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Adaptor-Mediated Trafficking of Tank Binding Kinase 1 During Diverse Cellular Processes

Swagatika Paul[1] | Sahitya Ranjan Biswas[2] | Julia P. Milner[3] | Porter L. Tomsick[3] | Alicia M. Pickrell[3]

[1]Graduate Program in Biomedical and Veterinary Sciences, Virginia-Maryland College of Veterinary Medicine, Blacksburg, Virginia, USA | [2]Translational Biology, Medicine, and Health Graduate Program, Virginia Polytechnic Institute and State University, Roanoke, Virginia, USA | [3]School of Neuroscience, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA

Correspondence: Alicia M. Pickrell (alicia.pickrell@vt.edu)

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ABSTRACT

The serine/threonine kinase, Tank Binding Kinase 1 (TBK1), drives distinct cellular processes like innate immune signaling, selective autophagy, and mitosis. It is suggested that the translocation and activation of TBK1 at different subcellular locations within the cell, downstream of diverse stimuli, are driven by TBK1 adaptor proteins forming a complex directly or indirectly with TBK1. Various TBK1 adaptors and associated proteins like NAP1, TANK, SINTBAD, p62, optineurin (OPTN), TAX1BP1, STING, and NDP52 have been identified in facilitating TBK1 activation and recruitment with varying overlapping redundancy. This review focuses on what is known about these proteins, their interactions with TBK1, and the functional consequences of these associations. We shed light on underexplored areas of research on these TBK1 binding partners while emphasizing how future research is required to understand the function and flexibility of TBK1 signaling and crosstalk or regulation between different biological processes.

1 | Introduction

TBK1 mediates diverse signaling pathways in the innate immune response [1–3], selective autophagy pathways such as mitophagy [4–9], xenophagy [10, 11], and lysophagy [12], and mitosis [13–16] by its activation at distinct subcellular locations. The first functional role ascribed to TBK1 was in the context of innate immune signaling [1, 2], playing a regulatory role in both NF-κB and Type I interferon pathways, which are triggered by a wide range of immunogenic stimuli [3, 10, 11, 17]. More recently, TBK1 has also been found to play key roles during selective autophagy, the removal of intracellular material for lysosomal degradation. TBK1 is recruited to ubiquitinated cargo by its direct or indirect binding of autophagy adaptors like p62, OPTN, NBR1, TAX1BP1, and NDP52 [4, 7–9, 12, 16, 18]. This association of TBK1 with these autophagy receptors is essential for the recruitment of additional factors to initiate autophagosome engulfment of this cargo [4, 16, 19, 20]. TBK1 was also discovered to participate in cell division, where its activation and localization on the centrosomes are necessary for proper mitotic progression [14, 15, 21].

Abbreviations: aa, amino acid; ALS, amyotrophic lateral sclerosis; CC, coiled coil; cDNA, complimentary DNA; cGAMP, cyclic GMP–AMP; cGAS, cGAMP synthase; CNS, central nervous system; CTD, C-terminal domain; EGF, epidermal growth factor; FTD, frontal temporal dementia; GM-DC, GM-CSF responsive dendritic cells; htt, huntingtin; IRF, interferon regulator factor; ISGs, interferon-stimulated genes; KD, kinase domain; KO, conditional knockout; KO, knockout; LBD, ligand binding domain; LIR, LC3 interacting region; MEFs, mouse embryonic fibroblasts; NAP1, nak-associated protein 1; NDP52, nuclear dot protein 52; NKT, natural killer T cells; OPTN, Optineurin; RLRs, RIG-I-like receptors; SDD, scaffolding dimerization domain; TANK, TRAF family member associated with NF-κB activator; TBD, TBK1 binding domain; TBK1, Tank binding kinase 1; TBKBP1, TBK1 binding protein 1; TDP-43, transactive response DNA binding protein 43; TICAM-1, TIR-containing adaptor molecule 1; TIR, Toll/interleukin 1 receptor; TLF, Toll-like receptor; UBAN, ubiquitin binding domain; ULD, ubiquitin-like domain.

Swagatika Paul and Sahitya Ranjan Biswas co-first authorship.

The crystal structure of TBK1 indicates that an adaptor protein is required for its activation [18, 22–24]. Binding of an adaptor protein induces a conformational change of TBK1, resulting in its autophosphorylation at serine 172 and activation of the kinase domain after dimerization [20, 22, 24]. Adaptor protein binding facilitates its activation at different subcellular locales [4, 25, 26]. Multiple adaptors like NAP1, TANK, SINTBAD, and OPTN contain a common TBK1 binding domain, which competitively binds to the C-terminal domain (CTD) of TBK1 during mitophagy and innate immune signaling [18, 27, 28]. In this review, we will highlight the work done in understanding how adaptors mediate activation and trafficking of TBK1 across different stimuli, what questions remain in the field, and how abnormal activation or subcellular localization of TBK1 contributes to one of the most common neurodegenerative diseases.

