Control of inflammatory gene expression at the step of transcription elongation

Yingli Shang[1], Teng He[2], Li Yu[1], Maddalena Coppo[3], Inez Rogatsky[3], Chao Tang[2] and Xiaoyu Hu[1]

Recruitment of RNA polymerase II (PolII) to target gene promoters to initiate RNA synthesis has long been considered the key step for gene regulation. However, recent genome-wide studies have revealed transcription of many genes are regulated post transcription initiation. Therefore, regulation of PolII transcription elongation, which is controlled by positive elongation factor complexes b (P-TEFb) and negative elongation factors (NELF), may be an important rate-limiting step in gene expression[1]. While many genes are known to be regulated at the PolII initiation step, little is known about how gene expression is modulated at the PolII elongation step.

Introduction

Pol II transcription elongation is regulated by positive elongation factor complexes b (P-TEFb) and negative elongation factors (NELF). Pol II pausing occurs shortly after transcription initiation and involves the association of pausing factors DSIF and NELF.(d)Pause release is triggered by the recruitment of the P-TEFb kinase. P-TEFb kinase phosphorylates the DSIF/NELF complex and CTD. Then the paused Pol II escapes into productive elongation.[2]

Results

We have found that LPS-induced expression of *Cxcl1*, a gene encoding a chemokine crucial for neutrophil recruitment, is regulated at the elongation step by transcription repressor hairy and enhancer of split 1 (Hes1) in mouse bone marrow-derived macrophages (BMDMs). Mechanically, Hes1 suppressed recruitment of the P-TEFb complex and subsequently attenuated e occupancy of serine 2-phosphorylated PolII at the *Cxcl1* gene locus. To directly evaluate PolII binding throughout the entire gene locus we analyzed genome-wide Pol II occupancy by chromatin immunoprecipitation followed by deep sequencing (ChIP-seq)in wildtype (WT) and Hes1-deficient BMDMs. Consistent with ChIP-PCR data, ChIP-seq data showed that Hes1 deficiency did not affect PolII binding near the transcription start site of the *Cxcl1* gene. Instead, PolII binding patterns at the *Cxcl1* gene body region significantly differed between WT and Hes1-deficient macrophages, validating our hypothesis that Hes1 indeed regulated *Cxcl1* gene transcription via targeting post-initiation steps by inhibiting transcription elongation.

By bioinformatic analysis of the PolII ChIP-seq data set, we wish to identify additional *Cxcl1*-like genes whose expression is regulated at thetranscription elongation step. In addition, we will further assess the role of transcription elongation in macrophages by genetically targeting P-TEFb and NELF complexes. We hope our results will elucidate mechanism and functional significance of regulation of inflammatory gene transcription at the elongation step, which may provide a rapid and efficient way for fine-tuning gene expression in response to environmental stimuli.

Summary and Future Plan

In this Study, Hes1 downregulated Cxcl1 gene transcription via targeting post-initiation steps by inhibiting transcription elongation. And we will further assess the role of transcription elongation in macrophages using RNAi to knockdown P-TEFb subunit (Cdk9) and NELFe or using NELFb knockout mice.

Reference

[1] Barboric, M., et al., NF-kappaB binds P-TEFb to stimulate transcriptional elongation by RNA polymerase II. Mol Cell, 2001. 8(2): p. 327-37.

[2]Adelman, K., et al., Immediate mediators of the inflammatory response are poised for gene activation through RNA polymerase II stalling. Proc Natl Acad Sci U S A, 2009. 106(43): p. 18207-12

[1] Institute for Immunology, Tsinghua University, Beijing

[2] Center for Life Sciences, Peking University, Beijing

[3] Weill Cornell Medical College, New York NY