The Role of the Phylogenetically Conserved Cochaperone Protein Droj2/DNAJA3 in NF-κB Signaling

Yoshiki Momiiuchi, Kohei Kumada, Takayuki Kuraishi, Takeshi Takagaki, Yoshihito Aigaki, Yoshiteru Oshima, and Shoichiro Kurata

From the Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai 980-8578, Japan, PRESTO Precursory Research for Embryonic Science and Technology, Japan Science and Technology Agency, Tokyo 332-0012, Japan, and the Department of Biological Sciences, Tokyo Metropolitan University, Tokyo 192-0397, Japan

Background: The NF-κB pathway is crucial for the regulation of immune responses, inflammation, and stress responses. The NF-κB pathway is a phylogenetically conserved signaling pathway with a central role in inflammatory and immune responses. Here we demonstrate that a cochaperone protein, Droj2/DNAJA3, is required to activate NF-κB signaling in flies and human cultured cells. Overexpression of Droj2 induced the expression of an antimicrobial peptide in Drosophila. Conversely, Droj2 knockdown resulted in reduced expression of antimicrobial peptides and higher susceptibility to Gram-negative bacterial infection in flies. Similarly, Toll-like receptor-stimulated IκB phosphorylation and NF-κB activation were suppressed by DNAJA3 knockdown in HEK293 cells. IκB kinase overexpression-induced NF-κB phosphorylation was also compromised in DNAJA3 knockdown cells. Our study reveals a novel conserved regulator of the NF-κB pathway at the level of IκB phosphorylation.

NF-κB signaling is crucial for the proper induction of innate and adaptive immune responses (1, 2). Pathogen-associated molecular patterns stimulate cell surface receptors, including Toll-like receptors (TLRs), to initiate a signaling cascade resulting in the activation of NF-κB (3). NF-κB drives the expression of target genes such as cytokines and costimulatory molecules that are required for the activation of adaptive immunity (4). Some cytokines again stimulate NF-κB signaling via cytokine receptors, driving the expression of genes that mediate cell proliferation, antimicrobial activity, and tissue repair (5). Inflammatory responses characterized by an influx of leukocytes, redness, pain, and swelling are the result of the NF-κB reactivation, and deviations in this process lead to chronic inflammation, autoimmune responses, and, ultimately, organ dysfunction (6).

Intracellular signaling of the NF-κB pathway is relatively well characterized. After engaging TLRs and cytokine receptors by extracellular stimulation, signaling through MyD88 and RIP1 and then tumor necrosis factor receptor-associated factors results in the phosphorylation of IκB kinase (IKK) and activation of the IKK complex kinase (7–9). IKK then phosphorylates IκB molecules that sequester NF-κB in the cytosol of resting cells and prevents its nuclear localization and transcriptional function (10, 11). Phosphorylated IκB promotes the degradation of NF-κB, releasing it to translocate to the nucleus where it transactivates target gene expression (12). Optimal induction of NF-κB target genes also requires phosphorylation of the NF-κB p65 subunit within the transactivation domain by a variety of kinases, including IKK (13). This NF-κB signaling pathway is called the canonical NF-κB pathway, in contrast to the non-canonical pathway that is activated by developmental stimuli to induce the NF-κB/RelB/p52 dimer to the nucleus (14). The canonical NF-κB pathway is positively and negatively regulated by many proteins and microRNAs (15–17), but the complete molecular mechanism is not known.

The fruit fly Drosophila melanogaster relies solely on innate immunity to fight against microbial infection (18, 19). Expression of antimicrobial peptides (AMPs) is a major effector mechanism for eliminating pathogenic microbes (20), and their expression is regulated by two distinct NF-κB pathways: the Toll and Imd pathways (19). The Toll pathway is activated by infection with Gram-positive bacteria and fungi (21, 22). Serine protease cascades are triggered in the hemolymph by infection, which results in the production of the Toll receptor ligand Spätzle (23–25). Binding of Spätzle to the Toll receptor induces receptor dimerization, thereby recruiting a signaling complex,
including the Drosophila homolog of MyD88 (dMyd88) (26, 27). This event leads to the phosphorylation of Cactus, a Drosophila homolog of IkB. Phosphorylated Cactus undergoes degradation by the ubiquitin-proteasome system (28, 29), leading to the release of the transcription factor Dif, a Drosophila NF-κB homolog (30). The released Dif enters the nucleus to activate the transcription of antimicrobial genes, such as the antifungal peptide Drosomycin (31).

