

Crystal structure of human transgelin

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Abstract

Transgelin (TAGLN), also known as smooth muscle protein 22 (SM22), is a highly conserved protein found in smooth muscle tissues of adult vertebrates. Abolition of transgelin gene expression by the oncogenic Ras may be an important early event in tumor progression and a diagnostic marker for breast and colon cancer development. Transgelin contains a single calponin homology (CH) domain. However, the question of whether this single CH domain can bind actin remains open. Here we report the 2.3 Å resolution crystal structure of full length human transgelin, whose main structural feature is confirmed to be a CH domain. Secondary structures of CH domains from different proteins were analyzed and conserved residues were identified that maintain similar tertiary structures.

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1. Introduction

Transgelin is a ubiquitous 22 kDa protein among smooth muscle tissues of normal adult vertebrates. It was first identified from chicken gizzard (Lees-Miller et al., 1987) and its human gene was cytogenetically localized to chromosome 11q23.2 (Camoretti-Mercado et al., 1998). Transgelin mRNA was detected at high levels by Northern blotting in the aorta, lung, uterus, intestine and in primary cultures of rat aortic smooth muscle cells (Solway et al., 1995). This protein has since been identified in many different species and given different names—TAGLN (Lawson

et al., 1997), SM22 (Kim et al., 1997), WS3-10 (Thweatt et al., 1992), and mouse p27 (Almendral et al., 1989)—although comparison of their near-identical cDNAs (within corresponding species) confirms their identity as transgelin. Transgelin plays an important role in the functions of the circulatory, genitourinary, respiratory and digestive systems.

Transgelin expression is one of the first markers of smooth muscle differentiation during embryogenesis. It is expressed exclusively in the smooth muscle cells of adult tissues transiently and in embryonic skeletal and cardiac tissues (Li et al., 1996). The expression of transgelin is abolished at the transcription level by oncogenic Ras in a large number of human colon and breast tumor samples (Shields et al., 2002), which implies that loss of transgelin gene expression may be an important early event in tumor progression and a diagnostic marker for breast and colon cancer development. Transgelin ablation in mouse reduces vascular contraction in response to depolarization, which is compensated by remodeling and increased adrenergic

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responsiveness (Zeidan et al., 2004). In hypercholesterolemic ApoE-deficient mice, genetic ablation of transgelin resulted in increased atherosclerotic lesion area and a higher proportion of proliferating smooth muscle cell derived plaque cells. These results indicate a role for transgelin in the regulation of smooth muscle cell phenotypes during atherogenesis (Feil et al., 2004).

Transgelin plays a role in cell differentiation by stabilizing the cytoskeleton through actin-binding (Camoretti-Mercado et al., 1998), and decorates contractile filament bundles within cultured tracheal smooth muscle cells that exhibit a differentiated phenotype (Halayko et al., 1999). Some investigators have also discovered that transgelin can bind to gel actin through a predicted actin-binding site that contains four positively charged amino acids (Prinjha et al., 1994; Shapland et al., 1993). This putative actin-binding domain, containing about 100 amino acids, was termed the “calponin homology (CH)” domain and has been identified in a variety of proteins, ranging from actin cross-linking proteins to signaling molecules proposed to function as autonomous actin-binding motifs (Castresana and Saraste, 1995). In some cases, two tandem CH domain repeats form an “actin-binding” domain (ABD).

CH domain-containing proteins can be characterized into three groups based on domain organization (Stradal et al., 1998): (1) the fimbrin family of monomeric actin cross-linking molecules containing two ABDs; (2) dimeric cross-linking proteins (α -actinin, β -spectrin, filamin etc.) and monomeric F-actin-binding proteins (dystrophin and utrophin), each containing one ABD; and (3) proteins containing only a single CH domain (calponin and transgelin). All known single CH-domain-containing proteins, including calponin, transgelin, IQGAP, and Vav, display a higher degree of sequence similarity with each other than to CH domains embedded in ABD (Gimona and Winder, 1998).

