J. Exp. Biomed. Sci. 12 (2006) 303-310

C-Terminal Region of Ankyrin-B Interact with Z-Line Portion of Titin

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Ankyrins are a ubiquitously expressed family of intracellular adaptor proteins involved in targeting diverse proteins to specialized membrane domains in both the plasma membrane and the endoplasmic reticulum. We described here that the C-terminal domain of ankyrin-B interact specifically with Z-line portion of titin in yeast two-hybrid analysis, *in vitro* pull-down assays and localization experiments in COS-7 cells. In this study we provide the first experimental evidence that Z-line portion of titin is necessary for the localization of ankyrin-B and ankyrin-B links between the sarcolemma and the myofibril in costameres.

Key Words: Ankyrin-B, Titin, Costameres, *In vitro* pull-down assay

INTRODUCTION

Ankyrins are a family of proteins involved in the organization of specific membrane domains by mediating the interactions between proteins of the plasma membrane and the subplasma membrane cytoskeleton (Bennett and Baines. 2001). Vertebrate ankyrin polypeptides fall into three classes, each containing multiple alternatively spliced variants: ankyrins-R (R for restricted distribution, and the prototypic ankyrin first characterized in erythrocytes; also expressed in a subset of neurons and striated muscle) encoded by *Ank1* on human chromosome 8p11; ankyrins-B (B for broadly expressed; first characterized in brain, but now recognized in most cell types) encoded by *Ank2* on human chromosome 4q25-27; and ankyrins-G (G for giant size and general expression, first characterized as a 480 kDa polypeptide in the nervous system; expressed in most cell types) encoded by *Ank3*, on human chromosome 10q21. Canonical ankyrins are 190~220 kDa proteins expressed in most tissues and cell types and comprise a membrane-binding domain (MBD) of 24 *ANK* repeats, a spectrin-binding domain, a death domain and a C-terminal domain. Whereas death domains in other proteins may function in activation of NF-B, caspase proteases and cell death, this domain has no known role within ankyrins.

Current views of ankyrin function are based on co-localization and biochemical interactions of ankyrin with other proteins. Ankyrin associates with a variety of membrane proteins including ion channels (Na^+/K^+ ATPase, H^+/K^+ ATPase, anion exchangers AE 1-3, voltage-sensitive Na^+ channels, Na^+/Ca^{2+}exchanger), calcium-release channels [ryanodine receptor, inositol (1,4,5)-triphosphate receptor], cell adhesion molecules [CD44, L1CAMs (L1, NgCAM, neurofascin, LAD-1, NrCAM, neuroglian)], as well as cytoplasmic proteins, including clathrin and tubulin (Bennett and Baines. 2001). Many of these interactions are mediated by *ANK* repeats within the MBD, although the Na^+/K^+ ATPase and H^+/K^+ ATPase associate at least in part with the spectrin-binding domain. Ankyrin-$B^{-/-}$ cardiomyocytes display down regulation and mis-sorting of calcium-release channels [ryanodine and inositol 1,4,5-trisphosphate IP_3 receptors] within the endoplasmic reticulum in cardiomyo-

*Received: December 1, 2006
Accepted after revision: December 11, 2006
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cytes that can be rescued by transfection with cDNA encoding ankyrin-B. Both ankyrin-G and ankyrin-R are expressed in cardiomyocytes, but cannot compensate for loss of ankyrin-B. Rescue studies with ankyrin-B/G chimeras have identified the C-terminal domain of ankyrin-B as the defining domain in specifying ankyrin-B activity (Mohler et al., 2002). A working hypothesis to explain the cellular basis for these phenotypes is that ankyrins play roles as chaperones or guides that direct vesicle transport of a variety of ion channels to sites in the plasma membrane as well as the endoplasmic reticulum.

Titin is the largest protein known, with a single polypeptide containing ~30,000 amino acid residues (Labeit and Kolmerer, 1995). It is encoded by a single gene that locates in both human and mouse on the long arm of chromosome 2 (Bang et al., 2001). From the titin gene, mRNA of up to 100 kb is transcribed (Freiburg et al., 2000). These mRNA are translated into 27,000 to 33,000 residue giant polypeptides, whose N- and C-termini are anchored within the Z-disc and M-line lattice of the sarcomere, respectively (Wu et al., 2002). The recent genomic analysis of human titin revealed 363 exons that code for a total of 38,138 amino acid residues with 4200 kDa. Titin's continuity along the sarcomere, and its early appearance during muscle development support the proposal that titin functions as a template for sarcomere formation (Gregorio et al., 1999). Also titin has two functions in striated muscle: as a "molecular blueprint" for sarcomeric protein assembly during myofibrillogenesis and as a "molecular spring" that maintains myofibrillar integrity during contraction, relaxation, and stretch (Trinick and Tskhovrebova, 1999; Labeit et al., 1997; Gregorio et al., 1998a).

