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The RAG recombinase: Beyond breaking

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ABSTRACT

DNA double-strand breaks (DSBs) are commonly seen as lesions that threaten genome integrity and contribute to cancer and aging processes. However, in the context of antigen receptor gene assembly, known as V(D)J recombination, DSBs are obligatory intermediates that allow the establishment of genetic diversity and adaptive immunity. V(D)J recombination is initiated when the lymphoid-restricted recombination-activating genes *RAG1* and *RAG2* are expressed and form a site-specific endonuclease (the RAG nuclease or RAG recombinase). Here, we discuss the ability of the RAG nuclease to minimize the risks of genome disruption by coupling the breakage and repair steps of the V(D)J reaction. This implies that the *RAG* genes, derived from an ancient transposon, have undergone strong selective pressure to prohibit transposition in favor of promoting controlled DNA end joining in *cis* by the ubiquitous DNA damage response and DNA repair machineries. We also discuss the idea that, in addition to being essential for the rearrangement of antigen receptor genes, RAG-mediated DSBs could impact cellular processes and outcomes by affecting genetic and epigenetic programs.

1. RAG structure

In vertebrates, including humans and mice, antigen receptor diversity is generated in immature lymphocytes and depends on a process of somatic rearrangement termed V(D)J recombination, a cut and paste mechanism which shuffles a large array of preexisting variable (V), diversity (D), and joining (J) gene fragments to create functional genes encoding for the variable regions of immunoglobulins (Ig) and T-cell receptor (TCR) proteins (Bassing et al., 2002; Lescale and Deriano, 2016). The recombination-activating gene (RAG) endonuclease or RAG recombinase composed of the RAG1 and RAG2 proteins, is the only lymphoid-specific protein complex required for V(D)J recombination (Oettinger et al., 1990; Schatz et al., 1989). Full-length murine RAG1 (1040 amino acids) and RAG2 (527 amino acids) are conserved polypeptides with specific properties (Fig. 1). Briefly, RAG1 contains recombination signal sequence (RSS)-binding domains, a region that interacts with RAG2, and an active site for DNA cleavage, which includes three essential acidic amino acids (D600, D708 and E962) that coordinate divalent metal ions and are essential for catalysis. RAG1 also contains two zinc finger regions (Zn) important for homodimerization (ZnA) and interaction with RAG2 (ZnB). ZnA also has ubiquitin ligase activity and interacts with and ubiquitylates histone H3 (Gellert, 2002; Jones and Simkus, 2009; Schatz and Swanson, 2011). Early mutagenesis studies defined the catalytic core of RAG1 and RAG2 proteins to residues 384–1008 and 1–383, respectively (Jones and Simkus, 2009; Sadofsky et al., 1994, 1993). More recent work has reduced the minimal region of RAG2 required to support recombination to residues 1–350 (Coussens et al., 2013; Kim et al., 2015), coinciding with the end of a region containing six kelch-like motifs predicted to adopt a six-bladed β-propeller-like structure (Callebaut and Mornon, 1998) termed the core domain. This core RAG2 region is essential for DNA cleavage activity, enhances RSS binding and interacts with RAG1. The C-terminal non-core region of RAG2 contains multiple regulatory motifs (Jones and Simkus, 2009) including a PHD finger that interacts with H3K4me3 (Callebaut and Mornon, 1998; Liu et al., 2007; Matthews et al., 2007), a cell cycle regulated protein degradation signal in which the phosphorylation of an essential threonine (T490) signals the periodic destruction of RAG2 at the G1-to-S transition and therefore assures proper coupling of V(D)J recombination to the cell cycle (Li et al., 1996; Zhang et al., 2011), and a highly conserved acidic "hinge" region that participates in post-cleavage DNA ends stabilization and DNA repair pathway choice (Coussens et al., 2013).

