

## SURVEY AND SUMMARY

# How does DNA break during chromosomal translocations?

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### ABSTRACT

**Chromosomal translocations are one of the most common types of genetic rearrangements and are molecular signatures for many types of cancers. They are considered as primary causes for cancers, especially lymphoma and leukemia. Although many translocations have been reported in the last four decades, the mechanism by which chromosomes break during a translocation remains largely unknown. In this review, we summarize recent advances made in understanding the molecular mechanism of chromosomal translocations.**

### INTRODUCTION

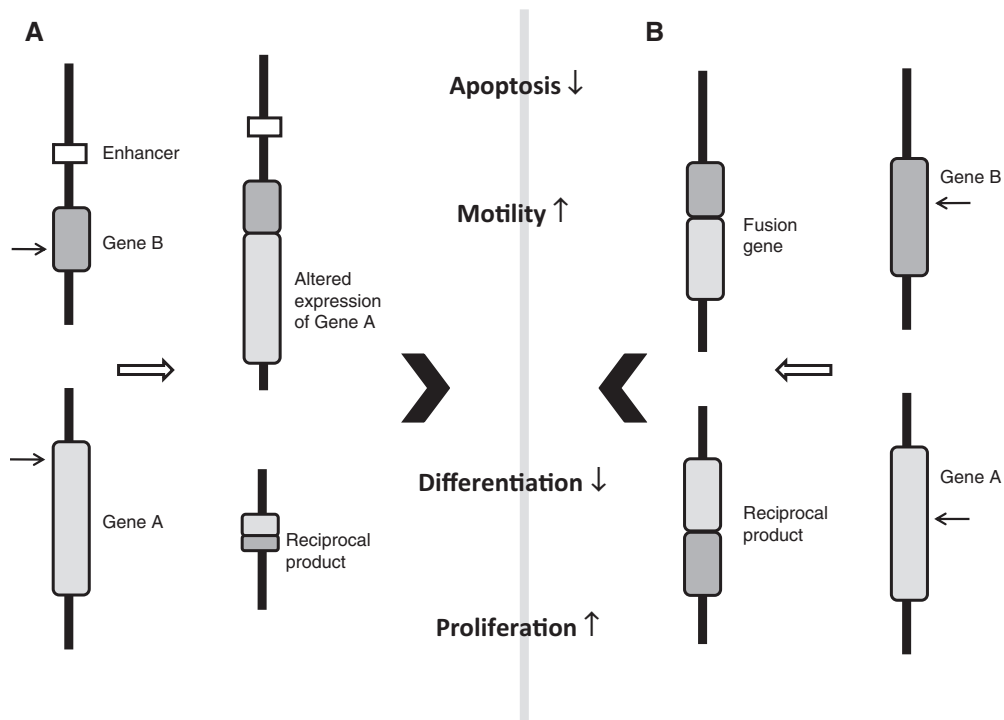
In 1914, Theodor Boveri first hypothesized that genetic aberrations could be the underlying cause of cancer (1,2). However, it took almost 50 years for the first chromosomal translocation to be discovered in any form of cancer. Nowell and Hungerford in 1961 showed the presence of a recurring chromosomal abnormality, named as the Philadelphia chromosome, in chronic myelogenous leukemia (CML) patients (3). Later it was identified as a translocation between chromosomes 9 and 22 (4). Following this, a translocation between chromosomes 8 and 14 was discovered in Burkitt's lymphoma (5). It was the first example, wherein, a chromosomal break was reported at an oncogene, *c-MYC*, and was shown to translocate to the immunoglobulin heavy chain (*IgH*) loci on chromosome 14 (6,7). Later, several other translocations were discovered and many of these were found to involve chromosome 14 as one of the partner chromosomes (8). Molecular studies of different translocations, especially cloning of the breakpoint junctions from patients, have further helped in the functional characterization of the genes involved and in understanding the mechanism of how these aberrations could cause neoplasia.

Translocations generally result from swapping of chromosomal arms between heterologous chromosomes

and hence are reciprocal in nature (Figure 1) (8,9). DNA double-strand breaks (DSBs) are prerequisites for such translocations, although little is known about their generation. Chromosomal translocations ultimately result in the deregulation of key cellular proteins, especially those coded by proto-oncogenes and tumor suppressor genes, which are critical functional regulators of the cell (10–12). This can happen in two ways. In the first case, the entire coding region of a gene can be juxtaposed to a transcriptionally active promoter or enhancer element of another gene on a different chromosome, thereby leading to an abnormal expression of the translocated gene (Figure 1A). In the second scenario, the translocation results in the formation of a unique fusion gene, which in turn codes for an activated form of the protein that affects the normal cellular physiology (Figure 1B). Alternatively, some translocations can inactivate tumor suppressor genes. For example, gene fusions such as *TEL1-AML* can repress the expression of *TEL1*, a tumor suppressor gene (13–15).