The question of whether or not transgelin is a direct actin-binding protein in smooth muscle cells remains controversial (Morgan and Gangopadhyay, 2001). Aorta transgelin homologs have been reported to bind simultaneously to six actin monomers to cause the gelation of actin *in vitro* and to stress fibers when added exogenously to permeabilized rat fibroblasts (Kobayashi et al., 1994; Shapland et al., 1993). Fu and colleagues (Fu et al., 2000) reported that transgelin co-sediments with actin at low ionic strength and co-localizes with actin in cultured smooth muscle cells. However, the problem is that actin and transgelin act from different species (Goodman et al., 2003). Gimona and Mital (Gimona and Mital, 1998) reported that transgelin failed to bind actin in co-sedimentation assays and did not co-localize with actin in transfected fibroblasts.

The crystal structures of several CH domains from ABDs comprising tandem pairs of CH domains have been reported (Goldsmith et al., 1997; Norwood et al., 2000). The three-dimensional solution structure of calponin, containing only a single CH domain, has also been determined (Bramham et al., 2002). However, no crystal structure has been reported

of a human protein containing a single CH domain to date. We have determined the crystal structure of transgelin, which contains a single CH domain, in order to investigate the functional role of this protein in actin binding.

2. Materials and methods

2.1. Construction, expression, and purification

The *TAGLN* gene, which encodes a mature protein of 201 amino acids, was amplified by PCR from a human liver cDNA library and cloned into the prokaryotic expression vector pGEX-6p-1 with BamHI and XhoI restriction enzyme sites. The transgelin protein was expressed in LB culture medium in *Escherichia coli* strain BL21 (DE3) and the selenomethionine transgelin derivative was expressed in M9 medium containing 60 mg l⁻¹ selenomethionine. Transformed cells were grown at 310 K in 2 × YT medium with 100 mg ml⁻¹ ampicillin added to an optical density of 0.5–0.65 (OD₆₀₀) before induction with 0.1 mM IPTG for 4 h at 289 K.

Bacterial cells were harvested by centrifugation at 5000g for 10 min at 277 K. Following harvesting, the cell pellets were resuspended in phosphate-buffered saline (PBS) solution and the cells were disrupted by sonication 200 times for 4 s periods with 6 s intervals. Cell debris was removed by centrifugation of the lysates at 15,000g for 30 min at 277 K. All of the following purification steps were performed at 289 K. The clear supernatant was loaded onto a Glutathione Sepharose 4B column (Pharmacia) equilibrated with PBS and the contaminated proteins were then eluted using PBS. Cleavage of the GST tag was achieved by PreScission protease overnight at 277 K and the target protein was further eluted with PBS. Further purification was achieved by gel filtration chromatography on Superdex 75 (Pharmacia) with 0.1 M Tris-HCl (pH 8.0) containing 0.15 M NaCl. The purified protein was analyzed by SDS-PAGE.

2.2. Determination of molecular weight

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method described by Laemmli (1970). Molecular weight standards used were: MBP-galactosidase (175 kDa), MBP-paramyosin (83 kDa), glutamic dehydrogenase (62 kDa), aldolase (47.5 kDa), triosephosphate isomerase (32.5 kDa), b-lactoglobulin A (25 kDa), lysozyme (16.5 kDa) and aprotinin (6.5 kDa).

2.3. Crystallization and data collection

The purified protein was concentrated to about 10 mg ml⁻¹. Initial screening was performed at 291 K by the hanging-drop vapor-diffusion method using sparse-matrix^[4] screen kits from Hampton Research (Crystal Screen reagent kits I and II) followed by a refinement of the conditions through the variation of precipitant, pH,

protein concentration and additives. Typically, droplets of 4 μ l were prepared on siliconized coverslips by mixing 2 μ l of protein solution and 2 μ l of the reservoir solution.