On the other hand, the Imd pathway is triggered when peptidoglycan recognition proteins (PGRP)-LC and PGRP-LE detect Gram-negative bacteria (32–34). Imdc has a function similar to that of mammalian RIP1, which works downstream of PGRPs (35). Upon bacterial recognition, Imdc is cleaved by the caspase-8-like protein Dredd to form a complex with the E3-ligase Diap2 and E2-ubiquitin-conjugating enzymes (36). The complex then activates transforming growth factor-β-activated kinase 1 (Tak1) and TAK1-binding protein 2 (Tab2), leading to activation of the Drosophila IKK complex, comprising Ir5d and kenny (36). The IKK complex then phosphorylates Relish, another Drosophila NF-κB homolog (37). The phosphorylated Relish protein is then cleaved by Dredd, and the N-terminal Relish translocates to the nucleus and induces the expression of antimicrobial genes, including Diptericin (38).

In this manner, the mammalian NF-κB pathway and the Drosophila Toll and Imd pathways exhibit remarkable similarity in intracellular signaling. Indeed, studies in Drosophila innate immunity have contributed substantially to our understanding of the mammalian NF-κB pathway (39). Here we report the identification of cochaperone Droj2/DNAJ3 as a newly identified factor that is required for IkB phosphorylation, thereby controlling NF-κB activity in Drosophila and humans.

Experimental Procedures

Infection and Survival Experiments—Bacterial infections were induced by injecting 70 nl/fly suspension of each pathogen strain. The optical density at 600 nm for each suspension was as follows: Ecc15, 1.0; Escherichia coli, 1.0; and Staphylococcus saprophyticus, 0.1. Survival experiments comprised ~60 flies of each genotype at 28 °C. Surviving flies were counted daily while being transferred to fresh vials.

Total RNA Isolation and Quantitative RT-PCR—Flies were infected as described above and collected at 24 h (S. saprophyticus) or 6 h (Erwinia carotovora carotovora (Ecc15 or Pectobacterium carotovorum subsp. carotovorum) and E. coli). Total RNAs were isolated from 10 flies of each genotype with TRIZol reagent (Ambion). Total RNA (1 mg) was used for cDNA synthesis with ReverTraAce reverse transcriptase (Toyobo) and oligo(dT) 15 primer (Promega). Using the first-strand cDNA synthesis with ReverTraAce reverse transcriptase (Toyobo) and oligo(dT) 15 primer (Promega). Using the first-strand cDNA synthesis with ReverTraAce reverse transcriptase (Toyobo) and oligo(dT) 15 primer (Promega). Using the first-strand cDNA synthesis with ReverTraAce reverse transcriptase (Toyobo). rpL32 was used as the internal control. The primers used for quantitative RT-PCR were as follows: rpL32, AGATCGTGAAGAAGCGCACCAAG (forward) and CACCAGGAACCTCTTTGTACCCCG (reverse); Drs, TTTGTGCGCCCTCTTTTCGCTTCT (forward) and GCATCTCTC-GACCACGACTTCA (reverse); Dpt, GTTCACATGGCC-GTGCCCTAAC (forward) and CCAAGTGTCTCCATATCC (reverse); and CG8863-RA, GTGAAACTCTCAGGAATCGTTCGCG (forward) and AATGGCCTTGAATTTCTCGC (reverse).

RNAi, Ligand Stimulation, and Luciferase Assay—S2 cells (4.0 × 10⁴) were plated in 96-well plates and transfected with 200 ng of dsRNA, 40 ng of pLI169-Attacin-firefly luciferase reporter vector, and 10 ng of pAc-lacZ transfection control vector together with the indicated in vitro expression vectors using the Effectene transfection reagent (Qiagen). 24 h after transfection, cells were stimulated with heat-killed E. coli for 48 h and lysed in Glo lysis buffer (Promega). Luciferase activity was measured with the One-Glo luciferase assay system (Promega) in a luminometer (SpectraMax L-TYA, Molecular Devices). β-Gal activity was measured with β-gal assay buffer (100 mM HEPES (pH 7.3), 150 mM NaCl, 10 mM MgCl₂, 0.5 mg/ml chlorphenol red-β-D-galactopyranoside (Roche)), and the values of the absorbance of free chlorophenol red at 595 nm were used to normalize the variability in transfection efficiency.

