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Title: How Z-DNA/RNA binding proteins shape homeostasis, inflammation, and immunity

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ABSTRACT

The right-handed double-helical structure of DNA (B-DNA), which follows the Watson-Crick model, is the canonical form of DNA existing in normal physiological settings. Even though an alternative left-handed structure of DNA (Z-DNA) was discovered in the late 1970s, Z-form nucleic acid has not received much attention from biologists, because it is extremely unstable under physiological conditions, has an ill-defined mechanism of its formation, and has obscure biological functions. The debate about the physiological relevance of Z-DNA was settled only after a class of proteins was found to potentially recognize the Z-form architecture of DNA. Interestingly, these Z-DNA binding proteins can bind not only the left-handed form of DNA but also the equivalent structure of RNA (Z-RNA). The Z-DNA/RNA binding proteins present from viruses to humans function as important regulators of biological processes. In particular, the proteins ADAR1 and ZBP1 are currently being extensively re-evaluated in the field to understand potential roles of the noncanonical Z-conformation of nucleic acids in host immune responses and human disease. Despite a growing body of evidence supporting the biological importance of Z-DNA/RNA, there remain many unanswered principal questions, such as when Z-form nucleic acids arise and how they signal to downstream pathways. Understanding Z-DNA/RNA and the sensors in different pathophysiological conditions will widen our view on the regulation of immune responses and open a new door of opportunity to develop novel types of immunomodulatory therapeutic possibilities.

INTRODUCTION

The well-known model of DNA, which was originally proposed by Watson and Crick, is a right-handed double-helical conformation of B-form DNA (B-DNA). Surprisingly, the first single-crystal structure study of DNA discovered that DNA adopted an unexpected zigzag shaped left-handed double helix (Z-DNA) instead of the established B-DNA (1). Later, it turned out that a DNA duplex with alternating purine-pyrimidine sequences such as poly(dG-dC) can stabilize the Z-DNA conformation under high salt concentrations (2). Since Z-DNA formation typically requires molar concentrations of salts, the significance of this structure under physiological conditions and its biological function has remained elusive.

In attempts to search for potential functions of Z-DNA in a biological system, double-stranded RNA-specific adenosine deaminase 1 (ADAR1) was identified as the first protein that contains a Z-DNA-binding domain (Zα) (3). Spectroscopic measurements revealed that when the Zα domain meets GC-repeated DNA, it can convert the B-DNA to Z-DNA and stabilize the left-handed conformation (4). Interestingly, structural studies revealed that Z-DNA binding to the Zα domain is specific not to a DNA sequence, but to the left-handed conformation (5, 6). Furthermore, the Z-DNA equivalent structure of RNA (Z-RNA) was found to bind to the Zα as well (6, 7). Soon after the Zα domain in ADAR1 was identified, sequence similarity search identified Z-DNA-binding protein 1 (ZBP1; also known as DLM1 and DAI) having similar motifs (8). In addition, a mammalian PKR-like kinase in fish, named PKZ, uses the Zα domain to promote immune responses (9). The Z-DNA/RNA-recognizing proteins exist not only in animals but also in certain viruses. Poxviruses, including vaccinia virus, are known to have the Zα domain bearing E3L protein (10). When the Zα domain of E3L was removed, the infection was attenuated, suggesting that Z-DNA/RNA binding activity is crucial for viral pathogenicity (10, 11). Taken together, the identification of a class of proteins that bind specifically to Z-DNA/RNA suggested that

noncanonical Z-form nucleic acids can have biological functions *in vivo*. In this mini-review, I discuss the recent advances in understanding the potential role of Z-form nucleic acid-recognizing proteins in cell death, inflammation, and immunity.

ADAR1

The ADAR1 gene expresses two isoforms of adenosine deaminases by taking advantage of its alternative promoters and start codons (12-14). The constitutively expressed short isoform, ADAR1p110, is mainly located in the nucleus, whereas the cytoplasm-predominant long isoform, ADAR1p150, is interferon (IFN)-inducible and shuttles between the nucleus and the cytoplasm (15, 16). Both ADAR1p110 and p150 isoforms share the deaminase domain, double-strand RNA-binding domains (RBD), and a putative Z-DNA-binding domain, Zβ. The isoform P150 has an extra Z-DNA/RNA-binding domain, Zα at its N-terminal end (17) (Fig. 1). Of note, despite having a similar amino-acid sequence and structural architecture with Zα, Zβ appears not to recognize Z-form nucleic acids (18). Currently, the function of Zβ is unclear.

