HHS Public Access

Author manuscript

DNA Repair (Amst). Author manuscript; available in PMC 2021 March 23.

Published in final edited form as:

DNA Repair (Amst). 2020 October ; 94: 102894. doi:10.1016/j.dnarep.2020.102894.

TREX1 – Apex predator of cytosolic DNA metabolism

Sean R. Simpson, **Wayne O. Hemphill**, **Teesha Hudson**, **Fred W. Perrino***

Department of Biochemistry, Center for Structural Biology, Wake Forest School of Medicine, Winston-Salem, NC 27157, United States

Abstract

The cytosolic Three prime Repair EXonuclease 1 (TREX1) is a powerful DNA-degrading enzyme required for clearing cytosolic DNA to prevent aberrant inflammation and autoimmunity. In the absence of TREX1 activity, cytosolic DNA pattern recognition receptors of the innate immune system are constitutively activated by undegraded TREX1 substrates. This triggers a chronic inflammatory response in humans expressing mutant *TREX1* alleles, eliciting a spectrum of rare autoimmune diseases dependent on the nature of the mutation. The precise origins of cytosolic DNA targeted by TREX1 continue to emerge, but DNA emerging from the nucleus or taken up by the cell could represent potential sources. In this Review, we explore the biochemical and immunological data supporting the role of TREX1 in suppressing cytosolic DNA sensing, and discuss the possibility that TREX1 may contribute to maintenance of genome integrity.

Keywords

TREX1; Cytosolic DNA metabolism; DNA sensing; Autoimmunity; Genome stability

1. Introduction

Infectious organisms utilize nucleic acid polymers to encode genetic information. DNA and RNA therefore represent excellent pathogen-associated molecular patterns (PAMPs) for innate immune recognition of microbial threats. Pattern recognition receptors (PRRs) have evolved to detect pathogen-derived nucleic acids within both the endosomal and cytosolic compartments. PRR ligation initiates signaling cascades leading to production of inflammatory signaling molecules, such as the type I interferons (IFN-I), which stimulate innate and adaptive immune responses against the perceived microbial threat (reviewed in [1]). DNA PRRs are generally not sequence-specific and provide broad detection coverage regardless of pathogen origin. This lack of specificity presents a potential danger, however. The innate immune system cannot distinguish self- from pathogen-derived DNA, so cells carefully manage and compartmentalize their own genetic material to prevent self-DNA sensing and aberrant immune activation. By sequestering DNA within the nucleus and

*Corresponding author at: Wake Forest Baptist Medical Center, Department of Biochemistry, 1 Medical Center Blvd, Winston-Salem, NC 27157, United States. fperrino@wakehealth.edu (F.W. Perrino).

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

mitochondria, cells establish the cytosol as a DNA-free space in which non-specific DNA-sensing molecules can be safely deployed for pathogen detection.

The Three-prime Repair EXonuclease 1 (TREX1) is required to maintain the cytosol as a DNA-free space. TREX1 is a potent 3′→5′ cytosolic exonuclease which rapidly degrades both double-stranded and single-stranded DNA (ds- and ssDNA) [2–5]. TREX1 was initially thought to participate in DNA repair, based on sequence and structural homology with bacterial DNA repair enzymes. However, *TREX1* ablation in mice demonstrated an aggressive, immune-activating phenotype and not a mutator phenotype [6]. *TREX1* mutations are now known to cause a spectrum of human autoimmune diseases associated with constitutive low-level IFN-I signaling in the absence of a microbial threat [7,8]. Murine models of TREX1 deficiency have demonstrated that the cyclic GMP-AMP Synthase - Stimulator of INterferon Genes (cGAS-STING) pathway of cytosolic DNA sensing is required for this inflammatory signaling [9,10], supporting a model in which TREX1 degrades cytosolic DNA to prevent inappropriate DNA PRR activation. TREX1 DNA substrates may arise from within the cell itself or from exogenous sources, but critically, must be cleared to maintain normal DNA compartmentalization and suppress persistent stimulation of cytoplasmic DNA sensors, inflammation, and autoimmunity.

The precise nature of TREX1 substrates continues to emerge, as do the specific molecular pathways of DNA sensing and inflammatory signaling underlying TREX1-mediated autoimmune pathogenesis. In this review, we summarize current knowledge regarding the activity, regulation, and function of the TREX1 enzyme. We explore the accumulated mouse and human immunological data demonstrating a role for TREX1 in suppression of cytosolic DNA sensing and inflammatory signaling. Additionally, we discuss current concepts regarding sources of TREX1 cytosolic DNA substrates, and the cell populations participating in DNA sensing to initiate inflammation. Finally, we examine data from other nucleic acid-processing enzymes which have been linked to autoimmunity, including *SAMHD1* and *RNASEH2* [11,12]. Despite acting on unique substrates in distinct nucleic acid metabolism processes, mutations in these genes cause forms of autoimmunity similar to TREX1-deficiency. We discuss the possibility that genome instability may represent a common source of DNA sensing in TREX1 dysfunction and other disorders.

2. TREX1 activity, structure, localization, and regulation

The *TREX1* gene is a single open reading frame located on chromosome 3p21.31 encoding a 314 amino acid polypeptide with a molecular weight of ~33 kDa [13]. *TREX1* homologs are found in metazoans, are well-conserved, and are expressed in most tissues [5,13,14], likely indicating an important house-keeping function in most cells. The gene encodes a magnesium-dependent, non-processive DNA exonuclease which degrades both double- and ssDNA from the 3′-terminus, cleaving the phosphodiester bond and releasing single nucleoside monophosphates [2–5,13]. The TREX1 N-terminal 242 amino acids contain all of the necessary structural elements for full catalytic activity, and the C-terminal region 72 amino acids are required for localization to the perinuclear space or endoplasmic reticulum (ER), where it is oriented with its active sites exposed to the cytosol (Fig. 1A) [15–22]. TREX1 is an obligate homodimer, with residues from one protomer reaching across the

dimer interface to contribute to catalysis in the opposing protomer. This structure is unique among exonucleases and is mediated by an extensive hydrogen-bonding network between protomers, including backbone contacts between β-strands, side-chain pairings, and hydrophobic packing of antiparallel helices (Fig. 1B). The strength of this interaction creates a kinetically stable dimer that does not dissociate and re-equilibrate at measurable rates [23–25].

The *TREX1* sequence contains the DEDD motif typical of many DNA exonucleases, and exhibits both sequence and structural homology with the *E. coli* 3′→5′ exonuclease domain of DNA Pol I and the DnaQ subunit of Pol III [4,5]. Because these bacterial enzymes play a critical role in DNA proofreading and repair, it was initially hypothesized that *TREX1* was a component of the mammalian DNA repair machinery. However, TREX1 exhibits very little degradative activity against DNA containing 3′ obstructive lesions that result from DNA damage, indicating that its primary substrate is undamaged DNA 3′-termini [26,27]. This notion is further supported by the crystal structures of TREX1 in complex with substrate or product, which reveal a nucleotide binding pocket within the active site too constrictive to allow binding of bulky 3′-modifications such as phosphate, phosphoglycolate, or tyrosyl residues (Fig. 1C) [15,20]. TREX1 has been reported to translocate to the nucleus following genotoxic stress [22,28] and to interact with the PARP1 complex [29], suggesting that it could contribute to DNA repair. However, TREX1 ablation in mice does not increase mutation rates or oncogenesis [6] and TREX1 does not co-localize with major DNA Damage Response (DDR) components γH2AX, pATR, or p53BP following genotoxic stress [28]. In addition, TREX1-deficient cells repair UV-induced dsDNA breaks and cyclobutane pyrimidine dimers at the same rate as TREX1 wild type (WT) cells [21]. Therefore, TREX1 does not appear to participate directly in DNA damage repair.

TREX1 exhibits complex and multi-layered regulation, suggesting a fluctuating level of exonuclease activity dependent upon the physiological status of the cell. *TREX1* transcription is induced by numerous DNA-damaging agents, including UV light, polycyclic aromatic hydrocarbons, and H_2O_2 [28] as well as high doses of gamma radiation [30] and the cytokine IFN-γ [31]. *TREX1* induction following DNA damage might indicate that TREX1 acts on DNA which “leaks” from the nucleus to the cytosol following DNA damage, a concept that will be revisited later in the review. Upregulation of *TREX1* in response to genotoxic stress or to IFN-γ requires c-Jun, a member of the AP-1 transcription factor complex, and the *TREX1* promoter contains an AP-1 binding site [28]. In addition, *TREX1* is induced by IFN-I signaling and is therefore considered an interferon-stimulated gene (ISG), suggesting a potential role for TREX1 in the anti-viral response [14,32]. The *TREX1* gene contains two splice sites, allowing for three potential splice variants [13]. At least one TREX1 splice variant results in truncation of key catalytic residues, revealing a potential pathway for post-transcriptional regulation. In addition, microRNA-mediated RNA interference has been shown to modulate *TREX1* message levels in endothelial cells [33]. Finally, our lab has demonstrated that the TREX1 C-terminus is post-translationally modified by ubiquitination, a modification which promotes shuttling to and degradation in the autophagosome [18]. Additional study of how and when TREX1 activity is modulated by the cell might contribute to our understanding of its normal physiological role.

