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Double-strand breaks and translocations in cancer

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Abstract. The correct repair of double-strand breaks (DSBs) is essential for the genomic integrity of a cell, as inappropriate repair can lead to chromosomal rearrangements such as translocations. In many hematologic cancers and sarcomas, translocations are the etiological factor in tumorigenesis, resulting in either the deregulation of a proto-oncogene or the expression of a fusion protein with transforming properties. Mammalian cells are able to repair DSBs by pathways involving homologous recombination and nonhomologous end-joining. The analysis of translocation breakpoints in a number of cancers and the development of model translocation systems are beginning to shed light on specific DSB repair pathway(s) responsible for the improper repair of broken chromosomes.

Key words. Translocations; double-strand breaks; DNA repair pathways; cancer; recombination.

Introduction

Cancer is associated with chromosomal abnormalities such as deletions, duplications, inversions and translocations. More than 640 recurrent balanced chromosomal rearrangements have been identified in malignancies, involving over 100 genes [1]. Recurrent reciprocal translocations in particular have been shown to be the cause of many hematologic tumors and certain solid tumors (i.e. sarcomas) [2, 3]. In reciprocal translocations, which are discussed in this review, an exact interchange of chromosomal segments occurs between partner chromosomes, resulting in two new derivative (der) chromosomes (fig. 1A). The result is either the dysregulation of a gene on one chromosome by juxtaposition to an antigen receptor gene on another chromosome, or the creation of a novel coding sequence by the fusion of sequences from genes on two different chromosomes. Nonreciprocal translocations result in only one der chromosome, and are often observed in solid tumors such as those of epithelial origin, which commonly contain a multitude of other cytogenetic changes.

Early in the 1900s Boveri proposed that a disturbance in the normal chromosomal balance of a cell might result in malignancy [4], which was later known as the somatic mutation theory of cancer. The prevailing view for many years, however, was that cancer was not the result of chromosomal abnormalities since the observed genetic changes in tumors seemed to be random [5–7]. Cytogenetic studies in the 1950s and 1960s characterized karyotypes by size, shape and number of chromosomes, but individual chromosomes could not be precisely identified. Nonetheless, a pivotal moment in cancer genetics occurred in 1960 with the discovery of the Philadelphia (Ph) chromosome in chronic myelogenous leukemia (CML) by Nowell and Hungerford in Philadelphia [8]. The Ph chromosome was a small, distinctive chromosome that was consistently associated with CML. Despite this discovery no consistent chromosomal abnormalities were found to be associated with a cancer during the following years, and again the view persisted that genetic changes were epiphenomena.

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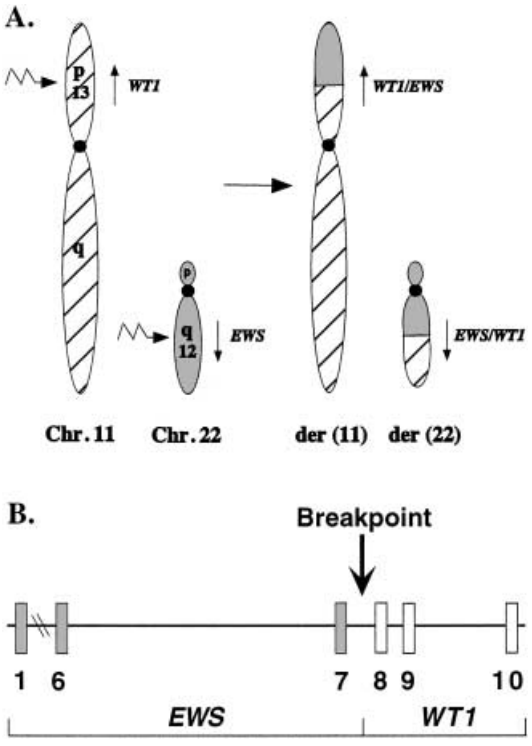

Figure 1. Example of a recurrent reciprocal translocation and a fusion gene. (*A*) Breakpoints (jagged arrows) in the Wilms tumor (*WT1*) gene on chromosome 11 and the Ewing sarcoma (*EWS*) gene on chromosome 22, resulting in the t(11;12) (p13;q12) reciprocal translocation of desmoplastic small round cell tumor (DSRCT), which produces two derivative (der) chromosomes. (*B*) The *EWS/WT*1 fusion gene is shown from der (22), which has oncogenic properties. Exons of *EWS* and *WT1* are represented by solid and open boxes, respectively, and are not drawn to scale.

In the 1970s cancer genetics was transformed by the development of techniques that allowed each chromosome to be identified based on its unique banding pattern [9]. This led to the discovery of a number of recurrent translocations in tumors. The first translocation to be identified was between chromosomes 8 and 21 in patients with acute myelogenous leukemia (AML) [10]. This was shortly followed by the discovery of a translocation between chromosomes 9 and 22 in CML [11]. The development of molecular genetics in the 1980s permitted the characterization of translocation breakpoints, and the first genes involved in a translocation were cloned. These genes were the *MYC* gene on chromosome 8 and the immunoglobulin heavy chain (*IgH*) gene on chromosome 14 in Burkitt's lymphoma [12, 13]. Since these discoveries additional techniques have been developed, permitting more precise analysis of translocations at the cytological level. Fluorescence in situ hybridization (FISH) provides fluorescent-labeled hybridization probes to identify subchromosomal regions [14, 15], and spectral karyotyping (SKY) allows the identification of each chromosome by DNA probes consisting of different fluorochromes [16, 17].

Table 1. Different DSB repair pathways in mammalian cells.

General types of DSB repair pathways	Some specific DSB repair pathways	Components
Nonhomologous recombination	illegitimate recombination	KU70/KU80, DNA-PK$_{cs}$, XRCC4/DNA ligase IV, MRE11/RAD50/NBS1
	V(D)J recombination	RAG1/RAG2, KU70/KU80, DNA-PK$_{cs}$, XRCC4/DNA ligase IV, MRE11/RAD50/NBS1(?)
Homologous recombination (HR)	gene conversion	RAD51, RAD52, RAD54, XRCC2, XRCC3, RAD51B, RAD51C, RAD51D, BRCA1, BRCA2, MRE11/ RAD50/NBS1
	single-strand annealing (SSA)	RAD52, RAD1/RAD10

See text for references.

Double-strand breaks (DSBs) have been suspected to be the causative lesion leading to translocations. DSBs can occur from exogenous DNA damaging agents as well as from endogenous cellular processes. An example of an exogenous agent is ionizing radiation, which directly causes DSBs as well as other lesions. Another agent is the antitumor drug etoposide, which causes a DSB by partially inhibiting the activity of DNA topoisomerase II (topo II) in the religation of cleaved strands [18]. Endogenous cellular processes and conditions resulting in DSBs include oxidative metabolism, which produces free radicals that damage DNA, mechanical stress on chromosomes and replication fork collapse. Finally, programmed DSBs also arise in specific tissue types, as during V(D)J recombination [19] and meiosis [20].

The repair of chromosomal DSBs in mammalian cells occurs by two general types of DNA repair pathways: homologous recombination (HR) and nonhomologous recombination, also known as nonhomologous end-joining (NHEJ) [21] (table 1). Consequently, DSBs are potent inducers of recombination in mammalian cells, stimulating both HR and NHEJ by three orders of magnitude or more [22]. Historically, NHEJ was presumed to be the dominant repair pathway in mammalian cells; however, recent studies have shown that HR is also a major pathway [23]. The contribution of each pathway in repairing a break on a chromosome may depend on such factors as the nature of the DSB and the particular cell cycle or developmental stage of the cell (i.e. embryo vs. adult) [24–26]. In general, HR and NHEJ are viewed as competing pathways [24, 25], but more recent evidence indicates that these two pathways have the potential to be coupled during the repair of a DSB [26]. Of key interest is determining how DSBs are formed that lead to translocations, and what factors and DSB repair

pathways are involved in generating translocations, the latter being the main focus of this review.

Translocations in hematologic and solid tumors

Translocations are a common cause of leukemias, lymphomas and solid tumors of mesenchymal origin (sarcomas). More is known about the chromosomal changes in hematologic tumors than in most solid tumors, despite the fact that solid tumors represent 95% of human cancers [3, 5]. One reason is that solid tumors are more difficult to analyze; for example, they generally have a more complex karyotype containing a multitude of abnormalities at the time of diagnosis, whereas hematologic tumors often contain only one or a few gross chromosomal rearrangements. In solid tumors, such as epithelial tumors of the breast and skin, it is difficult to differentiate between primary pathogenic events and secondary evolutionary and/or 'bystander' events. Although it is possible that a translocation or another chromosomal rearrangement is the etiological factor in these malignancies, it is also possible that a mutation in a caretaker gene, which makes a cell hypermutable, results in the observed global genomic instability.

