A Classic Zinc Finger from Friend of GATA Mediates an Interaction with the Coiled-coil of Transforming Acidic Coiled-coil 3*

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Classic zinc finger domains (cZFs) consist of a β-hairpin followed by an α-helix. They are among the most abundant of all protein domains and are often found in tandem arrays in DNA-binding proteins, with each finger contributing an α-helix to effect sequence-specific DNA recognition. Lone cZFs, not found in tandem arrays, have been postulated to function in protein interactions. We have studied the transcriptional co-regulator Friend of GATA (FOG), which contains nine zinc fingers. We have discovered that the third cZF of FOG contacts a coiled-coil domain in the centrosomal protein transforming acidic coiled-coil 3 (TACC3). Although FOG-ZF3 exhibited low solubility, we have used a combination of mutational mapping and protein engineering to generate a derivative that was suitable for in vitro and structural analysis. We report that the α-helix of FOG-ZF3 recognizes a C-terminal portion of the TACC3 coiled-coil. Remarkably, the α-helical surface utilized by FOG-ZF3 is the same surface responsible for the well established sequence-specific DNA-binding properties of many other cZFs. Our data demonstrate the versatility of cZFs and have implications for the analysis of many as yet uncharacterized cZF proteins.

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The abbreviations used are: cZF, classic zinc finger domain; FOG, Friend of GATA; TACC3, transforming acidic coiled-coil 3 protein; GST, glutathione S-transferase; CD, circular dichroism; NOESY, nuclear Overhauser effect spectroscopy; HSSQC, heteronuclear single quantum coherence; mFOG, murine Friend of GATA; TCEP, Tris(2-carboxyethyl)phosphine.

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39789

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39789
Characterization of the Interaction between FOG-1 and TACC3

Sequestering it in the cytoplasm. A decrease in TACC3 levels during erythroid maturation has been postulated to allow FOG to move into the nucleus to regulate genes required for erythroid differentiation (20).

We define the region of FOG required to mediate the interaction with TACC3 as FOG-ZF3. This domain, when expressed in isolation, showed poor solubility. Nevertheless, we used maltose binding protein (MBP) coupled to glutathione sepharose to obtain a derivative of FOG-ZF3/MBP that is soluble but maintains the TACC3-binding properties of FOG-ZF3. We confirm that this domain forms a genuine classic ZF. We further show that, remarkably, it is the α-helix of ZF3 that contacts TACC3. This is the same α-helix that, in well-characterized εZFs, contacts the major groove of DNA. The interacting domain of TACC3 forms a predominantly dimeric coiled-coil, and it is a small negatively charged C-terminal surface of this coiled-coil that interacts with FOG-ZF3. Our results are discussed in the context of the fields of genomic annotation, protein design, gene expression and hematopoiesis.

Materials and Methods

Yeast Two-hybrid Screening—The Clontech two-hybrid system was used according to the manufacturer’s instructions. A murine erythroleukemia cell cDNA library (8) in the Gal4 activation domain fusion vector, pGAD10. Gal4AD was transfected into the yeast strain Hf7C, harboring the bait plasmid pGBT9.mFOG (241–407). DNA was recovered from yeast colonies that grew on Trp/Leu/His-deficient media and used to transform Esherichia coli strain HB101. Plasmid DNA was purified and the inserts sequenced.

Interactions between FOG and TACC3 as well as TACC3 self-association were investigated by co-transforming HF7c with appropriate bait and prey plasmids. The resulting transformants were selected on Leu/Trp/His-deficient plates. Growth was monitored after incubation at 29 °C for 30 h.