Costameres, structures at the plasma membrane of skeletal muscle, are present in a rectilinear array that parallels the organization of the underlying contractile apparatus. Costameres are subsarcolemmal protein assemblies that circumferentially align in register with the Z-disk of peripheral myofibrils and physically couple force-generating sarcomeres with the sarcolemma in striated muscle cells (Ervasti, 2003). Costameres have three major functions: to keep the plasma membrane, or sarcolemma, aligned and in register with nearby contractile structures; to protect the sarcolemma against contraction-induced damage; and to transmit some of the forces of contraction laterally, to the extracellular matrix. These functions require that costameres link the contractile apparatus through the membrane to the extracellular matrix (Bloch et al., 2002). A variety of data indicate that costameres are a striated muscle-specific elaboration of the focal adhesions expressed by non-muscle cells. The canonical focal adhesion protein vinculin is also a founding member of costameres (Pardo et al., 1983), and its immunofluorescence staining pattern in striated muscle remains the standard by which many other costameric proteins have been identified. Other focal adhesion proteins found in costameres include talin, -actinin, and 1 integrins (Patel and Lieber, 1997). Although not restricted to costameres, immunoelectronmicrograph studies indicated that the intermediate filament protein desmin constitutes one of the physical links between the Z-disk and sarcolemma (Patel and Lieber, 1997).

A key question in the function of ankyrin is how the ankyrin and the membrane proteins become precisely aligned with the contractile apparatus. We have begun to address this question by identifying ligands of ankyrin-B, a costameric protein that, we hypothesize, helps to coordinate the alignment of the sarcolemma proteins with nearby Z-disc and M-line. Because titin serves as a molecular blueprint for the assembly of other myofibrillar elements, we postulated that it might also provide a site for anchoring the costameric protein at the level of the Z-disc and the M-line.

MATERIALS AND METHODS

1. Yeast two-hybrid interaction studies

1) Plasmid preparation

Yeast two-hybrid assays were performed utilizing the BD Matchmaker™ Pretransformed Libraries (BD Biosciences Clontech, Version No. PR3X398). The C-terminus of human 220 kDa ankyrin-B was amplified by PCR using appropriate primers containing EcoR I/BamH I. The reaction was performed on human skeletal muscle cDNA library with the 50X Advantage-HF 2 polymerase Mix, 10X HF 2 dNTP mix, and 10X HF 2 PCR buffer (BD Biosciences Clontech, Version No. PR15722). The size of the product was determined on agarose gel and stained by ethidium bromide (EtBr). Amplified PCR fragments were cloned into the bait vector pAS2-1 (Matchmaker system II; BD Biosciences Clontech) to obtain GAL4-BD fusion. In order to investigate potential ankyrin-B and titin interactions, the titin fragments were amplified by PCR using appropriate primers and

ligated into the pGAD424 vector (Matchmaker system I; BD Biosciences Clontech) to obtain GAL4-AD fusion.

2) Yeast transformation and mating

Interaction of the two test proteins reconstitutes the function of the GAL4 transcription factor and results in expression of the reporter genes, which are detected by assays for the reporter gene products. The lithium acetate (LiAc)-mediated method was performed to transform DNA into yeast. In this study, bait protein was expressed as a fusion protein with GAL4 DNA-BD in the AH109 strain and prey protein was expressed as a fusion protein with GAL4 DNA-AD in the yeast strain Y187. In brief, the yeast competent cells were prepared and suspended in LiAc solution with the plasmid DNA to be transformed, along with excess carrier DNA. Polyethylene glycol (PEG) with an appropriate amount of LiAc was added and the mixture of DNA and yeast was incubated at 30℃. After incubation, DMSO was added and the cells were heat shocked at 42℃. The AH109 strain was plated on SD/-Trp and the Y187 strain was plated on SD/-Leu to select positive transformants. Positive colonies appearing after 5-10 days at 30℃ were assayed for X-α-galactosidase activity on same SD/-Ade/-His/-Trp/-Leu plates supplemented with 1.5 mM 3-amino-1.2.4-triazole (3-AT). Hydrolysis of X-α-galactosidase yields a blue end product.

