Mechanisms that Promote and Suppress Chromosomal Translocations in Lymphocytes

Monica Gostissa,1 Frederick W. Alt,1 and Roberto Chiarle1,2

1Howard Hughes Medical Institute, Immune Disease Institute, Program in Cellular and Molecular Medicine, Children’s Hospital Boston and Departments of Genetics and Pediatrics, Harvard Medical School, Boston, Massachusetts 02115; email: alt@enders.tch.harvard.edu
2Department of Biomedical Sciences and Human Oncology and CERMS, University of Torino, 10126 Turin, Italy

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Abstract
Recurrent chromosomal translocations are characteristic features of many types of cancers, especially lymphomas and leukemias. Several basic mechanistic factors are required for the generation of most translocations. First, DNA double-strand breaks (DSBs) must be present simultaneously at the two participating loci. Second, the two broken loci must either be in proximity or be moved into proximity to be joined. Finally, cellular DNA repair pathways must be available to join the two broken loci to complete the translocation. These mechanistic factors can vary in different normal and mutant cells and, as a result, substantially influence the frequency at which particular translocations are generated in a given cell type. Ultimately, however, appearance of recurrent oncogenic translocations in tumors is, in most cases, strongly influenced by selection for the translocated oncogene during the tumorigenesis process. In this review, we discuss in depth the factors and pathways that contribute to the generation of translocations in lymphocytes and other cell types. We also discuss recent findings regarding mechanisms that underlie the appearance of recurrent translocations in tumors.
INTRODUCTION
Chromosomal translocations fuse two different chromosomes and are fundamental pathogenetic events in many cancers. Translocations are found in most hematopoietic malignancies (1) and also in various solid tumors, including prostate and lung cancers (2). Translocations can arise in normal cells and contribute to primary neoplastic transformation and can also arise in tumor cells and contribute to tumor progression. Certain tumor types, including many lymphoid malignancies, harbor clonal, recurrent translocations that appear in most cells and involve the same chromosomal loci. Studies of recurrent translocations in tumors have yielded substantial insight into mechanisms that generate them and how they contribute to oncogenesis. Several general mechanistic factors are implicated. First, most well-characterized translocations involve joining of DNA double-strand breaks (DSBs) in two separate chromosomal loci. Second, for a translocation to occur, the two participating DSBs must be present at the same time in the cell nucleus and they must be in physical proximity for joining. Thus, the DSBs must happen in chromosomal loci that lie close together in the nucleus or in loci that are brought together. Finally, the two DSBs that generate the translocation must be joined interchromosomally. The vast majority of characterized translocations appear to be mediated by an end-joining mechanism.

Most known recurrent translocations were first characterized cytogenetically and, thus, involve different chromosomes. There are several types of translocations. Balanced, reciprocal translocations involve breakage of two different chromosomes followed by fusion of the telomeric fragment of one to the centromeric fragment of the other and vice versa. Nonreciprocal translocations come in several forms. As one example, a nonreciprocal translocation might result from the fusion of the centromeric portion of two chromosomes to generate a dicentric chromosome with the telomeric (acentric) portions being lost. Dicentric chromosomes can serve as substrates for the generation of further cytogenetic complexity such as gene amplifications (3, 4). Translocations can also involve different locations along the same chromosome. Indeed, in some cases, chromosomal deletions, including those involved in the physiological process of immunoglobulin (Ig) heavy chain (IgH) class switch recombination (CSR) in B cells, may involve the same general mechanisms as interchromosomal translocations (5, 6). Also, the various forms of translocations likely arise by the same general processes.

Recurrent translocations are particularly common in leukemias and lymphomas (1), with most B lineage tumor translocations involving Ig loci as one partner and with most T lineage tumor translocations involving T cell receptor (TCR) loci as a partner. In this regard, DSBs are specifically introduced into Ig and TCR loci in developing B and T cells, respectively, during the V(D)J recombination process that assembles antigen receptor variable region exons. In addition, activated mature B cells introduce DSBs into their IgH loci in the context of CSR. These lymphocyte-specific programmed DSBs can generate one chromosomal partner in the clonal translocations commonly observed in lymphoid tumors. However, programmed DSBs in developing lymphocytes are not the only ones that contribute to translocations. DSBs in the loci that partner with Ig or TCR loci to form translocations may be generated by off-target V(D)J recombination or by CSR activities or may result from more general factors, such as oxidative metabolism or genotoxic agents (7).

DSBs are potentially harmful lesions for the cell. Thus, eukaryotic cells evolved mechanisms to efficiently repair DSBs or eliminate cells that harbor them. DNA DSB response pathways rapidly recognize DSBs in chromatin and generate foci of factors around DSBs that activate cellular checkpoints and promote repair (8). In mammalian cells, there are two well-characterized DSB repair pathways, one involving homologous recombination (HR) and the other involving nonhomologous DNA end-joining (NHEJ). HR requires a second locus with long stretches
of homology and is operative in postreplicative cells, where a homologous DNA template on a sister chromatid is available. NHEJ directly joins DNA ends that lack homology or that have short homologies, and it is operative throughout the cell cycle. At least two NHEJ pathways exist. Classical-NHEJ (C-NHEJ) is the primary mammalian end-joining pathway and is essential for promoting chromosomal integrity and suppressing translocations. In the absence of C-NHEJ, DSBs can still be joined by alternative end-joining (A-EJ), a process or processes that clearly can join DSBs that contribute to translocations.

Translocations happen spontaneously in normal cells but more often in cells deficient for DNA DSB response or repair pathways (see below). When a translocation involves oncogenes or tumor suppressor genes, it can be positively selected in the context of neoplastic transformation and ultimately appear clonally in tumor cells. Although selection likely plays the main role in the appearance of most clonal translocations in tumors, mechanistic factors can greatly influence this phenomenon, for example by promoting clonal translocations involving one of two equivalent cellular oncogenes (9). Most recurrent translocations activate cellular oncogenes, either by generating oncogenic fusion proteins, such as the BCR-ABL fusion protein found in human myeloid leukemias and certain early B cell leukemias (2) or by deregulating oncogene expression by linking it to strong transcriptional control elements such as in IgH to c-myc translocations found in many human Burkitt’s B cell lymphomas (1). Deregulated oncogene expression may involve increased expression, inappropriate expression (e.g., deregulated in the cell cycle), or both. Translocations that lead to deregulated oncogene expression are common in lymphoid malignancies.

In this review, we discuss recent advances in our understanding of the mechanistic features that generate recurrent translocations found in tumors. The basic factors that contribute to the initiation of recurrent translocations in B and T lineage cells are likely to be much the same, even though they involve different loci owing to the introduction of V(DJ) recombination-associated DSBs into different antigen receptor loci, differential activity of antigen receptor cis-regulatory elements, and/or differential activity and expression of potential target cellular oncogenes in the two lineages. Thus, although this review focuses primarily on factors and processes that lead to chromosomal translocations in B lineage cells, many of the general principles discussed are applicable to T lineage cells or even nonlymphoid cells.

ANTIGEN RECEPTOR GENE ASSEMBLY IN B CELL DEVELOPMENT

Overview

The basic subunit of Ig molecules is composed of a pair of identical IgH and a pair of identical Ig light (IgL) chains. Ig molecules serve as antigen recognition components of the B cell receptor (BCR) and secreted antibodies (10). IgL chains can be encoded by either the Igκ or the Igλ loci (10). The antigen-binding region of Ig molecules is contributed by the amino-terminal variable (V) regions of IgH and IgL chains, while the IgH constant region (CH) provides effector functions and determines antibody class. IgH and IgL variable regions (and those of TCR chains) are encoded in exons that are assembled from germ-line gene segments via V(D)J recombination. The initially expressed C41 exons (Cμ exons) also can be switched to exons that encode a different C41 by CSR. Both V(D)J recombination and CSR are initiated by lymphocyte-specific enzymes that introduce DSBs in specific targets and are both completed by DSB end-joining mechanisms that operate in all cells. Misrepair of DSBs introduced during V(D)J recombination or CSR can promote oncogenic translocations. In mouse and human B lineage tumors, such translocations often involve IgH, but some involve Igκ or Igλ.

V(D)J Recombination

IgH variable region exons are assembled from variable (VH), diversity (D), and joining (JH)
gene segments just upstream of Cμ exons (Figure 1). The mouse IgH locus spans several megabases (Mb) at the telomeric end of chromosome 12 and contains 4 JH, 13 D, and hundreds of VH segments. Mouse Igκ and Igλ variable regions are encoded only by V and J segments, with Igκ locus (which contains over 140 Vκ and 4 Jκ segments) and Igλ locus (which contains three functional Vλ and three functional Jλ segments) lying on chromosomes 6 and 16, respectively (Figure 1) (10). The human IgH (telomere of chromosome 14), Igκ (chromosome 2), and Igλ (chromosome 22) loci are organized similarly to those of mouse, although the human Igκ locus is much more complex (10).

V(D)J recombination is initiated by the products of recombination activating genes (RAG) 1 and 2 (11, 12). The RAG1 and RAG2 proteins form a complex (RAG) that is specifically expressed in developing B and T lymphocytes (10). RAG introduces DSBs at the borders of V, D, and J segments (13), which are then joined by C-NHEJ to form V(D)J exons (14). Thus, the complete V(D)J recombinase is composed of the RAG DSB-generating enzyme and the C-NHEJ DSB repair complex. RAG1 and RAG2 are both absolutely required for V(D)J recombination; deficiency for either causes a complete block of B and T cell development (severe combined immunodeficiency, SCID) because of an inability to generate initiating DSBs (15, 16). Likewise, a deficiency in any C-NHEJ components also causes an essentially complete block in B and T cell development because of an inability to join RAG-cleaved V, D, and J segments (17).