Production of Recombinant Proteins and GST-pull-down Assays—Recombinant GST-FOG and GST-TACC3 proteins were produced in the E. coli strain BL-21, purified as described previously (21) and immobilized on glutathione-agarose beads. The TACC3 coding region from pGAD10.TACC3 was transferred into the vector pGEX4X-2 and used to generate 15N-labeled FLAG-TACC3 using in vitro transcription and translation (Promega). 5 μl of 15N-labeled FLAG-TACC3 was mixed with 5 μl of agarose beads-protein complex equivalent to 5 μl of fusion protein in 300 μl of pull-down buffer (150 mM NaCl, 20 mM Tris, pH 7.5, 1% Nonidet P-40, 2.5 μg/ml bovine serum albumin, 10 μM ZnSO4, 0.1 μM l-mercaptoethanol, 1.5 mM phenylmethylsulfonyl fluoride). The mixture was incubated for 1 h at 4 °C, washed with pull-down buffer, resolved on a 10% polyacrylamide gel, and visualized using a Phosphor-Imager (Amersham Biosciences).

Immunoprecipitation and Western Blot Analysis—The FLAG expression construct FLAG-TACC3 was kindly provided by Drs F. Gergely and J. Raff. Human embryonal kidney 293T cells were transiently transfected with 0.75 μg of each expression construct plus the Transfection Reagent (Roche Applied Science). Cell extracts were prepared in whole cell lysis buffer (150 mM NaCl, 5 mM EDTA, 50 mM Tris, pH 7.5, 0.1% Nonidet P-40) containing Complete protease inhibitor (Roche Applied Science), 10 mM NaF, and 1 mM Na3VO4. Proteins were immunoprecipitated with anti-FLAG M2 (Sigma) and protein G-Sepharose (Amersham Biosciences) and separated by SDS-PAGE (Novex). After transfer to polyvinylidene difluoride membranes (Millipore), filters were blocked in phosphate-buffered saline containing 5% skim milk and 0.1% Tween 20 then probed with rabbit antisera against FOG-1 followed by horseradish peroxidase-coupled secondary antibodies (Amersham Biosciences) and developed by ECL (Amersham Biosciences). The intensities of the bands were quantified using ImageQuant software (Molecular Dynamics) and corrected for equal loading using an internal control (GAPDH) as determined by densitometry of duplicate experiments. Titrations were carried out on a Microcal VP-ITC microcalorimeter at 25 °C. For each titration, 15–20 injections were made at 5-min intervals, with each injection consisting of 15 μl of titrant. The reference power was set at 10 μcal s−1, and the cell was stirred continuously at 310 rpm. Data were analyzed using the Origin ITC Analysis software (Microcal Software, Northampton, MA). A linear fit of the isotherm of the protein injected into buffer alone was subtracted from the experimental isotherms to account for the heats of dilution and mixing. A non-linear least squares fit to a single binding site model was used to obtain values for the binding constant, stoichiometry, and dissociation rate constant.

Sedimentation Equilibrium—Sedimentation equilibrium experiments were carried out on TACC3-77 (dissolved in 10 mM sodium phosphate, 150 mM NaCl, 1 mM dithiothreitol, pH 7) at 4 °C using a Beckman Optima XL-A ultracentrifuge equipped with an AnTi-60 rotor. Absorbance (230 and 360 nm) versus radius scans (0.091-mm increments) were collected at 3-h intervals until the samples had reached equilibrium. Data were recorded at three different speeds (16,000, 20,000, and 24,000 rpm) and loading concentrations (9, 18, and 37 μM). Analysis of the data was carried out using the NONLIN software (25). The density of the solvent and the partial specific volume of the peptide were estimated using Sednterp (26). Final parameters were determined by non-linear least squares fit to several models using a program similar to that of residuals and χ2 values were used to determine the goodness of fit.

NMR Spectroscopy—Lyophilized FOG peptides were dissolved in a solution containing TCEP and ZnSO4 (1 mM each) to final concentrations of ~150–300 μM. The pH was adjusted to ~5.5 using 0.1 M NaOH, and D2O (5% v/v) and 2,2-dimethyl-2-silapentane-5-sulfonic acid (25 mM) were added. One-dimensional 1H NMR spectra were acquired at 2 °C on a Bruker DRX600 NMR spectrometer. For determination of the structure of ZF3KRA, homonuclear two-dimensional data were collected and analyzed as described (23, 27). The NOESY spectrum was recorded with a mixing time of 200 ms. 1H–15N HSQC, coupling constants were obtained from an HNHA as described previously (28). All NMR data were processed using XWINNMR (Bruker, Karlsruhe) and analyzed with the program XEASY (28).

