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Discrete Functions of TRAF1 and TRAF2 in *Drosophila* Mediated by JNK- and NF-κB-dependent Signaling Pathways

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The ligand-mediated activation of the tumor necrosis factor receptor (TNFR) superfamily can induce a wide spectrum of cellular responses, including apoptosis, cell proliferation, and differentiation. These functions are mostly mediated by a family of intracellular TNFR-binding proteins, the TNF receptor-associated factors (TRAFs). In humans and mice, TRAF family consists of six members (TRAF1 through TRAF6), and these proteins have a conserved stretch of amino acids near their C termini termed the TRAF domain. The TRAF domain is required for binding of these signal-transducing adaptor proteins to the members of TNFRs. Two additional functional domains, zinc finger domain and RING finger domain, are located at the N terminus of TRAF protein, and are proposed to be essential for the activation of specific downstream signaling components.The involvement of TRAF family proteins in a variety of signal transduction pathways and cellular responses has been extensively studied by numerous cell-culture based studies and several mouse genetic studies. From the previous reports, mammalian TRAF2 and TRAF6 were found to regulate the transcription of downstream target genes through the activation of two different intracellular signaling pathways, c-Jun N-terminal kinase (JNK) and nuclear factor-κB (NF-κB) signaling pathways. Even though many attempts were made to distinguish the major TRAF-mediated signaling pathways and to deduce the *in vivo* function of each TRAF, it has been hampered by highly redundant roles of mammalian TRAFs in correlation with their signaling mechanisms.Besides the intensive studies of TRAFs in the mammalian system, there were some pioneering studies to reveal the function of TRAFs in *Drosophila*. Two *Drosophila* homologues of mammalian TRAFs, DTRAF1 and DTRAF2, have been identified, and the biochemical and cell culture-based studies with these proteins have shown that TRAF-dependent signaling pathways are indeed highly conserved in *Drosophila*. DTRAF2, like mammalian TRAF6, interacts with *Drosophila* ECSIT and Pelle, and consequently activates NF-κB in Schneider cells. DTRAF1 interacts with *Drosophila* Ste20 kinase (Misshapen, *msn*) and induces a synergistic activation of JNK in mammalian cultured cells. However, there was a contradictory report showing the functional interactions between DTRAF1 and the NF-κB signaling pathway in cell culture-based experiments. Despite these efforts, *in vivo* studies with a whole animal to confirm these *in vitro* experiments and to further dissect the specific signaling mechanisms of DTRAFs regulating developmental and immunological functions remain to be accomplished.In the present study, we demonstrated that DTRAF1 is an *in vivo* regulator of JNK pathway in *Drosophila*. Ectopic expression of DTRAF1 in the developing eye induced apoptosis, thereby causing a rough eye phenotype. Further genetic interaction analyses revealed that the apoptosis in the eye imaginal disc and the abnormal eye morphogenesis induced by DTRAF1 are dependent on JNK and its upstream kinases, Hep and DTAK1. In support of these results, *DTRAF1*null mutant showed a remarkable reduction in JNK activity with an impaired development ofimaginal discs and a defective formation of photosensory neuron arrays. In contrast, DTRAF2 was demonstrated as an upstream activator of NF-κB. Ectopic expression of DTRAF2 induced nuclear translocation of *Drosophila* NF-κBs, DIF and Relish, consequently activating the transcription of anti-microbial peptide genes, *diptericin*, *diptericin-like protein* and *drosomycin*. Consistently, the null mutant of *DTRAF2* showed immune deficiencies in which NF-κB nuclear translocation and anti-microbial gene transcription against microbial infection were severely impaired. Collectively, our findings demonstrate that DTRAF1 and DTRAF2 play pivotal roles in *Drosophila* development and innate immunity by differentially regulating the JNK- and the NF-κB-dependent signaling pathway, respectively.