Among different cancers, mostly lymphoma and leukemia are characterized by the presence of unique chromosomal translocations (10–12,16). The t(14;18) is one of the most well studied chromosomal translocations and is characteristic of follicular lymphoma (FL) (17) (Table 1). It results in the overexpression of anti-apoptotic protein, *BCL2*, which provides the cells a survival advantage and a probability to acquire further deleterious mutations, ultimately leading to cancer (18,19). On the other hand, the t(9;22) translocation found in CML results in the deregulation of *ABL* gene present on chromosome 9 by *BCR* gene promoter on chromosome 22. This results in the formation of a unique in-frame fusion mRNA and protein (3,4,8,20–22) (Table 1). Examples of other major chromosomal translocations detected in patients include *BCL6* translocation involving chromosome 3 in diffuse large B-cell lymphoma, t(11;14) in mantle cell lymphoma and many others (7,23–30) (Table 1). In addition, interstitial deletions are also frequently found in T-cell neoplasia patients. Activation of the human *NOTCH1* protein

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**Figure 1.** Consequences of chromosomal translocations. A chromosomal translocation can broadly result in either juxtaposition of oncogenes near promoter/enhancer elements (A) or gene fusions (B). Both results in the deregulation of the expression of genes affecting various cellular and physiological processes like proliferation, differentiation, motility and apoptosis. Arrows at the genes depict the sites for double-strand break formation. Normally, the reciprocal joining does not lead to any functional product.

**Table 1.** Most common chromosomal translocations in cancer

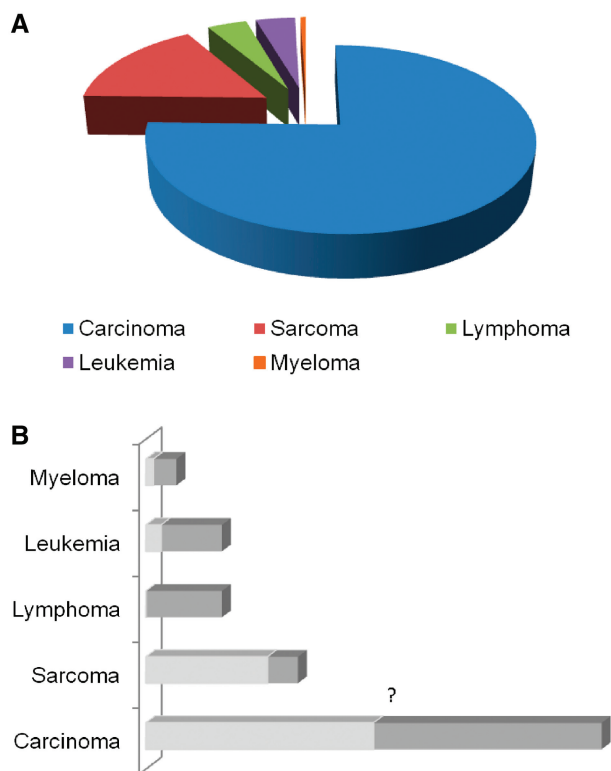
Translocation	Genes	Type of cells	Name of cancer
t(14;18)	<i>BCL2</i>	B-cells	Follicular lymphoma
t(8;14)	<i>c-MYC</i>	B-cells	Burkitt's lymphoma
t(3;14)	<i>BCL6</i>	B-cells	Diffuse large B-cell lymphoma
t(9;22)	<i>BCR</i> and <i>ABL</i>	Myeloid cells	Chronic myeloid leukemia
t(11;14)	<i>CCND1</i>	B-cells	Mantle cell lymphoma
t(10;14)	<i>HOX11</i>	T-cells	T-Acute lymphoblastic leukemia
t(2;5)	<i>NPM</i> and <i>ALK</i>	B-cells	Anaplastic large cell lymphoma
t(21;7)	<i>TMPRSS2</i> and <i>ETS</i>	Epithelial cells	Prostate carcinoma
t(12;15)	<i>ETV6</i> and <i>NTRK3</i>	Epithelial cells	Breast carcinoma
t(11;22)	<i>EWS</i> and <i>FLI-1</i>	Mesenchymal cells	Ewing's sarcoma

occurs upon truncation due to the removal of the extracellular domain, during t(7;9) translocation and this is observed in around 10% of T-cell acute lymphoblastic leukemia cases (31). Interstitial deletion leading to the juxtaposition of the *SCL* and *SIL* genes on chromosome 1 is another common genetic abnormality found in T-cell leukemia patients (32,33).

Earlier it was believed that chromosomal translocations are primarily restricted to the lymphoid cancers and a few sarcomas, despite carcinomas accounting for approximately 80% of all cancers (Figure 2A) (34,35). Around 90% of all lymphomas and more than 50% of the leukemias have been reported to possess translocations (36). Interestingly, recent studies have shown that carcinomas possess chromosomal translocations, making them very useful as biomarkers (37,38) (Figure 2B). This discovery

has been mainly due to the development of novel techniques, which have overcome the limitations faced earlier. However, unlike the hematological malignancies, where mostly the oncogenes juxtapose to the immunoglobulin loci, these cancers predominantly possess gene fusions (39–42) (Figures 1 and 2). This breakthrough has sparked a new interest in the search of translocations in various types of carcinomas.

There have been many attempts to decipher the reasons for fragility of chromosomes during translocations. However, the exact mechanism of most of the translocations is still elusive, except in very few cases. This review largely describes the various mechanistic aspects of generation of chromosomal translocations, focusing on the causes of DNA breaks at specific, confined regions of the genome.