An 15N-labeled titration was carried out by adding aliquots of unlabeled TACC3-77 (330 μM stock) to a solution of 15N-labeled FOG-ZF3KRA (initial concentration = 110 μM) in the same buffer (10 mM
Characterization of the Interaction between FOG-1 and TACC3

39791

sodium acetate, pH 5.5, containing 2 mM TCEP and 1 mM ZnSO4) and recording 15N-HSQC spectra after each addition. At some points during the titration, the mixture was concentrated using a centrifugal concentrator with a 1-kDa cut-off membrane.

**Structure Determination**—NOE-derived distance restraints were obtained from the two-dimensional 1H-NOESY spectra and calibrated using the CALIBA module of DYANA (29). 1J, 1H coupling constants were included in the calculations. Structure calculations were performed initially in DYANA and later in CNS (30) using the standard ARIA protocol (31, 32). Zinc coordination (33) was incorporated in the ARIA calculations, and calculations were carried out as described previously (34). ARIA found consistent assignments for all NOEs, and a passive model of TACC3 was constructed in Swi2 PDB Viewer and SwissModel (38) (www.expasy.org/spdbv), using the x-ray crystal structure of the coiled-coil from cortxinIII (PDB accession code 1d7n (39)) as a template.

The HADDOCK protocol (40) was used to dock FOG-ZF3KRA and a single chain of the homology model of TACC3. HADDOCK is a series of scripts that run “on top of” ARIA (31, 32) and CNS (30). Residues implicated in mediating the interaction from either NMR or mutagenesis experiments described herein (nine from FOG-ZF3 and six from TACC3) were used as “active residues” to generate ambiguous interaction restraints. Six and five residues from TACC3 and FOG-ZF3, respectively, were defined as “passive” using the criteria described Dominguez et al. (40). 200 structures were calculated, and the 10 with the lowest energy were refined in water using the standard ARIA protocol as implemented in HADDOCK.

**RESULTS**

**FOG-1 Interacts with the Centrosomal Protein TACC3**—Three of the classic cZFs of FOG (cZF2, 3, and 4) are clustered in a domain in the N terminus of the protein. Interestingly, although this region superficially resembles the arrays of DNA-binding cZFs in proteins such as TFIIIA, no DNA-binding function has been observed for this region of FOG (13). We chose a region encoding amino acids 241–407 of FOG, encompassing both this cluster and the variant cZF1 (which is known to bind GATA-1), as a bait for use in the yeast two-hybrid system (Fig. 1A). We screened a murine erythroleukemia cell cDNA library and recovered six clones that interacted specifically with the bait but neither with negative control (Gal4-DNA binding domain alone) or several irrelevant bait proteins (data not shown). Sequencing revealed that three of the positive clones encoded the known FOG partner protein GATA-1, whereas the three remaining clones encoded a C-terminal portion of TACC3 (349–637). The interaction was further verified using a GST-pull-down assay, where GST-FOG-(241–407) retained in vitro transcribed and translated TACC3-(349–637), but GST alone did not (Fig. 1B). A specific interaction between FOG-1 and TACC3 was confirmed in mammalian cells (Fig. 1C). Expression vectors encoding FOG-1 or TACC-3 carrying a FLAG tag at its N terminus were transfected into embryonal kidney epithelial cells, and TACC-3 was immunoprecipitated from whole cell lysates using anti-FLAG antibody. FOG-1 was detected in these immunoprecipitates but not in those using an isotype-matched control antibody.