2.4. Structure determination

Multiple wavelength anomalous dispersion data sets were collected by a spindle method using a MarCCD detector (Marresearch) with synchrotron radiation on beamline 3W1A of the Beijing Synchrotron Radiation Facility (<http://www.ihep.ac.cn/bsrf/english/main/main.htm>). Data were collected from a single selenomethionyl derivative crystal at peak (0.9798 Å), inflection (0.9800 Å), and remote (0.9000 Å) wavelengths to 2.8 Å resolution. Data collection from a native transgelin crystal was performed in-house on a Rigaku RU200 rotating anode X-ray generator (Rigaku Corporation) operated at 48 kV and 98 mA (Cu K α ; $\lambda = 1.5418$ Å) with a Mar345 image plate detector. The crystal was mounted on a nylon loop and flash-cooled at 100 K using an Oxford Cryosystems cold nitrogen gas stream with reservoir liquid as cryo-protection. Data were indexed and scaled using the DENZO and SCALEPACK programs (Otwinowski and Minor, 1997).

2.5. Phase determination and model refinement

The multiple-wavelength anomalous dispersion (MAD) method was used to determine the crystal structure of transgelin. For transgelin structure determination, initial heavy atom search and MAD phasing steps were performed using SOLVE (Terwilliger and Berendzen, 1999) and followed by density modification with RESOLVE (Terwilliger, 2000). The program O (Jones et al., 1991) was used for inspection of electron density maps and manual building. After refinement of the model against the native data set between 50 and 2.3 Å using simulated annealing, energy minimization, restrained individual B factor and addition of waters in the program CNS (Brunger et al., 1998), the R -factor and R_{free} dropped from 40.2% and 42.8% to 21.1% and 26.2%, respectively. Throughout refinement, the agreement between the model and the observed data was monitored by calculating R_{free} based on a subset of 5% of the unique reflections. No non-crystallographic symmetry (NCS) restraints were applied during refinement. The stereochemical quality of the refined structures was checked with the program PROCHECK (Laskowski et al., 1993) and none of the main chain torsion angles is located in disallowed regions of the Ramachandran plot. Statistics for the structure determination and refinement are summarized in Table 1.

3. Results and discussion

3.1. Expression and purification of transgelin

Transgelin fused with a His-tag was expressed as a soluble protein in *E. coli*. After a series of purification steps,

the purified transgelin protein was confirmed to be >95% pure on SDS-PAGE.

3.2. Crystallization and data collection

Rod-like crystals appeared after about two months from two different conditions of Crystal Screen kit I (Hampton Research) containing sodium formate or PEG4000 as precipitants (reagents 22 and 33 of kit I, respectively). The conditions were further optimized by variation of precipitants, buffer pH, additives and protein concentration, and larger rod-like crystals to a size of 0.1 \times 0.1 \times 0.4 mm were obtained, which are reproducible and suitable for X-ray diffraction. The crystals were grown from a reservoir solution comprising 4 M sodium formate.

The native human transgelin crystal has unit cell parameters of $a = 39.2$ Å, $b = 61.9$ Å, $c = 55.9$ Å, $\alpha = \gamma = 90^\circ$, $\beta = 90.1^\circ$, and belongs to the space group $P2_1$. There are two transgelin molecules per asymmetric unit with a Matthews coefficient of 2.24 Å³ Da⁻¹ and solvent content of 45.1%. The diffraction data extend to 2.3 Å resolution. The data collection statistics are summarized in Table 1.

3.3. Structure determination

The transgelin crystal structure was solved using multiple wavelength anomalous dispersion from a single selenomethionyl derivative crystal. Four selenium sites were located in one asymmetric unit using the program SOLVE. Initial phases were calculated from these four sites by SOLVE and density modification was performed with RESOLVE. The experimental electron density map was interpretable in helical regions. The model was further improved by cycles of manual building and refinement using the program O and CNS without NCS restraints. The structure was subsequently refined to a final 2.3 Å with an R -factor of 21.1% and R_{free} of 26.2%. No residues were located in disallowed regions of the Ramachandran plot.

Residues 1–20 and 156–201 were missing from the electron density map. Therefore, in order to improve the electron density map, NCS averaging of the electron density was performed with CNS. However, the results show that the density for residues 1–20 and 156–201 is still ambiguous and not favorable for tracing. Furthermore, from crystal packing, we found little free space available to accommodate the folding of residues 156–201. These results are consistent with molecular weight determination, which suggest that human transgelin may degrade in the buffer (Liao et al., 2007). Furthermore, these observations are also consistent with the $P2_12_12_1$ crystal form of transgelin (data not shown). The final model statistics are summarized in Table 1.