2. RAG-mediated cleavage

V(D)J recombination is initiated during the G1 phase of the cell cycle when the RAG recombinase introduces double-strand breaks (DSBs) between V, D and J coding gene segments and RSSs (Schatz

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and Swanson, 2011). RSSs consist of relatively conserved heptamer and nonamer elements, with respective consensus sequences of CACAGTG and ACAAAAACC, separated by an intervening spacer of either 12 (12-RSS) or 23 (23-RSS) nucleotides. Cleavage starts with the binding of the RAG proteins to one RSS and the formation of a synapsis in which a partner RSS is captured. HMG1 (high mobility group 1), a non-specific DNA bending protein, facilitates synapsis formation and subsequent cleavage. After binding, the RAG proteins nick one DNA strand precisely between the RSS heptamer and the coding segments. This generates a free 3′-OH that is used to attack the opposite strand in a *trans*-esterification reaction, forming a DNA DSB. RAG-mediated cleavage at a pair of RSSs generates four broken DNA ends: two blunt 5′ phosphorylated signal ends (SEs) which terminate in the RSS, and two covalently sealed (hairpin) coding ends (CEs). Efficient DSB formation requires 12/23-RSS pair assembly into a synaptic complex with the RAG proteins. This restriction, referred to as the "12/23 rule", directs asymmetrical recombination between appropriate gene segments within a given V(D)J locus (Schatz and Swanson, 2011).

3. RAG-mediated transposition

Mechanistic similarities between RAG-mediated DNA cleavage and that of some transposases support the idea that the RAG recombinase derives from a transposable element that entered the genome of a common ancestor of all jawed vertebrates (we encourage the reader interested in the topic to consult these recent articles: Fugmann, 2010; Huang et al., 2016; Kapitonov and Koonin, 2015; Litman et al., 2010; Teng and Schatz, 2015). This is also consistent with the observation that the RAG proteins (at least their truncated forms) are capable of transposition, the process of moving genetic information from one position in the host genome into another, *in vitro* (Agrawal et al., 1998; Hiom et al., 1998), and, to a much lesser extent, in living cells (Chatterji et al., 2006; Reddy et al., 2006). However, transposition is substantially divergent from RAG-mediated V(D)J recombination. In the latter, the RAG proteins participate in resealing of DNA breaks through the non-homologous end-joining pathway (NHEJ) and joins the two signal ends together instead of using them to integrate into a new target location (Fig. 2). This implies that the *RAG* genes have undergone strong selective pressure to prohibit transposition activities in favor of promoting controlled DNA end joining in *cis* by the host DNA damage response (DDR) and DNA repair machineries.

Transib and *Transib*-like transposition elements contain a single open reading frame that shares similarities with RAG1 (Fugmann, 2010). When co-expressed with RAG2, the *Transib* transposase has a latent ability to initiate V(D)J recombination and an increased transposase activity (Carmona et al., 2016), suggesting that the capture of an ancestral RAG2 might have contributed to the evolution of an ancient RAG1-like transposase. Consistent with this is the observation that although RAG1 alone allows very low levels of recombination, it differs from RAG1/2-mediated canonical V(D)J recombination in having lost the requirement for asymmetrical (12/23-RSS) DNA substrates, implicating RAG2 in the origin of the "12/23 rule" (Carmona et al., 2016). A RAG-related transposable element, *ProtoRAG*, was recently discovered in lancelet, a chordate that predates the origin of jawed vertebrates (Huang et al., 2014, 2016; Zhang et al., 2014). *ProtoRAG* encodes RAG1- and RAG2-like proteins that constitute an active endonuclease and transposase with mechanistic similarities to vertebrate RAG (Huang et al., 2016). Strikingly, lancelet RAG2 lacks the entire RAG2 C-terminal region (residues 351–527), suggesting that vertebrate RAG2 might have acquired its regulatory C-terminal domain independently. The observation that the C-terminal portion of RAG2 suppresses RAG transposition (Elkin et al., 2003; Tsai and Schatz, 2003), mis-cleavage/targeting activities (Lu et al., 2015; Mijuskovic et al., 2015; Teng et al., 2015) and aberrant repair of broken DNA ends (Lescale and Deriano, 2016; Roth, 2014) supports the idea that RAG2 might have largely contributed to the evolution of an ancient RAG1 transposase into a domesticated V(D)J recombinase essential for adaptive immunity of the host.

