

**Fig. 3** Expression of CD4 increases response to low doses of Daudi. Assays were carried out as described in Table 1. Data shown are from a representative experiment of three experiments that were carried out: 16M-9 (○), 16T4-3 (△), 16T4-9 (□).

on the 16M-9 and 16T4-9 cell lines. Antibody titration experiments carried out on these cell lines in parallel with the CD4 (L3T4)-dependent T-cell hybridoma 3DT52.5<sup>10</sup> showed that 1,000 ng ml<sup>-1</sup> of GK1.5 was more than tenfold in excess of the amount of antibody needed to inhibit the function of 3DT52.5 completely when stimulated with A20 (data not shown). These data suggest that if the murine CD4 expressed by the murine T-cell hybridomas is functional, it does not contribute substantially to the enhancement of responsiveness observed by the human CD4-expressing cell lines. The ability of antibodies to the human CD4 molecule to block the function of the CD4-expressing lines is not due to nonspecific steric effects of antibodies bound to the cell surface as antibodies to H-2 class I (M142)<sup>20</sup> and Thy 1.1 (22.1)<sup>21</sup>, both of which are expressed at higher densities than CD4 on all cell lines studied, had no significant effect on their function (data not shown). By155.16 has also been infected with a retroviral vector capable of imparting CD8 (T8) expression. The CD8-expressing lines isolated were found to produce approximately the same amount of IL-2 in response to the Daudi cell line as does 16M-9 (S. Ratnoffsky, *et al.*, manuscript in preparation). Thus, the enhanced responsiveness of the CD4-expressing lines is not simply due to an aberrant effect caused by the expression of foreign cell-surface protein in these cells.

It has been postulated that the increased avidity between the T cell and stimulator cell and/or the intracellular signal mediated by CD4 may be critical to T-cell responsiveness under conditions of suboptimal antigen. We investigated the effect of CD4 under conditions of suboptimal antigen by looking at the ability of the infectants to respond to limiting numbers of Daudi cells. In this case the level of antigen expressed on a single stimulator cell is not altered, but the probability that a stimulator cell will meet a T cell is decreased. Therefore, any alteration in the T cell that increases its ability to be activated should result in a greater response at lower levels of stimulator cells. Cell lines 16T4-3 and 16T4-9 respond better than 16M-9 to levels of Daudi cells ranging from 10<sup>4</sup> to 10<sup>6</sup>; 16T4-9 produces as much IL-2 in response to 10<sup>4</sup> Daudi cells as 16M-9 produces to 3 × 10<sup>5</sup> Daudi cells (Fig. 3). These data suggest that the expression of CD4 by the parent line By155.16 allows a response to 30-fold fewer stimulator cells.

The data presented here demonstrate that CD4 is capable of enhancing T-cell responsiveness and may play a critical role in T-cell activation under conditions of suboptimal antigen stimulation.

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## Genetic evidence that zinc is an essential co-factor in the DNA binding domain of GAL4 protein