3.4. Structure description

As with the structure of the CH domain reported by Bramham and colleagues (Bramham et al., 2002), the crystal structure of transgelin is globular and formed by six

Table 1
Data collection and model refinement statistics

Data collection statistics	Se-TAGLN			Native TAGLN
	Peak	Edge	Remote	
Space Group	$P2_12_12_1$			$P2_1$
Unit cell parameters	$a = 39.2 \text{ \AA}$, $b = 55.9 \text{ \AA}$, $c = 61.2 \text{ \AA}$, $\alpha = \beta = \gamma = 90^\circ$			$a = 39.2 \text{ \AA}$, $b = 61.9 \text{ \AA}$, $c = 55.9 \text{ \AA}$, $\alpha = \gamma = 90^\circ$, $\beta = 90.1^\circ$
Wave length (Å)	0.9798	0.9800	0.9000	1.5418
Resolution limit (Å)	2.8	2.8	2.8	2.3
Observed reflections	22,339	27,178	15,700	60,453
Unique reflections	3,621	3,625	3,604	11,519
Completeness (%)	99.8 (98.2)	100.0 (100.0)	99.7 (97.6)	99.6 (98.3)
($I/\sigma(I)$)	4.5 (2.1)	4.6 (3.1)	4.0 (1.4)	4.4 (2.5)
R_{merge} (%) ^a	14.3 (49.3)	15.3 (54.2)	13.5 (53.7)	13.3 (46.2)
Final refinement statistics				
Resolution range (Å)				50.0–2.3
R_{work} (%) ^b				21.1
R_{free} (%) ^b				26.2
Total reflections used				12,039
No. of reflections in working set				11,519
No. of reflections in test set				592
r.m.s.d. from ideal				
r.m.s.d. bonds (Å)				0.012
r.m.s.d. angles (°)				1.876
Average B factor (Å ²)				31.2

^a $R_{\text{merge}} = \sum_h \sum_i |I_i(h) - \langle I(h) \rangle| / \sum_h \sum_i \langle I_i(h) \rangle$, where $I_i(h)$ is an individual intensity measurement and $\langle I_i(h) \rangle$ is the average intensity for all measurements of the reflection.

^b $R_{\text{work}} = \sum ||F_{\text{obs}}| - |F_{\text{calc}}|| / \sum |F_{\text{obs}}|$, where F_{obs} and F_{calc} are the observed and calculated structure-factor amplitudes, respectively. R_{free} was calculated as R_{work} using a randomly selected subset containing 5% of unique reflections not used for structure refinement.

helices, termed helices I–VI (Fig. 1a). Among these six helices, helix III, IV and VI are relatively parallel and perpendicular to helix I, II and V. The core structure of human transgelin is comprised of four main α -helices: helix I (Asp23-Cys38), helix III (Gly59-Leu69), helix IV (Phe88-Ser104) and helix VI (Ala124-Asn141). Helix I is perpendicular to helix III, helix IV, and helix VI, and the remaining three core helices (III, IV and VI) form a triple-helix bundle. The other two helices, helix II (Gly48-Leu56) and helix V (Ser114-Gly120), are short and connected to each other by long loops. These two helices are located close to each other at the N-terminal of helices III and VI, respectively.

There are two transgelin molecules per asymmetric unit, which are near-identical with a root mean square deviation (r.m.s.d.) of 0.18 Å (Fig. 1b). There are no significant differences between the two molecules, which are related by non-crystallographic symmetry, and a total surface area of $\sim 330 \text{ \AA}^2$ is buried in the interface between the two transgelin molecules. Contacts are largely mediated between residues on helix I (Glu28, Ile31, Glu32) and the helix I-helix II loop (Pro40, Arg44, Arg47) of one monomer with residues on helix IV (Met86, Val87, Phe88, Met91, Glu92, Ala95) of the second monomer (Fig. 1c).

