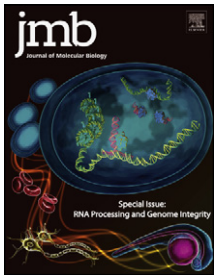

RNA Exosome and Non-coding RNA-Coupled Mechanisms in AID-Mediated Genomic Alterations

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Abstract

The eukaryotic RNA exosome is a well-conserved protein complex with ribonuclease activity implicated in RNA metabolism. Various families of non-coding RNAs have been identified as substrates of the complex, underscoring its role as a non-coding RNA processing/degradation unit. However, the role of RNA exosome and its RNA processing activity on DNA mutagenesis/alteration events have not been investigated until recently. B lymphocytes use two DNA alteration mechanisms, class switch recombination (CSR) and somatic hypermutation (SHM), to re-engineer their antibody gene expressing loci until a tailored antibody gene for a specific antigen is satisfactorily generated. CSR and SHM require the essential activity of the DNA activation-induced cytidine deaminase (AID). Causing collateral damage to the B-cell genome during CSR and SHM, AID induces unwanted (and sometimes oncogenic) mutations at numerous non-immunoglobulin gene sequences. Recent studies have revealed that AID's DNA mutator activity is regulated by the RNA exosome complex, thus providing an example of a mechanism that relates DNA mutagenesis to RNA processing. Here, we review the emergent functions of RNA exosome during CSR, SHM, and other chromosomal alterations in B cells, and discuss implications relevant to mechanisms that maintain B-cell genomic integrity.

Introduction

Genomic integrity is crucial for cell homeostasis and several mechanisms converge to detect and repair genomes to avoid deleterious effects [1]. Mutagenic agents can be environmental (UV, chemical, viral, etc.) or endogenous (ROS, replication error, etc.); importantly, transcription itself can induce genome instability by opening chromatin and exposing single-strand DNA (ssDNA) [2]. The immune system negates a potentially infinite number of pathogens *via* mechanisms requiring tailor-made receptors and antibody molecules that recognize pathogen-derived antigens (Ag) with high specificity. However, the number of genes is limited and vastly disproportionate to the number of encoded receptors. Evolution has selected elegant recombination mechanisms to generate Ag receptor diversity from a limited set of genes. In mammals, the first mechanism to create Ag receptor diversity is common to both types of T and B lymphocytes; occurs in the thymus "T" and bone marrow "B" respectively; and is called V(D)J recombination. During V(D)J recombination, one variable (V), eventually one diversity (D), and one junction (J) genes are randomly assembled by recombination activating genes (RAG) recombinases to shape the Ag binding site and a first Ag receptor repertoire [3]. These recombination steps are tightly regulated by numerous criteria including loci nuclear localization [4], transcription factor- and architectural protein-induced chromosomal looping which allows promoter/enhancer interactions and synapsis between recombination sequences [5], chromatin remodeling [6], and DNA demethylation [7]. Globally, these changes induce accessible and transcribed chromatin during VDJ recombination in B lymphocytes [8–13]. As B-cell development continues, B cells traverse to secondary lymphoid organs formed in the spleen, tonsil, or gut and undergo a second round of immunoglobulin (Ig) gene diversification steps that depends on the expression of the activation-induced cytidine deaminase (AID)

protein [14–16]. This enzyme initiates mutations and double-strand breaks (DSBs) for somatic hypermutation (SHM) and class switch recombination (CSR) processes, respectively, vastly increasing the diversity of B-cell Ag receptors (BCR) and antibodies. Again, several layers of regulation ensure spatial and temporal specificity of these recombination steps, now implying the RNA exosome as a master regulator.

SHM, CSR, and B-cell fate

Following V(D)J recombination, B cells express a membrane IgM molecule, associated with Igα and Igβ signaling subunits to form the BCR. Transcription initiates from the V promoters to polyadenylation sites of constant region exon(s); thereafter, RNA splicing joins VDJ to IgM (and IgD in immature B cells) exons or VJ to an IgL constant exon for IgH and IgL mature transcripts, respectively. Ig chains are then translated and associate as double heterodimer proteins, with the VDJ and VJ associated to form the variable region, creating an Ag binding site of predefined specificity, while the IgH constant region allows recruitment of specific immune system actors (Fig. 1, top). Antigen binding to the BCR, in coordination with co-stimulatory receptors, induces signaling, B-cell proliferation, and activation that eventually lead to AID expression. AID introduces mutations at VDJ and VJ genes leading to SHM, and a germinal center-based process leads to selection of somatically mutated B-cell clones that have the highest affinity for the Ag, allowing affinity maturation (Fig. 1, middle).

