Chapter 3

Recent Insights into the Formation of RAG-Induced Chromosomal Translocations

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Abstract

Chromosomal translocations are found in many types of tumors, where they may be either a cause or a result of malignant transformation. In lymphoid neoplasms, however, it is clear that pathogenesis is initiated by any of a number of recurrent DNA rearrangements. These particular translocations typically place an oncogene under the regulatory control of an Ig or TCR gene promoter, dysregulating cell growth, differentiation, or apoptosis. Given that physiological DNA rearrangements (V(D)J and class switch recombination) are integral to lymphocyte development, it is critical to understand how genomic stability is maintained during these processes. Recent advances in our understanding of DNA damage signaling and repair have provided clues to the kinds of mechanisms that lead to V(D)J-mediated translocations. In turn, investigations into the regulation of V(D)J joining have illuminated a formerly obscure pathway of DNA repair known as alternative NHEJ, which is error-prone and frequently involved in translocations. In this chapter we consider recent advances in our understanding of the functions of the RAG proteins, RAG interactions with DNA repair pathways, damage signaling and chromosome biology, all of which shed light on how mistakes at different stages of V(D)J recombination might lead to leukemias and lymphomas.

Introduction

Lymphoid neoplasms are among the most common malignancies in humans; mysteriously, they have become increasingly common in both adults and children over the past two decades, with the incidence of non-Hodgkin's lymphoma alone having doubled.[1] A number of factors are implicated in the etiology of these disorders, including ionizing radiation, chemical exposures, viral infection, autoimmune disease and acquired immunodeficiencies. Some of these conditions might directly create genetic mutations that initiate tumorigenesis; others may simply promote a favorable immune milieu by chronic antigenic stimulation or immunosuppression. It is fairly certain, however, that many lymphoid neoplasms are born of chromosomal translocations involving antigen receptor loci.[2,3] Up to 90% of cases of non-Hodgkin's lymphoma, for instance, bear such translocations.[1] These aberrant rearrangements most often exert their oncogenic effects by placing an oncogene under the regulatory control of a highly expressing Ig or TCR gene promoter, thereby dysregulating cell differentiation, proliferation, or survival.[3-5] Translocations also commonly

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fuse the coding sequences of two different genes, which then encode chimeric oncoproteins that activate oncogenic transcriptional programs.[6] Both types of events frequently bear signs of having originated through some error in V(D)J recombination, the process by which antigen receptor genes are rearranged.[2,3,7,8]

V(D)J recombination can be thought of as a special case of targeted, strictly regulated genomic instability. There are seven antigen receptor loci that encode the T-cell receptor (TCR) α, β, γ and δ chains and the immunoglobulin (Ig) H and L (κ and λ) chains. Groups of V, D and J coding segments are arrayed along the loci, flanked by recombination signal sequences (RSS). The lymphoid-specific recombinase, consisting of RAG1and RAG2 (the protein products of the recombination activating genes 1 and 2), selects a pair of signal sequences that may be many kilobases apart, cleaves the DNA at the signal sequence borders, and the resulting DNA double-strand breaks are joined by the ubiquitous nonhomologous end joining (NHEJ) proteins. Since antigen receptor gene rearrangement entails breaking and rejoining the chromosome several times before a complete Ig or TCR molecule can be expressed on the cell surface, the creation of a diverse repertoire of antigen receptors violates genomic integrity as a matter of course. It has been estimated that, each day, the human body creates 1×10^{11} B-cells.[9] Granted, most of these newly generated cells die because they form nonfunctional or self-reactive antigen receptors. Even so, an estimated 9×10^{9} cells survive this process every day.[9] These numbers are staggeringly large. An error rate of less than a thousandth of a percent would still yield a large number of cells bearing potentially oncogenic translocations. How is it that leukemias and lymphomas do not overcome us all? The mechanisms that preserve genomic integrity during rearrangement must be unusually reliable, multiply redundant, or both.