**Delineating the TACC3 Contact Surface of FOG**—We next used the yeast two-hybrid system to test a series of deletions of FOG and investigate whether any particular cZF was required for the interaction. The results indicated that a minimal region containing only ZF3 (328–360) was both necessary and sufficient for the interaction (Fig. 1A). To assess whether adjacent regions of FOG (e.g. ZFs 2 and 4) contribute to TACC3 binding, semi-quantitative yeast two-hybrid assays were performed with constructs containing either ZF1–4 or ZF3 alone. Resistance of the yeast to increasing 3-amino-1,2,4-triazole concentrations is a measure of the cellular levels of His3p (the HIS3 gene product) and therefore a reflection of the strength of the bait-prey interaction. A 3-amino-1,2,4-triazole titration showed that ZF1–4 and ZF3 bound TACC3 with similar affinities (data not shown), indicating that adjacent domains do not contribute markedly to the FOG-TACC3 interaction. We then carried out an alanine scan across ZF3, changing each residue to Ala, except for residues such as Ala346 (which was changed to Asp) and the highly conserved Cys, His, and hydrophobic core residues required for structural integrity. The results are shown in Fig. 1D. In summary, several residues in FOOG-ZF3, including Thr343, Thr344, Asn347, Arg350, and Val354, were implicated in binding to TACC3.

To establish how these residues relate to each other in the fold of FOG-ZF3, we sought to use standard NMR methods to determine the solution conformation of the domain. However, although the one-dimensional 1H NMR spectrum of FOG-ZF3 indicated that it took up a well defined structure (Fig. 2A), it was not sufficiently soluble to allow multidimensional experiments to be recorded. Consideration of the charge properties of the domain, together with the knowledge of the putative contact surface indicated by the alanine scan and knowledge of the main structural residues required for cZF folding, led us to construct a triple point mutant (FOG-ZF3KRA; Fig. 1E) that we expected would be more soluble but would preserve the cZF fold and the TACC3 contact surface. The introduction of two additional basic residues (E330K and L336R) and the removal of one glutamic acid (E349A) shifted the theoretical pI from 8.1 to 10.3. A one-dimensional 1H NMR spectrum of FOG-ZF3KRA (Fig. 2A) indicated that the mutant was folded and likely to have the same fold as the wild-type domain; additionally, the mutant proved to be substantially more soluble than the wild-type domain.

We therefore went on to solve the three-dimensional structure of FOG-ZF3KRA (Fig. 2B). As expected, the domain forms a classic zinc finger fold, comprising an N-terminal β-hairpin, a type-VI β-turn, and a 10-residue C-terminal α-helix, and it overlaps with the first cZF from Zif268 with a root mean square deviation of 2.9 Å (backbone Cα, N, and C atoms over the well ordered regions).

A titration of TACC3-77 into 15N-FOG-ZF3KRA was then carried out and monitored by recording 15N-HSQC spectra (Fig. 2C). Signals corresponding to several residues disappeared very early in the titration (Thr343, Ala346, Arg350, His351, Val354, Thr356, and Asp357), and several more reduced in intensity and/or underwent small shifts (−10 Hz for the HN proton) as the titration proceeded. Thus, the kinetics of the interaction are such that the interaction is in intermediate to fast exchange on the chemical shift timescale. Fitting of the chemical shift changes observed for signals in fast exchange (data not shown) indicated that the association constant for the interaction (Ka) is ~10^4 M^-1 and that a lower limit for the off-rate for the complex is ~30 s^-1. This estimate of Ka was confirmed using isothermal titration calorimetry, which yielded Ka = (1.4 ± 0.8) × 10^4 M^-1 (Fig. 2D).

Fig. 2E shows the amino acids responsible for mediating the interaction of FOG-ZF3 with TACC3, as deduced from mutational (right) and NMR titration (left) data, mapped onto the structure of FOG-ZF3KRA. In the case of the NMR data, the seven residues that underwent the largest changes in intensity are shown. The binding surface is clearly localized to the α-helix of FOG-ZF3, and the NMR and mutational data are completely consistent with each other.

