Published in final edited form as:

Nat Immunol. 2009 November ; 10(11): 1137–1139. doi:10.1038/ni1109-1137.

NDP52: the missing link between ubiquitinated bacteria and autophagy

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Abstract

Mammalian cells ubiquitinate bacteria that erroneously enter the cytosol, and target these intruding microbes for destruction by autophagy. New work shows that the protein NDP52 directly binds to ubiquitinated bacteria and facilitates the assembly of an autophagic membrane that surrounds these invaders.

Mammalian cells are capable of mounting autonomous defense responses that have the capacity to eliminate invading intracellular bacterial pathogens. These responses are diverse and can defend against pathogens that thrive within vacuolar compartments as well as microbes that escape into the nutrient rich milieu of the cytosol. An emerging defense mechanism capable of eliminating bacteria that gain access to the host cytosol involves ubiquitination of the pathogen and clearance of the organism by the host autophagy machinery. Until now, proteins directly involved in detecting ubiquitinated bacteria and targeting them for destruction by the autophagy pathway have remained unidentified. In this issue of *Nature Immunology*, Thurston *et. al.* report that the protein NDP52 functions as a receptor that recognizes ubiquitinated bacteria and coordinates their destruction by the autophagy pathway¹.

Salmonella enterica species are common food-borne pathogens that have the ability to induce their own uptake into mammalian cells that are normally non-phagocytic, such as enterocytes². Once internalized, *S. enterica* modulate the transport and fusion of the vacuoles in which they reside to create a specialized compartment that supports intracellular replication³. Occasionally, this *S. enterica*-containing vacuole will lyse, which results in the release of bacteria into the cytosol of the host cell, a location where these bacteria can proliferate rapidly. Vacuole lysis occurs spontaneously at a low frequency, and is greatly enhanced by disrupting the function of bacterial effector proteins critical for maintaining vacuole integrity⁴. Remarkably, it was observed that the surface of intact cytosolic *S. enterica* is targeted by the host ubiquitin conjugation system, whereas pathogenic bacteria that have evolved specific mechanisms to replicate in the cytosol manage to avoid host ubiquitination⁵. Additional studies revealed that ubiquitinated *S. enterica* were targeted by the host autophagy system, which sequestered the bacteria in autophagosomes and restricted their ability to replicate intracellularly⁶. Similar results were obtained using the gram-positive organism *Streptococcus pyogenes*⁷, demonstrating that the host autophagy pathway plays a general and important role in defending cells against cytosolic intruders.

Independent of studies on the role autophagy might play in clearance of cytosolic bacteria, it was reported that vacuole lysis and intracellular proliferation of *S. enterica*, as well as enteropathogenic *Escherichia coli* and *S. pyogenes*, was greatly enhanced in mouse embryonic fibroblasts deficient in TANK binding kinase (TBK1)⁸. This phenomenon was not connected

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with the established role of TBK1 in the canonical signaling pathway leading to type I interferon induction. In an effort to understand why TBK1 was important for maintaining the integrity of the Salmonella-containing vacuole (SCV), it was discovered that TBK1-deficient cells contain elevated amounts of aquaporin-19. Aquaporins comprise a family of channel proteins that control water homeostasis ⁹, suggesting that in the absence of TBK1 the ability of the cell to balance the water exchange between the cytosol and the SCV during infection was impaired, resulting in vacuolar disruption and enhanced entry of *S. enterica* into the cytosol. Thus, the hyperproliferation of cytosolic bacteria in the TBK1-deficient cells was thought to be a consequence primarily of enhanced vacuole disruption. The findings by Thurston *et al.*, however, indicate that TBK1 plays additional roles in preventing replication of cytosolic bacteria.

Thurston *et al.* made the important observation that the host proteins Nap1 and Sintbad co-localize with ubiquitinated *Salmonella*. Because Nap1 and Sintbad are upstream regulators of TBK1, these data suggested that TBK1 might also be involved in regulating host defense responses to *S. enterica* after vacuole lysis occurs. Nap1 and Sintbad were found to contain a homologous amino-terminal region required for *in vivo* interaction with ubiquitin. However, this amino-terminal region did not bind ubiquitin directly, suggesting that there must be adapter proteins that link the TBK1-binding proteins Nap1 and Sintbad to ubiquitinated bacteria. Affinity purification of host proteins capable of linking Nap1 and ubiquitin resulted in the identification of nuclear dot protein 52 (NDP52). *In vitro* binding studies revealed that a zinc finger domain in NDP52 interacted with ubiquitin and a SKICH domain engaged Nap1, demonstrating that NDP52 was functioning as an adapter linking ubiquitin and Nap1. Lastly, immunoprecipitation of NDP52 from mammalian cells recovered a complex that contained TBK1, IKK ϵ , Nap1 and Sintbad, demonstrating that NDP52 is a component of a TBK1 signaling complex (Figure 1).

