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Spatial genome organization in the formation of chromosomal translocations

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Abstract

Chromosomal translocations and genomic instability are universal hallmarks of tumor cells. While the molecular mechanisms leading to the formation of translocations are rapidly being elucidated, a cell biological understanding of how chromosomes undergo translocations in the context of the cell nucleus *in vivo* is largely lacking. The recent realization that genomes are non-randomly arranged within the nuclear space has profound consequences for mechanisms of chromosome translocations. We review here the emerging principles of spatial genome organization and discuss the implications of non-random spatial genome organization for the genesis and specificity of cancerous chromosomal translocations.

Keywords

chromosome territory; translocation; nuclear architecture; spatial organization; contact-first

Introduction

Chromosomal abnormalities are the most prevalent characteristic of tumor cells. Virtually all tumor types and tumor cells either show numerical abnormalities or chromosome rearrangements [1]. Chromosomal translocations, defined as the aberrant fusion of genetic material from two or more chromosomes, may either be merely a consequence of cancerous transformation, but, as clearly demonstrated in several cases, may also act as cause of tumorigenesis by formation of fusion genes which give rise to oncogenic chimeric fusion proteins or lead to misregulation of genes with oncogenic potential. The classic example of such a translocation-mediated fusion protein is the translocation of chromosomes 9 and 22 at the *BCR* and *ABL* genes, generating a constitutively active ABL tyrosine signaling kinase [2]. The t(9:22) is responsible for most cases of chronic myelogenous leukemia cases [2] (see Gollin, this issue).

The formation of a chromosome translocation is a multi-step process (Fig. 1). First, double strand breaks (DSB) must occur in a minimum of two chromosomes concomitantly. Second, the normally acting repair mechanism must fail to eliminate the DSBs. Third, broken chromosome ends must physically meet within the nuclear space and, finally, they must be illegitimately misjoined (Fig. 1). Two major repair pathways may contribute in the formation of chromosomal translocations. Homologous recombination (HR) requires extensive length of

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homology and happens usually through the sister chromatid or the homologous chromosome [3,4]. One mode of homologous repair, gene conversion that usually occurs between sister chromatids is an error free pathway and usually doesn't lead to chromosomal rearrangements. An alternative homologous pathway, single strand annealing (SSA) occurs between two unrelated homologous sequences and gives rise to translocations. On the other hand, in non homologous end joining (NHEJ), the broken ends are simply rejoined with the use of little or no homology. This is an error-prone pathway which frequently gives rise to chromosomal rearrangements [3,4].

The formation of translocations is inherently linked to the spatial arrangement of the involved chromosomes since it requires the physical interaction of the translocation partners. While much progress has been made in the elucidation of the molecular mechanisms of DNA repair and their relevance to formation of chromosomal translocations, the influence of the cellular and spatial arrangement of the genome on the formation of translocations is only poorly understood. The recent appreciation of the complexity of spatial genome organization and its emerging role in genome function strongly points to a critical role of higher order genome organization in the formation of chromosomal translocations [5]. We explore here the role spatial genome organization plays in the formation of chromosomal translocations.

Spatial genome organization in the interphase nucleus

Non-random spatial positioning of chromosomes

In the interphase nucleus, chromosomes occupy discrete three-dimensional regions, known as chromosome territories [6–8]. The territories are arranged in reproducibly non-random patterns [6–8]. When human chromosomes are mapped in relation to the nuclear periphery or the nuclear center (i.e. their radial position), each chromosome is arranged to a first approximation according to its overall gene density [9–13] or size [9,11,14,15] (Fig. 2A). In spherical human nuclei, such as from lymphocytes, gene rich chromosomes preferentially locate towards the nuclear interior and gene poor chromosomes preferentially locate towards the nuclear periphery, regardless of the proliferation status of the cell [9,11–13]. Similarly, in proliferating fibroblasts, which have ellipsoid nuclei, chromosomes are arranged according to gene density [9,13,16]. When these cells enter quiescence, however, chromosomes appear to be arranged in a size dependent manner, with the larger chromosomes more peripherally located [9,11, 14–16]. This change in positioning is reversible, such that when cells are allowed to re-enter the cell cycle human chromosome 18 (HSA18), which is small and gene poor, relocates back to the nuclear periphery from the interior of the nucleus, but only after the cell has been through mitosis [9]. Cremer and colleagues (2005) have also observed a size depended positioning of some chromosomes in early S-phase fibroblast nuclei [14].