2 | Discovery of TBK1

TBK1 was first discovered in 1999 during pulldown experiments designed to elucidate how TANK and TRAF2 modulated NF-κB activation [2]. Subsequently, another group isolated and cloned the cDNA of TBK1 and demonstrated that it is activated in both IκB and NF-κB pathways [1]. To understand its role in vivo, TBK1 knockout (KO) mice were generated [29], and while TBK1 heterozygous mice had no apparent phenotype, TBK1 KO mice resulted in embryonic lethality around embryonic day E14.5 caused by liver degeneration and cell death [29]. TBK1 protein consists of one N-terminal kinase domain necessary for its catalytic activity (KD; aa 9–300) [30]. The ubiquitin-like domain (ULD; aa 305–383) is important in regulating both the KD and SDD [30, 31]. The helical scaffold dimerization domain (SDD; aa 385–657) is a region critical for dimerization where the ULD of one TBK1 monomer interacts with the SDD of another, necessary for downstream signaling [22, 32]. The last 67 amino acids of the C-terminal domain (aa 662–729) [22] is where adaptor proteins bind to TBK1, regardless of dimerization status, similarly to other IKK-related kinases [33] (Figure 1).

TBK1, parallel to its homolog IKKε, induces the Type I interferon response by activating the transcription factor interferon regulator factor 3 (IRF-3) to trigger defense against viral infection downstream of the Toll-like receptor 3 (TLR-3) receptor [3, 34]. IKKε displays around 60% homology to TBK1 [1] with a similar, but distinct function to TBK1 in innate immunity [3, 35]. Since then, multiple groups have corroborated the fact that TBK1 is necessary for the transcription of several Type I interferon-stimulated genes (ISGs) in the context of both viral and bacterial infections [11, 36–38]. In each of these immune response pathways, TBK1 has multiple adaptor binding partners for its activation, like TANK, SINTBAD, NAP1/AZI2, OPTN, and STING [17, 23, 26, 28, 39–43] (Figure 1). TBK1-adaptor complexes phosphorylate downstream targets, such as IRF-3 and IRF-7, that control the ISG response [3, 44–46] inducing a specific immune response. In addition, many studies have also described the viral response in inactivating TBK1 to circumvent the immune response [47].

3 | TANK

TANK was originally discovered to be involved with NF-κB signaling as a TRAF binding protein, hence the name TRAF family member-associated NF-κB activator (TANK) [48]. However, its role as a TBK1 adaptor was first evident in a yeast two-hybrid screen to uncover novel TBK1 binding proteins [2]. Although TANK plays a role in activating TBK1 downstream of various types of immune stimuli in vitro [49], this view was challenged as TANK was found to be dispensable for IFN production via both TLR and RIG-I-like receptor (RLR) signaling pathways in vivo. Two independent studies investigating the physiological relevance of TANK found that TANK KO mice exhibited autoimmune glomerulonephritis and osteoporosis due to a heightened activation of immune cells and osteoclasts, which is fatal at 3 months of age [50, 51]. It is still unclear whether TBK1 activation and its function are affected due to the loss of TANK. In bone marrow–derived macrophages (BMDMs), TANK is phosphorylated after LPS stimulation, and interferon production is reduced in TANK KO BMDMs after LPS and poly I:C stimulation, but not abolished, as it appears to be compensated for by OPTN-TBK1 complexes [17]. In TLR signaling contexts, TANK does appear important in the early activation of TBK1 through its interaction and activation of IKKε, but activation of TBK1 is only reduced and not abolished when TANK is absent [39].

The discovery of other TBK1 adaptors like NAP1 and SINTBAD prompted sequence homology and mutagenesis experiments to identify that three of these adaptors have a common TBK1 binding domain (TBD) with high sequence similarity (Figure 1B) [28, 42]. This is also true of OPTN, which was reported later [52]. These adaptors use their TBD and compete to bind with the CTD of TBK1 constitutively in vitro [26]; however, due to the mostly dispensable nature of TANK in innate immune signaling, there has been less recent focus on this adaptor despite the phenotypes displayed by TANK KO mice.

4 | NAP1/AZI2

Nak-associated protein 1 (NAP1) was first identified when its cDNA was isolated in 1999 from a library prepared after stimulating mouse embryonic fibroblasts (MEFs) with a potent methyltransferase inhibitor, 5-azacytidine, and it is alternatively known as AZI2 [53]. Although this study initially characterized the 48kD cytoplasmic protein, its function in the innate immune response as a TBK1 binding protein was discovered in 2003 using a yeast two-hybrid screen with TBK1 as bait [42]. It was then renamed to NAK-associated protein 1 considering its function as a NAK (TBK1) binding protein. NAP1 mRNA was found to be ubiquitously expressed in all major human tissues with the highest expression levels in pancreas and testis [42, 54]. In vitro binding assays and mutagenesis experiments found that the amino acid sequence between 150 and 270 was the region necessary for TBK1 binding, and this binding enhanced the activity of NF-κB in a dose-dependent manner [42]. Thereafter, another study discovered that endogenous NAP1 binds to Toll/interleukin 1 receptor (TIR) containing adaptor molecule 1 (TICAM-1) and participates in the TLR-3-mediated IRF-3 activation pathway post poly-I:C stimulation in HeLa

