We describe a novel zinc finger protein, ZID (zinc finger protein with interaction domain). At its amino terminus ZID contains a 120-amino-acid conserved motif present in a large family of proteins that includes both the otherwise unrelated zinc finger proteins, such as Ttk, GAGA, and ZF5, and a group of poxvirus proteins: We therefore refer to this domain as the POZ (poxvirus and zinc finger) domain. The POZ domains of ZID, Ttk, and GAGA act to inhibit the interaction of their associated finger regions with DNA. This inhibitory effect is not dependent on interactions with other proteins and does not appear dependent on specific interactions between the POZ domain and the finger region. The POZ domain acts as a specific protein–protein interaction domain: The POZ domains of ZID and Ttk can interact with themselves but not with each other, POZ domains from ZF5, or the viral protein SalF17R. However, the POZ domain of GAGA can interact efficiently with the POZ domain of Ttk. In transfection experiments, the ZID POZ domain inhibits DNA binding in NIH-3T3 cells and appears to localize the protein to discrete regions of the nucleus. We discuss the implications of multimerization for the function of POZ domain proteins.

[Key Words: Zinc fingers; protein interactions; DNA binding]

Received April 11, 1994; revised version accepted June 2, 1994.
breakpoints in acute promyelocytic leukemia (APL) and non-Hodgkin's lymphoma, respectively (Chen et al. 1993; Kerckaert et al. 1993; Ye et al. 1993, Miki et al. 1994). In PLZF translocations the PLZF POZ domain and only two of its nine zinc fingers are joined to the retinoic acid receptor [RARα] gene, suggesting that it might be the PLZF POZ domain rather than its zinc finger region, which is instrumental in cellular transformation.

In this paper we report the characterization of a novel POZ domain zinc finger protein, ZID (zinc finger protein with interaction domain). We show that the POZ domain acts to inhibit DNA binding, both in ZID and other POZ domain zinc finger proteins and that it acts as a specific protein–protein interaction domain. These results suggest that protein–protein interactions play an important role in the function of POZ domain proteins.

Results

ZID, a novel zinc finger protein

We set out to isolate cDNAs encoding proteins that interact with the CArG box region of the human skeletal α-actin promoter (Muscat and Kedes 1987). A yeast strain containing a lacZ indicator gene controlled by four copies of a CArG box oligonucleotide was transformed with an activator-tagged human cDNA expression library [Dalton and Treisman 1992]. A colony color assay for lacZ expression identified three colonies expressing CArG box-binding activity, which each contained the same plasmid, conventional techniques were used to isolate additional cDNAs encoding the complete protein. RNase protection analysis indicated that ZID RNA is expressed in various tissues and cell lines. ZID RNA was found at approximately equivalent levels relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RNA in colon, kidney, lung, skin, spleen, and testes and in the HFF, HeLa, HepG2, and HT1080 cell lines; in brain, ZID RNA was expressed at about a sevenfold higher level (data not shown). The combined cDNA sequence encodes a protein of 424 amino acids that we term ZID (see below; Fig. 1). The sequence includes four C_{2}-H_{2}-type zinc finger motifs at its carboxyl terminus. In addition, 120 amino acids at the amino terminus constitute a preserved domain present in a family of proteins that includes many different C_{2}-H_{2} zinc finger proteins and a group of non-zinc finger poxvirus proteins (see Fig. 10, below). The function of this domain, which we term the POZ domain, is the subject of this paper.

Definition of ZID DNA-binding specificity

Preliminary experiments showed that the protein encoded by the original cDNA, Δ139ZID, prepared by cell-

![Figure 1. The ZID cDNA. The POZ domain is overlaid with a double-dashed line, the zinc fingers are overlaid with a single solid line. Five sequencing ambiguities are designated with IUPAC code. The in-frame termination codon at the 5' boundary of the open reading frame is overlaid. The POZ domain and the zinc fingers, defined by sequence searches with the tBlastn program (Altschul et al. 1990) are indicated schematically below. Sequences were combined from the following: the original HeLa VP16-tagged cDNA, nucleotides 465 (arrowhead)–1419, two VP16-tagged cDNAs recovered by the PCR, nucleotides 1–464 and 2836–3495; human placental cDNA, nucleotides 523–2835. The sequence is incomplete as it lacks a polyadenylation signal.](genesdev.cshlp.org)
The POZ domain inhibits DNA binding

We tested the DNA-binding activity of intact ZID using the gel mobility retardation assay. Surprisingly, intact ZID protein bound the consensus binding site weakly, generating complexes that appear heterogeneous (Fig. 3A, lane 2; see also Fig. 8A, lane 2, below). To map the ZID sequences responsible for this effect, we analyzed a set of ZID deletion derivatives. Precise removal of the entire POZ domain generated proteins that formed discrete high affinity complexes; more extensive amino-terminal deletions did not increase binding further [Fig. 3A, lanes 4–6]. Quantitation of the data by PhosphorImager indicated that these deletions increased binding affinity approximately eightfold. In contrast, deletion of either half of the POZ domain or sequences between the POZ domain and the zinc fingers had no effect [Fig. 3A, lanes 3,7,8].

We next examined DNA binding by two other POZ zinc finger proteins, GAGA and Trk (Harrison and Travers 1990; Soeller et al. 1993). These proteins and truncated derivatives lacking their POZ domains were prepared by cell-free translation, and appropriate DNA probes used to assess DNA binding by the gel mobility retardation assay. Neither protein bound DNA efficiently unless sequences including the POZ domain were removed [Fig. 3A, lanes 9–14]. Taken together, these data suggest that inhibition of DNA binding in vitro is a general property of POZ domains and is not specific to ZID.

To exclude the possibility that these results reflect the inability of complexes formed by the intact proteins to enter the gel, we also analyzed DNA binding using an indirect DNA immunoprecipitation assay. For this experiment, derivatives of the proteins including the free translation, bound only weakly to the CArG box oligonucleotide used for the screen. To define the binding specificity of the ZID zinc fingers in more detail, a binding-site selection experiment was performed. Oligonucleotides selected in this assay contained variants of the consensus sequence (C/T/G)GGCTYA(T/G)(C/G)A/G(Y/C), which mismatches the CArG oligonucleotide at two conserved positions [Fig. 2A]. We used various selected oligonucleotide probes in the gel mobility retardation assay to test whether this represents the bona fide ZID DNA-binding consensus. The protein efficiently and specifically bound a probe containing the consensus sequence; binding was insensitive to addition of excess unlabeled mutant consensus oligonucleotide carrying C → G transversions at the two most conserved positions [Fig. 2B, lanes 1–3]. DNA-binding analysis with other selected sequences showed that in general the more closely a probe matches the consensus, the more strongly it binds the protein [Fig. 2B, lanes 4–24]. The derived consensus therefore accurately reflects ZID zinc finger DNA-binding specificity in vitro. The binding consensus oligonucleotide probe was used to examine HeLa cell extracts for DNA-binding activity; no specific complexes were detectable [data not shown].