3.5. Comparison with other structures containing CH domains

Many proteins are known to contain CH domains. The structures of CH domains from a number of proteins have so far been determined by NMR or X-ray, including those of human β -spectrin, chicken calponin, mouse Eb-1, and human plastin 3 T-isoform. The transgelin CH domain has a very similar fold to other known CH domain structures. To compare the CH domains from different proteins, Fig. 2 shows the sequences of these CH domains aligned on the basis of their secondary structure by the program ClustalW (Thompson et al., 1994). From this alignment, it is evident that helix III is a significantly conserved region among CH domains and forms an essential part of the hydrophobic core of the protein (Gimona et al., 2002). A three-dimensional structure-based sequence alignment is more sensitive in terms of finding key residues in CH folds than purely primary sequence based alignment (e.g. BLAST). Based on the sequence alignment and conservation of residues alone, therefore, we conclude that helices I, III, and V should maintain the actin-binding sites or the CH domain tertiary structure (Fig. 2).

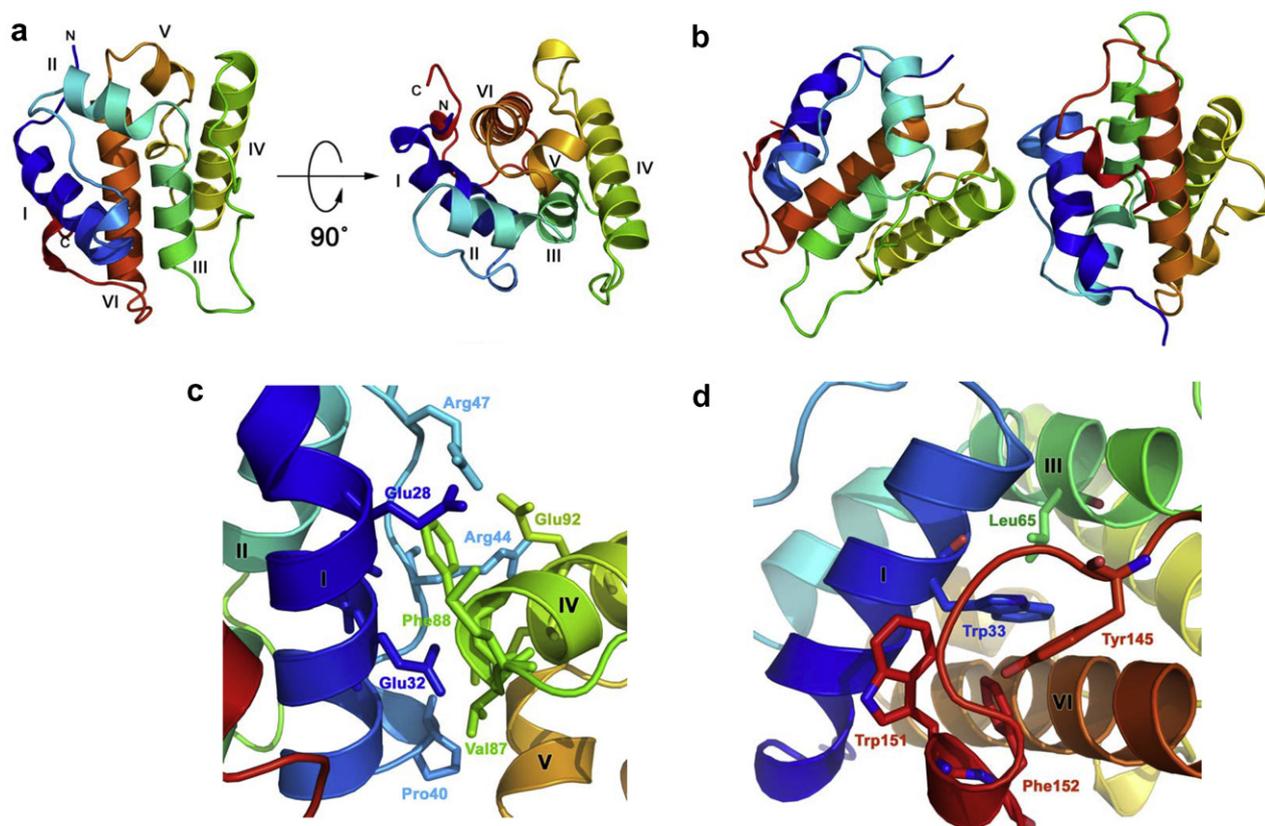


Fig. 1. Crystal structure of TAGLN. (a) Two views related by 90° of the crystal structure of human transgelin (TAGLN). The six helices are labeled from I to VI. The TAGLN structure is shown in ribbon representation and colored blue from the N-terminus to red at the C-terminus. (b) Ribbon representation showing the two molecules in an asymmetric unit. (c) Ribbon representation showing part of the interface between two molecules in an asymmetric unit. Helices I and II of molecule A are shown interacting with helices IV and V of molecule B. (d) Ribbon representation showing the role of key conserved residue Trp33 in structural stability. