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REVIEW
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The Happy Hopping of Transposons: The Origins of V(D)J Recombination in Adaptive Immunity

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Nearly 50% of the human genome is derived from transposable elements (TEs). Though dysregulated transposons are deleterious to humans and can lead to diseases, co-opted transposons play an important role in generating alternative or new DNA sequence combinations to perform novel cellular functions. The appearance of an adaptive immune system in jawed vertebrates, wherein the somatic rearrangement of T and B cells generates a repertoire of antibodies and receptors, is underpinned by Class II TEs. This review follows the evolution of recombination activation genes (RAGs), components of adaptive immunity, from TEs, focusing on the structural and mechanistic similarities between RAG recombinases and DNA transposases. As evolution occurred from a transposon precursor, DNA transposases developed a more targeted and constrained mechanism of mobilization. As DNA repair is integral to transposition and recombination, we note key similarities and differences in the choice of DNA repair pathways following these processes. Understanding the regulation of V(D)J recombination from its evolutionary origins may help future research to specifically target RAG proteins to rectify diseases associated with immune dysregulation.

Keywords: transposable elements, V(D)J recombination, adaptive immunity, RAG recombinase, DNA transposons

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INTRODUCTION

Transposable elements (TEs) are pervasive in all kingdoms of life. There are two major classes based on their mechanism of transposition (**Figure 1**): Class I TEs, also known as retrotransposons, and Class II TEs, also known as DNA transposons. Class I TEs replicate *via* a copy-and-paste mechanism through an RNA intermediate (Boeke et al., 1985; Finnegan, 1989; Kazazian, 2004). This class is further divided into two subclasses: LTRs (long terminal repeats) and non-LTRs. LTRs include endogenous retrovirus (ERV) retrotransposons and non-LTRs include both long and short interspersed nuclear elements (LINEs/L1s and SINEs). Class II DNA transposons mobilize through a cut-and-paste mechanism wherein the original sequence is excised from the genome before being inserted at another site. A subclass of DNA transposons, Helitrons, mobilize through a peel-and-paste mechanism with a circular DNA intermediate (Bourque et al., 2018).

Functionally, TEs have both negative and positive effects on host cell fitness. In this review, we present the beneficial and detrimental effects of TEs. In particular, we focus on how vertebrate adaptive immunity arose through harnessing genes that arose by TE insertion. These recombination activation genes (RAGs) are considered to be derived from Class II TEs (Huang et al., 2016). This statement is made on the basis that the structural domains and biochemistry of RAG action are strikingly similar to that of Class II TEs (Carmona and Schatz, 2017). In this review, we discuss the contribution of TEs in the evolution of V(D)J recombination, present structural and functional comparisons between transposase and V(D)J recombinase, put forth several unanswered questions, and finally discuss how this knowledge may aid treatment of immune disorders.

NEGATIVE AND POSITIVE EFFECTS OF TRANSPOSABLE ELEMENT ACTIVITY

Unconstrained TEs have been linked to many human diseases. A common origin for tumorigenic activity is by transposons hopping into essential genes, activating proto-oncogenes, or inactivating tumor suppressor genes. In this way, the new insertion of TEs can impact regulatory regions, altering or disrupting gene function and spatiotemporal dynamics. Another possibility is that ectopic recombination can lead to duplications, deletions, or insertions, all of which cause genomic instability (Economou-Pachnis and Tsichlis, 1985; Meischl and Roos, 1998; Meischl et al., 2000; Claverie-Martin et al., 2005; Burns and Boeke, 2012; Tarallo et al., 2012; Li et al., 2013; Burns, 2017; Guo et al., 2018; Ardeljan et al., 2020; Rodriguez-Martin et al., 2020). Further, while it was thought transposons are active only in the germ line with exceptions in the brain (Muotri et al., 2005; Goodier, 2014), next generation sequencing has demonstrated that retrotransposons can be reactivated in somatic tissues under pathological conditions (Hancks and Kazazian, 2016).

Reports of TE mobilization are not just confined to humans (Bourque et al., 2018). *Mus musculus*, *Danio rerio*, *Drosophila melanogaster*, *Escherichia coli,* and *Arabidopsis thaliana* (Huang et al., 2012; Moschetti et al., 2020; Chang et al., 2022), among other widely studied organisms, are known to have TE activity with both detrimental and beneficial functions. However, some TE functions have arisen uniquely for certain species over the course of evolution. Adaptive immunity, a crucial function that arose through TE co-option, is restricted in its species distribution (Mitra et al., 2013; Morales Poole et al., 2017).

