Syndrome***

Hutchinson–Gilford progeria syndrome (HGPS) is a severe premature aging disease that affects children. First described by Jonathan Hutchinson and Hastings Gilford in the 1800s, this disease is recognized by a group of characteristics indicative of premature aging. The most common of these include alopecia (hair loss), failure to thrive (short stature and low weight), lipodystrophy (loss of fat), scleroderma of the skin, and increased visibility of blood vessels. Initially, children appear unaffected, but symptoms usually present around 1 year of age, leading to a mean age of diagnosis of 2.9 years. Another main characteristic of HGPS is heart disease. Patients suffer from atherosclerosis/hardening of the arteries, which is sometimes associated with calcification. This change, in combination with loss of smooth muscle from blood vessels, leads to an increased risk of heart attacks and stroke. These are the main causes of death in this disease, with the average life expectancy of a HGPS patient being 13.5 years (Hennekam 2006).

HGPS is an extremely rare disease with an incidence of approximately 1 in every 4–8 million live births. Of these cases approximately 80 % are caused by the same de novo mutation in the *LMNA* gene (De Sandre-Giovannoli et al. 2003; Eriksson et al. 2003). This gene encodes both A-type lamins, making HGPS part of a group of diseases known as the laminopathies. The ‘classic’ mutation found in the majority of HGPS patients is the G608G mutation, which is a silent mutation at the protein level. At the DNA level, however, it causes activation of a cryptic splice donor site, which results in an interstitial deletion of 150 amino acids from exon 11. This deletion gives rise to a truncated protein, with a 50-amino-acid deletion, called Progerin (Eriksson et al. 2003). Pre-lamin A and progerin are subject to the same posttranslational modifications. The region deleted in progerin contained an important cleavage site for the enzyme ZMPSTE24, which removes the farnesylated N-terminus of the protein, freeing it from the membrane. Lacking the cleavage site, progerin therefore remains bound to the nuclear membrane. Interestingly, homozygous mutations in the ZMPSTE24 gene have also been found to cause an atypical form of HGPS. Progerin expression is thought to have a dominant negative effect on cell function; it has been shown to cause thickening of the nuclear envelope as well as nuclear shape abnormalities such as blebs and invaginations (Goldman et al. 2004; Bridger and Kill 2004).

The association of the nuclear lamina with the chromatin and chromosomes increased interest in genome organization and chromosome/chromatin localization in cases of disruption to the nuclear lamina through mutation in *LMNA*, such as that seen in HGPS. Genome organization has been shown to be disrupted in a number of cells with lamin A mutation or that lack lamin A completely (Galiova et al. 2008; Shimi et al. 2008; Taimen et al. 2009). Further, three studies have revealed mispositioning of whole chromosome territories in cells with *LMNA* mutations (Meaburn et al. 2007a; Mewborn et al. 2010; Mehta et al. 2011). Interestingly, using chromosome 10 positioning that had previously been shown to occupy different nuclear

locations in proliferating, quiescent, and senescent cells (Mehta et al. 2007, 2010), Mehta et al. (2011) revealed that HGPS cells had a quiescent-type distribution of this chromosome in proliferating HGPS fibroblasts. Complete reorganization of the genome was, however, not observed because the X chromosomes were found at the nuclear periphery in both control and HGPS cells. This mislocalization of chromosomes could be restored to normal when the HGPS cells were treated with farnesyl transferase inhibitors that prevent progerin from being farnesylated. A global genome-wide study of the sequences associated with progerin at the nuclear periphery in mouse cells confirms that A-type lamins are involved in chromatin and genome organization in nuclei. Kubben et al. (2012) show that in cells with progerin some genes have been relocated away from the nuclear periphery, whereas others have enhanced association.

## *Cancer*

Cancer is a disease characterised by genomic instability, resulting in unlimited cell replicative potential. Transformation is a multistep process usually encompassing many genetic modifications including aneuploidy, copy number variants, gene mutations, aberrant DNA methylation patterns, and chromosomal rearrangements. The majority of these changes promote increased oncogenic transcription, which stimulates proliferation and inhibits apoptosis.

With increased understanding of chromosome territories in the interphase nucleus, much work has gone into understanding the differences that emerge in neoplastic tissue compared with normal samples. The observed changes have been both on the global scale, such as loss of heterochromatin, and at the gene scale, such as the repositioning of tumor-associated genes in cancer formation (Zhu et al. 2011; Meaburn and Misteli 2008).