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Using the DALI server (Holm and Sander, 1997), we identified many proteins with similar CH domain folds. The most closely related proteins (with *Z*-score above 10) are calponin (PDB code 1h67, *Z*-score = 14.3, root-mean-square deviation (r.m.s.d.) = 2.0 Å), fimbrin (PDB code 1aoa, *Z*-score = 10.9, r.m.s.d. = 2.0 Å), β-spectrin (PDB code 1bkr, *Z*-score = 9.8, r.m.s.d. = 2.1 Å), ras GTPase-activating-like protein rng2 (PDB code 1p5s, *Z*-score = 9.2, r.m.s.d. = 2.3 Å), and *t*-plastin (PDB code 1wjo, *Z*-score = 7.7, r.m.s.d. = 2.3 Å). These proteins are known to be actin-binding proteins, or to bind to actin-like proteins, and share similar tertiary structures. Fig. 3 shows a comparison of the tertiary structures of CH domains.

3.6. Structural conservation

As shown in Fig. 2, sequence identity among CH domains is generally low but there are a number of similarities. Hydrophobic interactions within the core of CH domains maintain the stability of the structure and fold; in transgelin, this hydrophobic core is formed from helices I, III, and VI. In particular, a highly conserved tryptophan

has an important role in all CH domains. In transgelin, Trp33 intercalates between helices III and VI, where it is sandwiched between two hydrophobic clusters. On one side, it forms part of a cluster with three aromatic residues: Tyr145, Trp151 and Phe152 (Fig. 1d). The hydrophobic cluster on the other side of Trp33 involves Leu30, Ile34, Leu65, Val66, Leu130 and Leu133. Of these, residues Leu30, Ile34, Leu65 and Val66 are conserved in hydrophobic nature in other CH domains. Another highly conserved residue, Pro71, interrupts helix III, although this is substituted by glycine in some CH domains.

High conservation is also observed in position 58, which is mostly an aspartic acid in CH domains but is occupied by asparagine in transgelin. The role of Asn58 in transgelin is as an N-terminal cap for helix III, similar to other CH domain structures. As in β-spectrin and other CH domain structures, this Asn58 capping residue is followed by a glycine, Gly59, so as to avoid clashing with the facing Leu56 residue. Helix I has one fully conserved residue (Trp) in all actin-binding proteins. Helix III has a strongly conserved residue (Val, Ile or Leu) and a few weakly conserved residues, while helix V has two weakly conserved residues (Fig. 2).