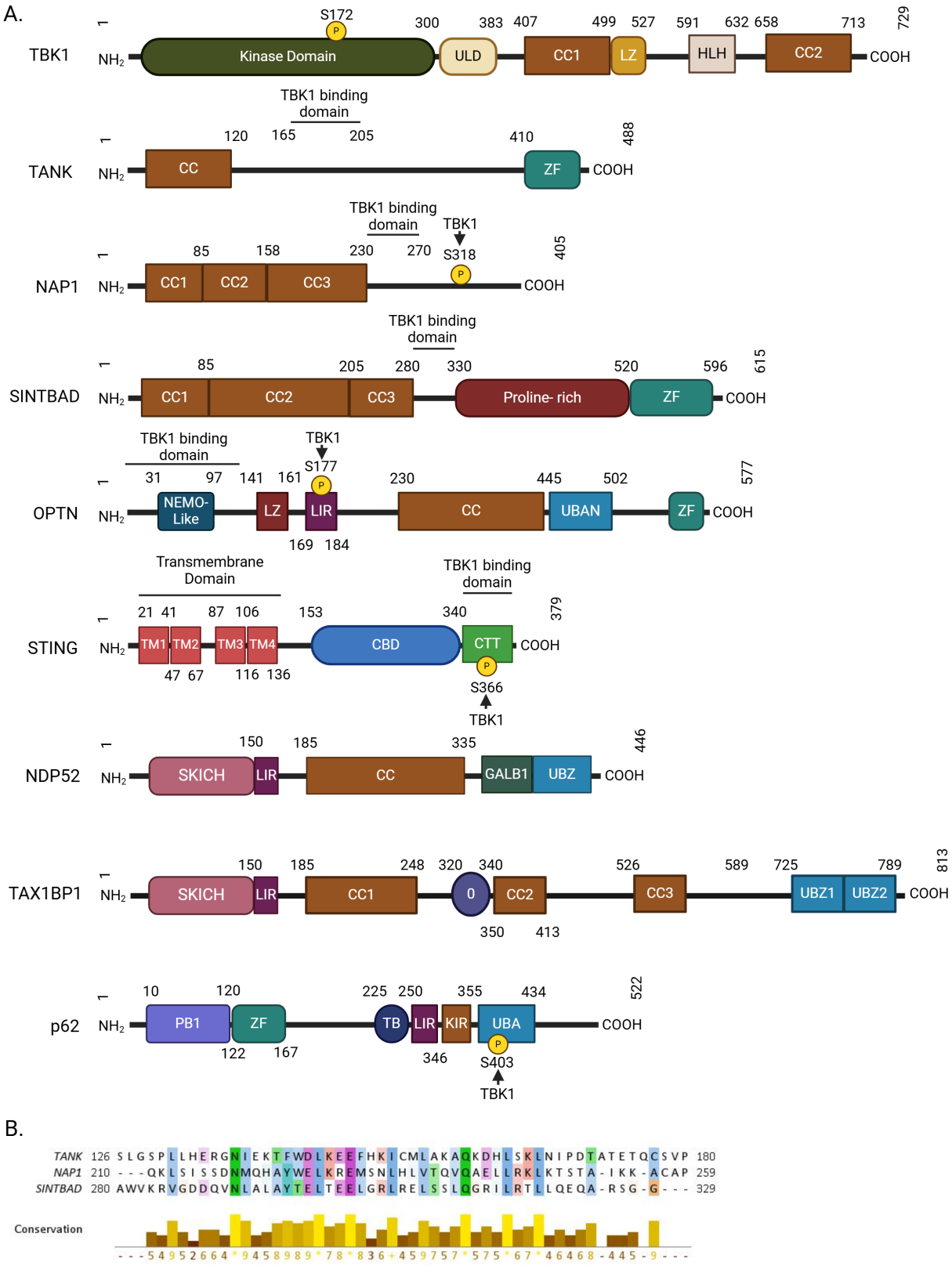

FIGURE 1 | Domain architecture of TBK1 and its adaptors. (A) Cartoon of TBK1 and adaptor proteins: ULD: Ubiquitin-like domain, CC: Coiled coil, LZ: Leucine zipper, HLH: Helix loop helix, ZF: Zinc finger, LIR: LC3-interacting region, UBD: Ubiquitin binding domain, TM: Transmembrane domain, CBD: Cyclic-di-GMP binding domain, CTT: C-terminal tail, PB1: Phox and Bem1p, KIR: Keap1-interacting region. Figure generated with Biorender. (B) The TBK1 interaction site is conserved across TANK, NAP1, and SINTBAD. Sequence alignment was performed using Clustal Omega, and the figure was generated with Jalview software.

cells [55]. This binding facilitated TBK1-mediated INFβ promoter activation, which was abolished in the case of truncated NAP1 lacking the TBK1 binding domain, and TANK did not immunoprecipitate with TICAM-1 either endogenously or when overexpressed [56]. This suggests that TANK cannot replace NAP1 to induce TICAM-1 mediated IFNβ. These results were also confirmed in vivo, infecting mice with PR8 H1N1 ssRNA virus. NAP1 KO mice displayed less interferon production, reduced survival, and increased lung severity scores, with TANK unable to compensate fully upon this type of viral challenge [57]. Full-length and truncated NAP1 can bind to the double-stranded RNA pattern recognition receptors RIG-1 and MDA5 to promote the IFN response, suggesting that this binding is not always dependent on the TBK1 binding domain of NAP1 [58]. NAP1 can induce transcription of INFβ by both the transmembrane receptor TLR-3 pathway and cytoplasmic pattern recognition receptors RIG-1 and MDA5 pathways [56, 58].