In fact, the obvious risks attendant upon sequential cutting and pasting of gene fragments are mitigated by numerous restrictions on the process, many of which have only just been appreciated (and many others of which, no doubt, remain to be discovered). Regulation of recombination requires deft orchestration of chromatin changes, trans-acting factors, transcription, selection of substrates for DNA cleavage and DNA double-strand break (DSB) repair machinery. There are excellent reviews in this volume that do greater justice to the topic of accessibility than we could in this chapter (see also refs. 10-12). Our focus will be on recent work elucidating the molecular mechanisms for maintaining the fidelity of DSB repair. We will begin the chapter by outlining the salient features of the V(D)J reaction. We will then consider those stages where mistakes often occur, with a focus on mechanisms that can lead, in theory at least, to translocations.

Overview of the V(D)J Recombination Reaction

Key steps in the reaction are outlined below. For comprehensive and elegant descriptions of the biochemistry, see references 7, 13 and 14.

The recombination signal sequences (RSS) that flank the V, D and J segments consist of conserved heptamer and nonamer elements separated by an intervening spacer of either 12 or 23 nucleotides. These recognition sequences are referred to as 12-RSS or 23-RSS, and efficient recombination requires that two complementary RSS (a 12/23 pair) be synapsed before cleavage can proceed.[15-17] The heptamer has the palindromic consensus sequence CACAGTG, but variations are common and the extent of deviation from the consensus influences the efficiency with which a site is cleaved. The AT-rich nonamer sequence is less conserved but still important for recombination[18], and even the spacer sequences influence the selection of an RSS.[19-22]

The RSS are recognized by the lymphoid-specific proteins RAG1 and RAG2 ("recombination activating genes 1 and 2"[23]), which together form a complex we will refer to as the V(D)J or RAG recombinase. HMGB1 (high mobility group box 1), a nonspecific DNA bending protein, facilitates synaptic complex formation and cleavage.[24,25] The RAG proteins nick one DNA strand precisely between the RSS heptamer and the coding segment. This generates a free 3'OH that is used to attack the opposite strand in a transesterification reaction, forming a double-strand break (DSB). The result is that the synapsed pair of RSS/coding segments yields four free DNA ends: two covalently sealed (hairpin) coding ends and two signal ends that terminate in a flush double-strand break.[26-30]

After coupled cleavage, the RAG proteins hold the DNA ends in a postcleavage complex, aligning them for proper joining by the nonhomologous end joining (NHEJ) machinery. The blunt-ended RSS undergo direct ligation (generally with no base loss) to form a signal joint, which is usually deleted as an extrachromosomal circular product that is lost during cell division. Less frequently, the orientation of the coding segments necessitates inversional recombination, in which the signal joint is retained in the chromosome. There is no known immunological function for signal joints, but in cases of inversional recombination their formation is necessary for preserving genomic integrity. Ligation of the two coding ends produces a coding joint that encodes the variable portion of the antigen receptor protein. Coding joints are typically imprecise, as the coding end hairpins must first be opened and often undergo loss or addition of nucleotides during processing. This junctional variability contributes further to antigen receptor diversity and is considered characteristic of repair by nonhomologous end-joining.

Potential Mechanisms of RAG-Mediated Translocations

Errors in recombination can be broadly classified into two categories. Those occurring during the early stage of the reaction (site selection and cleavage) can be conceptualized as cases of mistaken identity: they involve either (1) mixing of authentic but inappropriate antigen receptor loci (e.g., TCRβ and TCRγ segments) in interlocus recombination, or (2) the misappropriation of sequences that fortuitously resemble RSS (cryptic RSS). One mechanism for preventing such errors involves regulation of substrate accessibility; we will discuss this and related regulatory controls relevant to each type of substrate selection error in the following section. Errors that take place in later stages of the reaction (joining) can instead be conceived as involving renegade double-strand breaks. Broken DNA ends created in the context of V(D)J recombination might escape normal DNA repair through defects in the RAG postcleavage complex, use of an inappropriate repair pathway, or an impaired DNA damage signaling response. Mechanisms that act to curtail aberrant repair will be considered in the context of these deficits in subsequent sections.