3. Immunological consequences of TREX1 dysfunction

TREX1 mutations induce a chronic state of IFN-I signaling and inflammation, presumably due to the accumulation or persistence of undegraded DNA in the cytosol and constitutive activation of DNA PRRs. A multitude of redundant PRRs detect DNA in the cytoplasm of eukaryotic cells (reviewed in [34]). The key sensor of cytosolic DNA resulting from TREX1 dysfunction appears to cGAS, a sequence-independent obligate dsDNA sensor localized predominantly within the cytoplasm [35]. DNA binding induces cGAS dimerization and production of cyclic GMP-AMP (cGAMP), a small molecule ligand of the ER-localized signaling adaptor protein STING [36–39]. Binding of cGAMP to STING leads to STING dimerization, translocation to the Golgi apparatus, and recruitment of both IKK and TANK-binding kinase 1 (TBK1) [40,41]. IKK phosphorylates IκB, leading to its degradation, NF-κB activation, and the expression of pro-inflammatory cytokines such as TNF-α and IL-1 [42]. Binding of TBK1 to STING induces TBK1 auto-phosphorylation and subsequent phosphorylation of IRF3 [43]. IRF3 dimerizes, translocates to the nucleus, and stimulates production of type I interferons (IFN-I), secreted signaling molecules which induce inflammation and mediate anti-viral immunity. IFNs bind to the IFN-alpha receptor (IFNAR), expressed on all cells, inducing the expression of hundreds of ISGs as a coordinated anti-viral defense program. TREX1-deficient mice exhibit constitutive IFN-I signaling and ISG expression, and ablation of cGAS [9,44,45], STING [10,46,47], IRF3 [48], or IFNAR [47,48] completely abrogates this chronic inflammatory state. Inhibition of TBK1 in TREX1-deficient mice mediates similar effects [49]. Thus, TREX1-dependent inflammation likely arises from the inappropriate sensing of undegraded TREX1 substrates in the cytosol by cGAS, leading to chronic STING activation and constitutive IFN-I signaling (Fig. 2).

Inflammation arising from *TREX1* mutations causes a spectrum of autoimmune diseases in humans and mice, and the nature of the specific mutation determines the resulting disease phenotype. Certain *TREX1* alleles give rise to Aicardi-Goutières Syndrome (AGS), a severe neurodegenerative disorder typically presenting in infancy [7,8,50]. AGS *TREX1* mutations include single amino acid variants and frameshift mutations leading to early translational STOP codons and presumably dysfunctional TREX1 polypeptides. Most disease-causing alleles exhibit recessive genetics, but some dominant alleles have been identified. AGS patients demonstrate sterile IFN-I production, dramatic expansion of white blood cells, neural inflammation and calcification, skin lesions, multi-organ inflammation, and significant mortality early in life [51–54]. Similarly, *TREX1* ablation in mice leads to an aggressive lupus-like autoimmune phenotype, as indicated by robust ISG induction, extensive myocarditis, nuclear autoantibody production, and markedly reduced survival [9,10,44,46,55,56]. $TREX1^{-/-}$ mice do not exhibit a neuroinflammatory phenotype, but the severity and rapid course of disease in these animals mirrors the aggressiveness of AGS. In contrast, humans expressing catalytically inactive *TREX1* D18N develop less severe autosomal dominant Familial Chilblain Lupus (FCL) [8,57,58]. FCL patients exhibit temperature-sensitive chilblain lesions on the hands and feet as well as systemic IFN-I signaling, autoantibody production, and multi-organ inflammation. Neurological involvement is occasionally observed, leading to speculation that FCL may represent a

milder form of AGS [50]. Our laboratory generated a mouse model of FCL by replacing the TREX1 WT allele with the catalytically inactive *TREX1* D18N allele. This mouse model exhibits a less aggressive lupus-like phenotype relative to *TREX1*$^{-/-}$ mice, paralleling the human TREX1 disease spectrum [47,59,60]. Finally, frameshift mutations in *TREX1* that eliminate the C-terminal region from the protein cause mislocalization throughout the cytosol and autosomal dominant Retinal Vasculopathy with Cerebral Leukodystrophy (RVCL) [16,19,61]. RVCL most often presents in middle age as progressive vision loss and neural degeneration, likely through destruction of small retinal and neural blood vessels [19,62]. In contrast with AGS and FCL, systemic IFN-I signaling is not observed [63]. Curiously, mice expressing RVCL-causative *TREX1* C-terminal mutants develop elevated levels of autoantibodies, but not specifically against nuclear antigens. Furthermore, systemic ISG expression is not observed, but certain purified cell populations may exhibit ISG upregulation *in vitro*, potentially indicating a more localized IFN-I response [64,65]. This suggests a disease mechanism distinct from AGS and FCL.

Different TREX1 mutations cause a spectrum of human diseases, indicating that total loss of TREX1 protein, fractional or complete loss of catalytic activity, and enzyme mislocalization differentially impact disease phenotype. This implies that TREX1 possesses additional function(s) beyond cytosolic DNA degradation, and that the C-terminal region must be properly localized in the perinuclear space or ER to fulfill at least one of these functions. Supporting this concept are studies demonstrating TREX1 participation in the oligosaccharyltransferase (OST) complex [64,66]. OST is an ER-localized membrane protein complex responsible for transferring short sugar moieties from lipid-linked oligosaccharides (LLOs) to nascent proteins. TREX1 interacts with this complex through its hydrophobic C-terminal region, and appears to mediate proper OST function through a DNA catalysis-independent mechanism. Loss of the TREX1-OST interaction disrupts normal OST function, leading to an increase in non-LLO-linked free glycans in *TREX1*$^{-/-}$ cells. Glycan mismanagement has been previously linked to inflammation and lupus-like autoimmunity [67,68], and indeed, free glycans induced by TREX1 dysfunction have been shown to directly induce IFN-I signaling through a STING-TBK1-dependent mechanism [69]. Thus, the human and mouse disease spectrum resulting from different *TREX1* mutations likely indicates that multiple molecular sources of inflammation contribute to disease (Fig. 3). Furthermore, the severe phenotypes resulting from full *TREX1* ablation likely reflect the combined effects of failed DNA degradation and OST dysregulation.

Systemic TREX1 dysfunction in multiple cell populations could drive inflammation and trigger autoimmunity. The inflammatory myocarditis phenotype of the *TREX1*$^{-/-}$ mouse initially suggested cardiomyocytes as a key cell population sensing and responding to undegraded cytosolic DNA [6,46]. Consistent with this model, ISG induction was observed in *TREX1*$^{-/-}$ hearts early during development, suggesting active DNA sensing in cardiac tissue. However, cardiomyocytes do not appear to possess a functional cGAS-STING signaling axis [10], suggesting that DNA sensing and IFN-I production might originate within the immune cell infiltrates that develop in *TREX1*$^{-/-}$ mice. One model may be that TREX1-deficient cardiomyocytes release undegraded cytoplasmic DNA into the microenvironment, perhaps within exosomes, which could act as an attractant and source of cGAS-stimulatory DNA for circulating immune cells [70]. Potentially consistent with this

model, a recent study found that cardiomyocytes release DNA following ischemic injury, triggering an IRF3-dependent influx of IFN-I-producing phagocytic cells into cardiac tissue [71]. Multiple studies have reported an immune cell origin of inflammatory signaling in *TREX1*$^{-/-}$ mice, and evidence supports both monocytes [10] and dendritic cells [56] as sources of DNA sensing and IFN-I production. In addition to innate immune populations, our laboratory has recently demonstrated that T cells respond to TREX1 catalytic inactivity by producing IFN-I [47]. Using the TREX1 D18N model of FCL, we have observed T cell *IFNA* expression and TBK1/IRF3 phosphorylation *in vivo*, and demonstrate that TREX1 D18N T cells produce IFN-I protein when activated. This would suggest that TREX1-dysfunctional T cells can supply an autocrine inflammatory signal promoting autoimmunity. In addition, distinct cell types may be involved in the inflammatory responses generated by self-DNA sensing *versus* DNA-independent free glycan accumulation. Studies focused on isolating the variables of failed DNA catalysis (TREX1 D18N mice) *vs*. OST dysregulation (TREX1 C-terminus mutant mice) may contribute to separating the influence of these competing inflammatory pathways, leading to a better understanding of autoimmune pathogenesis in TREX1-linked human diseases.

4. TREX1 DNA substrates

The molecular targets of TREX1 degradation are an active area of research, and several potential DNA sources have been proposed. This may suggest that TREX1 acts on DNA arising from multiple independent physiological processes. One model posits that TREX1 acts in the SET complex to degrade genomic dsDNA during granzyme A-mediated cell death [72]. Granzyme A is released by cytolytic immune cells, initiating a death program in cells targeted for deletion. This process involves nuclear translocation of the SET complex, allowing the NM23-H1 endonuclease to cleave phosphodiester bonds in genomic DNA. TREX1 binds to the free 3′-hydroxyl termini of NM23-H1-generated nicks and fully dismantles the genome, killing the target. However, granzyme A-mediated cell killing is primarily linked to virally-infected cells and cancer cells, raising questions about the mechanism of constitutive inflammation detected from TREX1 dysfunction in humans and mice. Recently, our laboratory has proposed that NM23-H1 and TREX1 could act in concert to degrade the genomic DNA of developing erythroblasts [60]. Over ~200 billion enucleated erythrocytes generated in humans each day [73], so TREX1 dysfunction in this context could lead to the persistence of undegraded erythroblast DNA, dysregulated erythropoiesis, and constitutive self-DNA sensing.