Radial chromosome positioning is evolutionarily conserved. Several vertebrate species, including several primates [17,18], mice [19] and chickens [20,21], all have a gene density related radial distribution of chromosomes. The distribution of gene rich genome regions in pigs implies that the porcine genome is also organized according to gene density [22]. Moreover, homologues of gene poor HSA18 and gene rich HSA19 in primates [17,18,21] and chicken [17] have been demonstrated to preferentially locate towards the nuclear periphery and the nuclear interior, respectively, recapitulating the positioning found in humans [9,10, 12,13]. These data imply that the radial positioning of chromosomes with respect to gene density is evolutionarily conserved in vertebrates over more than 300 million years, irrespective of highly divergent karyotypes [17]. Furthermore, the position of genes within interphase nuclei is also conserved between species. HSA11p15 and HSA11p13 locate to similar positions with respect to their chromosome territory as their corresponding loci on mouse chromosome (MMU) 7 and MMU2 [23,24]. Finally, the position of genes relative to one another is also conserved; the *MLL* gene is more peripherally located than *AF4* and *AF9* in fibroblasts derived

from both humans and two species of Muntjac deer [25]. Such conserved arrangement of the genome strongly suggests a role of spatial positioning in the regulation of genome function [7].

Despite the various correlations between gene density and chromosome position it is clearly too simplistic to assume that chromosome positioning is determined by gene density alone. There are several cases where the position of chromosomes varies under circumstances when the gene density of chromatin has not changed, for example between proliferating and non-proliferating fibroblasts [9,16], during differentiation [26–29], in cells derived from different tissues and cell types [13,19,30], and, based on analysis of pericentric heterochromatin distribution, in early mouse embryos at the onset of gene transcription [31,32]. In all these examples, gene density obviously remains constant, but the expression profile changes considerably, thereby suggesting that the position of a chromosome is not so much determined by gene density alone but is dominantly affected by the functional status of each chromosome. This notion is supported by the observation that genome organization is most similar between cell types that share common differentiation pathways [30].

In addition to tissue specific genome organisation, changes in the spatial organisation of the genome have been observed in several diseases, including cancer [10,25,33–36], epilepsy [37], and laminopathies, a group of diseases caused by mutation in the nuclear proteins lamin A/C [16], further suggesting a link between positioning and genome activity rather than simply gene density.

Non-random spatial positioning of genes

In addition to whole chromosomes, genes are also spatially non-randomly positioned during interphase [38]. For example, in lymphoblasts, *MYC*, *BCL6* and *IGK* preferentially locate to the nuclear periphery, *IGL* and *CCND1* preferentially locate to the nuclear interior, whereas *IGH* resides in an intermediate radial position [34]. As for chromosomes, the mechanisms that determine gene positions are not entirely clear and likely involve multiple components. One factor that appears to contribute to the nuclear position of a gene is the gene density in its immediate vicinity. In hematopoietic cells, the local gene density within a ~2MB region is a fairly accurate predictor of the nuclear position of several genes [25]. *AF4* (HSA4), *AF6* (HSA6), *AF9* (HSA9) and *MLL* (HSA11) map to chromosomes with a similar gene density, however, *MLL*, which is located in a gene dense region of HSA11, resides in a more internal nuclear position than the other genes. However, as for entire chromosomes, local gene density is not the sole determinant of positioning. The location of various genes and transgenes also depend on the their activity and level of expression [26,29,39–50]. As a general rule, genes appear more internally localized in their active state compared to when they are inactive. For example, *IGH* and *CD4* preferentially locate to the nuclear periphery when not expressed, but become more internally localized upon activation [26,47,51]. CD8, on the other hand, becomes more peripherally located upon activation [26]. However, not all genes alter location with changes in activation [24,52,53]. For example, *RB1* remains preferentially located towards the nuclear periphery regardless of activity [52]. In further support of a link between positioning and their expression, adjacent genes can localize to different nuclear regions, in accordance with their individual transcriptional regulation [49]. Taken together, while correlations between activity and nuclear position can frequently be found for single genes, no generally applicable rules have emerged.

Genes often also have preferred positions with respect to their chromosome territory. In some cases, genes localize towards the periphery of the territory [43,52–55] whereas in other cases they preferentially localize to the interior of the territory [24]. In extreme cases where gene clusters are expressed at very high levels actively transcribing high gene density chromatin regions can even extend out from their chromosome territory, generally into a more internal

fraction of the nucleus [23,46,56–62], intermingling within the territory of another chromosome[62]. However, some genes including *PAX6*, *c-MYC*, and *TTN* do not significantly alter their location with respect to their chromosome territory in cells types with differing expression levels [24,52,53].

The internal structure of chromosome territories is also non-random. Remarkably, gene dense and gene poor regions of an individual chromosome appear to be clustered within a chromosome territory. In Drosophila, gene rich regions within a 7MB domain of chromosome 2 are spatially segregated from the interdispersed gene poor regions in the same chromosome domain and the gene-rich and gene-poor regions cluster amongst themselves [63]. Similarly, in mouse the gene rich regions of a 4.3MB region of MMU14 are spatially clustered in interphase nuclei, as are the gene poor regions [64]. The gene rich and gene poor clusters also have distinct preferential nuclear locations, in which the gene poor clusters located to the nuclear periphery and the gene rich clusters are more internally localized [64]. Separation of coding and non coding DNA is also apparent on a whole chromosome scale. Genes locate to the periphery of the human chromosome X territory, regardless of activity, with only non-coding genome regions residing in the interior of the territory [55].