Figure 2. ZID DNA-binding specificity. [A] Sequences of 35 oligonucleotides recovered by binding site selection were aligned with respect to the ZID DNA-binding site, and nucleotide frequency at each position is indicated. Of the 46 oligonucleotides sequenced, 40 contained consensus matches [5 overlapping the primer sequence] and 6 contained no match. Below the derived consensus is part of the sequence of the skeletal α-actin oligonucleotide used to select the ZID cDNA: [nA] C, G, or T; the boxes indicate mismatches to the consensus probe [bold type, underlined] and differences from probe A that still match the consensus (normal type). Probes are from the binding-site selection, and the context of the binding sites therefore differs in each. Competing synthetic oligonucleotides A and M are 21 bp.
The POZ protein–protein interaction domain

Figure 3. The POZ domain inhibits DNA binding. (A) DNA binding by ZID, Ttk, and GAGA proteins and deletion derivatives (diagramed in B) was tested using appropriate probes. (Lanes 1, 9, 12) Unprogrammed lysate. (B) Structures of the proteins tested in A. In this and subsequent figures all proteins are drawn to scale, with each parent protein and its derivatives shaded differently, and reference letter code for A on the left. POZ domains are shown as extended ovals and DNA-binding domains as rectangles. Protein names are at the right: Deletions are denoted by Δ, and numbers indicate the first amino acid that is present in the protein.

The POZ domain inhibits DNA binding by purified ZID

To test whether the inhibitory effect of the POZ domain requires its interaction with other proteins in the reticulocyte lysate, we analyzed DNA binding by purified ZID derivatives. ZID and a derivative lacking the POZ domain, Δ122Z, were expressed in *Escherichia coli* as carboxyl-terminal histidine-tagged fusion proteins, purified from inclusion bodies under denaturing conditions,

Figure 4. Indirect DNA immunoprecipitation assay of ZID and Ttk DNA binding. (A) [Lanes 1–4] One microliter of 9E10 epitope-tagged VP16–ZID and Ttk derivatives with (lanes 1, 3) and without (lanes 2, 4) the POZ domain fractionated by SDS-PAGE [10% gel]: note that ZID proteins migrate as a doublet (lanes 1, 2). [Lanes 5–10] Five microliter programmed (lanes 6, 7, 9, 10) or unprogrammed (lanes 5, 8) lysates were incubated with 32P-labeled ZID or Ttk probes and immunoprecipitated with 9E10 antibody, and recovered protein and DNA were fractionated by SDS-PAGE [lanes 5–7, ZID; lanes 8–10, Ttk]. Position of the recovered DNA is indicated by (●). [B] Structures of the proteins used in A; the 9E10 epitope is indicated [* and t].

epitope tag 9E10 (Evan et al. 1985; Munro and Pelham 1987) at the carboxyl terminus were constructed. Radiolabeled proteins either containing or lacking the amino-terminal POZ domain were incubated with appropriate radiolabeled DNA probes; protein–DNA complexes were recovered by immunoprecipitation and analyzed by SDS-PAGE. Quantitation of these data using the PhosphorImager showed that removal of their POZ domains increases the DNA-binding affinity of ZID and Ttk 5- and 10-fold, respectively (Fig. 4A, lanes 6, 7 and 9, 10).
and renatured. Protein $\Delta 122Z$, a polypeptide of 46 kD [Fig. 5A, lane 2], bound DNA efficiently in the gel mobility retardation assay [Fig. 5A, lane 5]. Purified full-length ZH protein preparations contained the expected polypeptide of 58 kD, but also always included a species of 44 kD [Fig. 5A, lane 1]. Because these proteins were purified under denaturing conditions via a carboxy-terminal histidine tag, the 44-kD protein must represent an amino-terminal truncation lacking the POZ domain. Complexes formed by intact ZH protein would be expected to migrate more slowly than $\Delta 122ZH$; however, with full-length ZH preparations only a complex of somewhat greater mobility than $\Delta 122ZH$ was detected, which presumably represents DNA binding by the proteolytic cleavage product [Fig. 5A, cf. lanes 4 and 5]. We conclude that interaction with additional proteins is not required for the inhibition of DNA binding by the POZ domain.

**The POZ domain inhibits activity of linked DNA-binding domains**

One way in which the ZID POZ domain might inhibit DNA binding is by a specific interaction with the ZID zinc finger region. To test this possibility we examined the DNA-binding properties of chimeric proteins, in which either the ZID POZ domain was replaced by heterologous POZ domains, or its zinc fingers were replaced by those from a non-POZ zinc finger protein [Fig. 6B]. POZ domains from the mouse zinc finger protein ZF5 [Numoto et al. 1993] and the vaccinia virus protein SALF17R, which is not known to bind DNA [Howard et al. 1991], both inhibited DNA binding by the ZID zinc fingers, as with ZID, heterogeneous slowly migrating complexes were detectable in both cases [Fig. 6A, lanes 1–6]. Moreover, the ZID POZ domain inhibited DNA binding when linked to the zinc finger region of SP1 [Kadonaga et al. 1987], a non-POZ zinc finger protein [Fig. 6A, lanes 7–9].

These experiments suggest that inhibition of DNA binding does not require a specific interaction between the POZ domain and the zinc finger region. We therefore tested whether the ZID POZ domain could interfere with DNA binding when linked to DNA-binding structures other than C$_2$–H$_2$ zinc fingers. Three different types of DNA-binding domains were linked to the ZID POZ domain: The Oct-1 POU/homeo domain [Sturm et al. 1988], the serum response factor [SRF] DNA-binding domain [Norman et al. 1988], and the Cys$_2$–Cys$_2$ zinc finger domain of RAR$\alpha$ [Giguere et al. 1987]. These proteins bind DNA as monomers, homodimers, and heterodimers [with retinoid X receptor (RXR)], respectively; the ZID/RAR$\alpha$ chimera was examined because it resembles the PLZF–RAR$\alpha$ chromosomal translocation (see Discussion). The DNA-binding activity of each chimera was compared with that of an appropriate derivative lacking the POZ domain. In each case, the ZID POZ domain greatly inhibited DNA binding by the chimeric protein [Fig. 6A, lanes 10–24]. Taken together, these results imply that the inhibitory effect of the POZ domain on DNA binding does not result from a specific interaction between it and the zinc finger region.

**The POZ domain is a protein–protein interaction motif**

We next considered the possibility that the POZ domain mediates oligomerization, and this somehow prevents access of the zinc fingers to DNA. To test this idea we used an immunoprecipitation assay, in which epitope-tagged and nontagged proteins were produced by cotranslation and immunoprecipitated using the tag antibody. In this assay, efficient coprecipitation of the nontagged protein is indicative of a stable interaction between the proteins. Two carboxy-terminally 9E10 epitope-tagged ZID derivatives were constructed: One comprised the amino-terminal half of ZID, whereas the second was otherwise identical but lacked the POZ domain [Fig. 7B, proteins A and B]. Interaction of these proteins with a variety of nontagged ZID derivatives was tested [Fig. 7B, proteins D–I]. Coprecipitation of the nontagged protein was only observed in cases where both proteins contain the intact ZID POZ domain [Fig. 7A, cf. lanes 1, 3, and 7]; similar results were seen when full-length tagged ZID was used [data not shown]. When nontagged and tagged ZID derivatives of differing sizes were mixed together...
rather than cotranslated, coprecipitation was inefficient, indicating that limited exchange took place and the interaction is kinetically stable (data not shown). The ZID POZ domain did not interact with ZID derivatives containing either the amino- or carboxy-terminal portions of the ZID POZ domain or the intact POZ domains from the ZF5, GAGA, or viral SalF17R proteins, showing that the interaction is specific (Fig. 7A, lanes 5,6,8; data not shown).