**Figure 2.** Frequency of chromosomal translocations in different cancers. (A) Distribution of different classes of cancers in terms of their incidence. Carcinoma constitutes the maximum percentage among all types of cancer followed by sarcoma. Leukemia and lymphoma together constitute around 8% of all cancers. (B) Percentage of occurrence of translocations within the different classes of cancer. Almost all lymphomas harbor translocations, whereas only one-fourth of all sarcomas (183) have been reported to possess the same. Gene fusions in carcinomas have been discovered recently. Although recent investigations suggest its presence in many carcinomas, the exact percentage of carcinoma bearing translocations is not yet clear (denoted by "?").

## RAG-MEDIATED CHROMOSOMAL TRANSLOCATIONS

### RAG cleavage based on consensus sequences

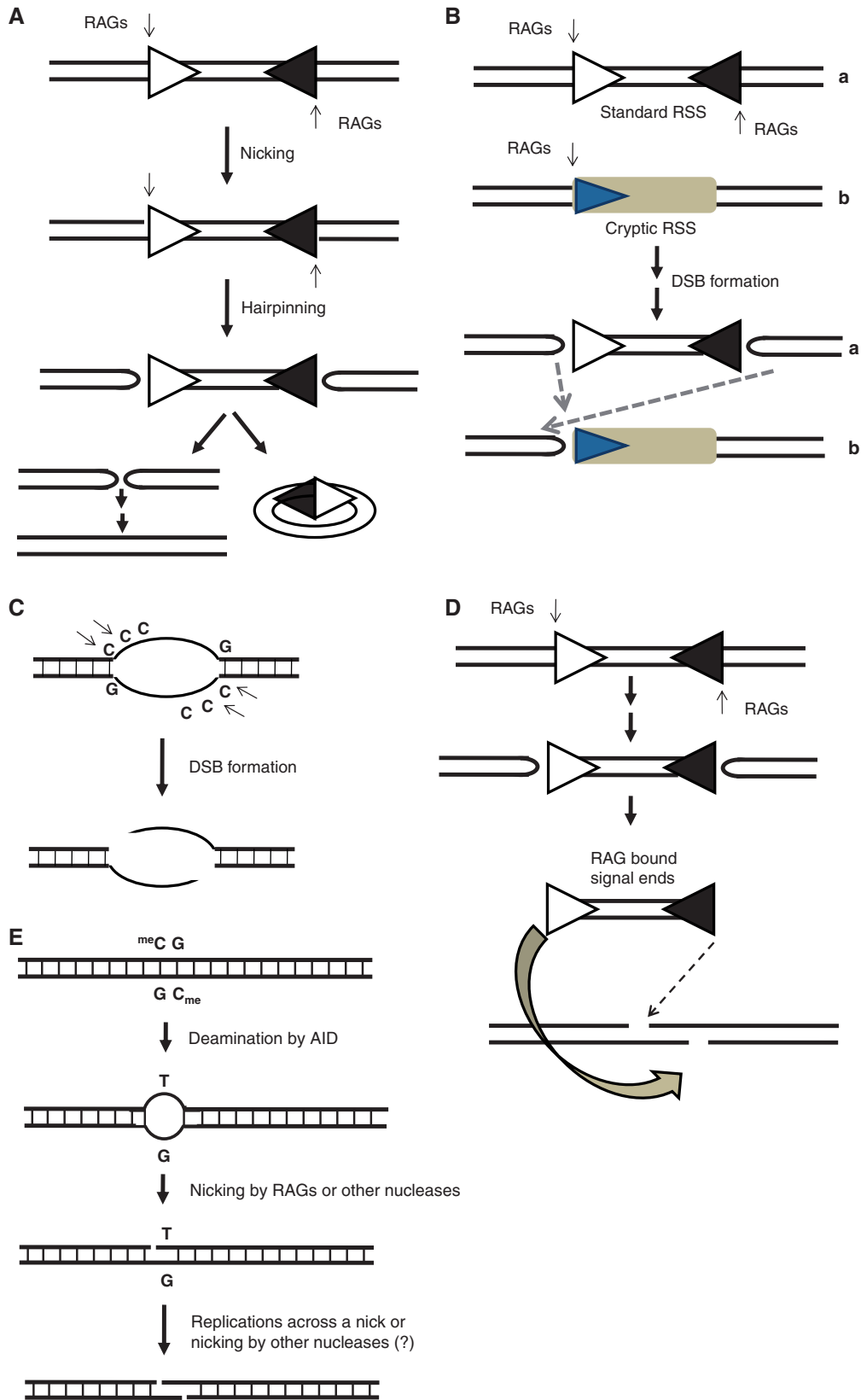
V(D)J recombination is responsible for the immense diversity of antibodies and T-cell receptors (TCRs) generated during the development of B and T lymphocytes (43,44). It is a site-specific recombination leading to the formation of variable region exon of the antigen receptor by rearranging the variable (V), diversity (D) and joining (J) subexons (45). The recombination activating genes, RAG1 and RAG2 (RAG complex), recognize the recombination signal sequences (RSSs) flanking the V, D and J subexons (46) (Figure 3A). RSSs are of two types, the 12 and 23 RSSs, based on the length of the spacer region interspersing the conserved heptamer and nonamer sequences. After binding to the RSS, RAGs induce nicks which are later converted to DSBs by DNAPKcs–Artemis complex through a hairpin intermediate (Figure 3A). Finally, the coding ends are joined by the non-homologous end joining (NHEJ), which is one of

the major DSB repair pathways in higher eukaryotes, to produce a functional antibody/TCR (47,48).

