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Epigenetic Changes Associated with Chromosomal Translocation in Leukemia

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1. Introduction

Chromosome translocation reflects an abnormality caused by rearrangement of DNA fragments between non-homologous chromosomes. These rearrangements can be visualized by cytogenetic analysis of affected cells. Non-random chromosomal translocations are frequently associated with a variety of cancers, particularly hematological malignancies and childhood sarcomas although recent evidence demonstrates that such translocations are also common in epithelial tumors (Aplan, 2006; Mitelman et al., 2005). Initially considered as random events that get selected, it has become increasingly apparent that chromosomal translocations are influenced by cell type, cell stage and genomic context. Most commonly, these non-random chromosomal translocations are associated with specific hematopoietic cell types. Such chromosomal translocations are commonly used as diagnostic tool and are increasingly utilized to guide therapeutic decisions. Although, the mechanism that causes chromosomal translocations remains largely unknown, it is commonly accepted that they arise from DNA double strand breaks (DSBs) that are misrepaired (Aplan, 2006; Digweed and Sperling, 2004; Betti et al., 2003; Povirk, 2006). For a translocation to occur there are several mechanistic factors required: First there needs to be at least two DSBs in different chromosomes. Second the DSBs must arise close enough both in three-dimensional space and in time. Finally, DNA repair pathways must be available to join the two broken DNA fragments to form the translocation. It is estimated that everyday a normal cell in our body is exposed to approximately 20,000 DNA damaging events (Ames and Shigenaga, 1992). A major source of DNA damage is oxygen free radicals; however there are other endogenous and exogenous sources of DNA damage such as replication, transcription, and genotoxic stress. All of these processes can induce DSBs, but for two DNA fragments to be joined they must necessarily come in close proximity of each other (less than 1.3μm) (Chen et al., 1996;

Misteli, 2010). These requirements are accommodated in two competing models to explain DSBs proximity in the nucleus: the position first and the breakage first (Figure 1). The position first model suggests that the DNA regions involved in the translocation are in close proximity before DSBs generation. Support for this model comes from the observation that translocation frequencies differ among tissues and is paralleled by tissue-specific organization of the involved chromosomes (Mitelman et al., 2007). For example, the frequency of c-myc translocations to IgH, Igκ, or Igλ locus in Burkitt's lymphomas correlates with reciprocal distance (Roix et al., 2003). In contrast, the breakage first model proposes that DSBs are produced far apart and then move into close proximity to be repaired. In yeast for example, independent DNA lesions move and colocalize in repair factories (Lisby et al., 2003a; Lisby et al., 2003b). In mammalian cells experimental evidence, ranging from no or limited movement to an extensive movement and clustering of these DSBs breaks, provides support for both models (Kruhlak et al., 2006).

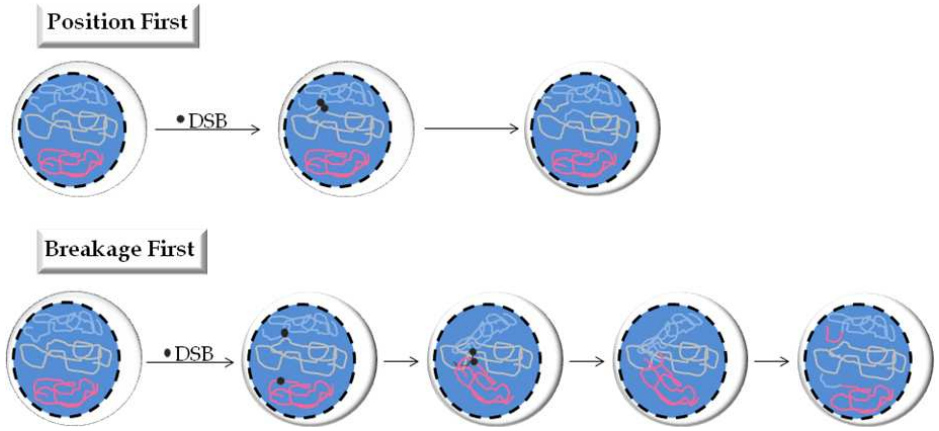

Fig. 1. **Models for Chromosomal Translocation Formation.** In the position first model two DSB that are nearby in the nuclei can be erroneously repair and give rise to a chromosomal translocation. In the breakage first model two DSB that are far apart in the nuclei are brought close together, probably to be repaired, resulting in a translocation.