Mounting evidence suggests that genomic DNA may move from the nucleus to the cytosol, perhaps resulting from DNA damage, repair processes, DNA replication, or turnover of endogenous retroelements. TREX1 likely acts as a general disposal mechanism for these DNA species to limit self-sensing. Two models have been proposed in which TREX1 degrades nucleus-derived ssDNA to prevent inflammation, but they differ on how ssDNA is generated. In one model, ssDNA fragments are thought to be generated in the nucleus during S phase following failed processing of aberrant replication intermediates [22,74,75]. *TREX1*$^{-/-}$ murine embryonic fibroblasts (MEFs) and primary fibroblasts from AGS patients exhibit cytosolic ssDNA accrual, and BrdU-pulse experiments suggest that this DNA is released from the nucleus during S phase. Cytosolic ssDNA accumulation in TREX1-

deficient cells is reportedly correlated with cell cycle defects, p53 activation and p21 induction, reduced CHK2 expression, and DNA comet formation, consistent with a state of elevated DNA damage and chronic DDR activation [21,22,76,77].The potential relationship between TREX1 inactivity and DNA damage will be discussed later in the review. Another model proposes a source of accumulating cytosolic ssDNA derived from retroviral replication. ssDNA retroelements were found to be more abundant in *TREX1*$^{-/-}$ *versus*WT hearts [48] and in TREX1-deficient cells [76,78,79], and overexpression of WT TREX1 but not catalytically-inactive TREX1 led to reduction in retrotransposition events *in vitro* [48]. Related to this idea, TREX1 also appears to exhibit degradation activity against HIV retroviral cDNA during infection. It has been proposed that TREX1 degradation of aborted, non-productive HIV cDNA contributes to HIV avoidance of detection by cGAS [80,81].

TREX1 dysfunction causes cGAS-STING activation, and cGAS requires dsDNA binding for activation [36,82–85]. Furthermore, TREX1$^{WT/D18N}$ heterodimers that could form in FCL patients exhibit no functional activity against dsDNA, but retain significant ssDNA degradative activity [123]. Inflammation arising from TREX1 dysfunction therefore likely reflects the sensing of dsDNA. Cytosolic ssDNA originating from the nucleus during DNA replication, reportedly ~60–65 nucleotides in length [22] would need to exhibit double-stranded structure to activate cGAS. HIV cDNAs have been shown to form hairpin structures permitting cGAS-DNA binding and activation [86], potentially suggesting that a similar property may extend to retroelement cDNAs, but attempts to relate retroelement activation of cGAS-STING to TREX1 dysfunction have generated conflicting results. The reverse transcriptase (RT) inhibitor AZT had no apparent effect on *TREX1*$^{-/-}$ mouse survival [55], but a marked improvement in survival was reported using a combination of Truvada and Viramune targeting a broader range of RT enzymes [87]. A more comprehensive study using broad-spectrum RT inhibitor cocktails found no change in survival, tissue inflammation scores, or IFN-I signaling between treated and untreated *TREX1*$^{-/-}$ animals [88]. Finally, the RT inhibitors 3TC and d4T appear to cause a reduction in inflammatory signaling within *TREX1*-mutant neuronal cell lines *in vitro* [79]. TREX1 substrates continue to emerge, and further research will likely reveal that TREX1 acts on many DNA species to maintain a DNA-free cytosol.

5. Nucleic acid metabolism, genome instability, and inflammation

Five genetic loci involved in nucleic acid-metabolism or detection are causally linked to AGS. In addition to *TREX1*, mutations in any of the three *RNASEH2* genes [12], *SAMHD1* [11], *ADAR1* [89], *and IFIH1* [90,91] are causative for AGS. Mutations in these genes can be partitioned into those involving cytosolic RNA sensing and those involving DNA sensing. ADAR1 is an RNA-editing enzyme which may target and destabilize endogenous dsRNA species, preventing chronic activation of the RIG-I/MDA5 RNA sensing pathway (reviewed in [92]). Likewise, *IFIH1* encodes MDA5 [93], and gain-of-function mutations in *IFIH1* cause AGS. Thus, one cluster of AGS mutations likely induce aberrant cytosolic RNA sensing. *TREX1*, *RNaseH2*, and *SAMHD1* mutations impinge on the cGAS-STING pathway of cytosolic DNA sensing. RNaseH2 excises misincorporated ribonucleotides from genomic DNA. These ribonucleotide lesions increase susceptibility to single- and double-stranded breaks (DSBs) and loss of RNaseH2 activity induces massive DNA damage,

impaired cell cycle progression, and perinatal lethality in mice dependent on cGAS-STING [94–96]. SAMHD1 is a triphosphohydrolase which regulates dNTP pools to maintain DNA replication fidelity [97,98]. SAMHD1 also likely contributes to dsDNA break repair by homologous recombination [99], and might promote resolution of stalled replication forks by MRE11 exonuclease activity [100]. SAMHD1 deficiency leads to chronic DNA damage, DDR activation, cellular senescence, and cell cycle defects [101], an increased mutational burden in cultured fibroblasts [102], and cGAS-STING-dependent IFN-I signaling [100,103]. Thus, AGS-causing mutations occur in multiple genes responsible for genome maintenance, suggesting a model in which failure to maintain genome integrity can lead to cGAS-STING activation and autoimmunity.

Ample experimental evidence supports the concept that genome instability can lead to the appearance of DNA in the cytosol and the activation of cGAS-STING. RNaseH2-deficient MEFs exhibit increased formation of micronuclei, extra-nuclear structures containing damaged chromatin that bud from the nucleus following missegregation of genetic material during mitosis [104,105]. Micronuclei are initially compartmentalized by a nuclear envelope but frequently rupture, allowing cGAS access to the damaged chromatin [104,106,107]. In addition, DNA damage caused by genotoxic agents, depletion of DNA repair factors, and the normal aging process are all associated with “leakage” of naked ss- and dsDNA fragments directly into the cytosol, which induces a cGAS-STING-dependent IFN-I response [30,108–113]. These observations suggest that cGAS-STING might participate in the detection of genotoxic stress in addition to PAMPs. Aging cells and cells exposed to genotoxic insults risk oncogenic transformation. cGAS-STING-dependent cytosolic DNA sensing and IFN-I production in these cells could act to promote immune recognition and clearance, as well as encourage senescence [114–116].

TREX1 does not appear to directly participate in DNA repair. It is therefore curious that *TREX1*$^{-/-}$ cells exhibit chronic DNA damage similar to RNaseH2 and SAMHD1-deficient cells [21,22,76,77]. It may be that the failure to degrade cytosolic DNA by TREX1 can cause genomic instability by triggering a “feedback” response in the nucleus. One model suggests that undegraded TREX1 ssDNA substrates in the cytosol can diffuse back into the nucleus, where they non-productively associate with DNA repair factors RPA and RAD51 [21]. Depletion of free RPA/RAD51 may chronically sensitize cells to DNA damage and genome instability. However, the structural and biochemical properties of TREX1 indicate that dsDNA is a key substrate, and genotoxic stress can lead to the appearance of double-stranded genomic DNA in the cytosol. The fate of this dsDNA after it leaves the nucleus could involve TREX1-mediated degradation. Consistent with a model of extranuclear dsDNA disposal, ablation of lysosomal endonuclease DNaseII in fibroblasts is associated with cytosolic accumulation of nucleus-derived dsDNA fragments [117]. Furthermore, these cells exhibit chronic DNA damage similar to AGS-mutant cells. DNaseII-deficient cells and mice also exhibit cGAS-STING-dependent IFN-I signaling [117–119]. This implies that **1)** dsDNA is regularly exported from and degraded outside the nucleus, and **2)** dysfunction in this process may lead to both cytosolic pattern recognition and DDR activation. A precise mechanism describing how failed cytosolic dsDNA degradation leads to DNA damage remains to be elucidated, but a similar model to that proposed for ssDNA TREX1 substrates

could apply. dsDNA fragments diffusing back into the nucleus would represent excellent substrates for DNA-PK, a nuclear kinase which mediates recruitment of repair factors to dsDNA breaks, and which is readily activated by fragments as small as 14 nucleotides *in vitro* [120–122]. The localization of TREX1 at the nuclear border, its high affinity for dsDNA, and the phenotypic similarities between TREX1 and DNaseII deficiency could suggest that both participate in disposal of leaked nuclear dsDNA to prevent genome instability and cytosolic DNA sensing.

6. Concluding remarks

TREX1 is required for efficient cytosolic DNA disposal to effectively prevent inappropriate cGAS-STING-mediated DNA sensing and immune activation. The consequences for human health resulting from dysfunction in this process include severe and debilitating autoimmune diseases. Many aspects of TREX1 biology remain to be fully elucidated. Additional work examining the precise molecular targets of TREX1 degradation is needed. Furthermore, additional clarification of the TREX1-deficient cell populations driving pro-autoimmune inflammatory signaling *in vivo* could lead to better, more targeted therapies for human diseases caused by *TREX1* mutation. Answers to these questions should provide a better understanding of how autoimmunity arises from failed nucleic acid processing, and how DNA waste products are normally managed to maintain cellular homeostasis and genome stability.

Acknowledgements

We apologize to any investigators whose research was not cited in the interest of generating a brief review. Research in the Perrino laboratory has been supported by the National Institute of Health (NIH, R01AI116725, T32AI007401, T32GM095440), the Alliance for Lupus Research, the Cowgill and Artom memorial fellowships, and the Comprehensive Cancer Center of Wake Forest University National Cancer Institute Cancer (Center Support Grant, P30CA012197).

