A novel pathway to detect and cope with exogenous dsDNA

Shouhei Kobayashi1 and Tokuko Haraguchi1,2,3,*

1Advanced ICT Research Institute Kobe; National Institute of Information and Communications Technology; Nishi-ku, Japan; 2Graduate School of Frontier Biosciences; Osaka University; Suita, Japan; 3Graduate School of Science; Osaka University; Toyonaka, Japan

How a living cell responds to exogenous materials is one of the fundamental questions in the life sciences. In particular, understanding the mechanisms by which a cell recognizes exogenous double-stranded DNA (dsDNA) is important for immunology research because it will facilitate the control of pathogen infections that entail the presence of exogenous dsDNA in the cytoplasm of host cells. Several cytosolic dsDNA sensor proteins that trigger innate immune responses have been identified and the downstream signaling pathways have been investigated. However, the events that occur at the site of exogenous dsDNA when it is exposed to the cytosol of the host cell remain unknown. Using dsDNA-coated polystyrene beads incorporated into living cells, we recently found that barrier-to-autointegration factor (BAF) binds to the exogenous dsDNA immediately after its appearance in the cytosol and plays a role in DNA avoidance of autophagy. Our findings reveal a novel pathway in which BAF plays a key role in the detection of and response to exogenous dsDNA.

The rapid detection of exogenous materials and subsequent responses are important for the survival of living cells in variable environments.1 A great deal of effort has been made to identify cellular factors that detect the invasion of exogenous dsDNA and mediate immune responses, such as type I interferon production.2-11 Downstream pathways after dsDNA detection have also been extensively investigated.12-13 Recently, stimulator of interferon genes (STING), which is an endoplasmic reticulum-associated protein that mediates type I interferon responses, has been identified as a critical factor in the regulation of an innate immune signaling pathway triggered by the invasion of exogenous dsDNA.14 However, cell biological information regarding when and where cytosolic dsDNA sensors detect exogenous dsDNA is lacking owing to the difficulties in monitoring exogenous dsDNA that enters the cytosol of cells.

Understanding intracellular fates of exogenous dsDNA after endosome breakdown is also important for the development of appropriate dsDNA carriers that ensure an efficient and yet safe nucleic acid delivery system. Many non-viral dsDNA carriers, including lipid-based and polymer-based DNA carriers, have been developed and are widely used for transgene expression.15,16 Most such dsDNA carriers were designed to induce efficient endosome breakdown because the endosome membrane is the first cellular barrier against transgene expression.17 However, even when free dsDNA is directly microinjected into the cytoplasm of a cell, only limited amount is effectively delivered to the nucleus,18,19 suggesting that other barriers to transgene expression exist in the cytoplasm. Because nuclear injection of dsDNAs is more effective than cytoplasmic injection for transgene expression from both non-viral dsDNA19 and cloned viral dsDNA,20 the nuclear envelope (NE) may act as a potent structural barrier for the delivery of dsDNA to the nucleus. Thus, understanding the fate of the exogenous dsDNA that invades the cytosol after endosome breakdown is important to achieve an efficient gene delivery system using non-viral carriers.

We recently reported the use of DNA-coated beads in which barrier-to-autointegration factor (BAF) acts as a cytosolic dsDNA sensor in mammalian cells.21 BAF, a highly conserved DNA binding

Keywords: autophagy, barrier-to-autointegration factor, DNA sensor, endosome breakdown, exogenous DNA, live CLEM, nuclear envelope

© Shouhei Kobayashi and Tokuko Haraguchi
*Correspondence to: Tokuko Haraguchi, Email: tokuko@nict.go.jp
Submitted: 06/12/2015
Revised: 06/16/2015
Accepted: 06/17/2015
http://dx.doi.org/10.1080/19420889.2015.1065361

This is an Open Access article distributed under the terms of the Creative Commons Attribution-Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/), which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited. The moral rights of the named author(s) have been asserted.