We next tested whether the Ttk POZ domain behaves similarly to that of ZID. For these experiments we used a Ttk derivative in which its amino-terminal sequences including the POZ domain are linked to a carboxy-terminal 9El0 epitope tag (Fig. 7B, protein C). In the immunoprecipitation assay this protein interacts efficiently with a nontagged Ttk derivative that contains its POZ domain but does not interact with the POZ domains of ZID and ZF5 (Fig. 7A, lanes 9–11; data not shown). In contrast, the Ttk POZ domain interacts with the GAGA POZ domain as efficiently as it interacts with itself (Fig. 7A, lanes 9–11; data not shown). The strict sequence specificity of these interactions strongly suggests that the proteins produced in vitro are correctly folded. These experiments demonstrate that the POZ domain is a protein–protein interaction domain and imply that it is protein–protein interactions mediated by the POZ domain that result in inhibition of DNA binding.

These observations raise the possibility that the multimeric forms of POZ zinc finger proteins bind specific DNA sequences distinct from those recognized by the finger region alone. However in site selection experiments, the intact proteins select, albeit more slowly, oligonucleotides containing the same consensus sequence selected by the fingers alone (data not shown; see Discussion).

**DNA binding by ZID–POZ heterodimers**

The experiments described above suggest a model in which efficient access of DNA to the zinc finger region of the protein is sterically hindered in the complex that results from POZ domain interactions. In this case, formation of a mixed complex between ZID and another POZ protein might result in increased accessibility of the ZID zinc fingers to DNA. To test the feasibility of this, we asked whether cotranslation of full-length ZID with partial ZID fragments containing the POZ domain resulted in the activation of ZID DNA-binding activity. Cotranslation of the central non-POZ, non-zinc finger region of ZID with intact ZID has no effect on DNA binding (Fig. 8A, lanes 2,3). In contrast, cotranslation of intact ZID with any one of three proteins containing an intact ZID POZ domain results in generation of discrete complexes, the mobility of which is proportional to the size of the ZID fragment (Fig. 8A, lanes 4,5,7). The DNA-binding activity generated in this fashion is weak compared with that of ZID derivatives lacking the POZ domain and requires the presence of an intact ZID POZ domain in the cotranslated protein (Fig. 8A, lane 8; data not shown). Because the cotranslated ZID derivatives

---

**Figure 6.** Specificity of POZ domain action. (A) DNA binding by the various chimeric proteins shown schematically in B was tested using appropriate probes. (Lanes 1–6) ZID; (lanes 7–9) SP1; (lanes 10–13) Oct-1; (lanes 14–16) SRF; (lanes 17–24) DR+51. (lanes 1,4, 7,1 O, 14,17) Unprogrammed lysate [nonspecific complexes vary between different batches]. RAR and RXR proteins were mixed after translation; the same result was seen if the proteins were cotranslated [not shown]. (B) Structures of the proteins assayed in A.
The POZ domain inhibits ZID DNA binding in vivo

Finally, we used transfection experiments to examine the activity of ZID in vivo. Reporter plasmids were constructed in which transcription of the chloramphenicol acetyltransferase (CAT) gene is controlled by one or three copies of the consensus ZID DNA-binding site or a mutated derivative. These reporter plasmids show similar levels of transcriptional activation upon transfection into NIH-3T3 and HeLa cells, indicating that the ZID binding site does not act as a positive or negative promoter element in these cells (Fig. 9A, lanes 1, 3, 5; data not shown). Cotransfection with expression plasmids expressing either intact ZID or a derivative lacking the POZ domain had no effect on reporter gene activity (data not shown). In contrast, cotransfection with an expression plasmid encoding VΔ139Z, in which the POZ domain is replaced by the VP16 transcriptional activation domain, efficiently and selectively activated the reporters containing the intact ZID DNA-binding sites (Fig. 9A, lanes 2–6).

Because ZID alone did not appear to activate transcription, we used ZID–VP16 fusion proteins to assay ZID DNA binding in vivo: The VP16 activation domain is lack the zinc fingers, it is likely that these complexes represent DNA binding by ZID hetero-oligomers. To examine the stoichiometry of these complexes, we cotranslated intact ZID with two different ZID derivatives containing the POZ domain. Two discrete protein–DNA complexes were generated, corresponding to those seen when the ZID derivatives were cotranslated individually with intact ZID. This suggests that the discrete DNA-binding species is a heterodimer composed of the intact ZID protein and the cotranslated derivative (Fig. 8A, cf. lanes 4–6).

Figure 7. The POZ domain is a specific protein–protein interaction domain. (A) Top] 15% SDS-PAGE analysis of 1 μl of [35S]methionine-labeled nontagged and 9E10 epitope-tagged proteins (shown schematically in B), produced by cotranslation in the combinations indicated above each lane. Some proteins migrate diffusely due to comigration of lysate proteins. [Bottom] SDS-PAGE analysis of proteins recovered from 5 μl of lysate by immunoprecipitation using 9E10 antibody. [B] Structures of the proteins tested in A. (s) The proteins terminate at amino acid position 308, ZID, 321, Ttk; 245, GAGA.

Figure 8. ZID DNA binding can be rescued via dimerization with an isolated POZ domain. (A) ZID and its derivatives were produced either alone or by cotranslation in the combinations indicated above each lane. DNA-binding activity was tested by gel mobility retardation assay with a ZID oligonucleotide probe. (Lane 1) Unprogrammed lysate. [B] Structures of the proteins.
We have characterized a novel zinc finger protein termed ZID. ZID is a member of a large family of proteins that contain a 120-amino-acid sequence motif, which we term the POZ domain. The POZ domain mediates protein oligomerization, and in all POZ proteins examined this interaction prevents high affinity DNA binding. It will be interesting to see whether other conserved motifs found in zinc finger proteins, such as the KRAB and FAX domains, are also involved in protein interactions (Knochel et al. 1989; Bellefroid et al. 1991; Rosati et al. 1991; Pengui et al. 1993).