These data suggest that the spatial arrangement of the genome may create specialized nuclear environments, such as preferentially active and silencing regions. Consistent with this, there is a correlation of silencing of genomic regions and their association with heterochromatin [39,41,44,48,49,51,52,65–69]. The nuclear periphery has also been implicated as a transcriptionally silencing region of the nucleus [5], although this is not a strict rule, given that in yeast several active genes are found to locate to nuclear pores [70–73]. Intriguingly, relocation of genes to nuclear pores was necessary for maximal expression of *HXK1* and *GAL* genes in yeast [72,73]. Expression of genes at the nuclear periphery has also been reported in mammalian cells [50,74]. Thus, despite the apparent lack of generalized rules to govern the spatial position of genes in interphase, the location of a gene within the nucleus appears linked to its controlled expression.

While single genes can be dynamic and relocalize within the nucleus, the position of entire chromosomes is largely maintained throughout the cell cycle [12,44,75–80]. Observations of chromatin motion *in vivo* demonstrate that nuclear envelope- or nucleoli-associated loci are more highly constrained that internally located loci, implying that these sites are physically tethered [81,82]. Interestingly more active chromatin loci at the nuclear periphery of *Saccharomyces cerevisiae* are more dynamic than inactive chromatin, although this movement appears constrained to two-dimensional sliding along the nuclear envelope [72,83].

Relative spatial positioning of the genome

It has become apparent from the studies on the positioning of genomic loci that single cells within a population show considerable variability in the positioning patterns. Thus the non-random positions of genes and chromosomes are not absolute, but rather probabilistic and the average position of a gene or a chromosome merely denotes its probabilistic preferred location [84]. As a consequence, spatial genome maps of radial chromosome positions suffer from very low resolution. An alternative to radial positioning maps is the mapping of preferred neighbors (relative positioning) of a given chromosome (Fig. 2B). This approach is more robust as it uses a single reference point for each measurement rather than the general framework of the nuclear diameter. In addition, relative positioning can be measured more accurately and more efficiently allowing high-throughput methods [85]. Using this approach, it has been demonstrated that mouse chromosomes have cell type-specific preferred neighbors [30,86], although investigating a different set of chromosomes, Mayer and colleagues (2006) found little evidence for preferred neighbors in mouse cells. This leads to the possibility that only certain chromosomes have preferred neighbors or may simply be a reflection of differing

analytical methods. Preferential relative positioning of chromosomes has also been suggested for human cells. The *major histocompatibility complex (MHC) class II* locus protrudes from the HSA6 chromosome territory when actively transcribing [56,62] and preferentially extends into HSA1, 2 and 9 but not the HSA8 territory [62], indicating preferred neighbors of HSA6 in fibroblasts. The analysis of radiation-induced translocations in human lymphocytes further supports preferred relative positioning of chromosomes [87–90]. However, some of these studies find a randomness with respect to relative positioning overall, but find a cluster of gene rich chromosomes (HSA1, 16, 17, 19, 22) in the nuclear interior [88,90]. Arsuaga et al., [88] found evidence of a cluster of acrocentric, nucleolus-associated, chromosomes, whereas Cornforth et al., [90] did not find a significant level of clustering for these chromosomes. Multi-color imaging experiments on non-irradiated fibroblasts suggest a random positioning of chromosomes with respect to each other, save for 3 pairs of chromosomes (HSA1-2, 2-3 and 13-15) [14].

Regardless of the differing data for whole chromosomes, specific genes and other genomic loci have preferred relative positions in interphase nuclei and their functional relevance is now becoming apparent. The classical example of non-random gene proximity are the ribosomal genes clustered in nucleoli [91]. The selective proximity of specific sequences in interphase nuclei may allow interactions with distal sequences from either the same chromosome in *cis* [92] or with sequences from another chromosome in *trans* [93–96]. These interactions have important functional implications. It is possible that clustering of co-regulated active genes, by forming around sites of specific regulatory factors or transcription factories, facilitates regulation and/or increases the efficiency of transcription and processing [92,94,97]. Interactions between spatially proximal loci can also have a direct role in regulating the alternative expression of genes [93,96], including imprinting events [95,98,99].

Translocation frequencies and interphase proximity

Aside from the important roles of the non-random genome organization in the correct regulation of genome expression, spatial proximity has become critically relevant for our understanding of how genomic instability and chromosomal aberrations occur. Cancers, particularly leukemia’s and lymphomas, frequently have disease specific chromosomal translocations associated with them [2,100,101]. An analysis of over 11,000 non-disease associated chromosomal abnormalities revealed, perhaps not surprisingly, that chromosome size influences translocation frequencies, most likely due to the more extensive target size of larger chromosomes [102]. A correlation between chromosome size and translocation frequency has also been reported for radiation induced translocations [103].

