

Chimeric murine interferon regulatory factor-2 (IRF-2) binds on IRF-E (IRF binding Element), VRE_β (Virus Response Element) but not binds on VRE_α

Krishna Prakash and Pramod C. Rath
Central University of Bihar, India

Background: IRF-2 is a multifunctional transcription factor having gene activation, repression and synergistic effect in conjunction with IRF-1. IRF-2 is also involved in type I IFN signaling by repressing INF_{β} gene. So far, the molecular mechanism of its DNA binding activity remains elusive.

Methods: We have done molecular sub-cloning, expression and EMSA study of chimeric murine IRF-2.

Result: Here we report expression of chimeric murine IRF-2 as GST-IRF-2 fusion protein in E.coli/BL21 cells and demonstrated DNA binding activity by Gel retardation technique using radio ^{32}P labeled IRF-E motif (GAAAGT)₄, Virus Response Element (VRE) of human INF_{β} and INF_{α} gene. We observed five different masses DNA/GST-IRF-2 complexes (1-5) with IRF-E motif, three different masses DNA/GST-IRF-2 complexes (1-3) with VRE_β, but we couldn't observe any complex of DNA/GST-IRF-2 with VRE_α. The specific binding on IRF-E motif was confirmed by carrying out 100-X fold cold competition with ^{32}P labeled IRF-E motif. In contrast to specific binding on VRE_β, we used negative control where we observed no binding complex, but we observed complexes with clones' IPTG-induced extract. As far as binding on VRE_α is concerned, we could not observe any complex in negative control as well as in IPTG-inducible clones extract.

Conclusions: Chimeric IRF-2 binds to IRF-E motif and VRE_β but not with VRE_α. This study is first of its kind and paves the way to understand the differential DNA binding and molecular mechanism of DNA binding activity of the IRF-2 molecule, which is crucial for its function(s).

Keywords: Interferon, Recombinant IRF-2, DNA binding domain, Gel retardation.

kpmolbio@gmail.com