A purine nucleoside, inosine, can be generated by deamination of adenosine. ADAR1 deaminates adenosine (A) to inosine (I) in dsRNA species, by employing its RBD and deaminase domain. Most of the A-to-I RNA editing by ADAR1 occurs within Alu elements embedded within mRNA (19). Mutations in the ADAR1 gene are implicated in Aicardi-Goutières syndrome (AGS), a fatal childhood autoimmune disease characterized by type I interferonopathies (20). Similar to human AGS, Adar1 KO and Adar1 deaminase-inactive mutant mice display embryonic lethality with high levels of expression of interferon-stimulated genes (ISG) (21-24). One of the antiviral defense pathways inducing IFN is a non-self dsRNA-recognition system that uses a sensor, MDA5, and an adaptor, MAVS, proteins. Ablation of MDA5 or MAVS rescues the embryonic lethality and the pathogenic type I IFN

production in Adar1 KO and Adar1 deaminase-inactive mutant animals (24, 25). Therefore, ADAR1-mediated RNA editing prevents MDA5-dependent recognition of self RNA and the following signal transduction to MAVS, suppressing uncontrolled IFN production (Fig. 2).

ADAR1p150-specific mutations are found in patients with dyschromatosis symmetrica hereditarian (DSH), which is characterized by irregular skin pigmentation (26). In contrast to mostly autosomal recessive AGS, DSH is an autosomal dominant disease. The mutations located on the upstream of the initiation codon of p110 generate early stop codons in ADAR1p150, but the expression of p110 is unaffected. DSH pathology seems to be determined by the insufficient level of Zα-containing ADAR1p150. In this regard, although ADAR1 has been studied mainly for its RBD and deaminase-mediated RNA editing, one can speculate that there is a Zα-specific function of ADAR1. Unfortunately, the role of the Zα domain in ADAR1p150 is currently ill-defined. One study suggests that Zα improves the RNA editing efficiency of ADAR1 when the target dsRNA has a Z-conformation-prone sequence proximal to A-to-I editing sequences (27). Also, the Zα domain was required for the localization to stress granules (SG), which is a transient cytoplasmic aggregate generated in response to translation-arresting cellular stresses (28). Since SG is mainly composed of mRNPs and certain RNA binding proteins, SG can interfere with viral replication by stopping the host translation machinery (29). Even though the physiological meaning of Zα-dependent localization to SG is unclear yet, SG-located ADAR1 may help resolve the aggregate by editing RNA duplexes produced by interactions between mRNAs (30).

ZBP1

Z-DNA binding protein 1(ZBP1) was initially found as a highly expressed gene in tumor stroma (31). Although one study proposed ZBP1 as a cytoplasmic DNA receptor activating type I interferon (IFN) and NF-κB signalling pathways, subsequent studies demonstrated that

ZBP1 was dispensable for cytosolic DNA-mediated host IFN responses (32-34). ZBP1 contains two N-terminal Zα domains, two RIP homotypic interaction motifs (RHIM), and one potential RHIM-like motif with a less-conserved core RHIM sequence (35). The RHIM provides a crucial interaction platform to establish necroptosis, which is a new form of caspase-independent programmed cell death (36) (Fig. 2). In mammals, RHIM is found in four proteins, Receptor Interacting Protein Kinase 1 (RIPK1), RIPK3, TIR-domain-containing adapter-inducing interferon-β (TRIF), and ZBP1 (37, 38). In contrast to apoptosis that does not result in inflammation, necroptosis is implicated in pathological inflammatory conditions. Upon necroptotic stimuli, particularly under conditions in which caspase-8 is inactive, RHIM-dependent protein interactions promote RIPK3-dependent phosphorylation of pseudokinase, Mixed Lineage Kinase Like (MLKL). The modification of MLKL drives its oligomerization, licensing MLKL to permeabilize cellular membranes (36).