RAG recognizes short recombination signal sequences (RSSs) that lie adjacent to V, D, and J segments. An RSS consists of a conserved heptamer and an AT-rich nonamer separated by a nonconserved spacer of either 12 or 23 nucleotides (10). RAG-mediated cleavage only takes place between gene segments that, respectively, have RSSs with 12-bp and 23-bp spacers (the 12/23 rule). In the IgH locus, V and J segments have 23-bp RSSs at their 3′ and 5′ ends, respectively, whereas D segments are flanked on both sides by 12-bp RSSs (10). Therefore, the 12/23 restriction directs joining by allowing JH and VH segments to rearrange only to D segments and not to each other. Igκ and Igλ V and J sequences, respectively, have 12-bp and 23-bp or 23-bp and 12-bp spacers that allow direct V to J joining. TCRβ V, D, and J gene segments are organized with RSSs that could permit both Dβ-to-Jβ and Vβ to either Dβ or Jβ joins, but direct Vβ-to-Jβ joining at this locus is prohibited by “beyond 12/23” restrictions (18). Overall, RSSs help direct proper and specific assembly of Ig and TCR variable region genes and provide specificity to the reaction. The 12/23 and beyond 12/23 restrictions may also be important in limiting RAG-initiated DSBs at spurious RSSs that occur throughout the genome (see the section on DSBs in Translocations).

RAG-generated DSBs at two participating gene segments form a pair of RSS ends in the form of blunt 5′-phosphorylated DSBs and a pair of hairpin-sealed coding ends (10). The RAG cleavage mechanism resembles that used in certain transposition reactions and, as such, likely reflects the evolutionary origin of RAG from a transposase (19). Truncated RAG proteins (termed core RAGs) carry out transposition-related reactions in biochemical assays, leading to the notion that RAG might generate translocations via such a reaction (20, 21). However, few examples of transposition-related RAG-mediated translocations have been found (22). In this regard, the regions deleted to generate core RAGs actually suppress the transposition-like activities of full-length RAGs (23–25). Cleaved RSS and

Figure 1
Schematic representation of the murine Ig loci and of the rearrangements that take place during V(D)J recombination. V, D, and J gene segments are represented by rectangles, 23-bp recombination signal sequences (RSSs) by gray triangles, 12-bp RSSs by black triangles, and enhancers by black ovals. See text for details.
Nonproductive rearrangement

Pro-B cells

Productive rearrangement

Pre-B cells

Nonproductive rearrangement

Pre-B cells
coding ends remain associated with RAG in a postcleavage synaptic complex (10), which may suppress end degradation and recruit DSB repair factors. In the latter context, RAG recruits C-NHEJ, while excluding A-EJ (26). In the V(D)J recombination joining step, RSS ends are directly joined to form precise RSS joins, while coding ends are processed, involving hairpin opening with potential loss of nucleotides, and de novo addition of N region nucleotides, which is important in antibody and TCR repertoire diversification (10). Opening and processing of hairpin coding ends also employ C-NHEJ factors (10).

Antigen receptor variable region exon assembly is controlled in a lineage- and developmental stage–specific fashion (18). V(D)J recombination is initiated at the IgH locus in progenitor B (pro-B) cells in the bone marrow (or fetal liver), with D-to-JH rearrangements occurring first and on both alleles, followed by appendage of a VH segment to the preexisting DJH complex (18). When a productive VH(D)JH exon is assembled and transcribed, it is linked via RNA processing to the Cμ constant region exons, allowing production of μ IgH chains (Figure 1) (18). Expression of μ chains signals cessation of further VH(D)JH rearrangement, ensuring allelic exclusion, and promotes progression to the precursor B (pre-B) cell stage, in which IgL variable regions are assembled, with rearrangement of Igκ variable region exons usually preceding that of Igλ (18). Assembly of a functional κ or λ IgL variable region exon leads to production of Igκ or Igλ chains that associate with μ chains to form IgM molecules, which are expressed on the surface of naive B lymphocytes (18). Expression of surface IgM can downregulate RAG expression and suppress further V(D)J recombination. However, if an autoreactive antibody is produced, RAG expression can be maintained in IgM+ B

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**Figure 2**

Schematic representation of the IgH constant region locus and of the rearrangements that take place during class switch recombination. Cμ genes are represented by rectangles, S regions by blue ovals, and enhancers by black ovals. See text for details.
cells to allow further IgL loci rearrangements in a process termed receptor editing (27). Receptor editing has been thought to occur primarily in bone marrow B cells, but it may also occur in some peripheral B cells, where it may also contribute to translocations (see below).

Additional aspects of V(D)J recombination may influence translocations. Substrate variable region gene segments are embedded within large chromosomal regions (up to several Mb in length) that, if considered in the context of linear DNA, would represent huge nuclear distances. Therefore, as for DSBs on different chromosomes that contribute to translocations, V, D, and J segments must be brought together for joining. Although RAG proteins contribute to tethering such segments in a synaptic complex, the actual synapsis mechanism might rely on programmed looping of antigen receptor loci (28), DSB response factors (29), and/or as yet uncharacterized RAG functions. Notably, RAG2 contains a C-terminal PHD domain that binds histone 3 methylated on lysine 4 (H3K4) (30–32). This modification is enriched in certain regions of antigen receptor loci but occurs widely in the genome, leading to broad binding of RAG2 (33). Such broad RAG2 binding may play an important role in V(D)J recombination, but overall targeting of RAG2 to its proper substrate must involve additional factors because core RAG2, which lacks the PHD domain, can still promote reduced but substantially normal V(D)J recombination in vivo (34, 35). Finally, functional RAG expression is limited to the G1 cell cycle phase by several mechanisms, including the degradation of RAG2 as cells go through the G1/S phase transition (36). Such regulation of RAG activity is likely important for minimizing potential translocation-promoting activities and provides an additional mechanism of ensuring repair of RAG-initiated DSBs by C-NHEJ. In mice, uncontrolled progression of C-NHEJ-deficient pro-B cells bearing unrepaired RAG-initiated DSBs into S phase owing to a G1/S checkpoint defect leads to pro-B cell lymphomas with oncogenic RAG-initiated translocations catalyzed by A-EJ (4).

IgH Class Switch Recombination
IgM-positive naive B cells migrate to peripheral lymphoid organs where, upon antigen stimulation, they can undergo somatic hypermutation (SHM) of IgH and IgL variable region exons and/or IgH CSR. Although different processes, SHM and CSR are both initiated by activation-induced cytidine deaminase (AID) (37). SHM generates point mutations, small deletions and insertions in targeted variable region exons (38–40). SHM occurs in specialized structures in peripheral lymphoid organs termed germinal centers (GCs), in which B cells interact with T cells and other immune cells in a process that promotes antigen exposure, induces AID, and allows the selection of B cells that express higher affinity BCRs (41). IgH CSR can also occur within the GC reaction, as well as in extrafollicular regions (41). CSR requires the generation of AID-initiated DSBs in the IgH C\(\mu\) locus; such breaks can be substrates for translocations (40). SHM generally does not lead to DSBs, although they may occasionally be generated as by-products of AID activity and may sometimes initiate translocations (40, 42, 43). Introduction of SHMs in a DNA sequence is often a telltale sign of AID activity, as SHMs are often found at CSR junctions and in translocation breakpoints from mature B cell lymphomas (43).

CSR is a deletional event that replaces C\(\mu\) exons with a downstream set of C\(\gamma\) exons (referred to as C\(\gamma\) genes) (Figure 2), allowing production of different antibody classes, such as IgG, IgE, or IgA (38). There are eight C\(\gamma\) genes in the mouse IgH, which span more than 200 kb; in the similarly organized human IgH there are nine C\(\gamma\) genes (Figure 2) (10). Long, repetitive, GC-rich sequences, termed switch (S) regions, that lie upstream of C\(\gamma\) genes mediate CSR (38). During CSR, multiple AID-initiated DSBs are introduced into S\(\mu\) (the donor) and into a downstream S region (the acceptor); these DSBs, which also must be synapsed, are then fused by end-joining. Of note, AID-initiated breaks in a given S region can also be joined to other AID-initiated DSBs within the same S
region to generate internal S region deletions (ISDs). C-NHEJ plays a pivotal role in joining S regions; however, in contrast to V(D)J recombination, A-EJ catalyzes CSR joining in the absence of C-NHEJ and potentially even in its presence (44, 45). Joining two broken S regions deletes the intervening chromosomal DNA and, thereby, juxtaposes a different CH gene to the expressed V(D)J exon (38). Individual S regions are preceded by noncoding exons (I regions) and promoters (I promoters), which can be differentially activated by specific signaling pathways via interactions with T cells and other lymphoid cells and their secreted cytokines (38, 46). Transcription through a given S region is required to target AID and, thereby, CSR. In this way, CSR is directed to specific S regions, allowing secreted antibodies to be tailored for particular pathogens or environments (46). This aspect of AID targeting influences the generation of particular tumors in specific contexts, such as the occurrence in gut-associated lymphoid tissues of IgA-secreting B lineage tumors (lymphomas and plasmacytomas) that have translocations involving the Cα S region of the IgH allele not used for IgA expression (47).

