Fas-Associated Factor 1 is a negative regulator of PYRIN-containing Apaf-1-like protein 1

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PYRIN-containing apoptotic protease-activating factor-1-like proteins (PYPAFs, also called NALPs) participate in inflammatory signaling by regulating NF-κB activation and cytokine processing, and have been implicated in autoimmune and inflammatory disorders. However, the precise mechanisms that regulate the signal pathway leading to NF-κB activation are not completely understood. Here, we used yeast-two hybrid assays to identify Fas associated factor 1 (FAF1) as a protein interacting with the pyrin domains of several PYPAFs. In these assays, FAF1 interacted strongly with PYPAF1, PYPAF3, and PYPAF7, moderately with PYPAF2 and PYNOD, but not at all with the pyrin domains of pyrin or the adaptor molecule ASC. The interaction between FAF1 and PYPAF1 in mammalian cells was confirmed by immunoprecipitation assays, and the Fas-interacting domain of FAF1 was critical for this interaction. When coexpressed in HEK293 cells, FAF1 interfere with NF-κB activation induced by PYPAF1 and ASC. A FAF1 mutant lacking the Fas-interacting domain showed significantly reduced ability to inhibit NF-κB activation. Furthermore, down-regulation of endogenous FAF1 protein augmented LPS-induced IL-8 production, a biological marker for NF-κB activation, in monocytic cells. Finally, the level of FAF1 expression in THP-1 cells increased in response to NF-κB stimulation. These findings suggest that FAF1 functions as a negative regulator of an NF-κB signal pathway that involves PYPAF1 and ASC.

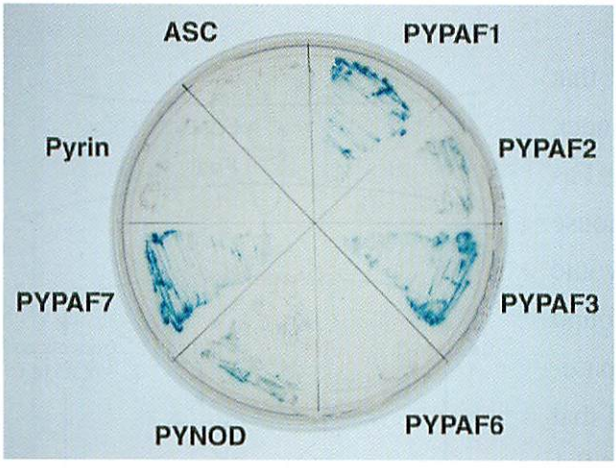

Figure 1. FAF1 interacts with the pyrin domains of PYPAF1, PYPAF2, PYPAF3, PYNOD, and PYPAF7, but not with those of pyrin, ASC and PYPAF6 in Yeast-two hybrid assays.

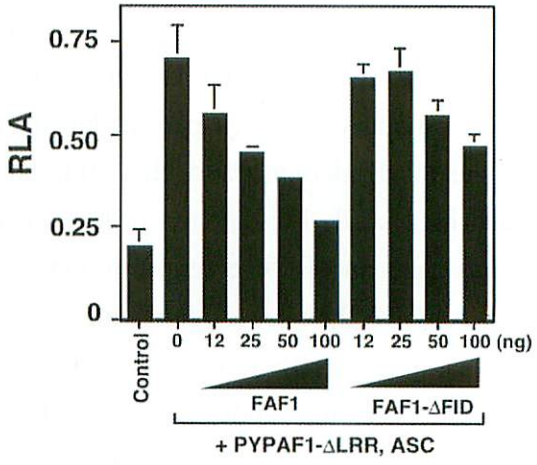

Figure 2. Cotransfection of a constitutive active mutant of PYPAF1 (PYPAF1- ΔLRR) and ASC induces NF-κB activation as revealed by a luciferase reporter assay in HEK293 cells. This NF-κB activation was inhibited by cotransfection of FAF1.