# Review Article

## Genome organization, instabilities, stem cells, and cancer

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## Abstract

It is now widely recognized that advances in exploring genome organization provide remarkable insights on the induction and progression of chromosome abnormalities. Much of what we know about how mutations evolve and consequently transform into genome instabilities has been characterized in the spatial organization context of chromatin. Nevertheless, many underlying concepts of impact of the chromatin organization on perpetuation of multiple mutations and on propagation of chromosomal aberrations remain to be investigated in detail. Genesis of genome instabilities from accumulation of multiple mutations that drive tumorigenesis is increasingly becoming a focal theme in cancer studies. This review focuses on structural alterations evolve to raise a variety of genome instabilities that are manifested at the nucleotide, gene or sub-chromosomal, and whole chromosome level of genome. Here we explore an underlying connection between genome instability and cancer in the light of genome architecture. This review is limited to studies directed towards spatial organizational aspects of origin and propagation of aberrations into genetically unstable tumors.

**Key words**; Nuclear architecture, spatial organization, chromatin structure, chromosomal aberrations, stem cells, genome instability, carcinogenesis

## 1. Introduction

One of the most fascinating aspects of genome biology is how the spatial organization of genome maintains the structural integrity and conversely how

mutations disrupt them at multitude levels to consequently lead to genome instabilities (GIs) over time. For decades, many meaningful insights have been gained by investigating the genesis of the chromosomal aberrations and other instabilities [1-5]. The current and yet transforming genome instability hypothesis suggests that the radiation like genotoxic stress can initiate and trigger a cascade of instability events at different levels of genome and eventually override many critical cell regulatory mechanisms [6, 7]. Mounting evidence indicate the emerging roles of epigenetic and chromosomal modifications in some solid tumors development wherein oncogenes and tumor suppressor genes play only a limited role [8-10]. Interestingly, recent studies elucidate that the origin of carcinogenesis is not restricted at genetic mutations level but rather extended to chromosomal or genome aberrations level in the genome [10, 11]. Besides, a large body of evidence demonstrates the profound impact of genome architecture on the genesis of genome instabilities [12-16]. In particular, several inherent features of genome organization such as chromosome territory arrangement as a determining factor in aberration mediated leukemia formation. Altogether, an emerging concept is that a group of genome instabilities not only evolved from simple deletions, duplications, inversions, translocations, but also encompass chromosome territories, somatic rearrangements and other genome's higher level architectural abnormalities. In other words, not only gene-level mutations but also higher-order level genome instabilities play critical roles in tumor initiation and development. More importantly, spatial organizational aspects of aberrations provide a structural basis to understand the origin of

tumor -specific rearrangements and aberrations in primary lymphocytes [12-15]. In addition to the initiation and progression of mutations and aberrations, DNA repair processes also depend on the chromatin organization. Thus, critical understanding of genome organization will shed a light on induction and perpetuation of multiple mutations [14, 15]. Our main intention is to highlight an emerging and predominantly important theme that remains underrepresented how genome organization profoundly influences initiation and propagation of genome instabilities and other pathological consequences. This review intend to focus only on the structural perspective of mutations, aberrations and cancer formation, thus will not address their genetic and pathological details.

Since the elucidation of lower to higher order spatial organization of genome, it is increasingly inevitable that radiation like mutagens induce lesions which are either left non-repaired or illegitimately rejoined to facilitate deleterious biological endpoints including gene rearrangements, cancer and apoptosis [6, 7, 9, 15]. Although mammalian cells possess several checkpoint mechanisms to maintain inherent genomic integrity, tumorigenesis efficiently overcomes the regulatory mechanisms to sustain genome instabilities [17]. In cancer cells, the high fidelity of DNA replication and segregation is often compromised by a cascade of genomic instability events. Here, an outstanding problem is how and when certain mutations amongst a large number of mutations could facilitate tumor induction and progression. Although our understanding of cancer formation has been greatly expanded during past decades, knowledge on mutation initiation, genetic instability maintenance and tumorigenesis are still unclear. In particular the spatial organization aspects that critically influence the initiation, progression of mutations and carcinogenesis are limited. Another area where there is paucity of knowledge is how certain structural aberrations evolve from corresponding genome architecture despite multitude of

surveillance mechanisms. In this review, we attempt to summarize the critical insights of implications of genome architecture on the induction and propagation of genome instabilities and their consequences per se.