AID is a single-strand (ss)-specific DNA cytidine deaminase, which catalyzes dC-to-dU deamination and displays a general preference for RGYW motifs (where R = purine, Y = pyrimidine, and W = A or T nucleotide) (38). Abnormal processing of dU:dG mismatches by base-excision repair or mismatch repair results in DNA nicks, which can lead to mutations or DSBs (40). The AID target RGYW motif and, in particular, the canonical palindromic motif AGCT are highly enriched in S regions and are also found in variable region exons. However, such motifs occur throughout the genome (38). Indeed, as for RAGs, AID activity has been linked to the generation of DSBs involved in translocations in both Ig and non-Ig loci (see below), and, although AID-introduced mutations are by far the most abundant in Ig loci, they are found in many other genomic sequences at lower levels (43, 48). Therefore, elucidation of mechanisms that control AID activity and preferentially target it to Ig genes is crucial for understanding how its tremendous mutagenic potential is restrained.

AID is targeted to duplex DNA by transcription (49). In this context, transcription through mammalian S regions, based on their unusual GC-rich sequence, results in the formation of stable RNA:DNA hybrids with displacement of the nontemplate strand as ss-DNA (R-loops) (50). R-loop formation allows AID to robustly target the nontemplate strand of transcribed S regions (49). R-loop formation also targets AID ectopically expressed in yeast to transcriptional promoters (51) and, in theory, could be associated with ectopic AID activity that may initiate prostate cancer translocations (52). However, R-loops are not formed in transcribed V(D)J exons, which are AID SHM substrates, or in transcribed amphibian (e.g., *Xenopus*) S regions, even though they can target CSR when inserted in place of mouse S regions (53). In this regard, AID phosphorylated on serine 38 (S38) interacts with the ss-DNA-binding protein RPA (replication protein A) to gain access to in vitro–transcribed non-R-loop-forming DNA sequences, such as *Xenopus* S regions (53, 54). Transcription/RPA-dependent AID access to substrate sequences still predominantly targets the nontemplate strand, indicating that additional mechanisms and cofactors exist that target AID activity to the template DNA strand, which is required for normal SHM patterns and CSR-associated DSBs (40).

Tight regulation of AID expression is critical for suppressing off-target activities. Loss of post-transcriptional downregulation of AID expression by miRNA155 leads to increased CSR, aberrant persistence of AID expression, and increased IgH/c-myc translocations (55, 56). S38 phosphorylation also regulates AID potency and could influence off-target activities (57, 58). In addition, most AID in B cells is in the cytoplasm, suggesting that regulation of nuclear accumulation might also harness AID activity (38, 59). Ectopic AID expression in mice led to tumorigenesis of B and non-B lineage cells (60, 61) and revealed novel AID translocation targets. Expression of AID is best characterized...
in activated mature B cells in the context SHM or CSR (60). However, AID is also expressed in bone marrow B cells and in pro- and pre-B cells and tumor lines, where it can induce SHM and CSR (62–64). In addition, AID expression has now been reported in several cell types, including germ and embryonic stem cells, where it can function in cytosine deamination (65).

DSBs IN TRANSLOCATIONS

Overview

Translocation breakpoint sequences in lymphomas often implicate RAG or AID involvement and also provide information regarding the developmental stage at which translocations occurred. For example, translocations that involve the IgH JH region most likely occurred in pro-B cells and translocations into the IgL J regions in pre-B cells. However, junctional sequences cannot always identify translocation-initiating mechanisms: For example, S region deletions associated with AID activity in Sμ sometimes extend into the adjacent Jμ region, and AID activity in the J region of IgH or IgL loci can lead to DSBs and translocations (1, 42). In addition, AID is active in bone marrow B lineage cells, and RAG activity was found in peripheral B cells. Indeed, recent studies suggest collaborations between RAG and AID in generating translocations. Finally, as mentioned above, not all DSBs that are precursors to translocations in lymphomas appear to have been initiated by RAG or AID (22).

DSBs Generated by RAG Activity

RAG could generate translocations via bona fide interchromosomal V(D)J recombination. In this case, RAG would recognize a set of 12/13 compatible RSSs lying on two different chromosomes, generate DSBs, and recruit C-NHEJ to join them. A hallmark of a true V(D)J recombination-mediated reciprocal translocation is the retention of fused coding ends at one translocation junction and fused RSS joins at the other (66). In this regard, V(D)J recombination-mediated translocations between the IgH and TCRα loci on different arms of human chromosome 14 lead to large inversions that can be detected even in non-transformed cells from patients suffering from Ataxia-telangiectasia (67). Although there are few clear-cut examples of translocations generated via intrachromosomal V(D)J recombination, there is abundant, albeit circumstantial, evidence for involvement of RAG-initiated DSBs in translocations (1, 22). RAG-generated breaks during early B or T cell development have been implicated in initiating translocations involving IgH or TCR loci that are found in certain B or T cell acute lymphoblastic leukemias (B- or T-ALLs) (68). Classic examples of putative RAG-initiated translocations are also found in mature B cell lymphomas, including the recurrent T(8;14) translocations involving IgH and c-myc in endemic Burkitt’s lymphoma, the recurrent T(11;14) translocations involving IgH and the bcl-1 locus found in a subset of mantle cell lymphomas; the recurrent T(14;18) translocation in follicular lymphoma that juxtaposes IgH and bcl-2; and the T(1;14) translocation found in mucosa-associated lymphoid tissue (MALT) lymphomas that links IgH to bcl-10 (1, 68). However, the exact developmental stage at which RAG generated the DSBs that initiated these mature B cell lymphoma translocations is still speculative (see below).

Mouse models directly demonstrate a role for RAG in the generation of oncogenic translocations. The most clear-cut examples come from mice deficient for various C-NHEJ factors [e.g., XRCC4, DNA Ligase IV (Lig4), or Ku80] that are also deficient for the p53 tumor suppressor. These mice uniformly develop pro-B cell lymphomas with clonal translocations that link the IgH JH region to sequences near c-myc on chromosome 15 (3, 4, 69). Breeding these mice onto a RAG-deficient background eliminated the pro-B cell tumors, demonstrating a RAG role in tumor formation (3, 4). Notably, elimination of RAG1 in another end-joining factor (DNA-PKcs)–deficient mouse that was also p53-deficient did not eliminate pro-B lymphomas, but instead.
DSBs Generated by AID Activity

Junctional analyses have implicated AID activity during IgH CSR in initiating DSBs that lead to B cell lymphoma translocations. Classic examples are the T(8;14) IgH/c-myc translocations found in sporadic Burkitt’s lymphoma, the T(4;19) IgH/bcl-3 translocation in chronic lymphocytic leukemia, the T(3;14) IgH/bcl-6 translocation in diffuse large B cell lymphoma (DLBCL), the T(9;14) IgH/Pax5 translocation in lymphoplasmacytic lymphoma, and the T(4;14), T(14;16), and T(6;14) translocations found in multiple myeloma (1, 2, 68). In these cases, the involved IgH S region is most frequently Sμ, but translocations in Sy and Sx are also observed. In addition, various studies have shown that AID activity initiates IgH locus DSBs that can lead to IgH chromosomal breaks and translocations in B cells that are deficient for various C-NHEJ or DSB response factors (45, 75–77). Likewise, AID is required for IgH/c-myc and other translocations in preneoplastic B cells from mice that are predisposed to B cell lymphomas harboring such translocations (78, 79).

Translocation partners of AID-initiated CSR DSBs may derive from various sources. Despite tight AID regulation, AID acts on non-Ig genes; indeed, comprehensive sequencing of the mouse genome from GC B cells has revealed off-target AID mutagenic action on about 25% of analyzed expressed genes (48). Notably, the mutation rate of most non-Ig genes is far below that of Ig variable region exons or IgH S regions in B cells in which these sequences are SHM or CSR targets (48). Given that AID-generated physiological DSBs occur mainly within specialized S region CSR targets, as opposed to variable region exon SHM targets, the potential for introducing AID-initiated DSBs in off-target loci may be expected to be low. However, even infrequent DSBs could contribute to oncogenic translocations that could be highly selected during tumorigenic processes. In this regard, in some human mature B cell tumors, such as DLBCL, a strong correlation is observed between hot spots of SHM in oncogenes (bcl-6, c-myc, and Pim1) and the propensity to be involved in translocations (43). Moreover, in experimental models, AID introduced both the IgH and c-myc DSBs that are used to generate IgH/c-myc translocations (80). Similarly,
AID has been shown to generate DSBs in Igβ, another locus that can translocate to IgH in activated B cells (81).

**AID and RAG May Partner in the Generation of Translocations**

RAG and AID have been suggested to partner to induce a single DSB in certain loci involved in recurrent translocations with antigen receptor loci in B and T cell lymphomas. In this context, AID can deaminate methyl-C to T, and thus induce C-to-T mutations at CpG sites. Furthermore, biochemical studies demonstrate that RAG could generate DSBs at T:G mismatches (22, 74). Proximity to CpG sites is a frequent feature of translocation junctions near bcl-2, bcl-1, and API in human follicular lymphoma, mantle cell lymphomas, and MALT lymphomas, respectively (82, 83). Notably, AID can be expressed at low levels in developing B cells that express RAG (62–64). Such findings led to the model that RAG and AID can collaborate to initiate translocations early in B cell development that become involved in oncogenesis in more mature cells in the periphery (74). AID and RAG also partner in the generation of translocations in C-NHEJ-deficient peripheral B cells by a different mechanism, with AID leading to DSBs in one partner locus (IgH) and RAG leading to DSBs in the other (IgL) (79). Notably, these same translocations appear recurrently in peripheral B cell lymphomas that arise from B cells deficient for both C-NHEJ and p53 (84), raising the possibility that RAG/AID collaborations that lead to translocations in some mature human B cell tumors might have happened in the periphery.