2. *In vitro* interaction studies

1) Construct preparation

The ankyrin B C-terminal domain sequences (4327~5517 bp) were digested using restriction enzymes (EcoR I and Xho I) in the clone encoding ankyrin-B C-terminal region and were inserted into expression vector, pGEX 4T-1 (Amersham Biosciences) to obtain GST fusions for *in vitro* pull-down assay. The titin ZIg1/2 domain sequences were amplified by PCR using appropriate primers containing Hind III/BamH I. The cDNA corresponding to titin MIg7/8 domains were amplified using the primer pair including Pst I/Sal I. All reactions were performed with Advantage-HF 2 PCR kit (BD Biosciences Clontech) with the clone encoding both titin ZIg1/2 and titin MIg7/8 domains as a template. The PCR products were digested using restriction enzymes and inserted into pSP64 Poly(A) (Promega). Prepared plasmids were used for *in vitro* transcription and translation.

2) GST-fusion protein preparation and purification

The chimeric fusion protein consisting of glutathione S transferase (GST) and the C-terminal region of human ankyrin-B was expressed after 0.4 mM isopropyl-β-D-thiogalactopyranoside (IPTG) induction in BL21 (DE3) bacteria for 3 h at 30℃ with shaking. The cells were re-suspended in PBS (140 mM NaCl, 2.7 mM KCl, 10.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4) containing 1 mM PMSF, 0.1 mM DTT, 10 mM EDTA, and 100 μM leupeptin. The cells were lysed by sonication after addition of 1% Triton X-100 and the cell lysate was clarified by centrifugation at 10,000 X g for 10 min. Three ml of the supernatant of bacterial sonicate was mixed with 1.5 ml of 50% glutathione Sepharose 4B beads (Amersham Biosciences) for 1 h at room temperature with shaking. The beads were washed three times with 6 ml of PBS and the GST fusion protein was eluted by mixing the beads with 600 μl of glutathione elution buffer (20 mM glutathione, 50 mM Tris-HCl, pH 7.5) at room temperature for 10 min. The elution was repeated twice and the three eluates were pooled. The purity of the protein was confirmed by SDS-PAGE. Then the elution samples were stored at -20℃.

3) *In vitro* transcription and translation

All *in vitro* transcription and translation experiments were performed using the TnT Quick Coupled Reticulocyte Lysate System (Promega). To label the peptides, the reactions were performed using the Transcend Non-Radioactive Translation Detection System as described by the manufacturer (Promega). The sizes of *in vitro* transcription and translation proteins were determined by SDS-PAGE and western blot analysis.

4) GST pull-down assay

The purified GST fusion proteins were incubated for 1 h at room temperature with the resin to immobilize the fusion proteins; glutathione-Sepharose 4B beads (Amersham Biosciences) for GST. The beads were washed three times with PBS by 10 folds of bed volume and the GST fusion protein was resuspended in the interaction buffer (PBS, 1% Triton X-100, 1% BSA). For binding experiments, 8 μl of biotin-labeled TnT protein extract was incubated with fusion protein (50 μl of beads, 50% slurry) in the interaction buffer for 1 h 30 min at room temperature with shaking. Beads were washed three times with wash buffer, PBS containing 1% Triton X-100.

Biotin-labeled proteins eluted in sample buffer (0.15 M

Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol) were separated by SDS-PAGE using 10~12% gels and transferred to a nitrocellulose membrane. Nitrocellulose blots were blocked with TBST (20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5% Tween 20) at room temperature for 1 h and probed with streptavidin conjugated to alkaline phosphatase in TBST for 1 h at room temperature with shaking. Blot was washed in TBST and water and was used with Western Blue Substrate for alkaline phosphatase for colorimetric detection as described by the manufacturer (Promega).

3. Cell staining

1) Construct preparation

The C-terminus of human 220 kDa ankyrin-B C-terminal (4327~5517 bp) were amplified by PCR using appropriate primers containing EcoR I/BamH I. The reaction was performed on Human skeletal muscle cDNA library with the 50X Advantage-HF 2 Polymerase Mix, 10X HF 2 dNTP mix, and 10X HF 2 PCR buffer (Biosciences Clontech, Version No. PR15722). The size of the product was determined on agarose gel and stained by EtBr. Amplified PCR fragments were cloned into pDsRed1-C1 (BD Biosciences Clontech). The titin ZIg1/2 domain sequences were amplified by PCR using appropriate primers containing Hind III/ BamH I. The cDNA corresponding to titin MIg7/8 domains were amplified using the primer pair including Pst I/BamH I. All reactions were performed with Advantage-HF 2 PCR kit (BD Biosciences Clontech) with the clone encoding both titin ZIg1/2 and titin MIg7/8 domains as a template. The PCR products were digested using restriction enzymes and inserted into pEGFP-C3 (BD Biosciences Clontech). Prepared plasmids were used for transfection into the COS-7 cell.