Most genes involved in translocations encode transcription factors, especially in acute leukemias and sarcomas, although some genes associated with translocations have different functions. For example, they can encode tyrosine kinases, cyclins, nucleopore proteins and anti-apoptotic proteins (see [2]). Overall, genes implicated in translocations are involved in an essential stage of cell growth, development or survival. Their oncogenic effect is often restricted to a particular cell type where the action of the fusion protein in a specific cell context contributes to incomplete differentiation and malignant transformation [27]. We discuss below three cancers and their translocation breakpoint regions, as well as their very different translocation gene products and the role of these products in tumorigenesis.

Burkitt's lymphoma (BL)

Burkitt's lymphoma is a hematologic cancer characterized by the t(8;14) (q24;q32) translocation in over 80% of patients, and involves the *MYC* and *IgH* genes on chromosomes 8 and 14, respectively. This translocation is restricted to B cells in which the *IgH* locus is subjected to developmental programmed chromosomal rearrangements and hypermutation. The normal *MYC* gene contains three exons which are all present in the t(8;14) translocation in the form of Burkitt's lymphoma endemic to Africa and New Guinea (fig. 2 A), whereas only exons 2 and 3 of *MYC* are present in the sporadic and AIDS-associated forms [28]. The *MYC* gene in these translocations is deregulated by the strong enhancer elements present at the *IgH* locus, such that the timing and level of its expression is changed substantially [29, 30]. In addition to these changes, negative regulatory elements are often abolished by point mutations in the first exon or intron of *MYC* [31]. The effect of MYC overexpression has been well-characterized [32–34]. An excess of MYC, a bHLH-LZIP tran-

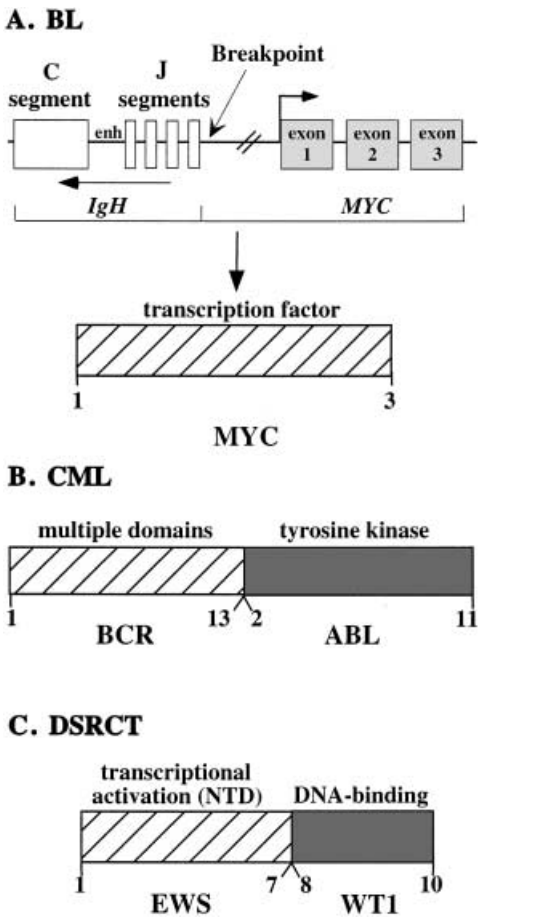

Figure 2. Schematic diagram of gene products from translocations associated with three cancers. (*A*) The t(8;14) (q24;q32) translocation typical of the endemic form of Burkitt's lymphoma (BL) results in the fusion of the immunoglobulin heavy chain (*IgH*) gene with the bHLH-LZIP transciption factor encoded by *MYC* as shown. Arrows indicate the direction of transcription, as well as the promoter region of *MYC*. In the *IgH* gene, enhancer elements (which are shown as enh) deregulate *MYC* expression. The protein is represented by a hatched box, and encodes exons 1–3, as does the wild-type protein. (*B*) The fusion protein of chronic myelogenous leukemia (CML). The breakpoints in BCR are generally restricted to exons 11–15 (also know as M-bcr exons b1–b5). The fusion protein shown here is encoded by exons 1–13 of BCR and exons 2–11 of ABL. This rearrangement changes the timing and expression of ABL, resulting in constitutive tyrosine kinase activity. (*C*) The fusion protein EWS/WT1 of desmoplastic small round cell tumor (DSRCT) comprises exons 1–7 of EWS and exons 8–10 of WT1. This fusion juxtaposes the amino terminal domain (NTD) of EWS containing transcriptional activation properties to the DNA binding domain of WT1, and results in a novel transcription factor. Exons and proteins are not drawn to scale.

scription factor, causes a shift in the equilibrium of dimers involving MYC and another bHLH-LZIP transcription factor, MAX. This overexpression apparently results in the inappropriate activation of downstream target genes, ultimately leading to malignant transformation of B cells. Two other translocations are associated with Burkitt's lymphoma, the t(2;8) and t(8;22) translocations, which also lead to the activation of *MYC* by juxtaposition to an *Ig* light chain gene [35, 36].

Similarly, in other B and T cell malignancies, translocation of *Ig* and T cell receptor (*TCR*) loci, respectively, causes deregulation of many different genes (see [2]). For example, in the t(3;14) translocation of diffuse B-cell lymphoma, *IgH* is juxtaposed to *BCL6*, which encodes a zinc-finger transcription factor [37]. Another example is in the t(10;14) translocation of T cell acute lymphoblastic leukemia (T cell ALL), where a *TCR* gene is fused to *HOX11*, which encodes a homeodomain protein [38, 39].

CML

CML is a hematologic cancer which is caused by the t(9;22) (q34;q11) translocation, producing the Ph chromosome and fusing the *BCR* gene on chromosome 22 with the *ABL* gene on chromosome 9 [40, 41]. In this type of translocation, the breakpoints are within the introns of both genes, and construct a novel gene in which *ABL* sequences are fused to *BCR* sequences and placed under the regulation of the *BCR* promoter [42].

The *BCR* gene has a central region of 5.8 kb that is termed the major breakpoint cluster region (M-bcr), for which the gene was named [43]. This gene has 23 exons with the M-bcr spanning exons 11–15. The breakpoints in the *ABL* gene extend over a larger region (≥200 kb) and occur either 5′ of the two alternative first exons or downstream of them in the first intron [44]. Although the *BCR* gene has identifiable protein domains and is ubiquitously expressed, its role in the cell is unknown [45]. The ABL protein is a nonreceptor tyrosine kinase that normally has very low constitutive kinase activity; however, the BCR/ABL fusion protein (fig. 2B) has constitutively elevated kinase activity [46, 47], and its expression has been shown to produce a CML-like disease in mice [48]. There are potentially multiple downstream targets through which the BCR/ABL fusion protein exerts its oncogenic effect, such as components of the RAS pathway, adhesion molecules, apoptotic factors and the cell-cycle-regulated proteins MYC and cyclin D1 [2, 45].

The *BCR* and *ABL* genes are involved in another t(9;22) translocation that causes ALL, accounting for over one-third of adult cases and a small portion of childhood cases [49, 50]. In this malignancy, the breakpoint in *BCR* occurs in the first intron in the minor breakpoint cluster region (m-bcr), whereas the breakpoints in *ABL* are the same as in CML. Splicing of the fusion gene in both CML and ALL retains exon 2 of ABL, resulting in the same portion of the ABL protein being expressed in both leukemias [51].

DSRCT

Desmoplastic small round cell tumor (DSRCT) is an aggressive solid tumor primarily of male adolescents that generally occurs in the peritoneal surfaces of the abdomen [52]. This malignancy is associated with the recurrent t(11;22) (p13;q12) translocation (fig. 1A) [53]. The breakpoints of this translocation usually occur in intron 7 of the Ewing sarcoma (*EWS*) gene and always in intron 7 of the Wilms Tumor (*WT1*) gene, with the *EWS/WT1* fusion gene from der (22) underlying the oncogenesis of DSRCT (fig. 1B) [54–56].

The EWS protein is a widely expressed protein consisting of an amino-terminal domain (NTD), which has transcriptional activational properties and shares some homology with RNA polymerase II, and a carboxyl-terminal domain, which contains an RNA recognition motif [57, 58]. The function of the EWS protein is unknown, but studies have suggested that it may be a RNA-binding protein involved in some facet of transcriptional regulation and processing [57–61]. The WT1 protein is a tumor-suppressor protein that belongs to the EGR family of zinc-finger transcription factors, and has been shown to be involved in transcriptional repression in vitro, but also has many other putative functions (see [62]). A subset of Wilms tumors is caused by mutations in WT1, some of which are loss-of-function mutations, whereas some are mutations in the amino terminus which convert WT1 into a transcriptional activator [62, 63].