**AID-Initiated Translocations in Nonlymphoid Tumors**

Recent studies have revealed unexpected roles for AID in generating translocations in solid tumors. Aberrant AID expression was found in gastric tumors associated with *Helicobacter pylori* infection (85) and in liver and colorectal cancers associated with inflammation (60, 85). Pluripotent tissues, such as oocytes and germ cells, also express AID, and a possible role for AID in testicular germ cell tumors has been proposed (60). Estrogen-induced AID expression in breast and ovary cells has suggested that AID-dependent genomic instability might underlie oncogenesis in these tissues (86). Finally, AID-initiated DSBs have been implicated in recurrent translocations in prostate cancers (52). The current findings in this area are of substantial interest; however, more work is required to firmly implicate AID in the generation of nonlymphoid cancer translocations.

**Other Types of DSBs Involved in Translocations**

Not all DSBs that contribute to recurrent translocations in lymphoid tumors are attributable to RAG or AID activity. Some involve RAG- or AID-initiated DSBs joined to a general DSB in a second locus. In this context, AID-initiated IgH DSBs join to I-SceI-initiated DSBs (6), including I-SceI-generated DSBs in c-myc (79, 80), providing an experimental demonstration of this mechanism. In addition, RAG or AID may not have any role in certain lymphoid tumor translocations, such as those with breakpoints in unrearranged IgL loci that lie far from known V(D)J or AID targets (22) or translocations that do not involve antigen receptor loci, such as ALK (anaplastic lymphoma kinase) translocations in certain B and T cell lymphomas (87, 88). In T-ALLs, a subset of recurrent translocation junctions involving SCL and LMO2 loci lack features that would implicate RAG in their generation (22).

A dividing cell may generate about ten DSBs per day (7). Fragile sites, non-Z DNA sequences, oxidative DNA damage, ionizing radiation, pharmacological inhibition of topoisomerases, and replication stress are among the potentially relevant DSB-generating factors (7, 22, 89). Common fragile sites can form gaps or breaks in metaphase chromosome spreads following inhibition of DNA synthesis; they are part of the structure of chromosomes in many species, and their instability correlates
with delayed DNA replication (90). Fragile sites FRA6E and FRA6F are found near breakpoints in ALL and acute myeloid leukemia translocations, respectively (91). Some c-myc translocation breakpoints in Burkitt’s lymphoma are close to FRA8C and FRA8D (90, 92). Topoisomerases introduce and repair transient DSBs. Thus, inhibition of topoisomerase activity (an approach that is widely used in cancer chemotherapy) could lead to persistent DSBs (93). In this context, anticancer drugs that inhibit topoisomerase II have been implicated in the development of secondary acute leukemia with recurrent translocations (94).

DSB RESPONSE AND END-JOINING PATHWAYS

Overview

Repair of chromosomal DSBs by end-joining is facilitated by the ATM-dependent DNA DSB response, which generates large foci of activated DSB response factors in chromatin surrounding DSBs (8, 95). The DSB response activates cell cycle checkpoints to allow for repair of the breaks and suppresses translocations (8, 95). The DSB response is important in diverse cell types for recognition and repair of a broad range of chromosomal DSBs, including DSBs involved in CSR and V(DJ) recombination (8, 95). Actual end-joining of DSBs can be carried out by more than one process. C-NHEJ fuses DSBs that do not share substantial homology at their ends, either as direct joints that lack junctional homology (also referred to as blunt joins) or DSBs with short stretches (1–5 bp) of microhomology (MH) at their ends to form MH-mediated joins. C-NHEJ factors were first discovered in mammalian cells based on their requisite role in V(DJ) recombination (14, 96). Subsequently, C-NHEJ was found to function in all eukaryotic cells, including yeast in which C-NHEJ plays a more minor role to HR (96). C-NHEJ is a major DSB repair pathway in somatic mammalian cells and is required for maintenance of genomic stability, including suppression of chromosomal translocations, in all analyzed somatic cell types. In the absence of C-NHEJ, DSBs can still be repaired by A-EJ. A-EJ has gained wide interest because of its potential role in catalyzing oncogenic chromosomal translocations (3, 4, 84, 97).

The ATM-Dependent DNA DSB Response

The ATM kinase is a member of the phosphatidylinositol 3-kinase-like kinase (PIKK) family, which also includes DNA-PKcs and Ataxia-telangiectasia- and Rad3-related (ATR) protein kinases (98). ATM has been termed a master regulator of the DSB response. Genetic deficiency for ATM in humans causes Ataxia-telangiectasia, which manifests as radiation sensitivity, genomic instability, immunodeficiency, and increased predisposition to T and B cell tumors with clonal antigen receptor loci translocations (98, 99). ATM is also somatically inactivated in a subset of human B and T cell lymphomas (99). Mutation of ATM in mice recapitulates many aspects of Ataxia-telangiectasia (98, 99). However, although ATM deficiency in mice predisposes them to thymic lymphomas with TCRα/δ locus translocations, ATM-deficient mice rarely develop B cell lymphomas (98, 99). Why ATM-deficient mice are spared from B cell lymphomas given that they have defects in V(DJ) recombination and CSR that lead to IgH and TCR locus breaks and translocations is unknown but of considerable interest.

The ATM-dependent DNA damage response, or DSB response, is activated by chromosomal DSBs and functions in both HR and C-NHEJ (8, 95, 98). The Mre11-Rad50-Nbs1 (MRN) protein complex senses DSBs and recruits ATM. ATM then phosphorylates a large set of additional proteins, including histone H2AX (H2AX), MDC1, 53BP1, and the Nbs1 component of the MRN complex, that are assembled over large regions of chromatin on both sides of a DSB to form IR-induced foci (IRIF) (8, 95, 98). Such IRIF may serve multiple functions, including activating checkpoints, tethering DSBs, and recruiting repair.
factors (see below). In response to DSBs, ATM also phosphorylates p53, Chk1, and Chk2 to regulate, respectively, the G1/M, intra-S, and G2/M cell cycle checkpoints (95, 98). The p53-dependent G1/S checkpoint is particularly important for detecting unrepaired RAG- or AID-initiated DSBs and leading to their repair or to elimination of cells that harbor them, because these DSBs are normally initiated in G1 (8).

Histone H2AX is a key orchestrator of IRIF formation. H2AX is a histone H2A variant that is incorporated into about 10% of nucleosomes, where it is poised to be activated in megabase regions that flank DSBs by phosphorylation on Ser139 of its C-terminal tail to form γ-H2AX (95, 100). ATM is a mediator of H2AX phosphorylation in response to DSBs in G1 (95, 100). However, H2AX can also be phosphorylated in response to DSBs by DNA-PKcs (a C-NHEJ component, see below) and by ATR during S phase in response to DSBs associated with replication stress (8, 95, 100). In this context, H2AX deficiency leads to chromatid breaks (prereplication) and chromosome breaks (postreplication), consistent with its key roles in both the G1 and S phases, whereas deficiencies for other DSB response factors (e.g., ATM and 53BP1) lead mainly to chromosome breaks, consistent with a major role in G1 (76). Phosphorylated H2AX binds other ATM substrates, such as MDC1, 53BP1, and Nbs1 (100). Recruitment of these factors to IRIF can happen in the absence of H2AX but is stabilized and amplified by γ-H2AX (101). In turn, MDC1 recruitment stabilizes ATM binding to DSBs and/or prevents γ-H2AX dephosphorylation, resulting in amplification of H2AX phosphorylation (100). In addition to potential signaling functions, IRIF are proposed to tether broken DNA ends to prevent separation and facilitate repair (95). Notably, ATM-dependent IRIF containing γ-H2AX and 53BP1 form in response to DSBs generated during V(D)J recombination and CSR (102). Correspondingly, ATM-deficiency in mice modestly impairs both processes and is associated with immunodeficiency and potential CSR defects in humans (98, 99). Deficiencies for DSB response factors lead to genomic instability in diverse cell types, although the severity of this phenotype is variable; for example, 53BP1 deficiency, other than in the context of CSR, predisposes mice to far less genomic instability than ATM or H2AX deficiency (76).

In ATM-deficient transformed mouse pro-B cell lines, a small subset of V(D)J recombination DSBs persists as broken ends or is improperly joined, indicating that ATM stabilizes RAG-initiated DSBs and promotes appropriate end-joining (103). However, it is not clear whether this function is indirect, for example via RAG phosphorylation, or direct. Deficiency for 53BP1 has a more modest effect on V(D)J recombination (29), whereas H2AX deficiency has no readily measurable effect on V(D)J recombination per se (104), suggesting that the more important ATM role might be independent of IRIF formation. However, in certain contexts, H2AX deficiency promotes B cell lymphomas with clonal IgH translocations, suggesting some influence on V(D)J recombination (105). Moreover, recent work indicates that ATM and H2AX can actually have a mechanistic influence on end-joining during V(D)J recombination that is masked by functional overlaps with the XLF end-joining factor (106).