In mammals, DNA transposons are generally not active, excepting a few species (Mitra et al., 2013; Gallus et al., 2015). In humans, DNA transposons occupy almost 3% of the genome and are inert and fossilized (Lander et al., 2001). By comparison, retroelements are more active in normal physiology. Out of 500,000 copies of L1, the only autonomously active Class I TE, around 100 are active in humans, and are associated with 124 disease-causing insertions (Hancks and Kazazian, 2012). L1 contains two open reading frames that encode ORF1p and ORF2p, both of which are required for retrotransposition. In addition to its own retrotransposition, ORF2p-encoded reverse transcriptase can also be hijacked by other RNAs in *trans* (*e.g.*, SINEs-Alu) to complete their retrotransposition activity (Stenz, 2021). These retroelements are controlled by the host at multiple stages of the transcription cycle, through transcriptional repression, RNA degradation, and other mechanisms (Bourque et al., 2018).

Since DNA transposons are not endogenously active in humans, their dysregulated activity is not known to be associated with disease; as retroelements are active, their disease associations are much clearer. Hemophilia was the first human disease shown to be caused by a *de novo* L1 insertion (Kazazian et al., 1988). Since this initial report, studies have elucidated similar disease-causing roles for retrotransposons in a wide range of disorders. For example, ORF1p has RNA chaperone activities (Martin, 2010), and its expression has been documented in various cancers. In specific, L1 integration into the adenomatous polyposis coli (*APC*) gene stratifies with colon cancer, and demethylation of a LINE promoter causes constitutive activation of the MET protein in bladder cancer (Ogino et al., 2008; Wolff et al., 2010). Further, L1 elements can evade genomic DNA methylation, enabling them to contribute new L1 insertions (Burns and Boeke, 2012; Hancks and Kazazian, 2016; Burns, 2017; Rodriguez-Martin et al., 2020). Moreover, a wide spectrum of neurodegenerative diseases also involve TEs (Saleh et al., 2019; Tam et al., 2019).

Despite their dangerous effects when unchecked, several TEs have become co-opted into the human genome over time. One explanation for the gradual loss of deleterious activity is that TEs were modified during domestication and lost their ability to autonomously transpose. In fact, several retroviruses have been repurposed into protein-coding genes and small RNAs (**Figure 2A**), providing important cellular functions. There are many examples of TE co-option and domestication, a few of which we present below. (i) Retrovirus-derived sequences modulate genetic circuits in embryonic development (Gerdes et al., 2016; Chuong, 2018). ERV gag-pol functions as regulators during placental development (Fu et al., 2019; **Figure 2B**). (ii) ERVs provide transcription regulatory regions for interferon genes. (iii) L1 retroelement mobilization in the brain is thought to diversify neuronal cell populations (Muotri et al., 2005; Coufal et al., 2009; Baillie et al., 2011). (iv) The neuronal gene *arc,* derived from gag proteins of the Ty3/gypsy retrotransposon, mediates synaptic plasticity (Pastuzyn et al., 2018; **Figure 2C**). (v) TEs affect the expression of genes at both transcriptional and post-transcriptional levels by providing alternative promoters or small RNAs (Chuong et al., 2016; Petri et al., 2019). (vi) TEs provide *cis*-regulatory sequences, which alter gene expression by acting as enhancers (Bejerano et al., 2006; Chuong et al., 2013, 2016), providing transcription factor binding sites (Wang et al., 2007; Sundaram et al., 2014; Ito et al., 2017), or acting as insulators (Bourque et al., 2008; **Figure 2D**). (vii) RAGs, derived from the protoRAG DNA transposon, catalyze V(D)J recombination in the vertebrate immune system (**Figure 2E**; Hencken et al., 2012). This allows for the generation of a vast repertoire of antigen binding receptors in lymphocytes.