The changes in the nuclear architecture of cancer cells are so robust they have been used in tumor diagnosis for more than 140 years. Since the first patient biopsy was examined in 1860, many advances have been made in understanding cancer. However, diagnosis still relies heavily on the analysis of cell morphology. Specific nuclear markers of cancer include changes in nuclear size and shape, nucleolus alterations, changes in chromatin organization, aberrantly shaped nuclear lamina, and alterations to promyelocytic leukemia (PML) bodies (Zink et al. 2004). Common nuclear shape changes include indentations and folds that are indicative of a wide variety of cancers. Nuclear morphological changes with specificity to certain cancer types include grooves or long clefts in the nuclear surface, which are associated with the expression of the papillary thyroid oncogene expressed exclusively in papillary thyroid carcinomas (Fischer et al. 1998). Enlarged nucleoli are associated with several cancer types; however, inconspicuous nucleoli are almost exclusively indicative of small-cell anaplastic lung carcinoma (Zink et al. 2004). Observed changes to chromatin structures include changes to heterochromatic foci, which are areas of the nucleus that contain highly compact chromatin structures usually

associated with gene silencing (Hahn et al. 2010). The changes to heterochromatic foci include loss, asymmetry, coarse appearance, and spreading throughout the nucleus (Zink et al. 2004). Several studies have identified silencing of tumor suppressor genes in cancer in parallel with changes to chromatin structure (Hahn et al. 2010). Tumor suppressor gene promoters showing heterochromatic markers such as H3K9 trimethylation have been identified in many cancer types (Lakshmikuttyamma et al. 2010). It is yet to be established whether changes in chromatin structure cause silencing of tumor suppressor genes and thus drive cancer. One study conversely found that knockdown mice lacking the tumor suppressor gene *BRCA1* resulted in changes to heterochromatin, including loss of foci from the nuclear periphery, leading to a more diffuse state of foci throughout the nucleus (Zhu et al. 2011). This finding suggests some sort of positive feedback whereby tumor suppressors regulate chromatin conformation; but once lost, aberrant chromatin changes promote cancer.

## Chromosome Positioning in Cancer

A vast number of diseases present with genetic defects that are often visible as chromosome rearrangements. The presence of chromosome abnormalities is a hallmark for many forms of cancer. In many cases the specific association of certain chromosome aberrations and type of tumor are considered of diagnostic and prognostic value.

Studies on interphase chromosome position in cancer cells were initiated by work where HT1080 fibrosarcoma cell transformation was chemically reversed. Acrocentric chromosomes that had been found through the nucleus were relocated more centrally, which seems to result from their association with the nucleoli and the coalescence of many smaller nucleoli to one prominent centrally located nucleolus after the treatment (Krystosek 1998). In a study from the Cremer laboratory, the differential positioning of human chromosomes 18 and 19 was much less obvious in colon adenocarcinoma cells, cervix carcinoma cells, and Hodgkin disease-derived cells (Cremer et al. 2003). The nuclear locations of chromosomes 10, 18, and 19 were assessed in normal thyroid tissue and compared to adenomatous goiters, papillary carcinomas, and undifferentiated carcinomas. There was no difference in chromosome position in the normal and goiter tissue with chromosomes 10 and 18 positioned toward the nuclear periphery; and chromosome 19 in a central location. However, in the papillary carcinoma tissue chromosome 19 was located centrally in statistically fewer cells. Further, in undifferentiated carcinomas all the chromosomes assessed were mislocalized (Murata et al. 2007). In a breast cancer cell line MCFCA1a differences in the distance between chromosomes 4 and 16 were found when compared to the control cell line MCF10A (Marella et al. 2009). Wiech et al. (2005) analyzed chromosome 8 positions in wax-embedded pancreatic cancer tissue samples. Radial distance indicated the repositioning of chromosome 8 to the nuclear periphery, which matched roundness scores showing a change in the shape