Abbreviations:

AGS	Aicardi-Goutieres syndrome
cGAMP	cyclic GMP-AMP
cGAS	cyclic GMP-AMP synthase
DDR	DNA damage response
DSBs	double-stranded breaks
dsDNA	double-stranded DNA
ER	endoplasmic reticulum
FCL	Familial Chilblain Lupus
IFN-I	Type I interferon
IFNAR	interferon-α/β receptor

ISG	interferon-stimulatedgene
LLOs	lipid-linked oligosaccharides
MEFs	murine embryonic fibroblasts
OST complex	oligosaccharyltransferase complex
PAMP	pathogen-associated molecular pattern
PRR	pattern recognition receptor
RVCL	retinal vasculopathy with cerebral leukodystrophy
RT	reverse transcriptase
ssDNA	single-stranded DNA
STING	stimulator of interferon genes
TBK1	TANK-binding kinase 1
TREX1	three-prime repair exonuclease 1
WT	wild type

References

[1]. Brubaker SW, Bonham KS, Zanoni I, Kagan JC, Innate immune pattern recognition: a cell biological perspective, Annu. Rev. Immunol 33 (1) (2015) 257–290, 10.1146/annurev-immunol-032414-112240. [PubMed: 25581309]

[2]. Lindahl T, Gally JA, Properties of Deoxyribonuclease III from Mammalian Tissues, J. Biol. Chem 244 (18) (1969) 7.

[3]. Perrino FW, Miller H, Ealey KA, Identification of a 3'–&5'-exonuclease that removes cytosine arabinoside monophosphate from 3' termini of DNA, J. Biol. Chem 269 (June (23)) (1994) 16357–16363. [PubMed: 8206943]

[4]. Mazur DJ, Perrino FW, Identification and expression of the TREX1 and TREX2 cDNA sequences encoding mammalian 3′→ 5′ exonucleases, J. Biol. Chem 274 (28) (1999) 19655–19660. [PubMed: 10391904]

[5]. Höss M, Robins P, Naven TJ, Pappin DJ, Sgouros J, Lindahl T, A human DNA editing enzyme homologous to the Escherichia coli DnaQ/MutD protein, EMBO J. 18 (July (13)) (1999) 3868–3875, 10.1093/emboj/18.13.3868. [PubMed: 10393201]

[6]. Morita M, et al., Gene-Targeted Mice Lacking the Trex1 (DNase III) 3′→5′ DNA Exonuclease Develop Inflammatory Myocarditis, Mol. Cell. Biol 24 (August (15)) (2004) 6719–6727, 10.1128/MCB.24.15.6719-6727.2004. [PubMed: 15254239]

[7]. Crow YJ, et al., Mutations in the gene encoding the 3'−5' DNA exonuclease TREX1 cause Aicardi-Goutieres syndrome at the AGS1 locus, Nat. Genet 38 (August (8)) (2006) 917–920, 10.1038/ng1845. [PubMed: 16845398]

[8]. Rice G, et al., Heterozygous Mutations in TREX1 Cause Familial Chilblain Lupus and Dominant Aicardi-Goutières Syndrome, Am. J. Hum. Genet 80 (April (4)) (2007) 811–815. [PubMed: 17357087]

[9]. Gao D, et al., Activation of cyclic GMP-AMP synthase by self-DNA causes autoimmune diseases, Proc. Natl. Acad. Sci 112 (October (42)) (2015) E5699–E5705, 10.1073/pnas.1516465112. [PubMed: 26371324]

[10]. Ahn J, Ruiz P, Barber GN, Intrinsic Self-DNA triggers inflammatory disease dependent on STING, J. Immunol 193 (9) (2014) 4634–4642, 10.4049/jimmunol.1401337. [PubMed: 25261479]

[11]. Rice GI, et al., Mutations involved in Aicardi-Goutières syndrome implicate SAMHD1 as regulator of the innate immune response, Nat. Genet 41 (7) (2009) 829–832, 10.1038/ng.373. [PubMed: 19525956]

[12]. Crow YJ, et al., Mutations in genes encoding ribonuclease H2 subunits cause Aicardi-Goutières syndrome and mimic congenital viral brain infection, Nat. Genet 38 (8) (2006) 910–916, 10.1038/ng1842. [PubMed: 16845400]

[13]. Mazur DJ, Perrino FW, Structure and expression of the TREX1 and TREX2 3'-&5' exonuclease genes, J. Biol. Chem 276 (18) (2001) 14718–14727, 10.1074/jbc.M010051200. [PubMed: 11278605]

[14]. Pereira-Lopes S, et al., The exonuclease Trex1 restrains macrophage prion-flammatory activation, J. Immunol 191 (12) (2013) 6128–6135, 10.4049/jimmunol.1301603. [PubMed: 24218451]

[15]. Brucet M, et al., Structure of the dimeric exonuclease TREX1 in complex with DNA displays a proline-rich binding site for WW domains, J. Biol. Chem 282 (19) (2007) 14547–14557, 10.1074/jbc.M700236200. [PubMed: 17355961]

[16]. DiFrancesco JC, et al., TREX1 C-terminal frameshift mutations in the systemic variant of retinal vasculopathy with cerebral leukodystrophy, Neurol. Sci 36 (2) (2015) 323–330, 10.1007/s10072-014-1944-9. [PubMed: 25213617]

[17]. Lee-Kirsch MA, et al., Mutations in the gene encoding the 3′−5′ DNA exonuclease TREX1 are associated with systemic lupus erythematosus, Nat. Genet 39 (9) (2007) 1065–1067, 10.1038/ng2091. [PubMed: 17660818]

[18]. Orebaugh CD, Fye JM, Harvey S, Hollis T, Wilkinson JC, Perrino FW, The TREX1 C-terminal region controls cellular localization through ubiquitination, J. Biol. Chem 288 (40) (2013) 28881–28892, 10.1074/jbc.M113.503391. [PubMed: 23979357]

[19]. Richards A, et al., C-terminal truncations in human 3′−5′ DNA exonuclease TREX1 cause autosomal dominant retinal vasculopathy with cerebral leukodystrophy, Nat. Genet 39 (9) (2007) 1068–1070, 10.1038/ng2082. [PubMed: 17660820]

[20]. de Silva U, Choudhury S, Bailey SL, Harvey S, Perrino FW, Hollis T, The crystal structure of TREX1 explains the 3′ nucleotide specificity and reveals a polyproline II Helix for protein partnering, J. Biol. Chem 282 (14) (2007) 10537–10543, 10.1074/jbc.M700039200. [PubMed: 17293595]

[21]. Wolf C, et al., RPA and Rad51 constitute a cell intrinsic mechanism to protect the cytosol from self DNA, Nat. Commun 7 (May) (2016) 11752, 10.1038/ncomms11752. [PubMed: 27230542]

[22]. Yang Y-G, Lindahl T, Barnes DE, Trex1 Exonuclease Degrades ssDNA to Prevent Chronic Checkpoint Activation and Autoimmune Disease, Cell 131 (5) (2007) 873–886, 10.1016/j.cell.2007.10.017. [PubMed: 18045533]

[23]. Orebaugh CD, Fye JM, Harvey S, Hollis T, Perrino FW, The TREX1 Exonuclease R114H mutation in Aicardi-Goutieres syndrome and lupus reveals Dimeric structure requirements for DNA degradation activity, J. Biol. Chem 286 (46) (2011) 40246–40254, 10.1074/jbc.M111.297903. [PubMed: 21937424]

[24]. Bailey SL, Harvey S, Perrino FW, Hollis T, Defects in DNA degradation revealed in crystal structures of TREX1 exonuclease mutations linked to autoimmune disease, DNA Repair 11 (1) (2012) 65–73, 10.1016/j.dnarep.2011.10.007. [PubMed: 22071149]

[25]. Fye JM, Coffin SR, Orebaugh CD, Hollis T, Perrino FW, The Arg-62 Residues of the TREX1 Exonuclease Act Across the Dimer Interface Contributing to Catalysis in the Opposing Protomers, J. Biol. Chem 289 (16) (2014) 11556–11565, 10.1074/jbc.M114.559252. [PubMed: 24616097]

[26]. Inamdar KV, Yu Y, Povirk LF, Resistance of 3′-Phosphoglycolate DNA Ends to Digestion by Mammalian DNase III, Radiat. Res 157 (3) (2002) 306–311, 10.1667/0033-7587(2002)157[0306:ROPDET]2.0.CO;2. [PubMed: 11839093]

[27]. Harrigan JA, Fan J, Momand J, Perrino FW, Bohr VA, Wilson DM, WRN exonuclease activity is blocked by DNA termini harboring 3' obstructive groups, Mech. Ageing Dev 128 (3) (2007) 259–266, 10.1016/j.mad.2006.12.005. [PubMed: 17224176]

[28]. Christmann M, Tomicic MT, Aasland D, Berdelle N, Kaina B, Three prime exonuclease I (TREX1) is Fos/AP-1 regulated by genotoxic stress and protects against ultraviolet light and benzo(a)pyrene-induced DNA damage, Nucleic Acids Res. 38 (19) (2010) 6418–6432, 10.1093/nar/gkq455. [PubMed: 20511593]

[29]. Miyazaki T, Kim Y-S, Yoon J, Wang H, Suzuki T, Morse HC, The 3'−5' DNA Exonuclease TREX1 Directly Interacts with Poly(ADP-ribose) Polymerase-1 (PARP1) during the DNA Damage Response, J. Biol. Chem 289 (47) (2014) 32548–32558, 10.1074/jbc.M114.547331. [PubMed: 25278026]

[30]. Vanpouille-Box C, et al., DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity, Nat. Commun 8 (June) (2017) 15618, 10.1038/ncomms15618. [PubMed: 28598415]

[31]. Serra M, Forcales S-V, Pereira-Lopes S, Lloberas J, Celada A, Characterization of Trex1 induction by IFN-γ in murine macrophages, J.I 186 (4) (2011) 2299–2308, 10.4049/jimmunol.1002364.

