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Crystal Structure of Mouse Elf3 C-terminal DNA-binding Domain in Complex with Type II TGF-β Receptor Promoter DNA

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The Ets family of transcription factors is composed of more than 30 members. One of its members, Elf3, is expressed in virtually all epithelial cells as well as in many tumors, including breast tumors. Several studies observed that the promoter of the type II TGF-β receptor gene (TβR-II) is strongly stimulated by Elf3 via two adjacent Elf3 binding sites, the A-site and the B-site. Here, we report the 2.2 Å resolution crystal structure of a mouse Elf3 C-terminal fragment, containing the DNA-binding Ets domain, in complex with the B-site of mouse type II TGF-β receptor promoter DNA ($mT\beta R\text{-}II_{DNA}$). Elf3 contacts the core GGAA motif of the B-site from a major groove similar to that of known Ets proteins. However, unlike other Ets proteins, Elf3 also contacts sequences of the A-site from the minor groove of the DNA. DNA binding experiments and cell-based transcription studies indicate that minor groove interaction by Arg349 located in the Ets domain is important for Elf3 function. Equally interesting, previous studies have shown that the C-terminal region of Elf3, which flanks the Ets domain, is required for Elf3 binding to DNA. In this study, we determined that Elf3 amino acid residues within this flanking region, including Trp361, are important for the structural integrity of the protein as well as for the Efl3 DNA binding and transactivation activity.

Keywords: Elf3; Ets domain; protein–DNA complex; type II TGF-β; receptor crystal structure

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Introduction

E74-like factor-3 (Elf3) is an E-twenty-six specific (Ets) transcription factor family member involved in the expression of at least 10 genes.[1] Elf3 works with other transcription factors to achieve specificity and to regulate genes involved in inflammation, differentiation, tumorigenesis, and metastasis.[2–11] Elf3 has been identified in a wide range of epithelial carcinoma cells, and it is aberrantly expressed in cancers of the lung and breast.[12] In recent years, studies in several cell culture model systems have shown that the promoter of the type II TGF-β-receptor (TβR-II) gene is transactivated by Elf3. The TβR-II gene behaves as a tumor suppressor gene in many contexts, and it is expressed in nearly all cell types. The TβR-II gene is strongly stimulated by Elf3 via two adjacent Elf3 binding sites (the A-site and the B-site) in differentiated cells derived from mouse F9 embryonal carcinoma cells.[13,14] Moreover, the forced expression of Elf3 in Hs578t breast cancer cells elevates the expression of TβR-II dramatically and decreases the tumorigenicity of these cells.[4] Given that both the A-site and the B-site are required for full promoter activity and the DNA binding domain of Elf3 appears to form a ternary complex *in vitro* with DNA containing both the A-site and the B-site, it is likely that the stoichiometry of binding for Elf3 to this promoter is 2:1.[13,14]

Elf3 is composed of five defined domains: a pointed domain, a transactivation domain (TAD), a serine and aspartic acid-rich (SAR) domain, an AT-hook

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Abbreviations used: TβR-II, type II TGF-β-receptor; TAD, transactivation domain; SAR, serine and aspartic acid-rich; Ets, E-twenty-six specific; EBS, Ets-binding site; mTβR-II, mouse TβR-II; dsDNA, double-stranded DNA; EMSA, electrophoretic mobility-shift assay.