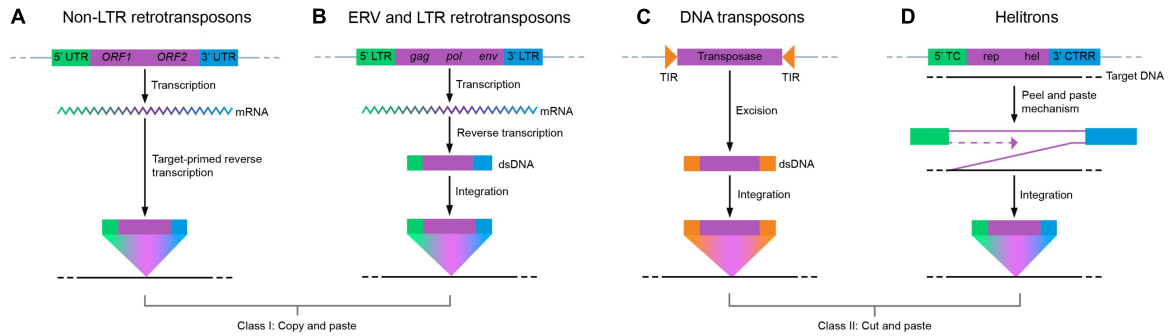

FIGURE 1 | Mechanism of action of the two classes of transposons. Class I TEs mobilize through an RNA intermediate. They can be further divided into two subcategories: non-LTRs (long terminal repeats) and LTRs/ERVs (endogenous retroviruses). **(A)** A prominent example of a non-LTR retrotransposon is LINE1/L1 (long interspersed nuclear element 1), which uses the process of target-primed reverse transcription carried out by the self-encoded proteins ORF1p and ORF2p. This process involves a nick in the target DNA by an endonuclease followed by reverse transcription and integration, both of which are enabled by the enzymatic activities of ORF2p. **(B)** ERV and LTR retrotransposons are flanked by LTRs and utilize a double-stranded DNA intermediate (dsDNA) prior to integration into the target site. Class II TEs require excision of genomic DNA with the help of self-encoded transposases. Subsequently, transposases help insert the excised DNA into a target site. **(C)** DNA transposons excise at the TIR, which is subsequently inserted as dsDNA in the target site. **(D)** Helitrons transpose by the rolling circle mechanism, involving strand invasion and DNA replication, without excision of the donor transposon DNA.

DNA TRANSPOSONS: A SUBSTRATE FOR THE EVOLUTION OF VERTEBRATE ADAPTIVE IMMUNITY

The appearance of V(D)J recombination in jawed vertebrates was a pathbreaking step in the evolution of the adaptive immune system (Jones and Gellert, 2004; Cooper and Alder, 2006). Crystallography and electron microscopy studies suggest that the transposase enzyme is the evolutionary progenitor of RAG1 and RAG2 (Liu et al., 2019). Functionally, RAG recombinases initiate V(D)J recombination in developing lymphocytes by generating DNA double stranded breaks (DSBs) at Recombination Signal Sequences (RSSs) (Schatz and Swanson, 2011), which are thought to have evolved from the terminal inverted repeats (TIRs) of Transib transposons (Huang et al., 2016). During V(D)J recombination, the 12 RSS and 23 RSS pair into a synaptic complex with RAG, which catalyzes nick formation at the RSSs and enables DSB formation (van Gent et al., 1996). Further, the endonuclease activity of RAG1/RAG2 utilizes RNase H-mediated *trans*-esterification chemistry that is shared with transposases. Such structural comparisons between DNA transposons and the V(D)J recombination machinery are summarized in **Figure 3**. Notably, unlike the cut-and-paste mechanism of DNA transposition, V(D)J recombination circularizes the ends of the excised segments in the signal joint to protect the genome against hazardous insertions.

Transib and protoRAG from *Branchiostoma belcheri* (common Lancelet) (Huang et al., 2016) are considered to be the two most promising RAG TE precursor candidates. Based on structural and functional analysis, Transib is an ancient evolutionary ancestor to RAGs, while protoRAG is a proximal ancestor with more features in common with RAG. While Transib and protoRAG both contain TIRs that are similar to RSSs, only protoRAG genes are homologous to both *RAG1* and *RAG2* and encode proteins with structural and functional similarities to vertebrate RAG.

REGULATION OF V(D)J RECOMBINATION

One of the drawbacks of the generation of a large repertoire of lymphocyte receptors with small energy investment in germline coding capacity is the generation of DNA DSBs. Therefore, to safeguard the genome and ensure that recombination is tightly restricted by cell type and developmental stage, multiple layers of DNA repair and regulation have evolved. In this section, we review important mechanisms that have evolved to regulate the activity of recombinases to preserve genomic integrity in adaptive immune system function.

One key characteristic of RAG function is its highly ordered catalysis. V(D)J recombination of immunoglobulin and T cell receptor (TCR) genes during B and T cell development requires specific recognition of the 12/23 asymmetric RSS by the RAG1 endonuclease. The RAG2 protein assists RAG1 in DNA binding and cleavage (Liu et al., 2019). Since there are often many V, D, and J domains to choose from, permutation of the V(D)J sequences results in diverse B and T cell receptors. These newly rearranged receptors then facilitate negative and positive selection (Klein et al., 2014). The elegant orchestration of these highly regulated processes culminates in a mature immune repertoire.