The EWS/WT1 fusion protein consists of the NTD of EWS and the carboxyl terminus of WT1, which contains the last three of four zinc fingers [56]. Thus in EWS/WT1, the RNA-binding domain of EWS is replaced with the DNA-binding domain of WT1, thereby creating a novel transcription factor (fig. 2C). Studies have shown that the chimeric protein activates putative WT1-responsive genes such as *PDGF-A, EGR-1* and *IGF-1R,* which are normally repressed by WT1 [64–66]. Further studies examining the effect of endogeneous expression of EWS/WT1 on the activation of native genes, however, are necessary to gain insight into EWS/WT1 downstream targets (see [62]).

The *EWS* gene is involved in translocations with many other genes besides *WT1*, resulting in a number of different sarcomas [3]. In each of these malignancies the *EWS* NTD is again fused to the DNA-binding domain of its translocation partner. This occurs in the t(11;22) (q24;q12) translocation of Ewing sarcoma in which the *EWS* gene is juxtaposed to the *FLI-1* gene, which encodes

a transcription factor with an ETS DNA-binding domain [67]. In another sarcoma, malignant melanoma of soft parts, the *EWS* gene is fused to *ATF-1*, which encodes a transcription factor in the bZIP family [68].

DSB repair pathways and translocations

The novel juxtaposition of DNA sequences at translocation breakpoints suggests the involvement of chromosomal breaks and their aberrant repair in the translocation process. Both NHEJ and HR pathways play major roles in the repair of DSBs in mammalian cells [21]. NHEJ involves the joining of two DNA ends with little or no homology to each other [69]. These ends may or may not undergo degradation, and if they are degraded, the degradation may be limited or extensive. Besides deletion of nucleotides, duplications, insertions and inversions can occur at these junctions. NHEJ also occurs at specific sequences, as during V(D)J recombination in which DSBs are introduced at recombination signal sequences (RSSs) by the RAG recombinase proteins [70]. The terms 'NHEJ' and 'illegitimate recombination' are often used interchangeably, but in this review illegitimate recombination denotes the joining of nonhomologous ends without the use of any site-specific sequences.

In contrast to NHEJ, HR relies upon homology of generally ≥ 200 bp for the repair of DSBs. Gene conversion, a specific type of HR, is a conservative process in which repair can precisely restore the original sequence that was present prior to the introduction of a DSB. This involves repair DNA synthesis in which the homologous sequence serves as a template for repair. Single-strand annealing (SSA) is another type of HR, but it is a nonconservative process due to the loss of genetic information during recombination. In SSA, a DSB occurs in or near two repeats, followed by annealing of complementary single strands of the repeats such that the intervening sequences are deleted and only one copy of the repeat remains. Components of NHEJ and HR DSB repair pathways are listed in table 1 [71].

Many potentially recombinogenic sequences have been associated with translocations (table 2). *Alu* repetitive elements appear abundant in regions that frequently undergo recombination (hot spots). For example, translocation breakpoint junctions often are composed of an *Alu* element joined to a unrelated sequence, as would be indicative of NHEJ. HR between *Alu* elements has been shown to be the cause of many intrachromosomal rearrangements associated with cancer and other diseases [72]; however, evidence for *Alu-Alu* HR involvement in chromosomal translocations is sparse [73]. Palindromic sequences have also been implicated in mediating translocations (see [74]). Palindromes can form hairpin DNA structures which can undergo breaks, making them genetically unstable and possibly recombinogenic [75].

Table 2. Some potential recombinogenic sequences or sites involved in translocations.

Illegitimate recombination
Alu repetitive elements
Palindromic sequences
DNA topo II cleavage sites
Scaffold-associated regions (SARs)
DNase I hypersensitive sites (DNase I HS sites)
Recombination involving immune system
Heptamer/nonamer motifs
Class switch regions
Somatic hypermutation regions
Homologous recombination
Alu repetitive elements (?)

See text for references.

Various elements such as topo II cleavage sites, scaffold-associated regions (SARs, also called MARs), and DNase I hypersensitive sites (DNase I HS sites) have also been found in breakpoint cluster regions of various genes and, therefore, have been predicted to play a role in translocations. Topo II cleavage sites have been shown to be involved in translocations both in vitro and in vivo [76–78]. Consistent with this, treatment of patients with topo II inhibitors (i.e. etoposides) can generate translocations leading to therapy-related leukemias [79]. SARs are AT-rich DNA regions of chromatin loops in the DNA scaffold, and DNase I HS sites represent accessible regions of DNA that could be potential sites for DSBs [80, 81]. Recent studies on translocations in AML have shown that the breakpoint regions of both the *AF9* gene and the *MLL* gene contain all three of these structural elements, which may promote the illegitimate recombination events between them [82].

In the following section we have highlighted specific DSB repair pathways implicated in the etiology of translocations in lymphomas and Ewing sarcoma. We also discuss a genetic system developed by our laboratory which is designed to determine the particular DSB repair pathway(s) involved in translocation events.

V(D)J recombination and other lymphoid developmental processes implicated in translocations

Specific translocations are often associated with tumors that arise from B and T cells where an oncogene on one chromosome is juxtaposed to an antigen receptor locus on another chromosome (see [2, 83, 84]). These translocations occur in immature lymphoid cells undergoing V(D)J recombination, as well as in more mature B cells undergoing isotype class switch recombination, somatic hypermutation and secondary V(D)J recombination events.

The *IgH* locus consists of V, D, J and C segments, and by combinatorial joining of these segments in B cells, antibody diversity is created [85, 86]. More specifically, the V(D)J recombination machinery joins the D segment to a J-Constant segment, followed by another recombination event joining the V segment to the DJ-Constant segment, resulting in a rearranged *IgH* gene (fig. 3A). At the recombining ends of each V, D, and J coding segment is a RSS which is composed of a heptamer and a nonamer separated by a spacer region of either 12 or 23 bp. To initiate V(D)J recombination, the RAG proteins make DSBs at the borders of the heptamers and the adjacent coding segments. The two ends containing the RSSs have blunt ends and are joined by NHEJ to form a circular molecule with a signal joint. By contrast, the two coding ends form hairpin ends which are opened prior to NHEJ to become a covalently linked coding joint.

One proposal as to how V(D)J recombination gives rise to a translocation is that the RAG proteins make a DSB at an antigen receptor locus and also at a cryptic RSS on another chromosome (fig. 3B) [87, 88]. If the cryptic site occurs near a proto-oncogene, the fusion to *Ig* enhancer elements results in the dysregulation of this gene. Although cryptic site usage has been documented in V(D)J recombination [89], often sequences approaching consensus RSS elements have not been found at translocation breakpoint junctions. Therefore, another mechanism has been proposed in which the second DSB is generated by some means other than the RAG proteins [90]. The RAG-cleaved end is then ligated to this other end in a process termed 'end-donation' (fig. 3B) [86].

A third proposal is that some translocations arise by RAG-mediated transposition in a mechanism termed one-ended transposition [91]. In its simplest form, this mechanism involves only one DSB at an antigen receptor locus rather than two DSBs, and has been proposed based on the activity of the RAG proteins in vitro using oligonucleotide substrates. The normal reaction occurs in two steps: the RAG proteins nick one strand at each heptamer junction, resulting in a strand with a 3′-OH and a 5′-P end, and then the 3′-OH end attacks the other strand in a transesterification reaction, creating a hairpin at the coding end and leaving a blunt end at the signal end. The two coding and two signal ends then become joined. In one-ended transposition the 3′-OH at a single signal end is proposed to attack a random site on another chromosome, which could be near a protooncogene. By a similar transesterification reaction as that found in the normal reaction, the reactive 3′-OH of this branched intermediate would attack the opposite strand to form a hairpin. A reciprocal translocation would culminate with the joining of the two hairpin ends produced in this reaction (fig. 3C).

Cryptic-site usage, end-donation and one-ended transposition are all possible mechanisms for the generation of reciprocal translocations in B and T cells due to inappropriate V(D)J recombination. Evidence supporting end-donation and possibly cryptic site usage has been suggested by sequence analysis of translocation breakpoints, although the relative contribution of each mechanism has yet to be conclusively determined.

In addition to V(D)J recombination, the maturation of B cells includes two other processes – isotype class switching and somatic hypermutation [92]. Through the introduction of point mutations into rearranged V regions, somatic hypermutation generates high-affinity antibodies, whereas isotype class-switching links rearranged V regions to different 3′ Constant regions, which alters antibody effector function. Both of these processes have been postulated to involve DSBs at the switching or mutating sequences [93 and references therein; 94], although details of these processes, including mechanisms of DSB formation, are as yet unknown. Consistent with DSB formation, translocation breakpoints have been associated with both switch regions and sequences undergoing hypermutation [95]. Of note, in Burkitt's lymphoma sequence analysis of breakpoints in the endemic form of the disease is consistent with translocations arising during somatic hypermutation, whereas analysis of breakpoints in the sporadic form of the disease is consistent with translocations arising during class switch recombination. Thus, aberrant repair mechanisms leading to translocations have been associated with all three developmental processes involving DNA alterations at the *Ig* and *TCR* loci.

