Identification of nuclear localization and nuclear export signals in Ets2 and their importance for the transcription of CTP:phosphocholine cytidylyltransferase α

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Introduction: Phosphatidylcholine (PC) is the major membrane phospholipid in mammalian cells and tissues and is produced in all nucleated cells via the CDP-choline pathway. An important rate-limiting and regulated enzyme in this pathway is CTP-phosphocholine cytidylyltransferase (CT), and the most active and important form of CT is CTα.

The transcriptional factors, Ets1 (440 amino acids) and Ets2 (468) were first identified based on its similarity to v-ets, a protein produced by the erythroid-myeloid transforming E26 avian retrovirus, and have a highly conserved Ets DNA binding domain (ETS domain) for binding to GGAA/T. Ets1 and Ets2 have been implicated in regulation of genes involved in proliferation, differentiation, apoptosis and senescence. Since CTα expression is regulated during cell proliferation and cell cycle, we were interested in the transcriptional regulation of CTα by Ets2.

The precise mechanism(s) by which Ets2 is localized within cells is not well understood. In this study, we generated enhanced green fluorescent protein (EGFP) fused, mutant versions of Ets1 and Ets2 and identified sub-domains in Ets2 with NLS and NES activity. Furthermore, we provide evidence that the NLS and NES in Ets2 are important for regulation of CTα transcription.

Materials and Methods: Tissue Culture and Transfection
COS-7 or NIH3T3 cells were grown in DMEM with 10% fetal bovine serum. For fluorescence microscopy, cells were grown overnight, then 1.0 μg of the constructed plasmids encoding mutant Ets1 or Ets2 proteins fused with EGFP was transfected using FuGENE 6®. After 24 h, cells were processed for microscopy. For luciferase assays, cells were grown overnight. 0.5 μg of CTα promoter-luciferase reporter, 0.002 μg of the pRL-CMV Renilla vector as a control; and 0.005 - 0.5 μg of wild-type, deleted or mutant forms of pcEts1 or pcEts2, or pcDNA (empty vector control) was prepared. For the electromobility gel-shift assay, cells were grown overnight and transfected with 2.5 μg of pcEts1 or pcEts2 with or without mutation or with pcDNA. Luciferase Assays

Either 24 or 48 h after transfection, cells were harvested and lysed, and the cell lysate was used in a dual-luciferase assay.

Preparation of Nuclear Extracts and Electromobility Gel-shift Assays
After 48 h, nuclear extracts were prepared. The labeled probe was separated from DNA-protein complexes by electrophoresis on a 6% non-denaturing polyacrylamide gel in Tris borate/EDTA buffer at 4°C. The gels were then dried and autoradiography was performed.

Results: Identification of the Ets2 NLS and NES
Ets2 (1-392) (which contains amino acids 1-392 of Ets2) was clearly localized in the cytoplasm (100%) of both NIH3T3 cells and COS-7 cells although Ets2 (1-229) was localized to the nucleus. EGFP fused, N-terminally deleted Ets2 (391-468) was localized to the nucleus but the shorter N-terminally deleted Ets2 (289-468) was homogenously distributed in all cells. Based on these results, we reasoned that a short, leucine-rich region of Ets2 (364-372) might be a NLS; moreover, we proposed that a short stretch of basic amino acids (404KRKNKPK410) found for Ets1 in the ETS domain is also present in Ets2.

Discussion: NLSs and NESs can determine the sub-cellular localization of proteins. NLSs are typically short basic regions or a bipartite basic sequences, whereas NESs are approximately ten amino acid sequences that are rich in hydrophobic residues, particularly leucine. As reported in this study, we identified a novel NLS in Ets2 consisting of the cluster of basic amino acids (404KRRKNKPK410) found for Ets1 in the ETS domain and surmise that this region is also important for Ets2 transcriptional function. Ets2 deleted at the C-terminus (1-392) was clearly localized to the cytoplasm. However, when an additional mutation at 364 and 372 amino acids was introduced, the deleted Ets2 was distributed in both the cytoplasm and the nucleus of cells. These results suggest that Ets2 contains a fragment with NES activity that is located in ETS domain. Among Ets transcriptional factors, only transcriptional repressors have previously been reported to contain both NLSs and NESs. Therefore, Ets2 provides the first example of a transcriptional activator that contains both NLS and NES functional domains. These results suggest that differential regulation of CTα transcription by Ets2 is mediated at least in part by movement of Ets2 through the nuclear membrane.

Recently, several papers have reported unique functions for Ets2, that related to osteoclast apoptosis, immune response at sites of inflammation, skeletal abnormalities and autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus and Sjogren’s syndrome. The transcription of CTα is regulated during cell cycle, and Ets2 enhanced the transcription of cyclin D1, and its activity was inhibited by cdk10. Colony-stimulating factor 1 enhanced the transcription of both CTα and Ets2. Taken together, these results suggest that Ets2 may regulate the transcription of CTα in physiological status.