In addition to chromosome size, a growing body of evidence points to a strong correlation between spatial proximity of chromosomes/genes in interphase nuclei and translocation frequencies, whereby more proximal chromosomes or genes undergo translocation events more frequently than distantly located genome regions (Fig. 3) [25,27,33,34,62,86,102,104–111]. The contribution of spatial proximity to translocation formation is illustration by the frequent formation of Robertsonian translocations arising by fusion of two acrocentric chromosomes, which in humans always contain nucleolar organizing regions (NORs) and cluster together in nucleoli [112–114]. Furthermore, translocations amongst peripheral chromosomes such as HSA4, 9, 13 and 18 occur at higher frequencies than with internally localized chromosomes, presumably due to their closer physical localization to each other [102]. In addition, high resolution microscopy analysis shows chromatin at the edges of chromosome territories to intermingle with neighboring chromosomes [62]. The degree of intermingling between pairs of chromosome territories in lymphocytes strongly correlates with their translocation frequencies [62]. Further examples of spatial proximity in interphase nuclei effects on cancerous translocation frequencies will be discussed in more detail below.

Tissue specific proximity and translocation frequencies

The fact that spatial genome organization is tissue specific may also be directly relevant to explain the observed tissue specificity of chromosomal translocations. When characterizing the tissue specific organization of mouse chromosomes, Parada and colleagues (2004) found that in hepatocytes, MMU5 and 6, which commonly translocate in hepatomas, formed a pair containing one homologue of each chromosome significantly more frequently than MMU12 and 15 which are not frequent translocation partners in hepatocytes. Likewise, MMU12 and 15, frequent translocation partners in lymphomas and plasmacytomas, commonly cluster in lymphocytes [86,107], but not in hepatocytes, in which this translation is not common [107]. Furthermore, γ-irradiation of normal human lymphocytes frequently induced translocation in chromosome pairs that are known to participate in leukemic translocations, suggesting these chromosomes are near neighbors in lymphocytes [103]. Along the same lines, HSA12 and 16, which commonly translocate in liposarcomas, shift position to come closer together during adipogenesis [27], suggestive of an proximity effect in the formation of t(12;16) in adipocyte cancers. Taken together, these considerations suggest that tissue specific proximity of chromosomes in interphase nuclei facilitates the formation of tissue-specific cancerous translocations.

Interphase gene proximity and translocation frequencies

While it is of interest that chromosome proximity reflects translocation frequencies, it is the positioning of the specific genes involved in the translocation event, rather than the entire chromosome, that is functionally important for an understanding of cancerous transformation. Thus, a more crucial question to address is whether translocation prone gene partners are frequently in close spatial proximity. This appears to be the case. The t(9;22)(q34;q11) translocation in chronic myeloid leukemia results in the fusion of *BCR* and *ABL* and these genes are in closer spatial proximity in normal heamatopoetic cells than other, non-translocating gene pairs [104–106,115,116]. Significantly, exposure to radiation, a known inducer of leukemia, results in *BCR* and *ABL* locating nearer to each other, in a more central part of the nucleus [104,105]. A translocation involving HSA15 and 17 and the consequential fusion of the *PML* and *RARα* genes is common in promyeloctic leukemia. *PML* and *RARα* are recurrently found in close proximity in normal B cells at a higher rate than control gene pairs [106]. An equivalent correlation between the proximity of genes and translocation frequency is observed in Burkitts lymphoma [34,105]. Notably, in B-cells the spatial proximity between *MYC* and any one of its multiple translocation partners (*IGH*, *IGL* or *IGK)* decreases as the clinically observed incidence of translocation for the given pair increases [34]. The same correlation between the incidence of the specific translocation and spatial proximity was observed for the *IGH* locus and other lymphoma translocation partners (*CCND1*, *BCL2* and *BCL6*) [34]. The analysis of four hematological cancer cells lines, where the chromosomes containing the genes of interest were unmodified, reveals somewhat different behavior for recurrent translocations involving *MLL* and multiple fusion partners [111]. The *ENL* gene is positioned closer to *MLL* than *AF4*, despite a higher frequency of *MLL:AF4* translocations compared to *MLL:ENL* detected in leukemia patients [111]. This does not rule out a role for spatial proximity, however, since the three most common translocation partners for *MLL*, *AF4*, *AF6* and *AF9*, all locate to the same radial position in hematopoietic cells [25]. Solid tumors, such as radiation induced cancers of the thyroid, also reveal a close spatial proximity of translocation-prone genes prior to a translocation event while these genes were further apart in normal lymphocytes and mammary epithelial cells [33,109,110].