Illegitimate recombination and translocations

Although multiple mechanisms have been proposed for the generation of reciprocal translocations involving antigen receptor genes, less is understood about translocations that do not involve these genes. In some leukemias, recombinogenic sequences have been proposed to play a role [82 and references therein; table 2]. However, even less is known about genetic events leading to translocations in sarcomas. To gain insight into the etiology of sarcomas, Zucman-Rossi et al. [96] determined the breakpoint junctions in 77 solid tumors carrying the t(11;22)(q24;q12) translocation characteristic of Ewing sarcoma. The translocation results in the fusion of the *EWS* gene to the *FLI-1* gene on chromosome 11, producing a fusion protein that is a novel transcription factor with potent transforming properties [58, 67]. Since the *EWS* gene has multiple translocation partners (e.g. *WT1*, *ERG*, *ETV1*, *ETV4* and *ATF1*) resulting in many different sarcomas [97], gaining insight into the etiology of the *EWS/FLI-1* translocation potentially has broad significance.

In this study the breakpoint regions of the unrearranged *EWS* and *FLI-1* genes were first sequenced, and then the der (22) breakpoint junctions in the 77 tumors were precisely determined. No consistent consensus sequence or

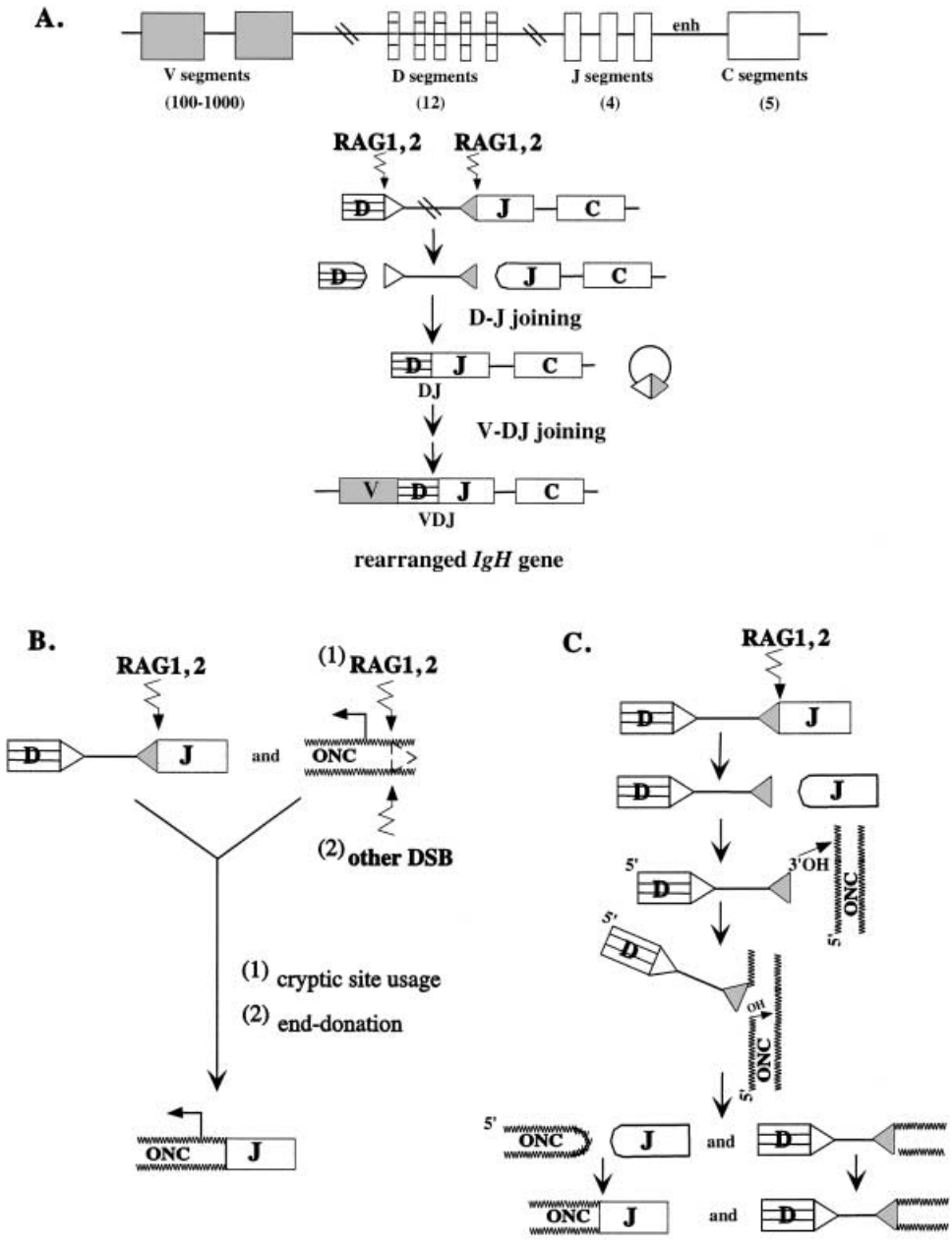

Figure 3. Mechanisms of V(D)J recombination leading to translocations. (*A*) Normal V(D)J recombination at RSSs adjacent to the D, J and V segments. The RAG proteins make DSBs (jagged arrows) at the junction of RSSs (which are shown as white or gray triangles) and coding segments. The blunt signal ends of the RSSs are ligated to form a circular molecule with a signal joint, whereas the D and J (or V and D) cleaved-coding ends first form hairpin ends that are then processed to form a coding joint. The number of V, D, J and C segments in the germline confirmation is indicated. (*B*) Two mechanisms leading to translocations are shown. In the first mechanism, cryptic-site usage (1), the V(D)J machinery erroneously makes a DSB at a cryptic heptamer (triangle with dashed lines) on another chromosome and then joins together the two broken chromosomes, resulting in a translocation in an otherwise normal V(D)J recombination event. In the second mechanism, end-donation (2), only one RAG-mediated DSB occurs, resulting in the fusion of an *IgH* end to a random chromosome which has also undergone a DSB by some other mechanism. (*C*) A third translocation mechanism is one-ended transposition which occurs when only one DSB at an RSS is made by the RAG proteins. The 3′-OH end of the cleaved RSS attacks a site on a chromosome which by chance is near an oncogene (ONC) (see text for details).

repetitive elements were found in the regions of the breakpoints. This was determined by examining 120 bp of normal *EWS* sequence 5′ of each of the 77 breakpoint junctions, and similarly 120 bp of normal *FLI-1* sequence 3′ of the junctions. The authors concluded that the t(11;22) translocation of Ewing sarcoma is due to illegitimate recombination that does not involve any known recombinogenic sequences (e.g. *Alu* elements or topo II sites).

Breakpoint junction sequences from the reciprocal der (11) were also determined in 36 of the 77 solid tumors. The results indicate that 30% (11/36) of the tumors had balanced junctions defined by ≤5 bp of gene sequence deleted or duplicated, whereas 58% (21/36) had undergone deletions (mean deletion of 56 bp and maximum of 8262 bp) or duplications (mean of 118 bp and maximum of 467 bp). Overall, almost every combination of balanced, deleted and duplicated ends of *EWS* and *FLI-1* sequences was observed at the breakpoint junctions. The remaining tumors, which represented 11% (4/36) of the tumors, had translocations with more complex patterns, and often involving the insertion of a locally derived inverted sequence (LDIS). These results indicate, therefore, that although recurrent reciprocal translocations appear to be balanced at the cytological level, at the fine structure level the majority of translocations are not balanced. The authors proposed a simple model of illegitimate recombination to account for all of the observed junctions (fig. 4). The model suggests that DSBs occur independently on two chromosomes, with most DSBs resulting in a staggered break. The protruding ends of the DSBs are then processed separately by DNA polymerase and/or 5′-to-3′ exonucleases. Depending on the ratio of these two activities, three basic categories of end structures can be generated: duplications, deletions and inversions (LDISs). A translocation is then created by the ligation of the processed ends of two chromosomes. Similar processing of DSB ends of translocation breakpoints have