We know little about the function of the ZID protein itself. Although we can detect ZID-specific RNA in many cell lines and tissues, we are unable to detect ZID-specific DNA-binding activity in cell extracts: This may reflect either failure to translate ZID RNA or, perhaps, POZ-dependent inhibition of ZID DNA-binding activity. It is unlikely that ZID plays a role in skeletal actin gene regulation, as the sequence used to identify ZID cDNAs represents a low affinity and evolutionarily nonconserved ZID binding site; moreover, a search of the sequence data base for conserved matches to the ZID consensus revealed no potential ZID DNA-binding sites of clear biological relevance. Several POZ zinc finger proteins have been proposed to be transcriptional repressors (Harrison and Travers 1990; Read et al. 1992; Brown and Wu 1993; Numoto et al. 1993; Xiong and Montell 1993): However, although an attractive view is that POZ domains act to inhibit transcriptional activation in vivo, we observed neither activation nor repression of transcription by ZID. Further study of the ZID protein in vivo will be required to provide insight into its biological role.
The POZ domain

The sequences of 28 POZ domains present in the data base at the time of submission are shown in Fig. 10. The first POZ domain proteins identified were encoded by poxviruses (Upton and McFadden 1986); however, the POZ domain was only recognized as a discrete protein domain at the time of submission are shown in Fig. 10. The sequences of 28 POZ domains present in the data base are as follows: M35233, M15806; SWC4L, and B (13 sheet); a helical region predicted in six of the nine proteins. Proteins and sequence accession numbers for the sequences are as follows: T6, T8, T9, M81919, F3L, C2L, M35027, EP65, Extremella virus protein p65, Z14256; kelch, open reading frame 1, L08632, C50C30.8, T16H12.6, C50C30.8, T16H12.5 and T16H12.6, C. elegans putative proteins, L14433, Z10662, GCL, M97933, protein A, X75498, BAB, U01333, EST1-5, expressed sequence tags, M85329, Z19233, M85793, T07956, T08942. POZ proteins not included in Fig.: EST1 and EST2 do not encode intact domain; Shope fibroma virus proteins T6, T8, T9, M14689, M12332, M15921 are nearly identical to the myxoma proteins. While under review two more POZ domain genes were submitted to the database: FBP, X79050 and Lola, U07607.

Figure 10. POZ domains. POZ domain sequences were aligned by the AMPS program (Barton and Sternberg 1987). Highly divergent domains are indicated (*). The consensus sequence is derived from positions where >85% of all POZ domains have the indicated amino acids. (Bottom) POZ domain motif search strings are shown. String A identifies all POZ proteins; string B all but the four more divergent proteins. Dark shading indicates positions with at least 85% identity, light shading for positions with only conservative amino acid substitutions. Structural predictions using a joint prediction program (E.E. Eliopoulos, University of Leeds, UK) for the 10 zinc finger POZ domains are shown. Regions scored by three or more algorithms in at least eight proteins are indicated by H (helix) and B (beta sheet); (h) a helical region predicted in six of the nine proteins. Proteins and sequence accession numbers for the sequences are as follows: Z13, T. Schulz and J. Wells (pers. comm.), LAZ3/Bcl-6, Z21943 and U00115; ZPFS, L16896, ZFS, L15325, PLZF, Z19002, KUP, X16576, Ttk X17121, BR-C X54663, GAGA, L22205, MT6, MT8, MT9 myxoma virus proteins T6, T8, T9, M81919, M35233, M15806, SWC4L, swine poxvirus protein C4L, L22013, VSalF 17R, VF3L, VC2L, vaccina virus proteins SalF17R, F3L, C2L, M35027, EP65, Extremella virus protein p65, Z14256; kelch, open reading frame 1, L08483; C50C30.8, T16H12.5 and T16H12.6, C. elegans putative proteins, L14433, Z10662, GCL, M97933, protein A, X75498, BAB, U01333, EST1-5, expressed sequence tags, M85329, Z19233, M85793, T07956, T08942. POZ proteins not included in Fig.: EST1 and EST2 do not encode intact domain; Shope fibroma virus proteins T6, T8, T9, M14689, M12332, M15921 are nearly identical to the myxoma proteins. While under review two more POZ domain genes were submitted to the data base: FBP, X79050 and Lola, U07607.
majority of which are hydrophobic, occur at 37 positions throughout the 120-amino-acid region; of these, 6 positions are identical in >85% of the sequences while at a further 11 positions only conservative substitutions are found. Additional positions show conservation among members of subfamilies such as the *Drosophila* zinc finger proteins or the viral proteins. Structural predictions for the POZ domains from the 10 zinc finger proteins suggest an alternation of helical and β-sheet structures; in the most highly conserved region a heptad repeat of hydrophobic residues is apparent. We have submitted to the Prosite motif data base two search strings that unequivocally identify the POZ family members in the current data base [Fig. 10].

The POZ domain mediates protein–protein interactions

Our data indicate that the ability to mediate protein interactions is a general property of POZ domains. Of the five POZ domains we have tested, those from ZID, ZF5, and the poxviruses exhibit only homomeric interactions, whereas those from the Ttk and GAGA proteins also participate in a heteromeric interaction. It should be possible to exploit the latter observation to map residues that determine the specificity of POZ domain interactions. The amino-terminal location of many POZ domains suggests that this potentially accessible location may be a prerequisite for interactions. However, several proteins contain sequences amino-terminal to the POZ domain, and the conservation of such sequences between the C50C3.8 and T16H12.5 proteins suggests that they are translated: Perhaps such proteins represent precursors containing a POZ domain that is exposed following proteolytic cleavage [see below].

We have not rigorously defined the stoichiometry of POZ domain interactions. Intact ZID protein generates heterogeneous complexes in the gel mobility retardation assay, which might result either from dissociation of multimers during electrophoresis or from unstable binding of dimers to two or more probe molecules. In contrast, simultaneous production of both intact ZID and a truncated derivative that retains the POZ domain but lacks the fingers, generates a heterodimeric, well-resolved DNA protein complex. Other data, however, are suggestive of multimeric interactions: The presence of the POZ domain allows the formation of large, uniform structures visible by electron microscopy [R. Newman, V. Bardwell, and R. Treisman, unpubl.], and sucrose velocity sedimentation experiments suggest that intact ZID and Ttk proteins are very large [V. Bardwell and R. Treisman, unpubl.].

An intact POZ domain appears necessary and sufficient for protein–protein interaction when tested in the coimmunoprecipitation assay or the DNA-binding “rescue” assay. In contrast, when efficiency of DNA binding was used to evaluate activity of the ZID amino-terminal sequences, deletion of either amino- or carboxy-terminal halves of the POZ domain failed to activate DNA binding. These observations suggest that subfragments of the POZ domain can exhibit residual interactions but that these must be weak compared with those of the intact domain.

Are POZ domain interactions regulated? A number of mechanisms for regulation can be envisaged. First, the POZ domain might be removed by specific proteolysis or by differential mRNA splicing [it should be noted, however, that of the various Ttk and BR-C mRNA isoforms identified, none encodes such a derivative [DiBello et al., 1991; Read and Manley, 1992]. Second, POZ domain interaction could be activated by proteolytic cleavage in which sequences amino-terminal to the POZ domain are removed. Third, POZ domain activity might be regulated by covalent modifications such as phosphorylation. Fourth, its interaction with other proteins either containing or lacking POZ domains might produce complexes with different functional properties. We are currently investigating these possibilities.