Mistaken Identities: Substrate Selection Errors

Interlocus Recombination

Normal V(D)J recombination is restricted by cell lineage (TCR loci rearrange in T-cells but not B-cells), developmental stage (e.g., TCRβ before TCRα) and, in many cells, to one allele (allelic exclusion). Since the RAG proteins, the RSS and the DNA repair machinery are the same in each case, this complex regulatory scheme depends in large part on the degree of accessibility allowed the recombinase to the various loci over time in different cells. For this reason, the packaging of TCR and Ig loci into chromatin differs in B- and T-cells and varies according to the activity of the loci, which is governed by developmental stage.

Nevertheless, some temporal overlap in the sequence of rearrangements does allow occasional interlocus (trans) recombination.[31-34] These rearrangements, which create a balanced translocation resulting in two derivative chromosomes, can generate functional chimeric receptor chains that appear in normal tissues.[33,34] As with recurrent oncogenic translocations, the system seems to favor rearrangements of particular sites: for example, it has been estimated that 1 in 10,000 normal human and mouse thymocytes carries the Dδ3-Jβ2.7 rearrangement.[32,35] These rearrangements, just like those that occur in *cis*, rely on RSS recognition, RAG-mediated cleavage and NHEJ repair. They are normal V(D)J reactions simply carried out with the wrong partner. Interlocus events do, however, exhibit recurrent base loss from signal joints[31,36] and difficulty forming coding joints.[37-39] These features suggest that trans rearrangements proceed through an abnormal pathway.

It is noteworthy that the incidence of interlocus recombination increases dramatically in cells bearing certain mutations (such as ATM deficiency) that predispose to lymphoid tumors.[32,40-42] These events have the appearance of simple substrate selection errors, but at least some of these rearrangements might arise from failures in DNA damage sensing and repair (see discussion of ATM defects below, in the section "The role of the DNA damage response in preventing translocations").

Cryptic RSS

The variability of RSS sequence entails considerable flexibility on the part of the RAG proteins. Unfortunately, this plasticity makes it possible for the RAG proteins to bind to fortuitous DNA sequences known as "cryptic RSS" that do not border antigen receptor gene segments but are sufficiently close to the consensus sequence to allow RAG recognition.[43,44] In one large review of oncogenic rearrangements from both B- and T-cell malignancies, most translocation breakpoints on the nonantigen receptor gene partner contained RSS-like sequences at or near the breakpoint, supporting "substrate selection error" as the responsible mechanism.[2] In addition, nontemplated nucleotides are frequently added to the junctions, suggesting TdT activity and therefore the involvement of V(D)J recombination.[2] The t(7; 9) (q34; q32) translocations found in T-cell lymphoblastic leukemia provide the clearest example. Chromosome 7 breakpoints are typically located at the RSS bordering Dβ segments, while breakpoints on chromosome 9 are flanked by consensus RSS heptamer sequences separated from AT-rich nonamer-like sequences by 11 or 12 base pairs.[45] The salient feature of substrate selection errors is that the V(D)J recombination reaction proceeds as normal except for partnering an RSS with an inauthentic sequence.

Preventing Errors by Controlling Accessibility

An RSS can deviate quite far from the consensus and still undergo recombination; Lewis et al defined the necessary features of cryptic RSS and suggested that even a weak signal, with a recombination frequency of 2×10^{-5} the canonical level, can have a physiological impact.[43] In light of estimates that the genome contains 10 million potential cryptic sites, approximately one every 1-2 Kb,[46] it is clear that RAG accessibility to target sites must be very tightly regulated.