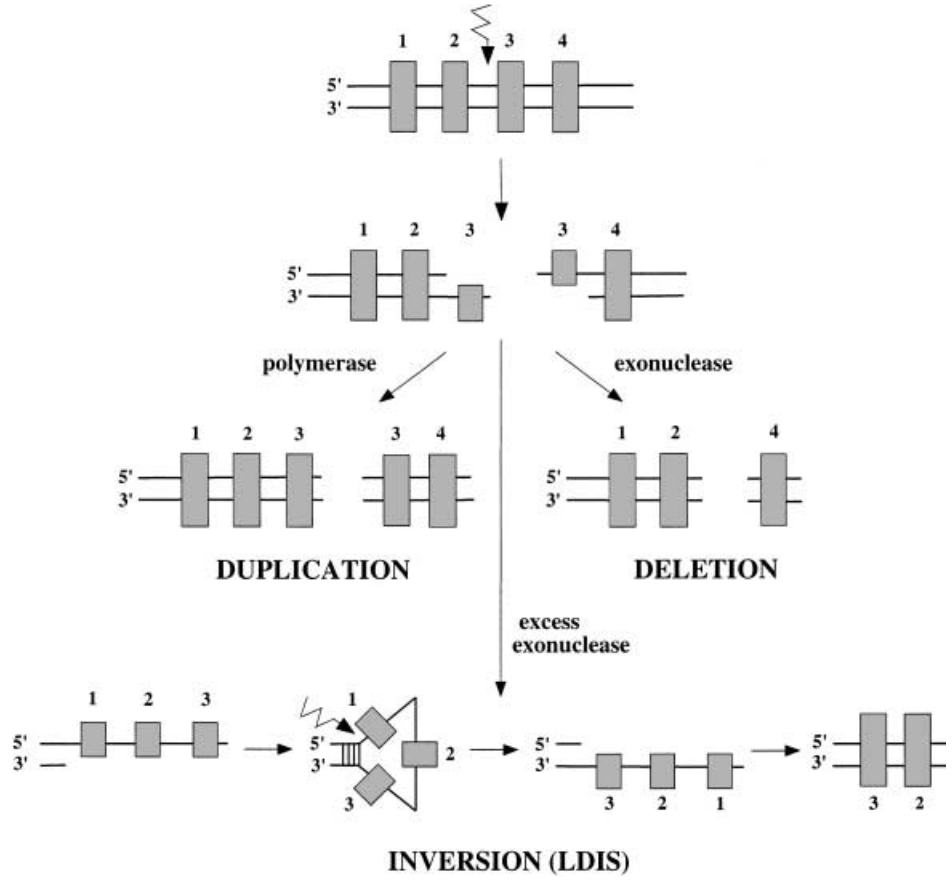

Figure 4. Model for processing of DSB ends which leads to duplications, deletions and inversions at translocation breakpoints. A DSB (jagged arrow) produces a staggered break in a chromosome. The ends can be filled in by polymerase, resulting in duplication of sequences at the two broken ends of that chromosome. Exonuclease activity can result in small deletions at the broken ends, whereas excess exonuclease activity can produce a locally derived inverted sequence (LDIS). During the formation of an LDIS, annealing at a small homology (shown as 4 bp) produces a hairpin end which is then released by a single strand nick. Polymerase and exonuclease activity results in an inversion. Only processing of one DSB end of a chromosome is shown for LDIS. A translocation then results from ligation of the processed ends of two chromsomes. Gray rectangles represent exon sequences.

been suggested in studies investigating different leukemias [98, 99].

Frequent translocations in a model system of DSB repair

Richardson and Jasin in 2000 [100] have recently shown that frequent reciprocal translocations can be generated by the repair of two DSBs, each on a different chromosome. This is in contrast to previous results using a similar interchromosomal system, where the repair of a single DSB did not produce a translocation [101]. DSBs were introduced into chromosomes using the rare-cutting I-*Sce*I endonuclease. These experiments were performed in mouse embryonic stem cells in which I-*Sce*I cleavage sites were placed in two defective neomycin phosphotransferase (*neo*$^-$) genes integrated into heterologous chromosomes (fig. 5). Upon I-*Sce*I endonuclease expression in this system, DSBs occur in each *neo*$^-$ gene, and HR repair by either gene conversion or SSA between these two repeats results in the formation of a *neo*$^+$ gene. Analysis of the *neo*$^+$ interchromosomal recombinant clones indicated that 79% (34/43) of these clones had undergone interchromosomal recombination by gene conversion and 21% (9/43) had undergone SSA. Of note, none of the gene conversion events led to translocations, whereas all of the SSA events led to reciprocal translocations. These translocation events occurred at a frequency of 1×10^{-4}.

In these experiments each of the reciprocal translocations recovered had a particular structure. The selected *neo*$^+$ gene generated by SSA was found at one breakpoint junction, whereas the second breakpoint junction was derived by NHEJ (fig. 5). Some clones had breakpoint junctions with minimal deletion of the broken chromosomal ends (13–26 bp), whereas other events had larger deletions (118 bp to ≥2.4 kb). These results indicate that ends produced from DSBs in two chromosomes can be readily joined. In addition, the ends can be joined to form the reciprocal translocation by two distinct repair (SSA and NHEJ) pathways.

NHEJ events have been presumed to be involved in translocations leading to sarcomas and leukemias, as mentioned earlier. Although SSA events between chromosomes results in frequent translocations in a model system in yeast [102] as in the system described above, the involvement of SSA in translocations in mammalian tumors has yet to be reported. Nevertheless, repetitive elements, such as *Alu* elements, make up a large fraction of the mammalian genome, and therefore would seem to be good candidates for HR events by SSA or even gene conversion (accompanied by the exchange of flanking markers). One possible explanation for this disparity is that DSBs at two *Alu* elements, rather than DSBs at one *Alu* element and at a second random sequence, may not occur frequently enough to generate a translocation. In addition, sequences undergoing DSB-promoted HR must be highly homologous for HR to occur efficiently [103,

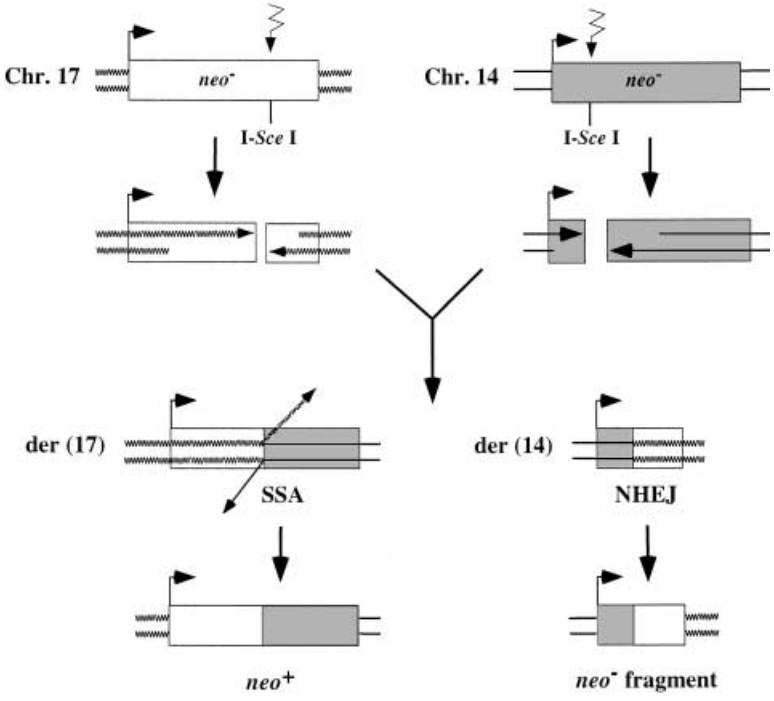

Figure 5. A reciprocal translocation produced by DSBs in a model translocation system. Integrated into two heterologous chromosomes are defective *neo* genes, each of which contains a rare-cutting I-*Sce*I cleavage site. DSBs (jagged arrows) are induced by expression of the I-*Sce*I endonuclease. SSA and NHEJ are two different DSB repair pathways involved in generating this translocation.

104], but *Alu* elements usually diverge from the *Alu* consensus sequence by 2–30% [105]. The proximity of the chromosomes containing the cleaved *Alu* elements may also play a role.

It is known that disruption of the conservative HR repair pathway, gene conversion, leads to a high level of chromosomal aberrations. For example, cells mutated for *BRCA1*, the hereditary breast cancer gene, are defective in HR of DSBs [106], and they show a high level of genomic instability [107]. Possibly, the loss of conservative HR causes a reliance on nonconservative DSB repair (i.e. SSA and NHEJ). Use of the I-*Sce*I-based genetic system for studying the formation of translocations should allow us to determine the effects of perturbating DSB repair pathways and to uncover other components involved in translocations in mammalian cells.