The POZ domain and DNA binding

We showed that in three zinc finger POZ proteins the presence of the POZ domain is associated with 5- to 10-fold reduction in DNA-binding affinity and the formation of heterogeneous complexes in the gel mobility retardation assay. Some DNA binding by the intact proteins is also detectable in immunoprecipitation, DNase I footprinting, and Southwestern blotting assays [V. Bardwell and R. Treisman, unpubl.]. The inhibitory effect of the POZ domain does not require interaction with other cellular proteins and does not appear to be mediated by specific interactions between the POZ domain and the zinc finger region concerned. Most strikingly, the zinc finger region of ZID can be replaced by heterologous DNA-binding structures without compromising the inhibitory effect of the POZ domain. The simplest interpretation of our data is that in the dimeric or multimeric complexes, the protein–protein interactions mediated by the POZ domain effectively prevent the interaction of the associated DNA-binding domain with DNA.

Our results are in apparent contrast to several studies that demonstrate DNA binding by the Ttk, GAGA, and ZF5 proteins [Brown et al., 1991; Read and Manley, 1992; Numoto et al., 1993; Soeller et al., 1993; Tsukiyama et al., 1994]. However, these studies did not explicitly address the effect of the POZ domain on DNA binding; the activity reported may correspond to the weak binding we detect with the intact proteins. Alternatively, it is possible that the inhibitory effect of the POZ domain might have been relieved by limited proteolysis during purification, or by the addition of amino-terminal sequences to enable expression of recombinant fusion proteins. Another potential explanation is that POZ domain proteins purified from natural sources might be in a form in which they bind DNA efficiently.

Our observations raise the possibility that POZ zinc finger proteins do bind DNA efficiently but with a specificity different from the isolated finger domains. In a simple model, homo-oligomers of POZ proteins might bind novel DNA sequences not recognized by the iso-
labeled finger regions. Our data do not support this idea. Although both the Ttk and ZF5 proteins have been reported to bind double targets containing two monomer sites separated by 29 and 16 bp, respectively (Brown et al. 1991; Numoto et al. 1993), intact Ttk does not efficiently bind such a target from the ftz promoter [V. Bardwell and R. Treisman, unpubl.]. Moreover, binding-site selection experiments with intact Ttk and ZID recovered only conventional monomer consensus sequences from a pool of 26-bp random sequence oligonucleotides. Finally, while it is attractive to propose that POZ-mediated oligomerization of GAGA, which contains only one zinc finger, allows efficient DNA binding (Soeller et al. 1993), in our experiments this does not appear to be the case. However, it remains possible that heteromeric complexes between different POZ proteins do bind DNA, and this possibility will require further study.

Non-zinc finger POZ proteins

Our results suggest that non-zinc finger POZ proteins also utilize this domain as an oligomerization motif and that this will be reflected in their activity. For example, we found that the viral POZ domain could effectively substitute for that of ZID to inhibit DNA binding. The Drosophila kelch protein is localized in specialized structures of the intercellular bridges that connect the nurse cells of the developing oocyte [Xue and Cooley 1993]; we speculate that oligomerization activity of the POZ domain is involved in this process. Perhaps the viral proteins, whose function is unknown but are apparently homologs of the kelch protein, form similar structures.

POZ domains and cancer

Two chromosomal translocations implicated in human cancer involve POZ domain zinc finger protein genes. One type of APL translocation fuses the POZ domain PLZF gene to that encoding the transcription factor RARα (Chen et al. 1993). This fusion protein is likely to be defective in DNA binding, as the ZID POZ domain inhibits DNA binding by many different types of DNA-binding domain, including that of RARα, when linked in cis. We suggest that the putative PLZF–RARα oncogene may act in a dominant-negative fashion by sequestering its partner RXR in an inactive multimeric complex. Consistent with this proposal, PLZF–RARα inhibits retinoic acid-induced transcriptional activation in transfection experiments [Chen et al. 1994]. A similar hypothesis has been advanced in the case of other, more common, APL translocations, which join the non-POZ domain gene PML to the RARα gene [Grignani et al. 1994]. Like the PLZF fusion, the PML–RARα resulting fusion contains a novel oligomerization domain, derived from PML, which both alters the DNA-binding properties of RARα (Perez et al. 1993) and causes the sequestration of RXR in large nuclear structures [Koken et al. 1994; Weis et al. 1994].

Up to 12% of non-Hodgkin’s lymphomas are associated with translocations involving the 3q27 locus, which encodes the POZ zinc finger protein LAZ3/Bcl6 (Kerckaert et al. 1993; Ye et al. 1993; Miki et al. 1994)], at least in one case, LAZ3 transcription is activated by its transcription into the IgH enhancer region [Kerckaert et al. 1993]. A conventional model would suggest that this leads to inappropriate activation of LAZ3/Bcl6 target genes in lymphoid cells, in turn leading to transformation. However, in light of our results, it is reasonable to suggest an alternative model in which transformation arises by a dominant-negative mechanism involving the sequestration of partner POZ domain proteins. Both the PZLF and LAZ/Bcl6 translocations should provide good model systems for studying POZ protein activity in a biological context.

Materials and methods

Yeast reporter strains

A skeletal α-actin CaR box 1–lacz reporter gene was constructed by insertion of four copies of the oligonucleotide 5’-tgacaCCACACCACAAAATATGCTCGAGAA-3’ into the XhoI site of pLGΔ178 [pVB4, oligonucleotide orientation + + + +]. The reporter gene was embedded in the Saccharomyces cerevisiae URA3+ coding region by insertion into the Stul site of pURA3 (G. Micklem, unpubl.) creating pVB25, which was used to generate an indicator yeast strain S25 by disruption of the genomic URA3 locus (Dalton and Treisman 1992).

Isolation of ZID cDNAs

S25 cells were transformed with a VP16 activator-tagged cDNA library [Dalton and Treisman 1992]. Transformation was as described (Hill et al. 1991) with 1.5-μg carrier DNA/μg library DNA; filter sheets [Dalton and Treisman 1992] were transferred from Ura− to galactose medium after 24–28 hr. Approximately 5×105 transformants were screened by the colony color assay [Dalton and Treisman 1992] and plasmids were recovered from cells displaying a plasmid-dependent blue phenotype. Further ZID cDNAs were recovered from the VP16 cDNA library by polymerase chain reaction (PCR) using nested primers or by use of ZID nucleotides 465–808 as a probe for the screening of a 3q27 placental CDNA library.

Plasmids

Construction was by standard methods; all plasmid structures were verified by appropriate restriction digest or sequencing. For in vitro transcription/translation, coding sequences were inserted into T7plink or derivatives that place the 9E10 Myc epitope tag at the amino or carboxyl terminus (Dalton and Treisman 1992). Coding sequences were inserted into MLVplink [Dalton and Treisman 1992] or pEF–BOS [Mizushima and Nagata 1990], and into pET-21d [Novagen] for expression in mammalian cells and bacteria, respectively. Details of plasmid construction are available on request. The single-letter code, with 9E10 epitope sequences underlined, is used throughout.