## 2. Exploring the 3D spatial organization of genome

Our understanding of spatio-temporal architecture of genome has advanced from an early theme that the genetic information within the primary sequence is three dimensionally (3D) organized as chromosomes to the current theme that a hierarchy of chromatin fibers folded to form morphologically distinct chromosome territories during interphase that are separated from each other by an inter-chromosome domain [16, 18-25]. During interphase, chromosomes within nuclei decondense into morphologically distinct units known as CTs during interphase. Whereas, positioning of chromosome territories (CTs) within nuclei is nonrandom and is influenced by chromosome size and/or gene density (for more, see section: "3D organization of genome"). Thus, the gene dense chromosomes preferentially located towards the center, whereas gene-poor chromosomes preferentially positioned towards the periphery of nuclei [22, 23, 26-30]. This emerging concept sheds light on the structural aspects of chromosome and confirms that both function and structure of the genome are interrelated. This also confirms that any disturbances in the structure of genome would inevitably afflict the function and possibly stability of genome [13, 15,16, 27-30]. As emphasized earlier, what we learn through 3D spatial organization studies of genome could inevitably pave the way for in-depth understanding of genome instabilities.

## 3. Implications of spatial organization of genome on the instabilities
### a) DNA breakpoints and genome instabilities: topology matters

Here we discuss the fascinating association between genome topology and the origin of many genome instabilities. The preferential spatio-temporal aspects of genome strongly implicate the genesis of specific chromatin alterations which lead to cancer-like endpoints in genome topology (refer supplementary figure 1). It is well established that 'sine qua non' of a vast number of chromosomal rearrangements is a juxtaposition of two or more broken chromatin ends within the optimum proximity in space and time. The broken chromatin ends from different chromosomes have mobility which facilitates illegitimate rejoining by repair factories while ends are within optimum proximity [32, 35]. The high-throughput image acquisition study shows that recurrent translocation gene loci, MYC:IGH, MYC:IGL and CCND1:IGH [35] are preferentially positioned in close proximity in normal lymphoblastic cells. At higher organization level this neighborhood impact is more prominent. For instance, the frequency of a cluster of chromosome territories (CTs #12, #14, and #15) in normal mouse splenocytes and lymphoma cell lines exhibit elevated level of translocations [14]. Both translocating chromosomes were in close spatial association, suggesting a conservation of CT arrangements between normal and derivative tumor cells. Besides, translocation-prone gene loci that are preferentially positioned in close proximity in normal cells suggest that locus proximity is usually a consequence of higher-order genome organization rather than a functional aspect of individual genes [15, 16, 23, 31 and 36].