Interestingly, in many chromosomal translocations, the most common partner is the *IgH* locus on chromosome 14 (11,26,49). Analyses of the translocation breakpoints suggest that V(D)J recombination may play a key role in the generation of such translocations, especially in the case of chromosome 14 (50,51) (Figure 3B). The breaks in the partner chromosomes are generally nonrandom. Genes such as *LMO2*, *TTG1*, *SIL* and *SCL* have been shown to possess cryptic RSS elements, which are misrecognized and cleaved by RAGs within cells, resulting in translocations or interstitial deletions (between *SIL* and *SCL*) (Figure 3B) (52–54). In addition, RAG-induced single-strand breaks have been suggested to stimulate homologous recombination (HR) or other error-prone DNA end joining pathways (55). The presence of mutations in RAGs that can destabilize postcleavage complex formation may facilitate generation of translocations by promoting aberrant repair (55,56). In addition, the presence of cryptic heptamers at translocation sites in which the critical nucleotides (4th and 5th positions) differ from the canonical heptamer may be pathogenic due to the formation of a weak postcleavage complex, thereby releasing the DSB ends prematurely (56). These unprotected DNA ends can then be repaired through pathways like alternative NHEJ (discussed below). Consistent with this, it has been speculated in the past that RAG-initiated DSBs might initiate HR between similar sequences located on non-homologous chromosomes and subsequently result in translocations (57,58). However, evidence for this hypothesis is still lacking.

### RAG cleavage based on DNA structures

Besides being a sequence-specific nuclease, recent studies have shown that RAGs can act as a DNA structure-specific enzyme (59–62). Studies have demonstrated that purified RAGs can cleave overhangs and flap structures within the DNA, *in vitro* (59,60). In addition, the major breakpoint region (MBR) at the 3' UTR in *BCL2* gene on chromosome 18, involved in t(14;18) translocation (18,19), was shown to exist in a non-B DNA structure, which can be cleaved by the RAG proteins. This was based on both *in vitro* cleavage assays using purified RAGs on plasmid DNA as well as *ex vivo* studies using extra-chromosomal DNA substrates in pre-B cells expressing endogenous RAGs (61). By *in vitro* studies, RAGs have also been shown to cleave other non-B DNA structures such as bubbles and heterologous loops (62,63). More recently, it was demonstrated that RAG cleavage on such non-B DNA structures is not only dependent on structure, but also influenced by the sequence (64). Cytosines were preferentially cleaved, when present at the single-stranded regions of altered DNA structures (Figure 3C). However, RAGs could not cleave heteroduplex DNA when adenines or guanines were present. A consensus sequence motif has also been proposed for such RAG-induced breaks at single-/double-strand DNA transitions (Figure 3C) (64). Thus, this new property of the RAG complex could explain some facets of genomic



**Figure 3.** Mechanism of RAG mediated translocations. **(A)** Standard V(D)J recombination is initiated by RAG induced nicks (indicated by thin arrows) at the 5' of the heptamer present within the standard recombination signal sequences (open triangle represents 12 RSS while closed triangle depicts 23 RSS). This is followed by hairpin formation using a transesterification reaction. The hairpins are further resolved and DSBs are joined using NHEJ protein machinery resulting in the formation of a coding joint. The free signal ends circularize to give the signal joint. **(B)** RAGs, in addition to nicking the standard recombination signals (a), can misrecognize sequences resembling standard RSS on other genes (b) and induce nicks,

(continued)

rearrangements including chromosomal translocations specific to lymphoid tissues, which were inexplicable thus far.

The exact nature of the non-B DNA structure at the *BCL2* MBR has been an area of active research (65,66). A recent study provided evidence for the formation of a parallel G-quadruplex structure at peak I and its upstream region, using multiple biochemical and biophysical methods, including nuclear magnetic resonance (NMR) spectroscopy (67). Since formation of the intramolecular G-quadruplex could result in the opposite strand essentially remaining single stranded, it could explain the sodium bisulfite reactivity of this region (61). Therefore, the presence of such altered DNA structures at translocation hotspots may explain the fragility at other genomic loci. However, more studies are needed in this direction.

### Other types of RAG cleavage mechanisms

RAG proteins possess transposase activity, during which a signal end generated after RAG cleavage gets inserted into a target site, with or without the characteristic 4–5-bp duplication (Figure 3D) (68,69). It has also been shown that such insertions could be facilitated, if the target DNA contained sequences that support cruciform structure formation (70). There are studies suggesting that RAG-mediated transposition and insertion events can occur in yeast and mammalian cells (71–74). Such studies add a new dimension to the potential of the RAG proteins in mediating genomic instability. In fact, many independent studies have proposed that RAG-mediated transposition could lead to chromosomal translocations, although there is no direct evidence yet. In this regard, a study showed the reinsertion of excised signal joints by RAGs both *ex vivo* and *in vivo*, by trans-V(D)J recombination at *IgH* or *TCR* target sites (75). Although this did not appear to be a transposition event, it was dependent on RAGs and led to integration of signal joints in an episomal system. It is also proposed that cryptic RSS, present near proto-oncogenes in some T-cell leukemias, can act as hotspots for such integration events.