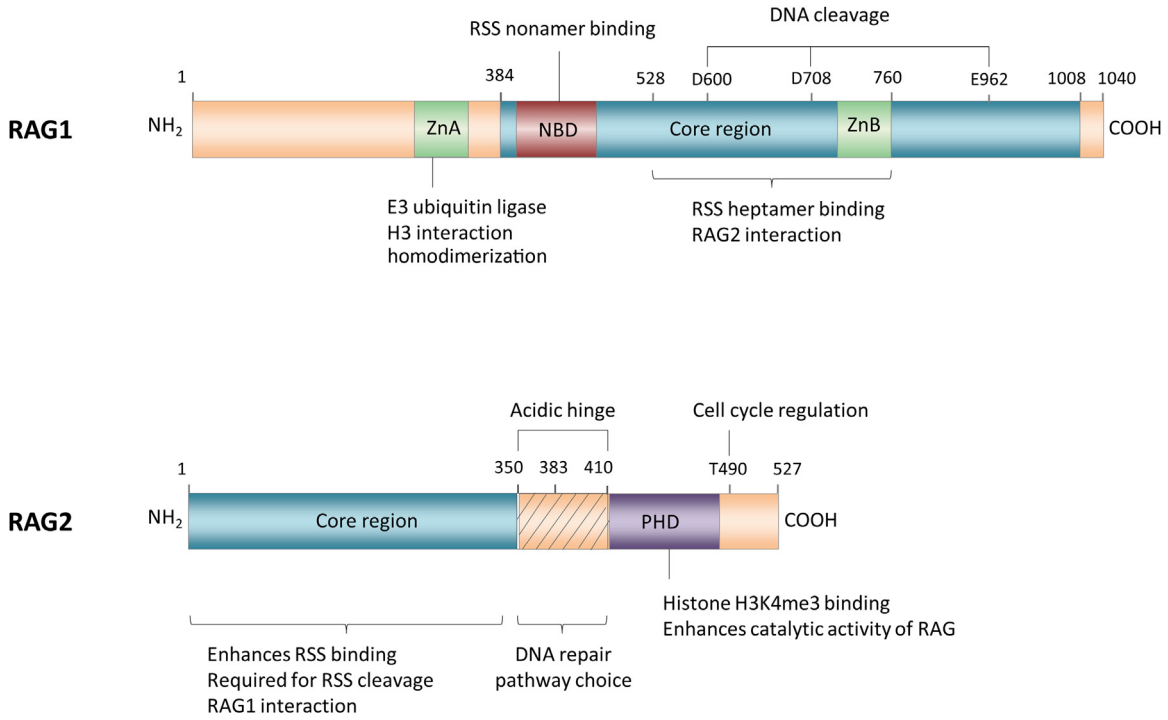

Fig. 1. Structure of RAG1 and RAG2 proteins. See text for details (From Lescale and Deriano, 2016). Zn: zing finger domain; NBD: nonamer binding domain; PHD: plant homeodomain.