HEK293 cells were plated in 96-well plates and transfected with 0.4 pmol of siRNA using Lipofectamine RNAiMAX (Invitrogen). After 24 h of siRNA transfection, cells were transfected with 100 ng of ELAM (endothelial leukocyte-adhesion molecule)-NF-κB luciferase and 20 ng of pRL-TK together with the expression vector using Lipofectamine LTX (Invitrogen). After 24 h of reporter transfection, cells were stimulated with flagellin (300 ng/ml), LPS (5 μg/ml), or dexamethasone (100 ng/ml) for 6 h. After stimulation, relative luciferase activity was measured using the Dual-Glo luciferase assay system (Promega).

Nuclear Extracts—Nuclear extracts of HEK293 cells were prepared using NE-PER nuclear and cytoplasmic extraction reagents according to the instructions of the manufacturer (Pierce). HEK293 cells in 6-well plates were transfected with ~3 nm/ml siRNA using Lipofectamine RNAiMAX (Invitrogen). After 24 h of transfection, cells were washed with PBS (pH 7.4), lysed with 200 μl of cytoplasmic extraction reagent 1, and vortexed for 15 s. After incubation on ice for 10 min, 11 μl of cytoplasmic extraction reagent 2 was added to the lysates. The lysates were vortexed for 5 s and incubated on ice for 1 min. After centrifugation (~16,000 g) for 5 min, the insoluble fraction, which contained nuclei, was suspended in nuclear extraction reagent and vortexed for 15 s every 10 min for a total of 40 min. After centrifugation (~16,000 g) for 10 min, the supernatant (nuclear extract) fraction was collected in a clean tube.

Western Blots—Cell lysates were prepared in SDS sample loading buffer (50 mM Tris-HCl, 200 mM β-mercaptoethanol, 2% SDS, 0.0125% bromophenol blue, and 10% glycerol) and resolved on 8%-10% SDS-PAGE. The protein bands were visualized using the Luminata Forte Western HRP substrate (Millipore) according to the protocol of the supplier, and the image was captured by ImageQuant LAS 4000 mini (GE Healthcare). The antibodies used were as follows: Tid-1 (RS13) mouse mAb (Cell Signaling Technology), MyD88 (D80F5) rabbit mAb (Cell Signaling Technology), IkBa (L35A5) mouse mAb (Cell Signaling Technology), phospho-1kB-α (Ser-32, 14D4) rabbit mAb (Cell Signaling Technology), NF-κB p65 (D14E12) XP rabbit mAb (Cell Signaling Technology), phospho-NF-κB p65 (Ser-535, 93H1) rabbit mAb (Cell Signaling Technology), IKKβ (D30C6) rabbit mAb (Cell Signaling Technology), phospho-1kB-α (Ser-32, D4F2) mouse mAb (Cell Signaling Technology), and phospho-NF-κB p65 (Ser-535, 93H1) rabbit mAb (Cell Signaling Technology).
IKKα (Ser-176)/IKKβ (Ser-177) (C84E11) rabbit mAb (Cell Signaling Technology), anti-actin antibody (ACTN05(04), Abcam), ECL™ anti-rabbit IgG and HRP-linked whole antibody (GE Healthcare), and ECL™ anti-mouse IgG and HRP-linked whole antibody (GE Healthcare).

Fly Strains—Drosophila stocks were maintained in standard cornmeal-yeast agar medium vials at 25 °C. Oregon R was used as the wild-type control. As a positive control of the Toll-susceptible lines, spzrm7 flies (40) were used, and for Imd susceptibility, RelishE20 flies (41) were used as the positive control. The transgenic strains Diptericin-lacZ (Dpt-lacZ) and Drosomycin-GFP (Drs-GFP) and lacZ staining have been described previously (42, 43). The data of the Gene Search (GS) strains (44) are available through FlyBase. hs-GAL4, NP2610-GAL4 (DGRC (Drosophila Genetic Resource Center), catalog no. 112982), UAS (Upstream Activating Sequence)-yellow (Bloomington, catalog no. 3043), Droj2-RNAi-2 (VDRC (Vienna Drosophila Resource Center), catalog no. 23638), and Droj2-RNAi-3 (VDRC, catalog no. 23637) using c564-GAL4 tub-GAL80ts flies were crossed at 18 °C, moved to 28 °C after eclosion, and kept for 5–7 days. The Droj2 cDNA was amplified by PCR with a GM13664 clone (DGRC) as a template and the following primers: 5’-CGGAGATTCATGGTTAGGAGACTGGA-3’ (forward) and 5’-CCGCTCGAGTTAACTCGATGTGCACTG-3’ (reverse). Amplified cDNA was subcloned into the pUAST vector of EcoRI and XhoI sites. After sequencing, the construct was injected into embryos to establish transgenic flies according to standard methods.