A new mechanism of RAG cleavage was explained recently based on the statistical correlation of presence of CpGs near some of the translocation breakpoint regions (76). The cytosines present at the CpGs, methylated or otherwise, could be a target for enzymes like activation-induced cytidine deaminase (AID), which can deaminate them, resulting in single-nucleotide mismatches. These can then be targeted by nucleases, one of which could be RAGs, thereby introducing nicks or DSBs at these sites (Figure 3E) (76). However, more

studies are required to understand this mechanism within the cells.

### *In vivo* evidence for RAG involvement in chromosomal translocations

Direct proof for the role of RAG proteins in causing translocations came from *in vivo* studies in mice lacking proteins involved in the NHEJ pathway. Although these mice cannot perform V(D)J recombination completely, they do not possess translocations, probably due to the activation of the p53-dependent checkpoint pathway. In agreement with this, mice lacking both p53 and NHEJ factors such as Ku80, Xrcc4 or Ligase IV developed lymphomas (77,78). Characterization of some of these lymphomas has shown that RAG-mediated breaks, which are unrepaired, initiate a break-induced replication, thereby leading to the translocations. These further are amplified due to the breakage–fusion–bridge mechanism (79). It was also observed that the integrity of the *Rag2* gene was essential for the development of lymphomas in such NHEJ/p53-deficient cells (58). In particular, the *RAG2* carboxy terminus was recently shown to play a critical role in the maintenance of genomic stability (80). The core *Rag2* homozygotic mice displayed a higher incidence of thymic lymphomas containing several genomic abnormalities including translocations. This observation was further supported by evidences that the periodic destruction of *RAG2* during G1 to S-phase cell-cycle transition could promote aberrant recombination in mice (81). This suggests that unresolved RAG-induced DNA DSBs caused during V(D)J recombination can lead to translocations and hence lymphomas. Deficiency of NHEJ factors such as Artemis or XLF (Cernunnos) proteins in murine embryonic stem cells were also shown to result in increased genomic instability and chromosomal translocations (82,83). Mice lacking DNA damage sensor proteins such as ATM developed thymic lymphomas, involving translocations of the *Tcr* loci (either *Tcr $\alpha$ / $\delta$*  (*Tcr $\alpha$ /d*) locus) (84–87). Recently, many such recurring translocations in thymic lymphomas were characterized in ATM-deficient mice (88). Unexpectedly, these lymphomas were found to be associated with V(D)J recombination errors in *Tcrd* and not *Tcra*, indicating that they were derived from T cells which have not attempted *Tcra* rearrangement. Aberrant resolution of the DSBs present in *Tcrd* due to misrepair in the absence of ATM leads to gene amplifications on chromosome 14 or the translocations involving chromosomes 12 and 14. However, the exact nature of the repair pathway, which joins these breaks leading to a translocation, is not yet clear, although the alternative NHEJ pathway could possibly be involved.

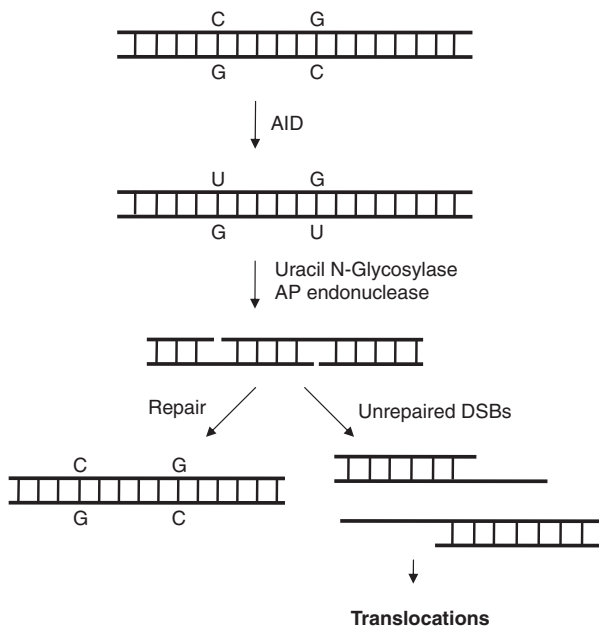
### Figure 3. Continued

which can be converted to hairpins, using the standard V(D)J-like mechanism. These hairpins can then be processed and joined using the components of the NHEJ pathway. (C) RAGs can also recognize single-stranded regions of non-B DNA structures, especially in the context of cytosines and induce nicks or DSBs. (D) RAG-mediated transposition can also introduce genomic instability and hence translocations. RAGs can act like a transposase and introduce signal ends into heterologous chromosomes at some other loci making them fragile. (E) CpGs are susceptible to deamination by enzymes like AID, resulting in single-nucleotide mismatches. These can in turn be recognized and cleaved by RAGs or other nucleases, to introduce nicks or DSBs. Such CpGs have been found at or near many chromosomal translocation breakpoint regions in lymphoid cancers.