**Figure 1**
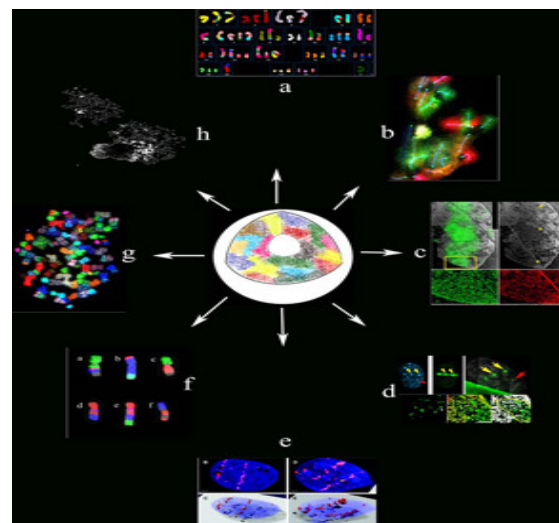
A schematic illustration of genesis of variety of genome instabilities evolved from different levels of genome organization in interphase nuclei. **Center panel:** Graphical representation of higher eukaryotic interphase nucleus with a segment cut away to reveal center spherical nucleolus and 3D distribution of different chromosomes (colored territories). N: Nucleus; CT: adjacent chromosome territories; nl: nucleolus. **a)** Extremely rearranged with 27 structural abnormalities obtained from adenocarcinoma, held at ATCC, obtained from Sanger Centre. (Courtesy: Edwards P; [99]). **b)** SKY-protein co-detection on meiotic mouse chromosomes whereas the synaptonemal complex (SC) and the centromere were highlighted in blue and green color respectively (Courtesy: H.H. Heng et al., [11]). **c)** Ultrastructure of chromatin containing DSBs labeled against γ-H2AX (green) in WT cells exposed to UV laser micro-irradiation. Bar, 500 nm. (Reproduced from The Journal of Cell Biology, 2006 [33]). **d)** (Top panel) Ultrastructure of chromatin containing DSBs in UV laser?irradiated H2AX?/?cells. (Top) Fluorescence images of Hoechst-stained DNA and Nbs1 (left and middle panels, respectively). (Down panel) the electron spectroscopic images (ESI) in WT cells. Bar, 380 nm. **e)** Spatial relations between γH2AX-CDs and Rad51 foci 60 minutes after irradiation. DNA, γH2AX, and Rad51 are shown in blue, red, and green, respectively. Scale bars, 2 μm (Reproduced from Science 2004; [34]). **f)** Comparison of chromosomal abnormalities in cancer cell lines with mutations in BRCA1, BRCA2, CHK2 and BUB1. (Courtesy: Edwards P; [99]). **g)** Examples of SKY-FISH co-detection (Courtesy: H.H. Heng et al., [11]). **h)** Examples of Chromosome fragmentation induced by drug treatment (Courtesy: H.H. Heng et al., [11]).

In higher eukaryotes, genome integrity is critically maintained and is under surveillance from a cell cycle checkpoints, thereby preventing proliferation of DNA damage. Nevertheless, the double strand breaks (DSBs) could overcome these critical defense mechanisms to induce a spectrum of genome instabilities [6, 7, 17 and 37]. Besides, DSBs could eventually trigger apoptosis or permanent cell-cycle arrest and perhaps undergo mis-rejoining, telomere-break fusion and telomere capping [37-41]. More importantly, loss or negligent DSB repair or checkpoint might trigger a multitude of irregulatory mechanism en route to tumorigenesis. First, DSB or chromosome breakage yields the opportunity for genomic rearrangements and thereby facilitating tumorigenesis. Second, chromosome breakage can amplify GI driving and bridge the gap between further genetic changes to metastatic tumor stage. This scenario becomes

potentially lethal with a mild DNA-repair defect accompanied by cell-cycle-checkpoint defects as in case of the AT and NBS like syndromes [39-42]. Likewise, several other instability syndromes are found to be strongly connected to defects in the DSB-repair pathways [43-45]. For instance, Bloom syndrome and Werner syndrome evolve in response to defects in BLM and WRN repair genes respectively [38]. Likely mutations in BRCA1 and BRCA2 cause defects in HR repair machinery that lead to breast cancers [42].

## b) Lower order genome architecture and rearrangements

Genomic variation and genetic heterogeneity are found to be universal features of cancers. Interestingly, various genetic disorders directly evolve from recurrent DNA rearrangements within unstable hotspots of genome organization [refer Table 1]. The molecular basis for these fragile sites usually stem from their inherent DNA secondary structures such that tri-nucleotide repeats (TNRs) and AT-rich mini-satellite aberrations evolve from their hairpins or DNA triplex secondary structures [46]. Besides, the common fragile sites are predisposed to chromosomal breakpoints in tumors and play a role in the in vivo occurrence of pathogenetic landmarks such as deletions and translocations, gene amplification, and integration of foreign DNA within 300-kb fragility region [47-51]. It is interesting to note that chromatin structural hindrance features rather than a cis-acting nucleotide mediated recombination facilitate potential crossovers. An open chromatin structure may expose DNA to DSBs or other damage that is then repaired in an aberrant fashion yielding rearrangements. For instance, chromatin organization at the hotspot regions might be prone to recombination such as the non-allelic homologous recombination (NAHR) strand exchange [52]. Some inversion polymorphisms, such as Angelman syndrome (AS) are shown to undergo NAHR at LCR spots that are positioned with inverted orientation [53]. Besides, some non-recurrent rearrangements are also associated with LCRs: translocation breakpoints are shown to predominantly occur within LCR regions. In

addition, LCRs have recently been recognized to be responsible also for non-recurrent rearrangements [13, 45-47]. These observations lead to the postulation that fragile sites which are susceptible to carcinogen-induced alterations could play a role in cancer cell-specific chromosomal rearrangements [46, 47, 53-56].

