Identification of novel regulators controlling Tristetraprolin stability

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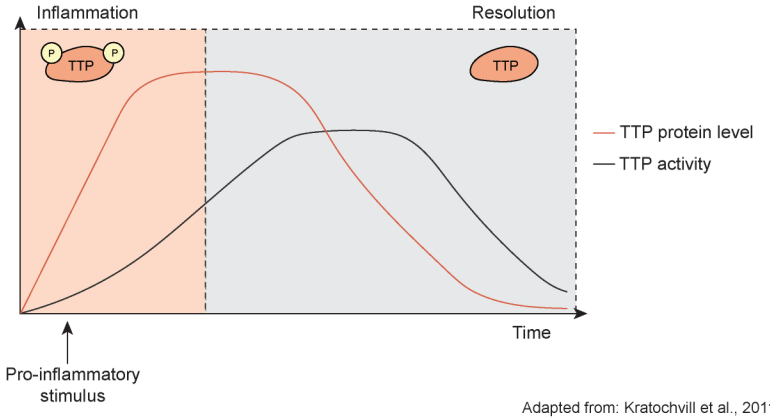
BACKGROUND:

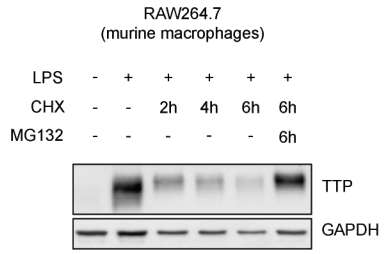
Fine-tuning of innate immune signaling is essential to restore homeostasis and to avoid the development of inflammatory disorders. In this context the RNA binding protein Tristetraprolin (TTP) plays a central role as gatekeeper of cellular homeostasis by promoting the degradation of several proinflammatory cytokines transcripts. Indeed, mice lacking TTP develop profound inflammatory syndromes. TTP activity and stability is critically controlled by its phosphorylation status and by the intrinsically unstructured nature of the protein itself.



Adapted from: Kratochvill et al., 2011

OPEN QUESTION:

TTP, as well as many intrinsically disordered proteins (IDPs), is rapidly degraded in a proteasome dependent manner. However, the molecular mechanism and the factors mediating its degradation have remained elusive.



MAIN AIMS:

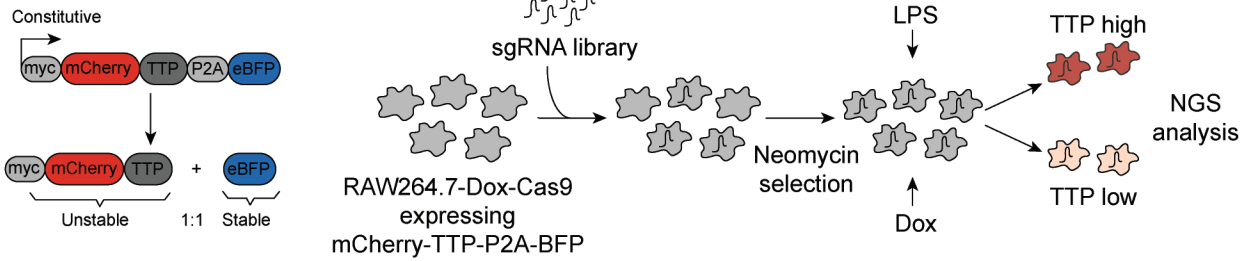
1. Identification of novel factors which specifically mediate TTP degradation using a CRISPR-based genetic screen.

2. Determine the relevance of identified 'hits' for controlling pro-inflammatory cytokine output,

3.How TTP degradation is achieved at the molecular level.

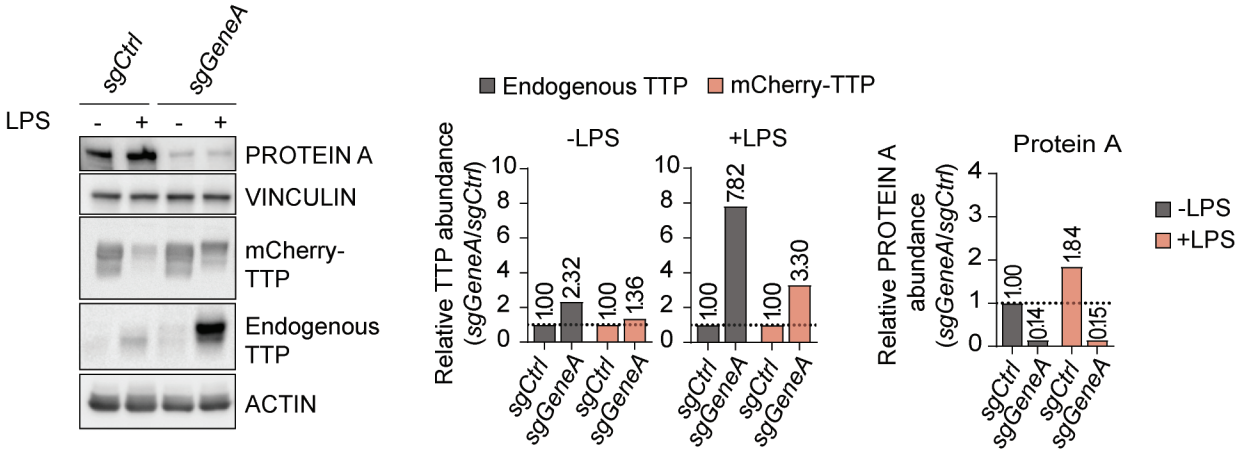
EXPERIMENTAL STRATEGY:

In order to identify factors controlling TTP stability, a genome-wide CRISPR/Cas9-genetic screen has been performed. The main premise of the designed gain-of-signal screening strategy is that cells, in which genes mediating TTP degradation are knocked-out, will show an increased stability of a mCherry-TTP reporter. The genes targeted in these population with an increased fluorescent signal will be determined, providing candidates for TTP degradation.



RESULTS:

Several TTP stability regulators have been identified and validated. PROTEIN A has been identified as the major regulator of TTP turn-over, providing a great starting point for role of this novel regulator in maintaining immune homeostasis and preventing inflammatory disease. The molecular mechanism controlling TTP degradation will then be elucidated.



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