Complete antigen receptor production involves two sequential gene segment rearrangements, one for each receptor chain locus. The immunoglobulin heavy chain and TCR β-chain loci are the first to be assembled in pre-B-cells and double negative thymocytes, respectively. Assembly occurs serially, with D to J rearrangement followed by V to DJ rearrangement. Following these steps, recombination of the immunoglobulin

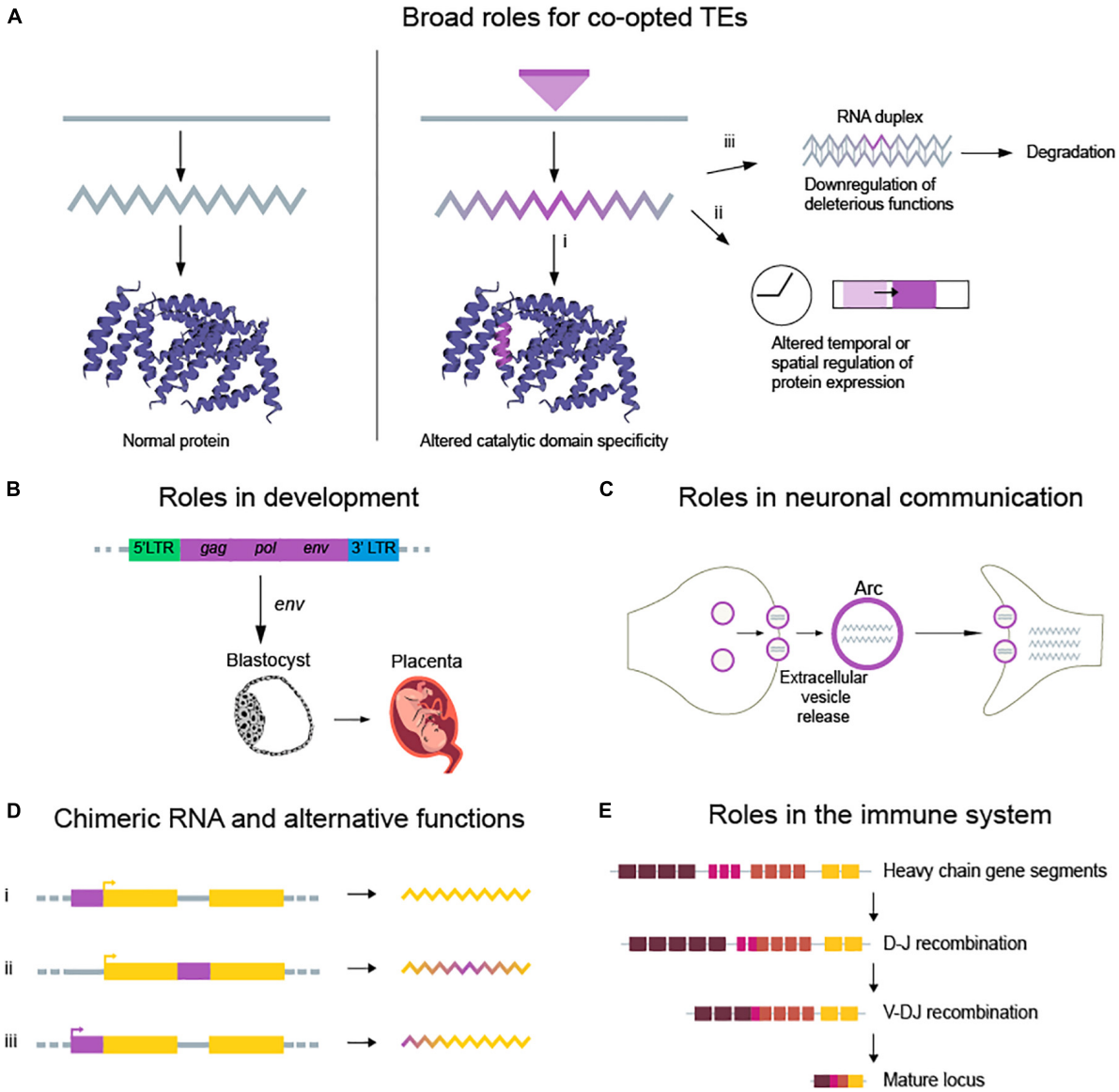

FIGURE 2 | Examples of beneficial TE functions that arose through co-option. **(A)** Beneficial TE functions can be broadly classified into three categories: introducing new gene functions, regulating existing functions, or downregulating negative functions. (i) New gene functions are typically introduced through co-option of TEs, repurposing natural cut/paste or copy/paste activities, which can result in new beneficial functions. (ii) Altered spatiotemporal gene expression occurs through insertion or modification of *cis*-regulatory elements, which can introduce binding sites for transcription and regulatory factors. (iii) Downregulation of deleterious genes can be achieved at transcriptional and post-transcriptional levels through small RNA hybridization and degradation of specific targets. **(B)** Upregulation of ERVs during embryonic development provides *cis* and *trans* elements for the expression of genes involved in pluripotency. **(C)** The Arc protein, derived from the gag protein of retrotransposons, forms capsid-like structures that deliver mRNA during neuronal communication. **(D)** TEs regulate the expression of neighboring genes in various ways (i) by acting as enhancers, (ii) by modulating splicing junctions, and (iii) by functioning as promoters. **(E)** *RAG1* and *RAG2*, thought to be derived from TEs, mediate V(D)J recombination. This process generates the diverse repertoire of antigen receptors in immune cells.