## c) Impact of higher order spatial organization and rearrangements

One fascinating aspect regarding the spatial organization is that translocation potential or inter-chromosomal rearrangements are best reflected in respect to their chromosome territories arrangement. The likelihood of undergoing illegitimate rejoining process is significantly correlated with spatial positioning of potential translocation partners at neighbor chromatin segments. In particular, the DSBs formed within contact or intermingling genome regions are more likely to translocate in human lymphocyte [16, 31, 32 35 and 37]. Given the non-random spatial positioning of genes and chromosomes, closer genome regions are subjected into higher probability of translocating each other. Yet another intriguing observation shows the relative preferential positioning of chromosomes undergoing translocations in mouse lymphoma cells is conserved [16]. This striking feature was observed in a variety of cancer cells that manifested with recurrent translocations and in other structural aberrations (refer- section: "Genome instability: a cancer connection"). Investigation on spatial distribution and shape of CTs #18 and #19 in cancer cells and normal diploid precursor cells suggests a radial higher order chromatin arrangement [22, 23]. Interestingly, the radial positioning of #18 and #19 was observed to be maintained in all tumor cell lines, irrespective of the nuclear shape or the occurrence. This suggests a basic stability of gene density related radial arrangement in the malignant cell types maintained. This confirms that, the higher order spatial genome distribution pattern potentially contributing for the acquisition of

chromosomal aberrations such as translocations, dicentrics and centrics.

## d) Structural alterations in cancers

It is now widely recognized that genome instability ("genetic instability" or "chromosomal instability) refers to abnormally high rates of genetic change occurring serially and spontaneously in cell-populations [57]. Genomic instability in cancer cells appears in three major forms: (i) aneuploidy, in which entire chromosomes are gained or lost, (ii) intrachromosomal instability, characterized by insertions, deletions, translocations, amplifications, and other forms all sharing the feature of utilizing DNA breakage as an early step, and (iii) point or oligobase mutations, which are rare except in DNA replication error inherited syndromes (hereditary nonpolyposis colorectal cancer) and in a small fraction of sporadic cancers. As mentioned earlier, cancer cells have event-driven changes, to the extent that they possess a mixture of primary and secondary changes with continuously changing karyotype. Interestingly, the distinct morphological features of nuclear architecture in tumor cells have shown to be altered even in the absence of karyotype changes during malignant transformation [29, 30]. Certain types of carcinogenesis are associated with gross chromosomal rearrangements such as large deletions, translocations or even complete chromosomal loss [58]. Specific morphological alterations include changes in the nuclear shape, spatial organization of chromatin, nucleoli and in other peri-nucleolar compartments [59]. Some rare premature disorders such as WS, HGPS and AT are associated with both the dramatic defects in nuclear architecture and a diverse set of causative genes that maintains the syndrome. For instance, WS is characterized by non functional RecQ helicase genome instability. Hutchinson Gilford progeria syndrome patients display a defective nuclear architecture with the loss of heterochromatin proteins in their nuclei [60]. Karyotypic aberrations including whole chromosomal loss or gain, ploidy changes and a variety of chromosomal aberrations are common in cancer cells. In addition, changes in DNA

content and chromosome number have shown to occur in pre-neoplastic lesions like oral leukoplakia, early cervical neoplasias and small benign colon tumors [61].