## AID-MEDIATED CHROMOSOMAL TRANSLOCATIONS

Apart from V(D)J recombination, processes such as somatic hypermutation and class switch recombination, also play a key role in generating antibody diversity (16). AID is the major enzyme required for both these processes (89–91). AID deaminates the cytosines present in single-stranded regions (during transcription or formation of R-loops) into uracil, which results in a mismatch. This can be further processed by uracil N-glycosylase/AP endonuclease, finally leading to either a mutation or a DSB (92–96) (Figure 4). The DSB generated is an intermediate for class switching and, therefore, if unrepaired, can be a suitable candidate for illegitimate joining (Figure 4). This is supported by recent studies, where it was demonstrated that the breaks at the *c-MYC* gene during t(8;14) translocation, characteristic of Burkitt's lymphoma, are induced by AID (16,97–99). In addition, *c-MYC* transgenic mice in a p53-deficient background rapidly developed B-cell lymphoma (100). The *c-MYC* region has also been suggested to form G-loop structures on plasmid DNA, which can be bound by AID (101). Taken together, these studies indicate that action of AID can result in the development of translocations, especially those involving immunoglobulin switch regions and hence lymphoma.

miR-155 has been recently shown to regulate the levels of AID (102). miR-155-deficient mice or those containing mutated miR-155 binding site on the mRNA exhibited t(8;14) translocation at a much higher frequency than that of wild type (102,103). This suggested an important



**Figure 4.** Mechanism of AID-mediated translocations. AID can deaminate cytosines leading to the formation of uracil, which is generally removed by Uracil N-Glycosylase. The nicks are usually repaired by the base excision repair mechanism. However, two unrepaired nicks in close vicinity can act as a double-strand break and thereby a substrate for chromosomal translocations.

role for AID during *c-MYC*-IgH translocations. Besides, deregulated expression of AID can induce tumors in mice and may further help in the progression of *c-MYC*-induced mature B-cell lymphomas (104,105).

AID has also been implicated in the occurrence of the translocations involving the androgen receptor (AR) target gene, *TMPRSS2* with ETS family of genes in prostate cancer. During the translocation, AR binds to the intronic region of both the genes involved, bringing together the loci involved in translocation. Furthermore, AID and LINE-1 repeat-encoded ORF2 endonuclease are recruited to the site, acting synergistically to generate site-specific DSBs, which can then be rejoined by repair pathways (106,107). AID, although a lymphoid specific protein and expressed at a very low level in prostate cancer cells, gets induced significantly upon treatment with AR agonists, genotoxic stress or by IR-irradiation (108). Interestingly, AID-induced DSBs have been detected in several non-Ig loci in the genome, many of which occur near regions of translocations, deletions and amplifications (109). This could help in explaining the role of AID in generation of chromosomal instability.

## CHROMOSOMAL TRANSLOCATIONS BY OTHER MECHANISMS

Both RAGs and AID are expressed transiently but predominantly in lymphoid cells (110,111). RAGs are expressed only in developing B and T cells, whereas AID is expressed in the germinal centre B cells (110,111). Therefore, not all translocations can be accounted by only these two proteins. Studies have shown that illegitimate V(D)J recombination may not be the only factor responsible for the generation of genomic alterations. *H2AX<sup>-/-</sup>p53<sup>-/-</sup>RAG2<sup>-/-</sup>* mice showed a similar genetic predisposition to thymic lymphoma as did the *H2AX<sup>-/-</sup>p53<sup>-/-</sup>* mice (112,113). In addition, an AID-independent pathway has been observed for *c-MYC*-IgH translocation, suggesting that a subset of switch region translocations can occur without AID (114).

Human genome can incur endogenous damage through free radicals or natural environmental radiations, which can also culminate into translocations (115,116). Breakage-induced replication (BIR) at a DSB has been shown to explain some chromosomal translocation events in yeast (117,118). Exogenously, DSBs can be caused by agents such as etoposide, which is a known topoisomerase II inhibitor and this can further help in the occurrence of secondary malignancies (119–122).

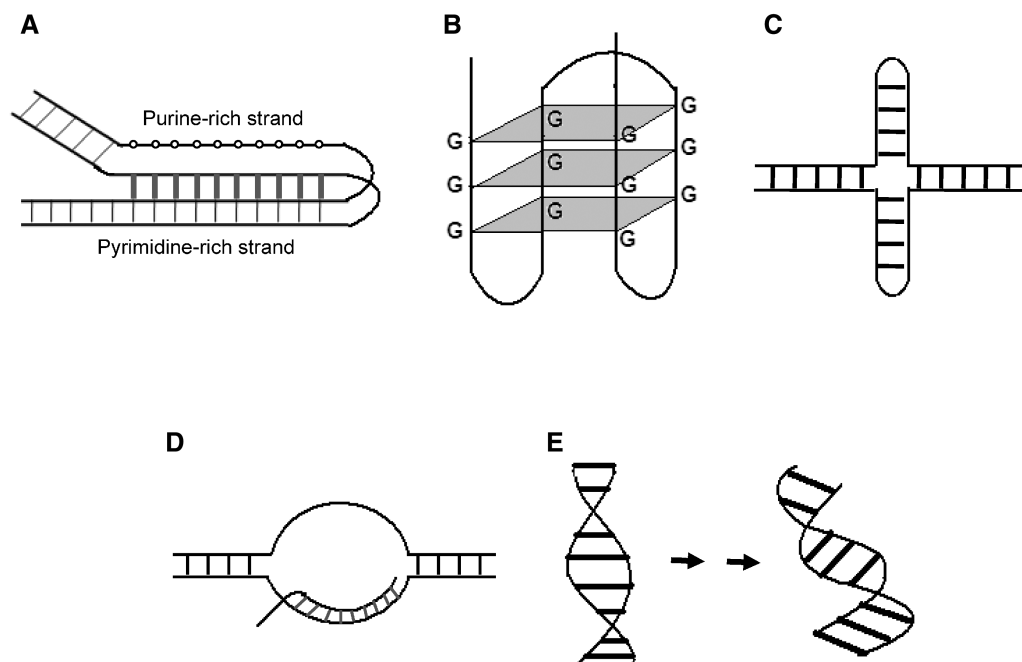
Recent studies have shown that the antiapoptotic protein, *BCL2*, can interact with KU proteins, resulting in the downregulation of NHEJ (123,124). Using mutagenesis studies, it was observed that KU70 can interact with BH1 and BH4 domains of *BCL2*, thereby reducing the efficiency of KU binding to the DNA ends (123). This is of particular relevance in cancer cells, wherein the levels of *BCL2* are elevated. It was observed that higher levels of *BCL2* led to a reduction in the efficiency of NHEJ in various cancer cell lines and vice versa (124). Since the *BCL2*-KU interaction downregulates NHEJ, a failure of