light chain and TCR α-chain loci is initiated. The highly ordered nature of recombination events creates important checkpoints in lymphocyte development to identify the formation of non-functional receptors. DNA cleavage by RAG recombinases is also regulated by chromatin structure, *trans* factors, the spatial localization of the antigen receptor loci, and transcription factors. *Cis* elements, such as promoters and enhancers, precisely direct transcription of V(D)J loci by interacting with transcription factors. Enhancers and promoters are so essential in this process that their deletion causes a complete block in recombination events and a loss of germline transcription. Further, transcriptional control restricts the expression of RAGs to the progenitor stages of B and T cell development, thereby ensuring that V(D)J recombination occurs solely in developing B and T lymphocytes (Gellert, 2002; Krangel, 2003; Schlissel, 2003; Del Blanco et al., 2011).

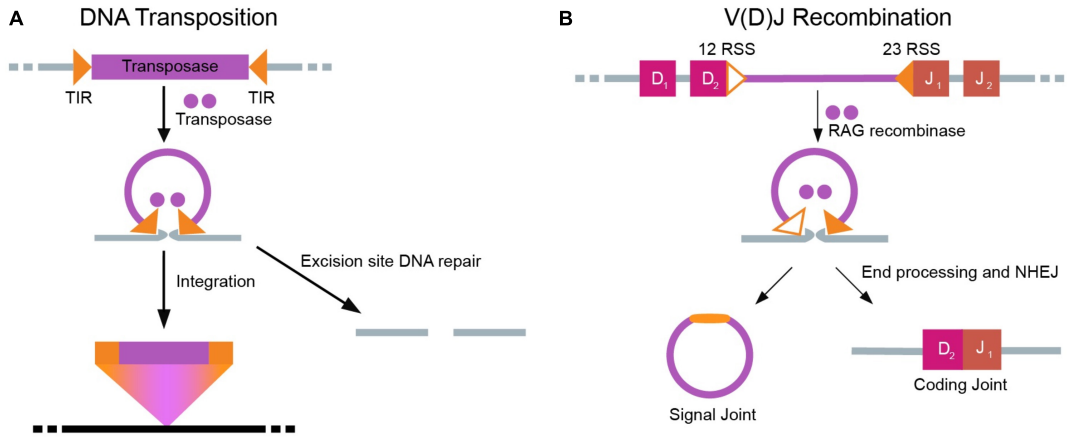

FIGURE 3 | Parallel mechanisms of DNA transposition and V(D)J recombination. **(A)** Self-encoded transposases excise a DNA transposon, including flanking terminal inverted repeats (TIRs). Subsequently, the transposon inserts into a new DNA sequence. The excision site must now be repaired through host DNA repair machinery. **(B)** In V(D)J recombination, V, D, and J segments are separated by intervening recombination signal sequences (RSSs) with similarity to transposons TIRs. RAG1/RAG2 recognize the RSS using the 12–23 rule, enabling cleavage of the intervening segment. The two ends of DNA that are separated (D and J ends as shown here) are subsequently joined through end processing and NHEJ to form the signal and coding joint.

DNA TRANSPOSASE PRECEDES RECOMBINATION ACTIVATION GENE RECOMBINASE: STRUCTURAL AND FUNCTIONAL JUSTIFICATIONS

The dominant hypothesis for RAG evolution explains the origin of adaptive immunity through the domestication and co-option of the protoRAG transposon. This hypothesis gained support from converging lines of evidence, including the *in vitro* ability of RAGs to mediate efficient transposition of RSS-flanked DNA insertions (Agrawal et al., 1998; Hiom et al., 1998). Structurally, the ProtoRAG DNA transposon contains BbRAG1L (homologous to RAG1) and BbRAG2L (homologous to RAG2). RAG1 contains several key domains, including an N-terminal RING/Zn finger, nonamer binding domain, and a catalytic core (Kim et al., 2015). BbRAG1L retains sequence similarity with the core domain of RAG1 (Carmona and Schatz, 2017). Importantly, the nonamer binding domain of RAG replaced the general DNA-binding domain of BbRAG1L (Zhang et al., 2019), suggesting that as the transposon was co-opted its function was gradually constrained. Vertebrate RAG2 is composed of a core domain (six Kelch beta-stranded motifs that together form a beta-propeller), an acid hinge, and a plant homeodomain (PHD) finger (Carmona and Schatz, 2017). BbRAG2L retains some similarity to the core domain but not the PHD finger of RAG2. The PHD confers the novel ability to read histone post-translational modifications to regulate gene expression (Jain et al., 2020), explaining the increased regulation of vertebrate RAG2. Structural comparisons cannot be complete without a discussion of substrates, and in fact it was the similarity between TIRs flanking DNA transposons and RSSs that prompted Tonegawa et al. to initially propose that RAGs originated by TE domestication (Sakano et al., 1979; Tonegawa, 1983; Fugmann, 2010; Huang et al., 2016). A good visualization of structural comparisons between protoRAG and RAG can be found in Zhang et al. (2019).