In eukaryotic cells, DSBs are repaired by two different repair pathways: homologous recombination (HR) and non-homologous end joining (NHEJ) (Figure 2). HR is a relative error-free repair pathway that required information from a template sequence to repair the damage. This pathway is active during S and G2 phases of the cell cycle when the sister chromatid template is easily available and it can also take place during both mitosis and meiosis. NHEJ is a template independent DSBs repair mechanism. It is an error-prone pathway active throughout the cell cycle and the most commonly used DBSs repair pathway in multicellular eukaryotes. NHEJ is subdivided in classical (C-NHEJ) and alternate (A-NHEJ). The C-NHEJ is responsible for individual intrachromosomal DSBs repair in an homology independent manner, while A-NHEJ works in a micro-homology dependent manner, is more error-prone and seems to be the primary pathway responsible for the generation of chromosomal translocations (Simsek and Jasin, 2010; Boboila et al., 2010).

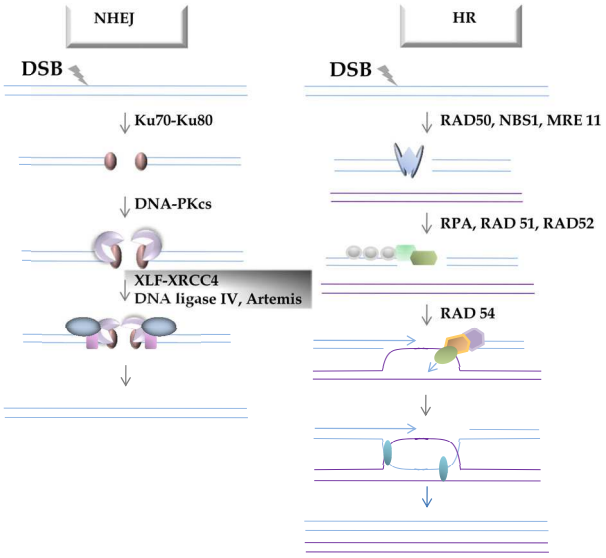

Fig. 2. **DNA Double Strand Break Repair Pathways**. Schematic representation of the two main DNA repair pathways: non homologous end joining (NHEJ) and homologous recombination (HR). The key regulatory proteins involved at multiple stages of each pathway are indicated.

Most of the chromosome translocations that have been analyzed to date show no consistent homologous sequences at the breakpoints regions. However, several structural features like DNase I hypersensitivity, topoisomerase II cleavage sites, DNA fragile sites and matrix or scaffold attachment regions (MARs or SARs) often colocalize with the mapped breakpoints. These observations suggest that chromatin organization may play a role in generation of translocations (Zhang et al., 2002; Strissel et al., 1998; Tanabe et al., 1996; Felix et al., 1995; Stanulla et al., 1997; Zhang and Rowley, 2006). Moreover, chromatin organization also has a prominent role in DNA repair process and therefore may influence both the selection of a DNA repair pathway and the eventual outcome of the DNA repair (Fernandez-Capetillo et al., 2004; Verger and Crossley, 2004; Tsukuda et al., 2005; Murr et al., 2006; Falk et al., 2008; Falk et al., 2010; Misteli and Soutoglou, 2009). Here we will focus on the current knowledge of the role that genomic structural features may have on formation of chromosomal translocations. Particularly we will analyze epigenetic marks associated with chromosome breakpoint regions and unusual DNA conformations among other structural features that may alter genomic structural stability.