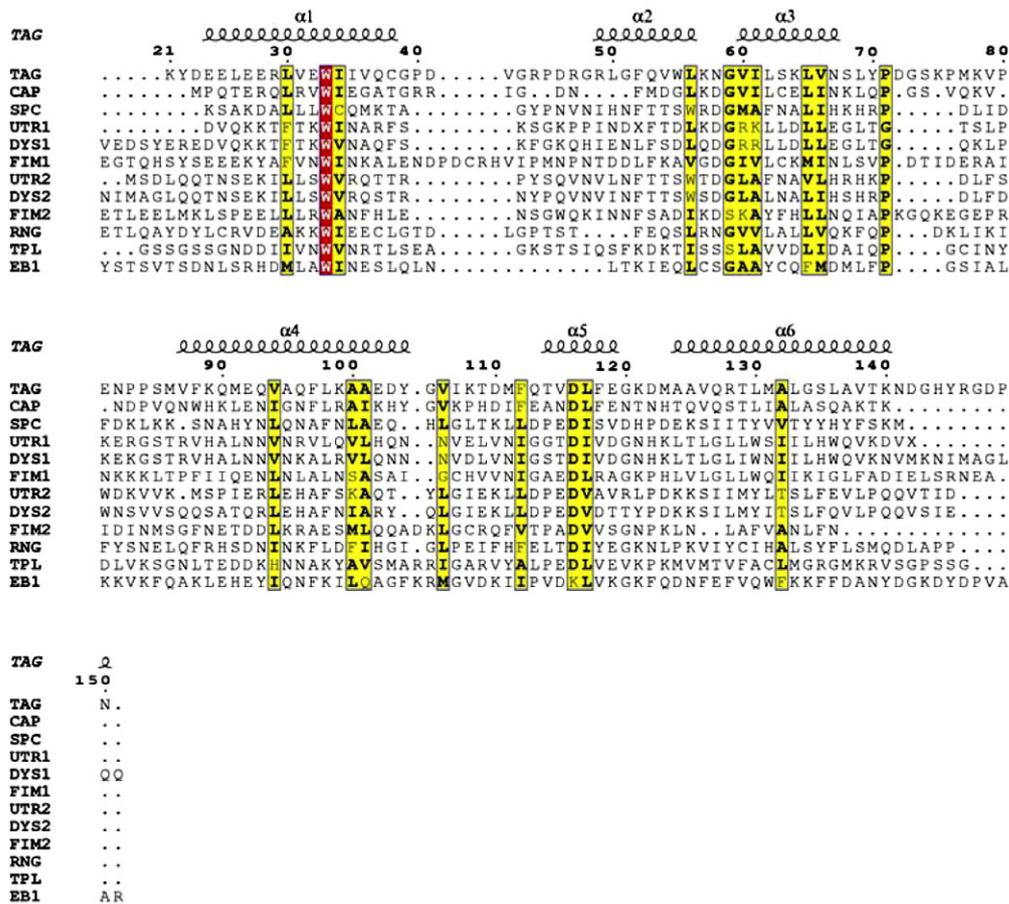


Fig. 2. Structure-based alignment of the sequences of CH domains with known structure. The helices from the determined structures are highlighted in yellow. Conserved residues are indicated as fully (red), strongly (pink) and weakly (cyan) conserved. The numbering scheme corresponds to that of human TAGLN. The names and residue numbers are as follows: TAG, human TAGLN (21–150); CAP, chicken calponin (27–134); SPC, human β -Spectrin (173–280); UTR1, human utrophin (135–248); DYS1, human dystrophin (7–133); FIM1, human fimbrin (115–250); UTR2, human utrophin (144–261); DYS2, human dystrophin (126–246); FIM2, human fimbrin (259–375); RNG, Ras GTPase-activating-like protein Rng2 (46–168); TPL, human plastin 3 T-isoform; EB1, end binding protein 1. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.7. Actin-binding

Although the structures of CH domain-containing proteins may vary significantly, the core CH domain fold is preserved in all crystal structures determined to date, with three α -helices forming a triple helix bundle and the fourth one lying perpendicular to them (Norwood et al., 2000). Despite their similarity in overall fold, CH domains from different proteins appear to have distinct functions. In ABDs, the N-terminal CH domain (CH1) alone is able to bind actin, although the affinity is lower than the full-length ABD (Winder et al., 1995). The C-terminal CH (CH2) domain alone has weak affinity for actin, but may contribute towards stabilizing the overall binding of the complete ABD (Banuelos et al., 1998). Linkers between CH domains are not directly involved in the interaction with actin filaments, but confer conformational flexibility to ABDs and play a role in arranging the CH domains to form an actin-binding surface (Olski et al., 2001). The crystal structure of utrophin suggests that these actin-binding

domains may be more flexible than was previously thought and that this flexibility may allow domain reorganization and plays a role in the actin-binding mechanism (Keep et al., 1999).