[32]. Xu J, Zoltick PW, Gamero AM, Gallucci S, TLR ligands up-regulate Trex1 expression in murine conventional dendritic cells through type I Interferon and NF-κB-dependent signaling pathways, J. Leukoc. Biol 96 (1) (2014) 93–103, 10.1189/jlb.2A0713-393RR. [PubMed: 24598055]

[33]. Wilson R, et al., MicroRNA regulation of endothelial TREX1 reprograms the tumour microenvironment, Nat. Commun 7 (2016), 10.1038/ncomms13597.

[34]. Vanpouille-Box C, Demaria S, Formenti SC, Galluzzi L, Cytosolic DNA sensing in organismal tumor control, Cancer Cell 34 (3) (2018) 361–378, 10.1016/j.ccell.2018.05.013. [PubMed: 30216189]

[35]. Sun L, Wu J, Du F, Chen X, Chen ZJ, Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway, Science 339 (6121) (2013) 786–791, 10.1126/science.1232458. [PubMed: 23258413]

[36]. Gao P, et al., Cyclic [G(2′,5′)pA(3′,5′)p] Is the Metazoan Second Messenger Produced by DNA-Activated Cyclic GMP-AMP Synthase, Cell 153 (5) (2013) 1094–1107, 10.1016/j.cell.2013.04.046. [PubMed: 23647843]

[37]. Diner EJ, et al., The Innate Immune DNA Sensor cGAS Produces a Noncanonical Cyclic Dinucleotide that Activates Human STING, Cell Rep. 3 (5) (2013) 1355–1361, 10.1016/j.celrep.2013.05.009. [PubMed: 23707065]

[38]. Ablasser A, et al., cGAS produces a 2′−5′-linked cyclic dinucleotide second messenger that activates STING, Nature 498 (7454) (2013) 380–384, 10.1038/nature12306. [PubMed: 23722158]

[39]. Ishikawa H, Barber GN, STING an endoplasmic reticulum adaptor that facilitates innate immune signaling, Nature 455 (7213) (2008) 674–678, 10.1038/nature07317. [PubMed: 18724357]

[40]. Dobbs N, Burnaevskiy N, Chen D, Gonugunta VK, Alto NM, Yan N, STING activation by translocation from the ER is associated with infection and autoinflammatory disease, Cell Host Microbe 18 (2) (2015) 157–168, 10.1016/j.chom.2015.07.001. [PubMed: 26235147]

[41]. Ishikawa H, Ma Z, Barber GN, STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity, Nature 461 (7265) (2009) 788–792, 10.1038/nature08476. [PubMed: 19776740]

[42]. Lawrence T, The Nuclear Factor NF-κB Pathway in Inflammation, Cold Spring Harb. Perspect. Biol 1 (6) (2009), 10.1101/cshperspect.a001651.

[43]. Tanaka Y, Chen ZJ, STING specifies IRF3 phosphorylation by TBK1 in the cytosolic DNA signaling pathway, Sci. Signal 5 (214) (2012), 10.1126/scisignal.2002521 pp. ra20–ra20. [PubMed: 22394562]

[44]. Gray EE, Treuting PM, Woodward JJ, Stetson DB, Cutting Edge: cGAS Is Required for Lethal Autoimmune Disease in the Trex1-Deficient Mouse Model of Aicardi–Goutières Syndrome, J. Immunol 195 (5) (2015) 1939–1943, 10.4049/jimmunol.1500969. [PubMed: 26223655]

[45]. Xiao N, et al., cGAS activation causes lupus-like autoimmune disorders in a TREX1 mutant mouse model, J. Autoimmun (March) (2019), 10.1016/j.jaut.2019.03.001.

[46]. Gall A, et al., Autoimmunity Initiates in Nonhematopoietic Cells and Progresses via Lymphocytes in an Interferon-Dependent Autoimmune Disease, Immunity 36 (1) (2012) 120–131, 10.1016/j.immuni.2011.11.018. [PubMed: 22284419]

[47]. Simpson SR, et al., T cells produce IFN-α in the TREX1 D18N model of lupus-like autoimmunity, J.I 204 (2) (2020) 348–359, 10.4049/jimmunol.1900220.

[48]. Stetson DB, Ko JS, Heidmann T, Medzhitov R, Trex1 prevents cell-intrinsic initiation of autoimmunity, Cell 134 (4) (2008) 587–598, 10.1016/j.cell.2008.06.032. [PubMed: 18724932]

[49]. Hasan M, et al., Cutting edge: inhibiting TBK1 by compound II ameliorates autoimmune disease in mice, J. Immunol 195 (10) (2015) 4573–4577, 10.4049/jimmunol.1500162. [PubMed: 26432890]

[50]. Rice GI, Rodero MP, Crow YJ, Human disease phenotypes associated with mutations in TREX1, J. Clin. Immunol 35 (3) (2015) 235–243, 10.1007/s10875-015-0147-3. [PubMed: 25731743]

[51]. Aicardi J, Goutières F, A Progressive familial encephalopathy in infancy with calcifications of the basal ganglia and chronic cerebrospinal fluid lymphocytosis, Ann. Neurol 15 (1) (1984) 49–54, 10.1002/ana.410150109. [PubMed: 6712192]

[52]. Lebon P, Badoual J, Ponsot G, Goutières F, Hémeury-Cukier F, Aicardi J, Intrathecal synthesis of interferon-alpha in infants with progressive familial encephalopathy, J. Neurol. Sci 84 (2–3) (1988) 201–208, 10.1016/0022-510X(88)90125-6. [PubMed: 2837539]

[53]. Rice G, et al., Clinical and Molecular Phenotype of Aicardi-Goutières Syndrome, Am. J. Hum. Genet 81 (4) (2007) 713–725, 10.1086/521373. [PubMed: 17846997]

[54]. Abe J, et al., Heterozygous TREX1 p.Asp18Asn mutation can cause variable neurological symptoms in a family with Aicardi-Goutieres syndrome/familial chilblain lupus, Rheumatology 52 (2) (2013) 406–408, 10.1093/rheumatology/kes181. [PubMed: 22829693]

[55]. Stetson DB, Ko JS, Heidmann T, Medzhitov R, Trex1 prevents cell-intrinsic initiation of autoimmunity, Cell 134 (4) (2008) 587–598, 10.1016/j.cell.2008.06.032. [PubMed: 18724932]

[56]. Peschke K, et al., Loss of Trex1 in dendritic cells is sufficient to trigger systemic autoimmunity, J. Immunol 197 (6) (2016) 2157–2166, 10.4049/jimmunol.1600722. [PubMed: 27511730]

[57]. Lee-Kirsch MA, et al., A mutation in TREX1 that impairs susceptibility to granzyme A-mediated cell death underlies familial chilblain lupus, J. Mol. Med 85 (5) (2007) 531–537, 10.1007/s00109-007-0199-9. [PubMed: 17440703]

[58]. Lehtinen DA, Harvey S, Mulcahy MJ, Hollis T, Perrino FW, The TREX1 double-stranded DNA degradation activity is defective in dominant mutations associated with autoimmune disease, J. Biol. Chem 283 (46) (2008) 31649–31656, 10.1074/jbc.M806155200. [PubMed: 18805785]

[59]. Grieves JL, Fye JM, Harvey S, Grayson JM, Hollis T, Perrino FW, Exonuclease TREX1 degrades double-stranded DNA to prevent spontaneous lupus-like inflammatory disease, Proc. Natl. Acad. Sci 112 (16) (2015) 5117–5122, 10.1073/pnas.1423804112. [PubMed: 25848017]

[60]. Rego SL, et al., TREX1 D18N mice fail to process erythroblast DNA resulting in inflammation and dysfunctional erythropoiesis, Autoimmunity 51 (7) (2018) 333–344, 10.1080/08916934.2018.1522305. [PubMed: 30422000]

[61]. Pelzer N, et al., Systemic features of retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations: a monogenic small vessel disease, J. Intern. Med 285 (3) (2019) 317–332, 10.1111/joim.12848. [PubMed: 30411414]

[62]. Mateen FJ, et al., Evolution of a tumor-like lesion in cerebroretinal vasculopathy and trex1 mutation, Neurology 75 (13) (2010) 1211–1213, 10.1212/WNL.0b013e3181f4d7ac. [PubMed: 20876473]

[63]. Rodero MP, et al., Detection of interferon alpha protein reveals differential levels and cellular sources in disease, J. Exp. Med 214 (5) (2017) 1547–1555, 10.1084/jem.20161451. [PubMed: 28420733]

[64]. Hasan M, et al., Cytosolic nuclease TREX1 regulates oligosaccharyltransferase activity independent of nuclease activity to suppress immune activation, Immunity 43 (3) (2015) 463–474, 10.1016/j.immuni.2015.07.022. [PubMed: 26320659]

[65]. Sakai T, et al., DNase-active TREX1 frame-shift mutants induce serologic autoimmunity in mice, J. Autoimmun 81 (July) (2017) 13–23, 10.1016/j.jaut.2017.03.001. [PubMed: 28325644]

[66]. Kucej M, Fermaintt CS, Yang K, Irizarry-Caro RA, Yan N, Mitotic Phosphorylation of TREX1 C Terminus Disrupts TREX1 Regulation of the Oligosaccharyltransferase Complex, Cell Rep. 18 (11) (2017) 2600–2607, 10.1016/j.celrep.2017.02.051. [PubMed: 28297665]

[67]. Chui D, et al., Genetic remodeling of protein glycosylation in vivo induces autoimmune disease, Proc Natl Acad Sci U S A 98 (3) (2001) 1142–1147. [PubMed: 11158608]

[68]. Green RS, Stone EL, Tenno M, Lehtonen E, Farquhar MG, Marth JD, Mammalian N-Glycan branching protects against innate immune self-recognition and inflammation in autoimmune disease pathogenesis, Immunity 27 (2) (2007) 308–320, 10.1016/j.immuni.2007.06.008. [PubMed: 17681821]

[69]. Fermaintt CS, et al., A bioactive mammalian disaccharide associated with autoimmunity activates STING-TBK1-dependent immune response, Nat. Commun 10 (May) (2019), 10.1038/s41467-019-10319-5.