of the territory. A subsequent paper also noted a reduction of the roundness of chromosome 8 territories, suggesting a thinner, more elongated territory in pancreatic carcinomas (Timme et al. 2011). The centromere and the gene encoding HER2 on chromosome 17 were also shown to compact in neoplastic breast tissue, conferring the repositioning of the centromere to a more internal location (Wiech et al. 2005). A subsequent study by Wiech et al. (2009) reported repositioning of chromosome 18 during cell differentiation of nonneoplastic cervical squamous epithelium, showing a move toward the nuclear interior. This finding was in contrast to the observations in cervical squamous carcinomas that showed a repositioning of chromosome 18 toward the nuclear periphery (Wiech et al. 2009). This study also analyzed the expression levels of *BCL2*, an inhibitor of apoptosis, which was shown to prolong cell survival and found to be unregulated in 54 % of cervical cancers. A reduction in *BCL2* expression has been found in the terminally differentiated cells on the outer layers of the cervical epithelium. In contrast, an increase in *BCL2* expression was found in the carcinomas, suggesting that relocation to the nuclear periphery increases *BCL2* transcription (Wiech et al. 2009).

## Gene Repositioning in Interphase of Cancer Cells

A comprehensive study into the nuclear organization in breast cancer by Meaburn et al. (2009) found that 8 of the 20 gene loci analyzed showed significant gene repositioning in cancer cells. All the gene loci studied have previously been implicated in cancer, with the most frequently repositioned gene locus being *HES5*, a transcription repressor that regulates cell differentiation. As the majority of genes were not repositioned, this finding suggests that the repositioning was gene specific rather than global genome reorganization. It was also concluded that gene repositioning was not associated with genome ploidy because the genes analyzed in this study had no changes in copy number. It was also observed that some genes were only repositioned in certain cancer types, suggesting that some gene repositioning is cancer type specific (Meaburn et al. 2009). This idea is supported by the findings of Wiech et al. (2005), who identified *BCL2* repositioning to the periphery in *BCL2*-positive cervical squamous cell carcinomas but not in *BCL2*-negative cancer cells. By analyzing gene position in normal cells and cells from noncancerous disease, breast hyperplasia or fibroadenoma, no significant difference was found (Meaburn et al. 2009). This result demonstrated that the rearrangements observed in cancer cells are cancer specific and cannot be seen in noncancerous diseased cells. The identification of cancer-specific genes repositioned in several types of breast cancer could prove a useful diagnostic tool. One problem this technique faces, however, is the intermingling of normal and diseased cells, which reduces the statistical power. It has been observed that the tissue directly adjacent to the cancerous tissue in patients has a normal pattern of gene organization that matched with the tissue from normal individuals (Meaburn et al. 2009). This finding is in agreement with previous reports

that the organization of genes does not differ between individuals, except in the case of disease (Wiech et al. 2005). Once validated in a larger number of patient samples, these problems should be overcome, yielding a useful diagnostic tool. Although the study by Meaburn et al. (2009) failed to show a relationship between gene repositioning and transcription levels, other studies investigating this aspect have shown that an altered positioning of specific genes in the nucleus is associated with altered transcription levels (Wiech et al. 2009). This finding is in contrast with a previous report that showed that gene repositioning occurs in early tumorigenesis and does not affect transcription levels (Meaburn and Misteli 2008). More work is needed in this area to understand why gene repositioning occurs, especially if it is not related to gene function. Seminal studies aimed at understanding differentiation and maturation of the lymphoid lineage have analyzed the relationship between gene positioning and activity. One of these studies showed preferential localization to the nuclear interior of the IGH and IGk loci during pro-B-cell lymphocyte development (Kosak et al. 2002). This repositioning event coincided with transcription, and subsequent recombination of these loci is required for the production of unique antibodies. This finding led the authors to conclude that chromatin rearrangement is a powerful mechanism for the control of transcription (Kosak et al. 2002). Further studies will elucidate the role of gene positioning within the nuclear architecture as an underlying condition for gene transcription and expression in cancer development and progression.

## **Formation of Chromosome Translocations in the Context of Nuclear Organization**

Cancer cells harbor a number of genetic abnormalities, of which chromosomal translocations are well-studied examples, especially in leukemia and lymphoma. The mechanisms of translocation formation are under study. Whether a multistep process or a simultaneous occurrence of several events (Forment et al. 2012), we expect an impact on genome organization and nuclear architecture. In any case, the exchange of chromosomal fragments requires the formation of two or more double-strand breaks (DSB). The incorrect repair of DSB leads to the fusion of nonhomologous chromosome ends, creating derivative chromosomes. The most error-prone pathway for the repair of DSB is nonhomologous end-joining, in which two chromosome ends in close proximity are religated. Within the nucleus there are error-free repair pathways for DSB resolution, such as homologous recombination; however, this process does require either a sister chromatid (post S-phase) or homologous chromosome (Meaburn et al. 2007b). There is an ever-growing list of cancer type-specific translocations, with the same rearrangement arising nonrandomly, and hence observed in the cancer cells of many individuals (Mitelman Database of Chromosome Aberrations in Cancer, <http://cgap.nci.nih.gov/Chromosomes/Mitelman>). These recurrent translocations are useful diagnostic