Statistical Analyses—Statistical analyses were performed using Student’s t test or the log-rank test, and p values of less than 0.05 were considered significant.

Results

Droj2 Overexpression Induces Drosomycin Expression in Epithelial Tissues of Drosophila Larvae—We previously performed gain-of-function genetic screening using a GS system library with the expression of two AMP reporters, Drs-GFP and Dpt-lacZ, as gain-of-function indicators (45). Ubiquitous expression was induced by heat shock-inducible hs-GAL4 in larvae, and GFP or lacZ expression was monitored. We reported the identification of two genes in this screening: PGRP-LE, which activates Imd-dependent AMP induction and autophagy (46–
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**FIGURE 2. Droj2 is required for host defense against Gram-negative bacterial infection.** A–D, the survival rate (percent) of c564-GAL4 tub-GAL80+/UAS-Droj2-RNAi (c564→Droj2-RNAi (2)) flies (A, C, and D) and the survival rate (percent) of c564-GAL4 tub-GAL80+/UAS-Droj2-RNAi (c564→Droj2-RNAi (2)) and c564-GAL4 tub-GAL80+/+; UAS-Droj2-RNAi/+ (c564→Droj2-RNAi (3)) flies (B). Flies injected with saline (A), Ecc15 (B). E. coli (C), and S. saprophyticus (D) were compared with wild-type flies and c564/UAS-yellow (c564→yellow) and flies mutant in either the Toll pathway (RelishE200) or the Imd pathway (Relish0.01). 60 flies were used in each experiment. **, p < 0.01; log-rank test; NS, difference not significant.

48), and Gyc76C, which mediates a cGMP-dependent signaling pathway involving downstream components of the Toll receptor.5 With continued screening, we found that the GS5242 line strongly induced Dpt expression in the malpighian tubules and 

Drosophila larvae (Fig. 1, A and B). Data from FlyBase indicate that a transposable element of the GS5242 line is inserted in the Droj2 gene region (Fig. 1C) (49). Indeed, RT-PCR analysis revealed that Droj2 mRNA expression was induced in GS5242 lines by hs-GAL4 (Fig. 1D). To confirm that Droj2 overexpression activates AMP induction, we generated the UAS-Droj2 line. As observed in the GS5242 line, UAS-Droj2 induced Dpt expression in the trachea by hs-GAL4 as well as NP2620-GAL4, which predominantly targets the tracheal epithelium (Fig. 1E). These findings strongly indicate that Droj2 overexpression induces AMP expression in larval epithelial tissues and suggest that Droj2 is a novel regulator of the humoral innate immune response in Drosophila.

Droj2 Mediates Imd Activation by Gram-negative Bacteria—Drosomycin expression in the trachea is controlled by the Imd pathway (50). The Imd pathway is essential for antimicrobial gene induction and survival upon infection with Gram-negative bacteria in adult flies (19). Data from FlyAtlas indicate that Droj2 is expressed ubiquitous throughout the body, including the fat body, which is responsible for AMP production, in adult flies (Fig. S1). Because Droj2 mutation causes embryonic lethal

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suggest a role for Droj2 in Imd pathway activation by Gram-negative bacteria in Drosophila.

Epistatic Positioning of Droj2—Even in the absence of infectious bacteria, overexpression of PGRP-LE, imd, Dredd, and Relish results in activation of the Imd pathway, inducing constitutive expression of Dpt (35, 41, 52, 53). We used this system to analyze the epistatic position of Droj2 in the Imd pathway. We found that the ability to induce Dpt expression varied among the overexpressed genes, the degree of which was consistent with a previous report (52). In Droj2-RNAi flies, PGRP-LE, imd-, Dredd-, and Relish-mediated induction of Dpt was blocked (Fig. 4, A–D), indicating that Droj2 is required for Dpt induction by overexpression of all these components of the Imd pathway. Therefore, Droj2 is likely to function downstream of Relish or regulate the activation of Relish.