2. Higher-order genome organization and translocations

The higher order organization of genomes architecture during interphase of the cell cycle forms chromosomes territories; which are defined as the nuclear space occupied by the DNA of a given chromosome (Cremer and Cremer, 2001; Cremer et al., 2006; Meaburn and Misteli, 2007; Misteli, 2007). Individual chromosomes are organized into open and closed chromatin domains that occupy different spatial compartments. A similar concept of

nonrandom nuclear positioning applies to single loci and may be relevant to their translocation potential. For example the BCR and ABL genes, located on chromosomes 9 and 22, whose translocation leads to formation of a fusion protein involved in leukemia, are located in close proximity in normal hematopoietic cells at much higher frequency than would be expected based on a random distribution (Lukasova et al., 1997; Neves et al., 1999; Bartova et al., 2000). The same is true for the human chromosomes 12 and 16, which are frequently translocated in liposarcoma and are found in close proximity in differentiated adipocytes (Kuroda et al., 2004). Similarly, the frequency of c-myc translocations to IgH, Igκ or Igλ locus in Burkitt's lymphomas correlates with reciprocal nuclear distance. However, it is important to note that clonal oncogenic translocations in tumors are highly selected and therefore cannot be used to unequivocally determine the actual translocation frequency. More unbiased examinations may yet reveal translocations between loci that are not frequently in close proximity. The proximity of two particular loci within the interphase nucleus can be cell-type or tissue-specific. In this context, substantial colocalization of IgH and Igλ occurs in activated splenic B cells but not in embryonic stem cells or thymocytes (Wang et al., 2009). Notably, colocalization with IgH is not a characteristic of the entire chromosome 16 on which Igλ locus resides. In fact, about 15Mb sequences on either side of Igλ do not colocalize; therefore, proximity can be determinant in the context of more narrow areas around specific genes and not with broad chromosome territories.

The internal structure of the chromosomal territories is poorly understood but most probably is formed by a network of looping chromatin fibers. This relatively open structure allows access to gene regulatory factors while simultaneously protect the DNA from the continuous attack of damaging agents. Supporting this view, data from several different loci involved in genomic rearrangements exhibit an altered chromatin conformation in their breakpoint regions. For example, the MLL gene exhibits a strong topoisomerase II cleavage site near exon 12 where genomic breakpoint from therapy related AML (t-AML) patients and infant leukemia patient with MLL translocation have been mapped. Moreover, the same region also exhibit hypersensitivity to DNaseI and is cleaved by S1 and Mung Bean nuclease which specifically recognize and cleave single-strand regions in supercoiled DNA (Strissel et al., 1998; Felix et al., 1995; Stanulla et al., 1997). These features however are not exclusive to MLL gene, as topoisomerase II and DNaseI hypersensitive sites has also been found at the breakpoint regions of AF9, BCL, ABL, *RUNX1*, ETO and CBP among other genes (Greaves, 1996; Zhang et al., 2002; Strissel et al., 1998; Aplan et al., 1996; Relling et al., 1998; Strick et al., 2006; Sperry et al., 1989). However, not all breakpoint regions identified to date for genes involved in translocations colocalize with either topoisomeraseII cleavage sites or DHS, suggesting that other chromatin structural properties maybe involved in determining the location of chromosome breakage.

3. Chromatin organization and DNA repair

Higher order chromatin structure is not only important for global susceptibility of DNA to damage but may also be relevant for DNA repair. It is well documented that the earliest response to a DSB is the phosphorylation of the histone H2A variant histone H2AX on its C-terminus. Within seconds of DSB formation, the phosphorylated H2AX is present over surrounding regions, spanning thousand to millions of base pairs (Rogakou et al., 1998; Rogakou et al., 1999; Leatherbarrow et al., 2006; Kinner et al., 2008). H2AX is not present