Although Z-DNA and Z-RNA are potential ligands of ZBP1, it remains unknown where, when, and how Z-form nucleic acids are generated to activate ZBP1-dependent cellular responses. Nevertheless, ZBP1 was shown to be triggered in response to several viral infections, including murine cytomegalovirus (MCMV), herpes simplex viruses (HSV) 1, and influenza A virus (IAV) (39-43). Furthermore, two independent studies demonstrated that in RIPK1 KO and RIPK1 RHIM mutant animals, ZBP1 serves as a potent inducer of necroptosis in response to unidentified endogenous cellular stress (44, 45). Taken together, ZBP1 protein proposes the existence of a Z-DNA/RNA-dependent signalling pathway that is important for anti-viral immunity, tissue homeostasis, and inflammation.

Current understanding of ZBP1 is limited to its function of inducing cell death. Intriguingly, an unbiased screening identified ZBP1 as one of the most inhibitory proteins against multiple +ssRNA virus infections (46). Since the screening was done in necroptosis-resistant Hela cells, the result indicates that ZBP1 has unidentified anti-viral functions apart

from its role in necroptosis. Indeed, a recent study revealed that zika virus infection-activated ZBP1 restricted viral replication by changing cellular metabolism rather than by inducing necroptosis (47). Furthermore, some studies demonstrated that overexpression of ZBP1 boosted effective cytotoxic T lymphocytes and long-lasting antitumor immunity *in vivo*, suggesting its potential role in host-adaptive immunity (48, 49).

Future Perspectives

Though known for decades, our understanding about the relationship between Z-DNA/RNA and the sensors ADAR1 and ZBP1 is largely limited to their biochemical aspects. The physiological origin of Z-form nucleic acids and the pathological effects of sensing Z-DNA/RNA remain still enigmatic. Since both ADAR1 and ZBP1 are interferon-inducible proteins, the potential cross talk between these two proteins in sensing Z-DNA/RNA is highly anticipated. The two proteins may compete for occupying Z-nucleic acids, or they may exhibit synergistic effects to establish inflammatory signaling pathways.

While I was preparing this manuscript, two studies reported that endogenous retroelements-derived dsRNA may serve as the agonist of ZBP1 in specific mouse models of skin and intestinal inflammation (50, 51). Nonetheless, since experimental data supporting that retroelements are direct targets of the Zα domain are lacking in these studies, other possibilities should remain valid. In fact, another recent study showed that in the mouse prefrontal cortex, the Zα domain of ADAR1 recognizes Z-DNA in host genomes to control gene expression during fear learning (52).

An emerging body of evidence proposes that there is a largely unexplored Z-DNA/RNA-activated signal transduction pathway that plays a role in host immunity, tissue homeostasis, inflammation, and cancer. We are still at the beginning of searching for the origin of Z-form nucleic acids and their potential functions in various physiological

conditions. Understanding the biological roles of Z-DNA/RNA and the sensors ADAR1 and ZBP1 is fundamental to fully leverage their therapeutic potentials.

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CONFLICTS OF INTEREST

The author declares no conflict of interest.

FIGURE LEGENDS

Figure 1. Functional Domains of Z-DNA/RNA binding proteins

The two Zα domain-containing ZBP1 establishes cell death by interaction with RIPK1 and RIPK3 via its RHIM domains. The ADAR1p150 isoform comprises a Zα domain, a nonfunctional Zβ, three RBD (dsRNA binding domain), and a deaminase domain that edits dsRNA. E3L from vaccinia virus has one Zα domain required for viral pathogenesis. PKZ is a fish kinase-recognizing Z-nucleic acid. The physiological role of Zα in all these Z-DNA/RNA sensing proteins is still largely unknown.

Figure 2. Z-nucleic acid-dependent inflammatory signaling pathway

ADAR1p150 and ZBP1 seem to recognize stress-induced Z-conformational nucleic acids. Although it is not experimentally demonstrated yet, the potential interplay between ADAR1p150 and ZBP1 is expected since both proteins are interferon-inducible and equally recognize Z-DNA/RNA through their Zα domains. The double-headed arrow indicates the nucleocytoplasmic shuttling of ADAR1p150 and ZBP1. While Z-nucleic acid-triggered ZBP1-dependent cell death pathway is currently actively being investigated, Z-DNA/RNA-dependent function of ADAR1p150 is still largely unknown. Some viruses such as vaccinia virus (VACV) can prevent host Z-DNA/RNA sensing by their Zα domain-bearing E3L protein. The mechanisms of signaling pathways with dashed lines are less well-established. Even though RIPK1-dependent NF-κB activation is well-known, how Z-DNA/RNA-dependent ZBP1 complex signals to RIPK1 remains elusive.