[70]. Diamond JM, et al., Exosomes Shuttle TREX1-Sensitive IFN-Stimulatory dsDNA from irradiated cancer cells to DCs, Cancer Immunol. Res (June) (2018), 10.1158/2326-6066.CIR-17-0581.

[71]. King KR, et al., IRF3 and type I interferons fuel a fatal response to myocardial infarction, Nat. Med 23 (12) (2017) 1481–1487, 10.1038/nm.4428. [PubMed: 29106401]

[72]. Chowdhury D, et al., The exonuclease TREX1 Is in the SET complex and acts in concert with NM23-H1 to degrade DNA during granzyme A-mediated cell death, Mol. Cell 23 (1) (2006) 133–142, 10.1016/j.molcel.2006.06.005. [PubMed: 16818237]

[73]. Gordon MY, Lewis JL, Marley SB, Of mice and men … and elephants, Blood 100 (13) (2002), 10.1182/blood-2002-08-2517 pp. 4679–4679. [PubMed: 12453884]

[74]. Lindahl T, Barnes DE, Yang Y-G, Robins P, Biochemical properties of mammalian TREX1 and its association with DNA replication and inherited inflammatory disease, Biochem. Soc. Trans 37 (3) (2009) 535–538, 10.1042/BST0370535. [PubMed: 19442247]

[75]. O'Driscoll M, TREX1 DNA exonuclease deficiency, accumulation of single stranded DNA and complex human genetic disorders, DNA Repair 7 (6) (2008) 997–1003, 10.1016/j.dnarep.2008.02.010. [PubMed: 18406216]

[76]. Li P, et al., Aicardi–Goutières syndrome protein TREX1 suppresses L1 and maintains genome integrity through exonuclease-independent ORF1p depletion, Nucleic Acids Res. 45 (8) (2017) 4619–4631, 10.1093/nar/gkx178. [PubMed: 28334850]

[77]. Prati B, et al., Three Prime Repair Exonuclease 1 (TREX1) expression correlates with cervical cancer cells growth in vitro and disease progression in vivo, Sci. Rep 9 (January) (2019), 10.1038/s41598-018-37064-x.

[78]. Nakaya Y, Lilue J, Stavrou S, Moran EA, Ross SR, AIM2-like receptors positively and negatively regulate the interferon response induced by cytosolic DNA, mBio 8 (4) (2017), 10.1128/mBio.00944-17 pp. e00944–17, /mbio/8/4/e00944–17.atom. [PubMed: 28679751]

[79]. Thomas CA, et al., Modeling of TREX1-dependent autoimmune disease using human stem cells highlights L1 accumulation as a source of neuroinflammation, Cell Stem Cell (August) (2017), 10.1016/j.stem.2017.07.009.

[80]. Kumar S, Morrison JH, Dingli D, Poeschla E, HIV-1 activation of innate immunity depends strongly on the intracellular level of TREX1 and sensing of incomplete reverse transcription products, J. Virol 92 (16) (2018), 10.1128/JVI.00001-18.

[81]. Yan N, Regalado-Magdos AD, Stiggelbout B, Lee-Kirsch MA, Lieberman J, The cytosolic exonuclease TREX1 inhibits the innate immune response to human immunodeficiency virus type 1, Nat. Immunol 11 (11) (2010) 1005–1013, 10.1038/ni.1941. [PubMed: 20871604]

[82]. Civril F, et al., Structural mechanism of cytosolic DNA sensing by cGAS, Nature 498 (7454) (2013) 332–337, 10.1038/nature12305. [PubMed: 23722159]

[83]. Du M, Chen ZJ, DNA-induced liquid phase condensation of cGAS activates innate immune signaling, Science 361 (6403) (2018) 704–709, 10.1126/science.aat1022. [PubMed: 29976794]

[84]. Li X, et al., Cyclic GMP-AMP Synthase is activated by double-stranded DNA-induced oligomerization, Immunity 39 (6) (2013), 10.1016/j.immuni.2013.10.019.

[85]. Luecke S, et al., cGAS is activated by DNA in a length-dependent manner, EMBO Rep. 18 (10) (2017) 1707–1715, 10.15252/embr.201744017. [PubMed: 28801534]

[86]. Herzner A-M, et al., Sequence-specific activation of the DNA sensor cGAS by Y-form DNA structures as found in primary HIV-1 cDNA, Nat. Immunol 16 (10) (2015) 1025–1033, 10.1038/ni.3267. [PubMed: 26343537]

[87]. Beck-Engeser GB, Eilat D, Wabl M, An autoimmune disease prevented by antiretroviral drugs, Retrovirology 8 (1) (2011) 91. [PubMed: 22067273]

[88]. Achleitner M, et al., Lack of Trex1 causes systemic autoimmunity despite the presence of antiretroviral drugs, J. Immunol (August) (2017) ji1700714, 10.4049/jimmunol.1700714.

[89]. Rice GI, et al., Mutations in ADAR1 cause Aicardi-Goutières syndrome associated with a type I interferon signature, Nat. Genet 44 (11) (2012) 1243–1248, 10.1038/ng.2414. [PubMed: 23001123]

[90]. Oda H, et al., Aicardi-Goutières syndrome is caused by IFIH1 mutations, Am. J. Hum. Genet 95 (1) (2014) 121–125, 10.1016/j.ajhg.2014.06.007. [PubMed: 24995871]

[91]. Rice GI, et al., Gain-of-function mutations in IFIH1 cause a spectrum of human disease phenotypes associated with upregulated type I interferon signaling, Nat. Genet 46 (5) (2014) 503–509, 10.1038/ng.2933. [PubMed: 24686847]

[92]. Lamers MM, van den Hoogen BG, Haagmans BL, ADAR1: 'Editor-in-Chief' of cytoplasmic innate immunity, Front. Immunol 10 (2019), 10.3389/fimmu.2019.01763.

[93]. Kato H, et al., Length-dependent recognition of double-stranded ribonucleic acids by retinoic acid–inducible gene-I and melanoma differentiation–associated gene 5, J. Exp. Med 205 (7) (2008) 1601–1610, 10.1084/jem.20080091. [PubMed: 18591409]

[94]. Hiller B, Achleitner M, Glage S, Naumann R, Behrendt R, Roers A, Mammalian RNase H2 removes ribonucleotides from DNA to maintain genome integrity, J. Exp. Med 209 (8) (2012) 1419–1426, 10.1084/jem.20120876. [PubMed: 22802351]

[95]. Pokatayev V, et al., RNase H2 catalytic core Aicardi-Goutières syndrome–related mutant invokes cGAS–STING innate immune-sensing pathway in mice, J. Exp. Med 213 (3) (2016) 329–336, 10.1084/jem.20151464. [PubMed: 26880576]

[96]. Reijns MAM, et al., Enzymatic removal of ribonucleotides from DNA is essential for mammalian genome integrity and development, Cell 149 (5) (2012) 1008–1022, 10.1016/j.cell.2012.04.011. [PubMed: 22579044]

[97]. Mauney CH, et al., The SAMHD1 dNTP triphosphohydrolase is controlled by a redox switch, Antioxid. Redox Signal 27 (16) (2017) 1317–1331, 10.1089/ars.2016.6888. [PubMed: 28398823]

[98]. Powell RD, Holland PJ, Hollis T, Perrino FW, Aicardi-Goutières Syndrome Gene and HIV-1 Restriction Factor SAMHD1 Is a dGTP-regulated Deoxynucleotide Triphosphohydrolase, J. Biol. Chem 286 (51) (2011) 43596–43600, 10.1074/jbc.C111.317628. [PubMed: 22069334]

[99]. Daddacha W, et al., SAMHD1 promotes DNA end resection to facilitate DNA repair by homologous recombination, Cell Rep. 20 (8) (2017) 1921–1935, 10.1016/j.celrep.2017.08.008. [PubMed: 28834754]

[100]. Coquel F, et al., SAMHD1 acts at stalled replication forks to prevent interferon induction, Nature 557 (7703) (2018) 57–61, 10.1038/s41586-018-0050-1. [PubMed: 29670289]