Mark Johnston

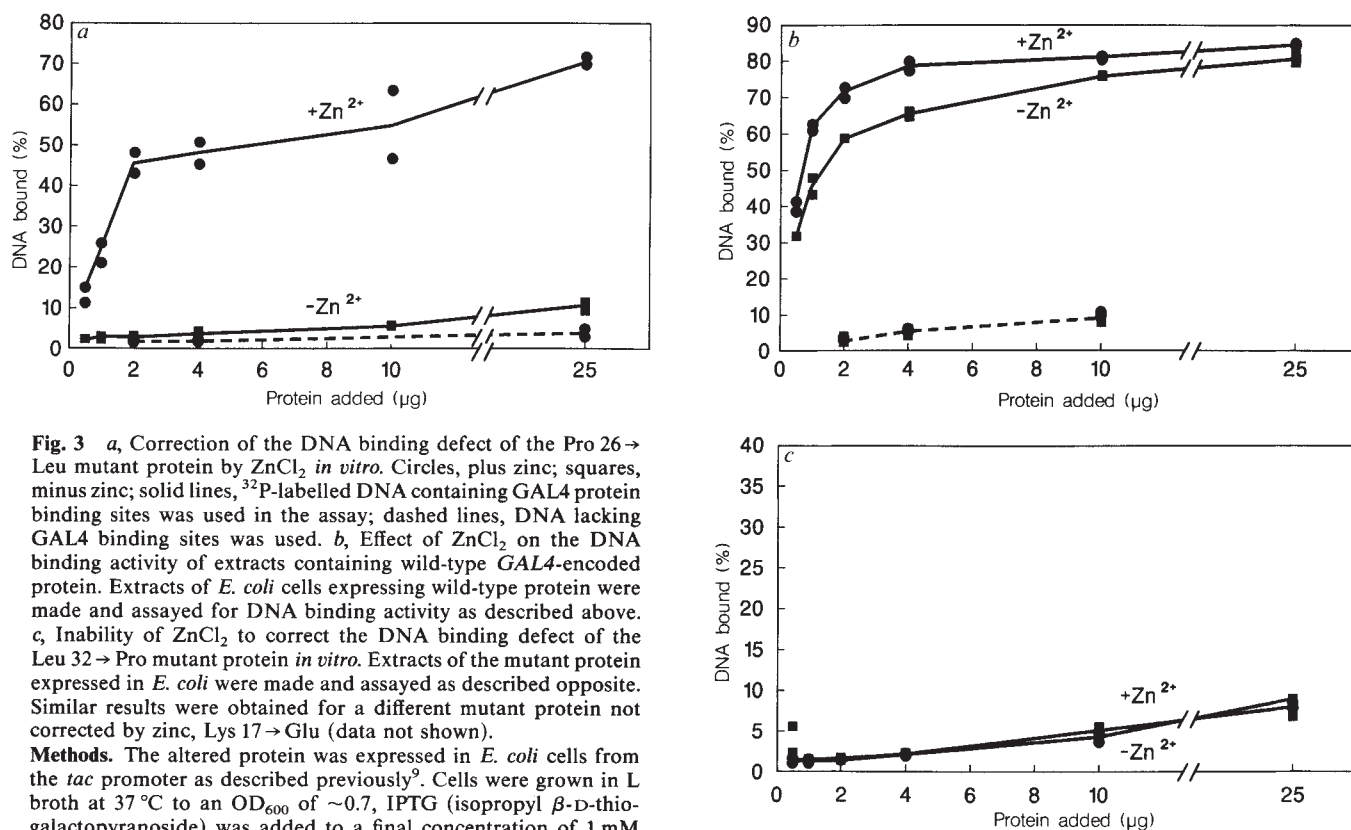
Department of Genetics, Washington University School of Medicine, St Louis, Missouri 63110, USA

The 'cysteine-zinc DNA binding finger' is a recently identified sequence motif that is present in a wide variety of transcriptional regulatory proteins and is thought to be directly involved in DNA binding<sup>1</sup>. It has been proposed that an essential component of this structure is a zinc ion bound between two pairs of cysteine residues. The *GAL4*-encoded protein of *Saccharomyces cerevisiae*, which binds to DNA and activates the transcription of several genes, contains this sequence motif. Here I describe a *gal4* mutant with an alteration in the cysteine-zinc DNA binding finger whose defect is corrected *in vivo* by high concentrations of ZnCl<sub>2</sub>. The DNA binding activity of the altered protein from this mutant is restored by ZnCl<sub>2</sub> *in vitro*. This is evidence that the GAL4 protein indeed contains zinc ions essential for its DNA binding activity.

It has been proposed that the 'zinc binding finger' contains a zinc ion bound between two pairs of cysteine (or histidine) residues (see Fig. 1a). The amino acids between the two pairs of cysteines (or histidines) are thought to form a loop or 'finger' of protein that contacts DNA. This hypothesis was initially suggested by the observation that the RNA polymerase III transcription factor A (TFIIIA) of *Xenopus laevis*<sup>2</sup>, which binds to both DNA and RNA, contains nine zinc binding finger motifs and approximately nine bound zinc ions<sup>3</sup>.