tools and are often associated with clinical outcome. A classical example is the Philadelphia chromosome derived from the t(9;22) and found in chronic myeloid leukemia (CML) and in some cases of acute lymphoblastic leukemia (ALL) (Goldman 2010). Another example is the t(12;21), found in approximately one third of pediatric patients with ALL and associated with a relatively good prognosis (Harrison et al. 2010). With increased understanding of the organization of the interphase nucleus, a number of studies have investigated the proximity of chromosomes involved in common translocations as well as the 3D positioning of their derivatives (Meaburn et al. 2007b) in certain cancers. There is evidence to support the hypothesis that translocations occur in interphase nuclei between chromosomes that occupy similar nuclear space (Kozubek et al. 1999; Parada et al. 2002; Kuroda et al. 2004; Gandhi et al. 2012); this may also be true for intrachromosomal fusions and genes that are at some distance linearly but may be placed together by chromosome folding (Gandhi et al. 2006). Nuclear position was also identified as a factor that contributed to translocation frequency, with peripherally located chromosomes such as 4, 13, and 18 being involved in a higher than expected number of translocations (Bickmore and Teague 2002). Later global screening studies have confirmed that nuclear position is fundamental in the selection of translocation partners (Engreitz et al. 2012; Roix et al. 2003), but transcriptional activity is also of fundamental importance (Klein et al. 2011). A number of studies have been undertaken to understand the interaction of different chromosome regions that favor the exchange of DNA fragments at the level of interphase nucleus, hence the formation of chromosome translocations (Branco and Pombo 2006; Murmann et al. 2005; Roix et al. 2003; Zhang et al. 2012). This point is supported by the finding that chromosome territories do not have neat borders and that neighboring territories do intermingle (Branco and Pombo 2006). It has been suggested that chromosomal translocations are events whose frequency is correlated to the spatial proximity of the loci involved, as described for some human lymphomas (Roix et al. 2003). One could speculate that a similar location in the nuclear environment is sufficient to facilitate an encounter and an exchange of chromosome fragments, a phenomenon described as chromosome kissing (Cavalli 2007). Also, gene loci located on the periphery of chromosome territories were found to be involved in more interchromosomal rearrangements than those deep within the territory (Gandhi et al. 2009). Internally located loci were more frequently involved in intrachromosomal aberrations (Gandhi et al. 2009).

Exposure to ionizing radiation results in DNA DSBs that permit nonhomologous chromosomes in close proximity to combine, creating complex rearrangements (Anderson et al. 2002). This rearrangement can cause several cancer types, in particular, radiation-induced thyroid tumors, such as papillary thyroid cancer. A common chromosome rearrangement observed in papillary thyroid cancer is the intrachromosomal inversion on chromosome 10 that creates the fusion gene *RET/PTC1*. Although the two genes involved in this inversion are on the same chromosome, they are 30 Mb apart. One study, however, showed that at least one copy of each gene colocalized in 35 % of normal thyroid tissues compared with only 6 %

in mammary epithelial tissue (Nikiforova et al. 2000); this explains the tendency to form inversions specifically in the thyroid. Another translocation that may arise from exposure to ionizing radiation is the t(9;22)(q34;q11). This translocation gives rise to the Philadelphia chromosome and the *BCR-ABL* fusion gene. The oncogenic chimeric protein produced drives the formation of CML as well as some cases of ALL. It has been shown that the *BCR* and *ABL* genes are found in distinct locations in the interphase nucleus of healthy stimulated and nonstimulated lymphocytes (Lukasova et al. 1997). In response to ionizing radiation, however, both genes were shown to move to a more internal location, reducing the distance between them to less than 1  $\mu\text{m}$  in 47.5 % of healthy donors (Lukasova et al. 1997; Kozubek et al. 1997). This finding suggests that rearrangement of chromatin in response to ionizing radiation brings into close proximity two genes known to be common translocation partners.