Human DNAJA3 Is a Functional Homolog of Drosophila Droj2—Molecular mechanisms that activate innate immune signaling are phylogenetically conserved from flies to humans (54). We sought to determine the human functional homolog of Drosophila Droj2 in the NF-κB pathway. Droj2 belongs to the DNAJ family of proteins, comprising a J domain, zinc-finger domain, and a C-terminal dimerization domain (49). The DNAJ family in humans has 49 members (55), and we comprehensively examined which DNAJ functionally corresponds to Drosophila Droj2. HEK293, a transfection-friendly cell line, highly expresses TLR5 whose NF-κB signaling is solely dependent on MyD88. The cells were transfected with NF-κB reporter together with siRNAs against human DNAJs and then stimulated with the TLR5 ligand flagellin. NF-κB reporter activity was reduced to less than 30% in cells treated with siRNAs against DNAJA3, DNAJB7, DNAJC4, DNAJC6, DNAJC8, or DNAJC27 (Fig. 5A). Among these, DNAJA3 (56), a type I DNAJ comprising an N-terminal J-domain, a glycine/phenylalanine-rich region, a cysteine repeat region, and a largely uncharacterized C terminus, had the most similar amino acid sequence to Drosophila Droj2, as analyzed by ClustalW and, therefore, was selected for further evaluation. We first tested another siRNA that targets different sequences of DNAJA3 to determine whether it suppresses TLR5-dependent NF-κB activation in HEK293 cells. Western blot analysis indicated that DNAJA3 protein was indeed decreased by siRNA treatment, and the reporter assay clearly suggested that another siRNA also inhibited NF-κB activation in flagellin-treated HEK293 cells (Fig. 5B). Fig. 5C shows that both siRNAs targeting DNAJA3 also suppressed LPS-stimulated TLR4-dependent NF-κB activation in HEK293 cells. In contrast, glucocorticoid activity stimulated by dexamethasone (Fig. 5D) was not suppressed in DNAJA3 knockdown cells. These findings, together, suggest that DNAJA3 is specifically required for activation of the NF-κB pathway.

FIGURE 3. Analysis of antimicrobial peptide expression in Droj2 knockdown flies and cells. A–C, Diptericin (A and B) or Drosomycin (C) gene expression was monitored by quantitative RT-PCR with total RNA extracted from wild-type, UAS-yellow, spzrm7, RelishE20, c564-GAL4 tub-GAL80ts/UAS-Droj2-RNAi (c564->Droj2-RNAi (2)) and c564-GAL4 tub-GAL80ts/UAS-Droj2-RNAi (c564->Droj2-RNAi (2) flies. D, Luciferase reporter assay in heat-killed E. coli-treated S2 cells transfected with Attacin-Luciferase and dsRNA of GFP, kenny (key), or Droj2. The experiments were repeated two to three times and yielded similar results. Data were analyzed using Student’s t test, and values represent the mean ± S.E. of triplicate samples. *, p < 0.05; NS, difference not significant.
DNAJA3 Is Required for IκB Phosphorylation—Western blot analysis of human DNAJA3 revealed that flagellin-induced NF-κB accumulation in the nucleus was suppressed in DNAJA3 knockdown cells (Fig. 6, A and B). This finding indicates that DNAJA3 functions upstream of NF-κB nuclear translocation. Analysis of Drosophila Droj2 suggested that Droj2 works genetically downstream of Relish or in the regulation of Relish (Fig. 4, A–D). Relish protein contains the N-terminal Rel homology domain and the C-terminal IκB-like ankyrin repeat domain (57). We hypothesized that human Droj2 functions around IκB and NF-κB. Phosphorylated IκB is subjected to protein degradation, and phosphorylation of the NF-κB p65 subunit occurs with IκB dissociation and is important for its nuclear translocation (13). Western blot analysis for the phosphorylated IκB and p65 revealed that phosphorylation was not observed in DNAJA3 knockdown cells (Fig. 6C). Consistent with this, the decrease in the amount of total IκBα protein following flagellin stimulation was not detected in DNAJA3 knockdown cells. Less IκB signal before stimulation in DNAJA3 knockdown cells would be due to the fact that IκB is a transcriptional target of NF-κB (58). These findings suggest that DNAJA3 is required for the phosphorylation of IκBα. IKK is the kinase responsible for NF-κB phosphorylation. Fig. 6D shows that overexpression of the catalytic IKKβ subunit induced the phosphorylation of p65 and that phosphorylation was attenuated in DNAJA3 knockdown cells. This finding indicates that DNAJA3 functions downstream of IKK.