in lower eukaryote, but the domain that is phosphorylated in response to DSB is present in the C-terminus of other H2A-family members like H2A in S. cerevisiae and H2AZ in D. melanogaster (Downs et al., 2007). Although loss of H2AX does not abrogate DNA-damage checkpoints or repair, it impairs the joining of programmed DNA lesions during immunoglobulin class-switch recombination. These observations suggest that chromatin modifications at a distance are required for bringing together the DNA ends (Petersen et al., 2001; Reina-San-Martin et al., 2003). Moreover, failing to rejoin these programmed DSB in the absence of H2AX result in frequent chromosomal abnormalities (Franco et al., 2006; Ramiro et al., 2006). In addition to phosphorylation, H2A is also modified by acetylation in its N-terminal tail by NuA4, a histone acetyl transferase (HAT). Acetylation seems to be important for the ability of cells to survive after DNA damage (Bird et al., 2002).

The findings that DSB induces a rapid local decrease in the density of the chromatin fiber (Kruhlak et al., 2006) and that nearby nucleosomes are repositioned (Shim et al., 2007) support the idea that ATP-dependent chromatin remodeling factors have an early role in the DNA damage response. Indeed, several reports have demonstrated that the ATP-dependent chromatin remodeling complexes RSC (Remodels the Structure of Chromatin), SWI/SNF (SWItch/Sucrose NonFermenting), INO80 (INOsitol requiring) and SWR (Sick with Rat8 ts) are recruited to DSB, although at different time after the DNA damage. The first complex recruited to the DSB is RSC, which mobilize the nucleosomes near a DSB to new positions. Interestingly, in the absence of RSC the phosphorylation of H2AX is delayed. The other three chromatin remodeling complexes, SWI/SNF, INO80 and SWR, are enriched at sites of DSB at later times suggesting that they are not required for the initial detection or signaling of the DSB, but for the subsequent stages of the repair process.

Additionally, acetylation of conserved residues in the N-terminal tails of H3 and H4 has been found to contribute to both homologous and non-homologous recombination processes. For example, in mammalian cells, the TIP60 and the HAT cofactor TRRAP (transformation/transcription-domain associated protein) are recruited to sites of DSB, where they induce acetylation of H4 and facilitate homologous recombination. Similarly, another HAT, MOF (also known as MYST1) contributes to irradiation-induced acetylation of H4 at lysine 16 (H4K16). Defects on H3 and H4 acetylation have been linked to sensitivity to ionizing radiation and alteration in cell cycle checkpoints (Gupta et al., 2005). Complexes catalyzing the reverse process, i.e. histone deacetylation, have been shown to be enriched at late times at the DSB regions. If the acetylation of histones in the vicinity of DNA damage facilitate the repair process, then it is possible that the role of these histone deacetylase complexes might be to restore the chromatin to its original state once the DNA has been repaired.

4. Histone post-translational modifications in chromosomal rearrangements

The role of histone modifications on genomic rearrangements has been extensively studied in the V(D)J recombination process. This assembly process depends on a series of site-specific recombination reactions that are initiated by DSBs produced by RAG1 and RAG2 complex (Bassing et al., 2002). Each rearranging gene segment is flanked by a recombination signal sequence (RSS). The recombinase complex recognizes pair of compatible RSS, introduce DSBs and then channel the reaction products to a DNA repair pathway. Aberrant

targeting of RAG proteins can produce chromosomal translocations that are associated with many forms of leukemia or lymphoma. In general, genes segments within recombinationally active loci are mark by the same histone modifications that characterize transcriptionally active genes, i.e. H3 and H4 acetylation as well as H3 trimethylation at lysine 4 (H3K4me3). More recently, it has become evident that the predominant effect of these histone modifications is to recruit the RAG complex to the RSS. In fact, RAG2 through its PHD domain specifically binds to H3K4me3 and mutations that abolish this binding results in greatly impaired V(D)J recombination activity (Liu et al., 2007; Matthews et al., 2007). Additional support for the epigenetic role on V(D)J recombination comes from the observation that in V genes H3 acetylation, although lower than in J genes, exhibit a gradient of enrichment that mirrored the rearrangement frequency. Interestingly, a reciprocal pattern is observed for the repressive modification H3 dimethylation at lysine 9 (H3K9me2) (Espinoza and Feeney, 2005; Espinoza and Feeney, 2007).

