Cell

# Linking Retroelements to Autoimmunity

**Vijay G. Bhoj[1] and Zhijian J. Chen[1,2,*]**
[1]Department of Molecular Biology
[2]Howard Hughes Medical Institute
University of Texas Southwestern Medical Center, Dallas, TX 75390-9148, USA
*Correspondence: zhijian.chen@utsouthwestern.edu
DOI 10.1016/j.cell.2008.08.010

**In this issue, Stetson et al. (2008) report a mechanism by which host cells avert an autoimmune response to self-nucleic acids. They show that the nuclease Trex1 prevents the accumulation of DNA derived from endogenous retroelements that, if left unchecked, trigger elevated production of type I interferons leading to autoimmunity.**

The detection of viral nucleic acids by pathogen recognition receptors leads to the production of type I interferons (IFNs) through the activation of NF-κB and interferon regulatory factors (IRFs) (Akira et al., 2006). Although they are a critical element in the response of mammalian cells to viral infection, uncontrolled production of IFNs can also have pathological consequences, including the development of autoimmune disease. In this issue, Stetson et al. (2008) report a new mechanism that suppresses the deleterious induction of IFNs in mice. They show that the nuclease Trex1 prevents the accumulation of reverse-transcribed DNA derived from endogenous retroelements that can trigger IFN production. These findings reveal an unexpected link between retroelements and the development of autoimmune disease.

Trex1 is an abundant 3′ > 5′ exonuclease known to digest single-stranded DNA (ssDNA) (Mazur and Perrino, 1999), and loss-of-function mutations of Trex1 in humans cause autoimmune diseases including Aicardi-Goutieres Syndrome (AGS) and familial chilblain lupus (Crow et al., 2006a). Recent studies have also identified mutations in Trex1 that are associated with systemic lupus erythematosus (Lee-Kirsch et al., 2007). Although mice lacking Trex1 do not develop Aicardi-Goutieres Syndrome or lupus, they have increased mortality due to inflammatory myocarditis (Morita et al., 2004).

To investigate the mechanism underlying the autoimmune diseases caused by Trex1 deficiency, Stetson et al. bred the Trex1-deficient mice with mice lacking IRF3, the IFN receptor (IFNαR1), or RAG2, a DNA recombinase required for the generation of functional lymphocytes. Strikingly, in each case, the mortality and cardiac inflammation observed in Trex1-deficient mice were nearly completely rescued. In addition, the induction of IFN-β by the loss of Trex1 was abrogated by compound deletion of IRF3 or IFNαR1. These results provide compelling evidence that the activation of the IRF3 pathway is responsible for the autoimmune diseases caused by Trex1 deficiency, at least in mice. However, IFN-β induction was observed in mice lacking both Trex1 and RAG2, suggesting that the induction of IFNs is not sufficient to cause the autoimmune phenotypes in the absence of lymphocytes.

How might the loss of Trex1 cause IRF3 activation? Given that Trex1 is a DNA nuclease, a reasonable hypothesis is that cells lacking Trex1 accumulate DNA that activates the IRF3 pathway. Sequence analysis of cytosolic DNA purified from hearts of mice lacking Trex1 showed that they had markedly more cytosolic DNA than wild-type hearts. Moreover, Stetson et al. observed a striking increase in the proportion of DNA corresponding to endogenous retroelements. Further, overexpression of Trex1 inhibited the retrotranscription and integration of two synthetic retroelements (LTR-type and L1). In contrast, Trex1 harboring catalytic site mutations associated with autosomal dominant Aicardi-Goutieres Syndrome were unable to prevent retrotransposition of an LTR-containing element although there was no effect on L1 retrotransposition. These results suggest that a class of Trex1 substrates is derived from retroelements.

The proposal that Trex1 metabolizes DNA derived from retroelements fits nicely with human genetic studies that identify several mutations in genes encoding subunits of RNase H2 in patients with Aicardi-Goutieres Syndrome (Crow et al., 2006b). Like carriers of Trex1 mutations, patients carrying RNase H2 mutations also have elevated levels of IFN-α and develop circulating antinuclear antibodies. That mutations in Trex1 and RNase H2 lead to very similar autoimmune diseases strongly suggests that they function in the same pathway. In light of the new data, it is likely that RNase H2 and Trex1 share a common substrate, the DNA:RNA hybrid derived from endogenous retroelements (Figure 1).