2) Cell culture and transfection

COS-7 cells, a monkey kidney fibroblast, were purchased from the KCLB® Korean Cell Line Bank (KCLB 21651). The cells were maintained in DMEM (Cambrex Bio Science Walkersville, Inc.) supplemented with 10% fetal bovine serum. COS-7 cells were transfected with various EGFP-titin fusions and DsRed-ankyrin B constructs using the LipoTaxi Mammalian Transfection Kit (Stratagene) according to the manufacturer's instructions. Briefly, cells were plated onto sterile coverslips in 60 mm dishes at 6×10^5 cells/ml 12 h prior to transfection. Complex formation solutions containing 10 μg DNA for fusion constructs and 60 ml of Lipotaxi reagent in 900 ml of serum-free DMEM were incubated 30 min at room temperature. Cells were washed once with serum-free DMEM and the 1.5 ml complex formation solution was added to the dish. After incubation under 5% CO_2 atmosphere at 37℃ for approximately 6 h, 2.5 ml DMEM with 20% serum was added to the dish and placed at 37℃ overnight. The medium was replaced with 5 ml of normal growth medium the following day, and the cells were incubated again at 37℃ for 48 h before processing.

3) EGFP and DsRed visualization

Fluorescence visualization of EGFP in cells was carried out as follows: cells were briefly washed in Delbecco's phosphate-buffered saline (DPBS; Sigma) and then fixed in DPBS/4% paraformaldehyde (Sigma) for 30 min at room temperature. The coverslips were then washed two times in DPBS and mounted on slides with mounting solution, universal solution and malinol solution, for 1 h (generous gift of Ul-San University Hospital). Cells were observed on a fluorescence microscope equipped with software.

RESULTS

1. Yeast two-hybrid experiments

We have shown that the C-terminal domain of ankyrin-B interacts with the two most N-terminal and the C-terminal immunoglobulin (Ig) domains of titin, ZIg1/2 and MIg7/8, which are present at the Z-disc and M-line respectively, by yeast two-hybrid screening (Fig. 1). To identify potential interaction partners for the ankyrin-B, specific oligonucleotides of titin MIg7/8 were synthesized by using PCR analysis. The "prey" cDNA fragments encoding the titin '79668~80627' bp (TitinC1) and '80628~80912' bp (TitinC2) were amplified using specific primers, and inserted into the pGAD424 prey vector. In these studies, we referred to the resulting plasmid as pGAD424+TitinC1 and pGAD424+TitinC2. The authenticity of the construct was verified by sequence analysis. Sequencing revealed that the TitinC1 and TitinC2 DNA were fused in the correct reading frame with the coding sequence of GAL4 DNA-binding domain (data not shown). Subsequently, prey plasmids were transformed into yeast Y187 strain. In order to identify potential interaction partners for the ankyrin-B, TitinC1 and TitinC2 subfragments were screened using the yeast two-hybrid