H3 trimethylation at lysine 4 (H3K4me3) is also implicated in meiotic recombination. In fact PRDM9, a zinc finger protein that catalyze the trimethylation of H3 at lysine 4, has recently been identified as a major determinant of sequence-specific meiotic recombination (Cheung et al., 2010).

Another histone modification, H3 methylation at lysine 79 (H3K79me), is associated with recombinationally active loci both in yeast and mammalian cell lines (Ng et al., 2003). Moreover, overexpression of DOT1L (a H3K79me specific methyltransferase) together with genotoxic stress and dihydrotestosterone significantly increases formation of chromosomal rearrangement involving the ETS genes, which are a distinguishing feature of prostate cancer (Kumar-Sinha et al., 2008; Lin et al., 2009).

Lin and colleagues (Lin et al., 2009), using prostate cancer as a model to study translocation mechanisms, have shown that after irradiation far more translocations are formed in androgen treated than in control cells. They also demonstrated that the translocation regions, TMPRSS2, ERG and ETV all contain binding sites for the androgen receptor (AR) near their breakpoints and that, after treatment with androgen, there is a rapid recruitment of AR to these sites. Moreover, AR recruitment induces changes in higher-order chromatin structure and epigenetic modifications establishing an open chromatin conformation characteristic of transcribed genes. Another consequence of AR binding was the recruitment of the activation-induced cytidine deaminase (AID), a key factor in somatic hypermutation (SHM) and class switch recombination (CSR) where it contributes to formation of DSB during the process of generating antibody diversity.

Additional data supporting the role of chromatin structure in genomic rearrangements comes from the analysis of the mechanisms involved in formation of t(2;5), a chromosomal translocation associated with anaplastic large cell lymphoma (ALCL). Mathas et al (Mathas et al., 2009) found up-regulation of several genes located near the ALCL translocation breakpoint, regardless of the presence of t(2;5) in the tumor. Moreover, their increased transcriptional activity promotes cell survival and repression of T-cell specific gene expression programs, both characteristics are a hallmark of ALCL (Mathas et al., 2009). Interestingly, cells isolated from ALCL patients lacking t(2;5) were more susceptible to form the (2;5) translocation than control cells. Together these data suggest that deregulation of breakpoint-proximal genes occurs before the formation of translocations and that similar to V(D)J recombination, transcriptional activity and altered chromatin structure predispose cells to chromosomal translocation.

This pattern of highly accessible chromatin structure characterized by H3 and H4 acetylation is also found at the breakpoint regions of other genes involved in translocations like MLL (Khobta et al., 2004), *RUNX1* (Stuardo et al., 2009) and ETO (our unpublished data). Using chromatin immunoprecipitation assays (ChIPs) we analyzed the chromatin structure at intrón 5 of the *RUNX1* gene, where all the translocation points for the (8;21) translocation has been mapped (Figure 3). Our results demonstrate that chromatin organization at intron 5 is completely different in HL-60 hematopoietic cells than in a non-hematopoietic cell (Stuardo et al., 2009). In fact, two distinct features mark the intron 5 in HL-60: a complete lack or significantly reduced levels of histone H1 and an increased association of hyperacetylated histone H3.