In addition to structural parallels, functional parallels exist between the two machineries. A key functional similarity between protoRAG and RAG is their end product (Huang et al., 2016). The cleaved TIR ends that are generated from protoRAG-induced DNA cleavage can undergo transposition or be joined together to form structures resembling the signal joints formed during V(D)J recombination. The non-TIR ends generated by the same mechanism resemble the coding joints formed during V(D)J recombination (Carmona and Schatz, 2017). Analysis of functional conservation lends further support to the notion that the co-option of protoRAG has added restrictions on the typically deleterious effects of unchecked transposition (Etchegaray et al., 2021). In particular, RAG-specific coupled cleavage activity, adherence to the 12/23 rule, and suppressed transposition, all impose restraints on the full extent of RAG action in vertebrate adaptive immunity (Zhang et al., 2019). These descriptions of structural and functional similarities, and the tighter regulation of RAG function, highlight that the evolutionary process of co-option was involved in the vertebrate acquisition of V(D)J recombination.

DNA REPAIR IN V(D)J RECOMBINATION AND TRANSPOSITION: HOW ARE THEY SIMILAR?

Since the core functionality of transposition and RAG-mediated recombination depends on the generation of (often double-stranded) chromosomal breaks, DNA repair mechanisms are essential at the excision site (Schlissel et al., 1993; Hedges and Deininger, 2007; Helmink and Sleckman, 2012). Transposon-induced breaks and RAG-induced breaks are different regarding how each is repaired; there are two pathways responsible for the repair of transposon-induced breaks, but only one pathway for the repair of RAG-induced

breaks (Izsvak et al., 2004; Munoz-Lopez and Garcia-Perez, 2010). Transposons have two options for DNA repair: (i) non-homologous end joining (NHEJ), which closes DSBs without great specificity, and (ii) homologous recombination (HR), which typically copies information present on the sister chromatid to fill the break (**Figure 4A**). In case either of these mechanisms is inhibited, the other can usually take its place. In contrast, the RAG machinery relies exclusively on NHEJ to repair DSBs (Lee et al., 2004; **Figure 4B**). This reliance on NHEJ facilitates the generation of a large assortment of B and T cell receptors. Use of the HR machinery would dampen this diversity. Irrespective of the cause of DSBs, both transposons and RAGs rely on the host's repair pathways to fix these breaks (Izsvak et al., 2004).

In greater detail, NHEJ typically involves DNA-dependent protein kinase (DNA-PK), DNA ligase IV, and X-ray repair cross-complementing protein 4 (XRCC4) (Drouet et al., 2005). DNA-PK is composed of a catalytic subunit (PKcs) and a Ku heterodimer that binds specifically to free DNA ends (Gottlieb and Jackson, 1993). Once the heterodimer binds to a DSB, it recruits other protein factors crucial for NHEJ. These factors subsequently phosphorylate various proteins in the NHEJ pathway, some of which are involved in the stability of the DNA complex, and others in end processing. DNA end processing occurs by various proteins depending on the nature of the break. In the case of lymphocytes, and therefore relevant to the RAG machinery, terminal deoxynucleotidyl transferase (Tdt) and the Artemis endonuclease add/remove nucleotides from DNA ends (Davis and Chen, 2013). Once end processing is complete, DNA ligase IV and XRCC4 (a scaffold protein) seal the breaks. Specific to the RAG machinery, these proteins operate by joining coding and signal ends; XRCC4 is strictly required for coding end joining, but signal end joining can proceed without it in some cases (Roy et al., 2012). Often, the steps are flexible and depend on the nature of the DSB, although the recruitment of the Ku heterodimer is a crucial first step (Helmink and Sleckman, 2012; Davis and Chen, 2013).

To repair breaks caused by DNA transposons, HR is also an option. HR strongly relies on Rad51 for appropriate functioning. This process involves trimming the DSB to a 3' overhang, after which Rad51 binds to the DNA, in a process termed pre-synapsis. Pre-synapsis is followed by synapsis, during which junctions form, eventually yielding a D-loop intermediate. From here, pathways diverge as to how the strands are processed. However, all routes use a template (typically a sister chromatid or homologous chromosome) to "refill" the DSB (Li and Heyer, 2008; Shrivastav et al., 2008).