In a prescient 1985 paper, Yancopoulos and Alt noted that rearranging segments are transcribed before (or coincident with) their activation for rearrangement and proposed that generating these germline transcripts altered chromatin structure so as to allow the recombinase access to a subset of appropriate substrates.[47] There are also other potential mechanisms for regulating locus accessibility that do not rely on transcription.[48] One approach to controlling access is through nucleosome packaging, which can block cleavage of specific RSS.[49] Proteins that enhance RAG interaction with RSSs[48,50,51] could conceivably recruit nucleosome remodeling complexes such as Swi/Snf that alter DNA-histone contacts within a nucleosome or alter the nucleosome's location.[52,53] The second approach is through covalent modifications of the tail domains of the histone proteins by acetylation of lysines, methylation of lysines and arginines, polyribosylation, serine phosphorylation and ubiquitylation.[54] Such posttranslational modifications can "open" chromatin by altering DNA-histone contacts within a nucleosome, histone-histone contacts between nucleosomes, or interactions between histones and other proteins. Accumulating evidence suggests that these reversible, epigenetic modifications comprise a "histone code" and that they associate with regulatory proteins known as code readers. Evolutionarily conserved domains within code-reader proteins bind to certain histone modifications with such specificity that they can distinguish the same modification at different residues (for example, trimethylation at K4 vs. K9).[54]

Several recent studies have shown that the plant homeodomain (PHD) finger, a methyl-lysine binding domain, serves as a code-reader: it can both promote and repress gene expression by interacting with trimethylated lysine 4 on histone 3 (H3K4).[55-58] Even more recently, the RAG2 PHD finger has been shown to recognize H3K4 trimethylation.[59-61] In these studies, the binding of RAG2 to H3K4 enhanced the selection and recombination of chromatinized gene segments in developing lymphocytes. The RAG complex, then, is not merely subject to chromatin structures determined by other factors, but must take an active role in recognizing substrates.

Other studies have shown that transcriptional cis-regulatory sequences, such as enhancers and promoters specific to each locus, are necessary for V(D)J recombination.[12,62] Furthermore, the RAG genes are regulated differently in B- and T-cells (for example, Foxp1 is required for B-cell-specific RAG expression[63]). Some DNA-binding transcription factors interact with RAG1/RAG2 and guide them to subsets of RSS; B-cell-specific V_H locus contraction, for instance, requires Pax5 to interact with both the V coding segments and the RAG complex.[64,65] The mechanisms of locus

contraction and looping remains poorly understood, but they are essential for promoting synapse formation between distal V and proximal D segments, which can be separated by distances of up to 3 megabases.[66] (In this regard, it is interesting to note that core RAG2 knock-in mice have difficulty with V to DJ rearrangements at the IgH and TCRβ loci.[67,68]) Whether nonantigen receptor loci are typically constrained by such complex regulatory schemes is not clear.

Signs That a Translocation Did Not Arise through Substrate Selection Error

Even granting the occasional chromatin loophole, three observations suggest that substrate selection errors do not account for the majority of RAG-mediated oncogenic translocations. First, many of the RSS-like sequences found at translocation breakpoints on the nonantigen receptor partner chromsome contain heptamers that are a poor match for the consensus, and a large fraction lack recognizable nonamer elements.[2,7] Previous work has shown that DNA cleavage in vivo requires both heptamer and nonamer; scrambling the nonamer or mutating a single critical nucleotide in the heptamer decreases cleavage by at least two orders of magnitude.[15,18,22,69] Therefore, the presence of sequences that deviate so much from the consensus on the partner (nonantigen receptor locus) chromosome might be merely coincidental.[2,3,7] The second argument against the use of some cryptic RSS in translocations is that the breakpoints are often not at the heptamer-coding flank border. This is incompatible with normal RAG-mediated cleavage, which is a very precise reaction. Finally, some translocations display short direct repeats,[8,70] suggesting that the cleavage event created a short single-stranded overhang. This, too, is inconsistent with normal cleavage by the V(D)J recombinase.

This is not to say that such events did not originate with a mistake in V(D)J recombination. If substrate selection error appears unlikely, there is an alternative model that better explains cases such as these. It is known as end donation and posits that the recombinase creates a double-strand break (DSB) at an authentic RSS that is then somehow joined to a random DSB that has been created through some unrelated process.[7] Until the past few years it has been difficult to conceive of a mechanism that would explain end donation, but recent work suggests that broken DNA ends created by RAG cleavage might escape their normal fate through defects in the RAG postcleavage complex, use of an inappropriate repair pathway, or an impaired DNA damage signaling response.