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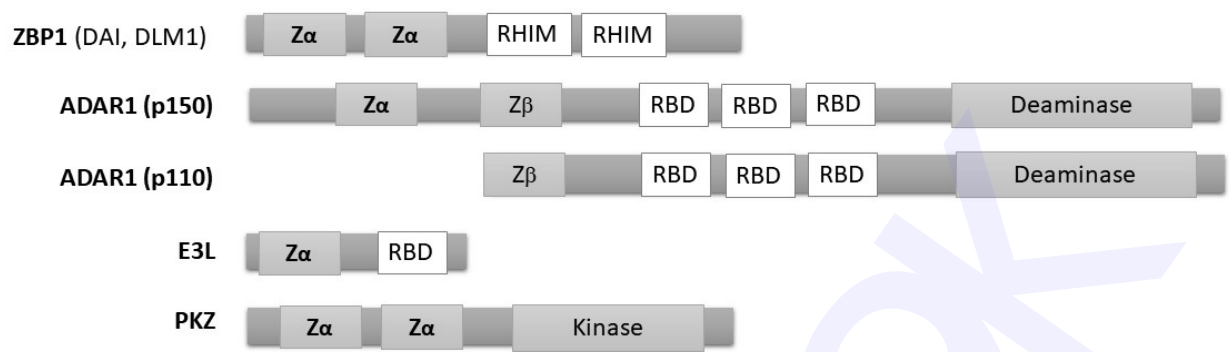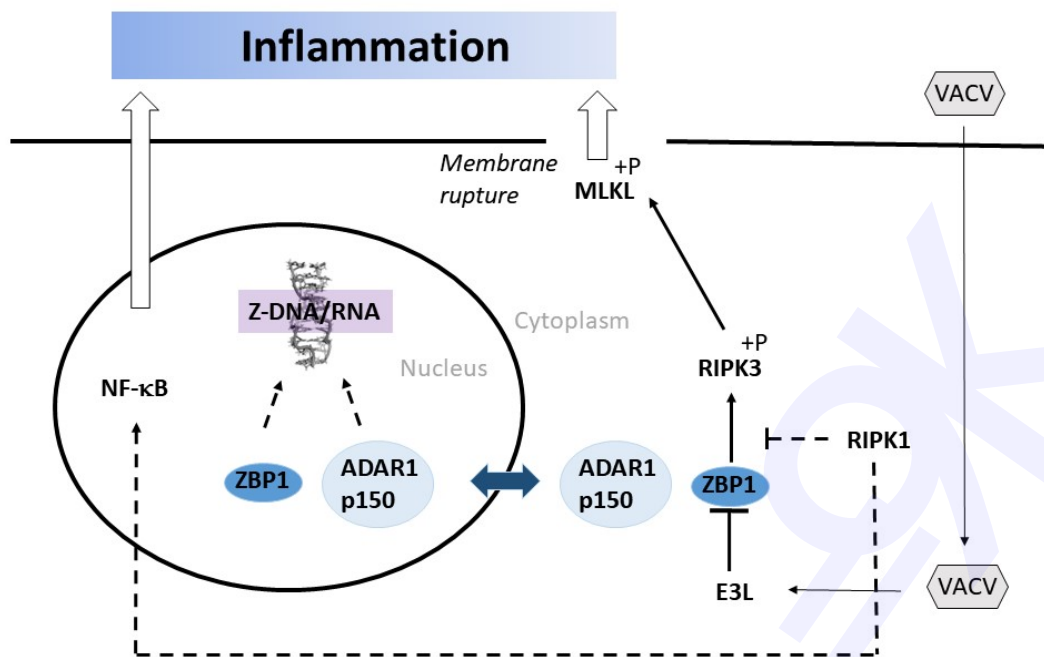
Fig. 1.

Fig. 2.