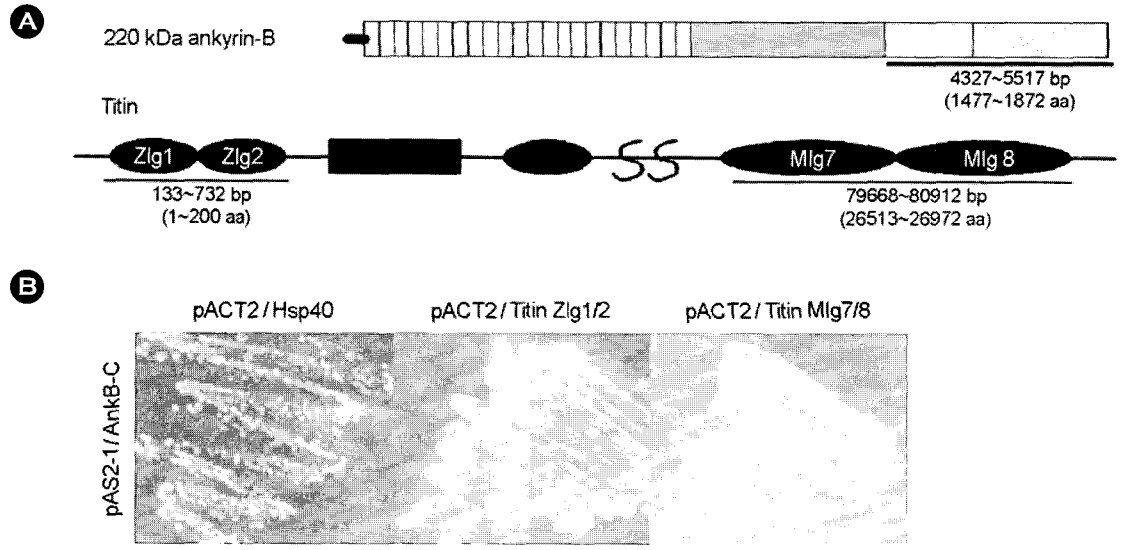

Fig. 1. Yeast two-hybrid screen identifies titin as a cytoplasmic ligand for ankyrin-B. **(A)** The 220 kDa ankyrin-B C-terminal region were inserted into the pAS2-1 bait vector. PCR fragments of Z-band region of titin (ZIg1/2) and M-line region of titin (MIg7/8) were inserted into the pACT2 prey vector. **(B)** As a positive control, Hsp40 was tested. Yeast two-hybrid analysis followed by X-α-galactosidase assay indicated that ankyrin-B specifically interacted with titin ZIg1/2 and MIg7/8.

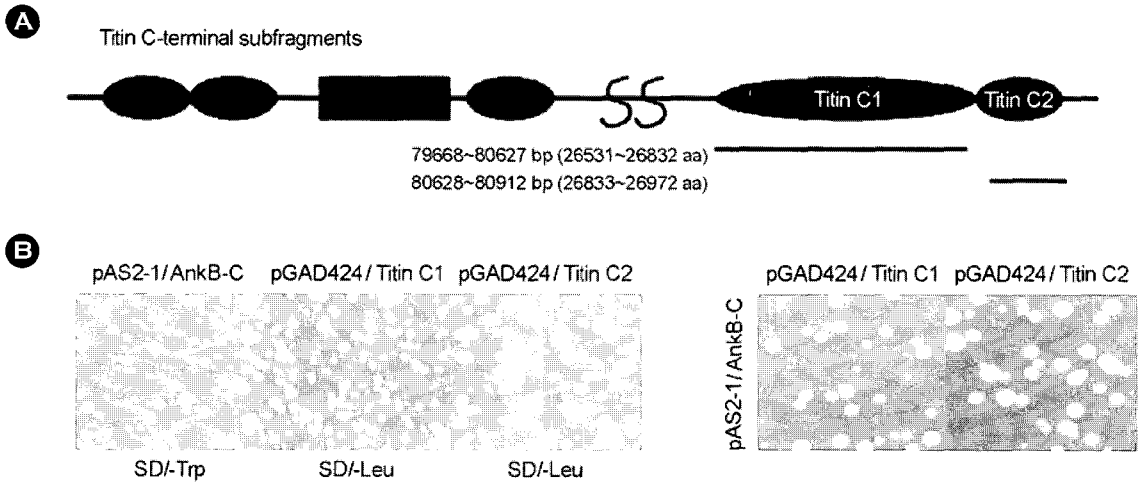

Fig. 2. Interaction of the C-terminal domain of ankyrin-B with titin subfragments. **(A)** Test of bait and prey plasmid. (a) Viability of yeast cells on SD/-Trp plate. (b)(c) Viability of yeast cells on SD/-Leu plate. **(B)** X-α-galactosidase assay using yeast mating. Yeast colonies grown on SD/-Ade/-His/-Trp/-Leu plates supplemented with 1.5 mM 3-AT. The end points were white. White colonies are false positive (i.e., contain noninteracting proteins).

technique and the C-terminal fragment of the ankyrin-B cDNA (AnkB-C) as bait (Fig. 2A). Yeast two-hybrid analysis followed by qualitative liquid X-α-galactosidase assay. No specific association between AnkB-C and Titin C1/C2 could be detected (Fig. 2B).