Discussion

Although the NF-κB signaling pathway is well studied because of its importance in many biologic processes, the complete picture of the complex molecular mechanisms of this pathway have not yet been elucidated. In this study, we identified the cochaperone Droj2, which is involved in the Imd pathway, a Drosophila NF-κB pathway, from our genome-wide gain-of-function screening in flies. Epistatic analysis revealed that Droj2 functions downstream of, or parallel to, NF-κB Relish, and a cell culture assay revealed that Droj2 is prerequisite to the nuclear translocation of Relish, suggesting that Droj2 func-
FIGURE 5. Human DNAJA3 is a functional homolog of Drosophila Droj2. A, luciferase reporter assay in HEK293 cells transfected with NF-κB-Luc and with the indicated dsRNAs targeting human DNAJ family genes and stimulated with flagellin. B, luciferase reporter assay in HEK293 cells transfected with NF-κB-Luc and dsMyD88, DNAJA3#1, or DNAJA3#2 and stimulated with flagellin. C, luciferase reporter assay in HEK293 cells transfected with NF-κB-Luc, TLR4, and MD2 and dsMyD88, DNAJA3#1, or DNAJA3#2. The cells were stimulated with LPS. DNAJA3#1 targets a different sequence of DNAJA3 than DNAJA3#2. D, luciferase reporter assay in HEK293 cells transfected with glucocorticoid receptor (GR)-Luc and NR3C1, DNAJA3#1, or DNAJA3#2. The cells were stimulated with dexamethasone. The experiments were repeated two to three times and yielded similar results. Data were analyzed using Student’s t test, and values represent the mean ± S.E. of triplicate samples. *, p < 0.05; **, p < 0.01.
tions with Relish protein. We then extended our analysis to the mammalian NF-κB/H9260 pathway and identified DNAJA3 as a human functional homolog of Droj2. Intriguingly, human DNAJA3 also works at the level of IκB/H9260 and NF-κB/H9260, suggesting that the role of Droj2 in NF-κB signaling is phylogenetically conserved.

Droj2 and DNAJA3 belong to the HSP40/DNAJ cochaperone family of proteins, which constitutes the largest and most diverse subgroup of the heat shock protein (HSP) family (55). In humans, at least 49 different DNAJ-encoding genes have been identified (55). HSP40s are widely known as regulators of HSP70 function, but they also have roles as cochaperones with HSP90 chaperones (59). Interestingly, recent studies suggest that a growing number of biologic functions of HSP40s are independent of either of these chaperones (60). DNAJ proteins are differentially expressed in human tissues, and, apparently, each DNAJ protein has its own biologic functions, although the exact cellular processes and molecular mechanisms involved are currently unknown for most of its members (55, 59, 60). Plant DNAJ proteins interact with viral coat and movement proteins to facilitate viral assembly and movement, respectively (61, 62).

With regard to the NF-κB pathway with DNAJ proteins, Cheng et al. (63, 64) have demonstrated that overexpression of DNAJA3 suppresses IκB phosphorylation by IKKβ and, thereby, concluded that DNAJA functions as a negative regulator of NF-κB. Our study also indicates a role for DNAJA3 in IκB phosphorylation, but our conclusion is opposite that of previous studies. DNAJA3 has two isoforms whose apoptosis-inducing functions apparently differ (56). One isoform is distributed dually to the mitochondria and cytosol (65), and another is associated with numerous proteins (66). Cheng et al. (63, 64) relied on the overexpression of DNAJA3 and its deletion mutants, but we mainly applied loss-of-function analyses to both Drosophila and human cultured cells. This difference may explain the contradictory interpretation of the role of DNAJA3 in NF-κB signaling.

Nuclear translocation of Relish in Drosophila is regulated by transglutaminase, which catalyzes the protein-protein cross-linking of Relish (67). Therefore, further studies are needed to evaluate the hypothesis that Droj2 functions with transglutaminase to regulate cross-linking reactions. The J-domain of DNAJ is critical for accelerating the ATPase activity of HSP70 (55).
Further studies should be performed to examine whether the co-chaperone activity of DNAJA3 is required for IκB phosphorylation, and, if so, to identify which HSP70 is the partner of DNAJA3. DNAJA3 binds to the IKK complex and IκBylation, and, if so, to identify which HSP70 is the partner of deletion mutant, are required.

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