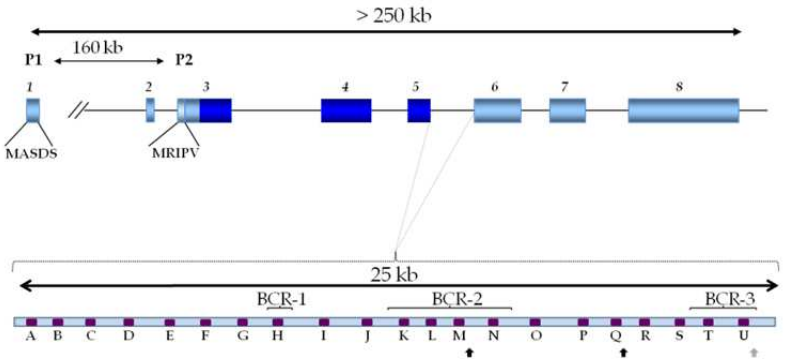

Fig. 3. **Diagrammatic representation of the *RUNX1* gene.** Top panel show the exon-intron organization of the gene as well as the two promoters that regulate its expression. Bottom panel show a magnification of intron 5 of the *RUNX1* gene. The three breakpoint clusters (BCR) are indicated as well as the amplification fragments analyzed by ChIP assays (purple blocks labeled A-U). Dark gray arrows indicate topoisomerase II sites and light gray arrow DNase I hypersensitive site.

The decreased association of histone H1, may indicate an overall enhanced accessibility and hence an increased availability to nucleases or DNA damaging agents. Notably, the region where the DNaseI hypersensitive site has been mapped presents one of the lowest rates of association of histone H1 in myeloid HL-60 cells (Figure 4, region U). Although the complete intron 5 is enriched in acetylated histone H3 (Figure 5), the chromatin organization is not homogeneous throughout the intron, suggesting that particular regions of intron 5 may play a regulatory role in transcription, subnuclear localization or compaction of the *RUNX1* gene. Interestingly, the chromatin organization at intron 5 resembles the chromatin structure adopted by the V(D)J gene segment. During V(D)J recombination, gene segments encoding the variable regions for immunoglobulins and T-cell receptors (TCR) are recombined and assembled in a new configuration. The same recombinase is present in both T and B cells, however recombination of immunoglobulins loci happens only in B cells while TCR loci rearrange only in T cells. Targeting of recombinase activity to specific gene segments is controlled largely by changes in chromatin accessibility in a spatio-temporal manner, and acetylation of histone tails has been shown to be a key event in this process. In fact, acetylation of histone H3 or H4 is elevated in B or T cell type at gene segments that can recombine, and reduced at segments that do not undergo recombination (Maes et al., 2006;

Maes et al., 2001; McMurry and Krangel, 2000). Moreover, hyperacetylation induced by inhibitors of histone deacetylase complexes (HDAC) rescues recombination defects caused by the elimination of extracellular signals that induce recombination (Durum et al., 1998). These studies suggest that histone hyperacetylation precedes recombination by opening chromatin and promoting access to the recombinase. Thus regardless of the molecular mechanism involved, it seems that an open chromatin conformation is a common requirement for a translocation to take place (Figure 6).

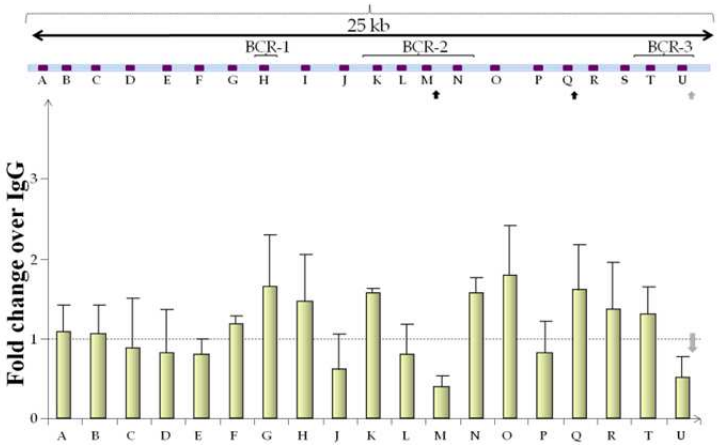

Fig. 4. **Intron 5 of *RUNX1* gene exhibit decreased association of histone H1.** Chromatin immunoprecipitation assays were performed with formaldehyde cross-linked chromatin isolated form HL-60 cells. Real-time PCR amplification of ChIP-DNA is shown as fold change over IgG in bar graph for immunoprecipitation with anti H1 antibody. Light gray arrow indicates DNaseI hypersensitive site.