2. Binding of titin in GST/AnkB-C in a pull-down assay

To confirm the specificity of interaction between ankyrin-B and titin, performed a GST-pull down assays. Titin ZIg1/2 and MIg7/8 domain were amplified by PCR using appropriate primers and then inserted into pSP64 poly(A) vector. Prepared plasmids were used for *in vitro* transcription and translation. All *in vitro* transcription and translation experiments were performed using TnT Quick Coupled Reticulocyte Lysate System and the Transcend Non-Radioactive Translation Detection System. *In vitro* translated proteins were determined by SDS-PAGE and western blotting (Fig. 3A). The molecular weight of proteins was about 21 kDa (Titin ZIg1/2) and 45 kDa (Titin MIg7/8).

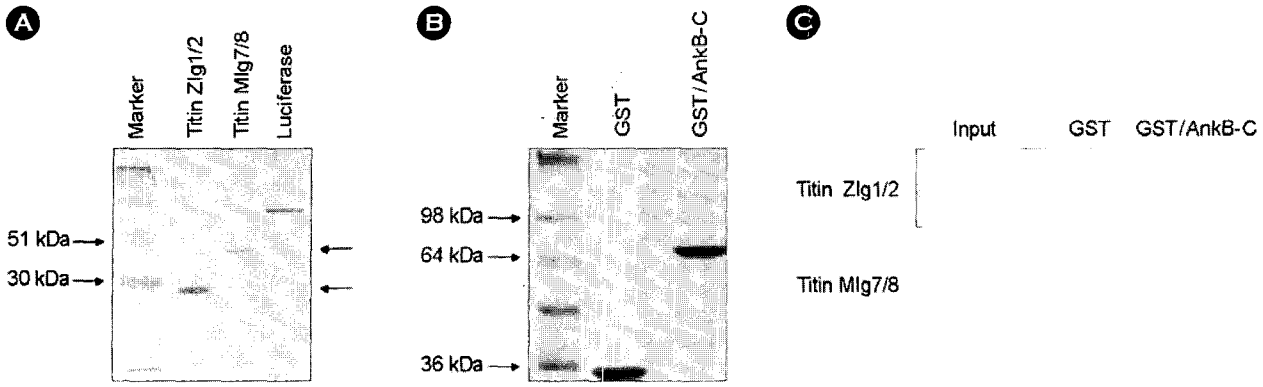

Fig. 3. A vector-based *in vitro* transcription and translation. **(A)** Titin ZIg1/2 and titin MIg7/8 domains were translated *in vitro* and detected using the Transcend Non-Radioactive Translation Detection System (*Marker*; COLORMARKER from Sigma, Luciferase; positive control). The molecular weight of proteins was about 21 kDa (Titin ZIg1/2) and 45 kDa (Titin MIg7/8). **(B)** Purification of GST/AnkB-C fusion proteins. GST-fusion proteins were inserted into pGEX 4T-1 expression vector and transformed into BL21. After purification, GST-fusion proteins subsequently subjected to SDS-PAGE analysis. *Marker* denotes SeeBlue® Plus2 Pre-Stained Standard Marker (Invitrogen). The expected molecular weights of the fusion proteins were about 26 kDa (GST), 71 kDa (GST/AnkB-C). **(C)** Titin ZIg1/2 interacted with C-terminal of ankyrin-B in GST pull-down assay. Titin fragments were translated *in vitro* (Input). In vitro-translated titin ZIg1/2 bound to glutathione-Sepharose 4B beads in the presence of GST/AnkB-C fusion peptide. No bindings were observed in the presence of titin MIg7/8 with GST/AnkB-C proteins. As a negative control, binding of GST to beads was tested.

Ankyrin-B was expressed as GST-fusion proteins. For purification of GST/AnkB-C fusion proteins, ankyrin fragments were inserted into pGEX 4T-1 expression vector and were induced to overexpress with 0.4 mM IPTG. The molecular weight of the fusion protein was about 26 kDa (GST) and 71 kDa (GST/AnkB-C), respectively. The purity of the proteins was confirmed by SDS-PAGE (Fig. 3B). After the cell lysates were mixed with glutathione Sepharose 4B beads, unbound proteins were removed and analyzed on SDS-PAGE. The beads were washed with PBS. GST/AnkB-C fusion proteins were eluted from the glutathione Sepharose 4B with glutathione elution buffer. The interaction of ankyrin-B C-terminal region and titin ZIg1/2 and MIg7/8 were tested by GST pull-down assays (Fig. 3C). Titin ZIg1/2 coprecipitated with the GST/AnkB-C, but did not coprecipitate with the GST alone. Titin MIg7/8 did not coprecipitate with both GST/AnkB-C and GST alone. This confirmed that the ankyrin-B C-terminal region specifically binds to titin ZIg1/2 domains.