Consequences of genetic instability on the spatial organization of the genome

While the spatial positioning of genome regions prior to the translocation event is clearly emerging as an important determinant of translocation outcome, it is less clear whether

alterations in the placement of genome regions resulting from translocation events have a role in tumorigenesis. This is a possibility that needs to be considered since spatial positioning has been linked to the control of gene expression [5]. Several studies on aneuploid and rearranged genomes, however, question whether genome rearrangements generally lead to alterations in the position of genetic material. First of all, it appears that the spatial organization of chromosomes is maintained with increasing copy number. HSA18 and 19 are in the same relative nuclear positions, when they are present in triplicate, compared to normal, diploid cells [12]. Moreover, chromosome X locates at the nuclear periphery in both male and female cells despite different copy numbers [13]. Interestingly, in diploid cells MMU12, 14 and 15 form a single cluster containing only one copy of each chromosome, whereas in tetraploid cells two clusters are formed [86]. A useful tool to address the regulation of spatial position of additional copies of a given chromosome are hybrid mouse fibroblasts containing a specific single human chromosome [117]. In such hybrid cells, the human acrocentic, NOR containing chromosomes associate with the host cell nucleoli [113]. When the position of all human chromosomes, with the exception of HSA12 and Y, were analyzed in hybrid cells, the majority did not locate to the radial position they occupy in human fibroblasts, instead appear to position similarly to their most syntenic mouse counterpart [117]. Taken together these data suggest that the spatial organization of additional copies of chromosomes are governed by the same mechanisms that determine the normal diploid genome.

Translocation events also do not generally alter the position of chromosomes. The clustering of chromosomes MMU12, 14 and 15 in hematopoeticly-derived cells is conserved during cancerous transformation, even with two translocations between these three chromosomes [86]. In agreement, no shifts in the nuclear positions of translocated derivative chromosomes, compared to the untranslocated chromosomes in normal cells, were detected for several human tumors [10]. Yet, the conservation of positions of translocated chromosomes may be specific to the chromosomes involved in the translocations and the differences in positions they normally reside, since differences in positions have been reported for derivative chromosomes. HSA18 and 19 occupy very different radial positions in lymphocyte nuclei, with HSA18 at the nuclear periphery and HSA19 at the nuclear interior [10–13], but derivative chromosomes t (18;19) occupy broadly similar positions to that of their normal counterpart [12]. However, the HSA19 genetic material on the derivative chromosomes orientates to a slightly more central position in 80% of cells [12]. In a Hodgkin lymphoma-derived cell line, where HSA19 is involved in a complex translocation with peripherally located HSA2 and 9, the distribution of HSA19 shifts to a more peripheral position [10]. Similarly, HSA18 genetic material involved in t(17;18) relocates to a more internal nuclear position, in keeping with the fact that HSA17 resides in the nuclear interior [10]. In tumors with gains or losses of parts of either HSA18 or HSA19, the radial positioning of these two chromosomes were largely maintained. Nevertheless, the difference in relative positioning between these chromosomes was less pronounced, with an increase in the frequency of cells where HSA19 was more peripherally located that HSA18 [10]. Thus it seems, in general, that chimeric chromosomes generated by fusion do not significantly relocate as a whole, but the translocated DNA orientates in the direction of where it normally locates.

Translocation events also sometimes lead to a shift in the position of the involved genes. The *BCR-ABL* fusion gene does not alter its position with respect to the un-rearranged copies [116]. Conversely, the position of other fused genes, such as *EWSR* and *FLL1* genes in Ewing sarcoma [108] and *MLL* with a subset of commonly translocation partner genes in leukemic cells [25], change to an intermediate position compared to their original location. In contrast, the un-rearranged copies of these genes did not alter their positions [108]. Moreover, the shift in positions of the fused genes is linked to alterations in the surrounding gene density in the proximity of the fusion event [25]. For example, the *MLL:AF9* fusion gene is in an intermediate position to that of the unrearranged copies of *MLL* and *AF9* and this relocation of the fusion

locus correlated with changes in the local gene density of the fusion gene brought about by the rearrangement event [25]. These data suggest that alterations in the local gene density are important for the relocation of the translocated genetic material.

Spatial genome organization and cellular mechanisms for formation of translocations

The non-random nature of higher order spatial genome organization directly affects the cellular mechanism by which chromosomal translocations form. Two models for how broken chromosomes may undergo illegitimate joining can be envisioned (Fig. 4). The “breakage-first” hypothesis postulates that breaks formed at distant locations are able to scan the nuclear space for potential partners and are brought together to produce translocations [118,119]. A prediction from this model is that single double strand breaks (DSBs) are required to undergo large-scale motions within the cell nucleus and must be able to roam the nuclear space in search of appropriate interaction partners. In contrast, a “contact-first” model proposes that joining of broken chromosomes can only take place when the breaks are created in chromatid fibers that colocalize at the time of DNA damage. In this model only limited, local positional motion of DSBs is expected [119,120].