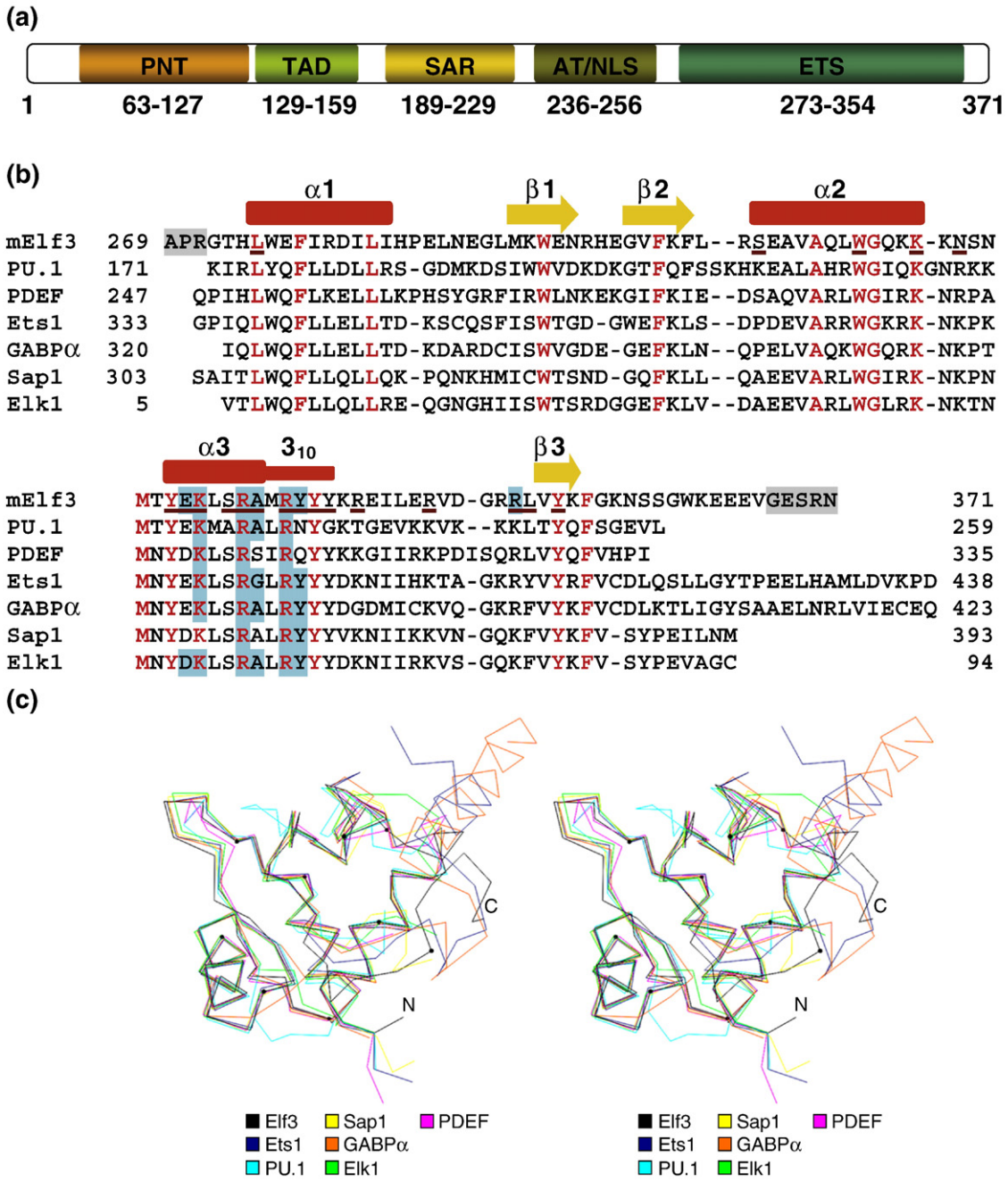

Fig. 1. Elf3 domain structure and sequence alignment. (a) A diagram showing the domains of Elf3. (b) Structure-based sequence alignment of the DNA-bound Ets domains of Elf3, PDEF (***1yo5***), PU.1 (***1pue***), Sap1 (***1k60***), Elk1 (***1dux***), Ets1 (***1k79***), and GABPα (***1awc***). The secondary structure elements of Elf3 are indicated above the corresponding sequence. Helices are depicted by red rectangles and β strands as yellow arrows. The α3 helix terminates with a distorted 3_{10} helix. Elf3 residues missing from the structure are highlighted in gray. Conserved residues are shown in red. The Elf3 residues interacting with DNA are underlined with brown. Amino acid residues of Ets domains in direct contact with DNA bases are highlighted in cyan. (c) A stereoview of the 3D alignment of Ets domains in the crystal structures of DNA-bound Elf3, PDEF, PU.1, Sap1, Elk1, Ets1, and GABPα. The α-carbon traces are shown as color-coded lines. The α-carbon position of every 10th amino acid residue of Elf3, starting from residue 280, is marked by a small circle.

domain, and an Ets domain (Fig. 1a).[1] The C-terminal Ets domain is a conserved DNA-binding domain approximately 85 amino acids in length that is shared among the members of the Ets family.[15–17] Ets domains bind specifically to a core GGAA/T motif of DNA often referred to as an Ets-binding site (EBS).[18–20] Structural analysis of Ets proteins reveals topological similarities in interactions with DNA;[21] however, the structural basis for the contribution of DNA sequences flanking the EBS is not well understood. To achieve a deeper understanding of the structural basis of the Ets domain binding to mouse TβR-II (mTβR-II) promoter DNA, we initiated structural studies of the mouse Elf3 Ets domain in