Regions from different chromosomes can also be brought into close proximity by association with specific nuclear structures such as nucleoli (Sullivan et al. 2001). The acrocentric chromosomes (13, 14, 15, 21, and 22) of the human genome all contain nucleolar organizer regions (NOR), which are composed of ribosomal gene repeats. Nucleoli form around these NOR elements after mitosis, and during cell-cycle progression the nucleoli fuse, creating fewer larger structures. However, it should be noted that this process is very rarely observed in cancer (Morgan et al. 1987), whereas it is more likely to affect gamete formation and offspring as a result of loss of genetic material. This process does, however, demonstrate that association with nuclear elements can increase the occurrence of translocations. One example specific to cancer is the translocation observed between the mouse chromosomes 12 and 15, which is present in 80 % of plasmacytomas (Osborne et al. 2007). The breakpoints in this translocation involve the *c-Myc* gene and immunoglobulin heavy chain locus (*IgH*). This translocation is mirrored in humans by that of t(8;14), which encompasses the same genes and is found in Burkitt's lymphoma as well as other forms of lymphoid cancers (Haluska et al. 1987). These genes are found in close proximity in only a third of human nuclei but are neighbors in mouse cells (Parada et al. 2004; Roix et al. 2003). A study by Osborne et al. (2007) found that upon activation both genes are recruited to the same transcription factories, increasing their physical proximity. As proximity has been shown to be a key factor in translocations, this increases our understanding of why the t(12;15) is observed so frequently. In support of this realization, it was observed that *c-Myc* colocalized and transcribed with *IgH* at the expected frequency to give rise to the observed level of translocations (Osborne et al. 2007). The other translocation partners of *c-Myc* in Burkitt's lymphoma and plasmacytoma include *IgK* and *IgL*. The colocalization of *c-Myc* to these genes was also analyzed and found to correlate with translocation frequencies. Therefore, this research suggests a correlation between the number of times genes come into close proximity and the likelihood of translocations (Osborne et al. 2007).

Proximity is only one factor thought to affect translocation frequencies; other factors to consider include chromosome size and gene density (Bickmore and

Teague 2002). One study found a correlation between chromosome size and translocation frequency in response to ionizing radiation (Cafourkova et al. 2001). Another comprehensive study that analyzed more than 11,000 non-disease-causing chromosome aberrations found that larger chromosomes were more frequently involved in translocations; this could, however, be ascribed to increased opportunity for translocations in large chromosomes because they are bigger targets. This study also identified that translocations appear to occur less frequently in highly dense regions of the genome (Bickmore and Teague 2002).

## Repositioning of Genes Affected by Translocation Events

It has been postulated that, because of a translocation event, specific genes might alter their position in the nucleus and therefore be more or less exposed to the transcription machinery. More precisely, certain genes could be activated or inactivated on the basis of the new environment they inhabit. This change would happen when two different regions characterized by different transcriptional activity become positioned next to one another (as in the case of a reciprocal translocation), resulting in an aberrant localization in the nucleus for one or both of the two regions. Studies on both constitutional syndromes and cancer have focused on the localization of the derivative chromosomes in the cell nucleus and also explored gene expression in the context of the newly established nuclear architecture (Ballabio et al. 2009; Harewood et al. 2010). A study on Ewing sarcoma cells has shown that the fusion genes derived from the cancer-associated rearrangement t(11;22) assume an intermediate nuclear position when compared to the wild-type *EWSR1* and *FLI1* genes (Taslerova et al. 2003). Murmann and coworkers observed that the change in position of loci affected by a translocation depends on the relative gene density of the 2-Mb window of the region considered. The study of wild-type *MLL* and five of its translocation partners showed that the resulting fusion genes changed their nuclear location according to the reciprocal gene density of the region involved (Murmann et al. 2005). More recently, a study on pediatric leukemia characterized by the presence of the acquired t(7;12) translocation has shown that an overexpression of the *HLXB9* gene (on chromosome 7q36) corresponded to an altered nuclear position of the derivative chromosome carrying the *HLXB9* gene itself (Ballabio et al. 2009). In this case, the translocated *HLXB9* gene localized more centrally than the wild-type allele. A larger study on the constitutional balanced translocation t(11;22) has shown on a larger scale that an altered spatial organization of the der(11) corresponds to an alteration of the expression profile of genes localized on the der(11). In the same study, chromosomes other than those involved in the rearrangement have also shown an altered nuclear position and altered gene expression profiles (Harewood et al. 2010). This finding shows that the global nuclear architecture and the location of various chromosomes are influenced by specific rearrangements. Altogether, nuclear positioning plays a functional role in regulating gene expression.