The wide variety of DNA binding proteins that contain the proposed zinc binding finger include several transcriptional regulatory proteins from the yeast *S. cerevisiae* and one from





**Fig. 3** *a*, Correction of the DNA binding defect of the Pro 26 → Leu mutant protein by ZnCl<sub>2</sub> *in vitro*. Circles, plus zinc; squares, minus zinc; solid lines, <sup>32</sup>P-labelled DNA containing GAL4 protein binding sites was used in the assay; dashed lines, DNA lacking GAL4 binding sites was used. *b*, Effect of ZnCl<sub>2</sub> on the DNA binding activity of extracts containing wild-type GAL4-encoded protein. Extracts of *E. coli* cells expressing wild-type protein were made and assayed for DNA binding activity as described above. *c*, Inability of ZnCl<sub>2</sub> to correct the DNA binding defect of the Leu 32 → Pro mutant protein *in vitro*. Extracts of the mutant protein expressed in *E. coli* were made and assayed as described opposite. Similar results were obtained for a different mutant protein not corrected by zinc, Lys 17 → Glu (data not shown).

**Methods.** The altered protein was expressed in *E. coli* cells from the *tac* promoter as described previously<sup>9</sup>. Cells were grown in L broth at 37 °C to an OD<sub>600</sub> of ~0.7, IPTG (isopropyl β-D-thiogalactopyranoside) was added to a final concentration of 1 mM (to induce expression from the *tac* promoter) either with or without 0.1 mM (final concentration) ZnCl<sub>2</sub>. Two hours later cells were harvested and lysed by sonication (10 bursts of 2 s, each followed by 10 s of cooling) in 1/100 volume of buffer D[200] either with or without 2 mM ZnCl<sub>2</sub>. (Buffer D is 25 mM Tris pH 7.5, 5 mM MgCl<sub>2</sub>, 1 mM EDTA, 12 mM 2-mercaptoethanol, 10% (w/v) glycerol. The number in square brackets is the KCl concentration in mM.) Cleared lysates, prepared by centrifugation in a Sorval SS34 rotor for 3 h at 15,000 r.p.m., were frozen in liquid N<sub>2</sub> and stored at -80 °C. Protein concentration of the extracts (usually ~20 mg ml<sup>-1</sup>) was determined by the method of Bradford<sup>12</sup>. The DNA binding activity of the extracts was determined by a nitrocellulose filter binding assay<sup>10</sup> using buffer D[50] either in the presence or absence of 2 mM ZnCl<sub>2</sub>, essentially as described previously<sup>9</sup>. Values are the proportion of <sup>32</sup>P-labelled DNA that is bound to the filters (%) as a function of extract protein added to the assay. Solid lines, pBM992 (ref. 9), consisting of pUC18 + the GAL1-10 control region, which contains 4 binding sites for the GAL4-encoded protein<sup>13,14</sup>; dotted lines, pUC18.

was assayed by a nitrocellulose filter binding assay<sup>10</sup>, as described previously<sup>9</sup>. Figure 3*a* shows that GAL4 protein from the Pro 26 → Leu mutant has substantial DNA binding activity when extracts of *E. coli* cells, induced for GAL4 expression in the presence of ZnCl<sub>2</sub>, are made in buffer containing ZnCl<sub>2</sub> and assayed in the presence of ZnCl<sub>2</sub>; extracts made and assayed without added ZnCl<sub>2</sub> have negligible DNA binding activity. The DNA binding activity of wild-type protein is unaffected by the presence of ZnCl<sub>2</sub> (Fig. 3*b*); ZnCl<sub>2</sub> has no effect on the *in vitro* DNA binding activity of GAL4 protein from a mutant whose growth defect is not corrected by zinc (Fig. 3*c*). These results strengthen the conclusion that the zinc binding finger of GAL4-encoded protein indeed contains zinc ions that are essential for its DNA binding activity. They also imply that sequences similar to the zinc binding finger of GAL4 protein in other DNA binding and transcriptional regulatory proteins are indeed zinc-containing DNA-binding domains.

Our results suggest that a GAL4 protein containing leucine instead of proline at position 26 binds zinc less readily than wild-type protein. It is not unreasonable to imagine that this proline residue causes a bend in the protein that brings the two pairs of cysteine residues close enough together to chelate a zinc ion. The protein that does not have this bend (Pro 26 → Leu) might have a lower affinity for zinc because close apposition of the pairs of cysteines is less favourable. We expect to find other *gal4* mutations that are corrected by high concentrations of zinc;

their analysis is likely to lend insight into the structure and function of the zinc binding finger. The analysis of zinc-remedial mutants provides a powerful tool for elucidating the structure and function of the zinc binding finger that complements information obtained from sequence homology searches. Furthermore, this genetic approach could be used to identify genes encoding other zinc-containing proteins whose sequences are presently unknown.

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