Conclusion

When a cell suffers a DSB, many DNA damage-response signaling and repair pathways are triggered. The cell then undergoes a cell-cycle arrest in order to repair the break or undergoes apoptosis. Inappropriate DSB repair has been proposed to generate the translocations that lead to leukemias, lymphomas and sarcomas. The outcome of a translocation is either dysregulation of a proto-oncogene or expression of a novel oncoprotein, both leading to tumorigenesis. The mechanisms of inappropriate repair are the subject of current investigation. Aberrant V(D)J recombination is proposed to lead to translocations by at least three different mechanisms. Other developmental processes in lymphoid cells, i.e. class switching and hypermutation, have also been implicated in translocations, but details of these processes are not known. A large-scale study of breakpoint junctions in translocations from tumors of Ewing sarcoma patients concluded that illegitimate recombination is the mechanism responsible for mediating these translocations. A genetic system used to induce translocations in mammalian cells has found that nonconservative HR and NHEJ repair, but not gene conversion, will frequently give rise to translocation events. It is becoming increasingly evident that defective repair of DSBs by many different types of DNA repair pathways [108], as well as defective DNA-damage-signaling pathways, play a major role in the development of cancer (reviewed in [109]).

In the future, the further characterization of the genes at translocation breakpoints and the fine mapping of larger sets of breakpoints will provide insight into new proto-oncogenes and different mechanisms that are involved in the development of cancer. In addition, mouse models have recently been developed using the Cre-*loxP* system to generate translocations in vivo and these are expected to provide new insight into the role of translocations in human cancers [110, 111]. The identification and characterization of translocations in various cancers will be expected to be used more frequently as genetic markers for the accurate diagnosis of patients, as well as prognostic tools when analyzing karyotypes of tumor cells. Furthermore, chimeric proteins from translocations provide unique therapeutic targets for novel treatments of cancer, such as the successful use of the new drug, STI-571, for the treatment of CML [112]. And finally, with more sophisticated cytogenetic techniques our understanding should greatly increase regarding the role of chromosomal aberrations in the etiology of solid tumors with complex karyotypes.

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1 Mitelman database of chromosome aberrations in cancer (2001) Mitelman F., Johansson B. and Mertens F. (eds), http://cgap.nci.nih.gov/Chromosomes/Mitelman
2 Look A. T. (1998) Genes altered by chromosomal translocations in leukemias and lymphomas. In: The genetic Basis of Human Cancer, pp. 109–141, Vogelstein B. and Kinzler K. W. (eds), McGraw-Hill, New York
3 Meltzer P. S. and Trent J. M. (1998) Chromosome rearrangements in human solid tumor. In: The Genetic Basis of Human Cancer, pp. 143–160, Vogelstein B. and Kinzler K. W. (eds), McGraw-Hill, New York
4 Boveri T. (1914) Zur Frage der Entstehung maligner Tumoren, Gustav Fischer, Jena
5 Mitelman F. (2000) Recurrent chromosome aberrations in cancer. Mutat. Res. **462:** 247–253
6 Rabbitts T. H. (1994) Chromosomal translocation in human cancer. Nature **372:** 143–149
7 Rowley J. D. (1998) The critical role of chromosome translocations in human leukemias. Annu. Rev. Genet. **32:** 495–519
8 Nowell P. C. and Hungerford D. A. (1960) A minute chromosome in human granulocytic leukemia. Science. **132:** 1497
9 Caspersson T., Zech L. and Johansson C. (1970) Differential binding of alkylating fluorochromes in human chromosomes. Exp. Cell. Res. **60:** 315–319
10 Rowley J. D. (1973) Identificaton of a translocation with quinacrine fluorescence in a patient with acute leukemia. Ann. Genet. **16:** 109–112
11 Rowley J. D. (1973) Letter: a new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature. **243:** 290–293
12 Dalla-Favera R., Bregni M., Erikson J., Patterson D., Gallo R. C., Croce C. M. et al. (1982) Human c-myc onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. Proc. Natl. Acad. Sci. USA **79:** 7824–2827
13 Taub R., Kirsch I., Morton C., Lenoir G., Swan D., Tronick S. et al. (1982) Translocation of the c-myc gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. Proc. Natl. Acad. Sci. USA **79:** 7837–7841
14 Cremer T., Lichter P., Borden J., Ward D. C. and Manuelidis L. (1988) Detection of chromosome aberrations in metaphase and interphase tumor cells by in situ hybridization using chromosome-specific library probes. Hum. Genet. **80:** 235–246

15 Gray J. W. and Pinkel D. (1992) Molecular cytogenetics in human cancer diagnosis. Cancer. **69:** 1536–1542
16 Schröck E., du Manoir S., Veldman T., Schoell B., Wienberg J., Ferguson-Smith M. A. et al. (1996) Multicolor spectral karyotyping of human chromosomes. Science **273:** 494–497
17 Speicher M. R., Ballard S. G. and Ward D. C. (1996) Karyotyping human chromosomes by combinatorial multi-fluor FISH. Nat. Genet. **12:** 368–375
18 Liu L. F. (1989) DNA topoisomerase poisons as antitumor drugs. Ann. Rev. Biochem. **58:** 351–375
19 Fugmann S. D., Lee A. I., Shockett P. E., Villey I. J. and Schatz D. G. (2000) The RAG proteins and V(D)J recombination: complexes, ends and transposition. Annu. Rev. Immunol. **18:** 495–527
20 Keeney S., Giroux C. N. and Kleckner N. (1997) Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. Cell **88:** 375–384
21 Jasin M. (2001) Double-strand break repair and homologous recombination in mammalian cells. In: DNA Damage and Repair: Advances from Phage to Humans, vol. 3, pp. 207–235, Nickoloff J. A. and Hoekstra M. F. (eds), Humana Press, Totowa, NJ
22 Rouet P., Smih F. and Jasin M. (1994) Introduction of double-strand breaks into the genome of mouse cells by expression of a rare-cutting endonuclease. Mol. Cell. Biol. **14:** 8096–8106
23 Liang F., Han M., Romanienko P. J. and Jasin M. (1998) Homology-directed repair is a major double-strand break repair pathway in mammalian cells. Proc. Natl. Acad. Sci. USA **95:** 5172–5177
24 Essers J., van Steeg H., de Wit J., Swagemakers S. M., Vermeij M., Hoeijmakers J. H. et al. (2000) Homologous and non-homologous recombination differentially affect DNA damage repair in mice. EMBO J. **19:** 1703–1710
25 Takata M., Sasaki M., Sonoda E., Morrison C., Hashimoto M., Utsumi H. et al. (1998) Homologous recombination and non-homologous end-joining pathways of DNA double-strand repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. EMBO J. **17:** 5497–5508
26 Richardson C. and Jasin M. (2000) Coupled homologous and nonhomologous repair of a double-strand break preserves genomic integrity in mammalian cells. Mol. Cell. Biol. **20:** 9068–9075
27 Rabbitts T. H. (1999) Perspective: chromosomal translocations can affect genes controlling gene expression and differentiation – why are these functions targeted? J. Pathol. **187:** 39–42
28 Neri A., Barriga F., Knowles D. M., Magrath I. T. and Dalla-Favera R. (1988) Different regions of the immunoglobulin heavy-chain locus are involved in chromosomal translocations in distinct pathogenetic forms of Burkitt lymphoma. Proc. Natl. Acad. Sci. USA **85:** 2748–2752
29 ar-Rushdi A., Nishikura K., Erikson J., Watt R., Rovera G. and Croce C. M. (1983) Differential expression of the translocated and the untranslocated c- myc oncogene in Burkitt lymphoma. Science **222:** 390–393
30 Hayday A. C., Gillies S. D., Saito H., Wood C., Wiman K., Hayward W. S. et al. (1984) Activation of a translocated human c-myc gene by an enhancer in the immunoglobulin heavy-chain locus. Nature **307:** 334–340
31 Cesarman E., Dalla-Favera R., Bentley D. and Groudine M. (1987) Mutations in the first exon are associated with altered transcription of c-myc in Burkitt lymphoma. Science **238:** 1272–1275
32 Amati B., Brooks M. W. and N. Levy. (1993) Oncogenic activity of the c-Myc protein requires dimerization with Max. Cell **72:** 233–245
33 Ayer D. E., Kretzner L. and Eisenman R. N. (1993) Mad: a heterodimeric partner for Max that antagonizes Myc transcriptional activity. Cell **72:** 211–222
34 Kato G. J., Lee W. M., Chen, L. L. and Dang C. V. (1992) Max: functional domains and interaction with c-Myc. Genes Dev. **6:** 81–92
35 Croce C. M., Thierfelder W., Erikson J., Nishikura K., Finan J., Lenoir G. M. et al. (1983) Transcriptional activation of an unrearranged and untranslocated c-myc oncogene by translocation of a C lambda locus in Burkitt. Proc. Natl. Acad. Sci. USA **80:** 6922–6926
36 Erikson J., Nishikura K., ar-Rushdi A., Finan J., Emanuel B., Lenoir G. et al. (1983) Translocation of an immunoglobulin kappa locus to a region 3′ of an unrearranged c-myc oncogene enhances c-myc transcription. Proc. Natl. Acad. Sci. USA **80:** 7581–7585
37 Ye B. H., Rao P. H., Chaganti R. S. and Dalla-Favera R. (1993) Cloning of bcl-6, the locus involved in chromosome translocations affecting band 3q27 in B-cell lymphoma. Cancer Res. **53:** 2732–2735
38 Hatano M., Roberts C. W., Minden M., Crist W. M. and Korsmeyer S. J. (1991) Deregulation of a homeobox gene, HOX11, by the t(10;14) in T cell leukemia. Science **253:** 79–82
39 Kennedy M. A., Gonzalez-Sarmiento R., Kees U. R., Lampert F., Dear N., Boehm T. et al. (1991) HOX11, a homeobox-containing T-cell oncogene on human chromosome 10q24. Proc. Natl. Acad. Sci. USA **88:** 8900–8904
40 Bartram C. R., de Klein A., Hagemeijer A., van Agthoven T., Geurts van Kessel A., Bootsma D. et al. (1983) Translocation of c-ab1 oncogene correlates with the presence of a Philadelphia chromosome in chronic myelocytic leukaemia. Nature **306:** 277–280
41 Shtivelman E., Lifshitz B., Gale R. P. and Canaani E. (1985) Fused transcript of abl and bcr genes in chronic myelogeneous leukemia. Nature **315:** 550–554
42 Shah N. P., Witte O. N. and Denny C. T. (1991) Characterization of the BCR promoter in Philadelphia chromosome-positive and -negative cell lines. Mol. Cell. Biol. **11:** 1854–1860
43 Heisterkamp N., Stam K., Groffen J., de Klein A. and Grosveld G. (1985) Structural organization of the bcr gene and its role in Ph^1 translocation. Nature **315:** 758–761
44 Bernards A., Rubin C. M., Westbrook C. A., Paskind M. and Baltimore D. (1987) The first intron in the human c-abl gene is at least 200 kilobases long and is a target for translocations in chronic myelogenous leukemia. Mol. Cell. Biol. **7:** 3231–3236
45 Laurent E., Talpaz M., Kantarjian H. and Kurzrock R. (2001) The BCR gene and Philadelphia chromosome-positive leukemogenesis. Cancer Res. **61:** 2343–2355
46 Konopka J. B., Watanabe S. M. and Witte O. N. (1984) An alteration of the human c-abl protein in K562 leukemia cells unmasks associated tyrosine kinase activity. Cell **37:** 1035–1042
47 Lugo T. G., Pendergas A. M., Muller A. J. and Witte O. N. (1990) Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. Science **247:** 1079–1082
48 Daley G. Q., Van Etten R. A. and Baltimore D. (1990) Induction of chronic myelogenous leukemia in mice by the P210bcr/abl gene of the Philadelphia chromosome. Science **247:** 824–830
49 Ribeiro R. C., Abromowitch M., Raimondi S. C., Murphy S. B., Behm F. and Williams D. L. (1987) Clinical and biologic hallmarks of the Philadelphia chromosome in childhood acute lymphoblastic leukemia. Blood **70:** 948–953
50 Rowley J. D. (1984) Biological implications of consistent chromosome rearrangements in leukemia and lymphoma. Cancer Res. **44:** 3159–3165
51 Chan L. C., Karhi K. K., Rayter S. I., Heisterkamp N., Eridani S., Powles R. et al. (1987) A novel abl protein expressed in Philadelphia chromosome positive acute lymphoblastic leukaemia. Nature **325:** 635–637