Since transposon-induced DSBs can be repaired using either NHEJ or HR, a decision must be made on which pathway to use. Between different transposons, differences exist regarding how each one prefers to close DSBs. For example, the *Sleeping Beauty* transposon excision site is repaired using NHEJ factors (including Ku) and ataxia-telangiectasia mutated (ATM) (a protein kinase with roles in DSB signaling). HR is used to close the break only in the absence of both Ku and ATM (Izsvak et al., 2004; Brandsma and Gent, 2012). Another common transposon is *Drosophila* P-elements, whose excision site strongly prefers HR for DSB repair (Gloor et al., 2000). This process is probably to avoid deleterious and/or non-templated mutations in the germline. NHEJ remains the dominant process by which breaks are repaired in mammalian somatic cells (Ochmann and Ivics, 2021).

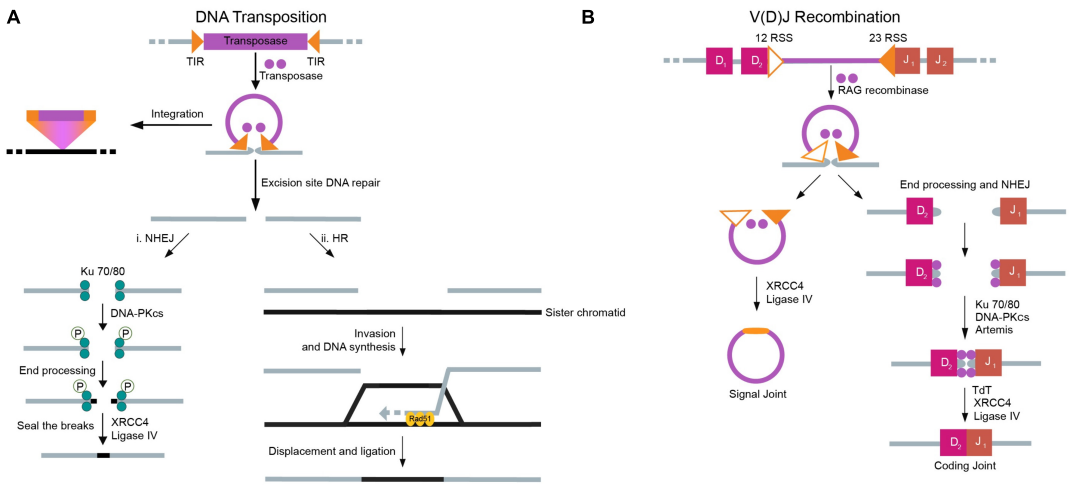

FIGURE 4 | Mechanisms of double-strand break repair for DNA transposition and V(D)J recombination. DNA repair occurs to seal double stranded breaks (DSBs) created in the donor strand due to DNA transposition and V(D)J recombination. **(A)** In DNA transposition, once the transposon has been excised, the DSB can be repaired using either NHEJ or HR, with preference generally given to NHEJ. (i) In NHEJ, the process starts with the binding of the Ku 70/80 heterodimer to the broken DNA ends. A phosphorylation cascade initiated by DNA PKcs results in the recruitment of various proteins in the NHEJ pathway that are responsible for both the stability of the DNA complex and end processing. Finally, DNA ligase IV and XRCC4 act to seal the breaks in the DNA strand. (ii) In the case of HR, a sister chromatid or homologous chromosome serves as a template to repair the DSB. The process is contingent on the DNA-binding activity of Rad51. After replication based on the template strand, the donor DNA reseals. **(B)** In V(D)J recombination, the DSB must be repaired using NHEJ. The NHEJ process in V(D)J recombination generally involves the same components as are used in the repair of DSBs caused by transposons. Proteins specific to the V(D)J recombination process are highlighted.

Just as a balance must be obtained between TE activity and genomic integrity, a balance must be struck between NHEJ and HR to repair DSBs. There are two reasons why NHEJ might be preferred over HR for non-co-opted TEs: (1) generation of increased diversity through error-prone joining, and (2) an increase in efficiency. Since NHEJ is inherently non-templated, there is potential for greater genetic diversity (Weterings and Chen, 2008), thereby greater functional diversity in the target sequence after insertion. Since transposons are evolutionarily thought of as parasitic, increased TE diversity maximizes their survival fitness. NHEJ also places greater emphasis on speed (Mao et al., 2008), trading quality for rapid sealing of potentially deleterious DSBs.

Since the priority for co-opted TEs (potentially including *Drosophila* P-elements that function in the fly germ line) is non-hazardous TE function, co-opted TEs have special requirements to preserve DNA. These TEs would only be selected for if the insertions were beneficial and did not damage the host, underscoring the preference for HR over NHEJ in situations of exaptation. It would be interesting to further dissect the possibility of the co-option of P-elements in *Drosophila* germline.