## e) Role of epigenetic alterations in genome instability

Another level of morphological change occurs epigenetically: the epigenetic influence in genome instability is linked to altered gene regulation either at the global level or at the level of specific oncogenes and tumor suppressor genes [62, 63]. Epigenetic gene silencing in cancer can actually predispose the DNA to mutations during carcinogenesis. In particular, sporadic cancer cells with micro-satellite instability are shown to have hyper-methylated genes such as MLH1 and MGMT [64, 65] and aberrant methylation on potential genes such as such as p16INK4a [66]. Besides, aberrant histone phosphorylation, decondensation and delayed replication timing have also been demonstrated in human tumor cell lines. Interestingly, epigenetically influenced tumor-suppressor genes are often observed to reside within instability susceptible genomic regions. Many tumor-suppressor genes are frequently associated with loss of heterozygosity (LOH) in several tumor types. Prominent chromosomal structural changes are observed in some cancers including the ICF (immunodeficiency centromeric instability and facial abnormalities) syndrome whereas the hypomethylation at specific centromeric regions along with the structural changes are well characterized. Therefore, epigenetic plasticity along with genetic lesions in genome architecture provides a major contribution towards tumor progression [67].

## 4. Tumorigenesis: a genome instability connection

The fascinating connection between GI and tumorigenesis has been under long-standing debate for decades [3]. Arguably, one of the most striking and direct consequences of the genome instabilities is carcinogenesis. Yet, the critical question of whether or not the GI is a prerequisite for cancer induction and or its propagation still remains open. Some studies favor the concept that the cancer genome is unstable and that a cascade of mutations results in instability which could possibly overcome survival mechanisms of genome [68-70]. On the other hand, as Sieber et al. noted, not all carcinogenesis are driven by GI. For instance, a mitotic recombination, not somatic BUB1 mutations precede APC mutations prior to tumor growth [70]. Besides, another likely caveat is that gene mutations not necessarily meant to initiate only tumorigenesis, they might be tumor suppressor type. Likewise, other studies argue that only a clonal evolution or selection not genetic alterations is driving force for tumor progression in colorectal adenomas. Here, it is worthy to mention that oncogenes are activated by specific alterations including translocations, gene amplifications or intragenic mutations and give a selective growth advantage to the cell. The opposite is true for tumor suppressor genes: inactivation leads to tumorigenesis.

Despite these contrasting reports, it is evident at least two levels of genome instabilities exist in a vast majority of cancers, one at the sequence level, and the other at a higher order chromatin level [68-72]. The fact that most cancers harbor many genetic or epigenetic changes suggests that sporadic tumors need to acquire some form of inherent genome instabilities. Interestingly, some mutations can lead to cancerous state only dependently on a selective advantage of presence of additional mutations [73]

A hallmark of tumorigenesis is accumulation of genetic lesions. It has been well established for years that cancer is an end product of the accumulation of multiple genetic lesions that could alter patterns of gene expression and cell proliferation [74 and 75]. Whereas the genesis of GIs in tumors typically arises through states of continuous, sustaining, and perpetuating novel chromosomal mutations, irrespective of cytogenetic complexity or heterogeneity and genomic integrity per se, at a rate higher than in normal cells [4, 5, 68 and 69]. While certain carcinogenesis depends solely on the aberration dependent induction, some cancers evolve by mechanisms with little impact of aberrations, clearly reflecting the

heterogeneity in tumor induction, genome stability and genotype patterns. The typical source of the cancer induction varies. For instance, some haematopoietic cancers like lymphomas are generally derived from translocations, while solid tumors are often associated with radically simple deletions [76]. Given the strong association towards human tumorigenesis, common endpoints such as delayed de novo chromosomal aberrations and mutations in specific target loci are critical markers for genomic instability [77].

## 5. Cancer stem cells

Does harboring a large number of mutations alone ensure the induction of tumorigenesis Despite a relatively vast number of multiple mutations, cancer induction probabilities are extremely low in cell population. Only small and phenotypically distinct subsets of cancer cells have the ability to proliferate extensively or to form diverse and consequent tumorigenesis. This phenomenon can be classically explained by the 'cancer stem cells or tumor initiating cells' (CSCs or TICs) hypothesis. This suggests that tumors are composed of a heterogeneous cell population, within which a small subset of cancer stem cells reside, driving the growth and propagation potential of the tumor in vivo [78]. Cancer stem cells share many of the characteristics of normal somatic stem cells, such as the ability to self-renew and to give rise of multiple types of differentiated cells. In addition, there is an increasing body of evidence suggests that some cancer stem cells are the direct descendants of normal tissue stem cells, indicating that normal stem cells can be the primary target of oncogenic transformation. This has been largely attributed to the longevity of tissue stem cells, which allows them to accrue more genomic alterations than their non-self-renewing progeny, leading to stem cell genetic instability, an engine for neoplastic transformation and development of malignancies. An elegant experiment carried out by Southam and Brunschwig demonstrates that only a very few cancer cells among the entire population, 1 in 1,000,000 cells are