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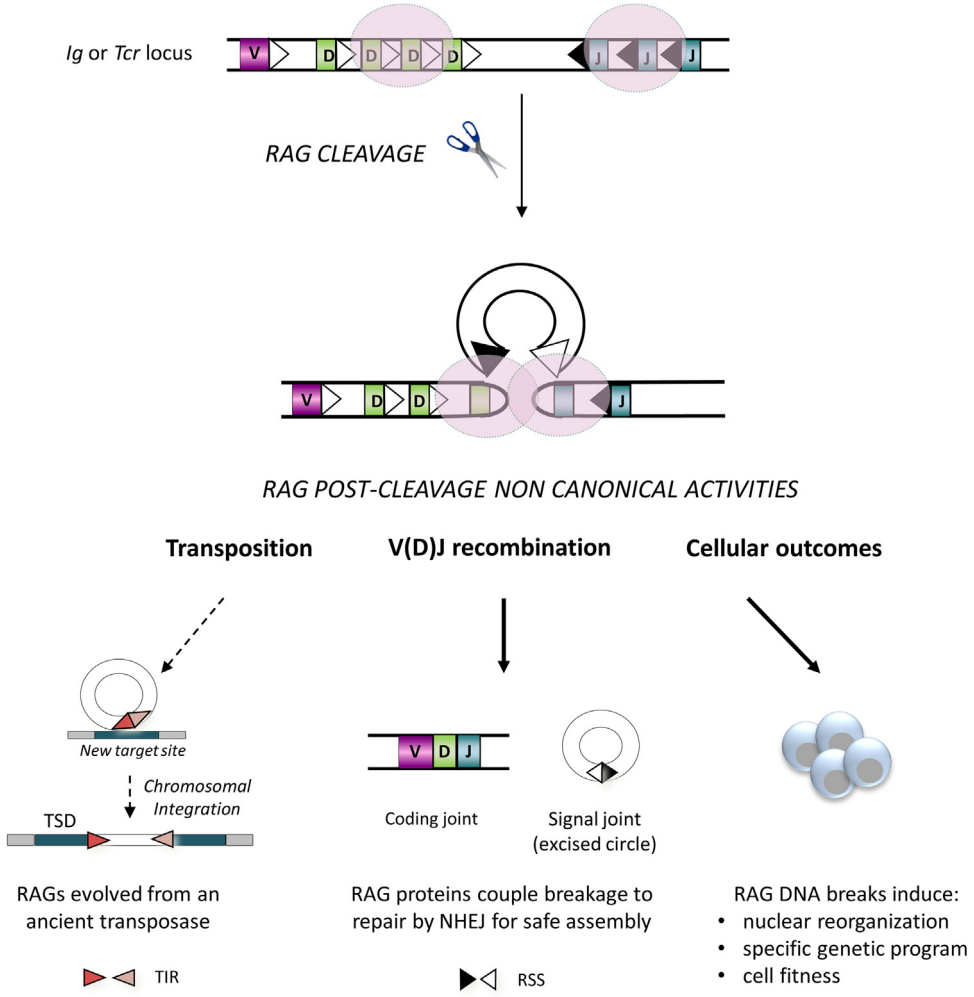

Fig. 2. The RAG Recombinase: Beyond Breaking. See text for details. Ig: Immunoglobulin; Tcr: T-cell receptor; TSD: Target Site duplication; TIR: Terminal Inverted Repeat, RSS: Recombination Signal sequence; NHEJ: Non Homologous End Joining.