The **FOG Contact Surface of TACC3**—The C-terminal region of TACC3 (349–637), which was present in the clones recovered from the yeast two-hybrid screen, contains a putative
The surface used by TACC3 to recognize FOG was examined using a mutational approach. An initial two-hybrid-based deletion analysis revealed that residues N-terminal to Glu591 had little effect on FOG binding, whereas a further deletion to Leu595 significantly reduced the interaction (Fig. 3A). Deletion of as few as ten residues from the C terminus of TACC3 (i.e., to Cys627) abolished the interaction. These results localize the contact region of TACC3 to between Glu591 and the C terminus Ile-637 (Fig. 3A).

We then carried out an alanine scan on this region. Pairs of residues in b, c, e, f, and g positions of the putative coiled-coil heptad were mutated to alanine, except where a residue was already alanine (Fig. 3B). In all, 13 mutant constructs were tested, and the results are shown in Fig. 3B. The mutations implicated a region toward the C terminus of TACC3 as the FOG contact surface.

**Characterization of the Interaction between FOG-1 and TACC3**

**A** schematic representation of Friend of GATA, showing the five variant Cys-Cys: His-Cys zinc fingers (V, four of which bind GATA-1) and the four classic zinc fingers (C) of unknown function. Constructs used in the yeast two-hybrid screen are indicated. The binding activity as measured using the yeast two-hybrid system is shown at the right; + indicates strong growth of yeast, and – indicates no growth or significantly reduced growth.

**B**, GST-pull-down experiment showing the interaction between GST-FOG-(241-407) and in vitro translated TACC3-(349-637). The top panel shows the amount of radiolabeled TACC3 retained by either GST-FOG-(241-407) or GST alone. The bottom panel is a Coomassie-stained SDS-PAGE demonstrating that both GST and GST-FOG-(241-407) were present. C, immunoprecipitation experiment demonstrating the interaction between TACC3 and FOG in mammalian cell nuclear extract. 293T cells were transfected with expression constructs encoding FOG-1 and FLAG-tagged TACC3. Whole cell lysates were prepared, and proteins were immunoprecipitated using anti-FLAG monoclonal antibody (FLAG) or control isotype-matched (C) monoclonal antibody. Western blotting, using α-FOG-1 antisera or anti-FLAG monoclonal antibody, confirmed expression of the FOG-1 and FLAG-TACC3 proteins. D, the results of the alanine scan across FOG zinc finger 3, carried out using the yeast two-hybrid assay. The interaction is shown as in A. E, amino acid sequence of FOG-ZF3. The three residues mutated in the FOG-ZF3 construct are indicated.

Coiled-coil domain. The surface used by TACC3 to recognize FOG was examined using a mutational approach. An initial two-hybrid-based deletion analysis revealed that residues N-terminal to Glu591 had little effect on FOG binding, whereas a further deletion to Leu595 significantly reduced the interaction (Fig. 3A). Deletion of as few as ten residues from the C terminus of TACC3 (i.e., to Cys627) abolished the interaction. These results localize the contact region of TACC3 to between Glu591 and the C terminus Ile-637 (Fig. 3A).

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**TACC3 Forms a Dimeric Coiled-coil with a FOG-binding Surface**—Two constructs were used in the biophysical characterization of TACC3: TACC3-103 (Ile535-Ile637) and TACC3-77 (Gln561-Ile637). Both of these constructs contain the most C-terminal region of TACC3 that is strongly predicted to form a dimeric coiled coil (MULTICOIL (41)). A far-UV CD spectrum of TACC3-77 (Fig. 4A) shows that this polypeptide is predominantly α-helical. Both yeast two-hybrid and glutaraldehyde cross-linking analyses showed that the C-terminal region of TACC3 is able to self-associate, probably as a dimer (data not shown). Sedimentation equilibrium data confirmed that TACC3-103 forms a dimer in solution (Fig. 4B), although results from gel filtration
Characterization of the Interaction between FOG-1 and TACC3

