

Meeting abstract

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## RNA-interference based screen identifies new factors important for NF-kappaB activation and termination

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from 12th Joint Meeting of the Signal Transduction Society (STS). Signal Transduction: Receptors, Mediators and Genes Weimar, Germany. 29–31 October 2008

Published: 26 February 2009

*Cell Communication and Signaling* 2009, **7**(Suppl 1):A42 doi:10.1186/1478-811X-7-S1-A42

This abstract is available from: <http://www.biosignaling.com/content/7/S1/A42>

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The transcription factor NF-kappaB is a key mediator of the innate immune system. Although tremendous research efforts over the past decades have led to a more and more detailed understanding of NF-kappaB signaling, there are still missing pieces in the puzzle, especially upstream of the IKK complex and in the termination of the signaling. To identify more of the factors important for this signaling pathway we have conducted an RNA-interference based screen.

For this purpose, we have developed an assay for high throughput analysis using a human epithelial cell line stably expressing a p65-GFP-fusion construct. The nuclear translocation of p65-GFP can be quantified by automated microscopic analysis. Three different stimuli were compared: the cytokines TNF-alpha and IL1-beta and the gastric pathogen *Helicobacter pylori*. We chose *H. pylori* as inducer because permanent infection with this bacterium can lead to chronic inflammation, ulceration and cancerogenesis and NF-kappaB is thought to be crucial in the promotion of this pathology. Furthermore, using different time points of the activation, we screened not only for factors important for activation, but also for termination of the signal.

In terms of activation, the screen identified known factors like IKKalpha and IKKbeta as well as factors so far not linked to the NF-kappaB pathway. Interestingly, two factors were identified that are specific for NF-kappaB activation after *H. pylori* infection and not necessary for NF-kappaB activation by the cytokines TNFalpha or IL-1beta. Regarding termination, the screen identified among other

factors an ubiquitin E3-Ligase so far not linked to the pathway. Upon down-regulation of this E3-Ligase, p65-GFP resides longer in the nucleus. This correlates with a strong degradation of IkappaBalpha. The screen was conducted with a library of siRNAs against 646 kinases and associated proteins, and is currently expanded to a genome wide scale.