4. Non-homologous end-joining of RAG DNA breaks

RAG-induced DNA breaks activate the ataxia telangiectasia mutated (ATM) kinase-dependent DDR that includes cell cycle checkpoint pathways, DNA repair pathways, and, when DSBs persist unrepaired, cell death pathways (Helmink and Sleckman, 2012; Shiloh and Ziv, 2013). Subsequently, the classical NHEJ pathway joins these DNA ends in a recombinant configuration, forming a coding joint (CJ) (the rearranged antigen receptor gene) and a reciprocal signal joint (SJ) (Deriano and Roth, 2013; Helmink and Sleckman, 2012; Schatz and Swanson, 2011) (Fig. 2). During NHEJ, the Ku70/80 heterodimer (Ku) binds DNA ends and recruits the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) to form the DNA-PK holoenzyme. DNA-PK phosphorylates multiple substrates, promoting synapsis of DNA ends and facilitating the recruitment of end processing and ligation enzymes. The hairpin-sealed coding ends are first opened by the Artemis endonuclease. Finally, the XLF-XRCC4-Ligase IV complex performs ligation of DNA ends (Deriano and Roth, 2013; Lieber, 2010). Recently, Jackson and colleagues identified a new component of the NHEJ machinery that they named PAXX for paralog of XRCC4 and XLF (Ochi et al., 2015). PAXX accumulates at sites of DNA damage and functions with XRCC4 and XLF to mediate DSB repair and cell survival in response to DSB-inducing agents. PAXX itself does not bind DNA but interacts with Ku and Ku-bound DNA to promote NHEJ factor assembly and enhance DNA end ligation (Craxton et al., 2015; Ochi et al., 2015; Roy et al., 2015; Xing et al., 2015). PAXX, similar to XLF (Li et al., 2008a), is dispensable for the repair of DSBs generated during V(D)J recombination (Kumar et al., 2016; Lescale et al., 2016b). However, while PAXX- and XLF-single deficient pro-B cell lines perform robust V(D)J recombination, PAXX and XLF double deficiency leads to a strong defect in joining coding and signal ends (Kumar et al., 2016; Lescale et al., 2016b). These results indicate that PAXX function in repairing RAG DSBs is masked by redundancy with its paralog XLF. Consistent with a role for PAXX in NHEJ-repair of RAG DNA breaks, PAXX function in V(D)J recombination depends on its interaction with Ku (Lescale et al., 2016b). XLF also has functional redundancy with several members of the ATM-DDR (Kumar et al., 2014; Zha et al., 2011) and with the RAG complex (Lescale et al., 2016a) during V(D)J recombination (see also below). In sharp contrast, PAXX is not redundant with ATM and the RAG complex in repairing DNA breaks (Kumar et al., 2016; Lescale et al., 2016b), indicating that PAXX and XLF paralogs have specific overlapping and non-overlapping functions during antigen receptor assembly.

5. The RAG post-cleavage complex: coupling breakage to repair for a safe assembly

During the past 20 years, a combination of biochemical, genetic, and *in vivo* studies have shed light on a role for the RAG proteins in the joining step of V(D)J recombination. After cleavage within a synaptic complex (Schatz and Swanson, 2011), the RAG proteins

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stay associated with the DNA ends in a post-cleavage complex (PCC), thought to provide a layer of regulation at the joining step. It is not known whether the four DNA ends all stay associated in a single complex or whether the two coding ends and two signal ends are maintained separately. The fact that the RAGs bind much more avidly to signal-end pairs than coding-end pairs indicates that RAG-signal end PCCs and RAG-coding end PCCs may have different properties (Agrawal and Schatz, 1997; Hiom and Gellert, 1997), and perhaps reflects the need for hairpin sealed coding ends to be accessible for processing before ligation. The discovery of separation of function mutants in RAG1 and RAG2 that are capable of cleavage but exhibit severe joining defects provided the first compelling evidence that the RAG PCC might serve a crucial function in joining both coding and signal ends (Roth, 2003). Additional studies showed that destabilization of the RAG post-cleavage complex in certain RAG mutants might lead to repair by homologous recombination and alternative NHEJ, an alternative DNA end-joining pathway that is implicated in the formation of chromosomal translocations (Corneo et al., 2007; Coussens et al., 2013; Deriano and Roth, 2013; Lee et al., 2004). These results led to a model in which the quite stable RAG PCC actively shepherds DNA ends to the NHEJ machinery for repair, thus protecting them from error prone end-joining pathways and aberrant recombination events (Deriano and Roth, 2013; Lee et al., 2004; Roth, 2003). This "stabilization/shepherding" model is consistent with the observation that PCCs that contain RSSs that diverge from the consensus (particularly in the heptamer) are also destabilized and allow for repair by alternative pathways (Arnal et al., 2010).

This model predicts that removal of such RAG regulatory function would affect genome integrity during V(D)J recombination. This is in fact the case, a mutant RAG2 in which the C-terminal residues 352–527 are deleted (core RAG2), is known to destabilize the RAG-PCC *in vitro* and to increase the rate of aberrant recombination product formation *in vivo* including interchromosomal translocations involving V(D)J loci (Corneo et al., 2007; Coussens et al., 2013; Curry and Schlissel, 2008; Deriano et al., 2011; Sekiguchi et al., 2001; Talukder et al., 2004). Additionally, core RAG2/p53-deficient mice display increased alternative NHEJ accompanied by genomic instability and accelerated lymphomagenesis, generating tumors bearing a complex landscape of chromosomal rearrangements (Deriano et al., 2011; Mijuskovic et al., 2012, 2015).