the latter could result in the generation of secondary chromosomal rearrangements including translocations in cancer cells.

Rearrangements have also been shown to occur in Alu repeats, which make up the largest family of repetitive elements, in several somatic and germline cells (125,126). These can join either with other Alu elements or with non-Alu sequences. Alu–Alu intrachromosomal recombination was seen in mixed lineage leukemia (MLL) gene, involved in acute myeloid leukemia (127). In the Philadelphia chromosome, *BCR* and *ABL1* genes contain certain Alu elements near their breakpoint regions, which may possibly lead to the occurrence of Alu-mediated DNA recombination, although this is yet to be proven (128). More recently, an intron-based system was used specifically to study induction of translocations at Alu elements (129). Induction of DSBs adjacent to identical Alu elements resulted in a higher frequency of translocations, predominantly via single strand annealing (SSA) pathway, whereas those near more divergent Alu elements utilized NHEJ.

A variety of genomic rearrangements can also arise due to palindrome-mediated genomic instability (130) (Figure 5C). A well-studied example is the recurrent t(11;22)(q23;q11) chromosomal translocation which harbors palindromic AT-rich repeat (PATRR) sequences on both the breakpoint regions (131). A few other translocations which have been reported to be mediated by palindromic sequences are t(1;22)(p21;q11), t(4;22)(q35;q11), t(17;22)(q11;q11) and t(X;22). PATRR11 on 11q23 can form a cruciform DNA structure *in vitro* (132) (Figure 5C). This gives rise to the possibility that a

cruciform structure may be the site of breakage due to action of nucleases or processes such as replication (133,134). Can such structures form within the cells? Some recent studies provide evidence for formation of such structures in bacteria and yeast. Plasmid DNA containing cruciform-forming sequences near promoter regions in bacteria were chemically probed for modification and were found to form cruciform structures (135). In an interesting study, evidences were provided for cruciform formation and its resolution within yeast (136). It was seen that plasmids bearing palindromic sequences, when transfected into *Saccharomyces cerevisiae*, were site-specifically cleaved by Mus81, which is considered as a DNA structure-specific endonuclease. Hence, the presence of non-B DNA structures at breakpoint regions suggests that they can make certain loci on the genome fragile, resulting in DSBs and further translocations by NHEJ or by altered NHEJ pathways (Figure 5). This is further confirmed in some cases of Burkitt's lymphoma, where breakpoints in the *c-MYC* gene are clustered around the H-DNA forming sequences in its promoter region (137) (Figure 5A). Many other forms of non-B DNA structures including Z-DNA, H-DNA, R-loop, G-quadruplex and triplex are now believed to be associated with various human diseases, as they are implicated in promoting genomic instability (17,138,139) (Figure 5). Even though several attempts are being made to prove the existence of such non-B DNA structures within the cells, more studies are required at the genomic level to show their presence and role in generating chromosomal translocations particularly within humans.



**Figure 5.** Non-B DNA structures involved in generation of chromosomal translocations. Recent studies have shown the importance of altered or non-B DNA structures in the generation of chromosomal translocations. Triplex DNA (A), G-quadruplexes (B), cruciform DNA (C), RNA-DNA hybrid (D) and B/A intermediate (E) DNA structures have been reported to be present at several common translocation breakpoint sites.