Human patients deficient for ATM, Mre11, and Nbs1 have mild CSR defects. Moreover, mouse B cells deficient for various DSB response factors (including ATM, H2AX, 53BP1, MDC1, Mre11, and Nbs1) show variably impaired CSR (8). In B cells deficient for these factors, a subset of AID-dependent IgH DSBs separates and progresses into chromosomal breaks and translocations (76, 77), clearly showing that these DSB response factors function in CSR end-joining. 53BP1 deficiency in mice nearly abrogates CSR but leads to similar levels of IgH breaks as for ATM or H2AX deficiency, implying that 53BP1 has an additional CSR role beyond any downstream of ATM (76). In this regard, although 53BP1-deficient B cells are severely impaired for CSR, which involves long-range joining of DSBs within two different S regions, they accumulate AID-dependent ISDs within Sμ and downstream S regions at
greatly increased frequency (107). This finding led to the proposal that 53BP1 is required for long-range DSB repair via a role in S region synopsis, although there are other interpretations (see below). Notably, 53BP1 copy number variations have been found in a subset of DLBCLs (108).

Mice deficient for H2AX, 53BP1, or MDC1 are not predisposed to lymphoid or other cancers, indicating that tumorigenesis in the ATM-deficient background reflects loss of ATM functions beyond phosphorylating these factors. In this regard, ATM deficiency leads to increased reactive oxygen species (ROS) levels that could generate DSBs (109). Indeed, high genomic instability in ATM/H2AX-double-deficient cells appears to result from a combination of ROS-induced S phase DSBs owing to ATM deficiency and an S phase DSB repair defect associated with H2AX deficiency (110). Another difference between ATM deficiency versus deficiencies in its downstream substrates is that ATM is involved both in DSB repair and, through p53 phosphorylation, in activating cell cycle checkpoints that monitor unrepaired DSBs. Thus, ATM deficiency results in a double whammy in which there are increased DSBs and the cells cannot respond to them. Because of this, ATM deficiency allows unrepaired V(D)J recombination DSBs to persist over multiple generations in developing lymphocytes and to be joined to other DSBs in peripheral B cells, giving rise to translocations (111). In contrast, dual deficiency for a DSB response factor or a C-NHEJ factor and p53 robustly promotes lymphomas and other cancers by similarly leading to unrepaired DSBs and eliminating checkpoints that monitor them (8, 17).

Mice deficient for p53 usually succumb, at about 6–8 months, to thymic lymphomas that are aneuploid and lack translocations (112). However, H2AX/p53-double-deficient mice rapidly succumb to thymic lymphomas with clonal translocations that usually do not involve TCR loci and usually do not appear RAG-dependent (105, 113). Less frequently, H2AX/p53-double-deficient mice succumb to pro-B cell tumors with clonal translocations essentially identical to those in tumors from C-NHEJ/p53-double-deficient mice (see below), suggesting some role for H2AX in repairing RAG-initiated DSBs (105). Finally, whereas 53BP1-deficient mice show little cancer predisposition, 53BP1/p53-double-deficient mice develop thymic lymphomas (and more rarely B cell lymphomas) at an accelerated pace compared with p53-deficient mice, and a subset of these mice have clonal translocations, including some that involve TCRα/δ (114, 115). The relatively infrequent appearance of T cell lymphomas with translocations involving the TCR loci in H2AX/p53- or 53BP1/p53-double-deficient mice versus ATM-deficient mice might reflect the more modest role for H2AX and 53BP1 in V(D)J recombination compared with ATM or other potential differences.

Classical Nonhomologous End-Joining

There are four core C-NHEJ factors: Ku70 and Ku80, which form the Ku DNA DSB recognition component, and DNA Lig4 and XRCC4, which function as the specific DNA ligation component. These four proteins are evolutionarily conserved and required for joining all forms of DSBs by C-NHEJ (7, 17). Additional C-NHEJ factors that are not evolutionarily conserved (e.g., not found in yeast) include the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) and the Artemis nuclease. These two factors are required for processing a substantial subset of ends to prepare them for joining by core C-NHEJ components (7, 17). Upon binding to DNA, the Ku70/Ku80 heterodimer undergoes a conformation shift and contributes to assembling other C-NHEJ factors (7). In particular, DSBR-bound Ku forms a complex with DNA-PKcs and activates its catalytic activity to form the DNA-PK holoenzyme (DNA-PK). DSBR-bound DNA-PK activates the endo- and exonuclease activities of Artemis, which are required for processing and joining a subset of DNA ends that cannot be directly ligated (7). Such ends include hairpin coding ends generated by RAG during V(D)J
recombination or ends containing oxidation-damaged nucleotides (116). DNA-PKcs and Artemis are largely dispensable for joining certain ends, such as the blunt 5′-phosphorylated RSS ends that can be directly ligated. As mentioned above, the XRCC4/Lig4 complex is responsible for the C-NHEJ ligation phase (7).

Mouse strains are available that are deficient for each known C-NHEJ factor. DNA-PKcs- and Artemis-deficient mice have a SCID phenotype owing to the inability to generate V(DJ) coding joins, but they are not markedly cancer prone and do not have other major phenotypes (17). Similar phenotypes are observed in the classical SCID mouse, which harbors a spontaneous DNA-PKcs mutation that only inactivates the kinase domain (17). Mice deficient for Ku70 or Ku80 are viable but show severe growth retardation and substantial neuronal apoptosis, in addition to a SCID phenotype owing to the inability to generate V(DJ) coding or RSS joins (17). The more severe phenotype of Ku-deficient mice compared with DNA-PKcs-deficient mice demonstrates that Ku has roles beyond those in the context of DNA-PK holoenzyme (17). XRCC4 or Lig4 deficiency results in late embryonic lethality owing to massive apoptosis of newly generated neurons, along with growth retardation and a SCID phenotype (17). The more severe phenotype of Ku-deficient mice compared with DNA-PKcs-deficient mice demonstrates that Ku has roles beyond those in the context of DNA-PK holoenzyme (17). XRCC4 or Lig4 deficiency results in late embryonic lethality owing to massive apoptosis of newly generated neurons, along with growth retardation and a SCID phenotype (17).

A subset of human RS-SCID patients has null mutations in Artemis (117). There are no known human patients with inactivating mutations of Ku70, Ku80, XRCC4, or Lig4, possibly because of the importance of C-NHEJ for mammalian cell survival. However, patients with Lig4 hypomorphic mutations are characterized by immunodeficiency, radiosensitivity, and, at least in some cases, immunological malignancies (118); a human Lig4 hypomorphic mutation has been modeled in mice and results in a similar phenotype (119). The XLF/Cernunnos protein also has been implicated as a C-NHEJ factor based on the facts that (a) it is mutated in certain radiosensitive human immunodeficient patients (120, 121) and (b) XLF-deficient human fibroblasts and mouse embryonic stem cells and fibroblasts are radiosensitive and defective for V(DJ) recombination in transient assays (120–123). In this regard, XLF binds XRCC4/Lig4 (120) and stimulates its activity toward incompatible ends (124). However, mice and mouse pro-B lines deficient for XLF show little if any V(DJ) recombination defect, although they are partially defective for CSR (122). In this regard, ATM has been found to have functional redundancies with XLF in the context of chromosomal V(DJ) recombination in developing lymphocytes (106).

Because of the V(DJ) recombination defect associated with C-NHEJ deficiency, studies of potential roles of C-NHEJ factors in CSR have been conducted either by introducing preassembled IgH and IgL variable region knock-in alleles into the various C-NHEJ-deficient backgrounds or by conditionally inactivating C-NHEJ factors in mature B cells. These studies showed that CSR occurs at up to 50% of wild-type levels in the absence of any given core C-NHEJ factor, although potentially with slower kinetics in some cases (45, 125, 126). There are only modest CSR defects in the absence of DNA-PKcs or Artemis (127), suggesting that many AID-initiated breaks may not require processing by these factors. However, IgH chromosomal breaks and translocations were observed in activated B cells deficient for any of the known C-NHEJ factors, and these breaks and translocations were AID dependent (45, 75, 79, 128). Thus, all C-NHEJ factors have a role in joining at least a subset of CSR DSBs and preventing them from undergoing translocations. The finding that CSR occurs in the absence of C-NHEJ factors provided the first demonstration that an A-EJ pathway can function in a physiological context.

**Alternative End-Joining**

A-EJ is best described as any form of end-joining that occurs in the absence of the core
C-NHEJ factors. Early evidence for A-EJ came from findings that Chinese hamster ovary cells deficient for Ku80 or XRCC4 could still join nonhomologous DNA ends (129, 130). Similar conclusions were reached from studies of human fibroblasts that lacked Lig4 (131, 132) and from biochemical studies that employed human cell extracts devoid of Ku or DNA-PKcs (133). A series of factors have been implicated in A-EJ, including Ku70, Nbs1, Mre11, CtIP, DNA Lig3, Parp1, and XRCC1 (102) (Figure 3). However, their exact role is not clear, and there are likely multiple A-EJ pathways (125). The relatively robust CSR in XRCC4- or Lig4-deficient B cells or B cell lines might reflect the recognition and processing components of the C-NHEJ pathway operating at reduced efficiency with another ligase (Lig1 or Lig3) substituting for Lig4 (7). In this case, it is debatable whether such joining should be called A-EJ or simply a form of C-NHEJ. However, Ku70- or Ku80-deficient B cells still undergo substantial CSR even though they lack the C-NHEJ DSB recognition component (125). Moreover, CSR occurs at substantial levels in B cells deficient for both Ku70 and Lig4, the DSB recognition and joining components of C-NHEJ, clearly confirming that A-EJ is a process (or processes) totally distinct from C-NHEJ (125) (Figure 3).