Interestingly, the lymphomas and translocations seen in core RAG2/p53-deficient animals closely resemble those of ATM deficient mice, suggesting that a similar DNA end destabilization/release mechanism might underlie genomic instability and lymphoma genesis in both mouse models (Deriano et al., 2011). Consistent with this, ATM, beyond its role in activating checkpoints, is important for the stability of RAG PCCs *in vivo* (Bredemeyer et al., 2006). ATM phosphorylates chromatin- and/or DNA-associated proteins, including the histone variant H2AX (forming γH2AX), p53 binding protein 1 (53BP1), mediator of DNA damage checkpoint 1 (MDC1) and factors of the MRE11 complex (MRE11, RAD50, and NBS1) that assemble over large DNA regions of the chromatin on both sides of DNA breaks to form so-called nuclear DNA repair foci. Because the stabilization function of ATM depends on its kinase activity, formation of ATM-dependent DNA repair foci has been proposed to tether DNA ends for proper joining via NHEJ. In fact, in ATM deficient cells undergoing V(D)J recombination, a fraction of coding ends evade the PCC and are occasionally joined aberrantly forming chromosomal deletions, inversions, and translocations (Bredemeyer et al., 2006; Helmink and Sleckman, 2012). Altogether, these results indicate that RAG2, and by extension the RAG-post cleavage complex, and ATM share mechanistic properties during V(D)J recombination, likely through stabilization of broken DNA-ends.

Additional insights into the mechanisms responsible for the stabilization of RAG-cleaved DNA ends within post-cleavage complexes came from the analysis of animal models doubly deficient for ATM/XLF or core RAG2/XLF. The NHEJ protein XLF was identified through both cDNA complementation of cells derived from an IR-sensitive immunodeficient patient (Buck et al., 2006) and through a yeast two-hybrid screen for XRCC4-interacting partners (Ahnesorg et al., 2006). XLF and XRCC4 are two distantly related members of the same protein family and share structural similarity (Andres et al., 2007; Callebaut et al., 2006; Li et al., 2008b). Together, they form long filaments, thought to help DNA end tethering during repair (Hammel et al., 2011; Reid et al., 2015; Riballo et al., 2009; Ropars et al., 2011; Tsai et al., 2007). In contrast to other NHEJ-deficient mice, XLF-deficient mice are not markedly immune-deficient and, as aforementioned, pro-B cell lines derived from these animals perform nearly normal V(D)J recombination. These observations led to the speculation that lymphocyte-specific factors/pathways compensate for XLF function during V(D)J recombination (Li et al., 2008a). This is supported by the analysis of V(D)J recombination in cells deficient for both ATM-dependent DDR and XLF; Combined deficiency of ATM, 53BP1, or H2AX with XLF deficiency results in a severe block in lymphocyte development with a significant defect in the repair of RAG-mediated DSBs, revealing functional redundancy between XLF and ATM-DDR factors during V(D)J recombination (Kumar et al., 2014; Zha et al., 2011). Recently, we showed that, similarly, core RAG2/XLF double deficiency leads to a profound lymphopenia associated with a severe defect in joining of RAG-cleaved DNA ends. Unrepaired DNA ends persist in the absence of p53-dependent cell cycle/apoptotic checkpoint, leading to high levels of genomic aberrations in core RAG2/XLF/p53-deficient cells and aggressive pro-B cell lymphomas harboring *Igh*/*c-myc* chromosomal translocations in mice (Lescale et al., 2016a). Interestingly, most translocation junctions contained short sequence homology suggesting aberrant repair of DNA ends by alternative NHEJ (Deriano and Roth, 2013; Lescale et al., 2016a). Therefore, in the absence of XLF, removal of the C-terminal portion of RAG2 abolishes NHEJ-mediated repair of RAG-DNA ends and promotes aberrant joining and genomic instability.