3. Ankyrin-B C-terminal domain colocalization with titin ZIg1/2 in cell study

To study at the cellular level the interaction between titin ZIg1/2 and the C-terminal region of ankyrin-B, the EGFP-tagged titin ZIg1/2 was transfected into COS-7 cells. In cells expressing only EGFP, fluorescence was observed throughout the cell, like in cells expressing only DsRed (Fig. 4A, B). In EGFP-tagged titin ZIg1/2, the flurescence signal was concentrated at the cytoplasm and the cell nucleus (Fig. 4D). By contrast, in cells expressing DsRed-tagged AnkB-C encoded by the multiple cloning site in the pDsRed1-C1 vector, it displayed a diffused cytosolic localization, like in cells expressing EGFP-tagged titin MIg7/8 (Fig. 4C-E). In cells cotransfected with AnkB-C, EGFP-titin ZIg1/2 staining was distributed almost completely to the same sites where AnkB-C was present and this suggest that titin ZIg1/2 associates with ankyrin-B (Fig. 4F-H). In agreement with previous results in yeast two-hybrid and pull-down experiments, no colocalization was observed when AnkB-C was cotransfected with the EGFP-titin MIg7/8 (Fig. 4I-K).

DISCUSSION

In this study, we provide evidence that Z-line portion of titin is necessary for the localization of ankyrin-B and ankyrin-B links between the sarcolemma and the myofibril in costameres. More specifically, the two most N-terminal immunoglobulin domains Z1 and Z2 form a binding site for ankyrin-B that requires the presence of both Ig-like domains. Both the ZIg1 and ZIg2 domains of titin are also required for binding to small ankyrin-1 (Kontrogianni-Konstantopoulos and Bloch, 2003) or telethonin (Mues et al., 1998).