These cells can also undergo CSR after AID activity on switch donor (Sμ) and switch acceptor regions (Sx), leading to DNA DSBs. For example, a Th2-orientated immune response induces CSR from IgM to IgG1 to produce high-affinity switched antibodies (Fig. 1, bottom). These IgG1 class-switched cells have the ability to undergo a second round of CSR to IgE, after re-exposure to Ag, to generate high-affinity IgE antibodies (Fig. 1, bottom), although cells can also directly switch from IgM to IgE to generate low-affinity IgE antibodies [17], these pathways resulting from a probabilistic transcription of Iγ and Iε non-coding transcripts [18]. Thus, from one V(D)J recombined clone, AID can generate many un-switched and switched sub-clones with increased Ag affinity, generating Ig diversity and optimal immune responses.

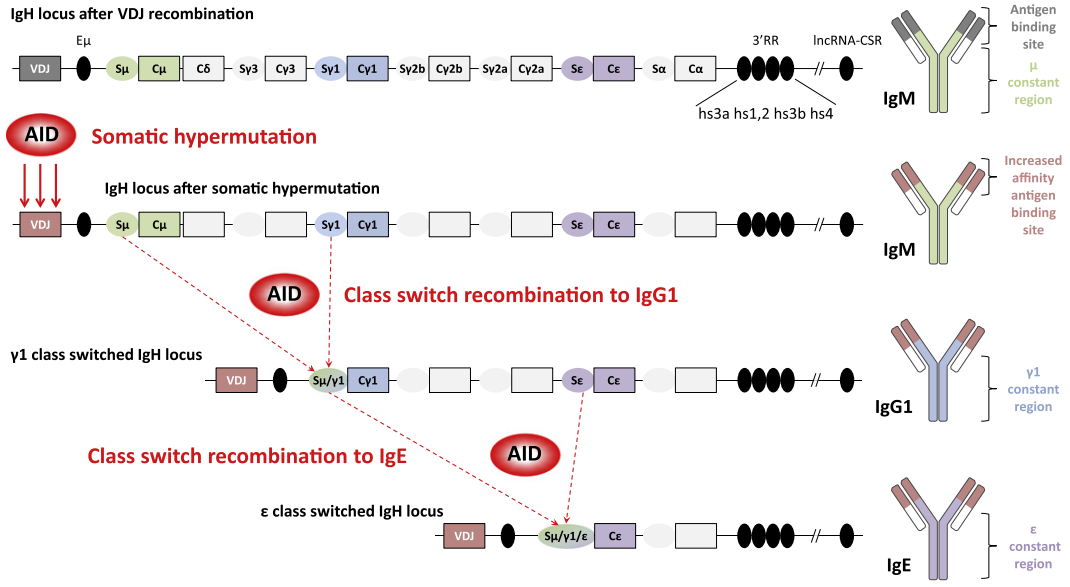

Fig. 1. Genomic organization of the IgH locus and immunoglobulin structure. Top: The mouse IgH locus is represented after VDJ recombination (not to scale). The recombined VDJ gene and constant (C) genes are represented as outlined boxes. Horizontally oriented ovals portray switch (S) regions (preceded by promoters and I exons, not shown) preceding each constant gene (excepted Cδ). Black ovals portray regulatory elements: intronic enhancer μ (Eμ), 3′RR, and the newly identified lncRNA-CSR region, located approximately 2.6 Mbp downstream of the 3′RR. The resulting immunoglobulin (Ig) protein is IgM, shown on the right. Middle: After B-cell activation, AID is expressed and induces its mutagenic activity on the VDJ gene, allowing production of IgM antibodies with increased affinity for the antigen. Then AID is targeted to S regions to initiate DSBs, thereby permitting recombination between two S regions, here Sμ and Sγ1. This process generates high-affinity IgG1 antibodies and a diversified B-cell repertoire. Bottom: Cells can re-express AID after re-exposure to antigen and undergo a second CSR event, from γ1 to ε here, to produce high-affinity IgE antibodies (direct class switching from μ to ε is also possible). All these AID-mediated events shape the Ig repertoire to improve antigen affinity and to adapt the Ig class for an optimal immune response.