ZID derivatives

ZID derivatives were inserted into T7plink or 9E10-tagged derivatives unless specified. ZID: [ZID codons 1–424 (complete protein)]. VA139Z: [MAGS][VP16 codons 410–490][ZID codons 139–424]; also in MLVplink. tA139Z: [MEQKLISEEDLNMAGSEFPLCSKE][ZID codons 139–424]. δ69Z: [M][ZID codons 69–424]: Δ122Z: [MAGS][ZID codons 122–424]. Δ139Z: [MAGSFPLCSKE][ZID codons 139–424].
\( \Delta 253Z: \) [MAGS][ZID codons 253–424]. Z69Δ139: [ZID codons 1–69]EFLPLSKE[ZID codons 139–424]. Z122Δ253: [ZID codons 1–122 and 253–424]. ZVert: [ZID codons 1–415][VP16 codons 411–490][EFLEQKLISEEDLN] (also in pEF–BOS). \( \Delta 122Z Vert (MAGS)[ZID codons 122–415][VP16 codons 411–490][EFLEQKLISEEDLN] \) (also in pEF–BOS). ZID derivatives in pEF–BOS. Z212Δ ([ZID codons 1–122]AGSEFLEQKLISEEDLN). Delta212ZΔ: ([ZID codons 1–122]AGSEFLEQKLISEEDLN). Delta222ZΔ: ([MAGS][ZID codons 122–212]AGSEFLEQKLISEEDLN). Z122A: ([ZID codons 1–122]SRSIRLVEASA). Z212Δ: [ZID codons 1–122]SRSIRLVEASA. Z212A: ([ZID codons 1–122]SRSIRLVEASA). Z122Z212Δ: ([MAGS][ZID codons 122–212]AGSEFLEQKLISEEDLN). ZVert ([ZID codons 1–415][VP16 codons 411–490][EFLEQKLISEEDLN] (also in pEF–BOS). Z212Δ: ([ZID codons 1–122]AGSEFLEQKLISEEDLN). Delta212ZΔ: ([ZID codons 1–122]AGSEFLEQKLISEEDLN). Delta222ZΔ: ([MAGS][ZID codons 122–212]AGSEFLEQKLISEEDLN). Z122A: ([ZID codons 1–122]SRSIRLVEASA). Z212Δ: ([ZID codons 1–122]SRSIRLVEASA). Z212A: ([ZID codons 1–122]SRSIRLVEASA).

\( \Delta 253Z: \) [MAGS][ZID codons 253–424]. Z69Δ139: [ZID codons 1–69]EFLPLSKE[ZID codons 139–424]. Z122Δ253: [ZID codons 1–122 and 253–424]. ZVert: [ZID codons 1–415][VP16 codons 411–490][EFLEQKLISEEDLN] (also in pEF–BOS). \( \Delta 122Z Vert (MAGS)[ZID codons 122–415][VP16 codons 411–490][EFLEQKLISEEDLN] \) (also in pEF–BOS). ZID derivatives in pEF–BOS. Z212Δ ([ZID codons 1–122]AGSEFLEQKLISEEDLN). Delta212ZΔ: ([ZID codons 1–122]AGSEFLEQKLISEEDLN). Delta222ZΔ: ([MAGS][ZID codons 122–212]AGSEFLEQKLISEEDLN). Z122A: ([ZID codons 1–122]SRSIRLVEASA). Z212Δ: ([ZID codons 1–122]SRSIRLVEASA). Z212A: ([ZID codons 1–122]SRSIRLVEASA). Z122Z212Δ: ([MAGS][ZID codons 122–212]AGSEFLEQKLISEEDLN). ZVert ([ZID codons 1–415][VP16 codons 411–490][EFLEQKLISEEDLN] (also in pEF–BOS). Z212Δ: ([ZID codons 1–122]AGSEFLEQKLISEEDLN). Delta212ZΔ: ([ZID codons 1–122]AGSEFLEQKLISEEDLN). Delta222ZΔ: ([MAGS][ZID codons 122–212]AGSEFLEQKLISEEDLN). Z122A: ([ZID codons 1–122]SRSIRLVEASA). Z212Δ: ([ZID codons 1–122]SRSIRLVEASA). Z212A: ([ZID codons 1–122]SRSIRLVEASA). Z122Z212Δ: ([MAGS][ZID codons 122–212]AGSEFLEQKLISEEDLN). ZVert ([ZID codons 1–415][VP16 codons 411–490][EFLEQKLISEEDLN] (also in pEF–BOS). Z212Δ: ([ZID codons 1–122]AGSEFLEQKLISEEDLN). Delta212ZΔ: ([ZID codons 1–122]AGSEFLEQKLISEEDLN). Delta222ZΔ: ([MAGS][ZID codons 122–212]AGSEFLEQKLISEEDLN). Z122A: ([ZID codons 1–122]SRSIRLVEASA). Z212Δ: ([ZID codons 1–122]SRSIRLVEASA). Z212A: ([ZID codons 1–122]SRSIRLVEASA).

The POZ protein–protein interaction domain

DNA-binding site probes

Oligonucleotides containing ZID, Ttk, SP1, Oct-1, and SRF sites were inserted into plasmid polylinkers and probes generated by PCR using appropriate primers. RAR/RXR probe (DR+5) was generated by labeling overlapping complimentary oligonucleotides. Protein-binding sites are underlined and linker sequences are in lowercase. TTK: 5’-CATTGTTAATGGAGCTATCTCTTTATATAGTGTAGTGGATGGCCA-3’, from the fushi tarazu enhancer 1610–1651 bp (Harrison and Travers 1990). GAGA: 5’-CTCGCCCTCCTGCTGTCGCTGCGATC3’, site C from the engrailed promoter 2301–2317 bp (Soeller et al. 1993). SP1: 5’-AATTCGACAGCCGCGCCGGCGCTCCAGGTAGC-3’, a synthetic site. Oct1: 5’-GATCCATGCAATGAGAGAAGTCTTA-3’, a synthetic site. SRF: 5’-ctagAGATGTCATATTAGGATCAGATC-3’, the c-fos serum response element (Hill et al. 1993). DR+5 5’-CTAGCTCATAGTGTGCAAGCATC-3’, a synthetic site.

Immunoprecipitation assays

Reactions (50 μl, containing 5 μl of reticulocyte lysate and 200 ng of 9E10 antibody) were set up as for DNA binding, but without DNA. Ten microliters of a 50% slurry of protein A–Sepharose (prebound with rabbit anti-mouse antibody in reaction buffer) was added and, after incubation for 1–2 hr at 4°C with rotation, the beads were recovered by centrifugation and washed twice with 0.5 ml of cold RIPA buffer (20 mM Tris-HCl at pH 8.0, 100 mM NaCl, 0.5% deoxycholate, 0.5% NP-40, 0.05% SDS, 10% glycerol, 10 mM EGTA, and protease inhibitors). Bound proteins were eluted by boiling beads in 2× SDS–gel loading buffer and fractionated on 15% SDS–polyacrylamide gels.