Upon *Salmonella* infection, NAP1 has been shown to participate in xenophagy. The NAP1-TBK1 complex is recruited onto ubiquitin-coated bacteria by a constitutive binding between the highly conserved N-terminal coil-coiled domain of NAP1 (aa 33–75) and the SKICH domain of an autophagy receptor NDP52, discussed in more detail below (aa 10–126) [11, 18]. Another study shows that the xenophagic removal of cytosolic bacteria activates ULK1-mediated autophagy where the conserved N-terminal coiled-coil domain of both the TBK1 adaptors SINTBAD and NAP1 can form trimeric complexes with NDP52 and FIP200 on the bacterial surface in response to cytosol-invading *Salmonella* [16, 20]. This direct interaction between the C-terminal of FIP200 (aa 1401–1591) and the N-terminal coil-coiled domain of NAP1(aa 33–75) was reported by a subsequent study, which characterized the noncanonical role of a core autophagy protein, FIP200, in tumor progression [59]. Loss of FIP200 correlated with NAP1-mediated overactivation of TBK1 [60], resulting in a heightened Type I IFN response and recruitment of infiltrating T cells at the site of mammary tumor, restricting tumor growth in mice; however, in the presence of FIP200, binding between FIP200 and NAP1 restricted TBK1 activation [59].

NAP1 KO mice display severe osteoporosis due to enhanced accumulation of osteoclasts during development [61]. While NAP1 KO impairs TLR and RLR mediated IFN production in bone marrow–derived dendritic cells cultured in the presence of the differentiation factor GM-CSF (GM-DC), it was dispensable in macrophages isolated from NAP1 KO mice [61]. It was found that GM-DCs lacking NAP1 had impaired differentiation, with a significantly lower number of mitotic cells in comparison to the WT due to the lack of activation of TBK1. This phenotype could not be rescued by the NAP1 mutant lacking the TBD, suggesting that NAP1-TBK1 binding is necessary for GM-DCs proliferation and growth [20, 62]. Although NAP1 is an established innate immune response protein, these studies provided some evidence that it also regulates cell survival and proliferation.

TBK1 was found to be necessary for proper progression through mitosis [14, 15, 21] where it was found to be activated at the centrosomes of dividing cancer-derived, immortalized cells [14]. Loss of TBK1 impairs mitosis, leading to aberrant cell division and the accumulation of multinucleated cells that were originally near diploid [63]. Loss of NAP1 phenocopies the mitotic defects observed in TBK1 KO cells and has been shown to participate in the activation of TBK1 during cell division, directly binding to TBK1, and in response, NAP1 is phosphorylated at S318 [63]. However, additional adaptor proteins are phosphorylated by TBK1 during mitosis at the centrosome, indicating that additional work is required to understand why these adaptor proteins are present at the centrosome and if they can compensate for the lack of NAP1 during mitosis.

5 | SINTBAD/TBKBP1

Large-scale proteomics identified SINTBAD as an innate immune signaling protein that participated in the TNFα/NF-κB pathway, also known as TBK1 binding protein 1 (TBKBP1), based on protein structure homology and protein–protein interaction maps [64]. Further validation found a direct interaction between the C-terminal adaptor binding domain of TBK1 and the TBK1 binding domain of SINTBAD (aa 280–330) by affinity purification [28]. SINTBAD, NAP1, and TANK competitively bind to TBK1 to facilitate its activation after an innate immune stimulus at the TBK1 binding domain [26, 28, 65]. Only NAP1 and SINTBAD have a homologous N-terminal coiled-coil region (CC1 and CC2) [28]. These homologous domains facilitate NAP1 and SINTBAD binding with the SKICH domain of the autophagy receptors NDP52 and TAX1BP1 to form a higher-order complex in the case of xenophagy [11, 16, 18, 20].

The generation of SINTBAD KO mice provided additional insight into the function of this adaptor protein. SINTBAD KO mice display significantly reduced numbers of natural killer T cells (NKT) in the thymus, liver, and spleen [66]. In this context, SINTBAD acts in coordination with autophagy machinery by forming a complex with ULK1 and FIP200 to facilitate NKT development, which is an interaction that also occurs during xenophagy [16, 66]. SINTBAD also plays a role in cell proliferation, as lack of SINTBAD inhibited the growth factor–stimulated TBK1 activation and downstream mTORC1 activation in A529 or primary lung cells [67]. Conditional SINTBAD KO (cKO) mice targeting lung epithelial cells corroborated data in culture, where TBK1-mediated epidermal growth factor (EGF)–stimulated mTORC1 signaling was impaired, and crossing these cKO SINTBAD mice with oncogenic KRAS LA2 mice demonstrated that the loss of SINTBAD reduced lung tumor growth, thus increasing survival [67]. However, it is unclear at which point during the cell cycle SINTBAD influences cell proliferation, so further investigation is still necessary.