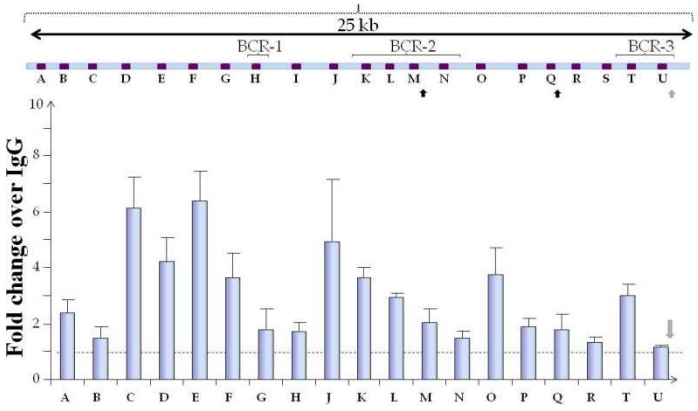

Fig. 5. **Intron 5 of *RUNX1* gene is enriched in acetylated histone H3.** Chromatin immunoprecipitation assays were performed with formaldehyde cross-linked chromatin isolated form HL-60 cells. Real-time PCR amplification of ChIP-DNA is shown as fold change over IgG for immunoprecipitation with anti acetylated H3 antibody. Light gray arrow indicates DNaseI hypersensitive site.

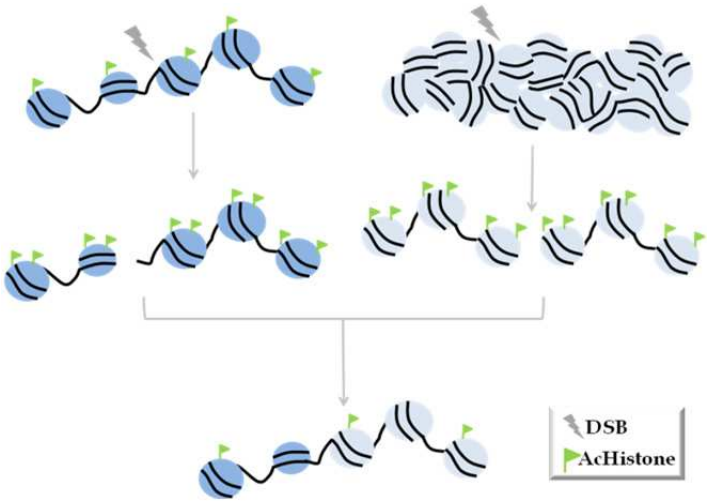

Fig. 6. **Summary of chromatin structure and histone modification in the regions involed in chromosomal translocation.** DSB can occur either in euchromatin or heterochromatin, but they must arise close enough both in time and in space to give rise to a chromosomal translocation. In both cases the regions surrounding the DSB will exhibit an open chromatin conformation either due to its presence in euchromatin or as result of the DNA repair process.

5. DNA conformation

In addition to the classical B-DNA structure described by Watson and Crick (Watson and Crick, 1953), more than 10 different DNA conformations are documented to date. These alternative DNA conformations include Z-DNA, hairpins/cruciforms, H-DNA (triplexes), slipped DNA and sticky DNA among others (Felsenfeld and RICH, 1957; Wang et al., 1979; Lilley, 1980; Panayotatos and Wells, 1981; Lyamichev et al., 1983; Sen and Gilbert, 1988; Mirkin, 2008). Several studies have shown that these non-canonical DNA structures affect DNA replication and transcription, and contribute to genome instability. For example non-B forming sequences located in c-MYC and BCL-2 genes localize with translocation breaking points. Studies have demonstrated that the H-DNA structure from the human c-MYC gene can induce DSBs in mammalian cells and stimulate genomic instability on mouse chromosomes in transgenic mice (Wang et al., 2008). However, the same sequences are not mutagenic in bacteria, suggesting a requirement for host-specific trans-acting factors to generate genomic instability.