However, the emergence of adaptive immunity further complicates the discussion (Cui and Meek, 2007). The critical need for an increased diversity of antigen receptors prompted the proto-RAG induced DSBs to choose NHEJ over HR. Mechanistically, one dominant hypothesis for why NHEJ is exclusively used is that the post-cleavage complex (composed of RAG proteins, DNA signal ends, and blunt DNA hairpin coding ends) shepherds the generated signal ends. The RAG proteins disallow signal ends from accessing the HR pathway by holding on to the ends rather than allowing for their early release (Lee et al., 2004). Further, V(D)J recombination is restricted to the G1 phase of the cell cycle, as the RAG2 enzyme that is necessary for the process is destroyed during the G1-S transition (Li et al., 1996). While NHEJ can happen during the G1 phase (Takata et al., 1998), HR cannot occur efficiently during G1 due to the lack of a sister chromatid (Johnson and Jasin, 2000). As such, only NHEJ is observed to repair V(D)J recombination-induced breaks.

UNANSWERED QUESTIONS AND FUTURE DIRECTIONS

In searching for whether and how V(D)J recombination retains structural and functional parallels to TEs, we looked at other processes necessary for adaptive immunity that involve DNA modifications: N/P-nucleotide addition (addition of palindromic sequences and non-genomic nucleotide sequences in the junctions between V, D and J gene segments of the immunoglobulins and T cells), somatic hypermutation, and isotype class switching. For each process, we reviewed the nature of the DNA modification to determine whether it might have originated from TE co-option.

Based on current knowledge, it appears that none of these processes have a proximal TE comparison. N/P-nucleotide addition generates diversity in joining regions between different domains of B-cell and T-cell receptors (Meier and Lewis, 1993; Repasky et al., 2004). This process involves NHEJ DNA repair through the action of Tdt, but no excision and repair steps directly resembling TEs (Repasky et al., 2004; Sandor et al., 2004). For further details on how N/P-nucleotide addition generates diversity see Srivastava and Robins (2012). Somatic hypermutation involves several factors that form a complex responsible for single nucleotide replacement to generate antibody diversity. This process, initiated by the targeting of cytidine deaminase to newly rearranged genes, does not involve excision and repair analogous to V(D)J recombination or TE action (Pilzecker and Jacobs, 2019). Isotype class switching is the process by which IgG, IgE, and IgA antibodies can be produced by mature B-cells. This process requires excision and repair, with the intervening constant domains deleted, which is quite similar to V(D)J recombination (Stavnezer et al., 2008). However, the complex structurally lacks specific recognition sites flanking the constant domains, and endonucleases perform a large portion of the cutting, splicing, and repair activity (Xu et al., 2014). As such, it is conceivable that V(D)J recombination stands on its own, uniquely having TE predecessors in basal organisms that were co-opted in jawed vertebrates over the course of evolution.

In writing this review, we have encountered numerous questions spanning several domains that warrant further study. These selected questions may prompt future studies on TEs and immunological processes:

- TEs are known to have a bias (Cutter et al., 2005; Green et al., 2012). Does the sequence of a TE contain a code for bias? If so, how can it be manipulated?
- As discussed previously, there are several protein components involved in appropriate NHEJ DSB repair. Which of these components are necessary and/or sufficient for DNA repair after transposition and V(D)J recombination? Does manipulation of these components influence the efficiency or functioning of TEs or RAGs?
- Can we repurpose transposase inhibitors as solutions to RAG-related immune dysregulation? More specifically, can we repurpose HIV reverse transcriptase inhibitors as therapeutic solutions to lymphoblastic leukemia and related cancers?
- Experimentally, is it possible to determine the conditions under which TEs will preferentially repair DSBs using HR over NHEJ?
- p53, a *trans*-repressor of transposons (Tiwari et al., 2017, 2018, 2020), regulates the DNA double strand break response. How might p53 and DNA transposons have interacted during the co-option of RAG recombinase?
- As this review has mentioned, studying how V(D)J recombination has arisen from TE co-option suggests that RAG predecessors have become increasingly regulated in the course of evolution. How can this knowledge of possible RAG origins be applied to human health and disease?

To this final question, our knowledge of TEs may be harnessed for therapeutic potential. For instance, many

diseases result from RAG mutations. In cases of immune deficiency, TEs synthetically targeted to the RAG locus might restore RAG recombinase function, thereby ameliorating immune system deficits. Another value in considering these lines of questioning is their contribution to basic science. Generating transgenic organisms has long been time-consuming and fraught with failure. Understanding and manipulating the insertion biases of TEs can generate tools to increase the efficiency of generating transgenic organisms. Collectively, the study of how TEs and immunological processes intersect sheds light on many unexplored areas and offers exciting possibilities for advancing basic science and therapeutics.

AUTHOR CONTRIBUTIONS

RS, NK, NC, and BT drafted and edited the manuscript. All the authors approved the final version.

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