## Aneuploidy and DNA Copy Number Alterations

Gain and loss of genetic material is another common feature of cancer cells. Microscopically, this defect can be visualized as complete loss or gain of entire chromosomes caused by missegregation during mitosis and resulting in aneuploidy. Other imbalances are visible as loss or gain of certain chromosomal regions: these are known as deletions, duplications, amplifications, or more generally as DNA copy number alterations (CNA). These changes have an impact in diagnosis and are relevant at the prognostic level. For example, extra copies of chromosome 3q define the difference between cervical dysplasia and invasive cervical carcinoma resulting from human papilloma virus (HPV) infection, whereas complete or partial loss of chromosomes 5 and 7 are the most commonly observed alterations in acute myeloid leukaemia (AML) (Zhang et al. 2011). It is assumed that the presence of additional genetic material corresponds to increased expression levels of the overrepresented sequences. This assumption is supported by a study on highly hyperdiploid pediatric ALL, showing that the presence of additional chromosomal material corresponded to an increased expression of the amplified loci (Gruszka-Westwood et al. 2004).

Very few studies have addressed the issue of chromosome organization in cases of aneuploidy. Croft et al. (1999) did not see any repositioning of an extra chromosome 18 in Edward syndrome cells with a trisomy 18 (Croft et al. 1999). This finding is supported by that of Koutna et al. (2000), who investigated specific trisomic loci within the HT-29 colon cancer cell line. They concluded that the location of the third copy of a specific locus is not significantly relocated when compared to the two loci present in a noncancerous tissue (Koutna et al. 2000). Although gene amplification resulting from aneuploidy has the ability to drive cancer formation it does not alter the organization of chromosome territories. Therefore, according to these studies, tumorigenesis appears to be independent of chromosome position. In another study, additional copies of chromosomes 7, 18, or 19 were artificially introduced in immortalized or cancer cell lines, and their position in the nucleus was observed and correlated with altered expression levels. It was ascertained that the presence of additional chromosomes increased transcription from the trisomic loci. However, a shift in positioning was noted for chromosomes 18 and 19, but not for chromosome 7. The authors proposed that positioning within the nucleus is determined by a unique chromosome-specific ‘zip code’ that might be independent from the transcriptional activity of the sequences that compose it (Sengupta et al. 2007).

## Other Disease Situations

In 1988, Manuelidis and Borden published their seminal work demonstrating that specific chromosomal domains were located to specific regions of the nuclei of neurons and glial cells. In large neurons, probes delineating chromosomes 9, 1, and Y were most commonly found adjacent to nucleoli. However, in astrocytes these

same regions were found at the nuclear membrane and not specifically associated with nucleoli. These data indicate that nonrandom chromosome positioning is of importance to the cell even in terminally differentiated cells such as nerve cells. Manuelidis was the first to show in the human cortex the spatial repositioning of chromosome in interphase nuclei in disease. She found that chromosome X had become relocated from the nuclear edge to the nuclear interior in seizure foci in epileptic patients. This study is significant because it links chromosome positioning with ill health. In this study we do not know if the repositioning affects gene expression on the X chromosome. However, one of the master regulator genes for epilepsy has been identified on the X chromosome (Stromme et al. 2002).

Very few studies have concerned chromosome and gene repositioning after an infection. However, genes have been observed to relocate within cells of hosts that are exposed to infectious agents. In *Biomphalaria glabrata* cells, the secondary host organism of the human parasitic disease schistosomiasis, commonly known as bilharzia, specific genes involved in the infection become relocated within the interphase nuclei at the same time that quantitative polymerase chain reaction (PCR) reveals that they are being expressed (Knight et al. 2011; Arican, Ittisprasert, Bridger, and Knight, manuscript in preparation). One other study revealed chromosome 17 and not 18 changed nuclear location over time after an Epstein–Barr virus (EBV) infection (Li et al. 2010).

## Concluding Remarks

As more laboratories consider the 3D and 4D nuclear organization of the genome in their studies on genome function, it is becoming clearer that chromosome position and association with nuclear structure matter a great deal with respect to regulating gene expression in healthy cells and affect the functioning of diseased cells when misorganization of the chromosomes and genes is apparent. Furthermore, misorganization and misplacement of chromosomes and their gene loci may be responsible for some disease situations.

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