Expression of ZID in E. coli

Expression and purification of histidine-tagged recombinant proteins was carried out using Novagen protocols. Purified proteins were dialyzed against 100 volumes of 50% buffer D0.1 M NaCl, 1 mM DTT, and 4 μM urea for 1 hr, against the same buffer with 2 μM urea (1 hr), then no urea (1 hr), and again overnight without urea. Protein concentration was estimated by Coomassie staining after SDS–PAGE.

Other methods

In vitro transcription was as described previously, for cell-free translation, rabbit reticulocyte lysate [Promega] was used according to the manufacturer’s instructions and included 50 μM ZnCl2. Gel mobility retardation assays were carried out as described (Pollock and Treisman 1990; Marais et al. 1993) using 0.1 mg/ml of poly[d(C–C)] (0.05 mg/ml for RAR/RXR), 2.5 or 5 μM of probe and 0.75–1.5 μl of reticulocyte lysate or 1 ng of purified protein. Equal amounts of proteins and lysate were used in any given experiment. For whole cell extracts from transfected cells, 15 μg was used. Gel mobility retardation assays with GAGA were carried out at 4°C. Site selections and DNA coimmunoprecipitations were carried out essentially as described (Pollock and Treisman 1990, 1991). The PhosphorImager was used for quantitation where necessary. Sequencing, SDS–PAGE, and immunoblotting with detection by enhanced chemiluminescence assay were carried out by standard methods.
Acknowledgments

We thank I. Goldsmith for oligonucleotide synthesis, M. Ginsberg, A. Whittaker, M. Mitchell, and P. Freemont for computing assistance, P. Jordan for confocal microscopy, A. Travers and L. Fairall for Ttk cDNA and binding site, G.L. Smith for SalF17R DNA, A. Dejean for RARα cDNA, R. Evans for RXR cDNA, S. Goodbourn and J. Eloranta for Oct-1 oligonucleotides and cDNA, M. Parker and A. Butler for DR+5 oligonucleotides, M. Fried for use of a gel reader, and F. Watt and G. Evan for use of fluorescence microscopes. We thank S. Dalton for the VP16 cDNA library and advice on yeast techniques. We thank J. Ahringer, G. Evan, N. Jones, S. Munro, D. Zarkower, and members of the laboratory for helpful discussion and comments on the manuscript. V.B. was supported by the Human Frontier Science Program and the Medical Research Council of Canada. R.T. is an international scholar of the Howard Hughes Medical Institute.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 USC section 1734 solely to indicate this fact.

References

Altschul, S.F., W. Gish, W. Miller, E.W. Myers, and D.L. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. 215: 403–410.

Barton, G.J. and M.J. Sternberg. 1987. A strategy for rapid multiple alignment of protein sequences. Confidence from tertiary structure comparisons. J. Mol. Biol. 198: 327–337.

Bellefroid, E.J., D.A. Poncelet, P.J. Lecocq, O. Revelant, and J.A. Martial. 1991. The evolutionarily conserved Kruppel-associated box domain defines a subfamily of eukaryotic multifingered proteins. Proc. Natl. Acad. Sci. 88: 3608–3612.

Brown, J.L. and C. Wu. 1993. Repression of Drosophila pair-rule segmentation genes by ectopic expression of tramtrack. Development 117: 45–58.

Brown, J.L., S. Sonoda, H. Ueda, M.P. Scott, and C. Wu. 1991. Repression of the Drosophila fushi tarazu [ftz] segmentation gene. EMBO J. 10: 665–674.

Chen, Z., N.J. Brand, A. Chen, S.J. Chen, J.H. Tong, Z.Y. Wang, S. Waxman, and A. Zelent. 1993. Fusion between a novel Kruppel-like zinc finger gene and the retinoic acid receptor-alpha locus due to a variant t(11;17) translocation associated with acute promyelocytic leukemia. EMBO J. 12: 1161–1167.

Chen, Z., F. Guidez, P. Rousselot, A. Agadir, S.-J. Chen, Z.-Y. Wang, L. Degos, A. Zelent, S. Waxman, and C. Chomienne. 1994. PLZF-RARA fusion proteins generated from the variant t(11;17) translocation in acute promyelocytic leukemia inhibit ligand-dependent transactivation of wild-type retinoic acid receptors. Proc. Natl. Acad. Sci. 91: 1178–1182.

Dalton, S. and R. Treisman. 1992. Characterization of SAP-1, a protein recruited by serum response factor to the c-fos serum response element. Cell 68: 597–612.

DiBello, P.R., D.A. Withers, C.A. Bayer, J.W. Fristrom, and G.M. Guild. 1991. The Drosophila Broad-Complex encodes a family of related proteins containing zinc fingers. Genetics 129: 385–397.

Dorn, R., V. Krauss, G. Reuter, and H. Saumweber. 1993. The enhancer of position-effect variegation of Drosophila, E(var)3-93D, codes for a chromatin protein containing a conserved domain common to several transcriptional regulators. Proc. Natl. Acad. Sci. 90: 11376–11380.

Evans, G.I., G.K. Lewis, G. Ramsay, and J.M. Bishop. 1985. Isolation of monoclonal antibodies specific for human c-myc proto-oncogene product. Mol. Cell. Biol. 5: 3610–3616.

Giguere, V., E.S. Ong, P. Segui, and R.M. Evans. 1987. Identification of a receptor for the morphogen retinoic acid. Nature 330: 624–629.

Gotzmann, D., J.-L. Couderc, S.E. Cranston, and F.A. Laski. 1993. Pattern formation in the limbs of Drosophila: bric a brac is expressed in both a gradient and wave-like pattern and is required for specification and proper segmentation of the tarsus. Development 119: 799–812.

Grignani, F., M. Fagioli, M. Alcalay, L. Longo, P.P. Pandolfo, E. Danti, O. Biondi, F.L. Coco, F. Grignani, and P.G. Pelicci. 1994. Acute promyelocytic leukemia: From genetics to treatment. Blood 83: 10–25.

Harrison, S.D. and A.A. Travers. 1990. The tramtrack gene encodes a Drosophila finger protein that interacts with the ftz transcriptional regulatory region and shows a novel embryonic expression pattern. EMBO J. 9: 207–216.

Hill, C.S., R. Marais, S. John, J. Wynne, S. Dalton, and R. Treisman. 1993. Functional analysis of a growth factor-responsive transcription factor complex. Cell 73: 395–406.

Hill, J., K.A. Ian, I.G. Donalk, and D. Griffiths. 1991. DMSO-enhanced whole cell yeast transformation. Nucleic Acids Res. 19: 5791.

Howard, S.T., Y.S. Chan, and G.L. Smith. 1991. Vaccinia virus homologues of the Shope fibroma virus inverted terminal repeat proteins and a discontinuous ORF related to the tumor necrosis factor receptor family. Virology 180: 633–647.