It is tempting to speculate that the RAG endonuclease, perhaps through the co-option of full length RAG2, has evolved to optimize repair of generated DNA breaks within post-cleavage complexes, providing an additional layer of protection against aberrant joining, genomic instability and cell transformation during the process of antigen receptor assembly. As the RAG proteins are only expressed in developing lymphocytes, such a role would necessarily overlap with the ubiquitous DDR and NHEJ machineries – XLF (and possibly the XRCC4/XLF tethering filament) might represent one component of the NHEJ pathway with which the RAG complex may have functionally co-evolved.

Coupling cleavage and repair to diversify safely might also extend to other biological processes requiring programmed DNA breakage. Immunoglobulin class switching is initiated in germinal center B cells when the B-cell specific activation-induced cytidine deaminase (AID) enzyme deaminates deoxycytidine to deoxyuridine at the immunoglobulin loci and together with the uracyl DNA glycosylase or components of the mismatch repair pathway creates DSBs. Interestingly, the C-terminal domain of AID is required to prevent excessive end resection and to promote end joining during class switch recombination (CSR), indicating that, similar to RAG, AID might participate in the repair phase of CSR (Zahn et al., 2014). Another example of orchestrated breakage and repair reaction is meiosis. Meiotic recombination starts with formation of programmed DNA breaks at many places across the genome (Keeney et al., 2014). Meiotic DSBs are formed by the topoisomerase-related SPO11 protein (Robert et al., 2016) via a covalent protein-DNA intermediate that is endonucleolytically cleaved to release SPO11

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attached to a short oligonucleotide. Removal of the covalently bound SPO11 is mediated by the MRE11 complex and is followed by extensive exonucleolytic resection of the DSB ends, which provides ssDNA tails for homologous recombination. Interestingly, SPO11-bound DSBs might act as a barrier to binding by Ku, thus preventing NHEJ in favor of homologous recombination (Keeney et al., 2014). It will be of most interest to understand to which extent SPO11 acts as a canonical type II topoisomerase versus a more specialized enzyme that has evolved to couple initiation and repair of meiotic DSBs.

6. RAG DNA breaks and allelic exclusion

Antigen receptor allelic exclusion assures the surface expression of immunoglobulin or T cell receptor chains from a single allelic copy of corresponding gene loci. RAG-DSBs have been shown to participate in allelic exclusion by modulating the positioning of antigen receptor gene loci within the nucleus. Specifically, RAG cleavage on one allele promotes repositioning of the second allele to pericentromeric heterochromatin. This nuclear repositioning to a repressive environment inhibits transcription and thus access of the RAG recombinase to the un-cleaved allele during repair of the first break, therefore participating in the enforcement of allelic exclusion. Although the precise mechanism responsible for this phenomenon is unknown, it depends on the C-terminal regulatory domain of RAG2 and ATM and likely requires specific signals/pathways emanating from RAG PCCs (Chaumeil et al., 2013a, 2013b; Chaumeil and Skok, 2013; Hewitt et al., 2009). Interestingly, the control of the number of DSBs generated by SPO11 also depends on ATM, indicating that such negative feedback regulation might also occur during meiosis (Keeney et al., 2014; Lange et al., 2011).