Insights into the molecular requirements of A-EJ have been provided by sequence analyses of joins generated in the absence of C-NHEJ factors. In this regard, CSR junctions isolated

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**Figure 3**

Diagram illustrating potential NHEJ pathways in mammalian cells. See text for further details.
from XRCC4- or Lig4-deficient B cells are almost completely MH-mediated, often with longer stretches of MH compared with CSR junctions from C-NHEJ-proficient cells (44, 45, 125). However, MH is not a requirement for A-EJ. Thus, A-EJ-mediated junctions of I-SceI-initiated breaks in Ku80-deficient cells have a substantial portion of direct joins (134, 135). In addition, CSR junctions generated in the absence of Ku70 or of both Ku70 and Lig4, while biased toward MH, have a significant fraction of direct joins (125). Therefore, the appearance of MH in junctions is not necessarily a sign of A-EJ, and the appearance of direct junctions is not necessarily a sign of C-NHEJ. Also, there may be forms of A-EJ that are more biased toward the MH usage (125).

The sequence of regions flanking DSBs likely plays an important role in determining the contribution of MH to A-EJ. For example, DSBs in regions with long stretches of MH (such as IgH S regions) might be resolved by A-EJ into junctions that contain MH more often than would DSBs that occur in regions lacking MH. In this context, although CSR junctions generated in the absence of Ku, XRCC4, or Lig4, the end-joining of AID-initiated DSBs via ISD actually increases (75). Thus, given the highly repetitive nature of individual S regions, the high level of ISD catalyzed by A-EJ may be promoted by the abundant MH found within a given S region (75). The use of MHs by A-EJ might also be reflected by the kinetics of DSB repair, given that a potential kinetic advantage of C-NHEJ over A-EJ has been suggested (125, 126, 132, 136), and A-EJ has sometimes been associated with increased deletions and end degradation (130, 137, 138). When a DSB is not repaired promptly, there may be more end resection and, therefore, more opportunity for A-EJ to find MH.

Role of C-NHEJ and A-EJ Pathways in Suppressing Oncogenic Translocations

Mouse embryonic stem cells, embryonic fibroblasts, and activated B cells deficient for Ku70, Ku80, XRCC4, Lig4, DNA-PKcs, Artemis, or XLF show substantial numbers of chromosomal breaks and translocations (17, 45, 75, 122, 123, 128, 139). Thus, C-NHEJ plays a pivotal role in suppressing genomic instability. In most cases, the nature of the chromosomal breaks in C-NHEJ-deficient cells (i.e., chromosome versus chromatid breaks) suggests that they arose in G1, where C-NHEJ is dominant. Notably, in cells with multiple DSBs, C-NHEJ has a preference for joining a DSB to another on the same chromosome as opposed to joining to DSBs on a different chromosome, a restriction that may allow C-NHEJ to suppress chromosomal translocations (139). How this restriction is achieved is not known, but it may result from C-NHEJ being tied into the DSB response that tethers DSB ends within a chromosome (75). In this context, the putative translocation-prone nature of A-EJ might be explained by the lack of such a restriction.

C-NHEJ-deficient mice are not markedly cancer prone given that cells carrying unrepaired DSBs are eliminated via the p53-dependent G1/S checkpoint (17). However, mice doubly deficient for p53 and either XRCC4, Lig4, Ku80, or DNA-PKcs rapidly succumb to pro-B cell lymphomas, with clonal RAG-initiated translocations of chromosome 12 and 15 that link the IgH JH region to a chromosomal region downstream of c-myc, which results in dicentric chromosomes that amplify c-myc via breakage-fusion-bridge cycles (3, 4, 17). The generation of such translocations has been linked to the persistence of the RAG-initiated JH DSBs into S phase (owing to the absence of p53) where they can be replicated and participate in translocations (4). Conditional elimination of XRCC4 in p53-deficient (CXP) peripheral B cells routinely leads to B cell lymphomas that arise in mesenteric lymph nodes, most of which harbor clonal T(12;15) translocations that fuse IgH S regions directly upstream of c-myc and lead to c-myc ectopic activation via attachment to the IgH 3’ regulatory region (84, 140). These CXP B cell tumors also often harbor a second clonal translocation that links RAG-initiated DSBs at the Igα locus.
to AID-initiated breaks in IgH (84). The latter translocation has no known oncogenic activity and may appear clonally in tumors because it occurs very frequently in XRCC4-deficient peripheral B cell tumor progenitors (79).

In the case of Artemis/p53 deficiency, IgH/c-myc translocations are present only in a subset of tumors; instead, most amplify N-myc in the context of chromosome 12 dicentrics that are generated from the fusion of RAG-initiated DSBs at the IgH JH locus, near the telomere of chromosome 12, to sequences around N-myc, near the chromosome 12 centromere (141). The reason Artemis/p53-double-deficient pro-B cell tumors harbor chromosome 12 dicentrics and N-myc amplifications is unknown but may be related to the specific role of Artemis in end processing. Notably, XLF/p53-double-deficient mice do not develop pro-B cell lymphomas, likely reflecting the fact that V(D)J recombination is not significantly affected in these mice; they do develop medulloblastomas, however, reflecting the more general role of XLF in DNA repair (122). In this context, although mice deficient for other C-NHEJ factors and p53 succumb to pro-B cell lymphomas, they also commonly have medulloblastomas in situ at the time of death (4, 141, 142). Correspondingly, conditional inactivation of XRCC4 in the nervous system of p53-deficient mice routinely leads to medulloblastomas with recurrent translocations (97).

Sequences of translocation junctions from tumors derived from C-NHEJ-deficient mice have shown that all were generated via end-joining, providing the first evidence that A-EJ can catalyze oncogenic translocations (4, 84). Other studies have shown that I-Sce1-mediated translocations in C-NHEJ-deficient cells were mediated by A-EJ (134, 135). A-EJ-mediated translocation junctions often show MH (4, 84, 135). In this regard, I-Sce1-mediated translocation junctions in wild-type cells also show a bias toward MH usage (80, 143), as do many translocation junctions from human cancer cells (68, 144). Given that A-EJ is not restricted to MH usage, possible explanations for the frequent MHs in translocation break-points include a potential translocation-prone MH-biased A-EJ subpathway or that translocations result from DSBs that undergo substantial processing, allowing more frequent exposure of MHs. Overall, C-NHEJ must suppress translocation formation either by eliminating the unrepaired DSBs necessary for their formation or by suppressing a putative translocation-promoting activity of A-EJ, or both. Further elucidation of factors and pathways involved in A-EJ are necessary to understand better the mechanisms of translocations and the degree to which C-NHEJ participates in their formation.

**ROLE OF NUCLEAR ARCHITECTURE AND DNA MOVEMENTS IN TRANSLOCATIONS**

**Overview**

To form a translocation, DSBs generated in different loci must be juxtaposed (synapsed) for joining. In this context, two DSBs can originate in loci that already are in proximity, or alternatively, DSBs introduced into loci that are not proximal might be brought together. Similar principles might also apply to joining DSBs located in distant positions on the same chromosome, for example in the context of V(D)J recombination and CSR or intrachromosomal translocations. The role of nuclear positioning in translocations has been articulated in two disparate models. The contact-first model suggests that translocations are only formed between two loci that are proximal when DSBs are generated. The breakage-first model proposes that DSBs introduced into loci that are not proximal can move into close proximity and then be joined (145) (Figure 4). These two models describe extreme possibilities; there may still be situations in which both are applicable and, as such, are not mutually exclusive. For example, even loci that are proximal as judged by microscopy may still not be in direct physical contact and, thus, require movement to achieve synopsis.
Role of Nuclear Proximity and Chromosome Dynamics in Translocations

Chromosomes are not randomly distributed in the nucleus but rather occupy distinct territories (146, 147). Individual chromosomes are organized into open and closed chromatin domains that occupy different spatial compartments, and their structure resembles fractal globules (148). The mechanisms that govern formation of chromosomal territories have not been elucidated but may reflect spatial arrangements of genes, local chromatin structure, and activity of transcriptional or other regulatory elements (149). A similar concept of nonrandom nuclear positioning applies to single loci and may be relevant to their translocation potential. Thus, the frequency of c-myc translocations to IgH, Igκ, or Igλ locus in Burkitt’s lymphomas correlates with reciprocal nuclear distance (150). Similar correlations on translocation frequencies were found with respect to the distance separating IgH and the CCND1, bcl-2, or bcl-6 loci (150). IgH and c-myc often lie in close proximity in activated mouse B cells, which again correlates with the high frequency with which they participate in translocations in a mouse B cell lymphoma model (79). However, clonal oncogenic translocations in tumors are highly selected, and, therefore, such correlations cannot be used to determine unequivocally the contribution of mechanistic aspects (proximity, DSB frequency, etc.) to actual translocation frequency. More unbiased examinations may yet reveal translocations between loci that are not frequently in close proximity.