FIG. 2. Structural characterization of the TACC3-binding domain of FOG. A, one-dimensional $^1$H NMR spectra of FOG-ZF3 and of the triple mutant FOG-ZF3KRA. Both domains are well folded, and conservation of the positions of the highly shifted methyl groups at 0.28 and 0.53 ppm (arrows) indicates that both domains take up the same overall fold. B, the three-dimensional structure of FOG-ZF3KRA. A stereo diagram of the 20 lowest energy structures from ARIA is shown as backbone traces (upper), and the lowest energy structure is shown as a ribbon diagram (lower). Zinc ligands are shown in yellow, and the zinc ions are shown in gray. The well ordered region is shown in blue in the backbone traces. C, interaction between $^{15}$N-FOG-ZF3KRA and TACC3-77 as monitored by $^{15}$N-HSQC experiments. A portion of the HSQC spectrum of $^{15}$N-FOG-ZF3KRA is shown as a function of the concentration of added TACC3-77. Each spectrum has been shifted as indicated by the arrow to show the changes more clearly. Black = 0 µM, purple = 82 µM, red = 127 µM, orange = 159 µM TACC3-77. The concentration of $^{15}$N-FOG-ZF3KRA was 107 µM at the beginning of the titration. It can be seen that the signals from Val354 and Arg350 disappear early on in the titration. D, determination of the association constant for the FOG/TACC3 interaction using isothermal titration calorimetry. Raw (upper trace) and integrated (lower trace) data from a titration of FOG-ZF3KRA into TACC3 are shown. The fit to a simple 1:1 binding model is also shown. E, the TACC3-binding surface of FOG-ZF3. On the left, the amino acids that underwent the most significant changes during the $^{15}$N-HSQC titration are shown in blue, whereas on the right the amino acids implicated in TACC binding from mutagenesis are indicated.
chromatography coupled to a multiangle laser light scattering detector indicated that, at higher concentrations, additional higher order species begin to form (data not shown). These data show that the C-terminal region of TACC3 forms a structure that is dimeric and predominantly α-helical (>70–75%), consistent with the MULTICOIL prediction.

Consistent with the interconversion of dimer and higher order oligomers on a microsecond to millisecond timescale, a 15N-HSQC of 15N-TACC3-77 showed very few signals. Remarkably, however, titration of FOG-ZF3 KRA into 15N-TACC3-77 resulted in a dramatic increase in the number of signals (data not shown), indicating that the binding of FOG induced TACC3-77 to take up a single well defined solution conformation. The titration could not, however, be driven to completion (despite the addition of 6 molar equivalents of FOG-ZF3 KRA), precluding us from making chemical shift assignments. Taken together, the results of the yeast and in vitro experiments suggest that TACC3 can self-associate and exist predominately as a simple dimeric coiled-coil.

To illustrate the putative contact surface on TACC3 we mapped the TACC3 residues implicated by mutagenesis onto a homology model of TACC3-77 generated using the coiled-coil domain of cortexillin (PDB accession code 1d7n (39)) as a template. As shown in Fig. 4C, the results of the alanine scan implicating a 9- to 10-residue segment of the coiled-coil as the docking site for the α-helix of FOG-ZF3. This surface is composed of h, c, f, and g residues and is very negatively charged. Note that a second molecule of FOG-ZF3 can in principle bind to the symmetry related site on the TACC3 dimer. Finally, we used the recently described protein docking protocol HADDOCK (40) to generate a representation of the FOG-TACC3 complex (Fig. 5A). An overlay of the 10 lowest energy structures from this procedure showed that the docking calculation converged well and reveals a representation of the structure of the complex. A total of ~1000 Å² of surface area is buried in this model of the complex, around half the average for protein complexes (42). This is consistent with the relatively weak association constant. A number of specific electrostatic contacts are also observed along the length of the interface, and these remain to be verified by direct experimental means.