However, retroelement-derived DNA may not be the only substrate metabolized by Trex1. A previous study found that Trex1-deficient mouse embryonic fibroblasts (MEFs) had cell-cycle defects resulting from chronic activation of a DNA-damage checkpoint (Yang et al., 2007). Interestingly, single-stranded DNA (ssDNA) accumulates in the endoplasmic reticulum (ER) in MEFs lacking Trex1 but not in wild-type MEFs. This may be significant as Trex1 is localized to the ER membrane and a missense mutation that generates a truncated protein lacking the C-terminal ER localization domain has been linked to autosomal dominant retinal vasculopathy with cerebral leukodystrophy (Chowdhury et al., 2006; Richards et al., 2007). Isolation of cytoplasmic DNA from Trex1-deficient MEFs revealed a discrete band corresponding to ssDNA of 60–65 nucleotides that is absent in

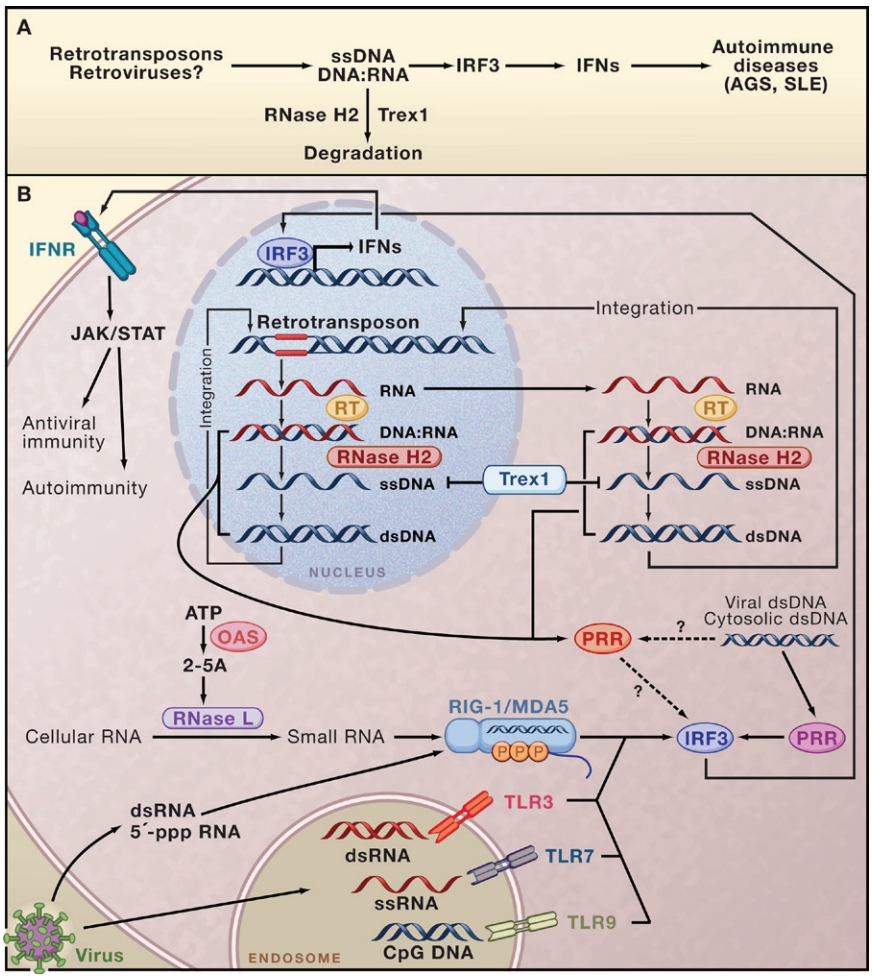


**Figure 1. Nucleic Acid Sensing and Autoimmunity**

(A) Single-stranded DNA (ssDNA) and DNA:RNA hybrids generated during the replication of retroviruses or endogenous retrotransposons may normally be degraded by the RNase H2 and Trex1 nucleases. Mutations inactivating either enzyme result in the accumulation of these nucleic acids, which through putative sensors lead to inappropriate activation of IRF3. Activated IRF3 induces the synthesis of type I interferons (IFNs), which stimulate the immune system leading to autoimmune diseases including Aicardi-Goutieres Syndrome (AGS) and systemic lupus erythematosus (SLE).

(B) Toll-like receptors (TLRs) 3, 7/8, and 9 detect double-stranded RNA (dsRNA), single-stranded RNA (ssRNA), and CpG DNA, respectively, which are derived from pathogens or phagocytosed apoptotic cells. Upon binding to these ligands within endosomes, TLRs activate a signaling cascade leading to the activation of IRF3. Activated IRF3 enters the nucleus and together with other activated transcription factors induces IFN production. In the cytosol, virally derived RNAs bind to the RNA helicases, RIG-I and MDA5, which then activate IRF3, resulting in IFN production. A feedforward loop that stimulates this RNA-sensing pathway is induced by IFNs. 2′-5′ oligoadenylate synthetase (2′-5′OAS), an IFN-induced protein, synthesizes 2′-5′ linked oligoadenylate (OA) from ATP. OA binds to and stimulates another IFN-induced protein, RNaseL, which processes cytosolic host RNA into small RNA to activate RIG-I and MDA5. Cytosolic dsDNA derived from bacteria, viruses, or the host also activate IRF3 through an unidentified receptor. Recent data implicating the DNA exonuclease, Trex1, in the regulation of IRF3 suggest the existence of a receptor system that detects DNA-containing species generated during the replication of endogenous retrotransposons and retroviruses. In cells lacking functional Trex1, these DNA species accumulate and activate IRF3 and IFN synthesis. Appropriate stimulation by IFNs during infections leads to antiviral immune responses while inappropriate activation, sometimes in the absence of infection, can lead to autoimmunity. PRR: pattern recognition receptor; 5′pppRNA: 5′-triphosphorylated RNA. RT: reverse transcriptase.