The Ends That Got Away: Errors in Joining

DSBs are potentially so damaging that cells have evolved complex networks of proteins to sense the presence and precise location of DNA damage, regulate the cell cycle and repair the breaks. Mounting evidence suggests that V(D)J recombination enjoys at least two layers of protection that even its DNA-rearranging cousin, class switch recombination, does not:[71] an end joining pathway that discourages translocations (classical NHEJ) and the RAG postcleavage complex, which is thought to ensure joining through this pathway and exclude other, error-prone repair. Yet another layer of protection is provided by ATM, part of the DNA damage signaling machinery, which may have a role in stabilizing the postcleavage complex but also can lead cells with unrepaired breaks to undertake apoptosis.

Genome Guardians: The Classical NHEJ Factors

The basic outline of NHEJ seems simple enough: a set of enzymes captures the two ends of the broken DNA molecule, a molecular bridge is formed to juxtapose the ends, and the break is religated.[72] In reality the process is rather complex and many aspects remain poorly understood (see refs. 72 and 73). A key component of NHEJ is the DNA-dependent protein kinase (DNA-PK) complex, which comprises the DNA-PK catalytic subunit (DNA-PKcs) and the Ku70 and Ku80 nuclear antigens.[74] Nonhomologous repair is initiated when the Ku70/80 heterodimer encircles a broken end,[75,76] creating a scaffold for the recruitment of other factors. Ku attracts DNA-PKcs to the break, where it might serve multiple roles, including the formation of a synaptic complex to bring the ends together.[72] Activated DNA-PKcs recruits XRCC4, DNA Ligase IV and Artemis. DNA-PKcs phosphorylation of Artemis converts the latter from an exonuclease to an endonuclease and allows it to open the hairpinned coding ends.[77,78] Since Artemis cannot process every type of

nonligatable end, other types of end-processing enzymes are also recruited. Polymerase activity, for example, is likely supplied by the DNA polymerase Mu, which associates with XRCC4, and terminal deoxynucleotidyl transferase (TdT) adds nontemplated nucleotides to increase junctional diversity.[79,80] Finally, XRCC4 and DNA Ligase IV ligate the ends.[81-83] The most recently discovered NHEJ factor, known as Cernunnos or XLF (for XRCC4-like factor), is also recruited by Ku and interacts with both XRCC4 and Ligase IV to ligate mismatched and noncohesive ends.[84-88] The order in which all these factors are recruited might be flexible, according to the specific nature of the break.[89]

Genetic ablation of Ku, DNA-PKcs, DNA Ligase IV, XRCC4, Artemis, or Cernunnos in mice prevents the completion of V(D)J recombination, arresting B- and T-cell development at an early stage and leading to a SCID (severe combined immunodeficiency) phenotype. The overall defect in DNA repair also produces sensitivity to ionizing radiation, a marked tendency to translocations and development of lymphoma (though in some cases, only on a p53-deficient background).[90-97] (By contrast, NHEJ-proficient mammalian cells reconstitute their chromosomes with remarkable accuracy after being exposed to doses of ionizing radiation large enough to induce massive chromosome fragmentation.[98,99]) Some NHEJ-deficient lines develop nonlymphoid tumors as well.[90,100,101] The discovery that a deficiency of NHEJ factors promotes oncogenesis revealed a crucial role for these proteins as genome guardians.[94,95]

Error-Prone End Joining: Alternative NHEJ

Despite their obvious defects in DNA repair, NHEJ-deficient mice (and humans[97,102,103]) can survive long enough to develop malignancy. The mouse tumors frequently show gene fusions between the IgH locus and c-Myc but can display many other nonreciprocal translocations. There must, then, be alternative mechanisms capable of repairing DSB without Ku, DNA-PKcs, Ligase IV, or XRCC4. And, in fact, there is, although it was not recognized as an alternative pathway when it was originally described in mammalian cells in the 1980s.[104-106]