6 | NDP52/CALCOCO2 and TAX1BP1

Nuclear Dot Protein 52 (NDP52), also known as CALCOCO2, and TAX1BP1 are ubiquitin-binding autophagy receptors that share similar protein domains [9]. NDP52 was first named after its protein distribution pattern in the nucleus [68, 69]; however, due to its interaction with TBK1, which is cytoplasmic, this review will focus on its cytoplasmic roles [11]. TAX1BP1 was first discovered as an interacting protein of human T cell leukemia virus type 1 (Tax1) in a yeast two-hybrid screen and hence named Tax1 Binding Protein 1 [70]. Both proteins' association

with TBK1 has been reported in basal autophagy [71, 72], xenophagy [11, 71, 73–75], and mitophagy [7, 76].

Often in these cellular contexts, both NDP52 and TAX1BP1 work in tandem or in coordination with other adaptors to facilitate TBK1 activation and trafficking. During xenophagy, NDP52 and TAX1BP1 recruit the TBK1 adaptors NAP1 and SINTBAD using their SKICH domain and form a higher-order complex with TBK1 [11, 16, 18, 76, 77]. During mitophagy, the activated TBK1 recruited on the depolarized mitochondria then directly phosphorylates NDP52 [4]. NDP52 has been shown to directly bind with FIP200 using an unbiased yeast two-hybrid approach [16]. During both xenophagy and mitophagy, the SKICH domain of NDP52 recruits the autophagy initiation ULK1 complex by binding with the C-terminal coiled-coil region of FIP200 (aa 1286–1395) [9, 16, 19, 20]. This recruitment of the ULK1 complex on the ubiquitinated cargo was found to be influenced by the availability of activated TBK1 due to the formation of a stable higher adaptor–TBK1–NDP52 complex [4, 9, 16, 20]. However, this complex between NDP52 and TBK1 is not direct and uses NAP1 as an intermediary [78, 79]. Similarly, in lysophagy, the SKICH domain of TAX1BP1 was also found to interact with FIP200, driven by the activated TBK1 recruitment at the site of damaged lysosomes in HeLa and induced pluripotent-derived neurons [12]. TBK1 recruitment at the site of autophagy occurs due to the binding between the SKICH domain of autophagy receptors NDP52 and TAXBP1 and TBK1 adaptors NAP1 and SINTBAD. This TBK1 complex with NDP52 and TAXBP1 then further promotes the recruitment of the autophagy initiation ULK1 complex.

TAX1BP1 also has an additional role in selective autophagy pathways. Although TAX1BP1 is ubiquitously expressed in all tissues [80], it is highly expressed in the brain and essential for the autophagic removal of neurodegenerative disease defining protein aggregates such as huntingtin (htt) and transactive response DNA binding protein 43 (TDP-43) to eliminate proteotoxic stress [81]. Further mechanistic insight has revealed that TAX1BP1 recruiting TBK1 to p62 ubiquitin condensates with the help of SINTBAD and NAP1, where TBK1 then phosphorylates p62 to enhance oligomerization [82]. Recently, in vitro experiments implicated a dynamic competition between TAX1BP1 and p62/NBR1 at ubiquitinated sites of fibrillar pathogenic tau species [83], but TBK1 was not further examined. Future work for specific disease aggregates is warranted to understand autophagic removal, whether it be specific or unselective. TAX1BP1 has also been implicated in the removal of TRIF, in its role as an autophagy receptor, to regulate interferon production [57, 84]. TAX1BP1's own stability, akin to NAP1 during mitosis, appears to be dependent on phosphorylation events by TBK1 and IKKε, basally and under poly I:C stimulation, through autophagic mechanisms [85].

7 | OPTN and p62/SQSTM1

OPTN was first discovered to interact with TBK1 in a yeast two-hybrid screen, where they found that OPTN binding activates TBK1 and is enhanced by a mutant version of OPTN E50K [52]. OPTN uses its N-terminal tank-binding domain, homologous with other TBK1 adaptors NAP1, SINTBAD, and TANK, to interact with TBK1 [52]. p62, alternatively known as sequestosome1 (SQSTM1), was originally named for its propensity to form aggregates [86]. p62 was initially found to be a part of inclusion bodies containing polyubiquitin-coated protein aggregates in central nervous system (CNS) and liver proteinopathies [87–90]. Later, it was reported that p62 simultaneously binds with ATG8 proteins and the ubiquitin chains during autophagy [91, 92]. p62 KO mice display an age-related accumulation of polyubiquitinated protein aggregates in the brain [93].

OPTN-mediated TBK1 activation in the innate immune response pathway was studied in a polyubiquitin binding defective mutant of an OPTN knock-in mouse model where OPTN was replaced with $OPTN^{D477N/D477N}$. Bone marrow–derived macrophages from this mutant mouse showed that although binding between TBK1 and OPTN was not affected by this OPTN mutation, TBK1-mediated INFβ activation and IRF3 phosphorylation were severely compromised post LPS and poly I:C stimulation [43]. Another study corroborated this finding in bone marrow–derived macrophages and bone marrow–derived dendritic cells isolated from mice with a C-terminal truncation of OPTN, finding a significantly lower TBK1-mediated activation of INFβ and IRF3 after LPS stimulation [94]. The global loss of OPTN in mice displays no overt phenotypes but displays differential phenotypes when challenged with different inflammatory inducing stimuli such as acute colitis or *Salmonella* infection [95].