wild-type MEFs. Based on these observations, Yang et al. proposed that Trex1 is involved in the metabolism of ssDNA generated as a result of aberrant DNA replication (Yang et al., 2007). Surprisingly, Stetson et al. found no apparent cell-cycle defect or activation of the DNA-damage checkpoint in primary Trex1-deficient MEFs. These differing results warrant further study of the role of Trex1 in the surveillance of DNA replication and damage products. Given that the disease in Trex1-deficient mice can be "cured" by deleting IRF3, IFN$\alpha$R1, or RAG2, aberrant activation of the IRF3 pathway would appear to be the major contributor to the phenotype. However, retroelement-derived DNA may not be the sole activator of the IRF3 pathway in the case of Trex1 deficiency. It is possible that the distinct ER-associated ssDNA identified by Yang et al., which may or may not be encoded by retroelements, could contribute to IRF3 activation. It is also possible that ssDNA-induced DNA damage leads to IRF3 activation. IRF3 and IFNs are also known to activate p53, which may cause cell-cycle arrest, senescence, or apoptosis. Further complicating the issue, Trex1 has been shown to be a part of a nuclease complex known as SET that is localized to the ER. SET degrades ssDNA to facilitate caspase-independent cell death by granzyme A and perforin (Chowdhury et al., 2006). Thus, multiple substrates of Trex1 may accumulate in the absence of Trex1 and activate the IRF3 pathway.

The work of Stetson et al. raises several important questions. What are the ligands that activate the IRF3 pathway? In principle, these ligands could include DNA:RNA hybrids, single-stranded DNA, or double-stranded DNA (Figure 1). What and where is the sensor (or sensors) that detects the retroelement-derived ligand(s)? Where does the catabolism of retroelement-derived DNA take place in the cell and how does reverse-transcribed DNA gain access to Trex1 or a putative DNA sensor? Do retroviral infections induce IFN production through the same mechanism as the endogenous retroelements? Finally, can blocking reverse transcription of retroelements prevent autoimmunity caused by the loss of Trex1 or RNase H2? Stetson et al. began to address this question and found that AZT, a reverse transcriptase inhibitor, did not alleviate the disease phenotypes in Trex1-deficient mice; however, as they pointed out, AZT may not inhibit all reverse transcriptases in cells. Further investigation into these questions should yield deep insights into this cell-intrinsic mechanism of autoimmunity.

## ACKNOWLEDGMENTS

We thank J. Cabrera for expert assistance in preparation of the figure. Research in the Chen laboratory is supported by grants from the NIH and the Welch Foundation. Z.J.C. is an HHMI investigator.

## REFERENCES

Akira, S., Uematsu, S., and Takeuchi, O. (2006). Cell *124*, 783–801.

Chowdhury, D., Beresford, P.J., Zhu, P., Zhang, D., Sung, J.S., Demple, B., Perrino, F.W., and Lieberman, J. (2006). Mol. Cell *23*, 133–142.

Crow, Y.J., Hayward, B.E., Parmar, R., Robins, P., Leitch, A., Ali, M., Black, D.N., van Bokhoven, H., Brunner, H.G., Hamel, B.C., et al. (2006a). Nat. Genet. *38*, 917–920.

Crow, Y.J., Leitch, A., Hayward, B.E., Garner, A., Parmar, R., Griffith, E., Ali, M., Semple, C., Aicardi, J., Babul-Hirji, R., et al. (2006b). Nat. Genet. *38*, 910–916.

Lee-Kirsch, M.A., Gong, M., Chowdhury, D., Senenko, L., Engel, K., Lee, Y.A., de Silva, U., Bailey, S.L., Witte, T., Vyse, T.J., et al. (2007). Nat. Genet. *39*, 1065–1067.

Mazur, D.J., and Perrino, F.W. (1999). J. Biol. Chem. *274*, 19655–19660.

Morita, M., Stamp, G., Robins, P., Dulic, A., Rosewell, I., Hrivnak, G., Daly, G., Lindahl, T., and Barnes, D.E. (2004). Mol. Cell. Biol. *24*, 6719–6727.

Richards, A., van den Maagdenberg, A.M., Jen, J.C., Kavanagh, D., Bertram, P., Spitzer, D., Liszewski, M.K., Barilla-Labarca, M.L., Terwindt, G.M., Kasai, Y., et al. (2007). Nat. Genet. *39*, 1068–1070.

Stetson, D.B., Ko, J.S., Heidmann, T., and Medzhitov, R. (2008). Cell, this issue.

Yang, Y.G., Lindahl, T., and Barnes, D.E. (2007). Cell *131*, 873–886.