Evidence for both models exists. The breakage-first model was first supported by a study in *S. cerevisiae* demonstrating that intrachromosomal single strand annealing of homologous sequences occurs with the same frequency as interchromosomal translocations [121]. This observation indicates that broken ends of chromosomes are able to search for homologous regions throughout the genome implying that there is no constraint imposed on the ability genome regions to roam the nucleus in search of homologous sequences. This promiscuous rejoining of broken chromosome ends also suggests, in stark contrast to mammalian cells, a lack of chromosome territoriality in yeast [121]. Along the same lines, direct visualization of two independent DSBs revealed long-range motion of broken chromosome ends and coalescence into common repair focus [122]. While these observations suggest the ability of broken chromosome ends to migrate within the yeast nucleus, it is less clear how DSBs behave in the mammalian nucleus which contains territorially organized chromosomes. The ability of broken chromosome ends to roam the nuclear space in mammalian cells is suggested by Aten et al. showing extensive migration and subsequent clustering of different DSBs induced by α-particle source irradiation [118]. Juxtaposition of the DSB-containing chromosomes was more apparent in G1 phase of the cell cycle and depended on the repair factor Mre11, one of several factors implicated in interconnecting damaged chromatin fibers [118].

On the other hand there is evidence to support the contact-first model. Analysis of mitotic recombination in *S. cerevisiae* shows a significant increase in frequency when the two alleles are situated on the same chromosome arm, and thus by definition are in close proximity, compared to interchromosomal events [123]. In addition, observations on donor preference during *S. cerevisiae* mating type switching further supports a role in spatial proximity in recombination events [124]. MATa cells preferentially use the left-arm donor locus (HMLa) as a template for recombination with MAT, whereas the right-arm donor (HMR α) is preferred for MAT switching in MAT α. Following the motion of the two loci in living cells has revealed that the preferred donor locus in each strain is on average in closer proximity than the non-preferred one with the MAT locus and undergoes a higher degree of motion increasing the probability of interacting with MAT [124].

Furthermore, proximity seems to play a role in recombination events in mammalian cells. This is illustrated by the fact that the choice of a double strand repair partner of a broken Line 1 element amongst several integrated in different positions in the genome is highly specific and conserved in a cell population suggesting that the chosen sites are in close proximity in the

nucleus [125]. Accordingly, HR between two homologous sites, one sustaining a DSB, occurs with higher frequency when they are located in the same chromosome than in distinct chromosomes [126–128]. Consistent with this notion, in situ visualization of DSBs induced by ultrasoft X-rays showed that such DSBs do not undergo long range motions but rather remain fixed in position several hours after the induction of the damage [129]. The same positional stability was exerted by UV-laser or γ–irradiation induced double strand breaks followed for several hours after the induction of the breaks [130]. The key experiment to distinguish between the breakage-first and the contact-first model will be to visualize the behavior of single DSBs in living mammalian cells and to ask whether they are able to roam the nuclear space or whether they are constrained at the site of breakage. The technology to perform such experiments is now available and the result will represent a major step forward in our understanding of the cell biology of chromosomal translocations.

Conclusions

It is becoming increasingly evident that the genome is highly and non-randomly spatially organized within the interphase nucleus and that this organization is linked to the correct expression of the genome as well as to the understanding how genomic instability develops. Studying how and why the genome is spatially organized has given us valuable insights into how translocations are formed. The reverse is also true; the analysis of the positions of the genomic instability products is providing important clues as to the rules governing the higher order spatial organization of the genome in its normal physiological state. Since the position of a gene appears to be linked to its expression behavior, a critical next step will be to address whether the mislocalization of fusion genes leads to alterations in gene expression of the surrounding genes, and thus whether translocations have other pathological effects apart from the generation of detrimental fusion proteins. The study of these types of effects of spatial genome organization has literally added a new dimension to our understanding of chromosomal translocations.