Addendum to Shouhei Kobayashi, Takako Koujin, Tomoko Kojidani, Hiroko Osakada, Chie Mori, Yusuke Hiraoka, and Tokuko Haraguchi. BAF is a cytosolic DNA sensor that leads to exogenous DNA avoiding autophagy. Proc. Natl. Acad. Sci. USA, 2015; 112 (22): 7027–7032, published ahead of print May 19, 2015, doi:10.1073/pnas.1501235112

www.tandfonline.com
Communicative & Integrative Biology

Communicative & Integrative Biology 8:5, e1065361; September/October 2015; Published with license by Taylor & Francis Group, LLC
protein, was first discovered as a cellular factor that prevents retroviral DNA from suicidal auto-integration and ensures its integration into host DNA. In addition to this virus-related function, BAF is known to function in host cell NE assembly and in S-phase progression. We found that when dsDNA-coated beads were incorporated into HeLa cells with a transfection reagent, BAF accumulated at the site of exogenous dsDNA immediately after its appearance in the cytosol at endosome breakdown (Fig. 1A). BAF also accumulated at the site of microinjected dsDNAs, independent of their forms (linearized, circularized, or complexed with a transfection reagent) (Fig. 1C). Our findings suggest that BAF is able to detect various forms of dsDNA in living cells, even when it forms a complex with other molecules. Our finding is consistent with those of previous reports indicating that BAF binds to dsDNA in a sequence-nonspecific manner in vitro. Interestingly, our experimental system using DNA beads revealed that the NE-like membrane started to assemble around dsDNA within several minutes after its detection by BAF, and enwrapped the dsDNA within another 10–15 min in a BAF-dependent manner (compare Fig. 1A and B). This BAF-mediated NE-like membrane assembly at the dsDNA protected the dsDNA from autophagy (Fig. 1A). This was the first direct evidence of intracellular membrane remodeling that is triggered at the site of exogenous dsDNA invasion. The physiological significance of this BAF-mediated sequestration of exogenous dsDNA from autophagy remains unknown. Because autophagy is believed to act as an intracellular defense system that leads the targets to degradation, BAF acts in a somewhat opposite manner from its defense function. We speculate that BAF-mediated NE-like membrane assembly may function as a stronger defense system against exogenous dsDNAs than.

Figure 1. BAF-mediated pathway to detect and cope with exogenous dsDNA in mammalian cells. (A) When dsDNA-coated beads were incorporated into cells with transfection reagents, BAF detected exogenous dsDNA immediately after endosome breakdown around the beads, and then induced assembly of nuclear envelope (NE)-like membranes. This NE-like membrane assembly leads to the avoidance of exogenous dsDNA from autophagy that targets the remnants of endosome membranes. (B) Knockdown of BAF caused a significant decrease in the assembly of NE-like membranes and increased the formation of autophagic membranes around the DNA-beads. (C) When dsDNAs were microinjected into cells, BAF immediately accumulated at the site of injected dsDNA and induced NE-like membrane assembly at the region. Symbols are common in Figs. 1A–C.
autophagy by rapidly compacting and isolating the DNA to prevent gene expression and replication. In fact, it is known that BAF can compact viral DNAs by bridging them in vitro,27-29 and that BAF inhibits viral DNA replication in vaccinia virus infections by binding to the DNA,30 supporting our idea. Another possibility is that BAF-mediated NE-like membrane assembly unexpectedly assists exogenous dsDNA, conferring protection from degradation, and this may allow for the exogenous dsDNA to be imported into the nucleus during cell cycle progression. Further studies are needed to understand this enigmatic behavior of BAF with respect to exogenous dsDNA, and to understand the relationship between BAF-mediated NE-like membrane assembly and autophagic membrane assembly.