At the time, it was known that eukaryotic cells are able to repair DNA ends by both homologous and nonhomologous means. In the case of V(D)J recombination intermediates, homology-based mechanisms seemed unlikely, as little or no homology is present between coding ends; moreover, rearranged coding segments underwent a curious addition and loss of nucleotides at the junction.[107] The mechanism for nonhomologous repair, however, had not yet been discovered and the field struggled to understand how "unrelated DNA ends are joined together willy-nilly with high efficiency".[104] The similarity of these junctions to coding joints hinted that the DNA breaks generated by the V(D)J recombinase might be repaired by the same mechanism.[106] Within several years, studies of V(D)J recombination in various radiosensitive cell lines made it possible to identify components of the NHEJ pathway.[108-112] Our understanding of NHEJ thus grew out of our understanding of V(D)J recombination—and because the wild-type RAG complex guides DNA ends to the classical pathway, not the alternative pathway (see below), the latter settled into quiet obscurity. Only recently, in fact, has it been realized that the two pathways are distinct.[113-115]

The hallmarks of junctions formed by alternative NHEJ are excessive deletions and a reliance on short sequence homologies (microhomologies).[106,113,115] Even blunt-ended plasmids in Ku80-deficient cells undergo resection and annealing of microhomologous sequences rather than simply being joined at the blunt ends.[115] It is worth noting that these microhomologies are present at oncogenic translocations from NHEJ-deficient cells.[96] Therefore, although alternative NHEJ provides enough repair activity to allow cell survival, it appears to be error-prone and predisposes the cell to genomic instability.

But if alternative NHEJ is relatively efficient, why does NHEJ deficiency virtually obliterate V(D)J recombination?

The RAG Postcleavage Complex Governs Choice of Repair Pathway

The observation that both nucleotide addition and deletion could occur prior to joining of coding ends indicated that the DNA ends must remain in one place long enough to allow processing by polymerases and endonucleases.[116] Thus, even before the discovery of RAG1 and RAG2, it

seemed that a stable protein-DNA complex must exist to allow the ends to be accessible to such modifying enzymes after cleavage.[116] When studies showed that cells deficient in Ku or DNA-PK could not resolve V(D)J intermediates, it seemed reasonable to think that, by analogy with the Mu transposase, a very stable postcleavage complex would make DNA ends inaccessible.[117] As the field's understanding of NHEJ repair grew, so did curiosity about how a RAG postcleavage complex might participate in joining.

Lacking a viable in vitro system to study joining, we turned to genetics. Separation-of-function mutants in RAG-1 and RAG-2 that are capable of cleavage but exhibit severe joining defects provided compelling evidence that the postcleavage complex serves a crucial function in joining both coding and signal ends.[118-120] These data lent support to the notion that the RAG proteins form a scaffold that holds the ends together to facilitate joining. Joining mutants could alter the architecture of the complex, facilitating premature release of ends or, conversely, creating a too-stable complex or hindering the recruitment of NHEJ factors.[118-121] Intriguingly, two RAG-1 mutants phenocopied NHEJ mutants: the rare joints they did manage to form exhibited the excessive deletions and short sequence homologies characteristic of alternative NHEJ.[118] These mutants led us to propose that the RAG proteins might function as genome guardians within the context of V(D)J recombination.

We pursued this hypothesis further by examining whether RAG-generated ends could be made available to repair pathways other than NHEJ. (Although homologous recombination and NHEJ predominate at different phases of the cell cycle, accumulating evidence suggests that they can act at the same time and even cooperate to repair a DSB.[73,122]) Using an in vivo system to assay for repair of signal ends by homologous recombination, Lee et al showed that two joining-impaired RAG1 mutants destabilize the RAG postcleavage complex, allowing the DNA ends to be available for repair by homologous recombination.[123] Wild-type postcleavage complexes, by contrast, stimulated no homologous recombination. This led us to propose a model in which the normally quite stable RAG postcleavage complex actively directs DNA ends to the NHEJ machinery for repair.[123] The question remained: how do the rare coding joints produced in NHEJ-deficient cells manage to be formed by the alternative NHEJ pathway?