Small ankyrin-1 is a transmembrane protein of the SR

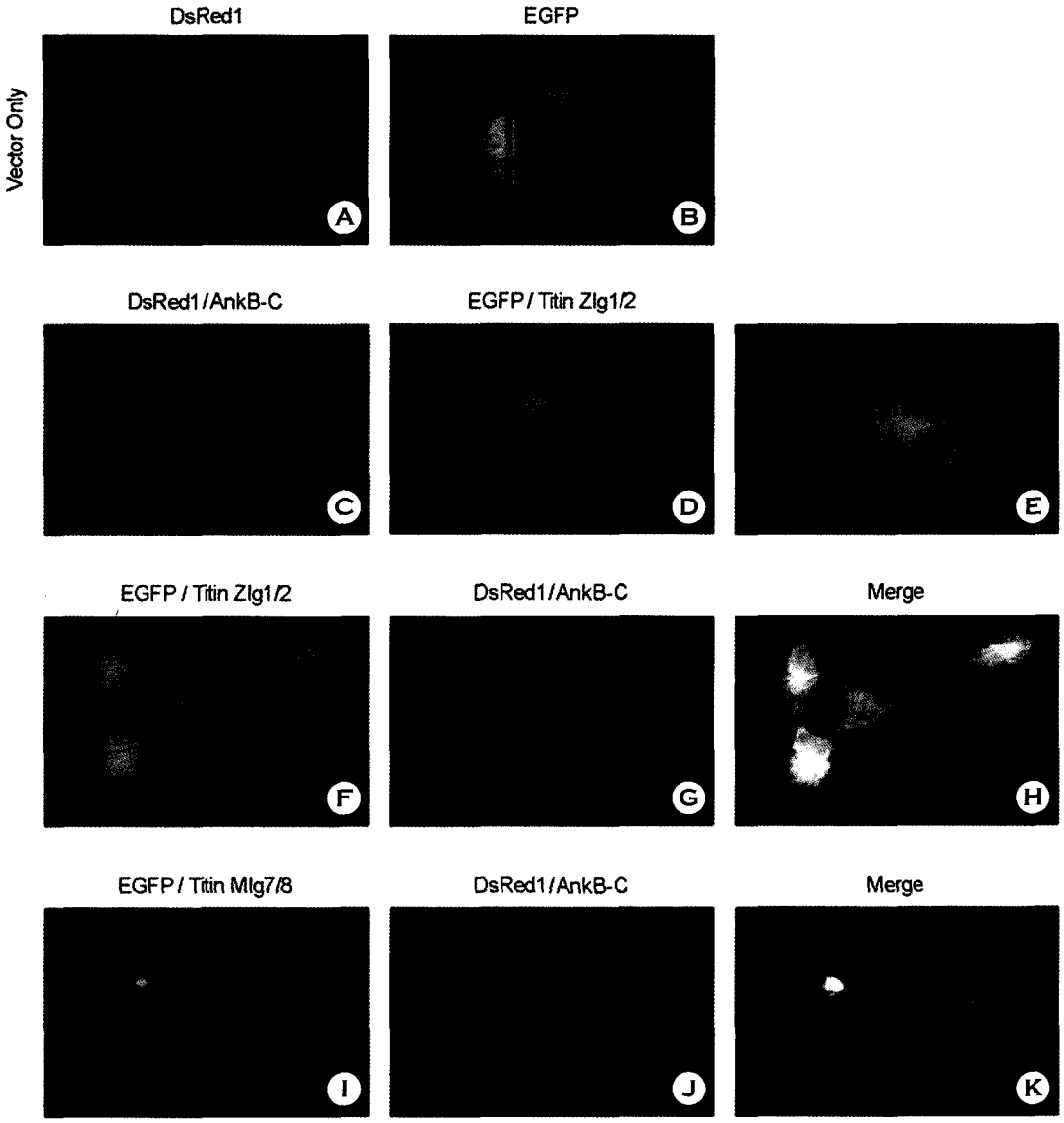

Fig. 4. Effect of AnkB-C on the localization of titin in COS-7 cells. **(A, B)** COS-7 cells were transfected with vector only. **(C-E)** COS-7 cells were transfected with vector encoded AnkB-C, titin ZIg1/2 and titin MIg7/8. **(F-H)** In cells cotransfected with AnkB-C, EGFP-titin ZIg1/2 staining was distributed almost completely to the same sites where AnkB-C was present and this suggest that titin ZIg1/2 associated with ankyrin B. **(I-K)** In agreement with previous results in pull-down experiments, no co-localization was observed when AnkB-C was cotransfected with the EGFP-titin MIg7/8.

and its interaction with titin's N-terminus may position the SR around the Z-disc of each sarcomere. A further role for titin in organizing the SR is suggested by the interaction between titin's near Z-disc domains Z9-Z10 and obscurin (Gregorio et al., 1998b). These interactions with titin's Z-disc region may be involved in positioning the SR and T-tubular membrane systems in close proximity to the I-band region of the sarcomere. Furthermore, it also ensures that these membrane systems move with the Z-disc, thereby preventing excessive strains, which possibly could tear membranes when the sarcomeres shorten during construction. Thus, during construction-relaxation cycles, titin maintains the structural organization of the sarcomere as well as the organization of the SR and T-tubular systems. Previous studies have shown that the hydrophilic sequence of Small ankyrin-1 (29~155 aa), which extends from the SR membrane into the sarcoplasm, specifically and directly interacted with the two most N-terminal Ig domains of titin, ZIg1/2 (Kontrogianni-Konstantopoulos and Bloch, 2003). Comparison of Small ankyrin-1 '29~155' aa and ankyrin-B

'1477~1872' aa sequences showed extensive homology.

The two N-terminal Ig domains of titin were previously shown to contain the binding site of a Z-disk protein referred to as telethonin (Mues et al., 1998). Similar to small ankyrin-1, the binding of telethonin to titin requires the presence of both ZIg1 and ZIg2. Titin ZIg1/2 can bind simultaneously to ankyrin-B, small ankyrin-1 and telethonin, indicating that these proteins can form a multiple complex.

The subcellular location of these proteins places limits on where this complex could form. Telethonin and titin ZIg1/2 co-localize at the edge of the Z-disk lattice, where their binding is believed to anchor the N-terminal portion of titin to the Z-disk. By contrast, small ankyrin-1 is limited to the SR at the periphery of the Z-disk, where its C-terminal hydrophilic sequence extends from the SR membrane. However ankyrin-B is limited to the costameres at the periphery of Z-disk, where its N-terminal membrane binding domain links sarcolemma proteins. Ankyrin-B or small ankyrin-1 is therefore likely to interact with titin and possibly form a three-way complex with T-cap only at the periphery of the Z-disk.

Acknowledgements

This work was supported partly by the 2004 Inje University Research Grant.

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