Cytogenetically, the proximity of particular loci within the interphase nucleus can be cell type–specific or tissue-specific. In this context,
substantial colocalization of \(\text{IgH}\) and \(\text{Ig}\lambda\) occurs in activated splenic B cells but not in embryonic stem cells or thymocytes (79). However, it is notable that colocalization with \(\text{IgH}\) is not a characteristic of all of chromosome 16 on which the \(\text{Ig}\lambda\) locus is located; sequences about 15 Mb on either side of \(\text{Ig}\lambda\) do not show such colocalization (79). Thus, proximity can be determined in the context of more narrow areas around specific genes and not just associated with broad chromosomal territories. The factors that mediate such focal and cell-specific locus proximity, as well as the organization of chromosome territories, have not been elucidated. In this context, \(\text{IgH}\) and \(\text{c-myc}\) have been observed to colocalize in activated B cells within so-called transcription factories (151). Likewise, \(\text{RET}\) and \(\text{H4}\), two genes located 30 Mb apart on the same chromosome, are transcribed and often translocated in papillary thyroid tumors and are found in spatial proximity only in thyroid cells and not in mammary epithelia (152). Androgen-induced transcription has been shown also to mediate interaction of genes involved in recurrent translocations in prostate tumors (52).

**Factors that Influence Synapsis of Chromosomal DSBs for Joining**

In pro-B cells, the \(\text{IgH}\) is folded into an organization that enhances the three-dimensional proximity of V, D, and J segments, which otherwise are too distant to be cytogenetically proximal (28). Such folding may contribute to their synapsis in the context of V(D)J recombination. The overall mechanisms that achieve such folding are unknown but are speculated to involve factors such as CTCF, which has binding sites scattered throughout the variable region of \(\text{IgH}\) (153). Various studies have also implicated transcriptional control elements and factors such as CTCF and cohesins in mediating interchromosomal interactions of various loci, including cytokine genes in lymphoid cells and olfactory receptors in neurons (154, 155). Theoretically, similar mechanisms might contribute to the proximity of certain RAG translocation targets on other chromosomes to antigen receptor loci, and RAG may then also function to ensure stability of cleaved complexes before joining.

During CSR, transcription-dependent interactions among promoters and enhancers may promote a looped conformation of the \(\text{C}_{\text{H}1}\) region of \(\text{IgH}\) that could favor synopsis of different S regions (156). In addition, investigators have speculated that AID and individual S region sequences play roles in the synopsis of two different S regions. However, CSR occurs at significant levels when S regions are deleted and I-SceI endonuclease DSBs are introduced at their former locations (6). These latter studies have concluded that the synopsis of DSBs separated by substantial distances (e.g., 100 kb apart) on a chromosome might be promoted by general mechanisms that evolved to favor intrachromosomal DSB joining and suppress interchromosomal DSB joining (6). Support for this model was provided by a large-scale screen of RAG-initiated translocations in pro-B cell lines (5). Finally, the DSB response has been speculated to mediate S region synopsis during CSR and potentially to promote intrachromosomal versus interchromosomal DSB repair (95). In this regard, investigators have proposed, on the basis of decreased CSR with increased ISDs in 53BP1-deficient B cells, that 53BP1 plays a synopsis role during CSR (107). However, more recently, the same phenotype was found for B cells deficient for various C-NHEJ factors, suggesting an alternative interpretation that 53BP1 deficiency may favor resolution of DSBs via A-EJ (e.g., ISD) versus C-NHEJ (e.g., CSR) (75). Accordingly, 53BP1 deletion leads to extensive resection of broken DNA ends by an ATM-dependent process that might promote A-EJ by exposing MHs (157).

There has been considerable debate as to whether DSBs move in the context of repair. In yeast, independent DNA lesions move and colocalize in repair factories (158). In addition, persistent unrepaired yeast DSBs migrate from the nuclear interior to the periphery, where they are tethered to the nuclear pore complex (159, 160). In mammalian cells, some studies
of introduced DSBs have revealed quite limited movements (161, 162), whereas others have found DSB movement and clustering, especially in G1 phase cells (163). An elegant study that followed a specific I-SceI DSB in real time concluded that DSBs are relatively stable in the nucleus and do not aggregate or converge to shared DNA repair foci (164). However, a potential caveat of this conclusion is that the experiment required visualization via the generation of large complexes of fluorescent proteins assembled around DSBs, which might influence mobility. In certain contexts, it still seems possible that DSBs might move; in particular, some proximal DSBs on different chromosomes might need to move over subcytogenetic distances for synapsis before joining. In that context, recent studies have demonstrated a role for 53BP1 in movement and joining of uncapped telomeres (165).

MECHANISMS OF ONCOGENIC SELECTION OF TRANSLOCATIONS IN B CELL LYMPHOMAS

Overview

In tumors, driver mutations are mutations that allow cells to escape normal cell cycle or differentiation control mechanisms and confer a proliferative advantage during oncogenesis. A large fraction of human and mouse lymphoid tumors contain driver mutations in the form of translocations that fuse *Ig* or *TCR* loci to a cellular oncogene, leading to its ectopic activation. In this section, we discuss recent progress in elucidating potential roles of transcriptional regulatory elements within *IgH* and *TCRa/β* loci in activating oncogenes subsequent to translocation.

Potential Roles of *IgH* Locus Enhancers

The *IgH* locus contains two known major transcriptional enhancer regions. The intronic enhancer (iEμ) lies between the variable region exons and Cμ and operates at relatively short range to promote optimal V(D)J recombination in developing B cells (166). The *IgH* 3′ regulatory region (iEμ/IGH3′RR) is a series of enhancers that lies just downstream of the most 3′ set of Cμ exons (Cα) and modulates CSR in mature B cells by long-range (over 100 kb) activation of certain I promoters (167). The IGH3′RR does not gain full enhancer activity until late in B cell development (167). Experiments with transgenic mice bearing *c-myc* fused to either iEμ or the IGH3′RR have demonstrated that both mediate *c-myc* overexpression and promote B lineage tumors. The iEμ/c-myc transgenes usually predisposed mice to pre-B cell lymphomas (47), whereas IGH3′RR/c-myc transgenes predisposed mice to more mature B cell tumors (168–170). However, in many mature B cell lymphomas with CSR-associated translocation breakpoints, iEμ is not linked to *c-myc* in the oncogenic T(12;15) but instead is contained in the reciprocal T15;12 (1), suggesting that *IgH* transcriptional regulatory elements other than iEμ play a key role in activation of translocated oncogenes (Figure 5).

To test the role of the IGH3′RR in oncogenic translocations definitively, a mutation that inactivates the IGH3′RR was bred into the CXP mouse model that routinely develops peripheral B cell tumors with *IgH* S region to *c-myc* translocations. Strikingly, peripheral B cell lymphomas were abrogated in CXP mice homozygous for the IGH3′RR inactivating mutation, even though *IgH/c-myc* translocations occurred at normal frequency in nontransformed CXP B cells (140). This study concluded that the IGH3′RR is not necessary for the occurrence of *IgH/c-myc* translocations but that it is required to transcriptionally activate *c-myc* subsequent to translocation. The IGH3′RR activated *c-myc* in translocations that involved the Sμ region, which lies nearly 200 kb upstream, indicating that this element can activate oncogene expression over large distances (140).

The iEμ/c-myc transgenic mice that develop pre-B lymphomas frequently harbor multiple copies of the transgenes, which could
contribute to overexpression. Moreover, a single-copy c-myc cDNA inserted between the JH and iEμ does not lead to pre-B lymphomas but instead leads to more mature B cell lymphomas and plasmacytomas (47). Several interpretations for this finding are possible, but a likely one is that oncogenic activity of the single-copy c-myc is not sufficiently activated by iEμ, and, thus, tumor development does not occur until later developmental stages when the IgH3′RR becomes fully active. Still, a significant percentage of DLBCL carry CSR-mediated translocations that fuse bcl-6 to the telomeric portion of chromosome 14 and retain iEμ but not the IgH3′RR (171). Bcl6 expression is not highly upregulated, but mainly deregulated, in most such tumors (171), suggesting that its deregulated expression might be potentially mediated by a nonphysiological activity of the Iμ exon promoter.

The potential low oncogenic activity of iEμ suggested by the experiments outlined above may have significant implications for IgH3′RR roles in human mature B cell lymphomas with translocations mediated by aberrant V(D)J recombination (thought to occur only in pro- or pre-B cells). In human endemic Burkitt’s lymphomas, IgH/c-myc translocations ascribed to aberrant IgH V(D)J recombination retain iEμ near the translocation breakpoint; however, c-myc breakpoints may occur several hundred kb upstream of c-myc (1). Such IgH/c-myc translocations may arise in early B cell developmental stages but remain oncogenically silent until the IgH3′RR becomes fully active at the mature B cell stage. A similar hypothesis

Figure 5
Long-range activation of translocated oncogenes. Left: translocations involving aberrant joining of IgH DSBs generated during V(D)J recombination to other DSBs generated by RAG activity on cRSS or other mechanisms. Translocated oncogene expression might be influenced by the iEμ and/or the IgH3′RR. Right: Translocation involving aberrant joining of a S region DSB initiated by AID during CSR to other DSBs generated by AID activity or other mechanisms. Although iEμ is not linked to the oncogene, IgH3′RR activates a translocated oncogene over long distances (see text for further details). (Abbreviations: AID, activation-induced cytidine deaminase; CSR, class switch recombination; DSB, double-strand break; iEμ, intronic enhancer; IgH3′RR, IgH 3′ regulatory region; RAG, complex of recombination activating gene 1 and 2 products.)
may apply to follicular lymphomas that frequently carry V(D)J recombination-initiated IgH/bcl-2 translocations (1). However, alternative explanations are conceivable. One is that development of mature B cell tumors, rather than pro- or pre-B cell malignancies, from cells carrying V(D)J-mediated translocations might reflect the time required for the accumulation of secondary mutations necessary for transformation. Another is that translocations may be generated directly in mature B cells, either by V(D)J breaks arising in pro-B cells and persisting through development or by breaks generated by RAG action in peripheral B cells (see the section on DSBs in Translocations).

