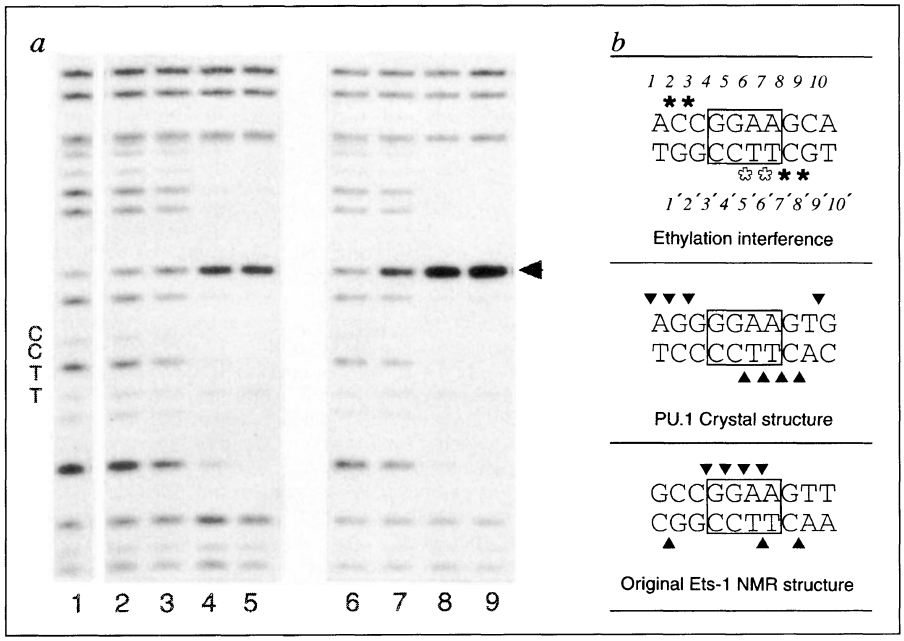
DNA binding by the ETS domain

SIR — The *ets* gene family encodes eukaryotic transcription factors that bind specific DNA sequences through the highly conserved, 85-residue ETS domain. The structures of two ETS-domain proteins, PU.1 and Ets-1, in complex with DNA, were described recently by crystallographic and by NMR spectroscopic methods, respectively[1,2]. These reports were apparently contradictory, although an erratum to ref. 2 now resolves this controversy. Our biochemical analyses of ETS-domain interactions with DNA (refs 3, 4 and work reported here) provide an independent resolution to this controversy and a verification of the current model of ETS-domain–DNA interactions.



Biochemical analyses distinguish between proposed models of ETS-domain–DNA binding. *a*, Similar modes of DNA binding of Ets-1 and PU.1 as detected by DNaseI footprints. DNaseI protection assays were performed with Ets-1 (lanes 2–5) and PU.1 (lanes 6–9); lane 1 displays the pattern of DNaseI cleavage in the absence of protein. Minimal fragments bearing the ETS domain were used (Ets-1, residues 331–440; PU.1, residues 167–271). Use of the high-affinity binding site SC1 (ref. 3) enabled this direct comparison between PU.1 and Ets-1 as this sequence 5′-ACCGGAAGCA-3′ is recognized by many ETS-domain proteins[4]. Arrowhead, DNaseI hypersensitive site between two guanine residues on the TTCC strand. *b*, Phosphate contacts between ETS domain and DNA as implicated by ethylation-interference analysis[3,4], the crystal structure of PU.1–DNA (Fig. 2 of ref. 1), and the original NMR-based structure of Ets-1–DNA (Fig. 5 of ref. 2). The revised Ets-1 NMR structure shows contacts similar to those observed with PU.1. Coordinates identify the 5′ phosphates of the 10 base pairs of each duplex; asterisks, sites of ethylation interference; arrowheads, phosphate contacts from structural studies. The contacts at phosphate 1 and 10 are made by PU.1 residues not conserved in other ETS domains. Phosphates 5′ and 6′ were not identified in the ethylation-interference analysis of Elf-1 (open asterisks)[4]. The lysine that contacts these positions in PU.1 is highly conserved within the family, but not present in Elf-1.

The two structural studies[1,2] confirm that the ETS domain is composed of three helices (H1, H2, H3) and a four-stranded, antiparallel β-sheet, placing the ETS-domain proteins in the winged helix–turn–helix (HTH) structural family[5–7]. Further, in both structures the HTH (helices H2 and H3) recognizes the 5′-GGAA-3′ consensus sequence in the major groove of the DNA. However, the original NMR-based study positioned the HTH on DNA in an orientation rotated by nearly 180°, opposite to that seen in the crystal structure. The two structures also differed in the roles that helix H1 and the β-sheet play in DNA binding.

It seemed unlikely that the homologous DNA-binding domains of PU.1 and Ets-1 would bind DNA differently. Indeed, similar DNA contacts are observed in biochemical analyses of several ETS-domain proteins. In DNaseI footprinting studies, a hypersensitive site on the TTCC strand at a precise location is an invariant feature of ETS-domain–DNA interactions. Using a binding site recognized by many ETS-domain proteins, we show here that the DNaseI footprints of Ets-1 and PU.1 are identical (*a* in the figure). DNaseI footprints of other ETS-domain proteins, Fli-1, GABPα and Elf-1, are also the same[4].

Ethylation-interference analyses also suggest a similar mode of DNA binding for ETS-domain proteins. Six major sites of ethylation interference (phosphate numbers 2, 3, 5′, 6′, 7′ and 8′) are detected consistently in the analyses of ETS-domain proteins Ets-1, Fli-1 and GABPα (refs 3, 4; *b* in the figure). As exemplified by several previous structural studies (including *Eco*RI[8] and λ repressor[9]), this technique identifies phosphates likely to be involved in DNA–protein interactions. Thus, the ethylation-interference data provide a signature pattern of contacts for evaluation of any model of ETS-domain–DNA interactions.

The pattern of phosphate contacts identified by ethylation interference implicates the PU.1 crystal structure and the revised Ets-1 NMR structure as the appropriate model for ETS-domain–DNA interactions. The figure compares the sites of ethylation interference with the phosphate contacts reported for the PU.1 and the original Ets-1 complexes. The pattern of backbone contacts identified in the crystal structure of PU.1 accounts for all predicted phosphate contacts[1]. Lack of complete concordance is likely to reflect variation among ETS domains (see figure legend). Phosphate contacts implicated in the revised Ets-1 NMR structure are also similar to the sites of ethylation interference (ref. 2 erratum, Brookhaven protein data bank, accession number 2STT; M. Werner, G. M. Clore and A. M. Gronenborn, personal communication). These findings support the ETS-domain mode of DNA binding that involves both the β-sheet 'wing' and HTH motif. Furthermore, these analyses illustrate how structural and biochemical approaches can be used in combination to evaluate models of protein–DNA interactions.

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