During selective autophagy, both p62 and OPTN associate with autophagy ATG8 proteins with the help of the LC3 interacting region (LIR) necessary for linking the cargo and the growing autophagophore for engulfment [96–98]. During mitophagy, the N-terminal region of OPTN interacts with TBK1 and links with ATG9A vesicles and ATG8 family proteins to initiate autophagosome formation around damaged mitochondria [99]. Interestingly, this binding is promoted by the phosphorylation of OPTN on serine 177 for mitophagy and phosphorylation of p62 on serine 403 by TBK1 in other selective autophagy contexts [10, 100–102]. In the case of *Salmonella* infection, TBK1 is recruited by the ubiquitin binding domain (UBAN) of OPTN to facilitate the phosphorylation of xenophagy proteins to restrict bacterial growth in host cells [10]. Several studies have also reported that both OPTN and p62 during mitophagy are phosphorylated by TBK1 on several residues [4, 8, 103]. In general, much of the mechanistic detail on OPTN, p62, and NDP52 has been elucidated in the context of selective autophagy pathways and their relationship with TBK1, while more recent studies focus on the roles of the other TBK1 binding autophagy adaptors such as SINTBAD, NAP1, and TAX1BP1 in these contexts.

8 | STING

STING was first identified [104–106] as a mediator of the Type I IFN response. TBK1 was shown to be necessary for STING activation [104], likely due to TBK1 phosphorylation [105]. Following the discovery that STING binds cyclic dinucleotides [107], it was confirmed that cyclic GMP–AMP (cGAMP) strongly binds STING to promote its activation and downstream signaling [108]. cGAMP synthase (cGAS), a previously uncharacterized nucleotidyltransferase, is the protein responsible for binding DNA and synthesizing cGAMP [109]. cGAMP binds

the ligand binding domain (LBD) of the endoplasmic reticulum resident STING dimer with high affinity [108]. This binding results in STING oligomerization and translocation to the Golgi [110, 111]. At the Golgi, TBK1 is recruited and activated by STING, resulting in STING phosphorylation, which facilitates recruitment and phosphorylation of IRF-3 [23, 41, 112, 113]. TBK1 phosphorylation of STING also results in endolysosomal recycling to turn off STING signaling [114]. This mechanism differs from the newly described role as a proton channel where STING functions in autophagy and inflammasome signaling (not discussed in detail here) [115].

STING has also been directly shown to bind and interact with TBK1. Cryo-electron microscopy experiments revealed that the TBK1 dimer interacts with the cytosolic ligand binding domain dimer at the C-terminal between aa residues 369 and 377 of STING [23]. Loss of these C-terminal residues resulted in a loss of TBK1 colocalization with STING after cGAMP stimuli, abolishing the interferon response [116]. Loss of phosphorylation of STING with the S365A mutation does not affect TBK1 recruitment [44] and only abolishes the interferon and not the NF-κB signaling pathway, but loss of TBK1 recruitment with the L373A mutation causes the loss of both innate immune pathways, similar to the C-terminal deletion STING mutants [117].

9 | Involvement of TBK1 Adaptor Proteins in Disease

TBK1 adaptor mutations have been found or associated with rare diseases [118–120] and more common neurodegenerative disorders as highlighted here. Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are two distinct but associated neurodegenerative diseases with clinical overlap, with the disorders existing across a spectrum. ALS is a progressive neuromuscular disorder that affects upper and lower motor neurons [121]. Neurodegeneration results in skeletal muscle atrophy, with ALS patients having a median survival of 3–5 years after diagnosis [122]. FTD is a similarly devastating neurodegenerative disease affecting multiple cortical regions, causing alterations in executive function, behavior, and language [123]. In 2009, it was recognized that there were considerable convergent symptomatic similarities that occurred in several patients [124]. On the histopathological level, TDP-43 is an aggregated protein that has undergone many types of post-translational modifications found in post-mortem ALS/FTD brains [125–127]. A majority of cases are sporadic; however, mutations in adaptor proteins p62 and OPTN alongside TBK1 have been reported.

The discovery of these loss-of-function mutations in both OPTN and p62 [128, 129] indicates that disruption of the trafficking, association with, and activation of TBK1 signaling also contribute to the pathophysiological changes in ALS/FTD (Tables 1 and 2). TBK1 itself has also been found to have mutations associated with ALS/FTD [130]. It is worth noting that some of these mutations exist as heterozygous mutations. Considering that p62 and OPTN are implicated in selective autophagy pathways and OPTN is involved in innate immunity, why these phenotypes occur in age-related neurodegenerative disease is of interest since it is likely that other cell types would be impaired by these global mutations. However, the redundancy of adaptors and potential compensatory mechanisms that occur could account for the vulnerability of post-mitotic neurons as opposed to cell types that can self-renew.

TABLE 1 | The outcome of relevant ALS/FTD mutations that affect OPTN.