## ROLE OF ALTERNATIVE NHEJ IN CHROMOSOMAL TRANSLOCATIONS

DNA DSBs, which are incurred by mammalian cells, are normally repaired using either the non-homologous DNA end joining or homologous recombination. However, in the absence of these canonical pathways, an 'alternative NHEJ' pathway has been described in mammalian cells (140–143). Although its exact molecular components are not very well understood, this pathway has been implicated in the generation of chromosomal translocations (140,141). It has been observed that alternative NHEJ operates mostly in the absence of classical NHEJ pathway as shown in Ku, Xrcc4 or Ligase IV-deficient mice (141,144). It appears that alternative NHEJ may not be a single multi-component pathway, but could be further categorized into multiple subsets. The most predominant mechanism among them is the microhomology-mediated end joining (MMEJ). This pathway utilizes a short region of homology for joining the junctions, has extensive deletions and can be seen in cells lacking NHEJ proteins such as XRCC4 and Ligase IV (145–147). Although even classical NHEJ sometimes utilizes microhomology for the end joining, the exact length of the homology required for classical and alternative NHEJ is not very clear. Other mechanisms of alternative NHEJ are primarily Ku or DNA-PKcs independent joining, which do not require microhomology and SSA (148–151). Truncation of murine core RAG2 proteins also resulted in a robust alternative NHEJ activity in Xrcc4-deficient cells (140). However, it was seen that such deletions in RAG2 could support alternative NHEJ even in wild-type cells. Here, they can lead to aberrant joining of the DSBs, generated during either V(D)J recombination or class switch recombination, ultimately resulting in chromosomal translocations (140,141). Previous studies on core RAG transgenic mice showed that some junctions contained regions of microhomology and extensive deletions, like the alternative NHEJ junctions (152). This and other observations confirm that alternative NHEJ can occur even in normal cells in the presence of classical NHEJ. However, its efficiency might be low and may depend on other factors. It is becoming increasingly clear now that alternative NHEJ could be the primary mediator in the formation of translocations in mammalian cells, since the classical NHEJ suppresses generation of such rearrangements (153–155). The role of NBS1 in preventing alternative NHEJ and thereby genomic instability has also been suggested (142). Further, PARP1 and PARP2 proteins, which usually act as DNA damage sensors, were shown to have contrasting roles in mediating alternative NHEJ (156). This seems to be consistent with studies on mice with mutated MRE11 complex, where increased translocations involving the TCR $\alpha$  loci were observed (157,158).

Can alternative NHEJ be detected in human neoplasia? Urothelial carcinoma extracts have been shown to utilize MMEJ as the predominant pathway of DSB repair, as opposed to classical NHEJ. The repair was independent on Ku70, DNA-PKcs and XRCC4 proteins, which would explain the extent of genomic instability present in such

cancers (159). Interestingly, the error-prone alternative NHEJ pathway correlated with the invasiveness of the cancer. In particular, end-joining fidelity was shown to be compromised with both the increase in the grade of cancer and the reduced activity of the Ku proteins (160). In another study, fidelity of NHEJ products was found to shift from classical to alternative pathways in B-cell leukemic cells that were resistant to DNA-damage-induced apoptosis (161). These studies suggest that deviations in the delicate balance between classical and alternative NHEJ could push the cell toward the latter; thus contributing to genomic instability and development of cancer.

Recently, we attempted to understand whether the efficiency of the NHEJ pathway plays any role in the generation of tissue-specific genomic instability. Upon studying the mechanism of NHEJ in cell free extracts of various rat tissues, we observed that apart from testis, lungs showed the maximum NHEJ efficiency, whereas terminally differentiated organs, such as heart, liver and kidney, showed the least (162,163). This finding is of interest as it correlates with the higher incidence of cancers in lungs, which could arise due to mutations in the genes involved in the NHEJ pathway. However, more studies are required to understand the role of NHEJ and alternative NHEJ in tissue-related chromosomal abnormalities in cancer.

## CHROMOSOMAL TRANSLOCATIONS IN HEALTHY INDIVIDUALS

Previously, it was believed that chromosomal translocations were associated only with cancer. However, many studies have shown the incidence of translocations in the peripheral blood of healthy individuals in the past decade. So far, the translocations, which have been detected among the healthy individuals, include *BCL2-IgH*, *BCR-ABL*, *NPM-ALK*, *BCL6-IgH* and *BCL1-IgH* translocations (164–170).

Among different chromosomal translocations, the t(14;18) is the most commonly reported translocation in healthy individuals. It has been detected with a prevalence of around 30–60% based on data obtained from Europe and America (171–177). Our studies in healthy Indian individuals showed that around 34% of the volunteers analyzed were positive for t(14;18) (178). The sequences of the breakpoint junctions from both healthy individuals and patients are similar, suggesting that the translocations arising in healthy individuals may not be mechanistically different from that in patients (171,179). Few studies have attempted to establish the long-term clonal persistence of such t(14;18) bearing cells in healthy individuals, by performing follow-up studies on the positive cases after a period of few years (171). It was observed that though there is an initial clonal expansion and persistence of a single t(14;18) bearing clone, with time, independent hits may lead to the formation of multiple cells bearing different t(14;18) translocation breakpoint junctions. Recently, it was also shown that the t(14;18) bearing cells in the healthy individuals act as FL-like B cells, since they were enriched in IgM memory cells and CD27 positive.



Such cells could, therefore, act as novel intermediates during the early stages of lymphomagenesis (180,181). Another translocation which has been detected in healthy individuals, albeit at a very low frequency is the t(11;14) translocation, involving the *BCL1* gene known to occur in mantle cell lymphoma (MCL) (178,182). Therefore, it will be of interest to see how a normal cell bearing a translocation transforms into a cancerous cell and this would undoubtedly be a topic of active research in the coming years.

## CONCLUDING REMARKS

Studies thus far suggest that RAGs and AID are the two major enzymes that induce chromosomal fragility in lymphoid cancers. The process of V(D)J recombination causes breaks mostly during generation of antibody and T-cell receptor diversity. In case of the partner chromosomes, RAGs induce breaks, when cryptic signal sequences or non-B DNA structures are present. AID can deaminate cytosines, leading to chromosomal breaks, especially during t(8;14) translocation. However, this is just the tip of the iceberg. Further studies will unravel the mechanism of chromosomal breaks during many other translocations in lymphoid as well as non-lymphoid tissues. In addition, the presence of such translocations in healthy individuals suggests that these translocations alone are not enough to cause cancer. The nature of the additional factors responsible for the transformation of the normal cells bearing translocations into malignant cells is an area which needs to be explored.

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