[101]. Kretschmer S, et al., SAMHD1 prevents autoimmunity by maintaining genome stability, Ann. Rheum. Dis 74 (3) (2015), 10.1136/annrheumdis-2013-204845 pp. e17–e17. [PubMed: 24445253]

[102]. Franzolin E, et al., SAMHD1-deficient fibroblasts from Aicardi-Goutières Syndrome patients can escape senescence and accumulate mutations, Faseb J. 34 (1) (2020) 631–647, 10.1096/fj.201902508R. [PubMed: 31914608]

[103]. Maelfait J, Bridgeman A, Benlahrech A, Cursi C, Rehwinkel J, Restriction by SAMHD1 limits cGAS/STING-Dependent innate and adaptive immune responses to HIV-1, Cell Rep. 16 (6) (2016) 1492–1501, 10.1016/j.celrep.2016.07.002. [PubMed: 27477283]

[104]. Mackenzie KJ, et al., cGAS surveillance of micronuclei links genome instability to innate immunity, Nature 548 (7668) (2017) 461–465, 10.1038/nature23449. [PubMed: 28738408]

[105]. Bartsch K, et al., Absence of RNase H2 triggers generation of immunogenic micronuclei removed by autophagy, Hum. Mol. Genet 26 (20) (2017) 3960–3972, 10.1093/hmg/ddx283. [PubMed: 29016854]

[106]. Harding SM, Benci JL, Irianto J, Discher DE, Minn AJ, Greenberg RA, Mitotic progression following DNA damage enables pattern recognition within micronuclei, Nature 548 (7668) (2017) 466–470, 10.1038/nature23470. [PubMed: 28759889]

[107]. Hatch EM, Fischer AH, Deerinck TJ, Hetzer MW, Catastrophic nuclear envelope collapse in Cancer cell micronuclei, Cell 154 (1) (2013) 47–60, 10.1016/j.cell.2013.06.007. [PubMed: 23827674]

[108]. Ahn J, Xia T, Konno H, Konno K, Ruiz P, Barber GN, Inflammation-driven carcinogenesis is mediated through STING, Nat. Commun 5 (1) (2014) 5166, 10.1038/ncomms6166. [PubMed: 25300616]

[109]. Bhattacharya S, et al., RAD51 interconnects between DNA replication, DNA repair and immunity, Nucleic Acids Res. 45 (8) (2017) 4590–4605, 10.1093/nar/gkx126. [PubMed: 28334891]

[110]. Erdal E, Haider S, Rehwinkel J, Harris AL, McHugh PJ, A prosurvival DNA damage-induced cytoplasmic interferon response is mediated by end resection factors and is limited by Trex1, Genes Dev. 31 (4) (2017) 353–369, 10.1101/gad.289769.116. [PubMed: 28279982]

[111]. Glück S, et al., Innate immune sensing of cytosolic chromatin fragments through cGAS promotes senescence, Nat. Cell Biol 19 (9) (2017) 1061–1070, 10.1038/ncb3586. [PubMed: 28759028]

[112]. Härtlova A, et al., DNA damage primes the type I interferon system via the cytosolic DNA sensor STING to promote anti-microbial innate immunity, Immunity 42 (2) (2015) 332–343, 10.1016/j.immuni.2015.01.012. [PubMed: 25692705]

[113]. Lan YY, et al., Extranuclear DNA accumulates in aged cells and contributes to senescence and inflammation, Aging Cell (January) (2019) e12901, 10.1111/acel.12901. [PubMed: 30706626]

[114]. Moiseeva O, Mallette FA, Mukhopadhyay UK, Moores A, Ferbeyre G, DNA damage signaling and p53-dependent senescence after prolonged β-Interferon stimulation, Mol. Biol. Cell 17 (4) (2006) 1583–1592, 10.1091/mbc.E05-09-0858. [PubMed: 16436515]

[115]. Yu Q, et al., DNA damage-induced type I interferon promotes senescence and inhibits stem cell function, Cell Rep. 11 (5) (2015) 785–797, 10.1016/j.celrep.2015.03.069. [PubMed: 25921537]

[116]. Baz-Martínez M, et al., Cell senescence is an antiviral defense mechanism, Sci. Rep 6 (November) (2016), 10.1038/srep37007.

[117]. Lan YY, Londoño D, Bouley R, Rooney MS, Hacohen N, Dnase2a deficiency uncovers lysosomal clearance of damaged nuclear DNA via autophagy, Cell Rep. 9 (1) (2014) 180–192, 10.1016/j.celrep.2014.08.074. [PubMed: 25284779]

[118]. Yoshida H, Okabe Y, Kawane K, Fukuyama H, Nagata S, Lethal anemia caused by interferon-β produced in mouse embryos carrying undigested DNA, Nat. Immunol 6 (January (1)) (2005) 49–56, 10.1038/ni1146. [PubMed: 15568025]

[119]. Kato Y, et al., Apoptosis-derived membrane vesicles drive the cGAS–STING pathway and enhance type I IFN production in systemic lupus erythematosus, Ann. Rheum. Dis (June) (2018), 10.1136/annrheumdis-2018-212988p.annrheumdis-2018-212988.

[120]. Falzon M, Fewell JW, Kuff EL, EBP-80, a transcription factor closely resembling the human autoantigen Ku, recognizes single- to double-strand transitions in DNA, J. Biol. Chem 268 (May (14)) (1993) 10546–10552. [PubMed: 8486707]

[121]. Yaneva M, Kowalewski T, Lieber MR, Interaction of DNA-dependent protein kinase with DNA and with Ku: biochemical and atomic-force microscopy studies, EMBO J. 16 (August (16)) (1997) 5098–5112, 10.1093/emboj/16.16.5098. [PubMed: 9305651]

[122]. Pang D, et al., Radiation-generated short DNA fragments may perturb non-homologous end-joining and induce genomic instability, J. Radiat. Res 52 (3) (2011) 309–319. [PubMed: 21628845]

[123]. Fye JM, Orebaugh CD, Coffin SR, Hollis T, Perrino FW, Dominant mutations of the TREX1 exonuclease gene in lupus and aicardi-goutieres syndrome, J. Bio. Chem 286 (37) (2011) 32373–32382, 10.1074/jbc.M111.276287. [PubMed: 21808053]

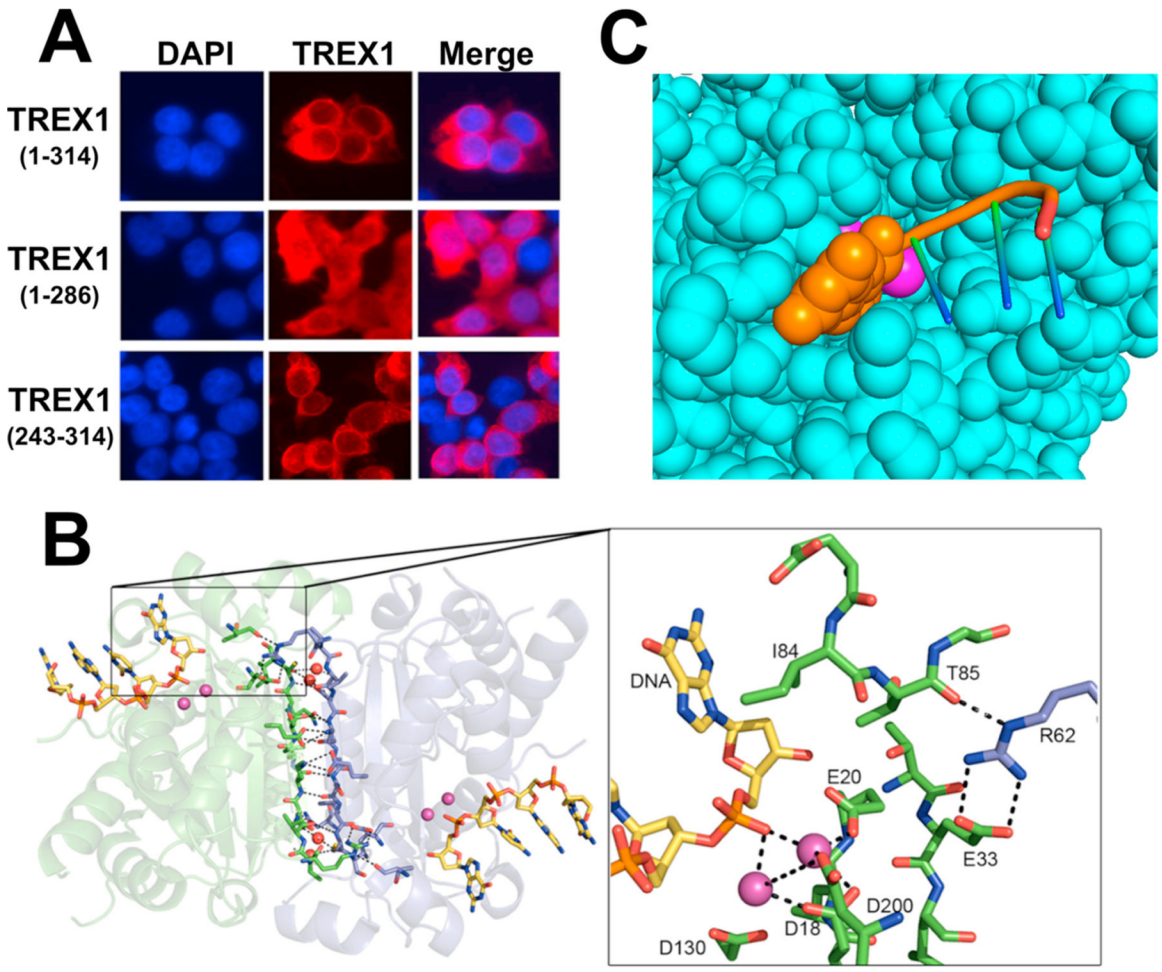

Fig. 1. TREX1 localization and structure.