52 Gerald W. L., Miller H. K., Battifora H., Miettinen M., Silva E. G. and Rosai J. (1991) Intra-abdominal desmoplastic small round-cell tumor. Report of 19 cases of a distinctive type of high-grade polyphenotypic malignancy affecting young individuals. Am. J. Surg. Pathol. **15:** 499–513
53 Sawyer J. R., Tryka A. F. and Lewis J. M. (1992) A novel reciprocal chromosome translocation t(11;22) (p13;q12) in an intraabdominal desmoplastic small round-cell tumor. Am. J. Surg. Pathol. **16:** 411–416
54 Ladanyi M. and Gerald W. (1994) Fusion of the EWS and WT1 genes in the desmoplastic small round cell tumor. Cancer Res. **54:** 2837–2840
55 Kim J., Lee K. and Pelletier J. (1998) The desmoplastic small round cell tumor t(11;22) translocation produces EWS/WT1 isoforms with differing oncogenic properties. Oncogene **16:** 1973–1979
56 Gerald W. L., Rosai J. and Ladanyi M. (1995) Characterization of the genomic breakpoint and chimeric transcripts in the EWS-WT1 gene fusion of desmoplastic small round cell tumor. Proc. Natl. Acad. Sci. USA **92:** 1028–1032
57 Ohno T., Ouchida M., Lee L., Gatalica Z., Rao V. N. and Reddy E. S. (1994) The EWS gene, involved in Ewing family of tumors, malignant melanoma of soft parts and desmoplastic small round cell tumors, codes for an RNA binding protein with novel regulatory domains. Oncogene **9:** 3087–3097
58 May W. A., Lessnick S. L., Braun B. S., Klemsz M., Lewis B. C., Lunsford L. B. et al. (1993) The Ewing's sarcoma EWS/FLI-1 fusion gene encodes a more potent transcriptional activator and is a more powerful transforming gene than FLI-1. Mol. Cell. Biol. **13:** 7393–7398
59 Bertolotti A., Lutz Y., Heard D. J., Chambon P. and Tora L. (1996) hTAF(II)68, a novel RNA/ssDNA-binding protein with homology to the pro-oncoproteins TLS/FUS and EWS is associated with both TFIID and RNA polymerase II. EMBO J. **15:** 5022–5031
60 Crozat A., Aman P., Mandahl N. and Ron D. (1993) Fusion of CHOP to a novel RNA-binding protein in human myxoid liposarcoma. Nature **363:** 640–644
61 Zinszner H., Albalat R. and Ron D. (1994) A novel effector domain from the RNA-binding protein TLS or EWS is required for oncogenic transformation by CHOP. Genes Dev. **8:** 2513–2526
62 Lee S. B. and Haber D. A. (2001) Wilms tumor and the WT1 gene. Exp. Cell. Res. **264:** 74–99
63 Haber D. A., Park S., Maheswaran S., Englert C., Re G. G., Hazen-Martin D. J. et al. (1993) WT1-mediated growth suppression of Wilms tumor cells expressing a WT1 splicing variant. Science **262:** 2057–2059
64 Rauscher F. J., Benjamin L. E., Fredericks W. J. and Morris J. R. (1994) Novel oncogenic mutations in the WT1 Wilms' tumor suppressor gene: a t(11;22) fuses the Ewing's sarcoma gene, EWS1 to WT1 in desmoplastic small round cell tumor. Cold Spring Harb. Symp. Quant. Biol. **59:** 137–146
65 Karnieli E., Werner H., Rauscher F. J., Benjamin L. E. and LeRoith D. (1996) The IGF-1 receptor gene promoter is a molecular target for the Ewing's sarcoma-Wilms' tumor 1 fusion protein. J. Biol. Chem. **271:** 19304–19309
66 Lee S. B., Kolquist K. A., Nichols K., Englert C., Maheswaran S., Ladanyi M. et al. (1997) The EWS-WT1 translocation product induces PDGFA in desmoplastic small round-cell tumour. Nat. Genet. **17:** 309–313
67 Delattre O., Zucman J., Plougastel B., Desmaze C., Melott T., Peter M. et al. (1992) Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumours. Nature **359:** 162–165
68 Zucman J., Delattre O., Desmaze C., Epstein A. L., Stenman G., Speleman F. et al. (1993) EWS and ATF-1 gene fusion induced by t(12;22) translocation in malignant melanoma of soft parts. Nat. Genet. **4:** 341–345
69 Jeggo P. A. (1998) DNA breakage and repair. Adv. Genet. **38:** 185–218.
70 Bogue M. and Roth D. B. (1996) Mechanism of V(D)J recombination. Curr. Opin. Immunol. **8:** 175–180
71 van Gent D. C., Hoeijmakers J. H. and Kanaar R. (2001) Chromosomal stability and the DNA double-stranded break connection. Nat. Rev. Genet. **2:** 196–206
72 Cooper D. N., Krawczak M. and Antonarakis S. E. (1998) The nature of human gene mutation. In: The Genetic Basis of Human Cancer, pp. 65–94, Vogelstein B. and Kinzler K. W. (eds), McGraw-Hill, New York
73 Onno M., Nakamura T., Hillova J. and Hill M. (1992) Rearrangement of the human tre oncogene by homologous recombination between Alu repeats of nucleotide sequences from two different chromosomes. Oncogene **7:** 2519–2523
74 Edelmann L., Spiteri E., Koren K., Pulijaal V., Bialer M. G., Shanske A. et al. (2001) AT-rich palindromes mediate the constitutional t(11;22) translocation. Am. J. Hum. Genet. **68:** 1–13
75 Akgün E., Zahn J., Baumes S., Brown G., Liang F., Romanienko P. J. et al. (1997) Palindrome resolution and recombination in the mammalian germ line. Mol. Cell. Biol. **17:** 5559–5570
76 Bae Y. S., Kawasaki I., Ikeda H. and Liu L. F. (1988) Illegitimate recombination mediated by calf thymus DNA topoisomerase II in vitro. Proc. Natl. Acad. Sci. USA **85:** 2076–2080
77 Aplan P. D., Chervinsky D. S., Stanulla M. and Burhans W. C. (1996) Site-specific DNA cleavage within the MLL breakpoint cluster region induced by topoisomerase II inhibitors. Blood **87:** 2649–2658
78 Zhou R. H., Wang P., Zou Y., Jackson-Cook C. K. and Povirk L. F. (1997) A precise interchromosomal reciprocal exchange between hot spots for cleavable complex formation by topoisomerase II in amsacrine-treated Chinese hamster ovary cells. Cancer Res. **57:** 4699–5702
79 Felix C. A. (1998) Secondary leukemias induced by topoisomerase-targeted drugs. Biochim. Biophys. Acta **1400:** 233–255
80 Kaye J. S., Bellard M., Dretzen G., Bellard F. and Chambon P. (1984) A close association between sites of DNase I hypersensitivity and sites of enhanced cleavage by micrococcal nuclease in the 5′-flanking region of the actively transcribed ovalbumin gene. EMBO J. **3:** 1137–1144
81 Sperry A. O., Blasquez V. C. and Garrard W. T. (1989) Dysfunction of chromosomal loop attachment sites: illegitimate recombination linked to matrix association regions and topoisomerase II. Proc. Natl. Acad. Sci. USA **86:** 5497–5501
82 Strissel P. L., Strick R., Tomek R. J., Roe B. A., Rowley J. D. and Zeleznik-Le N. J. (2000) DNA structural properties of AF9 are similar to MLL and could act as recombination hot spots resulting in MLL/AF9 translocations and leukemogenesis. Hum. Mol. Genet. **9:** 1671–1679
83 Korsmeyer S. J. (1992) Chromosomal translocations in lymphoid malignancies reveal novel proto-oncogenes. Ann. Rev. Immunol. **10:** 785–807
84 Tycko B. and Sklar J. (1990) Chromosomal translocations in lymphoid neoplasia: a reappraisal of the recombinase model. Cancer Cells **2:** 1–8
85 Gellert M. (1997) Recent advances in understanding V(D)J recombination. Adv. Immunol. **64:** 39–64
86 Lewis S. (1994) The mechanism of V(D)J joining: lessons from molecular, immunological and comparative analyses. Adv. Immunol. **56:** 27–150
87 Tsujimoto Y., Gorham J., Cossman J., Jaffe E. and Croce C. M. (1985) The t(14;18) chromosome translocations involved in B-cell neoplasms result from mistakes in VDJ joining. Science **229:** 1390–1393
88 Raghavan S. C., Kirsch I. R. and Lieber M. R. (2001) Analysis of the V(D)J recombination efficiency at lymphoid chro-