In conclusion, direct visualization of BAF-mediated detection of exogenous dsDNA exposed to the cytosol of host cells shows an intimate link between the detection of exogenous dsDNA and subsequent intracellular responses. Our bead-mediated method will provide new insight into such responses to the entry of exogenous materials into a cell.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Funding
This study is supported by CREST of JST to T.H., and by JSPS Kakenhi Grant Numbers 22770205, 25650073 to S.K., and 21370094 to T.H.

References
1. Kawasaki T, Kawai T, Akira S. Recognition of nucleic acids by pattern-recognition receptors and its relevance in autoimmunity. Immuno Rev 2011; 243:61-73; PMID:21884167; http://dx.doi.org/10.1111/j.1600-065X.2011.01048.x
2. Takaoka A, Wang Z, Choi MK, Yanai H, Negishi H, Ban T, Lu Y, Miyagishi M, Kodama T, Honda K, et al. DAI (DM1-12BP1) is a cytosolic DNA sensor and an activator of innate immune response. Nature 2007; 448:501-5; PMID:17618271; http://dx.doi.org/10.1038/nature06013
3. Fernandes-Alnemri T, Yu JW, Darra P, Wu J, Alnemri ES. AIM2 activates the inflammasome and cell death in response to cytoplasmic DNA. Nature 2009; 458:509-13; PMID:19158676; http://dx.doi.org/10.1038/nature087710
4. Hornung V, Ablaser A, Charrel-Dennis M, Barenfend F, Horvarth G, Caffrey DR, Latz E, Fritzgerald KA. AIM2 recognizes cytosolic dsDNA and forms a caspase-1-activating inflammasome with ASC. Nature 2009; 458:514-18; PMID:19158675; http://dx.doi.org/10.1038/nature07725
5. Chiu YH, Macmillan JR, Chen ZJ. RNA polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway. Cell 2009; 138:576-91; PMID:19963170; http://dx.doi.org/10.1016/j.cell.2009.06.015
6. Yanai H, Ban T, Wang Z, Choi MK, Kawamura T, Negishi H, Nakasato M, Lu Y, Hangai S, Koshiba R, et al. HMGB proteins function as universal sentinels for nucleic-acid-mediated innate immune responses. Nature 2009; 462:299-103; PMID:19899330; http://dx.doi.org/10.1038/nature08512
7. Kobiyama K, Takeishi F, Jouani N, Sakaue-Sawano A, Miyawaki A, Ishii KJ, Kawai T, Suzaki S, Hiranu H, Ishii N, et al. Extrachromosomal histone H2B mediates innate antiviral immune responses induced by intracellular double-stranded DNA. J Virol 2010; 84:822-32; PMID:19960922; http://dx.doi.org/10.1128/JVI.01339-09
8. Yang Z, An H, Liu X, Wen M, Zheng Y, Rui Y, Cao X. The cytosolic nucleic acid sensor LRFRP1 mediates the production of type I interferon via a beta-catenin-dependent pathway. Nat Immunol 2010; 11:487-94; PMID:20453884; http://dx.doi.org/10.1038/ni.1876
9. Unterholzer L, Keating SE, Baran M, Horan KA, Jenson SB, Sharma S, Siros CM, Jin T, Latz E, Xiao TS, et al. IFI16 is an innate immune sensor for intracellular DNA. Nat Immunol 2010; 11:997-1004; PMID:20757534; http://dx.doi.org/10.1038/ni.1938
10. Zhang Z, Yuan B, Mao M, Lu N, Kim T, Liu YJ. The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells. Nat Immunol 2011; 12:959-65; PMID:21892174; http://dx.doi.org/10.1038/ni.2035
11. 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 2013; 339:786-91; PMID:23558413; http://dx.doi.org/10.1126/science.1232458
12. Keating SE, Baran M, Bowie AG. Cytosolic DNA sensors regulating type I interferon induction. Trends Immunol 2011; 32:574-81; PMID:21940216; http://dx.doi.org/10.1016/j.it.2011.08.004