(A) Microscopy images demonstrating full-length protein (AA 1–314) and C-terminal region (AA 243–314) localizing to perinuclear region/ER, and diffuse localization of TREX1 C-terminal truncation mutant (AA 1–286). Images borrowed from published data [18]. **(B)** The dimer interface and active site of TREX1 with DNA bound (yellow and orange). Magnesium ions are denoted as magenta spheres. Protomers are differentiated by color. Interactions along the dimer interface are represented by dashed lines. Catalysis in the highlighted TREX1 active site (right) is mediated by several key residues in the green protomer, including D18 and D200, as well as residues from the blue protomer, including R62 and R114 (not shown). Image was borrowed from published data [25]. **(C)** A space-filling model of substrate binding in the TREX1 active-site. The 3′-terminal nucleotide (orange spheres) of the 4-mer oligonucleotide substrate is tightly constrained inside the nucleotide binding pocket, such that bulky DNA damage adducts would be sterically hindered. TREX1 atoms are represented as cyan spheres. Graphic was generated in PyMol using Protein Data Bank structure ‘2OA8’.

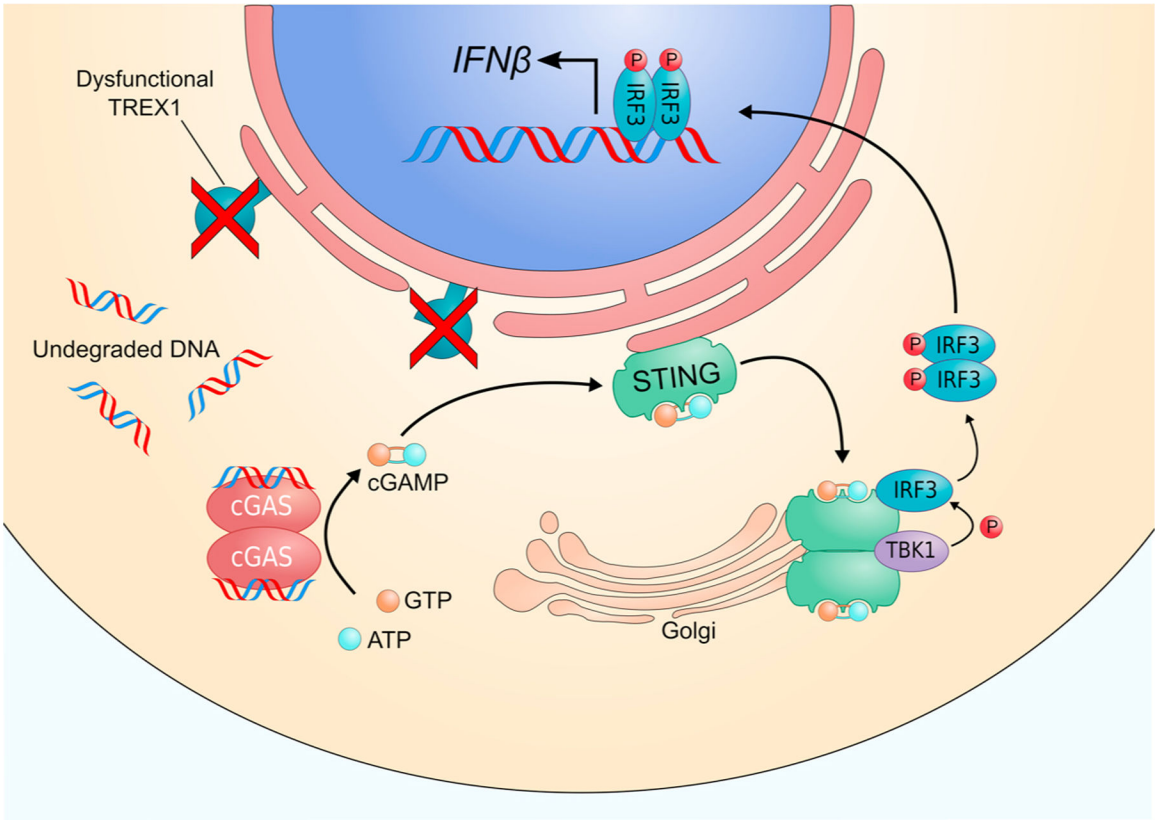

Fig. 2. TREX1 dysfunction triggers the cGAS-STING cytosolic DNA sensing pathway.

Model of how TREX1 catalytic inactivity initiates IFN-I-dependent inflammation. TREX1 dysfunction allows cytosolic accrual of undegraded DNA, cGAS-dependent sensing, cGAMP synthesis, and activation of STING. STING recruitment of TBK1 and TBK1-dependent IRF3 phosphorylation triggers transcription of IFN-I genes, leading to chronic inflammation and autoimmunity.

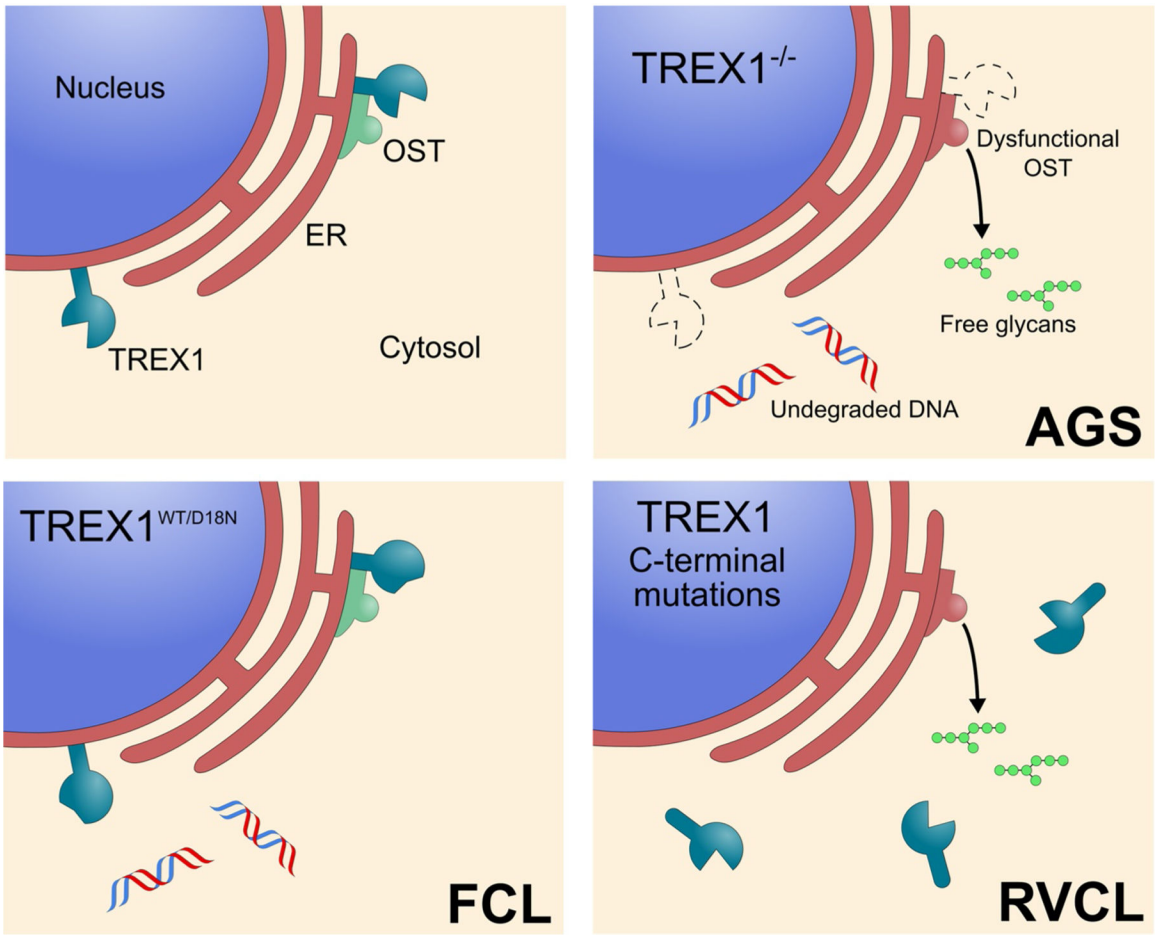

Fig. 3. Heterogeneity in TREX1-linked human diseases likely relates to distinct sources of inflammation.

Cartoon models depicting active sources of inflammation caused by *TREX1* mutations. Full ablation of *TREX1* or highly deleterious mutations that result in no polypeptide being produced (upper right) lead to both failed cytosolic DNA degradation and OST dysregulation, and are associated with the severe AGS phenotype. Point mutation D18N (lower left) renders TREX1 catalytically inactive and leads to failed cytosolic DNA degradation, but preserves OST interactions, consistent with the more mild FCL phenotype observed in humans expressing the D18N allele. C-terminal mutations (lower right) induce TREX1 mislocalization throughout the cytosol but preserve catalytic activity, likely allowing clearance of cytosolic DNA but negatively impacting OST regulation. C-terminal mutations cause RVCL in humans, but a direct link between RVCL and free glycan mismanagement has yet to be definitively established.