mosomal translocation breakpoints. J. Biol. Chem. **276:** 29126–29133
89 Lewis S. M., Agard E., Suh S. and Czyzyk L. (1997) Cryptic signals and the fidelity of V(D)J joining. Mol. Cell. Biol. **17:** 3125–3136
90 Bakhshi A., Wright J. J., Graninger W., Seto M., Owens J., Cossman J. et al. (1987) Mechanism of the t(14;18) chromosomal translocation: structural analysis of both derivative 14 and 18 reciprocal partners. Proc. Natl. Acad. Sci. USA **84:** 2396–2400
91 Hiom K., Melek M. and Gellert M. (1998) DNA transposition by the RAG1 and RAG2 proteins: a possible source of oncogenic translocations. Cell **94:** 463–470
92 Rajewsky K. (1996) Clonal selection and learning in the antibody system. Nature **381:** 751–758
93 Casellas R., Nussenzweig A., Wuerffel R., Pelanda R., Reichlin A., Suh H. et al. (1998) Ku80 is required for immunoglobulin isotype switching. EMBO J. **17:** 2404–2411
94 Jacobs H. and Bross L. (2001) Towards an understanding of somatic hypermutation. Curr. Opin. Immunol. **13:** 201–218
95 Kuppers R., Klein U., Hansmann M. L. and Rajewsky K. (1999) Cellular origin of human B-cell lymphomas. N. Engl. J. Med. **314:** 1520–1529
96 Zucman-Rossi J., Legoix P., Victor J-M., Lopez B. and Thomas G. (1998) Chromosome translocation based on illegitimate recombination in human tumors. Proc. Natl. Acad. Sci. USA **95:** 11786–11791
97 de Alava E. and Gerald W. L. (2000) Molecular biology of the Ewing's sarcoma/primitive neuroectodermal tumor family. J. Clin. Oncol. **18:** 204–213
98 Gillert E., Leis T., Repp R., Reichel M., Hosch A., Breitenlohner I. et al. (1999) A DNA damage repair mechanism is involved in the origin of chromosomal translocations t(4;11) in primary leukemic cells. Oncogene **18:** 4663–4671
99 Super H. G., Strissel P. L., Sobulo O. M., Burian D., Reshmi S. C., Roe B. et al. (1997) Identification of complex genomic breakpoint junctions in the t(9;11) MLL-AF9 fusion gene in acute leukemia. Genes Chromosomes Cancer **20:** 185–195
100 Richardson C. and Jasin M. (2000) Frequent chromosomal translocations induced by DNA double-strand breaks. Nature **405:** 697–700
101 Richardson C., Moynahan M. E. and Jasin M. (1998) Double-strand break repair by interchromosomal recombination: suppression of chromosomal translocations. Genes Dev. **12:** 3831–3842
102 Haber J. E. and Leung W. Y. (1996) Lack of chromosome territoriality in yeast: promiscuous rejoining of broken chromosome ends. Proc. Natl. Acad. Sci. USA **93:** 13949–13954
103 Elliott B. and Jasin M. (2001) Repair of double-strand breaks by homologous recombination in mismatch repair-defective mammalian cells. Mol. Cell. Biol. **21:** 2671–2682
104 Elliott B., Richardson C., Winderbaum J., Nickoloff J. and Jasin M. (1998) Gene conversion tracts from double-strand break repair in mammalian cells. Mol. Cell. Biol. **18:** 93–101
105 Schmid C. W. (1996) Alu: structure, origin, evolution, significance and function of one-tenth of human DNA. Prog. Nucleic Acid. Res. Mol. Biol. **53:** 283–319
106 Moynahan M. E., Chiu J. W., Koller B. H. and Jasin M. (1999) Brca1 controls homology-directed DNA repair. Mol. Cell. **4:** 511–518
107 Xu X., Weaver Z., Linke S. P., Li C., Gotay J., Wang X. W. et al. (1999) Centrosome amplification and a defective G2-M cell cycle checkpoint induce genetic instability in BRCA1 exon 11 isoform-deficient cells. Mol. Cell. **3:** 389–395
108 Pâques F. and Haber J. E. (1999) Multiple pathways of recombination induced by double-strand breaks in *Saccharomyces cerevisiae*. Microbiol. Mol. Biol. Rev. **63:** 349–404
109 Khanna K. K. and Jackson S. P. (2001) DNA double-strand breaks: signaling, repair and the cancer connection. Nat. Genet. **27:** 247–254
110 Buchholz F., Refaeli Y., Trumpp A. and Bishop J. M. (2000) Inducible chromosomal translocation of AML1 and ETO genes through Cre/loxP-mediated recombination in the mouse. EMBO Rep. **1:** 133–139
111 Collins E. C., Pannell R., Simpson E. M., Forster A. and Rabbitts T. H. (2000) Inter-chromosomal recombination of Mll and Af9 genes mediated by cre-loxP in mouse development. EMBO Rep. **1:** 127–132
112 Druker B. J., Talpaz M., Resta D. J., Peng B., Buchdunger E., Ford J. M. et al. (2001) Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N. Engl. J. Med. **344:** 1031–1037