C/EBPBeta and Elk-1 synergistically transactivate the c-fos serum response element

ABSTRACT

These data show that C/EBPβ and Elk-1 synergize in SRF dependent transcription of both a Gal-4 reporter and the SRE. This suggests that SRF, TCF, and C/EBPβ are all necessary for maximal induction of the c-fos SRE in response to mitogenic signaling by Ras.

INTRODUCTION

Introduction c-fos is a member of the family of immediate early genes, and its transcription is transiently induced in response to mitogenic signals. The serum response element (SRE) is located approximately 300 bp upstream of the transcriptional start site in the c-fos promoter and is necessary for serum induction of c-fos. The SRE binds a transcription factor named serum response factor (SRF) which was found to be necessary, but not sufficient, for serum induction of the SRE. In vivo footprinting analysis shows that SRF is constitutively bound to the SRE in both quiescent and growth factor stimulated cells. This suggests that it is the transcriptional activation of a complex of SRF and its accessory proteins that is regulated rather than regulation of SRF DNA binding. The ternary complex factors (TCFs) are members of the ets family of transcription factors. The TCF family members Elk-1, SAP-1, and SAP-2/ERP/NET have been found to have a role in regulating the SRE. TCFs cannot bind the SRE autonomously, but require protein-protein interactions with SRF in order to bind the SRE. The TCFs contain 3 conserved motifs termed the A, B, and C boxes. The N-terminal A-box (amino acids (aa) 1-90 of Elk-1) is necessary to bind DNA, while the central B-box (aa 148-168) is the SRF interaction domain. The C-terminal C-box (aa 352-399), harboring the transactivation domain, contains several consensus mitogen activated protein kinase (MAPK) phosphorylation sites. Accordingly, the TCFs have been found to be targets of the Ras-Raf-MAPK signal transduction pathway. In addition, the TCFs have been found to be targets for all three families of MAPKs: the extracellular signal-regulated kinases 1/2 (ERK1/2), the jun-N-terminal kinases/stress activated protein kinases (JNK/SAPK), and the p38 kinase. The transcriptional activity of the TCFs are stimulated by phosphorylation of the C-terminal MAPK sites. Another transcription factor that is involved in regulation of the c-fos SRE is CCAAT/Enhancer binding protein-beta (C/EBPβ). C/EBPβ (also known as NF-IL6, LAP, NF-M, AGP/EBP, and CRP2) is a member of the basic-leucine zipper family of transcription factors. The C/EBPβ mRNA contains three in-frame methionines which give rise to three different translation products: p38, p35, and p20-C/EBPβ. p38 and p35-C/EBPβ both contain an N-terminal transactivation domain and a C-terminal DNA binding/dimerization domain. p20-C/EBPβ lacks the N-terminal transactivation domain and therefore acts as a repressor of transcription. Our lab has previously shown that p35-C/EBPβ activates an SRE-driven reporter construct while p20-C/EBPβ inhibits serum stimulation of the same reporter. We have also shown that both p35-C/EBPβ and p20-C/EBPβ could interact with SRF in vivo and that the interaction between SRF and p35-C/EBPβ, but not between SRF and p20-C/EBPβ, is stimulated by activated Ras. The target for this Ras stimulation is Thr235 in a consensus MAPK site in C/EBPβ. Therefore, C/EBPβ is a target of a Ras-dependent signaling pathway that regulates its interaction with SRF. Based on the observations that TCF factors as well as p35-C/EBPβ: (1) interact with SRF (2) transactivate the SRE and (3) are responsive to Ras-dependent signaling pathways, we tested the possibility

that both TCF and p35-C/EBPβ are necessary for maximal induction of the SRE in response to mitogenic stimulation. In this study, we show that p35-C/EBPβ and the TCF family member Elk-1 synergize in transactivation of SRF dependent transcription of both a Gal4 dependent reporter and an SRE-driven reporter construct, but only in response to mitogenic stimulation by Ras. We further show that Elk-1 and p35-C/EBPβ interact in vitro in a glutathione-S-transferase (GST)-pulldown assay as well as in an in vivo coimmunoprecipitation assay. The in vivo interaction is dependent on the presence of activated Ras. Finally, we show that the C-terminal domain of C/EBPβ is sufficient to interact with Elk-1 while the N-terminal A-box of Elk-1 is necessary to interact with C/EBPβ. These results suggest a cooperative role between the TCF and C/EBPβ transcription factors in regulation of the c-fos SRE in response to Ras-dependent signaling pathways.

CONCLUSION

Conclusions This report demonstrates a new model for c-fos SRE activation in response to Ras-dependent signaling pathways. We show that SRF, Elk-1, and p35-C/EBPβ are all necessary for maximal Ras-stimulated transactivation of the SRE.