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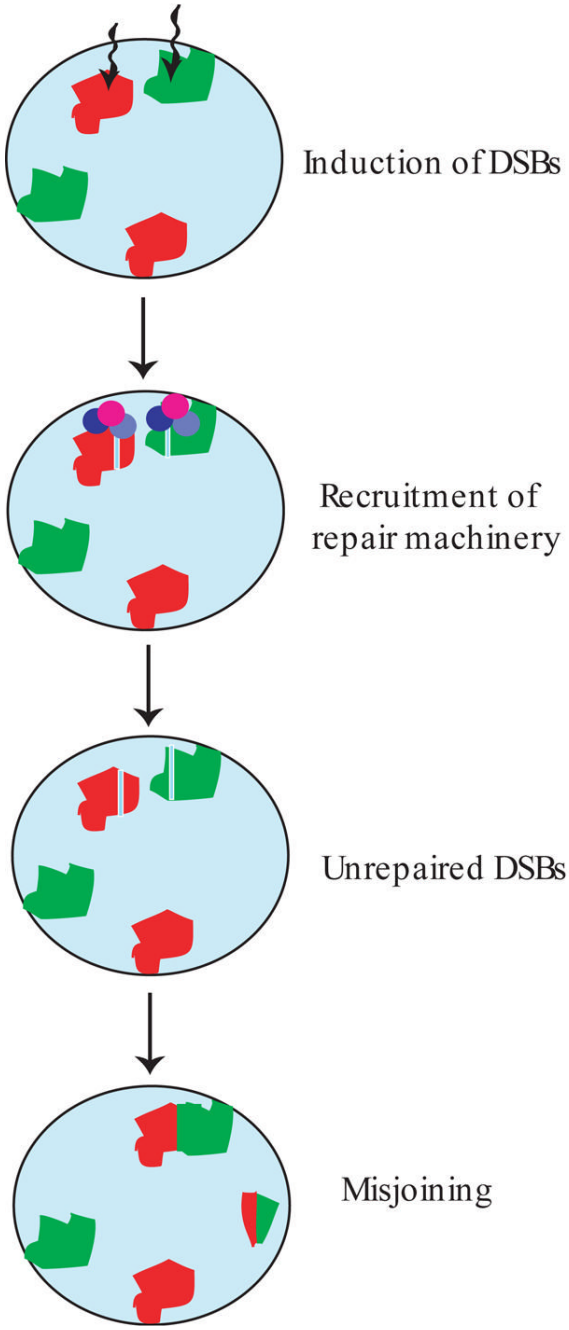

Figure 1. Formation of chromosomal translocations is a multi-step process

Chromosomal translocations form after double strand breaks (arrows) often inflicted by radiation or genotoxic chemicals. The repair machinery (purple, blue) is recruited to the double strand breaks, but if it fails to repair the damaged chromosome territories (red, green), they undergo illegitimate misjoining to form chimeric chromosomes.

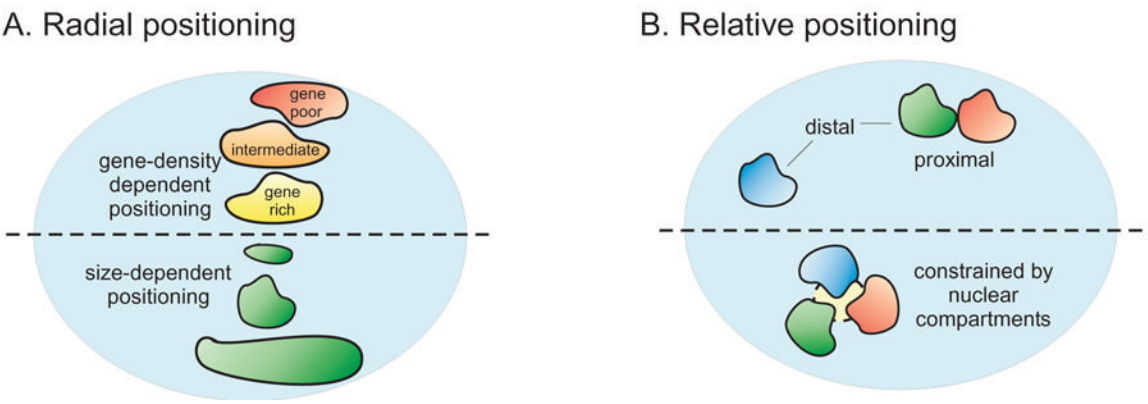

Figure 2. Spatial organization of genomes

The non-random arrangement of chromosomes and genes can be described as (A) radial position relative to the nuclear center or (B) relative position with respect to other chromosomes or genes. (A) The radial position of chromosomes has been correlated with either gene density (top) or chromosome size (bottom). (B) Chromosomes and genes may either be proximally or distally located relative to each other (top) and their position may be constrained by intranuclear structures such as the nucleolus (yellow).

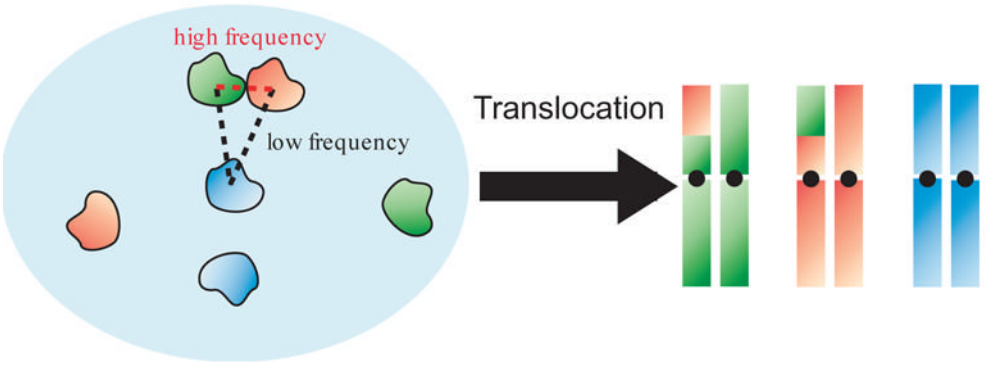

Figure 3. Spatial proximity in the formation of translocations

Increasing evidence suggests a link between relative spatial positioning of chromosomes and their frequency of translocation. Proximally positioned chromosome (red, green) undergo translocations at a higher frequency than distally positioned chromosomes (blue).