Kadonaga, J.T., K.R. Carner, F.R. Masiarz, and R. Tjian. 1987. Isolation of cDNA encoding transcription factor Sp1 and functional analysis of the DNA binding domain. Cell 51: 1079–1090.

Kerckaert, J.P., C. Deweindt, H. Tilly, S. Quied, G. Lecocq, and C. Bastard. 1993. LAZ3, a novel zinc-finger encoding gene, is disrupted by recurring chromosome 3q27 translocations in human lymphomas. Nat. Genet. 5: 66–70.

Knochel, W., A. Potting, M. Koster, B.T. el, W. Nietfeld, T. Bouwmeester, and T. Pieler. 1989. Evolutionary conserved modules associated with zinc fingers in Xenopus laevis. Proc. Natl. Acad. Sci. 86: 6097–6100.

Koken, M.H.M., F. Puvion-Dutilleul, M.C. Guillemin, A. Viron, G. Linares-Cruz, N. Stuurman, L. de Jong, C. Szostecki, F. Calvo, C. Chomienne, L. Degos, E. Puvion, and H. de The. 1994. The t(15;17) translocation alters a nuclear body in a retinoic acid-reversible fashion. EMBO J. 13: 1073–1083.

Koonin, E.V., T.G. Senkevich, and V.I. Chernov. 1992. A family of DNA virus genes that consists of fused portions of unrelated cellular genes. Trends Biochem. Sci. 17: 213–214.

Liou, H.C. and D. Baltimore. 1993. Regulation of the NF-kappa B/rel transcription factor and I kappa B inhibitor system. Curr. Opin. Cell Biol. 5: 477–487.

Marais, R., J. Wynne, and R. Treisman. 1993. The SRF accessory protein Elk-1 contains a growth factor-regulated transcriptional activation domain. Cell 73: 381–393.

McKnight, S.L. and K.R. Yamamoto, eds. 1992. Transcriptional regulation. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Miki, T., N. Kawamata, S. Hirosawa, and N. Aoki. 1994. Gene involved in the 3q27 translocation associated with B-cell lymphoma, BCL5, encodes a Kruppel-like zinc-finger protein. Blood 83: 26–32.

Mizushima, S. and S. Nagata. 1990. pEF-BOS, a powerful mammalian expression vector. Nucleic Acids Res. 18: 5322.

Munro, S. and H.R. Pelham. 1987. A C-terminal signal prevents secretion of luminal ER proteins. Cell 48: 899–907.

Muscat, G.E. and L. Kedes. 1987. Multiple 5' flanking regions of

1676 GENES & DEVELOPMENT
the human alpha-skeletal actin gene synergistically modulate muscle-specific expression. Mol. Cell. Biol. 7: 4089–4099.

Norman, C., M. Runswick, R. Pollock, and R. Treisman. 1988. Isolation and properties of cDNA clones encoding SRF, a transcription factor that binds to the c-fos serum response element. Cell 55: 989–1003.

Numoto, M., O. Niwa, J. Kaplan, K.K. Wong, K. Merrell, K. Kamiya, K. Yanagihara, and K. Calame. 1993. Transcriptional repressor ZF5 identifies a new conserved domain in zinc finger proteins. Nucleic Acids Res. 21: 3767–3775.

Pengui, G., V. Calabro, P. Cannada-Bartoli, P. De Luca, T. Esposito, P. Taillon-Miller, S. LaForgia, T. Druck, K. Huchner, M. D’Urso, and L. Lanis. 1993. YAC-assisted cloning of transcribed sequences from the human chromosome 3p21. Hum. Mol. Gen. 2: 791–796.

Perez, A., P. Kastner, S. Sethi, Y. Lutz, C. Reibel, and P. Chambron. 1993. PMLRAR homodimers: Distinct DNA binding properties and heteromeric interactions with RXR. EMBO J. 12: 3171–3182.

Pollock, R. and R. Treisman. 1990. A sensitive method for the determination of protein-DNA binding specificities. Nucleic Acids Res. 18: 6197–6204.

———. 1991. Human SRF-related proteins: DNA-binding properties and potential regulatory targets. Genes & Dev. 5: 2327–2341.

Read, D. and J.L. Manley. 1992. Alternatively spliced transcripts of the Drosophila tramtrack gene encode zinc finger proteins with distinct DNA binding specificities. EMBO J. 11: 1035–1044.

Read, D., M. Levine, and J.L. Manley. 1992. Ectopic expression of the Drosophila tramtrack gene results in multiple embryonic defects, including repression of even-skipped and fushi tarazu. Mech. Dev. 38: 183–195.

Rosati, M., M. Marino, A. Franz, A. Tramontano, and G. Grimaldi. 1991. Members of the zinc finger protein gene family sharing a conserved amino-terminal module. Nucleic Acids Res. 19: 5661–5667.

Senkevich, T.G., G.L. Muravnik, S.G. Pozdnyakov, V.E. Chizhikov, O.I. Ryazankina, S.N. Shchelkunov, E.V. Kooin, and V.I. Chernos. 1993. Nucleotide sequence of XhoI O fragment of ectromelia virus DNA reveals significant differences from vaccinia virus. Virus Res. 30: 73–88.

Soeller, W.C., C.E. Oh, and T.B. Kornberg. 1993. Isolation of cDNAs encoding the Drosophila GAGA transcription factor. Mol. Cell. Biol. 13: 7961–7970.

Sturm, R.A., G. Das, and W. Herr. 1988. The ubiquitous octamer-binding protein oct-1 contains a POU domain with a homeo subdomain. Genes & Dev. 2: 1582–1599.

Tsukiyama, T., P.B. Becker, and C. Wu. 1994. ATP-dependent nucleosome disruption at a heat-shock promoter mediated by binding of gaga transcription factor. Nature 367: 525–532.

Upton, C. and G. Mcfadden. 1986. Tumorigenic poxviruses: Analysis of viral DNA sequences implicated in the tumorigenicity of Shope fibroma virus and malignant rabbit virus. Virology 152: 308–321.

Weis, K., S. Rambaud, C. Lavaud, J. Jansen, T. Carvalho, M. Carmo-Fonseca, A. Lamond, and A. Dejene. 1994. Retinoic acid regulates aberrant nuclear localization of FMS-RARα in acute promyelocytic leukemia cells. Cell 76: 345–356.

Xiong, W.C. and C. Montell. 1993. tramtrack is a transcriptional repressor required for cell fate determination in the Drosophila eye. Genes & Dev. 7: 1085–1096.

Xue, F. and L. Cooley. 1993. kelch encodes a component of intercellular bridges in Drosophila egg chambers. Cell 72: 681–693.
The POZ domain: a conserved protein-protein interaction motif.

V J Bardwell and R Treisman

Genes Dev. 1994, 8:
Access the most recent version at doi:10.1101/gad.8.14.1664

References
This article cites 47 articles, 16 of which can be accessed free at:
http://genesdev.cshlp.org/content/8/14/1664.full.html#ref-list-1

License

Email Alerting Service
Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or click here.