Some proteins containing a single CH domain have been shown to bind proteins other than actin through their CH domains. For instance, EB1 (end-binding protein 1) has been demonstrated to bind to microtubules through both hydrophobic and electrostatic interactions (Hayashi and Ikura, 2003). The structure of calponin, also containing one CH domain, provides an example of interaction between F-actin and CH domain-containing proteins (Bramham et al., 2002). However, the question of whether the single CH domain from transgelin can bind actin remains the subject of debate. Mutagenesis analysis has shown that C-terminal truncation mutants [transgelin-(1–186) and transgelin-(1–166)] exhibited markedly reduced co-sedimentation with actin, and no actin-binding of transgelin-(1–151) could be detected. Internal deletion of a putative actin-binding site (154-KKAQEHLK-161) par-

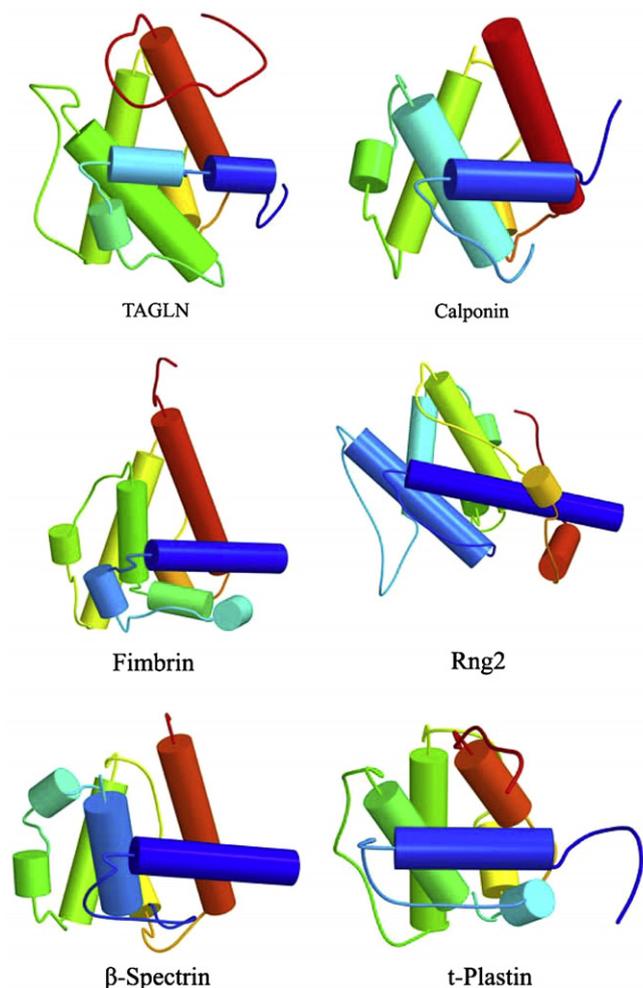


Fig. 3. Structural comparison of CH domains from different proteins. Structures are colored from blue at the N-terminus to red at the C-terminus. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

tially prevented actin-binding, as did point mutations to neutralize either or both pairs of positively charged residues at the ends of this region (KK154LL and/or KR160LL). Internal deletion of amino acids 170–180 or 170–186 was found to partially or almost completely inhibit actin co-sedimentation, respectively. These data suggest that multiple regions within the C-terminal domain are required for full actin affinity (Fu et al., 2000).

4. Conclusions

We have determined the 2.3 Å resolution crystal structure of human transgelin and confirmed that it possesses a “calponin homology (CH)” domain. Comparison with other structures containing CH domains has enabled us to identify conserved structural elements that might play a role in maintaining the tertiary structure. The putative actin-binding region of transgelin resides in the C-terminal of the protein, which is unfortunately absent from the structure reported here. Therefore, further work is needed

to confirm the interaction between the transgelin CH domain and actin, either by determination of their complex structure or by other mutagenesis based biochemical–biophysical methods.

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