OPTN mutations	Effect	Reference
E478G	OPTN inclusions/aggregates	Maruyama et al. 2010
E478G	Increased proinflammatory cytokine response	Lie et al. 2018
E478G	Autophagy disruption and perturbed inclusion body clearance	Shen et al. 2015
E478G Q398X	OPTN failed to translocate to damaged mitochondria	Moore and Holzbaur, 2015
E478G Q398X	Autophagolysome defects, endoplasmic reticulum stress	Sundaramoorthy et al. 2015
E478G Q398X	Dysregulated reactive oxygen species, antioxidant enzymes, and decreased mitochondrial membrane potential	Zhu et al. 2016
K59N, A93P, R96L, E478G, K557T, V295F and R545Q	Stress granule clearance defects	Kakihana et al. 2021
Q235/A481V	TBK1 mRNA and protein reduction	Pottier et al. 2015
F178A	OPTN translocation unperturbed, LC3 autophagosome formation, and mitophagy disrupted	Wong and Holzbaur, 2014
R217X	TBK1 mRNA levels not altered	Nolan et al. 2021
V295F	Golgi fragmentation and ER stress	Fifita et al. 2016
K489E	Increased TBK1 and p62 protein levels	Mishra et al. 2023

10 | Conclusion

Activation of TBK1 is reliant upon binding to adaptor proteins, which initiates higher-order oligomerization of the TBK1 adaptor complex and leads to transautophosphorylation at serine 172 [18, 22, 131] (Figure 2). Multiple adaptors like NAP1/AZI2, TANK, SINTBAD, and OPTN contain a common TBK1 binding domain, which competitively binds to the C-terminal domain (CTD) of TBK1 during mitophagy and innate immune signaling [18, 27, 28]. These adaptors appear in different cell signaling contexts. For example, OPTN binding to TBK1 during viral infection drives Type I interferon production [132], and it also interacts with TBK1 during mitophagy at the outer mitochondrial membrane [8]. Loss of these adaptors/associated proteins has revealed the mechanism by which TBK1 drives different cellular processes. However, considerable compensation for the loss of a single adaptor by other adaptors in innate immunity and selective autophagy pathways occurs. Therefore, identifying the

TABLE 2 | The outcome of relevant ALS/FTD mutations that affect p62.

p62 mutations	Effect	Reference
G427R, P394L, G413S	Ubiquitin binding deficient	Deng et al. 2019
P348L, G351A	Decreased NRF-2 transcriptional activity	Goode et al. 2016
R110C, P348L	Decreased NRF-2 transcriptional activity, phosphorylation of S403, solubility	Foster et al. 2019
L341V	Reduced binding to ATG8 family members in vitro	Brennan et al. 2021
L341V	Reduced binding to LC3B	Goode et al. 2016