Palindromic sequences, including palindromic AT-rich repeats (PATRRs), have the potential to form stem-loop structures by intrastrand base pairing within single-stranded DNA. In fact, PATRRs mapped on chromosome 22q11 and other chromosomes, such as 11q23 and 17q11, were found to cause non random chromosomal translocation in sperm cells in the general population (Kato et al., 2006) as well as in cell culture (Inagaki et al., 2009). Polymorphisms within the PATRRs affect the susceptibility to translocation *in vitro*, with longer and more symmetric PATRRs showing a stronger predisposition to translocation events (Kato et al., 2006; Kogo et al., 2007; Inagaki et al., 2009). Therefore, it has been

proposed that the secondary structures adopted by palindromic DNA induce a greater susceptibility to DSBs thus leading to translocations in human (Kurahashi et al., 2000).
There have also been identified chromosomal fragile sites, which are genomic regions especially susceptible to DNA breakage. These fragile sites are non-random specific loci that are stable under normal conditions, but under conditions of partial replication stress can form visible gaps or breaks in metaphase chromosomes (Durkin and Glover, 2007; Richards, 2001). Many different studies have established a connection between DNA fragile sites and the formation of cancer-specific genome rearrangements. However, only recently there has been direct evidence linking breakage at DNA fragile sites to the formation of a cancer specific translocation. Using as model RET/PTC rearrangements; which are commonly found in the papillary thyroid carcinoma (PTC) and in all cases result in the fusion of the tyrosine kinase domain of RET (rearranged in transformation) to the 5′ portion of various unrelated genes (Nikiforov, 2008) Gandhi et al (Gandhi et al., 2010) demonstrate that fragile site-inducing chemicals can create DNA breaks within the RET/PTC partner genes and ultimately lead to the formation of RET/PTC rearrangements. Moreover, aphidicolin induced DNA breaks at RET gene were located within intron 11, which is the breakpoint cluster region identified in thyroid cells. Clinical studies have shown that two different rearrangements, RET/PTC1 and RET/PTC3, are more frequent in sporadic and radiation-induced tumors respectively (Fenton et al., 2000; Fugazzola et al., 1995; Nikiforov et al., 1997; Motomura et al., 1998). Interestingly, treatment of cells with aphidicolin (APH), 2-aminopurine (2-AP) and 5-bromodeoxyuridine (BrdU) resulted in the generation of RET/PTC1 but not RET/PTC3 suggesting that sporadic PTC tumors may result from breakage at fragile sites. Although no consensus sequence have been identified in the fragile sites until now, the majority of them can form highly stable non-B DNA structures.

6. Concluding remarks

Traditionally it has been assumed that translocations arise randomly by stochastic DSB and that enrichment of particular translocations was result of the survival advantage acquired by the cells bearing the translocation. However, more recent results suggest that breaks in the genome occur in a nonrandom fashion and that higher-order chromatin organization maybe, at least in part, responsible for the formation of recurrent translocations. Although, significant progresses have been made in understanding formation of chromosomal translocation, particularly in the area of VDJ recombination, many more questions remain unanswered. For instance, it is still not known what proteins or pathways are involved in formation and maintenance of structure at the breakpoint regions, if these regions have a role in some cellular processes and what signaling pathways or environmental conditions promote chromosomal translocations. The response to these basic research questions may greatly improve diagnostic and therapeutic and may help to develop preventive measures for disorders associated with genomic instability such as chromosomal translocations.

7. Acknowledgments

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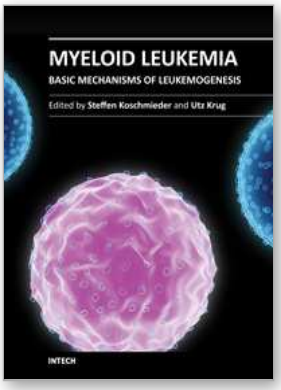

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