13. Barber GN. Cytoplasmic DNA innate immune pathways. Immuno Rev 2011; 243:99-108; PMID:21884170; http://dx.doi.org/10.1111/j.1600-065X.2011.01051.x
14. Ishikawa H, Barber GN. The STING pathway and regulation of innate immune signaling in response to DNA pathogens. Cell Mol Life Sci 2011; 68:1157-65; PMID:21611320; http://dx.doi.org/10.1007/s00018-010-0605-2
15. Zhou R, Geger RC, Dean DA. Intracellular trafficking of nucleic acids. Expert Opin Drug Deliv 2004; 1:127-40; PMID:16296725; http://dx.doi.org/10.1517/17425247.1.1.127
16. Pichon C, Billiet L, Midoux P. Chemical vectors for nuclear delivery of biologicals. J Control Release 2011; 151:220-8; PMID:21078351; http://dx.doi.org/10.1016/j.jconrel.2011.10.004
17. Zhang B, Friedrich H, Dehne J, Reiter K, Usadel M, Kaul C, Kremmer E, Kissel T. B1 barrier-to-autointegration factor is a coiled-coil DNA sensor that leads to exogenous DNA avoiding autophagy. Proc Natl Acad Sci USA 2011; 108:1727-32; PMID:21591860; http://dx.doi.org/10.1073/pnas.1011352108
18. Lee MS, Craigie R. A previously unidentified host protein protects retroviral DNA from autointegration. Proc Natl Acad Sci USA 1998; 95:1528-33; PMID:9645094; http://dx.doi.org/10.1073/pnas.951528
19. Jacques JM, Stevenson M. The inner-nuclear-envelope protein emerin regulates HIV-1 infectivity. Nature 2006; 441:641-5; PMID:16680152; http://dx.doi.org/10.1038/nature04682
20. Haraguchi T, Joujin T, Segura-Totten M, Lee KK, Matsuoka Y, Yoneda Y, Wilson KL, Hirazuka Y. BAF is required for emerin assembly into the reforming nuclear envelope. J Cell Sci 2001; 114:4575-85; PMID:11792882
21. Haraguchi T, Joujin T, Kojijani T, Kojijin T, Shimoi T, Osakada H, Mori C, Yamamoto A, Hirazuka Y. Live cell imaging and electron microscopy reveal dynamic processes of BAF-directed nuclear envelope assembly. J Cell Sci 2008; 121:2540-54; PMID:18628300; http://dx.doi.org/10.1242/jcs.034597
22. Haraguchi T, Joujin T, Osakada H, Kojijin T, Mori C, Masuda H, Hirazuka Y. Nuclear localization of barrier-to-autointegration factor is correlated with progression of S phase in hela cells. J Cell Sci 2007; 120:1967-77; PMID:17519288; http://dx.doi.org/10.1242/jcs.03461
23. Zheng R, Ghirlando R, Lee MS, Mizuuchi K, Krause M, Craigie R. Barrier-to-autointegration factor (BAF) bridges DNA in a discrete, higher-order nucleoprotein complex. Proc Natl Acad Sci USA 2000; 97:8997-9002; PMID:10908652; http://dx.doi.org/10.1073/pnas.1501235112
24. Bradley CM, Ronning DR, Ghirlando R, Cragie R. The inner-nuclear-envelope barrier-to-autointegration factor. Nat Struct Mol Biol 2007; 12:935-6; PMID:17482380; http://dx.doi.org/10.1038/nsmb989
25. Lee MS, Craigie R. A previously unidentified host protein protects retroviral DNA from autointegration. Proc Natl Acad Sci USA 1998; 95:1528-33; PMID:9645094; http://dx.doi.org/10.1073/pnas.951528
26. Wolfe MS, Trakman P. Porcine B1 kinase overcomes barrier to autointegration factor, a host defense against virus replication. Cell Host Microbe 2007; 1:187-97; PMID:18005698; http://dx.doi.org/10.1016/j.chom.2007.03.007

www.tandfonline.com
Communicative & Integrative Biology
e1065361-3