## [S2-1] [4/18/2005(Mon) 09:30-10:00/Gumungo Hall A]

# JNK Down Regulate The Expression of Antimicrobial Peptide Genes Activated by NF-kB During Innate Immune Response

**Young-Joon Kim[1], Taeil Kim[1], Yong Hwa Song[2], Se Nyun Kim[2], Jeong Ho Yoon[2], Ji-Hwan Rhee[3], Jeongsil Kim-Ha[3]**

*[1]Department of Biochemistry, Yonsei University, Seoul, Korea,*
*[2]Digital Genomics Inc. Seoul, Korea, 3Department of Molecular Biology, Sejong University, Seoul, Korea*

Drosophila innate immune system is a potent first-line host defense in response to various microbial pathogens. This system is composed of several molecular signaling modules which were well conserved evolutionarily. JAK/STAT, JNK and IKK-NF-kB signaling modules play pivotal roles in the initial production of immune effector molecules, but how these signailing pathways were integrated to elicit immediate immune response is now know well. We have found that Drosophila JNK pathway can down regulate the expression of IKK/NF-kB dependent antimicrobial peptide genes. We show that this repressive effect is relayed through a Drosophila histone deacetylase complex. Furthermore, we show that when JNK activity was compromised, the acetylation levels of histone at the promoter region of antimicrobial peptide genes were increased drastically. In this report, we demonstrate that signaling modules such as JNK and IKK-NF-kB pathways have a regulatory crosstalk to respond effectively to environmental pathogens.

Innate immunity is the first-line defense system of multicellular organisms in response to various microbial invaders[11-13]. The recognition of pathogen-associated molecular patterns (PAMPs) by germline-encoded receptors initiates the signaling cascades leading to the activation of various genes encoding antimicrobial peptides (AMPs), cytokines, inflammatory mediators, and regulators of phagocytosis. In Drosophila two distinctive signaling pathways-the Toll and IMD pathways-play specific roles to induce PAMP-dependent innate immune responses.

Toll signaling pathway was initially identified to control embryonic dorsoventral patterning in Drosophila, but later found to control the innate immune response against fungal and Gram-positive bacterial infections. Spaezle, a ligand for Toll, is activated by proteolytic cleavage by a serine protease upon infection and the binding of Spaezle to Toll receptor activates a signaling cascade through dMyD88, Pelle, and Tube. This attains the degradation of kB-like Cactus followed by the nuclear translocation of NF-□B homologs Dorsal and Dif to activate distinctive anti-fungal and anti-

Gram-positive bacterial immune reactions[25-28]. On the other hand, immune response to Gram-negative bacterial infection is controlled by IMD pathway, which culminates in the expression of AMPs such as *Attacin*, *Cecropin* and *Diptericin*. Lipolpolysccharide (LPS) was initially thought as the triggering ligand for the Imd pathway, but peptidoglycan (PGN) contaminated in the purified LPS fraction was shown recently as the genuine stimulant for the IMD pathway. IMD mutant flies showed severe defects in resistance to Gram-negative bacterial infection while being normal in response to fungal and Gram-positive bacterial infection. Recognition of Gram negative bacteria PGN by a peptidoglycan recognition protein (PGRP) recruits and activates IMD, a homolog of mammalian RIP (TNF-receptor-interacting protein), dTAK1 (TGF-activated kinase 1), and IKK (kB kinase) complex. Phosphorylation of Relish by IKK, and the subsequent nuclear translocation of the processed Relish activates the expression of the IMD pathway-specific AMP genes. In addition to the activation of IKK, which leads to the synthesis of high levels of AMPs, the activated dTAK1 also stimulates the JNK signaling module. JNK activation has been implicated in the control of diverse biological processes including developmental morphogenesis, inflammation and apoptosis. In particular, the LPS/PGN induced JNK activation in the IMD pathway are involved in the synthesis of various cytokines and cytoskeletal remodeling needed for phagocytotic responses. Recently, several laboratories led the possibilities that mammalian NF-kB negatively regulates the JNK pathway in TNF-a induced apoptosis. This type of negative crosstalk has also been found in Drosophila challenged with LPS fraction and shown to involve proteasomal degradation of dTAK1 by Relish-dependent genes[10]. The inhibition of JNK module by NF-kB allows only a transient JNK activation, thus prevents over-expression of inflammatory molecules. However, whether a similar negative crosstalk in the opposite direction works to control the activation of NF-kB signaling module is not known.

In order to understand the regulatory circuits of the IMD pathway during the innate immune response against the Gram-negative bacteria, we identified the genes required for the dynamic expression patterns of the LPS/PGN-induced genes using expression profile analysis coupled with a specific gene knockdown by RNA interference (RNAi). We show that knockdown of Drosophila JNK or AP1 enhanced the expression of IKK/NF-kB dependent genes, whereas their overexpressions were inhibitory for the expression of NF-kB target genes. The negative effect of JNK module was mediated by the AP1 transcription factors recruited to the activated NF-kB target promoters at the later stage of induction, which in turn recruits a specific histone deacetylase complex to modify local histone acetylation levels. These results demonstrate a reciprocal regulation between signaling modules leading to a proper termination of IMD pathway signaling induced with LPS/PGN.