Since the homologous recombination assay was unable to map the fate of coding ends and we had identified mutations in RAG2 that affected joining without destabilizing the postcleavage complex, we again took a genetic approach. We identified a truncated RAG2 allele that allows substantial coding and signal joint formation to occur in cells deficient for DNA-PKcs or XRCC4.[124] Junction sequences revealed a tendency toward large deletions and microhomology use. Surprisingly, this RAG2 mutant also revealed alternative NHEJ to be active even in wild-type cells.[124] These studies, along with work from the Alt and de Villartay labs studying the use of alternative NHEJ in class switch recombination,[125,126] make it clear that alternative NHEJ is quite robust, albeit error-prone. Thus, we have come full circle: V(D)J recombination allowed the discovery of classical NHEJ and now has brought attention back to alternative NHEJ.

Why is classical NHEJ less prone to translocations than the alternative pathway? Perhaps components of the classical NHEJ pathway interact with chromatin (or chromosome) components to maintain the chromosomal identity of broken ends (see below). In addition, studies of NHEJ have revealed that repair is biphasic: most repair occurs quite rapidly upon induction of a DSB, but there is a slow component that might correspond to alternative pathways and which continues at the same level when the classical pathway is disabled.[127] Thus, it seems the rapidity of classical NHEJ repair ensures that most DSBs are healed within a few hours; those lesions that cannot be repaired in this time will be subject to alternative end joining. It is conceivable that difficult-to-repair DSBs lingering in the nucleus might, over time, separate or drift to a different chromosome territory in the course of other cellular processes (but see below).

How Do Chromosome Ends Meet?

Mammalian chromosomes occupy discrete three-dimensional regions in the nucleus known as chromosome territories. These territories are not fixed, but are specific to different cell types.[128] In

order for a translocation to occur, there must be DSBs in (at least) two chromosomes at the same time; the DSBs must have escaped the normal repair mechanisms; the broken chromosome ends must physically meet and they must be illegitimately repaired. An obvious question arises: do the DSBs roam the nucleus, looking for a partner, or do they stay put?

Two hypotheses have been put forth. The breakage-first model posits that breaks are able to traverse the nuclear space, searching for potential partners, and come together to produce translocations. The contact-first model, on the other hand, proposes that since chromosomes occupy territories in the nucleus, breaks on distinct chromosomes will meet only if they occupy nearby or intermingling domains.[128] To test these possibilities, Soutoglou et al developed a cell system in which they could induce one DSB at a defined site and follow the fate of each of the damaged DNA ends in real time by observing specific fluorescent tags on either side of the break.[129] The authors demonstrated that a single DSB in mammalian cells is positionally stable, with only slight motion of the DNA break.[129] This stability required the end-binding Ku80/Ku70 heterodimer but, surprisingly, was independent of other DNA repair factors, the structural proteins H2AX and SMC1, the cohesin complex and even the Mre11 complex, which has been strongly implicated in anchoring ends. Whether other factors will turn out to be necessary for this immobilization of a break—or whether the cause of the breakage, or the number of breaks induced at the same time, influence this positional stability—remains to be seen.

These results have striking implications for understanding how translocations form in vivo. First, they demonstrate that chromosomal positional stability is related to genomic stability. (At least in mammals; yeast do not have chromosome territories. DSBs in yeast migrate to any of several small nuclear sites that act as damage repair centers.[130]) Second, the data support a contact-first model in mammalian cells and are consistent with the emerging motion that nonrandom, higher order spatial organization of chromosomes accounts in large part for the recurrence of specific translocations. Ten years ago, experiments showed that γ-irradiation of normal human lymphocytes induces translocations in chromosome pairs that have been observed in leukemias, suggesting that these chromosomes are near neighbors in lymphocytes.[131,132] Several frequent translocation partners, including Myc-Igh and BCR-ABL, have been found to exist in close spatial proximity to each other in normal cells before the formation of translocations.[128] The misjoining of proximally positioned chromosome regions supports the observed correlation between the degree of chromosome intermingling and the likelihood of translocations.[133] The frequency of translocations involving antigen receptor loci likely reflects the fact that more gene-rich chromosomes undergo less compaction and more intermingling.[133]