Oncogenic Translocations and Gene Amplification

Murine pro-B cell lymphomas that arise in the context of germ-line deficiency for C-NHEJ and p53 routinely harbor complex chromosomal rearrangements (referred to as complicons) in which IgH and c-myc are highly coamplified (3, 4, 17). In these mouse models, complicons originate from translocations that join unrepaired RAG-induced breaks in the IgH V region to sequences far downstream of c-myc, leading to generation of dicentrics. These dicentrics are unstable and in the next cell cycle break again, entering the so-called breakage-fusion-bridge cycle, which can lead to gene amplification (3, 4). The translocations in these tumors sometimes do not contain iEμ on the IgH/c-myc translocated chromosome. In such cases, one may speculate that if translocations were to occur directly into c-myc they would not sufficiently activate its expression to oncogenic levels (owing to the absence or low activity of iEμ). Thus, there might be a selection for oncogenic translocations that occur downstream of c-myc and that generate dicentrics, leading to c-myc gene amplification. Translocations in ATM-deficient mouse T cell lymphomas were thought to involve aberrant V(D)J recombination of the TCRα locus and ectopic activation of an oncogene via fusion to the TCRα enhancer. However, recent experiments have demonstrated that such translocations arise during TRCβ locus V(D)J recombination, are independent of the TCRα locus enhancer, and lead to the generation of complicons that amplify a small region of chromosome 14 upstream of TCRαβ (172). The latter finding might suggest that an as yet unidentified oncogene may be located in the amplified region. In human B lineage tumors, complex translocations/amplifications have been described during late-stage tumor progression, for example in multiple myeloma (173, 174) and GC-derived B cell lymphomas (175).

Potential Relevance of Activation Mechanism to Therapy

Understanding the detailed molecular mechanisms responsible of aberrant oncogene activation following translocation has proven crucial for the development of novel targeted anticancer therapies. This is the case, for example, for the drug imatinib, which is successfully used to treat chronic myelogenous leukemia and which specifically targets the translocation product, the BCR-ABL fusion protein (176). Further studies on other common translocations may, therefore, lead to other potential therapies. For example, given the similar organization of the murine and human IgH loci, identification of drugs that block the long-range activity of the B cell–specific IgH3′RR may provide a novel tool to counteract the oncogenic activity of c-myc or other oncogenes activated by this element in the context of IgH locus translocations.

SUMMARY POINTS

1. Recurrent chromosomal translocations are fundamental pathogenetic events in lymphomas and leukemias and also in many other tumor types.
2. Recurrent translocations most often represent low-frequency events that are highly selected in the context of tumor formation or tumor progression. Transcriptional control elements in antigen receptor loci can contribute to the oncogenicity of a translocation.

3. Most translocations in mice and humans are initiated by DNA double-strand breaks (DSBs) and are completed by a repair process that involves DSB end-joining.

4. Factors that influence the formation of a translocation (mechanistic factors) can also contribute to the appearance of particular recurrent translocations in a given tumor; these include DSB frequency at target loci, proximity of loci in the nucleus for joining, and availability of end-joining pathways that can join them across chromosomes.

5. Two general types of DNA end-joining pathways are present in mammalian cells. C-NHEJ is the major pathway and is essential for suppressing translocations and promoting genomic stability. An alternative end-joining pathway or pathways has been found to join ends in the absence of C-NHEJ, including ends that are intermediates in IgH class switch recombination and ends involved in translocations.

FUTURE ISSUES

1. Characterization of factors participating in A-EJ is mandatory for understanding the exact contribution of both C-NHEJ and A-EJ pathways to the generation of translocations.

2. The precise mechanisms by which DSBs on different chromosomes are juxtaposed before joining is an important question in the field. Future studies that clarify the molecular pathways that can lead to synopsis of distant chromosomal DSBs and further studies of the dynamics of DSBs in living cells should help answer this question.

3. The influence of mechanistic factors on the generation of translocations is masked by cellular selection. Development of techniques to analyze the occurrence of translocations in a manner that is unbiased by selection is a prerequisite for elucidating the potential roles of mechanistic factors in translocation frequency. In this regard, the ability to discriminate oncogenic driver mutations from passenger mutations in tumors might be relevant for interpreting data from cancer genome sequencing projects and for the design of targeted therapies.

DISCLOSURE STATEMENT

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Contents

Innate Antifungal Immunity: The Key Role of Phagocytes
Gordon D. Brown ..................................................... 1

Stromal Cell–Immune Cell Interactions
Ramon Roozendaal and Reina E. Mebius ................................................................. 23

Nonredundant Roles of Basophils in Immunity
Hajime Karasuyama, Kaori Mukai, Kazusige Obata, Yusuke Tsujimura,
and Takeshi Wada ................................................................................................. 45

Regulation and Functions of IL-10 Family of Cytokines
in Inflammation and Disease
Wenjun Ouyang, Sascha Rutz, Natasha K. Crellin, Patricia A. Valdez,
and Sarah G. Hymowitz ......................................................................................... 71

Prevention and Treatment of Papillomavirus-Related Cancers
Through Immunization
Ian H. Frazer, Graham R. Leggatt, and Stephen R. Mattarollo ......................... 111

HMGB1 Is a Therapeutic Target for Sterile Inflammation and Infection
Ulf Andersson and Kevin J. Tracey ........................................................................ 139

Plasmacytoid Dendritic Cells: Recent Progress and Open Questions
Boris Reizis, Anna Bunin, Hiyaa S. Ghosh, Kanako L. Lewis, and Vanja Sisirak ..... 163

Nucleic Acid Recognition by the Innate Immune System
Roman Barbalat, Sarab E. Ewald, Maria L. Mouchess, and Gregory M. Barton ...... 185

 Trafficking of B Cell Antigen in Lymph Nodes
Santiago F. Gonzalez, Søren E. Degn, Lisa A. Pitcher, Matthew Woodruff,
Balthasar A. Heesters, and Michael C. Carroll ..................................................... 215

Natural Innate and Adaptive Immunity to Cancer
Matthew D. Vesely, Michael H. Kershaw, Robert D. Schreiber,
and Mark J. Smyth ................................................................................................. 235

Immunoglobulin Responses at the Mucosal Interface
Andrea Cerutti, Kang Chen, and Alejo Chorny .................................................... 273

HLA/KIR Restraint of HIV: Surviving the Fittest
Arman A. Babirova, Rasmi Thomas, and Mary Carrington ................................. 295
Mechanisms that Promote and Suppress Chromosomal Translocations in Lymphocytes
Monica Gostissa, Frederick W. Alt, and Roberto Chiarle ........................................... 319

Pathogenesis and Host Control of Gammaherpesviruses: Lessons from the Mouse
Erik Barton, Pratyusha Mandal, and Samuel H. Speck .................................................. 351

Genetic Defects in Severe Congenital Neutropenia: Emerging Insights into Life and Death of Human Neutrophil Granulocytes
Christoph Klein ................................................................................................................. 399

Inflammatory Mechanisms in Obesity
Margaret F. Gregor and Gökhan S. Hotamisligil .............................................................. 415

Human TLRs and IL-1Rs in Host Defense: Natural Insights from Evolutionary, Epidemiological, and Clinical Genetics
Jean-Laurent Casanova, Laurent Abel, and Lluis Quintana-Murci .................................. 447

Integration of Genetic and Immunological Insights into a Model of Celiac Disease Pathogenesis
Valérie Abadie, Ludvig M. Söllid, Luis B. Barreiro, and Bana Jabri .................................. 493

Systems Biology in Immunology: A Computational Modeling Perspective
Ronald N. Germain, Martin Meier-Schellersheim, Aleksandra Nita-Lazar, and Iain D.C. Fraser ................................................................. 527

Immune Response to Dengue Virus and Prospects for a Vaccine
Brian R. Murphy and Stephen S. Whitehead .................................................................... 587

Follicular Helper CD4 T Cells (T<sub>FH</sub>)
Shane Crotty ....................................................................................................................... 621

SLAM Family Receptors and SAP Adaptors in Immunity
Jennifer L. Cannons, Stuart G. Tingye, and Pamela L. Schwartzberg ............................... 665

The Inflammasome NLRs in Immunity, Inflammation, and Associated Diseases
Beckley K. Davis, Haitao Wen, and Jenny P.-Y. Ting ......................................................... 707

Indexes

Cumulative Index of Contributing Authors, Volumes 19–29 ............................................ 737
Cumulative Index of Chapter Titles, Volumes 19–29 ......................................................... 744

Errata

An online log of corrections to Annual Review of Immunology articles may be found at http://immunol.annualreviews.org/errata.shtml