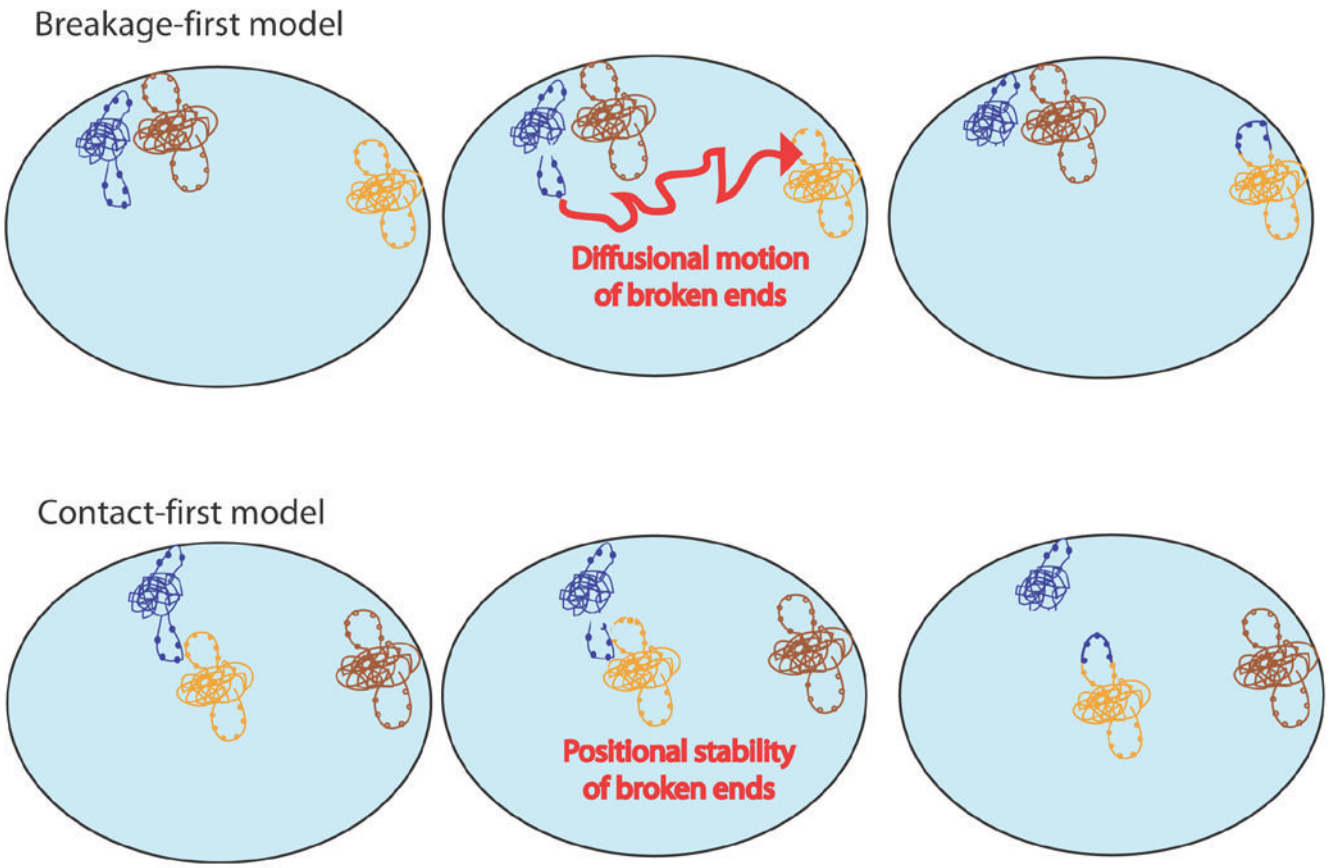

Figure 4. "Breakage-first" and "Contact-first" models for formation of chromosome translocations

(top) Breakage-first: Chromosomal translocations may either form by joining of DSBs in distantly located chromosomes. In this model the broken chromosome ends are able to diffuse over large distances to roam the nuclear space for possible translocation partners. (bottom) Contact-first: Translocations may preferentially form between already proximally positioned genome regions in which breaks occur.