7. RAG DNA breaks and cellular outcomes

RAG-induced DNA breaks trigger a broad genetic program that includes the canonical ATM/p53-dependent DDR that promotes G1/S cell cycle checkpoint and cell death and the NF-kB pathway that enhances the expression of pro-survival genes (Helmink and Sleckman, 2012). More surprising is the observation that RAG DSBs also regulate many genes not directly involved in canonical DDR, through both ATM-dependent and ATM-independent pathways (Bednarski et al., 2012, 2016; Bednarski and Sleckman, 2012; Bredemeyer et al., 2008). For instance, in small pre-B cells undergoing *Igk* gene rearrangement, RAG-DSBs activate an ATM-dependent cell type-specific checkpoint pathway that opposes the pre-B cell receptor (BCR) proliferative signals and prevents cells from prematurely entering into S phase. Inhibition of pre-BCR signals also leads to a reduction in *Igk* germline transcription and inhibition of additional DSBs at the *Igk* locus (Bednarski et al., 2016). Therefore, in a sort of negative feedback mechanism, RAG DNA breaks provide cell specific signals that limit the number of RAG DSBs and prevent the cell from entering cycle until the initial rearrangement has been completed and ATM signaling is terminated (Bednarski et al., 2016). Additionally, RAG DNA breaks provide cellular cues that might regulate lymphocyte migration and homing within specific bone marrow niches, for example through the up-expression of CD62L, CD69, and SWAP70 (Bredemeyer et al., 2008). Interestingly, AID-DNA breaks generated in mature B cells during CSR also activate ATM and a genetic program important for plasma cell differentiation (Sherman et al., 2011, 2010).Therefore, programmed DSBs might trigger specific genetic programs essential for the physiological maturation of lymphocytes.

It was recently discovered that the RAG proteins can impact cellular differentiation and fitness of natural killer (NK) cells (Karo et al., 2014; Karo and Sun, 2015). NK cells represent a lineage of innate lymphoid cells (ILCs) that do not require antigen receptor assembly for their development or function. However, NK cells arise from common lymphoid progenitors (CLPs) that express low levels of RAG and are known to contain rearrangements at several antigen receptor loci (Borghesi et al., 2004). In fact, a substantial fraction (20 to 30%) of mature NK cells derive from RAG-expressing CLPs. These RAG-experienced NK cells, as compared to their RAG-inexperienced counterparts, exhibit increased capacity to survive virus-driven proliferation and enhanced DNA break repair activity (Karo et al., 2014). This fitness advantage depends on RAG cleavage (Karo et al., 2014) and might require the induction of a specific gene expression program, epigenetic reprogramming and other RAG-DSB mediated cellular changes in CLPs and/or developing ILCs (Karo and Sun, 2015).

Interestingly, RAG binds to thousands of places on the genome outside of antigen receptor loci (Ji et al., 2010; Maman et al., 2016; Teng et al., 2015; Teng and Schatz, 2015). Although RAG binding at ectopic locations might represent a threat for genome integrity (Teng et al., 2015), these regions might also represent physiological targets for modulating gene expression. Consistent with this, RAG ectopic binding is driven by interactions between the RAG proteins and active transcription-associated chromatin marks including H3K4me3 and H3K27Ac (Ji et al., 2010; Maman et al., 2016; Teng et al., 2015; Teng and Schatz, 2015). Although RAG binding to transcription-associated chromatin marks *per se* does not correlate with global changes in the expression of their associated genes (Teng et al., 2015), RAG-mediated cleavage at some of these sites might lead to specific epigenetic/gene expression changes and somatic mutations. In that regard, it will be interesting to determine the location and frequency of RAG-DSBs in specific hematopoietic cell subsets and their correlation to RAG-binding sites, epigenetic and gene expression changes.

8. Concluding remarks

As illustrated in this review, RAG-mediated recombination represents a paradigm to study the regulation and physiological consequences of more sporadic DNA breakage events. It will likely shed light on the interesting question of whether and to what extent the occurrence of DSBs, for instance during replication, mitosis, cell migration or neurogenesis, contribute to somatic mosaicism and to genetic and epigenetic program changes that might affect cell function and fate. The existence of multiple DSB response pathways that are activated by diverse cellular events and that generate distinct signaling outcomes (Burgess and Misteli, 2015) supports the idea that DSBs, apart from endangering genome integrity, serve essential physiological functions. If this is the case, the pathological consequences of aberrant DNA breakage, sensing and repair could be much broader than commonly thought.

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