DISCUSSION

cZFs as Protein-binding Domains—Classic ZFs contact DNA via their α-helix, with each cZF contacting around three contiguous base pairs (43), and it is known that particular positions in the α-helix of cZFs (the −1, 1, 2, 3, and 6 positions of the helix) are important for making contacts with DNA. In contrast, almost nothing is known about the protein-binding abilities of cZFs. Here, we demonstrate that the α-helix of FOG-ZF3 contacts a small region of the C-terminal coiled-coil domain of TACC3. Remarkably, the cZF residues that contact TACC3 lie in exactly the same positions as those that are usually used in DNA recognition (Fig. 5B), although it is notable that the surface used by FOG-ZF3 to contact TACC3 is more extensive than surfaces typically used in DNA binding (Fig.
Thus, the TACC3-binding surface extends along the entire length of the α-helix, consistent with the observation that the TACC3 coiled-coil is larger than the major groove of double-stranded DNA; the latter does not permit contacts to be made by the entire α-helix of a cZF.

The affinity of the FOG-TACC3 interaction is relatively low. However, it is becoming increasingly clear that weak interactions play an important role in biology. For example, many of the protein-protein interactions that take place during electron transport exhibit association constants as low as $10^3$ to $10^4$ M$^{-1}$ (44). Similarly, we have shown recently that the N-terminal zinc finger of GATA-1 interacts with specific ZnFs in FOG family proteins with affinities between $10^4$ and $10^5$ M$^{-1}$ (23, 45). This latter interaction has been demonstrated to be essential for normal erythropoiesis (46). It is possible that the strengths of these interactions are effectively higher in the cellular environment, as a consequence of factors such as high local protein concentrations due to nuclear compartmentalization, recruitment by other factors, or molecular crowding. Both FOG and TACC3 are known to interact with other proteins, and it is conceivable that cooperation between these different interactions gives rise to more persistent FOG-TACC3 complexes. On the other hand, it is quite possible that low affinity interactions play a useful biological role, because they would allow rapid re-assembly of protein complexes in response to environmental stimuli.

By analogy to the well characterized DNA-binding properties of cZFs, these results hint at the possibility of understanding the protein-binding capacity of cZFs in terms of interactions between α-helices; however, it is likely that protein-protein interactions involving cZFs will prove to be more diverse, given the generally non-repetitive nature of protein structure. These findings do, however, underline the importance of searching for potential protein binding activity as well as nucleic acid-binding activities when studying novel cZF proteins. This is an increasingly important consideration with the availability of vast amounts of sequence data. In this context, it is also interesting that many cZFs appear to be found in isolation in protein sequences (that is, not part of one of the tandem arrays usually associated with DNA binding). It seems likely that such domains may, in many cases, act as protein recognition motifs, and this raises the question as to whether primordial cZFs are bound to proteins or to nucleic acids.

Functional Implications—In vivo experiments indicate that FOG proteins are essential partners for various GATA transcription factors (11, 47). The founding member of the FOG family, the mammalian protein FOG-1, interacts with GATA-1 and is required for erythroid and megakaryocytic development (48). The finding that FOG-1 interacts with TACC3 is of particular interest as the murine TACC3 homologue was originally identified as erythropoietin-induced cDNA (ERIC) and is thus also implicated in the control of erythropoiesis (14). TACC3 knockout experiments in the mouse have also suggested that TACC3 is involved in the control of hematopoiesis (49). Recent work has shown that TACC3 can inhibit FOG activity in gene reporter assays. This
result together with the observations that TACC3 can sequester FOG in the cytoplasm and that TACC3 levels decrease upon differentiation in murine erythroleukemia cells has led to the suggestion that TACC3 plays a role in controlling red cell differentiation by inhibiting FOG (20).

In summary, classic zinc fingers must now be considered not only as nucleic acid binding motifs but also as protein contact domains. The possibility clearly exists now for designer zinc fingers to target protein rather than DNA substrates. Moreover, when dealing with artificial cZFs tailored to bind specific DNA sequences, the possibility of protein binding activity must not be overlooked. Finally, the existence of a specific interaction between FOG and TACC3 extends the activities of both proteins and suggests a tantalizing link between promoter driven transcriptional regulation and higher order control of cell proliferation.
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A Classic Zinc Finger from Friend of GATA Mediates an Interaction with the Coiled-coil of Transforming Acidic Coiled-coil 3
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