capable of initiating tumorigenesis when injected back into the same patient subcutaneously [79]. The alternative possibility to 'cancer stem cell' hypothesis is postulated by stochastic model, in which all tumor cells have self-renewal and proliferation capability, but the probability for any cancer cell to enter into sustainable tumorigenesis phase is quite low [80].

## 6. DNA repair and genome organization

One of the preeminent aspects that can radically change the aberration pattern is DNA repair, as even a minute defect in the DNA repair system could drastically and persistently alter the genome's stability. Marked biological importance of the repair mechanism is exemplified by the fact that many lethal genetic syndromes developed as a consequences of inherited defects in the DNA-repair genes. Inevitably, the mutations in ATM, ATR, BRCA1, Chk2, and p53 lead to a lethal level of CIN. Defects in both Mec1 and Tel1 lead to a 13,000-fold increase in gross chromosomal rearrangement (GCR) rates in mec1 tel1 double mutant cell lines [81]. Compared to non-mismatch repair (non-MMR) defective colorectal tumors, MMR defective colorectal tumor cell lines exhibit 1000-fold increase in mutation rate [81 and 82]. The heterogeneity of DNA repair within the genomic DNA depends upon the higher-order organization, in particular, the compartmentalization of transcriptional activity in different spatial domains of the interphase nucleus such that the actively transcribing genes are proximal to the nucleoskeleton [83]. Thus, this might influence the kinetics of excision repair across genome. Repair analysis after UV irradiation indicates that the excision repair activity is indeed a nonrandom process, preferentially initiated at the nuclear matrix regions in close association with transcription sites subsequently spreading into the loop regions of chromatin [84]. By using a quasi-in-vitro chromatin assay, Balajee SA et al., have shown that the repair in Chinese hamster cells initiates at the nuclear matrix in close association with transcription sites [85]. Interestingly, studies demonstrate that

the DSB repair response is not hindered or limited by accessibility complexities in nuclear architecture; recruitment of TFIIH like large DSB complex factors to locally damaged sites is transient to even in dense segments of chromatin [86]. Thus these interesting studies confirm that chromatin architecture not only defines the way how mutations initiate, propagate and transform into lethal aberrations but also dictate the efficiency of repairing those DNA breaks.

## 7. Conclusions and perspectives

Given the complexity of carcinogenesis origin from the genome, it is indeed critical to gain meaningful understanding of the impact of chromatin architecture on rearrangement and mutations formation. Current studies exploring the genome aberrations via genome organization raise many interesting questions and challenges. For instance, which genome abnormalities, if not all, are critical for initiating genome instabilities How and why do the proliferation and differentiation aspects of cancer stem cells behave in a typical pattern than the surrounding normal cells Is propagation of genome instability critically influenced by chromatin and or chromosome arrangement within nuclei Is the number or rate of multiple mutations correlated with induction probability of carcinogenesis It is apparent that current understanding of how genome instabilities sustain withstanding cell survival mechanisms is limited. Despite a vast number of cytogenetic approaches to diagnose genome instabilities, there are prominent questions yet to be answered. In the forthcoming years, central attention will be on the underlying connection between how chromatin influences the occurrence of recurrent mutations that critically alter the gene patterns.

## Conflicts of Interest

The authors declare that there are no conflicts of interest.

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## Table Legend (click to view the table)

A list of selective examples of genetic aberrations evolves at different levels of genome. Genome instabilities induced by translocations, genetic mutations, whole or sub-chromosomal rearrangements, breakage syndromes and, epigenetic mediated instabilities are provided.

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