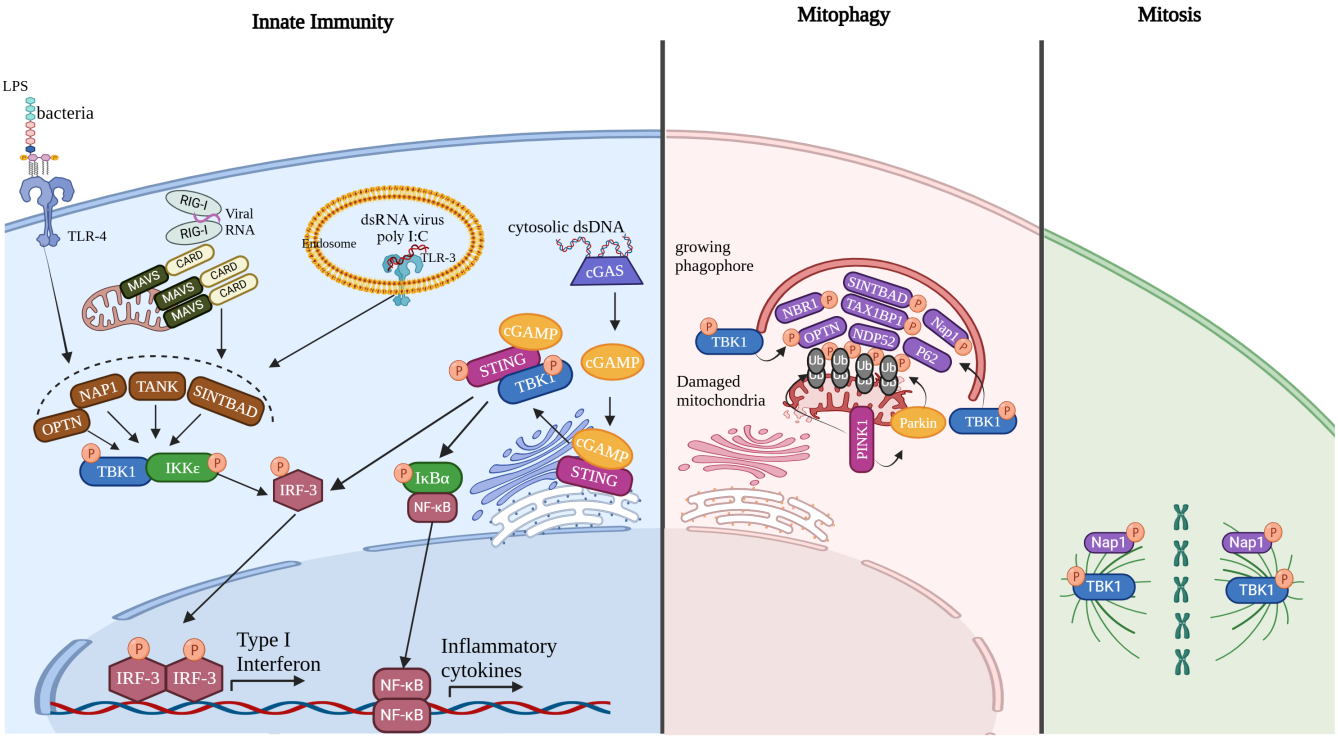

FIGURE 2 | Graphical depiction of TBK1 signaling and its adaptors in select pathways such as innate immune signaling, mitophagy, and mitosis. TBK1 and IKKε act as noncanonical IKKs downstream of TLRs and RLRs. Upon activation, TLRs recruit adaptor proteins, including TRIF and TRAF3, which activate TBK1 within a complex comprising its adapters NAP1, SINTBAD, TANK, and OPTN. Activated TBK1 and IKKε phosphorylate transcription factors like IRF-3, promoting their nuclear translocation. This induces the production of type I interferons (IFNs) and inflammatory cytokines. Additionally, TBK1 plays a key role in the cGAS–STING pathway, where the activated STING recruits TBK1 to catalyze the phosphorylation of IRF-3 and stimulate NF-κB signaling, further driving the inflammatory response. Damaged mitochondria accumulate PINK1 on their outer membrane, where it phosphorylates outer mitochondrial membrane (OMM) proteins, creating docking sites for Parkin. Activated Parkin ubiquitinates the damaged mitochondria, enabling recognition by cargo receptors, including OPTN, p62, and NDP52. The adaptors NAP1, SINTBAD, and TAX1BP1 mediate the interaction between TBK1 and the cargo receptors. Clustered TBK1 phosphorylates and activates the cargo receptors and coreceptors, enhancing their affinity for ubiquitin and ATG8 family members. This process promotes the recruitment of downstream autophagy complexes leading to the engulfment and degradation of the damaged organelle. During mitosis, TBK1 is activated through its association with NAP1 at the centrosomes, enabling the phosphorylation of multiple proteins essential for mitosis and cytokinesis. Additionally, NAP1 serves as a substrate for TBK1, which regulates its stability. Figure generated with Biorender.

unknown activator(s) of TBK1 in all contexts, such as in mitosis, can help us understand the mechanisms by which TBK1 is associated with and possibly drives cancer progression [133–135]. Also, this redundancy brings possibilities in the existence of crosstalk or regulation across innate immunity, mitosis, mitophagy, and other TBK1-regulated pathways. This is most strongly evident during mitophagy and viral infection where cell division is perturbed upon the activation of one pathway or another [13, 15]. However, these are most likely not the only pathways that intersect. In addition, the breadth of substrates [136–138] (that are not adaptors) with which TBK1 has been associated intersects with multiple cellular processes, adding another layer of complexity.

It is also important to note that conflicting results on adaptor function have been described. For example, triple KO of NAP1/SINTBAD/TANK in HEK293T cells exposed to SeV infection did not disrupt phosphorylation of IRF3, but different KO clones did display divergent responses to IFN production [139]. Other groups have demonstrated that TLR4 TBK1 signaling inhibits canonical IKK NF-κB signaling [39, 140, 141], which challenges the initial findings described above [1, 2], which were heavily reliant on overexpression constructs. Differences between mouse and human cells [142, 143] to different immunogenic stimuli as well as specific cell type responses all may be possible contributors to the differential responses described as well.

The sequence of events for TBK1 activation and localization is not always clear in every signaling context. Whether an adaptor first binds to TBK1 to activate it, and this TBK1 adaptor complex is recruited at the site of necessity, or the adaptor arrives at the required site, which recruits TBK1 to facilitate phosphorylation of the substrates, is ambiguous [144]. It is known that the TBK1 adaptor OPTN and the associated autophagy proteins NDP52, p62, and TAX1BP1 are phosphorylated on various residues at the site of xenophagy, selective autophagy pathways, and mitophagy [4, 7, 8, 16, 85, 101, 103]. However, the precise temporal organization of these complex events is beginning to be elucidated. One potential reason for this is that it is unclear where activated versus inactivated TBK1 are subcellularly located at a given time [15]. Adding another layer of complexity, NAP1, SINTBAD, and TBK1 are found as liquid condensates after rabies virus infection, heat shock, or transfection of dsRNA, which appear to precede activation and are dynamic, but whether they are present under other stimuli is unknown [145, 146]. These mechanistic details are also difficult to parse out, considering that in many contexts, more than one adaptor is bound to TBK1 during activation. In any case, future studies into the dynamic signaling of TBK1 have the potential to provide great biological insight into a multitude of cell signaling pathways.

Author Contributions

Conceptualization: Swagatika Paul and Alicia M. Pickrell. Writing – original draft: Swagatika Paul and Alicia M. Pickrell. Graphical figures and legends: Porter L. Tomsick, Julia P. Milner, Sahitya Ranjan Biswas. Writing – review and editing: Julia P. Milner, Porter L. Tomsick, Sahitya Ranjan Biswas, and Alicia M. Pickrell. All authors have read and approved the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data used to support this study are available upon request from the corresponding author, AMP.

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