In addressing the role of NDP52 in detecting cytosolic bacteria, it was found that NDP52 co-localized with ubiquitinated *S. enterica*, whereas a NDP52 paralog called CoCoA, which lacks a ubiquitin binding domain, did not decorate the surface of cytosolic bacteria. Although immunofluorescence microscopy was successful at verifying a signaling complex containing NDP52, Nap1, Sintbad, and TBK1 surrounding cytosolic *S. enterica*, localization studies were insufficient to conclude that NDP52 function was necessary for a host response that protects against cytosolic bacteria. This question was resolved by silencing NDP52 expression. The total number of bacteria and the number of ubiquitin-positive bacteria increased in *S. enterica*-infected cells expressing NDP52-specific siRNA. Additionally, it was found that NDP52 was important for restricting cytosolic replication of *S. pyogenes*, which is ubiquitinated if it gains access to the cytosol, but not *Shigella flexneri*, which has evolved mechanisms to efficiently replicate in the cytosol that include the ability to avoid being ubiquitinated. Thus, Thurston *et al.* establish that NDP52 is a *bone fide* innate immune receptor that is involved in cytosolic surveillance.

But what is the mechanism by which NDP52 restricts the replication of cytosolic bacteria? Because the clearance of cytosolic bacteria involves the autophagy machinery, a possible role for NDP52 in regulating autophagy of bacteria was investigated. Microtubule-associated protein 1 light chain 3 (LC3) is recruited to autophagic membranes at an early stage of autophagosome biogenesis ¹⁰. NDP52 and LC3 were found to co-localize around cytosolic *S. enterica*, suggesting a connection between these factors. Reducing NDP52 expression resulted in a decrease in the number of autophagosomes containing *S. enterica*, and NDP52 bound directly to LC3 *in vitro*. Together, these findings provide strong evidence that NDP52 is a critical adapter capable of directing the formation of an autophagic membrane around ubiquitinated bacteria in the cytosol of mammalian cells.

The unique features of NDP52 that are important for detecting cytosolic bacteria and activating cell autonomous responses remain to be determined. It is possible that a specific pattern of ubiquitin linkages displayed on the surface of cytosolic bacteria is bound preferentially by NDP52; this would confer specificity upon the recruitment of NDP52 to the poly-ubiquitin coat displayed by these microbes. The ability of NDP52 to serve as an adapter for TBK1 also appears to be critical in the cell autonomous response, but what role TBK1 plays in association with NDP52 remains to be determined. Importantly, enhanced replication of *S. enterica* was observed in cells overexpressing aquaporin-1 and treated with TBK1-specific or NDP52-specific siRNA, implying a role for TBK1 not only in regulating aquaporin-1 abundance, but also in regulating cellular functions by a mechanism that involves NDP52 and that occurs after bacteria gain access to the cytosol.

It has not been determined whether TBK1 in association with NDP52 is important for the initiation of autophagosome formation, so it remains a formal possibility that TBK1 promotes the assembly or maturation of the autophagic vacuole that eventually will sequester ubiquitinated cytosolic bacteria. It would also be worthwhile investigating whether the protective functions conferred by TBK1 are related in any way to activities requiring the proteasome. Early studies that focused on ubiquitination of *S. enterica* in the cytosol showed that treating host cells with proteasome inhibitors enhanced bacterial replication⁵. The exact role the proteasome plays in restricting bacterial replication in the cytosol is not well understood, although it has been suggested that in macrophages the proteasome may play a direct role in degrading cytosolic bacteria. Thus, TBK1 may be involved in regulating proteasome-dependent events occurring at the surface of ubiquitinated bacteria. Of course there exist many other possibilities of how TBK1 may promote cell autonomous responses directed against cytosolic bacteria, making future investigations into this pathway of innate defense an exciting and important research venture.

Acknowledgments

This work was supported by NIH grant R01AI41699 (CRR) and F32GM084485 (SI).

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Figure 1. Host response to cytosolic bacteria

Upon contact with host cells *S. enterica* stimulate uptake and trafficking through the activities of bacterial effector proteins delivered by type III secretion systems. The vacuolar compartment in which *S. enterica* resides (SCV) matures into a specialized organelle that supports bacterial replication. Vacuole maintenance failures result in a small but significant number of *S. enterica* gaining access to the host cytosol. Cytosolic bacteria are detected and targeted for ubiquitination by an unknown ubiquitin ligase enzyme (UBL). The autophagic receptor NDP52 binds ubiquitinated bacteria in the cytosol. NDP52 functions as a scaffold for the assembly of a TBK1-Sintbad-Nap1 signaling complex, and recruits autophagic membranes through an interaction with LC3. TBK1 function is also important for regulating abundance of aquaporin-1, which maintains SCV integrity, and in activating a host type 1 interferon response that assists in clearing cytosolic bacteria.