The Role of the DNA Damage Response in Preventing Translocations

The DNA damage sensing pathway was not initially thought to be involved in V(D)J recombination, as damage checkpoints are not activated during the process; in fact, it was assumed that the RAG postcleavage complex sequestered the DSB from the DNA damage sensing machinery. It thus came as a surprise to find that ATM, γ-H2AX and the Mre11 complex localize to RAG-mediated DNA breaks.[134,135] The mystery was deepened by the first studies to investigate whether these factors had any role in V(D)J recombination: the answer, apparently, was no.[136,137] Further probing unearthed a greater tendency to TCR α/δ interlocus recombination in mice deficient for ATM, Mre11, Nbs1, or 53BP1.[42,138-141] Mice deficient in ATM, Rad50, or H2AX develop thymic lymphomas, as do H2AX- and Mre11-deficient mice on a p53 null background.[136-139] Many of these tumors harbor translocations thought to derive from errors in V(D)J recombination, and tumorigenesis is reduced or delayed in mice when ATM deficiency is coupled with RAG1 or RAG2 deficiency.[142,143] Mutations in ATM, Nbs1 and Mre11 cause Ataxia-Telangiectasia, Nijmegen Breakage syndrome and Ataxia-Telangiectasia-Like disorder, respectively; like the mice, patients with these diseases have a predisposition to lymphoid malignancies and harbor frequent translocations between the TCR and Ig loci.

Recent studies provide insight into the role played by ATM (and perhaps, by extension, other damage sensors) in V(D)J recombination and why this role is virtually invisible under normal

circumstances. In addition to its newly discovered role in stabilizing DSB complexes during V(D)J recombination,[144] ATM has a checkpoint function to prevent the propagation of DSBs caused either by RAG or low-dose gamma irradiation to daughter cells.[145] Callen and colleagues posit that ATM$^{-/-}$ lymphocytes that fail primary V(D)J assembly, leaving a DSB on one allele, can still achieve productive rearrangement through independent recombination of the second allele. The presence of the DSB in ATM-deficient cells would not prevent pre-B-cells from undergoing the maturational process. Therefore, DSBs produced in precursor cells would persist in mature B-cells in peripheral lymphoid tissues, where they would then undergo class switching and be subject to further (AID-mediated) DNA breakage.[145] The initial RAG-mediated break could persist for many days, ultimately to be joined to another chromosome in a progeny cell.

This model puts an interesting twist on extant models of how chromosome ends meet in the nucleus and undergo misrepair, forming a translocation. The work of Callen and colleagues supports a contact-first model but suggests that a DSB could migrate from its original position in the chromosome territories and participate in a repair event with another chromosome broken in a progeny cell.[145] One might think of this as diachronic end donation. With regard to physiological relevance, it is striking that up to 50% of mantle cell lymphomas have mutations or deletions in ATM.[146] Callen et al suggest that ATM mutation is likely to be an early event in the malignant transformation.[145]

The foregoing studies emphasize that creating (or preventing) a translocation is a complex process. One has to consider not only the nature of repair factors and the ordered assembly and disassembly of DNA-protein complexes, but the fact that these processes take place in three dimensions and over time. Understanding the spatiotemporal regulation of these repair processes and their coordination with chromosome dynamics, changes in chromatin structure, DNA damage signaling, the cell cycle and other physiological processes represents one of the major challenges to unraveling the puzzle of aberrant V(D)J recombination events. Indeed, the recent discovery that over 700 proteins interact with ATM and ATR in the DNA damage response[147] indicates that this story is likely to get much more complicated.

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