# TNF-alpha mRNA levels in RAW264.7 cells show a biphasic expression pattern that results from both transcriptional and post-transcriptional processes

Michael P. Woods, Shelly Waggoner, Yi-Shun Lin, Tony Giordano, and Gretchen L. Temeles

Message Pharmaceuticals, Malvern, PA 19355, USA

Message Pharmaceuticals is a drug discovery company focused on the identification and development of small molecules that alter RNA/protein interactions. In the course of establishing a TNF-alpha screening program, it was essential to characterize the timing of post-transcriptional regulatory events for the TNF-alpha mRNA. The production of TNF-alpha, a critical mediator of inflammation, is induced by many different external stimuli including lipopolysaccharide (LPS). TNF-alpha biosynthesis is regulated at the transcriptional, post-transcriptional and post-translational levels, although the relative contribution of each remains controversial. We have found that TNF-alpha mRNA in RAW264.7 cells showed a previously unreported biphasic expression pattern following LPS stimulation. Before levels of secreted TNF-alpha protein began their steady rise, TNF-alpha mRNA levels increased by 10-fold within 30-60 minutes after LPS stimulation. The mRNA levels then decreased sharply, followed by a second rise between 90-120 minutes, then declined over 24 hours. In contrast, levels of the primary hnRNA transcript rose within 15 minutes and remained relatively constant at the same time that levels of mature mRNA changed dramatically. Measurement of the half-life of TNF-alpha mRNA over this period indicated that the stability of the mRNA also demonstrated a pattern of rapid and consistent changes, suggesting that the levels of mature mRNA are the result of a dynamic and complex interaction between transcriptional and post-transcriptional mechanisms.